Chemoreception in invasive rusty crayfish (*Orconectes rusticus*): learning and adaptation in aquatic ecosystems of Northwestern Ontario

A thesis presented to

The Faculty of Graduate Studies

of

Lakehead University

by

Cassidy Douglas Weisbord

In partial fulfillment of requirements
for the degree of
Master of Science in Biology

March 5th, 2011

Abstract

Crayfish utilize chemosensory cues, in addition to other sensory inputs, to mediate a variety of fundamental life processes. Exotic species, like the rusty crayfish (*Orconectes rusticus*), are known to employ a broader range of chemosensory stimuli owing to their superior adaptability and behavioural plasticity relative to native crayfish species. The ability to respond rapidly to changing biotic and abiotic conditions contributes to the successful establishment of many introduced species in newly adopted ecosystems. I report two behavioural studies designed to measure chemically mediated associative learning, and environment-specific chemical cue utilization, in rusty crayfish. I found that rusty crayfish could quickly and easily form a learned attraction to a walleye (*Sander vitreus*) egg cue when paired with a food stimulus using a single, two-hour exposure. I also found that rusty crayfish from two ecologically distinct habitats responded differently to sympatric *v.* allopatric conspecifc injury cues. Specifically, both populations tested were attracted to injury cues from a lake where crayfish were likely to cannibalize with higher frequency, but showed no response to the same cue from the other study lake. My results help describe how aquatic invasive species use chemical information in their environment to facilitate adaptive responses and survival in new and unfamiliar ecosystems. Observations are discussed in the context of relevant literature and theory.

Dedication

To my grandfather, Sydney Weisbord, and my Mom, Susan Cannon. Thank you for the quiet strength.

Acknowledgements

I offer my deepest and most sincere appreciation to my supervisor Dr. Greg Pyle, whose unconditional support and encouragement has helped foster in me a standard of methodological rigour that gives me an invaluable sense of professional confidence. Your passion for aquatic organisms, ecosystems, and applied science is infectious, and I am immensely grateful for having walked down this road together. I hope it leads us to new and exciting challenges ahead. I thank my committee members, Dr. Joe Carney and Dr. Stephen Hecnar for your suggestions and guidance during this study.

My thanks go out to all members of the Aquatic Biotechnology and Ecotoxicology Laboratory (ABEL) of Lakehead University (LU) who offered valuable input and criticism at crucial steps in the evolution of this project. Particular recognition goes out to David T. Callaghan who was instrumental in the collection of data reported in these studies. I thank Dr. Walter Momot who, at my behest, provided mentorship and guidance of greater value than I could ever articulate. I am further grateful to my colleagues in graduate studies here at LU whose support, both purposeful and unintentional, has made this an all the more enriching experience.

Thanks are also due to Mr. John Charbeneau and the Atikokan Sportsman's Conservation Club as well as the Ontario Ministry of Natural Resources (OMNR), with special recognition to Mr. Sohail Siddiqui and the OMNR Dorion Fish Culture station for help procuring samples that helped make this study possible.

Finally, I offer heartfelt thanks to my friends and family who have been so immensely encouraging of my academic pursuits. In particular, I want to thank my Dad, Hyman Weisbord, who has reminded me that my foray in the biological sciences is only an adult manifestation of my childhood fascinations. I also thank Liane Kandler, my partner in academia as in life, your unconditional love and support as a fellow graduate student has made this experience so much more meaningful and enjoyable. Your energy and fervour for excellence are a constant inspiration to me. Lâche-pas!

Table of Contents

List of Tables

List of Figures

Chapter 1

Chapter 3

Chapter 4

Chapter 1. Introduction

Chemical stimuli provide crucial sensory information in the life of aquatic organisms. This chemical information governs many aspects of interspecies and intraspecies communications affecting such behaviours as feeding and prey location (Mackie 1973; Derby and Atema 1982, 1988; Tierney and Atema 1988; Hazlett 1994a; Steele *et al*. 1999; Adams and Moore 2003), predator avoidance (Hazlett 1994b; Hazlett and Schoolmaster 1998; Kats and Dill 1998; Acquistapace *et al*. 2004), kin or mate recognition and reproduction (Zulandt Schneider *et al*. 2001), as well as territory recognition, adoption and site fidelity (Foster 1985; Bronte *et al*. 2002; Mirza and Chivers 2002). Chemosensory acuity is especially important in low-light, benthic environments where inhabiting organisms rely heavily on chemical information, in the absence of other sensory cues, in service of food location, predator avoidance, and other social behaviour. Enhanced ability to use environmental chemosensory information to survival benefit, relative to native species, allows introduced (invasive) aquatic organisms to increase survival in new and unfamiliar water bodies (Hazlett 2000; Hazlett *et al*. 2002; Hazlett *et al*. 2003). Aquatic invasive species (AIS) demonstrate remarkable behavioural plasticity, which greatly facilitates colonization and diminishes the probability of extinction following introduction (Parker *et al*. 2001; Sakai *et al.* 2001; Allendorf and Lundquist 2003). This adaptability allows AIS to respond rapidly to novel biotic and abiotic conditions and varying degrees of propagule pressure (Kolar and Lodge 2001; Lockwood *et al*. 2005). Current research suggests that introduced populations can still adapt rapidly, in spite of losses in allelic richness and heterozygosity that might reduce adaptive potential, to overcome population bottlenecks at the outset of

colonization (Dlugosch and Parker 2008). The survival advantages conferred to AIS through chemosensation, chemically-mediated behaviour, and associative learning processes, allow them to overcome novel pressures while competing for, and capitalizing on, novel resources (Dickinson 1980; Hazlett 1994a; 1994b; Hazlett and Schoolmaster 1998; Hazlett *et al.* 2002; Hazlett 2003; Hazlett *et al.* 2003; Pecor *et al*. 2010). Understanding the factors underlying behavioural plasticity and the role of chemically mediated behaviour, informs on the subtle, and sometimes not so subtle, ways by which AIS exert an ecological impact. This information is crucial to the efforts of conservation biologists charged with the prevention and management of introduced invasive species populations (Allendorf and Lundquist 2003).

Of the more than 350 species of crayfish in North America, sixty-five belong to the genus *Orconectes*. In general, crayfish are keystone species in benthic invertebrate communities, accounting for 40-60% of the total zoobenthic biomass in both lentic and lotic freshwater habitats (Dorn and Wojdak 2004; Dorn and Mittelbach 1999; Momot 1995; Momot *et al*. 1978). Keystone species are characterized as having a considerable impact on their habitat relative to their respective biomass (Paine 1995). The rusty crayfish (*Orconectes rusticus*, Girard 1852), native to the Ohio River basin (Momot *et al*. 1978), has colonized surrounding waters in the United States (Wilson *et al*. 2004; Olden *et al*. 2006; Bobeldyk and Lamberti 2008) and is now considered an invasive species in Canadian watersheds (Momot *et al*. 1988; Wilson *et al*. 2004; Lake Simcoe Science Advisory Committee 2008; Phillips *et al.* 2009; Phillips 2010). Rusty crayfish settle on a variety of substrates including rock, sand, gravel, silt and clay; they are generally non-burrowing, but do prefer refuges that offer rocks, logs and other debris as cover (Phillips

2010). Rusty crayfish often colonize the littoral zone thereby assimilating resources and competing directly with other sympatrically distributed species (Wilson *et al*. 2004) Above all, *O. rusticus* demonstrates a high degree of adaptability to new environments owing to their aggressive behaviour, ability to out-compete native crayfish species and, higher metabolic rate and appetite than native crayfish species (Jones and Momot 1983; Phillips *et al*. 2009; Phillips 2010). Consequently, rusty crayfish grow larger, hide less from predators, and feed more rapaciously than native crayfish in Northern Ontario. In addition, invasive crayfish species such as *Procambarus clarkii* (Girard 1852), *Orconectes limosus* (Rafinesque 1817) and *O. rusticus* are sensitive to a broader range of heterospecific and conspecific danger signals, than native crayfish species (Hazlett 2000; Hazlett *et al.* 2003). This heightened sensitivity to hemolymph derived "alarm cues" (Acquistapace *et al*. 2005) confers upon *O. rusticus* an improved capacity for danger detection relative to locally adapted indigenous crayfish. Increased sensitivity to diverse alarm cues increases the probability of avoiding predation (Mathis and Smith 1993), that could contribute to successful territory adoption and adaptability to novel predators. Moreover, Hazlett *et al.* (2002) established that invasive crayfish species have a better memory, presenting greater retention for learned indicators of predation risk relative to native species after a two-hour exposure to a paired alarm cue with a novel predator odour. Collectively, these attributes confer upon *O. rusticus* many survival advantages and help to illustrate why invasive species can be so successful at out-competing locally adapted heterospecifics and quickly reaching high population densities (Stein 1977; Schweitzer and Larson 1999).

Though generally considered opportunistic generalist omnivores, a more accurate characterization of most crayfish would be preferential carnivores that, in searching for and consuming animal protein, demonstrate incidental detritivorous and herbivorous feeding tendencies (Momot 1995). Crayfish like *O. rusticus* have been implicated in the decline of fish populations through egg predation and destruction of macrophyte beds that serve as juvenile fish habitats (Horns and Magnuson 1981; Chambers *et al.* 1990; Dorn and Mittlebach 1999; Dorn and Wojdak 2004; Jonas *et al*. 2005; Ellrott *et al*. 2007). Some research suggests that persistent spawning-associated metabolites may serve as chemical labels to fish species that exhibit a high degree of perennial spawning site fidelity (Foster 1985; Bronte *et al*. 2002). These same info-chemicals may also serve as feeding cues to aquatic organisms that practice fish egg predation such as various benthic fish and invertebrates. Crayfish are known to modify the structure and composition of the littoral zone, which can readily impact macrophyte, macroinvertebrate, as well as, fish communities (Chambers *et al.* 1990). By unfortunate coincidence, the lentic and lotic shallow waters that *O. rusticus* colonize in Northern Ontario are characteristically very similar to the spawning grounds perennially used by scatter-spawning species such as lake trout (*Salvelinus namaycush*, Walbaum 1792) or walleye (*Sander vitreus*, Mitchill 1818) (Scott and Crossman 1973; Foster 1985; Hara 1994; Gunn 1995). Therefore, scatter-spawning fish species that favour littoral spawning sites, and reproductive salmonids that build nests in shallow streams, may face considerable risk to recruitment when living sympatrically with rusty crayfish. Many species of crayfish including rusty crayfish are already known to affect a wide variety of other sympatric organisms within the littoral food web. Existing studies have described significant impacts on littoral

congeners, amphibians, macrophytes, gastropds, macroinvertebrates, periphyton, as well as filamentous algae (Creed 1994; Lodge *et al*. 1994; Perry *et al*. 1997; Nyström *et al*. 2001; McCarthy *et al*. 2006; Phillips *et al*. 2009; Olsen *et al.* 2011).

Fitzsimons *et al.* (2002) estimated that lake trout egg consumption by crayfish (*Orconectes* spp.) for a standardized 30 day period after the date of peak spawning, ranged from 0 – 65 eggs per m^2, or as much as 82% of the potential egg abundance at eight established spawning reefs in Lake Ontario, Canada. Research by Claramunt *et al*. (2005), reporting on the relationship of interstitial lake trout egg predator density to egg mortality, supports the notion that eggs are most vulnerable just after deposition and that latency after deposition is directly proportional to decreasing egg mortality. Claramunt *et al*. (2005) reported that egg mortality was extremely high early in the spawning period such that 40% of eggs were lost after two days, and 80% of eggs were lost after two weeks of seeding into a protected, near shore spawning area in Lake Michigan, USA. Similarly, Dittman *et al.* (1998) established that chemical cues, and not visual cues, emanating from salmon eggs (*Oncorhynchus* spp.) act as the putative stimuli driving egg predation by coastrange and slimy sculpin (*Cottus aleuticus*, Gilbert 1896 and *C. cognatus*, Richardson 1836). Moreover, this study established a narrow window of approximately 24 hours during which sculpins used chemosensory cues to detect and consume newly fertilized salmon eggs. This study further suggested that attractive substances were likely derived from egg materials relative to other spawning associated metabolites by demonstrating no attraction to gravid female ovarian fluid. This was among the first studies to look at chemosensory cues involved in egg predation. Leading from this study, Mirza and Chivers (2002) looked at attraction of slimy sculpin to

chemical cues derived from brook charr (*Salvelinus fontinalis*, Mitchill 1814) eggs. They reported that slimy sculpin showed a distinct attraction to egg rinse solutions over control water under laboratory conditions; sculpin were equally attracted to freshly fertilized and water hardened egg rinse odour, but did prefer injured egg water to hardened-egg water. These findings also suggest that fish egg predators such as *Cottus* spp. use chemical cues to locate and consume salmonid eggs. Mirza and Chivers (2002) also suggest, that chemical cues alone, believed to be more persistent in aquatic environments (Hara 1994), might provide more reliable information than visual or mechanical cues when preying upon fish eggs. Salmonids often build their nests (termed "redds") atop underground springs, which help oxygenate eggs via upwelling (Scott and Crossman 1973). This tendency may, in turn, facilitate the dispersal of sufficient concentrations of egg-derived chemical cues into the environment to attract egg predators. By detecting these cues, fish egg predators such as crayfish may be quite successful at locating redds and preying upon fish eggs contained within. As crayfish macerate eggs in the process of consumption, additional environmental cues may be released analogous to the responses to damaged egg cues observed in Mirza and Chivers' (2002) study. Consequently, more egg predators may be attracted to the nest and hasten the rate of egg consumption. Ultimately, benthic invertebrate egg predators may be more successful in using the same chemical cues as vertebrates to locate and consume fish eggs by virtue of their chemosensory acuity.

A growing body of knowledge is uncovering the significant role that chemical information plays in mediating spawning site fidelity, spawning behaviour, as well as fish egg predation. Research suggests that persistent, spawning-associated metabolites may serve as site labels in fish species that exhibit a high degree of perennial spawning site

fidelity (Foster 1985; Dittman *et al*. 1998; Yambi and Yamazaki 2001; Zhang *et al.* 2001; Bronte *et al*. 2002; Doving *et al*. 2006). These same info-chemicals may also serve as chemoattractants to invertebrate fish egg predators in the same way they attract vertebrate egg predators (Ditman *et al*. 1998; Mirza and Chivers 2002). Understandably then, as *O. rusticus* continues to adopt sympatric distributions with indigenous fish species of Northern Ontario, a better understanding of chemical signals mediating egg predation, potentially the very same involved in spawning site imprinting and fidelity, is increasingly warranted. Understanding how crayfish use chemical cues to prey upon fish eggs could help inform remediation strategies to mitigate impacts on native fish populations. In order to properly study chemically mediated behaviour in crustaceans such as crayfish, an appreciation of the physiology and sensitivity of the chemosensory system is useful.

Decapod crustaceans are good candidate organisms for chemosensory research owing to the well-understood morphological, physiological, and functional features of their sensory biology (Ache 1982). Antennules are generally accepted as the principal chemosensory receptors in crustaceans, and are functionally very similar to the olfactory systems of terrestrial arthropods and vertebrates (Ache 1982; Anderson and Ache 1984; Carr *et al*. 1987). These olfactory structures interact with the environment via aesthetasc sensilla, which project from the cuticle wall along the lateral filaments of the crustacean's biramous antennules (Figure 1). Each antennule-based sensillum is innervated by cilia-bearing dendrites of bipolar olfactory receptor neurons (ORN) housed within the protective cuticle. The cuticle is perforated by microscopic pores, which allow the passage of odour molecules from the environment to the olfactory system (Farbman

1992). Antennules are distinctly different from antennae, which play a role in olfaction but are principally considered tactile organs. Additional chemosensory sensilla are found on the maxillipeds, chelae, and pereiopods of decapod crustaceans (Carr *et al*. 1987).

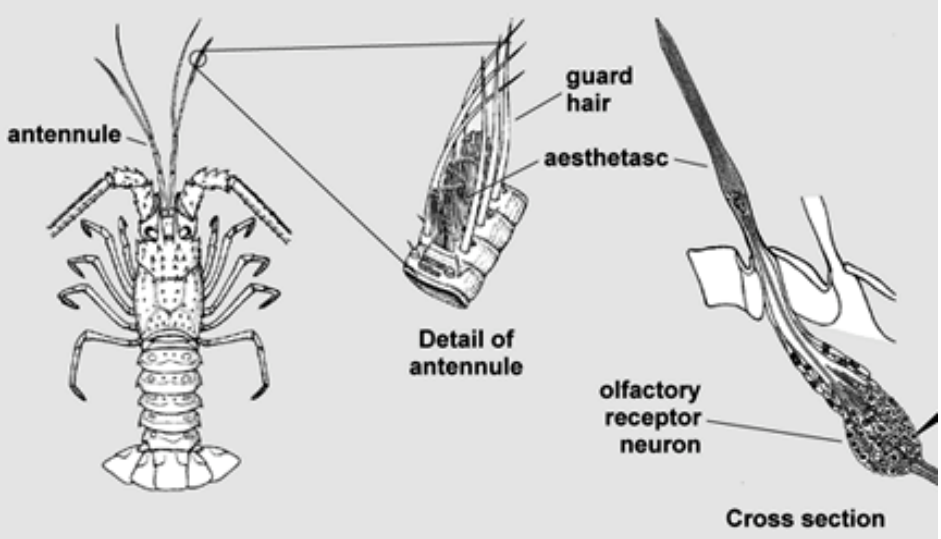


Figure 1. Illustration of decapod crustacean chemosensory macro, micro and ultrastructures (adapted from Ache 2003).

When an odour molecule interacts with a receptor molecule on the cilia, a signal transduction cascade is activated leading to the opening of ion channels across the ORN cell membrane. The opening of ion channels causes an influx of positive ions (usually sodium or calcium) and initiates an extracellular depolarization across the cell membrane, which ultimately generates an excitatory response or action potential (Farbman 1992). The action potential carries the olfaction-generated nerve impulses to the central nervous system, and can perform at a rate of discharge proportional to stimulus concentration (Carr *et al*. 1987; Buch *et al*. 1991; Farbman 1992). The chemoreceptive neuron

effectively acts as a transducer, converting the chemical information derived from receptor-analyte interaction into electrical impulses through the aforementioned mode of action (Buch *et al*. 1991). Furthermore, antennular chemoreceptors have a very low detection threshold capable of perceiving certain amino acids at picomolar concentrations (Thompson and Ache 1980). Studies generally support a link between chemoattraction to amino acids and food detection in decapod crustaceans (Johnson and Ache 1978; Ache 1982; Zimmer-Faust *et al*. 1984; Johnson and Atema 1986; Tierney and Atema 1988). Chemosensory cues are a driving force in predator prey interactions among aquatic species (Kats and Dill 1998), but also govern a well-established link between olfaction, prey location and feeding in decapod crustaceans (Ache 1982; Derby and Atema 1982; Tierney and Atema 1988; Hazlett 1996; Dittman *et al*. 1998).

While the previously mentioned studies provide some circumstantial evidence that *O. rusticus* is a fish egg predator, little is known about the potential shared usage of chemical cues as spawning site labels in fish and feeding cues in crayfish. Furthermore, although the aptitude for fish egg predation by species such as sculpins (*C. cognatus* and *C bairdi*), round goby (*Neogobius melanostomus*, Pallas 1814), and crayfish (*Orconectes* spp.) has received some study, the response of crayfish to fish egg cues has received little attention. Given the potential impact rusty crayfish may have on sympatric fish populations, studying the subtle motivating influences of egg predation serves to describe potential pressures on recruitment in relevant aquatic species.

From this reasoning, a project was designed to determine whether or not rusty crayfish were attracted to dissolved molecular components (< 0.45 μm) in egg cues from various potentially sympatric fish species. These efforts culminated in the preliminary

trial work reported in Chapter 2. From what was learned during this first study, two subsequent projects were developed and are reported in Chapters 3 and 4. Respectively, they explore associative learning and conditioned behavioural response in rusty crayfish to walleye egg cues, and the interaction of environment and life history on selective utilization of conspecific injury cues in two isolated rusty crayfish populations. Relevant discussion and background are offered in each of Chapters 3 and 4 while final comments and discussion are presented in Chapter 5.

Chapter 2. Preliminary trials.

2.1 Summary

To investigate whether or not crayfish were attracted to fish egg cues, male rusty crayfish were collected and maintained under laboratory conditions as reported in Chapter 3.3.1. Male *O. rusticus* were chosen in keeping with existing chemosensory behavioural research methods suggesting that ecologically relevant observations can be made using males alone (Adams and Moore 2003; Acquistapace*, et al*. 2004). However, the crayfish population used for preliminary trials were collected in the summer of 2009, and communally held at high densities (150-200 crayfish/ 70 L holding tank) without physical or mechanical isolation from each other. Consequently, high incidences of cannibalism (generally related to moulting of in-tank conspecifics) led to a steady decline in the population collected and maintained for this study. This challenge to maintaining a stable experimental population fostered the development of improved husbandry strategies as reported in Chapter 3.3.1. From the available population, a crayfish was randomly chosen for each behavioural trial (described in Chapter 3.3.2) and then returned to the population following each trial set. This procedure ensured that each crayfish was not reused during a trial set but could be selected during subsequent trial sets. Fish egg cues were generated from walleye (*Sander vitreums*), lake trout (*Salvelinus namaycush*), and brook trout (*Salvelinus fontinalis*) as detailed in Appendix A.1 (SOP: Egg rinse preparation). Fertilized and unfertilized walleye eggs were obtained in the spring of 2009 from the Atikokan (Ontario, Canada) Sportsman's Conservation Club in conjunction with annual wild fish collection and spawning activities. Fertilized and unfertilized lake trout and brook trout eggs, used to generate salmonid egg cues were obtained in the fall of

2008 during annual spawning activities carried out at the Ontario Ministry of Natural Resources, Dorion Fish Culture station (DFSC). The latter, therefore, were from hatchery populations.

A series of food cue trials were conducted to ensure I could measure a behavioural response to exogenous chemical stimuli in my experimental population. Results from these trials are reported in Chapter 3.3.4 and provided a good baseline characterization for an attraction response pattern in my rusty crayfish population.

Data were analyzed as reported in Chapter 3.3.6. All statistical tests conducted were subject to appropriate power analyses to ensure my measures were robust and responses could be measured amid highly variable data. Wherever significance was declared, all statistical tests had a probability of 80% or greater of rejecting the null hypothesis when in fact it should be rejected.

Based on findings by Mirza and Chivers (2002), who established that damaged egg cues were more attractive to egg predators than cues from intact fish eggs, I first tested filtered, damaged lake trout and walleye egg cues (n = 16 and 20 respectively). I found however, that crayfish showed no response to either cue according to any of the employed behavioural endpoints (described in Chapter 3.3.2; first choice, last position, time in the stimulus *v.* control arms, and latency to first arm choice, $P > 0.05$). Given the possibility that the filtering process may have removed putative chemoattractive components from my egg rinses, I further decided to test some additional egg cues kept on hand that had not been filtered prior to freezing. I therefore tested an unfiltered cue generated from intact water hardened brook trout eggs (n = 20). Again, no response was

measured according to any behavioural endpoints (first choice, last position, time in the stimulus *v.* control arms, and latency to first arm choice, $P > 0.05$).

Because none of the fish species I used to generate egg cues are present in Pounsford Lake, from which my experimental population of crayfish had been obtained, I wondered if, and how, learning could play a role in obtaining behaviourally relevant information from novel chemosensory stimuli. This objective lead to the study presented in the following chapter. I also wanted to see how separate, geographically isolated populations of rusty crayfish species might respond differently to stimuli of crucial survival importance such as conspecific "alarm cues." This objective culminated in the study reported in Chapter 4.

Chapter 3. Associative learning in male rusty crayfish (*Orconectes rusticus*): conditioned behavioural response to a walleye (*Sander vitreus*) egg cue

3.1 Abstract

Chemical information mediates communication and learning in aquatic organisms. Crayfish use chemoreception to establish social hierarchies, avoid predation and locate food resources. The means employed by crayfish to locate and consume fish eggs is only circumstantially understood. Fish eggs release recognizable chemoattractants for vertebrate egg predators that may motivate crayfish to engage in egg predation. I hypothesized that male rusty crayfish (*Orconectes rusticus*), from a walleye-free (*Sander vitreus*) lake would not possess an innate recognition of a walleye egg cue. However, if conditioned by employing a single two-hour paired stimulus exposure (known food cue + egg cue), then male *O. rusticus* would be attracted to the same egg cue upon subsequent exposure. A Y-maze behavioural arena was used to assay crayfish response to walleye egg cue before and after conditioning. Indices of behavioural response included: first choice, final position, time in the stimulus *v.* control arms, and latency to first arm entry. Once conditioned, crayfish took significantly less time to choose the arm containing the egg cue alone relative to a control. Results from this study suggest that male *O. rusticus* quickly and easily learn to identify novel odour stimuli from fish eggs.

3.2 Introduction

Chemoreception and chemical stimuli provide crucial sensory cues to a diverse array of aquatic taxa (Carr 1988; Rittschof 1992; Hara 1994; Kats and Dill 1998; Krieger and Breer 1999). This chemical information governs many aspects of intra- and inter-species communications affecting such behaviours as prey location and feeding, predator avoidance, kin, mate or territory recognition, reproduction, and social hierarchy (Foster 1985; Lima and Dill 1990; Bronte *et al*. 2002; Simon and Moore 2007; Aquiloni and Gherardi 2010). This is especially true in low-light, benthic environments where inhabiting organisms rely on chemical information in the absence of other sensory cues or where resources must be located from afar (Mackie 1973; Derby and Atema 1982; Tierney and Atema 1988; Steele *et al.* 1999). Superior behavioural plasticity relative to native species allows aquatic invasive species to improve survival and facilitate territory adoption in new and unfamiliar ecosystems (Hazlett *et al*. 2003).

Aquatic invasive species cause acute and pervasive biodiversity consequences in the ecosystems they invade (Strayer 2010). Canada is currently facing several invasive fresh and saltwater crustaceans including the European green crab (*Carcinus maenas* L. 1758) and rusty crayfish (*Orconectes rusticus*, Girard 1852) (DFO 2010). Crayfish are ubiquitous members of lentic and lotic freshwater invertebrate communities accounting for 40-60% of the total zoobenthic biomass (Momot *et al*. 1978; Momot 1995; Dorn and Mittelbach 1999; Dorn and Wojdak 2004). Though generally considered opportunistic, polytrophic, generalist predators (Hobbs 1993), crayfish are far from being indiscriminate omnivores. They are more accurately characterized as preferential carnivores that, in searching for and consuming animal protein, demonstrate incidental detritivorous and

herbivorous feeding tendencies (Momot 1995). Through their rapacious foraging habits, crayfish have an incredible capacity to modify their habitat that may in turn exact an ecological impact with potentially far reaching trophic cascades (Flint and Goldman 1975; Chambers *et al.* 1990). *Orconectes rusticus*, native to the Ohio River basin (Momot *et al*. 1978), has heavily colonized surrounding waters in the United States (Olden *et al*. 2006; Bobeldyk and Lamberti 2008) and is now considered invasive in the boreal aquatic ecosystems of Northern Ontario (Momot *et al*. 1988; Wilson *et al*. 2004; Lake Simcoe Science Advisory Committee 2008; Phillips *et al.* 2009; Phillips 2010). Invasive crayfish species like *O. rusticus* often demonstrate a high degree of adaptability to new environments owing to their aggressive behaviour, superior memory, adaptability to new food resources, higher metabolic rate and hence appetite, sensitivity to a broader range of chemical alarm signals, and superior behavioural plasticity relative to native crayfish species (Jones and Momot 1983; Hazlett 2000; Hazlett *et al*. 2002, 2003). In addition, female *O. rusticus* are able to lay eggs at much lower temperatures relative to congeners giving them a seasonal population growth advantage (Momot 1966; Aiken 1968; Berrill and Arsenault 1982). Collectively, these attributes confer upon *O. rusticus* many survival advantages, and may help explain consequential native species displacement, as well as successful, ongoing, range expansion (Schweitzer and Larson 1999). Moreover, the shallow waters preferred by crayfish like *O. rusticus* are commonly used by fish species as spawning grounds and where fish eggs and young are particularly vulnerable to predation (Dorn and Wojdak 2004).

Crayfish like *O. rusticus* have been implicated in the decline of fish populations through egg predation and destruction of macrophyte beds that serve as nest sites,

spawning grounds, and juvenile fish nurseries (Horns and Magnuson 1981; Chambers *et al.* 1990; Dorn and Mittlebach 1999; Dorn and Wojdak 2004; Jonas *et al*. 2005; Ellrott *et al*. 2007). Through active foraging, crayfish modify the structure and composition of the littoral zone to the detriment of macrophyte, macroinvertebrate, and ultimately, sympatric fish communities (Chambers *et al.* 1990; Olsen *et al.* 1991; Phillips *et al.* 2009). While some studies suggest that fish eggs release chemoattractants that are enticing to vertebrate egg predators (Dittman *et al*. 1998; Mirza and Chivers 2002; Fitzsimons *et al*. 2006, 2002), their usage by invertebrate egg predators like crayfish remains less understood. Perhaps the same "info-chemicals" that motivate vertebrate fish egg-predators also motivate crayfish to prey upon fish eggs. Identifying which adaptive behaviours drive invertebrate fish-egg predators will help promote a better understanding of the more subtle means by which *O. rusticus*, and aquatic invasive species in general, exert their ecological impacts.

There are various learning mechanisms by which organisms can use past experience to modify behaviour. Associative learning is the process by which an organism forms an association by simultaneously experiencing two sensory stimuli such that subsequently experiencing one helps to then recall the other (Dickinson 1980). This process is well-documented in relation to recognition of novel predators (Hazlett and Schoolmaster 1998; Hazlett 2003) and adoption of new food source (Hazlett 1994a) in both *Orconectes* sp. and *Cambarus* sp. crayfish.

The following study was designed to measure the conditioned behavioural response of *O. rusticus* to unfamiliar chemical stimuli. I hypothesized that a male *O. rusticus* population, with decades of existence without walleye (*Sander vitreus*), would

possess no innate recognition to the smell of walleye eggs. However, once associated with an established food source through a single paired-stimulus conditioning event, these same crayfish would be attracted to the same egg cue upon subsequent exposure. While many existing studies have looked at various crayfish behavioural responses to various single or paired chemical stimuli in terms of change in posture (Hazlett 1994b), grooming or nonlocomotory movement (Hazlett and Schoolmaster 1998), this study sought to characterize behavioural response in terms of choice, speed of choice as a measure of interest, and appeal of test stimuli (Adams and Moore 2003).

3.3 Materials and Methods

3.3.1 Crayfish collection and holding

Male rusty crayfish, *Orconectes rusticus,* were collected (Appendix A.2 SOP: Collection and transportation of rusty crayfish) from Pounsford Lake (48º 29.5274' N, 88º 46.3475' W) in Sleeping Giant Provincial Park, Ontario in spring of 2010. Female crayfish are generally egg-laden at this time of year and not actively foraging, hence, only males were principally caught and retained for study. Male *O. rusticus* alone have also been used successfully in chemosensory behavioural research (Adams and Moore 2003; Acquistapace*, et al*. 2004). To control for variability in size class and life history in my experimental population, a random sample of 150 fully intact crayfish were retained from the 700 crayfish that were trapped. These 150 crayfish (mean weight, cephalothorax length $\pm$ SD; 12.1 $\pm$ 3.3 g, 34.3 $\pm$ 3.0 mm) were transported to Lakehead University (Thunder Bay, ON) in aerated native lake water, and acclimated to laboratory conditions for two weeks prior to experimental use. Fifty to sixty crayfish were communally held in flow-through (3 L/min.$^{-1}$) 70 L laundry tubs, supplied with supplemental aeration, and a

submersible power head to facilitate water mixing and turnover. Each individual crayfish was housed in a numbered green plastic (10 cm ID) flowerpot, covered with a flowerpot saucer, and held closed with an elastic band. Drainage perforations in the bottom allowed for adequate water exchange. Crayfish were held at 20 (±1)º C, provided a photoperiod of 16:8 L:D and fed weekly into each flowerpot with commercial trout pellet food (Unifeed, Pro-Form Aquapride, 5 mm) to provide nutrition but also create a learned association with the odour as a food source (Hazlett 1994a; Hazlett and Schoolmaster 1998). Thus, crayfish were provided three independent feedings, and fasted for 48 hours, prior to experimental use.

3.3.2 Y-maze assay

A static Y-maze behavioural arena (74 L X 39 W cm, Appendix A.3 SOP: Manual static Y-maze assay) was used to test crayfish response to chemical stimuli before and after a paired-stimulus conditioning event. A corrugated plastic barrier running two thirds of the arena length provided chemical separation of the stimulus and control arms. A clear barrier, perforated to allow chemical exchange, separated an acclimation zone from the two arms of the Y-maze. Stimuli and controls were randomly assigned to either the left or right delivery arm for each trial, and observers were not aware which side contained the stimulus. All experimental systems were visually isolated, and observers recorded behaviour through a small (4 X 10 cm) horizontal viewing window. Trials were conducted by first filling the arena with 10 l of holding temperature-matched, dechlorinated Thunder Bay municipal water. A randomly selected crayfish was carefully delivered into the acclimation zone and left for 20 minutes to adjust to maze conditions. Following acclimation, 20 ml of both stimulus and controls

were gently delivered to each opposing end of the delivery arms using a 50 ml syringe connected to airline tubing and a 9 cm Pasteur pipette, operated remotely from behind a visual barrier. Preliminary trials using commercial food colouring revealed that aqueous mixtures delivered to one end of either arm diffused to the acclimation chamber gate within 5 minutes. Following a 5-minute stimulus delivery period, the perforated barrier was gently raised sufficient to allow the crayfish to pass through, and behavioural endpoints were recorded for an 8-minute observation period. Behavioural endpoints were chosen in keeping with Adams and Moore (2003) including: first choice, final position, time in the stimulus *v.* control arms, and latency to first arm choice. For a given trial, first choice was defined as the first arm (stimulus or control) that crayfish entered, while final position was defined as the final location occupied by the crayfish (stimulus, control, or acclimation chamber) following the 8 minute observation period. Y-mazes were thoroughly washed using a mild detergent (Sparkleen, Fisherbrand) solution and rinsed with dechlorinated water following each trial set to ensure no stimulus contamination would confound subsequent trials.

3.3.3 Walleye egg cue

A fish egg cue was produced following Mirza and Chivers (2002) to approximate odour signals given off by walleye (*S. vitreus*) eggs from spawning grounds. Minor methodological modifications were employed to address standardization and chemical variability from single male-female pairings during artificial fertilization. Fish egg stimulus was produced in conjunction with the annual walleye spawning activities of the Atikokan Sportsman's Conservation Club (Atikokan, Ontario). Gametes used for spawning were from wild fish, and fertilized eggs came from an artificial fertilization

using the roe of 5 females and the milt from 3 males. Eggs were not "mudded" during the process of fertilization to provide for as unadulterated a stimulus as possible. Forty-five grams of freshly fertilized eggs were left to water harden in 2 l of dechlorinated water for 3 hours. After 3 hours, the water-hardened eggs were transferred to 1 l of dechlorinated tap water and left to soak for 30 minutes. Eggs were then removed and additional dechlorinated water was added to bring the final volume up to 5 l. This stock solution was brought back to the laboratory and frozen (-20º C) in 100 ml aliquots. A dechlorinated water control was treated, produced, and frozen in exactly the same way as the egg cue but without the egg soak.

3.3.4 Food cue

Food cue preliminary trials using the same Y-maze assay were conducted to ensure I could measure a behavioural response in my experimental population. A food cue was prepared by homogenizing 10 g of commercial trout pellet food in 500 ml dechlorinated water. The homogenate was stirred for 20 minutes, and then filtered through coarse filter wool to remove any particulate matter. Food cue was produced and used daily for behavioural trials. During preliminary trials, crayfish spent significantly more time in the food cue arm when paired with a dechlorinated water control (paired *t*-test; $t = 2.13$, df = 28, $P = 0.042$). This suggested an established recognition of, and attraction to, dissolved components from the trout food as a result of feeding experience (Tierney and Atema 1988).

3.3.5 Conditioning procedure

Conditioning procedures were modeled on the works of Hazlett *et al.* (2002, 2003). Crayfish were conditioned together for two hours using a paired-stimuli exposure (walleye egg cue + food cue) in a 100 l tank of dechlorinated water (20º C). Each crayfish was visually and mechanically isolated in its flowerpot enclosure and the tank containing all flowerpot enclosures was provided supplemental aeration to facilitate mixing. Once introduced into the tank, crayfish were left to acclimate for 1 hour. Following acclimation, 200 ml of each paired stimulus (walleye egg cue + food cue) was slowly added to the tank. Ninety minutes later, a second 200 ml aliquot of each stimulus was added in the same manner and the crayfish were left for another 30 min. Following conditioning, animals were returned to their holding tanks. Behavioural trials were conducted 48 hours following conditioning.

3.3.6 Analysis

A paired *t*-test (paired-*t*) or Wilcoxon signed rank test (*V*) was used to compare mean time crayfish spent in the stimulus *v*. control arms, while a two sample *t*-test (*t*) or a Wilcoxon rank sum test (*W*) with continuity correction was used to evaluate differences in mean latency to initial arm selection, depending on normality of the data. A chi-square (χ^2) test was used to compare first arm choice and, last position within the Y-maze following the observation period, with a respective 50:50 and 33:33:33 expectation due to random chance alone. For all analyses, results were considered statistically significant when $P < 0.05$. All statistical analyses were conducted in R version 2.10.1 (R Development Core team 2009).

3.4 Results

As expected, crayfish showed no response to the walleye egg cue prior to conditioning according to any measured endpoints (first choice, last position, time in the stimulus *v.* control arms, and latency to first arm choice, $P > 0.05$, $n = 20$). The Pounsford Lake rusty crayfish population has no life history experience with olfactory cues from walleye. Hence, chemical stimuli associated with walleye life processes are not likely to provide behaviourally relevant information to the extant crayfish population. Once conditioned however, the same crayfish took significantly less time to choose the walleye egg cue arm than they took to choose the control arm ($t = 3.04$, df = 16, $P = 0.007$). Following conditioning, crayfish took on average, 17 seconds to choose the stimulus arm and two minutes to choose the control arm (Figure 2). Other behavioural responses did not vary significantly between the stimulus and control arms due to a high degree of variability in the measured endpoints (first choice, final position, time in the stimulus *v.* control arms, $P > 0.05$).

3.5 Discussion

Results from this study confirm my hypothesis regarding the behavioural response of conditioned male *O. rusticus* to a walleye egg cue. Male *O. rusticus* can quickly and easily form a learned association between a novel odour stimulus (walleye egg cue) and a known food cue. Once formed, this learned association results in attraction. By extension, once an association is formed between olfactory and gustatory stimulation through walleye egg contact, likely during random foraging, male *O. rusticus* should readily respond with attraction to egg cues from walleye spawning grounds under field

conditions. These results are consistent with Hazlett (1994a) who successfully conditioned *O. rusticus* to produce a feeding response to the novel food odour of zebra

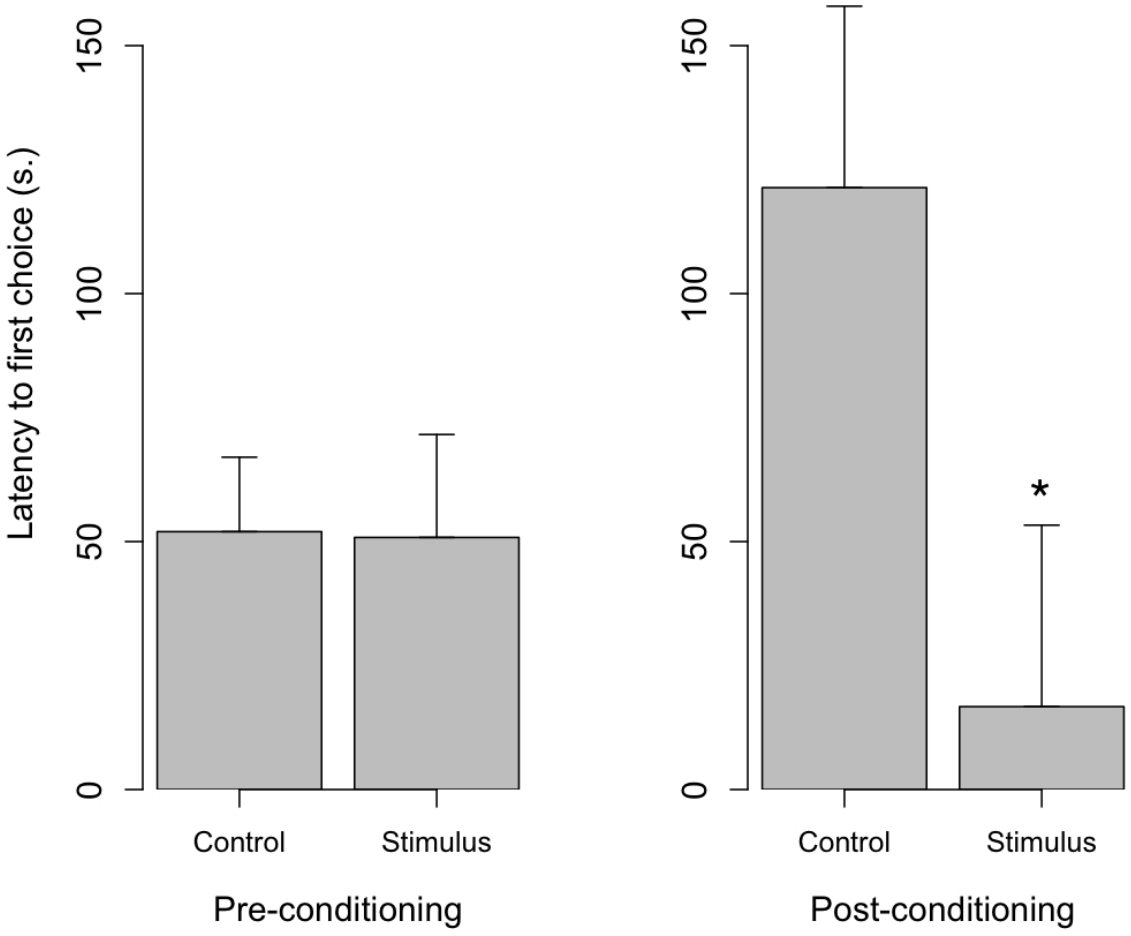


Figure 2. Mean + (SE) latency of rusty crayfish to first arm choice before (n = 20) and after a 2 hour paired-stimulus (food cue + walleye egg cue) conditioning event. Asterisk (*) denotes significant difference from control (*t*-test; $t = 3.04$, df = 16, $P = 0.007$).

mussel (*Dreissena polymorpha*, Pallas 1771). However, while Hazlett (1994a) proposed that the mechanism of learning involves the formation of an association between food odour and food taste, my results suggest that association of olfactory stimuli alone, is sufficient for associative learning to take place. The facility with which *O. rusticus* can learn new chemical signals in its environment highlights the adaptive benefit of associative learning seen in this study. Being an omnivorous, polytrophic forager, invasive crayfish like *O. rusticus* must be able to quickly adapt to changing resources over spatial and temporal gradients. While foraging in new environments, crayfish are exposed to numerous unfamiliar chemical signals. The learned association between an established food cue and unfamiliar (potential) food stimuli described in this study is only one possible mechanism by which *O. rusticus* form associations that allow them to capitalize on novel resources. The capacity for associative learning confers an advantage to the individual (or species) that possesses this ability by allowing them to adapt to changes in its environment crucial to its survival in contrast to the individual (or species) that does not possess this ability. Should *O. rusticus* come to prey upon walleye eggs, a learned association could occur that would increase *O. rusticus'* predisposition for any future chemically mediated homing to walleye spawning grounds.

This result has clear implications for recruitment or rehabilitation of walleye and other important sport fish in lakes invaded by *O. rusticus*. Pounsford Lake is unlikely to contain any extant populations of predator-guild species following failed Ontario Ministry of Natural Resources (OMNR) introductions of largemouth bass (*Micropterus salmoides* Lacépède, 1802) in the early 1950's (Momot *et al.* 1988), and of walleye

around 1964 (Werner 1983. A preliminary report on fisheries data for Sibley Provincial Park. Ontario Ministry of Natural Resources, Thunder Bay District, unpublished data).

The most recent account of walleye is from 1982 when two were caught during OMNR sampling activities (Werner 1983. A preliminary report on fisheries data for Sibley Provincial Park. Ontario Ministry of Natural Resources, Thunder Bay District, unpublished data). However, today, only a minimal largemouth bass population remains (personal observation). Many other lakes in Northern Ontario, currently under colonization, do however contain native walleye populations (Rosenberg *et al.* 2010). My study suggests that within these lakes, a single exposure event could suffice for *O. rusticus* to associate walleye eggs with food. Once formed, this association should persist (Hazlett *et al*. 2002) and additional encounters would reinforce this association (Dickinson 1980). However, learned associations may attenuate within 3 and 6 weeks (Hazlett *et al.* 2002).

Fitzsimons *et al*. (2002) estimated that lake trout (*Salvelinus namaycush* Walbaum in Artedi, 1792) egg consumption by crayfish (*Orconectes* spp.) for a standardized 30 day period after the date of peak spawning, ranged from 0 – 65 eggs consumed per m^2. This represented as much as 82% of the potential egg abundance at eight established spawning reefs in Lake Ontario. Moreover, in areas of low egg abundance (<100 eggs/m^2), characteristic of 5 of the 8 spawning sites observed, crayfish and sculpin (*Cottus* spp.) density was sufficiently high to cause nearly 100% mortality in lake trout eggs. Thus, scatter spawning species such as walleye and lake trout (Scott and Crossman 1973), that favour littoral spawning sites, risk high egg mortality when living sympatrically with *O. rusticus*.

While compelling, there was a high degree of variability in the data. This prevented any further response characterization according to the employed behavioural endpoints. Findings by Kraus-Epley and Moore (2002) suggest that bilateral and unilateral antennal lesions alter the orientation abilities of *O. rusticus* to chemosensory cues. Crayfish with complete or partial antennal lesions demonstrate an impaired ability to successfully orient to an odour source and underscores the importance of spatial comparison during orientation behaviour. In spite of being physically and mechanically isolated during holding, close inspection revealed that a small percentage of my study population possessed damaged antennules. Thus, these animals may have spent more time searching, and were less adept at orienting to the directional or concentration gradient of the odour plume within the Y-maze. Recent research by Gardiner and Atema (2010) suggests that because odour plumes show chaotic intermittency in the aquatic environment, bilateral time differences in stimulus detection trump odour concentration during orienting activities. Hence, over the course of the observation period, as mixing progressed at the interface of the arms and acclimation zone of the Y-maze, observed crayfish were progressively less able to rely on directional stimulus signals thereby obfuscating a clear response pattern.

Future studies might explore biochemical analysis to try and elucidate and characterize the putative attractive components released by fish eggs. In addition, determining whether *O. rusticus* are similarly amenable to associating fish egg cues from other valued game species (e.g., salmonids) with food, would serve to further expand the range of ecological impact exerted by such aquatic invasive species. Nonetheless, this is the first study to explore the role of associative learning in egg predation and provides

evidence to the use of chemical cues by invertebrate fish egg predators. It joins an ever-growing body of knowledge surrounding the intricate mechanisms by which aquatic invasive species interact with new ecosystems.

Chapter 4. Behavioural response of two rusty crayfish (*Orconectes rusticus*) populations to injury cues from sympatric and allopatric conspecifics

4.1 Abstract

Generalist predators like crayfish consume a variety of protein sources, including conspecifics. Cannibalism in aquatic food webs occurs in response to resource limitation, nutritional imbalance, intraspecific competition or predation, and population density increases. Many of these conditions confront aquatic invasive species within adopted ecosystems. Allopatric populations of these invasive species may utilize conspecific chemical signals in response to the unique conditions found in their respective environments. I hypothesized that two invasive rusty crayfish (*Orconectes rusticus*, Girard 1852) populations, living in two ecologically distinct lake systems (Whitefish and Pounsford), would differentially respond to chemical cues from injured allopatric and sympatric conspecifics. Crayfish behavioural response to chemical stimuli (first choice, final position, time in the stimulus *v.* control arms, and latency to first arm entry) was measured using a Y-maze assay. Neither population responded to stimuli from injured Whitefish Lake crayfish, however, both populations spent significantly more time in the arm containing the Pounsford Lake crayfish injury cue. These results suggest chemical cues alone can account for this discernable difference. The uniquely attractive nature of injury cues from injured Pounsford Lake rusty crayfish to both study populations reflects localized adaptation of the Pounsford Lake community. This may be consequential to changing selective pressures brought on by colonization.

4.2 Introduction

The rusty crayfish (*Orconectes rusticus*) is a crustacean native to the Ohio River basin (Momot *et al*. 1978; Olden *et al*. 2006). Originally documented 30 years ago, it is now invasive in the boreal aquatic ecosystems of Northern Ontario, Canada (Momot *et al*. 1988; Wilson *et al*. 2004; Lake Simcoe Science Advisory Committee 2008; Phillips *et al.* 2009; Phillips 2010). Understanding its ethology necessitates consideration of the role of sensory ecology in mediating various life processes and trophic interactions (Carr 1988). Chemosensory acuity is especially important to nocturnal, benthic organisms such as many crayfish, which rely primarily on chemical information relative to other sensory cues in search for essential resources (Mackie 1973; Derby and Atema 1982; Tierney and Atema, 1988; Steele *et al.* 1999). Crayfish social behaviour exploits chemosensory information extensively (Dunham and Oh 1992; Zulandt Schneider *et al*. 2001; Bergman and Moore 2005; Simon and Moore 2007; Berry and Breithaupt 2008; 2010; Aquiloni and Gherardi 2010). This is especially evident when evaluating predation risk (Hazlett 1994a, 2003; Hazlett and Schoolmaster 1998; Hazlett *et al*. 2003; Acquistapace *et al*. 2004), detecting food (Hazlett 1994b), or discerning the physiological condition of other crayfish (Hazlett 1985; Adams and Moore 2003). Invasive crayfish species display superior utilization of environmental chemosensory cues, relative to native species (Hazlett 2000; Hazlett *et al*. 2002; Hazlett *et al*. 2003). Chemically mediated behavioural plasticity allows invasive crayfish species to improve survival during the occupation of unfamiliar ecosystems. Phenotypic plasticity, rather than genetic diversity, may be a better short-term survival strategy for invasive species to overcome population

bottlenecks when the number of initial colonists or frequency of introductions is low (Sakai *et al*. 2001; Allendorf and Lundquist 2003).

Typically, aquatic organisms utilize chemosensory cues from injured conspecifics to assess predation risk (Kats and Dill 1998). Under laboratory conditions, crayfish demonstrate innate "anti-predator" behaviours when detecting chemical signals from crushed conspecifics paired with a food stimulus (Hazlett and Schoolmaster 1998; Hazlett 2007). Such behaviours are observed as a suppression of movement or feeding activity, with concomitant postural changes or increased shelter use. Chemically mediated activation of such behaviours increases the likelihood of survival in the receiver organism. Acquistapace *et al*. (2005) proposed that peptides in the hemolymph clotting processes provide the putative components in conspecific chemical "alarm cues" which prompt anti-predator behaviour.

In contrast, Adams and Moore (2003) demonstrated that intermoult male *O. rusticus* showed preference and attraction to conspecific moult cues, spending more time in the presence of a moult stimulus when paired with a control. During ecdysis, crustaceans undergo biochemical changes that facilitate assimilation of inorganic chemicals and ions involved in the loosening of the old, and generation of a new exoskeleton (Waddy *et al*. 1995). These changes include increased ecdysone and 20-hydroxyecdysone (Chang 1995), as well as hemocyanin and glucose concentrations, in the hemolymph (Galindo *et al*. 2009). Understandably, crayfish are particularly vulnerable to predation during ecdysis and hence may use chemical information from moulting conspecifics to exploit

cannibalistic opportunities (Adams and Moore 2003). Seemingly, components of chemical cues from injured conspecifics may warn crayfish of potential predation under certain circumstances, while signalling an easy meal under others. Response to conspecific chemical stimuli is specific to the physiological condition of other crayfish, may vary according to environment (i.e., habitat and diet) or life history, and is influenced by detection of other concurrent stimuli (Hazlett and Schoolmaster 1998; Adams and Moore 2003; Bergman and Moore 2005; Hazlett 2007).

Similar behavioural and physiological changes occur in other aquatic species populations in response to shifting community structure or resource availability. Walleye physiologically optimize their aerobic foraging behaviour when prey species are larger or more abundant (Kaufman *et al.* 2006). Metabolically, walleye become less active overall, but maintain aerobic capacities when chasing larger prey less frequently to satisfy energetic needs. Kaufman *et al*. (2006) suggest that behaviourally adapted species can modify their biochemical physiology when living under dissimilar community structures or practicing alternate feeding strategies. Seasonal variation in food abundance will modify crayfish diet (Abrahamson 1966; Guan and Wiles 1998). During summer when crayfish are most active, and resources plentiful, animal protein accounts for a significant proportion of the diet (30% whole dietary wet weight) (Guan and Wiles 1998). This observation corroborates with Momot (1995) who suggested that crayfish are preferential carnivores that consume detritus and plant material opportunistically in pursuit of animal protein. Because the summer represents the season of greatest population growth but also increased competition, crayfish will cannibalize at a frequency sufficient to satisfy their

energetic requirements. Persistent intraspecific predation may in turn modify the physiology of cannibalistic populations.

Cannibalism, ubiquitous in natural animal populations (Fox 1975; Elgar and Crespi 1994), often occurs in response to increased competition and resource limitation (Nyström and Granéli 1996). Intraguild predation (IGP), the act of killing and eating potential competitors, is widely observed across a diverse array of taxa and trophic levels under a variety of conditions (Polis *et al.* 1989; Holt and Polis 1997). Cannibalism, a form of IGP, can affect the distribution, abundance, and evolution of the species concerned. Intraspecific predation can often be related to fluctuating levels of environmental productivity, seasonal or otherwise (Polis *et al.* 1989; Elgar and Crespi 1994; Holt and Polis 1997). Because invasive species can rapidly impact the invaded ecosystem, possessing the ability to rapidly adapt to changes brought on by the process of colonization, is adaptively beneficial. While IGP is a well-recognized foraging strategy in terrestrial arthropods, and crayfish are well known to cannibalize (Nyström 2002; Wise 2006), very little is known about the role of chemosensation in mediating intraspecific predation in crayfish.

The following study measured the behavioural response of two *O. rusticus* populations, from two ecologically unique habitats, to injury cues from novel (allopatric) and sympatric conspecifics. I hypothesized that given differences in biotic and abiotic lake characteristics, and time since invasion, *O. rusticus* from these two lakes would respond differentially to stimuli from injured crayfish. To test this hypothesis, I collected *O. rusticus* from two lakes in the Thunder Bay District of Northern Ontario; Pounsford

Lake is a small, deep, oligotrophic lake with a well-established (~ 50 yrs.) crayfish population, and Whitefish Lake, a large, shallow, mesotrophic, and recently colonized lake (< 10 yrs) (Table 1). Existing studies have characterized crayfish behavioural response to conspecific "alarm cues" to illustrate anti-predator behaviour (Hazlett 1994b; Hazlett and Schoolmaster 1998). However, I was interested in the interaction of environment and population history on selective utilization of behaviourally relevant chemical stimuli in invasive species populations. Studies of population biology and sensory ecology can provide crucial ethological insights that inform conservation biologists on local adaptation and short-term evolution of both invasive and resident species alike (Allendorf and Lundquist 2003).

4.3 Materials and Methods

4.3.1 Crayfish collection & holding

Male rusty crayfish, *Orconectes rusticus* (Girard, 1852), were collected from Pounsford Lake (48º 29.5' N, 88º 46.3' W) in Sleeping Giant Provincial Park, and Whitefish Lake (48º 13.6' N, 89º 59.3' W) Ontario, in July of 2010. A random sample of 150 fully intact crayfish was retained from approx. 700 trapped in Pounsford Lake, while 90 fully intact crayfish were retained from approx. 400 trapped in Whitefish Lake. These 150 (mean weight, cephalothorax length $\pm$ SD; 12.1 $\pm$ 3.3 g, 34.3 $\pm$ 3.0 mm) and 90 (11.6 $\pm$ 6.6 g, 31.6 $\pm$ 5.0 mm) crayfish were separately transported to Lakehead University (Thunder Bay, ON) in aerated lake water, and acclimated to laboratory conditions for two weeks prior to experimental use. Forty to fifty crayfish were

Table 1. Comparison of limnological characteristics between Pounsford and Whitefish Lakes, Thunder Bay District, Northwestern Ontario.

	Pounsford Lake	Whitefish Lake
Approximate, linear inter-lake distance (km)	95	
Characteristics	small/deep, depauperate, oligotrophic [2]	large/shallow, biodiverse, mesotrophic [1]
Location	48º 29' N, 88º 46' W	48º 13' N, 89º 59' W
Elevation (m)	259	405
Volume (m3*106)	NA	60.3 [1]
Surface Area (ha)	127 [2]	3015 [3]
Mean Depth (m)	7 [2]	1.8 [3]
Max Depth (m)	11 [1]	6.4 [3]
alkalinity (CaCO3-mg/l)	61.6 [2]	NA
Total Nitrogen (mg/l)	NA	0.522 [1]
Total Phosphorus (mg/l)	NA	0.027 [1]
Total dissolved solids (mg/l)	83.25 [4]	66 [1]
Secchi depth (m)	4.3 [1]	1.5 [1]
Predator species	*Micropterus salmoides, Perca flavescens* [2]	*Micropterus dolomieu, P. flavescens, Esox lucius, Sander vitreus* [3]
Fishing pressure	minimal; Provincial park, motor-less watercraft only [5]	significant; motor boat access, lakeside urbanization [3]
Benthos	mud, silt, rock, gravel, minimal aquatic vegetation [1,2,3]	sand gravel, large macrophyte beds [1,3,5]
First *O. rusticus* reporting:	1985 [2] (see comments in text)	2003 [3]
Source of *O. rusticus*	OMNR fish introductions [2,4]	bait bucket introductions [3]
Orconectes virilis	extirpated [5]	present [3]

1. Quetico Milles Lacs Fish Assessment Unit. 1981. Whitefish Lake Synopsis. Northwest Science and Information Publications. Ontario Ministry of Natural Resources, District of Thunder Bay. Unpublished. 2. Momot *et al.* 1988. 3. Berube and Kraft 2010. 4. Werner, R. 1983. A preliminary report on fisheries data for Sibley Provincial Park. Ontario Ministry of Natural Resources, District of Thunder Bay. Unpublished. 5. Personal observation. NA: Not available.

communally segregated according to lake origin in flow-through (2 l/min.$^{-1}$, dechlorinated Thunder Bay municipal water) 70 l laundry tubs, supplied with supplemental aeration, and a submersible circulation pump to facilitate water mixing and turnover. Individuals were housed in numbered green plastic (10 cm ID) flowerpots, covered with a flowerpot saucer, and held closed with an elastic band. Drainage holes in the bottom allowed for adequate water exchange. This allowed us to mitigate injury from agonistic interaction, while controlling for exposure to conspecific injury cues during acclimation and holding. Crayfish were held at 20 ($\pm$1)º C, provided a photoperiod of 16:8 (L:D), fed weekly with commercial trout pellet food (Unifeed, Pro-Form Aquapride, 5 mm), and fasted for 48 hours prior to experimental use.

4.3.2 Stimuli

Injury cues were produced from either Pounsford or Whitefish Lake male rusty crayfish to characterize the type of odour signals that might be transmitted during a predation event or an agonistic interaction with conspecifics. Similar to established methods (Hazlett 1999; Aquistapace *et al*. 2004; Hazlett *et al*. 2006), one medium sized (mean weight $\pm$ SD, 12.6 $\pm$ 3.3 g) adult male crayfish from either lake was macerated for 5 seconds in a small electric blender, then gently mixed in 500 ml of dechlorinated water for 20 minutes, and filtered through coarse filter wool to remove particulate matter. This injury cue was produced anew for each day's trials, and used within 6 hours as the putative components may degrade over time (Hazlett 1999; Aquistapace *et al*. 2005). Dechlorinated water which had been blended, mixed, and filtered was used as control.

Food cue preliminary trials using the same Y-maze assay were conducted to obtain a baseline behavioural response to chemoattractants. This stimulus was prepared

by stirring 10 g of crushed commercial trout pellet food in 500 ml dechlorinated water for 20 minutes, then filtering the homogenate through coarse filter wool to remove particulate matter (Pecor *et al*. 2010). Dechlorinated water which had been stirred and filtered was used as control.

4.3.3 Behavioural trials

Behavioural trials were conducted as reported in Chapter 3.3.2 to measure crayfish response to chemical stimuli. A static Y-maze (74 l X 39 W cm), filled with 10 l of holding temperature-matched, dechlorinated Thunder Bay municipal water, was used to test Pounsford and Whitefish Lake crayfish response to conspecific chemical stimuli generated from either sympatric or allopatric crayfish. A clear barrier, perforated to allow chemical exchange, separated an acclimation zone from the two arms of the Y-maze. A bisected, 10 cm length of 10 cm diameter PVC pipe was put into the acclimation zone to provide the animal shelter and facilitate acclimation. Stimuli and controls were randomly assigned to either the left or right delivery arm for each trial, and observers were unaware as to which side contained the stimulus. All experimental systems were visually isolated, and observers recorded behaviour through a small (4 X 10 cm) horizontal viewing window. A randomly selected crayfish from a given lake population, was delivered into the acclimation zone and left for 20 minutes to adjust to maze conditions. Following acclimation, 20 ml of both stimulus and controls were simultaneously delivered to each opposing end of the delivery arms using a 50 ml syringe connected to airline tubing and a 9 cm Pasteur pipette, operated remotely from behind a visual barrier. Preliminary dye trials revealed that aqueous mixtures delivered to one end of either arm diffused to the acclimation chamber gate within 5 minutes. Following a 5-minute stimulus delivery

period, the perforated barrier was gently raised sufficiently to allow the crayfish to pass through and behavioural endpoints were recorded for an 8-minute observation period. Behavioural endpoints were chosen in keeping with Adams and Moore (2003) including first choice, final position, time in the stimulus *v.* control arms, and latency to first arm entry. For a given trial, first choice was defined as the first arm (stimulus or control) that crayfish entered, while final position was defined as the final location occupied by the crayfish (stimulus, control, or acclimation chamber) following the 8 minute observation period. Crayfish from either Pounsford or Whitefish Lake were used only once for a given trial series and not reused in subsequent trials for this study. Y-mazes were thoroughly washed using a mild detergent (Sparkleen, Fisherbrand) solution and rinsed with dechlorinated water following each trial set to ensure no stimulus contamination would confound subsequent trials.

4.3.4 Analysis

A square root transformation (x'= sqrt(x + 0.375)) was used to normalize the distribution of the time variables prior to analysis. A paired *t*-test (paired-*t*) was then used to compare mean time crayfish spent in the stimulus *v*. control arms. A two sample *t*-test (*t*) was used to evaluate differences in mean latency to initial arm selection (stim. *v.* control). A chi-square (χ^2) test was used to compare first arm choice and last position within the Y-maze following the observation period with a respective 50:50 and 33:33:33 expectation due to random chance alone. Initial arm choice was defined as the first arm (stimulus or control) that crayfish entered while last position was defined as the final position of the crayfish (stimulus, control or acclimation chamber) following the 8 minute observation period. Any trial in which crayfish did not make an initial arm choice during

the observation period was omitted from analysis. This *a priori* data-censoring rule resulted in two replicates from the Pounsford Lake population response to a Pounsford Lake injury cue being removed from my dataset. For all analyses, results were considered statistically significant when $P < 0.05$. All tests were conducted and graphics produced using R ver. 2.10.1 (R Development Core team 2009).

4.4 Results

During the preliminary trial series, crayfish spent significantly more time in the food cue arm when paired with a dechlorinated water control (paired *t*-test; $t = 2.13$, df = 28, $P = 0.042$). This response pattern suggested crayfish recognized, and were attracted to dissolved components from the trout food as a result of feeding experience (Tierney and Atema 1988).

I also found that neither Pounsford Lake ($n = 20$), nor Whitefish Lake male rusty crayfish ($n = 20$) showed any response to injury cues from Whitefish Lake conspecifics according to the behavioural endpoints (first choice, last position, time in the stimulus *v.* control arms, latency to first arm entry, $P > 0.05$). I did observe however, that male rusty crayfish from both populations spent significantly more time in the arm containing a Pounsford Lake crayfish injury cue when paired with a control (Whitefish Lake crayfish: paired-$t = -2.67$, df = 18, $P = 0.015$; Pounsford lake crayfish: paired-$t = -2.37$, df = 21, $P = 0.027$). On average, Pounsford Lake crayfish spent twice as long, and Whitefish Lake crayfish almost three times as long, in the Pounsford Lake crayfish stimulus arm as they did the control arm (Figure 3.). This response pattern closely resembles the attraction response characterized during preliminary food cue trials.

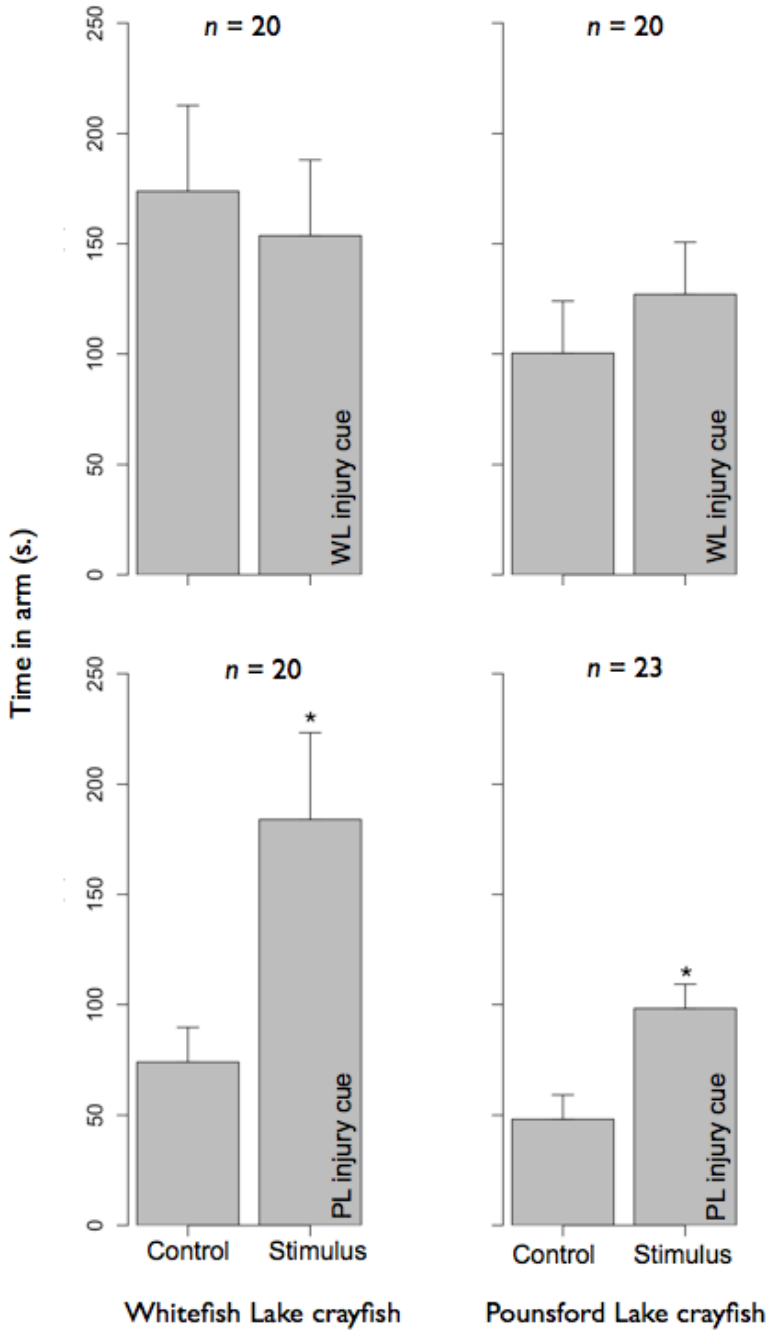


Figure 3. Mean (+SEM) time Whitefish and Pounsford Lake rusty crayfish (Orconectes rusticus) spent in the stimulus v. control arms in a Y-maze behavioral assay when presented with stimuli from either crushed Whitefish Lake (WL) or Pounsford Lake (PL) conspecifics. Asterisks (*) indicate significant difference from control when $P \leq 0.05$.

4.5 Discussion

Results suggest that allopatric populations of invasive rusty crayfish may develop ecosystem-specific behavioural responses to conspecific chemosensory stimuli. I found that crayfish from two geographically isolated *O. rusticus* populations, from two ecologically unique habitats, at two different stages of colonization, were responding the same way to conspecific injury cues depending on the origin of the injured crayfish. Specifically, crayfish from both Pounsford and Whitefish Lake did not respond to stimuli from damaged Whitefish Lake conspecifics and both populations were attracted to the same stimulus generated from Pounsford Lake crayfish. My results suggest there may be a difference, discernable by chemical composition alone, between the Pounsford Lake and Whitefish Lake rusty crayfish populations such that the former are uniquely identifiable (possibly as a food source) relative to the latter. Due to conditions of resource limitation, increased competition, and high population densities, Pounsford Lake crayfish may have come to rely more heavily on cannibalism to satisfy their energetic needs and control population dynamics. Similar to the physiological adaptations of walleye reported by Kaufman *et al.* (2006), the practice of intraspecific predation in Pounsford Lake rusty crayfish may be reflected at a biochemical level. Research by Adams and Moore (2003) describes how and why rusty crayfish readily distinguish conspecific moult cues. I suggest that injury cues from Pounsford Lake crayfish convey a different signal to other rusty crayfish because of a unique biochemical profile resulting from the history of cannibalism within that population. While current research suggests that invasive species enter new ecosystems with the ability to respond to a broader range of chemical predation

risk cues (Pecor *et al*. 2010), my study provides compelling evidence to suggest that under certain conditions, adaptive foraging behaviour may lead to novel employment of conspecific chemosensory stimuli in certain aquatic invasive species.

Interestingly, Pounsford Lake is without an established predator species fish community following several, failed Ontario Ministry of Natural Resource introductions of largemouth bass (*Micropterus salmoides* Lacépède, 1802) in the early 1950's (Momot *et al*. 1988), and of walleye (*Sander vitreus*) around 1964 (Werner 1983. A preliminary report on fisheries data for Sibley Provincial Park. Ontario Ministry of Natural Resources, Thunder Bay District, unpublished data) (Table 1). These introductions were the most likely vector by which *O. rusticus* came to establish in Pounsford Lake (W. Momot 2011, personal communication). By comparison, Whitefish Lake has a well-established, multi-species predator fish community. As a consequence, Whitefish Lake rusty crayfish may still actively rely on sympatric conspecific "alarm cues" to help avoid predation. Under the community structure of Pounsford Lake however, conspecific injury cues provide information of a different behavioural relevance; rather than warning of an active predator, they are more likely to signal a potential meal. Therefore, Whitefish Lake crayfish respond in the same way as Pounsford Lake crayfish, to Pounsford Lake crayfish injury cues. Given the degree of establishment of the Pounsford Lake (~ 50 yrs.) relative to the Whitefish Lake rusty crayfish population (< 10 yrs.), the former may have adapted collectively to maximize resource utilization, while managing recruitment, population density, and competition (Fox 1975a; b; Figiel *et al*. 1991; Skurdal and Taugbøl 2002). Increased reliance on conspecifics to satisfy energetic or nutritional requirements is well recognized under a variety of conditions including depauperate ecosystems or areas of

low productivity (Fox 1975a; 1975b; Wise 2006). Under the conditions of high population density and low ecological productivity found in Pounsford Lake, intraguild predation provides a framework upon which to understand ingrained cannibalistic tendencies of the extant rusty crayfish population. Findings by Olsen *et al*. (1991), Wilson (2004), and colleagues describe that over the long-term, *O. rusticus* invasion can significantly reduce littoral zone biota, as well as macrophyte species richness and biomass in an invaded water body. A similar, dramatic, ecosystem-wide impact can be observed in Pounsford Lake. At the outset of colonization, rusty crayfish introduced into Pounsford Lake may have adopted a greater propensity towards cannibalism as a "life-boat strategy" to overcome population bottlenecks and reduce the probability of extinction (Polis 1981). In time, the colonizing rusty crayfish population may have come to further rely on intraspecific predation to mitigate increasing competition and food resource limitations (Wise 2006). These hypotheses would be consistent with observations on cannibalism in other natural populations including both aquatic and terrestrial arthropods (Fox 1975a; 1975b; Elgar and Crespi 1994; Wise 2006). It is also consistent with published observations on the adaptive differences of local populations of invasive species tailored to the unique selective pressures of their adopted ecosystems (Sakai *et al.* 2001; Allendorf and Lundquist 2003). The ability to switch from a generalist predator to an intraspecific specialist predator, seasonally, ontogenetically, or in response to environmental conditions, characterizes the adaptive benefit of phenotypic plasticity common to successful invasive species (Gray 1986; Davidson *et al*. 2011). Recent findings by Drown *et al*. (2011) strongly suggest that invasive aquatic genotypes tend to be opportunistic specialists, which enables them to capitalize on niche resource

opportunities relative to locally adapted species. Phenotypic plasticity has also been suggested to help mediate climate change responses among invasive terrestrial arthropods (Chown *et al*. 2007). Therefore phenotypic plasticity can facilitate invasive species to rapidly adapt and survive in new and unfamiliar ecosystems or respond to environmental perturbations in ways local species cannot.

With my experimental design, possibly the crayfish were simply curious about the test stimuli and therefore ended up spending more time in the stimulus arm than the control arm of the maze. However, consistencies in response trends between the injury cue stimuli and food cue preliminary trials, which established a baseline attraction response, suggest otherwise. The same trend, spending more time in the stimulus arm when paired with a control, is seen when comparing behavioural response of both *O. rusticus* populations to a food cue and to that of a conspecific injury cue of Pounsford Lake origin. Findings by Adams and Moore (2003), reporting Y-maze methods using stimuli paired with a control, support my results. Adams and Moore (2003) suggested the ability to identify and locate a moulted crayfish using chemoreception alone would facilitate a resource-limited, hungry crayfish in capitalizing on an easy meal. Although cannibalism is only one of several possible explanations (for positive chemotaxis to conspecific moult cues) presented by the authors, assimilating resources previously acquired by the moulted individuals is clearly advantageous for crayfish. Polis (1981) suggested that low food resource availability, and increased competition under conditions of high population density, leads to an increase in foraging activity, especially in the absence of predators. Because most social behaviour in crayfish is mediated through agonistic interaction, which can result in injury or death, increased foraging activity

increases the probability of physical injury incurred from agonistic interactions with competitors (Capelli and Munjal 1982; Bechler *et al*. 1988; Woodlock and Reynolds 1988; Bergman and Moore 2005). Under conditions of high population density and resource limitation (i.e., food, shelter), plasticity in foraging behaviour that incorporates intraspecific predation confers an adaptive advantage to species with this predisposition.

Determining which specific components of Pounsford Lake rusty crayfish injury cues may serve as the putative chemoattractants warrants further investigation. Elucidating unique chemoattractants, using biochemical fractionation techniques and behavioural assays, could help inform on selective remediation strategies for conservation biologists working with *Orconectes rusticus*. Furthermore, evaluating the differences in rusty crayfish population density, and size class structure as well as comparative productivity indexes (e.g., total phosphorus (TP), chlorophyll *a* (Chl *a*) concentration, zooplankton density, and biomass) between Pounsford and Whitefish Lakes would help better characterize the ecological disparities between these two ecosystems. A comparison of size class distributions between Pounsford Lake rusty crayfish and those in other lakes might demonstrate a stunted population, which would indicate overpopulation. Finally, it would be interesting to see whether seasonal food scarcity and abundance patterns, or size class could influence cannibalistic activity in the Pounsford Lake rusty crayfish population. Depending on life stage or season, I would expect crayfish to be at varying degrees of metabolic activity with corollary energetic requirements that lead to ontogenetic changes in feeding behaviour (Abrahamsson 1966; Guan and Wiles 1998).

Chapter 5. Discussion

Species considered exotic yesterday, and invasive today, become indigenous tomorrow. It is all a matter of time. The commonly used categorization of species as "invasive" belies the incredible adaptive capabilities that carry a population through varying degrees of succession in the process of becoming established where once they were not. Findings reported in Chapters 3 and 4 of this thesis both complement, and expand, our understanding of how crayfish species use chemical information in ways that facilitate survival and new resource adoption in novel aquatic ecosystems. Indeed, studying the rusty crayfish in Northwestern Ontario can provide unique examples of short-term (and long-term) adaptation commonly observed in many invasive species (Sakai *et al.* 2001; Allendorf and Lundquist 2003; Dlugosch and Parker 2008).

Findings reported in Chapter 3 complement a growing knowledge base on chemically mediated associative learning processes in invasive crayfish species. My results are consistent with published studies, which support the conclusion that invasive crayfish species use a broader range of chemical stimuli, and rely on associative learning processes, to confer survival advantages when facing novel predators or when capitalizing on novel resources (Hazlett 1994a; 1994b; Hazlett and Schoolmaster 1998; Hazlett 2000; Hazlett *et al.* 2002; Adams and Moore 2003; Hazlett 2003; Hazlet *et al*. 2003; Acquistapace *et al*. 2004; Hazelett 2007; Pecor *et al*. 2010). Results discussed in Chapter 3 resonate in particular with findings reported by Dittman *et al.* (1998) and Mirza and Chivers (2002), supporting the idea that chemical cues have a role to play in fish egg predation, whether it involves innate or learned recognition on behalf of the predator. Whereas invasive crayfish tend to respond innately to injury cues from

conspecific or heterospecifc cohabitants, identification of novel predators or resources requires a learning process to take place (Hazlett 1994a; Hazelett and Schoolmaster 1998; Hazlett 2003; Hazlett *et al*. 2003). However, this learning process can occur very quickly and have a lasting effect on the behaviour of the organism thereby allowing it to adjust and adapt quickly to changing biotic and abiotic conditions (Hazlett *et al*. 2002). This phenotypic plasticity is what allows so many invasive species to be successful in spite of the many challenges brought on during territory adoption (Sakai *et al*. 2001; Allendorf and Lundquist 2003; Davidson *et al*. 2011; Drown *et al.* 2011). The ability to condition a walleye-naïve rusty crayfish population to become attracted to a walleye egg cue using chemosensory stimuli alone, offers a compelling glimpse into the remarkable adaptability of chemically mediated learning processes in rusty crayfish. This study also serves to expand our scope of knowledge regarding the types of chemosensory cues employed by invasive crayfish, while hinting at the implications of these invasions for sympatric fish species.

Chapter 4 reports unique observations about ecosystem-specific utilization of conspecific injury cues in crayfish. This study resonates strongly with what is known about the conditions that give rise to cannibalism in terrestrial arthropod populations (Wise 2006). My observations also provide an interesting complement to those of Adams and Moore (2003) with respect to rusty crayfish response to conspecific chemical stimuli. In Chapter 4, both study populations responded similarly to injury cues from a rusty crayfish population likely to cannibalize with greater frequency, as Adams and Moore's (2003) crayfish responded to conspecific moult cues. Rusty crayfish may possess a predisposition towards cannibalism that is governed, among other things, by chemical

cues that convey the physiological condition of conspecifics. Moreover, while triggers for intraspecific predation are often chemical, other biotic and abiotic conditions will also promote specialist foraging strategies. This ability to shift foraging tactics to suit, or capitalize on, available resources further underscores the behavioural plasticity considered common to invasive species (Sakai *et al*. 2001; Allendorf and Lundquist 2003; Dlugosch and Parker 2008; Davidson *et al*. 2011; Drown *et al.* 2011).

Collectively, the observations reported are unique in the field of chemosensory research in crayfish. Chapter 3 is the first study to suggest the role of associative learning as a possible mechanism for mediating egg predation by a recognized invertebrate predator. Findings reported in Chapter 4 are the first to describe an ecosystem-specific behavioural response to conspecifc injury cues in rusty crayfish. Together, these observations help describe the adaptive behaviours of rusty crayfish while laying the foundation for continued research into the sensory ecology, and associated behaviour, of invasive crayfish species. The story of the rusty crayfish in Northwestern Ontario is one of classic biological invasion. If we read between the lines of conservation dogma, we can appreciate the infinitely complex and dynamic flexibility of natural systems and their inhabitants at work.

Literature Cited

Abrahamsson, S.A.A. 1966. Dynamics of an isolated population of the crayfish *Astacus astacus* Linné. Oikos. **17**(1): 96-107.

Ache, B. W. 1982. Chemoreception and thermoreception. *In* The Biology of Crustacea. Vol. 3, *Neurobiology: Structure and Function*, *Edited by* H. L. Atwood and D. C. Sandeman. Academic Press, New York. pp. 369- 398

Ache, B.W. 2003. Crustacean olfaction [online]. *In* AccessScience. McGraw-Hill Companies. Available from: http://accessscience.proxy.mpcc.edu/content.aspx?id=YB030360 [accessed on 21 March 2011].

Acquistapace, P., Daniels, W.H., and Gherardi, F. 2004. Behavioral responses to 'alarm odors' in potentially invasive and non-invasive crayfish species from aquaculture ponds. Behaviour. **141**(6): 691-702.

Acquistapace, P., Calamai, L., Hazlett, B.A., and Gherardi, F. 2005. Source of alarm substances in crayfish and their preliminary chemical characterization. Canadian Journal of Zoology. **83**(12): 1624-1630.

Adams, J.A. and Moore, P.A. 2003. Discrimination of conspecific male molt odor signals by male crayfish, *Orconectes rusticus*. Journal of Crustacean Biology. **23**(1): 7-14.

Aiken, D.E. 1968. The crayfish *Orconectes virilis*: survival in a region with severe winter conditions. Canadian Journal of Zoology. **46**(2): 207-211.

Allendorf , F.W. and Lundquist, L.L. 2003. Introduction: population biology, evolution, and control of invasive species. Population Biology. **17**(1): 24-30.

Anderson, P.A.V. and Ache, B.W. 1985. Voltage- and current-clamp recordings of the receptor potential in olfactory receptor cells *in situ*. Brain Research. **338**(2): 263-280.

Aquiloni, L. and Gherardi, F. 2010. Crayfish females eavesdrop on fighting males and use smell and sight to recognize the identity of the winner. Animal Behaviour. **79**(2): 265-269.

Bechler, O.L., Deng, X., and McDonald B. 1988. Interspecific pheromonal communication between sympatric crayfish of the genus *Procambarus* (Decapoda, Astacidae). Crustaceana. **54**(2): 153-162.

Bergman, D.A. and Moore, P.A. 2005. The role of chemical signals in the social behavior of crayfish. Chemical Senses. **30**(1): 305-306.

Berrill, M. and Arsenault, M.R. 1982. Spring breeding of a northern temperate crayfish *Orconectes rusticus*. Canadian Journal of Zoology. **60**(11): 2641-2645.

Berry, F.C. and Breithaupt, T. 2008. Development of behavioural and physiological assays to assess discrimination of male and female odours in crayfish, *Pacifastacus leniusculus*. Behaviour. **145**(10): 1427-1446.

Berry, F.C. and Breithaupt, T. 2010. To signal or not to signal? Chemical communication by urine-borne signals mirrors sexual conflict in crayfish. BMC Biology. **8**(25): 1-11

Berube, D. and Kraft, L. 2010. Invasion of rusty crayfish (*Orconectes rusticus*) into Whitefish Lake. Ontario Mininistry of Natural Resources, Northwest Science & Information. NWSI Aquatic Update 2010-3. 8 pp.

Bobeldyk, A.M. and Lamberti, G.A. 2008. A decade after invasion: evaluating the continuing effects of rusty crayfish on a Michigan river. Journal of Great Lakes Research. **34**(2): 265–275.

Bronte, C.R., Schram S.T., Selgeby, J.H., and Swanson, B.L. 2002. Reestablishing a spawning population of lake trout in Lake Superior with fertilized eggs in artificial turf incubators. North American Journal of Fisheries Management. **22**(3): 796-805.

Buch, R.M., Barker, T.Q., and Rechnitz, G.A. 1991. Intact chemoreceptor biosensors based on Hawaiian aquatic species. Analytica. Chimica. Acta. **243**(2): 157-166.

Capelli, G.M. and Munjal, B.L. 1982. Aggressive interactions and resource competition in relation to species displacement among crayfish of the genus *Orconectes*. Journal of Crustacean Biology. **2**(4): 486-492.

Capelli, G.M. and Magnuson, J.J. 1983. Morphoedaphic and biogeographic analyses of crayfish distribution in Northern Wisconsin. Journal of Crustacean Biology. **3**: 548-564.

Carr, W.E.S. 1988. The molecular nature of chemical stimuli in the aquatic environment. *In* Sensory Biology of Aquatic Animals. *Edited by* J. Atema *et al.* Springer-Verlag, New York. pp. 3-27.

Carr, W.E.S., Ache, B.W., and Gleeson, R.A. 1987. Chemoreceptors of crustaceans: similarities to receptors for neuroactive substances in internal tissues. Environmental Health Perspectives. **71**: 31-46.

Chambers, P.A., Hanson, J.M., Burke, J.M. and Prepas, E.E. 1990. The impact of the crayfish *Orconectes virilis* on aquatic macrophytes. Freshwater Biology. **24**(1): 81-91.

Chang, E.S. 1995. Physiological and biochemical changes during the molt cycle in decapod crustaceans: an overview. Journal of Experimental Marine Biology and Ecology. **193**(1-2): 1-14.

Chown, S.L., Slabber, S., McGeouch, M., Janion, C., and Leinaas, H.P. 2007. Phenotypic plasticity mediates climate change responses among invasive and indigenous arthropods. Proceedings of the Royal Society. Biological Sciences. **274**(1625): 2531-2537.

Claramunt, R.M., Jonas, J.L., Fitzsimons, J.D., and Marsden, J.E. 2005. Influences of spawning habitat characteristics and interstitial predators on lake trout egg deposition and mortality. Transactions of the American Fisheries Society. **134**: 1048-1057.

Creed, R.P.Jr. 1994. Direct and indirect effects of crayfish grazing in a stream community. Ecology. **75**(7): 2091-2103.

Davidson, A.M., Jennions, M., and Nicotra, A.B. 2011. Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis. Ecology Letters. **14**(2): 1-13.

Derby, C.D. and Atema, J. 1982. The function of chemo- and mechanoreceptors in lobster (*Homarus americanus*) feeding behaviour. Journal of Experimental Biology. **98**: 317-327.

Derby, C.D. and Atema, J. 1988. Chemoreceptor cells in aquatic invertebrates: peripheral mechanisms of chemical signal processing in decapod crustaceans. *In* Sensory Biology of Aquatic Animals. *Edited by* J. Atema *et al.* Springer Verlag, New York. pp. 365-386.

DFO. 2010. Aquatic invasive species [online]. Fisheries and Oceans Canada. Available from: http://www.dfo-mpo.gc.ca/science/enviro/ais-eae/index-eng.htm [accessed 24 October 2010].

Dickinson, A. 1980. Contemporary Animal Learning Theory. Cambridge University Press, New York, N.Y.

Dittman, A.H., Brown, G.S. and Foote, C.J. 1998. The role of chemoreception in salmon egg predation by coastrange (*Cottus aleuticus*) and slimy (*C. cognatus*) sculpins in Iliamna Lake, Alaska. Canadian Journal of Zoology. **76**(3): 406–413.

Dlugosch, K.M. and Parker, I.M. 2008. Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. Molecular Ecology. **17**(1): 431–449.

Dorn, N.J. and Mittelbach, G.G. 1999. More than predator and prey: a review of interactions between fish and crayfish. Vie et Milieu. **49**(4): 229-237.

Dorn, J.D. and Wojdak, J.M. 2004. The role of omnivorous crayfish in littoral communities. Oecologia. **140**(1): 150–159.

Doving, K.B., Stabell, O.B., Ostlund-Nilsson, S., and Fisher, R. 2006. Site fidelity and homing in tropical coral reef cardinalfish: are they using olfactory cues? Chemical Senses. **31**(3): 265-272.

Drown, D.M., Levri, E.P., and Dybdahl, M.F. 2011. Invasive genotypes are opportunistic specialists not general purpose genotypes. Evolutionary Applications. **4**(1): 132-143.

Dunham, D.W. and Oh, J.W. 1992. Chemical sex discrimination in crayfish *Procambarus clarkii*: role of antennules. Journal of Chemical Ecology. **18**(12): 2363-2372.

Elgar, M.A. and Crespi, B.J. 1994. Cannibalism: ecology and evolution among diverse taxa. Journal of Evolutionary Biology. **7**(1): 121-123.

Ellrott, B.J., Marsden, J.E., Fitzsimons, J.D., Jonas, J.L., and Claramunt, R.M. 2007. Effects of temperature and density on consumption of trout eggs by *Orconectes* propinquus and *O. rusticus*. Journal of Great Lakes Research. **33**(1): 7–14.

Farbman, A.I., 1992. Cell Biology of Olfaction. Cambridge University Press, Cambridge.

Figiel, C.R. Jr., Babb, J.G., and Payne, J.F. 1991. Population regulation in young of the year crayfish, *Procambarus clarkii* (Girard, 1852) (Decapoda, Cambaridae). Crustaceana. **61**(3): 301-307.

Fitzsimons, J.D., Perkins, D.L. and Krueger, C.C. 2002. Sculpins and crayfish in lake trout spawning areas in Lake Ontario: estimates of abundance and egg predation on lake trout eggs. Journal of Great Lakes Research. **28**(3): 421-436.

Fitzsimons, J.D., Williston, B., Williston, G., Bravener, G., Jonas, J.L., Claramunt, R.M., Marsden, J.E. and Ellrott, B.J. 2006. Laboratory estimates of salmonine egg predation by round gobies (*Neogobius melanostomus*), sculpins (*Cottus cognatus* and *C. bairdi*), and crayfish (*Orconectes propinquus*). Journal of Great Lakes Research. **32**(2): 227–241.

Flint, R.W. and Goldman, C.R. 1975. The effects of a benthic grazer on the primary productivity of the littoral zone of Lake Tahoe. Limnology Oceanography. **20**(6): 935-944.

Foster, N.R. 1985. Lake trout reproductive behavior: influence of chemosensory cues from young-of-the-year by-products. Transactions of the American Fisheries Society. **114**(6): 794–803.

Fox, L.R. 1975a. Cannibalism in natural populations. Annual Review of Ecology and Systematics. **6**: 87-106.

Fox, L.R. 1975b. Factors influencing cannibalism, a mechanism of population limitation in the predator *Notonecta hoffmanni*. Ecology. **56**(4): 933-941.

Galindo, C., Gaxiola, G., Cuzon, G., and Chiappa-Carrara, X. 2009. Physiological and biochemical variations during the molt cycle in juvenile *Litopenaeus vannamei* under laboratory conditions. Journal of Crustacean Biology. **29**(4): 544-549.

Gardiner, J.M., and Atema, J. 2010. The function of bilateral odor arrival time differences in olfactory orientation of sharks. Current Biology. **20**(13): 1187-1191.

Gray, A.J. 1986. Do invading species have definable genetic characteristics? Philosophical Transactions of the Royal Society of London. Series B. **314**(1167): 655-672.

Guan, R. and Wiles, P.R. 1998. Feeding ecology of the signal crayfish *Pacifastacus leniusculus* in a British lowland river. Aquaculture. **169**(3): 177-193.

Gunn, J.M. 1995. Spawning behavior of lake trout: effect on colonizationability. Journal of Great Lakes Research. **21**(Suppl. 1): 323–329.

Gunn, G.M. and Sein, R. 2000. Effects of forestry roads on reproductive habitat and exploitation of lake trout (*Salvelinus namaycush*) in three experimental lakes. Impacts of major watershed perturbations on aquatic ecosystems. Canadian Journal of Fisheries and Aquatic Sciences. **57**(S2): 97–104.

Hara, T.J. 1994. The diversity of chemical stimulation in fish olfaction and gustation. Reviews of Fish Biology and Fisheries. **4**(1): 1–35.

Hazlett, B.A. 1985. Chemical detection of sex and condition in the crayfish *Orconectes virilis*. Journal of Chemical Ecology. **11**(2): 181-189.

Hazlett, B.A. 1994a. Crayfish feeding responses to zebra mussels depend on microorganisms and learning. Journal of Chemical Ecology. **20**(10): 2623-2630.

Hazlett, B.A. 1994b. Alarm response in the crayfish *Orconectes virilis* and *Orconectes propinquus*. Journal of Chemical Ecology. **20**(7): 1525-1535.

Hazlett, B.A. 1996. Organization of hermit crab behaviour: Responses to multiple chemical inputs. Behaviour. **133**(7): 619-642.

Hazlett, B.A. 1999. Responses to multiple chemical cues by the crayfish *Orconectes virilis*. Behaviour. **136**(2): 161-177.

Hazlett, B.A. 2000. Information use by an invading species: do invaders respond more to alarm odors than native species? Biological Invasions. **2**(4): 289-294.

Hazlett, B.A., Acquistapace, P., and Gherardi, F. 2002. Differences in memory capabilities in invasive and native crayfish. Journal of Crustacean Biology. **22**(2): 439-448.

Hazlett, B.A. 2003. Predator recognition and learned irrelevance in the crayfish *Orconectes virilis*. Ethology. **109**(9): 765-780.

Hazlett, B.A. 2007. Conditioned reinforcement in the crayfish *Orconectes rusticus*. Behaviour. **144**(7): 847-859.

Hazlett, B.A., Burba, A., Gherardi, F. and Acquistapace, P. 2003. Invasive species of crayfish use a broader range of predation-risk cues than native species. Biological Invasions. **5**(3): 223-228.

Hazlett, B.A. and Schoolmaster, D.R. 1998. Responses of cambarid crayfish to predator odor. Journal of Chemical Ecology. **24**(11): 1757-1770.

Hobbs, H.H, III. 1993. Trophic relationships of North American freshwater crayfishes and shrimps. Contributions in Biology and Geology / Milwaukee Public Museum. **85**: 1-110.

Holt, R.D. and Polis, G.A. 1997. A theoretical framework for intraguild predation. American Naturalist. **149**(4): 745–764.

Horns, W.H. and Magnuson, J.J. 1981. Crayfish predation on lake trout eggs in Trout Lake, Wisconsin. Rapports et Proces-Verbaux des Reunions Conseil International pour l' Exploration de la Mer. **178**: 299-303.

Johnson, B.R. and Ache, B.W. 1978. Antennular chemosensitivity in the spiny lobster, *Panulirus argus*: amino acids as feeding stimuli. Marine Behavior and Physiology. **154**(2): 226-240.

Johnson, B.R. and Atema, J. 1986. Chemical stimulants for a component of feeding behavior in the common gulf-weed shrimp *Leander tenuicornis* (Say). Biological Bulletin. **170**: 1-10.

Jonas, J.L., Claramunt, R.M., Fitzsimons, J.D., Marsden, J.E. and Ellrott, B.J. 2005. Estimates of egg deposition and effects of lake trout (*Salvelinus namaycush*) egg predators in three regions of the Great Lakes. Canadian Journal of Fisheries and Aquatic Sciences. **62**(10): 2254-2264.

Jones, P.D. and Momot, W.T. 1983. The bioenergetics of crayfish in two pothole lakes. Freshwater Crayfish. **5**: 193-209.

Kats, L.B. and Dill, L.M. 1998. The scent of death: chemosensory assessment of predation risk by prey animals. Ecoscience. **5**(3): 361-394.

Kaufman, S.D., Gunn, J.M., Morgan, G.E., and Couture, P. 2006. Muscle enzymes reveal walleye (*Sander vitreus*) are less active when larger prey (cisco, *Coregonus artedi*) are present. Canadian Journal of Fisheries and Aquatic Sciences. **63**(5): 970-979.

Kolar, C.S. and Lodge, D.M. 2001. Progress in invasion biology: predicting invaders. Trends in Ecology and Evolution. **16**(4): 199–204.

Kraus-Epley, K.E. and Moore, P.A. 2002. Bilateral and unilateral antennal lesions alter orientation abilities of the crayfish, *Orconectes rusticus*. Chemical Senses **27**(1): 49-55.

Krieger, J. and Breer, H. 1999. Olfactory reception in invertebrates. Science. **286**(5440): 720-723.

Lake Simcoe Science Advisory Committee. 2008. Lake Simcoe and its watersheds: Report to the Ministry of the Environment, Ontario, Canada [online]. Available from http://www.ene.gov.on.ca/en/water/lakesimcoe/docs/PublicReport-watershed.pdf [accessed 24 October 2010].

Lima, S.L. and Dill, L.M. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. Canadian Journal of Zoology. **68**(4): 619–640.

Lockwood, J.L., Cassey, P., and Blackburn, T. 2005. The role of propagule pressure in explaining species invasions. Trends in Ecology and Evolution. **20**(5): 223–228.

Lodge, D.M., Kershner, M.W., and Aloi, J.E. 1994. Effects of an omnivorous crayfish (*Orconectes rusticus*) on a freshwater littoral food web. Ecology. **75**(5): 1265-1281.

Mackie, A.M. 1973. The chemical basis of food detection in the lobster *Homarus gammarus*. Marine Biology. **21**(2): 103-108.

Mathis, A., and Smith, R.J.F. 1993. Chemical alarm signals increase the survival time of fathead minnows (*Pimephales promelas*) during encounters with northern pike (*Esox lucius*). Behavioral Ecology. **4**(3): 260-265.

McCarthy, M.J., Hein, C.L. Olden, J.D., and Vander Zanden, M.J. 2006. Coupling long-term studies with meta-analysis to investigate impacts of non-native crayfish on zoobenthic communities. Freshwater Biology. **51**(2): 224-235.

Mirza, R.S. and Chivers, D.P. 2002. Attraction of slimy sculpins to chemical cues of brook charr eggs. Journal of Fish Biology. **61**(3): 532-539.

Momot, W.T. 1966. Upstream movement of crayfish in an intermittent Oklahoma stream. American Midland Naturalist. **75**(1): 150-159.

Momot, W.T. 1984. Crayfish production: a reflection of community energetics. Journal of Crustacean Biology. **4**(1):35–54.

Momot, W.T., Gowing, H. and Jones, P.D. 1978. The dynamics of crayfish and their role in ecosystems. American Midland Naturalist. **99**(1): 10-35.

Momot, W.T. 1992. Further range extensions of the crayfish Orconectes rusticus in the Lake Superior basin of northwestern Ontario. Canadian Field Naturalist. **106**: 397-399.

Momot, W.T. 1995. Redefining the role of crayfish in aquatic ecosystems. Reviews in Fisheries Science. **3**(1): 33–63.

Momot, W.T., Hartviksen, C. and Morgan, G. 1988. A range extension for the crayfish *Orconectes rusticus*: Sibley Provincial Park, northwestern Ontario (Canada). Canadian Field Naturalist. **102**(3): 547–548.

Nyström, P. and Granéli, W. 1996. The effect of food availability on survival, growth, activity and the number of mature females in crayfish populations. Freshwater Crayfish. **11**(4): 170-181.

Nyström, P., Svensson, O., Lardner, B., Brönmark, C., and Granéli, W. 2001. The influence of multiple introduced predators on a littoral pond community. Ecology **82**(4):1023–1039.

Nyström, P. 2002. Ecology. *In* Biology of Freshwater Crayfish. *Edited by* D.M. Holdich. MPG Books Ltd, Bodmin, Cornwall, Great Britain. pp. 192-235.

Olden, J.D., McCarthy, J.M., Maxted, J.T., Fetzer, W.W. and Vander Zanden, M.J. 2006. The rapid spread of rusty crayfish (*Orconectes rusticus*) with observations on native crayfish declines in Wisconsin (USA) over the past 130 years. Biological Invasions. **8**(8): 1621-1628.

Olsen, T.M., Lodge, D.M., Capelli, G.M., and Houlihan, R.J. 1991. Mechanisms of impact of an introduced crayfish (*Orconectes rusticus*) on littoral congeners, snails, and macrophytes. Canadian Journal of Fisheries and Aquatic Sciences. **48**(10): 1853–1861.

Paine, R.T. 1995. A conversation on refining the concept of keystone species. Conservation Biology **9** (4): 962–964.

Parker I.M., Thompson, J.N., and Weller, S.G. 2001. The population biology of invasive species. Annual Review of Ecology Evolution and Systematics. **32**(1): 305–332.

Pecor, K.W., Deering, C.M., Firnberg, M.T., Pastino, A. K., and Wolfson, S.J. 2010. The use of conspecific and heterospecific alarm cues by virile crayfish (*Orconectes virilis*) from an exotic population. Marine and Freshwater Behaviour and Physiology. **43**(1): 37-44.

Perry, W.L., Lodge, D.M., and Lamberti, G.A. 1997. Impact of crayfish predation on exotic zebra mussels and native invertebrates in a lake-outlet stream. Canadian Journal of Fisheries and Aquatic Sciences. **54**(1): 120-125.

Phillips, I.D. 2010. Biological synopsis of the Rusty Crayfish (*Orconectes rusticus*). Canadian Manuscript Report of Fisheries and Aquatic Sciences. **2923**: 1-17.

Phillips, I.D., Vinebrooke, R.D. and Turner, M.A. 2009. Ecosystem consequences of potential range expansions *of Orconectes virilis* and *Orconectes rusticus* crayfish in Canada – a review. Environmental Reviews. **17**: 235-248.

Polis, G.A. 1981. The evolution and dynamics of intraspecific predation. Annual Review of Ecology and Systematics. **12**: 225-251.

Polis, G.A., Myers, C.A., and Holt, R.D. 1989. The ecology and evolution of intraguild predation: potential competitors that eat each other. Annual Review of Ecology and Systematics. **20**: 297–330.

R Development Core Team. 2009. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Rittschof, D. 1992. Chemosensation in the daily life of crabs. American Zoologist. **32**(3): 363-369.

Rosenberg, D.M., Turner, M.A., Jansen, W., Mosindy, T. and Watkinson, D.A. 2010. Threats to Lake of the Woods and the Winnipeg River by the rusty crayfish (*Orconectes rusticus*), an aquatic invader. Workshop Rpt. TWR-005, Science and Information Section, NWSI Tech, Ontario Ministry of Natural Resources, Thunder Bay, ON. pp. 1-54 + append.

Sakai, A. K., Allendorf, W F., Holt, J.S., Lodge, D.M., Molofsky, J., With, K.A., Baughman, S., Cabin, R.J., Cohen, J.E., Ellstrand, N.C., McCauley, D.E., O'Neil, P., Parker I.M., Thompson, J.N., and Weller, S.G. 2001. The population biology of invasive species. Annual Review of Ecology and Systematics. **32**(1): 305–332.

Schweitzer, J.A. and Larson, K.C. 1999. Greater morphological plasticity of exotic honeysuckle species may make them better invaders than native species. Journal of the Torrey Botanical Society. **126**(1): 15-23.

Scott, W.B. and Crossman, E.J. 1973. Freshwater Fishes of Canada. Bulletin of the Fisheries Research Board of Canada. No. 184.

Simon, J.L. and Moore, P.A. 2007. Male-female communication in the crayfish *Orconectes rusticus*: The use of urinary signals in reproductive and non-reproductive pairings. Ethology. **113**(8): 740-754.

Skurdal, J. and Taugbøl, T. 2002. Astacus. *In* Biology of Freshwater Crayfish. *Edited by* D.M. Holdich. MPG Books Ltd, Bodmin, Cornwall, Great Britain. pp. 467-510.

Steele, C., Skinner, C., Steele, C., Alberstadt, P., and Mathewson, C. 1999. Organization of chemically activated food search behavior in *Procambarus clarkii* Girard and *Orconectes rusticus* Girard crayfishes. Biological Bulletin. **196**(3): 295-302.

Stein. R.A. 1977. Selective predation, optimal foraging and the predator-prey interaction between fish and crayfish. Journal of Ecology. **58**(6): 1237-1253.

Strayer, D.L. 2010. Alien species in fresh waters: ecological effects, interactions with other stressors, and prospects for the future. Freshwater Biology. **55**(1): 152-174.

Thompson, H. and Ache, B. W. 1980. Threshold determination for olfactory receptors of the spiny lobster. Marine Behaviour and Physiology. **7**: 249-260.

Tierney, A.J. and Atema, J. 1988. Behavioral responses of crayfish *(Orconectes virilis* and *Orconectes rusticus)* to chemical feeding stimulants. Journal of Chemical Ecology. **14**(1): 123-133.

Waddy, L.L., Aiken, D.E., and De Kleijn, D.P.V. 1995. Control of growth and reproduction. *In* Biology of the Lobster. *Edited by* J.R. Factor. Academic Press, Inc., San Diego, California. pp. 217-266.

Wilson, K.A., Magnuson, J.J., Lodge, D.M., Hill, A.M., Kratz, T.K., Perry, W.L. and Willis, T.V. 2004. A long-term rusty crayfish (*Orconectes rusticus*) invasion: dispersal patterns and community change in a north temperate lake. Canadian Journal of Fisheries and Aquatic Sciences. **61**(11): 2255–2266.

Wise, D.H. 2006. Cannibalism, food limitation, intraspecific competition, and the regulation of spider populations. Annual Review of Entomology. **51**: 441-465.

Woodlock, B. and Reynolds, J.D. 1988. Laboratory breeding studies of freshwater crayfish *Austropotamobius pallipes* (Lereboullet). Freshwater Biology. **19**(1): 71

Yambe, H. and Yamazaki, F. 2001. Species-specific releaser effect of urine from ovulated female masu salmon and rainbow trout. Journal of Fish Biology. **59**(6): 1455-1464.

Zhang, C., Brown, S.B., and Hara, T.J. 2001. Biochemical and physiological evidence that bile acids produced and released by lake char (*Salvelinus namaycush*) function as chemical signals. Journal of Comparative Physiology, B: Biochemical, Systematic, and Environmental Physiology. **171**(2): 161-171.

Zimmer-Faust, R.K., Tyre, J.E., Michel, W.C., and Case, J.F. 1984. Chemical mediation of appetitive feeding in a marine decapod crustacean: the importance of suppression and synergism. Biological Bulletin. **167**(2): 339-353.

Zulandt Schneider, R.A., Huber, R., and Moore, P.A. 2001. Individual and status recognition in the crayfish, *Orconectes rusticus*: the effects of urine release on fight dynamics. Behaviour. **138**(2): 137-153.

Appendix A.

A.1 Standard Operating Procedure (SOP) for Egg Rinse Preparation Approximating Egg Mass

Purpose: The objective is to approximate the weight in milligrams of eggs collected for a species-specific egg rinse preparation.

Using a sufficiently sensitive measuring device, count out and weight 50 eggs.

Divide total weight by 50: X weight/egg mg = XTotal/50

NOTE: To approximate egg mass of water hardened eggs; weigh eggs only after water hardening stage (i.e. > 3 hours after fertilization).

Egg Rinse Control

For each type of egg rinse for each species an equal volume of control must be produced.

1. Obtain 5 litres of dechlorinated water and immerse the same implement used for egg soaking into the control water for 30 minutes.

2. All controls should be filtered as described in Egg Rinse direction 5.

3. Water should then be pipetted into 100 ml uniquely labelled aliquots and frozen at – 20 º C as soon as possible.

4. Water can be stored temporarily (less than 6 hours) at 4 º C if necessary (i.e. using a cooler and frozen ice packs).

5. For damaged egg rinse controls, 1 litre of dechlorinated water should be agitated and filtered in the same manner as with the actual damaged egg preparation and then topped off to bring the final volume to 6) litres. Proceed to Egg Rinse Control Steps 2-4.**Egg Rinse**

- This procedure will generate 5 litres of egg rinse stock solution (scale as appropriate).

- Intact or damaged fertilized egg cues should be produced immediately after stripping and fertilization, be from a minimum of three male-female pairings, and soaked less than 5 minutes after fertilization.
- Intact or damaged unfertilized eggs cues should be from a minimum of three females and soaked less than 5 minutes after stripping.
- Intact and damaged fertilized water hardened eggs cues should be from a minimum of three male-female pairings, and prepared after 3 hours of water hardening.

NOTE: All eggs should be used prior to any egg disinfection. All fertilization should be dry (done in the absence of water as much as possible or with a minimal amount of the same dechlorinated water used to generate the cue).

1. Using a sufficiently sensitive measuring device, weigh out 45 grams of fertilized, unfertilized or water hardened eggs. Handle eggs delicately.

2. Immerse 45 grams of eggs in 5 litres of dechlorinated water.

3. Let eggs soak in water for 30 minutes.

4. Remove the eggs and retain supernatant.

5. Filter suspension by fine filtration (i.e. through a 0.45 µm filter membrane) to retain only dissolved materials.

6. Pipette into 100 ml uniquely labelled aliquots and freeze at – 20º C as soon as possible (5 L/100 mL aliquots = 50 aliquots).

7. Supernatant can be stored temporarily (less than 6 hours) at 4ºC if necessary.

Damaged fertilized, unfertilized, and water hardened egg Using a sufficiently sensitive measuring device, weigh out 45 grams of fertilized, unfertilized or water hardened eggs. Handle eggs delicately.

1. Immerse 45 grams of eggs in 5 litres of dechlorinated water.

2. Gently and manually macerate eggs using an appropriate hand-held implement (such as a potato masher) inside the 1 litre of water for approximately 30 seconds so as to simulate in-stream egg damage. Complete homogenization is not necessary and may, in fact, be too aggressive to yield an ecologically-relevant cue.

3. Filter suspension appropriately to remove large particles (course filtration) followed by fine filtration (i.e. through a 0.45 μm filter membrane) to retain only dissolved materials.

4. Retain filtrate and proceed with Egg Rinse directions 6-7.

Record Keeping

Record the following details for each collection: Date, time, location, species, an estimate of individual egg mass, water temperature, delay until freezing, comments on collection, handling or sample preparation.

A.2 Standard Operating Procedure (SOP) for Collection and Transportation of Rusty Crayfish

Species: *Orconectes rusticus*

1. **Purpose:**

   1.1. This method is used to collect a population of 100-400 individual live rusty crayfish for experimental purposes.

2. **Responsibility:**

   2.1. All collection should be carried out by the researcher(s) identified on the Ontario Ministry of Natural Resources (OMNR) Scientific Collection Permit obtained in advance (Appendix A.).

3. **Minimum Qualifications/Training Required:**

   3.1. CCAC animal care Modules 1-5 (pending review Winter 2011)

   3.2. WHMIS

   3.3. BAF facility orientation

   3.4. First Aid (recommended for all field work)

   3.5. G level drivers permit

4. **Materials:**

   4.1. OMNR Scientific Collection Permit.

   4.2. If collection will occur in a provincial park, a permit to conduct research in a provincial park is required (contact: Steve Kingston Zone Ecologist (NW Zone) (807) 475-1761 steve.kingston@ontario.ca, current as of March 2011).

   4.3. 8-10 wire mesh conventional minnow traps with fastening clasps.

4.4. Bait; conventional wet cat food of the seafood, fish or shellfish variety works well. One individual pouch or container for every trap to be baited.

4.5. Approx. 300 m of synthetic rope or heavy twine.

4.6. Plastic jugs or containers (with caps) to act as marker buoys. One for every line (trap) to be set.

4.7. Handheld clicker counter for crayfish enumeration.

4.8. Two large capacity (60-100 L) coolers for collection, sorting, and transport. Larger is better.

5. Procedure:

5.1. Identify each marker buoy with the following information: collection permit number, name, contact number, date, university affiliation, trap number (i.e., 1/8, 2/8, 3/8, etc.)

5.2. Attach approx. 30 m of rope or heavy twine to each minnow trap clasp with the marker buoy attached roughly 3 m from the free (shore-side) end of the twine.

5.3. Perforate one individual bait pouch or container several times to facilitate odour dispersal and place into minnow trap.

5.4. Deploy each minnow trap in 1-4 m water, spaced at least 20 m apart within the littoral zone and attach well to shore structure.

5.5. Traps should be left to "soak" for 12-24 hours but not longer as crayfish can escape once trapped inside.

5.6. Prior to collection, fill each cooler with lake water; this is best done in the shade if it is sunny and/or warm out.

5.7. Empty each minnow trap's catch into one of the two coolers.

5.8. Using a randomly generated list of numbers, of a sample size (*n*) equal to the number of crayfish to be retained, and ranging from 1- the number of crayfish

anticipated to be caught (500-1000 would be appropriate for the number of traps described here in one of the two locations listed below), systematically remove all crayfish from cooler 1, while placing the random number listed crayfish into the second cooler as one counts through the catch. Crayfish not retained should be retuned to the lake from which they were caught. Good practice would include enumerating the number of males versus females as well as taking weight (g) and cephalothorax length (mm) from a sample no smaller than 10% of the final population. This is useful morphometric information to help characterizes the population. Either males or females or both can equally be collected in this manner.

6. **Notes:** Collection in this manner has been successfully conducted at Pounsford Lake (Sleeping Giant Provincial Park, 48º 29.5274' N, 88º 46.3475' W) and Whitefish Lake (48º 13.6' N, 89º 59.3' W) in the Thunder Bay District of Northern Ontario in June, July, August and October, in 2009 and 2010 where abundant populations are available. Crayfish can be trapped year round however, they are more active and more accessible during the summer months. Crayfish collected in this fashion can be kept under stable laboratory conditions for 3-4 months (See Crayfish Holding (ABEL-INTERNAL-SOP-2011-03)).

7. Transportation:

7.1. No more than 200 adult crayfish can be transported for less than 2 hours in 100 L coolers filled to at least 75% capacity with lake water. Vigorous supplemental aeration must be provided to each holding vessel immediately and transit time to acclimation at the receiving aquatics facility should be minimized.

Review/Approval:

Author: _____

Veterinarian:_____

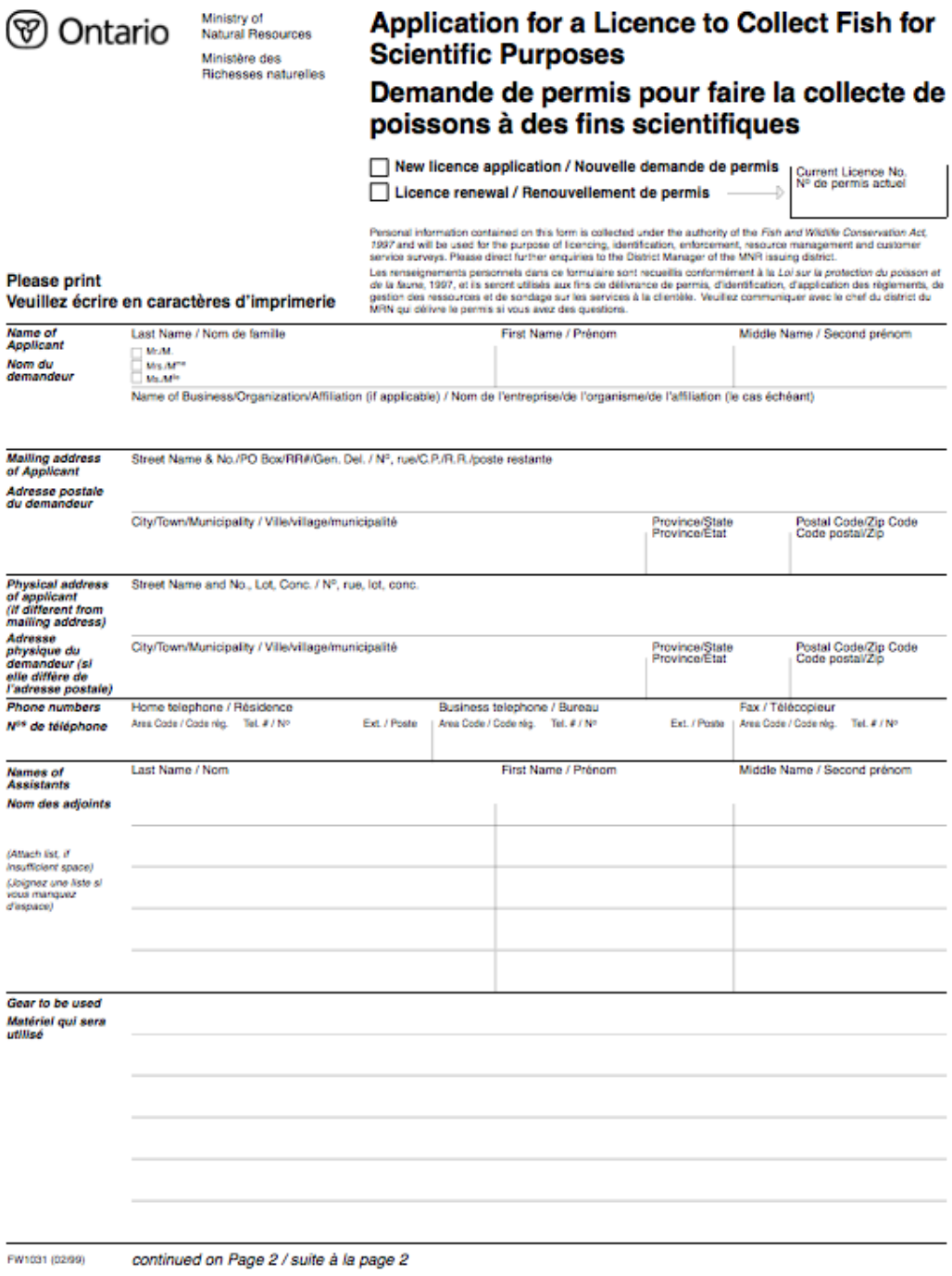
Date of review: _____

ACC Chair: _____

Date of approval: _____

Appendix A.

Ontario Ministry of Natural Resources Collection Permit



Ontario

Ministry of Natural Resources

Ministère des Richesses naturelles

Application for a Licence to Collect Fish for Scientific Purposes

Demande de permis pour faire la collecte de poissons à des fins scientifiques

☐ New licence application / Nouvelle demande de permis

☐ Licence renewal / Renouvellement de permis → Current Licence No. / N° de permis actuel

Personal information contained on this form is collected under the authority of the *Fish and Wildlife Conservation Act, 1997* and will be used for the purpose of licencing, identification, enforcement, resource management and customer service surveys. Please direct further enquiries to the District Manager of the MNR issuing district.

Les renseignements personnels dans ce formulaire sont recueillis conformément à la *Loi sur la protection du poisson et de la faune, 1997*, et ils seront utilisés aux fins de délivrance de permis, d'identification, d'application des règlements, de gestion des ressources et de sondage sur les services à la clientèle. Veuillez communiquer avec le chef du district du MRN qui délivre le permis si vous avez des questions.

Please print
Veuillez écrire en caractères d'imprimerie

Name of Applicant / ***Nom du demandeur***	Last Name / Nom de famille	First Name / Prénom	Middle Name / Second prénom
	☐ Mr./M. ☐ Mrs./Mme ☐ Ms./Mlle		
	Name of Business/Organization/Affiliation (if applicable) / Nom de l'entreprise/de l'organisme/de l'affiliation (le cas échéant)		
Mailing address of Applicant / ***Adresse postale du demandeur***	Street Name & No./PO Box/RR#/Gen. Del. / N°, rue/C.P./R.R./poste restante		
	City/Town/Municipality / Ville/village/municipalité	Province/State / Province/Etat	Postal Code/Zip Code / Code postal/Zip
Physical address of applicant (if different from mailing address) / ***Adresse physique du demandeur (si elle diffère de l'adresse postale)***	Street Name and No., Lot, Conc. / N°, rue, lot, conc.		
	City/Town/Municipality / Ville/village/municipalité	Province/State / Province/Etat	Postal Code/Zip Code / Code postal/Zip
Phone numbers / ***N°s de téléphone***	Home telephone / Résidence: Area Code / Code rég. Tel. # / N° Ext. / Poste	Business telephone / Bureau: Area Code / Code rég. Tel. # / N° Ext. / Poste	Fax / Télécopieur: Area Code / Code rég. Tel. # / N°
Names of Assistants / ***Noms des adjoints***	Last Name / Nom	First Name / Prénom	Middle Name / Second prénom
(Attach list, if insufficient space) / *(Joignez une liste si vous manquez d'espace)*			
Gear to be used / ***Matériel qui sera utilisé***			

FW1031 (02/99)

continued on Page 2 / suite à la page 2

Collection information

Données sur la collecte

Date/Collection Period *From-To* / Date/période de collecte *De - à*

Y/A Y/A Y/A Y/A M M D/J D/J Y/A Y/A Y/A Y/A M M D/J D/J

(Attach list, if insufficient space)

(Joignez une liste si vous manquez d'espace)

Species Espèce	Specify Size (eggs, fry, adults) Précisez le stade (oeuf, fretin, adulte)	Numbers Nombre	MNR District District du MRN	Name of Waterbody Nom de l'étendue d'eau

Attachments

Pièces jointes

Yes/Oui ☐ No/Non ☐

An outline of the proposed project/collection and the objectives of the study. Or if an educational institution, a statement from an authorized officer (department head) of the institution indicating the educational institution's endorsement of the project and an outline of the objectives of the study.

Aperçu du projet/de la collecte proposé(e) et des objectifs de l'étude. S'il s'agit d'un établissement d'enseignement, déclaration d'une personne autorisée (chef de service) de l'établissement attestant son appui pour le projet et aperçu des objectifs de l'étude

I certify that the information provided in this application is true.

Je certifie que les renseignements fournis dans cette demande sont véridiques.

Signature of Applicant / Signature du demandeur

Date of application / Date de la demande

Y/A Y/A Y/A Y/A M M D/J D/J

A.3 Standard Operating Procedure (SOP) for Rusty Crayfish Holding

Species: rusty crayfish (*Orconectes rusticus*)

1. **Purpose:**

   1.1. This method is used to house and provide proper husbandry for a population of rusty crayfish for long-term holding (3-4 months).

2. **Responsibility:**

   2.1. The acting Biology Aquatics Facility (BAF) Aquatics Technician, assisted when necessary by the researcher using the experimental population, should care for the crayfish population.

3. **Minimum Qualifications/Training Required:**

   3.1. CCAC animal care Modules 1-5 (pending review Winter 2011)

   3.2. WHMIS

   3.3. BAF facility orientation

4. **Materials:**

   4.1. 80 L utility tubs with a length of PVC of a sufficient diameter to completely seal into the tub drain. This length of PVC pipe will act as the standpipe and should be cut to a length sufficient to hold the tub full at approximately 80% capacity.

   4.2. A supply of temperature regulated dechlorinated water (DO, pH, hardness, alkalinity, temperature, ammonia and acceptable concentrations of dissolved inorganic substances should be consistent with Boyde's (1998; 1990) "Water Quality for Pond Aquaculture" parameters for finfish).

   4.3. A stable photoperiod (e.g., 16:8 h, light: dark).

   4.4. A supply of clean (i.e., free of oil and other contaminants) compressed air, airline tubing, and air stones to supply supplemental aeration to holding tubs.

   4.5. A submersible circulation pump for each tub to facilitate water mixing and turnover.

4.6. One plastic flowerpot (10 cm O.D.), two size-matched plastic flowerpot saucers, and one medium sized elastic band for every crayfish to be held. Flowerpots should have drainage holes in the bottom to facilitate water exchange. This is suitable for crayfish with a mean weight, cephalothorax length $\pm$ SD; 12.1 $\pm$ 3.3 g, 34.3 $\pm$ 3.0 mm or smaller. For larger crayfish, a larger flowerpot should be used.

4.7. Suitable foods including commercial trout pellet food (Unifeed, Pro-Form Aquapride, 5 mm, www.proformfeeds.com), frozen finfish (cod, tilapia, trout), or dried cat food (fish and shellfish varieties are best).

4.8. Daily log (see example in **Appendix A**. Table 1.).

5. Procedure:

5.1. Crayfish should be chemically isolated (i.e., different tanks) by sex and by origin, and physically isolated from each other within those tanks.

5.2. Flow dechlorinated water through the utility tubs at a rate of 3-5 L/ minute^{-1}. Supply each tank with ample aeration using supplemental airlines and at least two air stones per tub.

5.3. Install submersible water pump to circulate water within tub.

5.4. Place each crayfish into a numbered flowerpot with a saucer covering the opening, held closed with an elastic band. This will act as a housing enclosure for each crayfish and provide mechanically isolation from tank mates. Fifty to sixty crayfish can be stocked per tub in this manner (**Appendix A.** Figure 1.).

5.5. Twice a week feed two 5 mm trout food pellets into each housing enclosure through the drainage holes in the bottom and cover with a second flowerpot saucer (**Appendix A.** Figure 1). Remove the second saucer 24 hours later, rinse flowerpot briefly to remove any residual food material, and replace lid. Note: crayfish are ectothermic and relatively inactive held in this way; therefore they require less caloric intake than more active animals.

5.6. Record temperature and check for mortality daily. Conduct more comprehensive water quality monitoring at a predetermined interval sufficient to ensure adequate ongoing environmental conditions. Parameters such as pH, ammonia, nitrite, nitrate, hardness and alkalinity should be checked at least once a week or sooner.

5.7. Dispose of mortalities by first wrapping them in a brown paper towel, then wrapping them in cellophane, and freezing them in the facility chest freezer. Mortalities should be collected from the freezer and disposed of monthly in accordance with Lakehead University's Office of Human Resources Health and Safety Policy (http://hr.lakeheadu.ca/wp/?pg=140#biosafety).

6. References:

6.1. Boyd, C.E. 1998. Water Quality for Pond Aquaculture. Research and Development Series No. 43. International Center for Aquaculture and Aquatic Environments, Alabama Agricultural Experiment Station, Auburn University, Alabama.

6.2. Boyd, C.E. 1990. Water Quality in Ponds for Aquaculture. Birmingham Publishing Company, Birmingham, Alabama.

Review/Approval:

Author: Cassidy Weisbord

Veterinarian: _____

Date of review: _____

ACC Chair: _____

Date of approval: _____

Appendix A.

Table 1. Example: water quality and daily log sheet

Date	Initials	Feeding	Temp. (º C)	Ammonia (ppm)	Nitrite (ppm)	Nitrate (ppm)	pH	Mortality	Notes

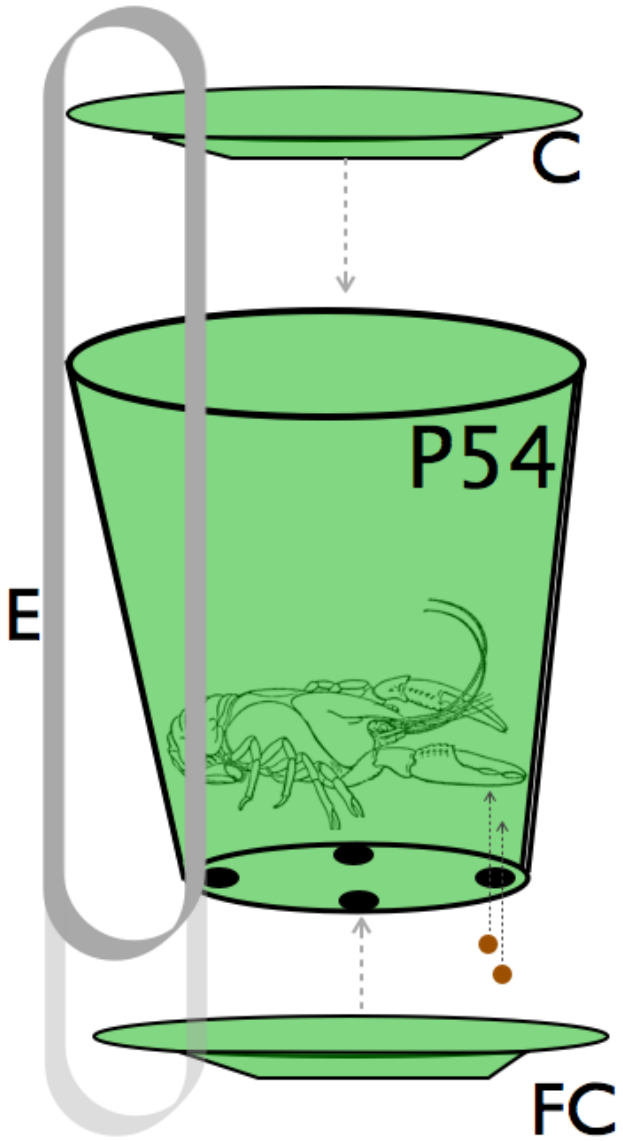


Figure 1. Flowerpot crayfish housing enclosure with positioning of cover (**C**), elastic band (**E**), and feeding cover (**FC**) for bottom.

A.4 Standard Operating Procedure (SOP) for Manual Static Y-maze Assay

Species: rusty crayfish (*Orconectes rusticus*)

1. Purpose:

1.1. This method is used to measure rusty crayfish response to a "chemoattractant". A chemoattractant is defined here as any aqueous solution of single or combined components of interest, at a standardized concentration, which elicits an attraction response when paired with a control, in a test population.

2. Responsibility:

2.1. Execution of this method and the collection of behavioural data thereby generated is the responsibility of the researcher(s) themselves.

3. Minimum Qualifications/Training Required:

3.1. CCAC animal care Modules 1-5 (pending review Winter 2011)

3.2. WHMIS

3.3. BAF facility orientation

4. Materials:

4.1. A Y-maze behavioural (74 L X 39 W cm) arena (Appendix A., Figure 1), including a (corrugated plastic) barrier running two thirds down the middle of the arena to provide chemical separation of the stimulus (s.) and control (c.) arms. A removable, clear, perforated barrier separates the acclimation chamber (a.) from the two arms while allowing chemical exchange to occur.

4.2. A visual barrier sufficient to visually isolate observer from behavioural arena. A small viewing window should provide sufficient visual access to the test animal(s) while minimizing the likelihood of being seen.

4.3. Airline tubing, disposable glass Pasteur pipettes (9 inch, 22.86 cm), 50 ml syringes (depending on volume of stimulus to be delivered). Some type of mounting structure (retort stand with clamps) to hold the syringes will help with stimulus loading and delivery while minimizing disturbance to the behavioural set up.

4.4. Test stimuli at a standardized concentration and a control prepared in the exact same way as the test stimulus minus the treatment.

4.5. A laboratory acclimated (at least two weeks after arrival) test population.

4.6. A temperature controlled dechlorinated water source.

4.7. Hand timers, bench sheets for recording observations (see example below Appendix A.).

5. Procedure:

5.1. Fill Y-maze with a sufficient amount of holding temperature matched, dechlorinated water sufficient to cover the animal comfortably while minimizing a vertical water column (approx. 10 L for the described Y-maze dimensions). This will facilitate the test animal moving in a 2 (rather than 3) dimensional plane.

5.2. Randomly assign stimulus and control to either the left or right delivery arm for every trial and load a designated volume of each. Stimuli and controls should be delivered via a Pasteur pipette positioned over the end of each arm of the arena, connected to a length of airline tubing, and a syringe for remote operation from behind the visual barrier.

5.3. Gently place a randomly designated animal into the acclimation chamber and allow it to acclimate for 8-20 minutes. Longer acclimation periods are better.

5.4. Slowly deliver s. and c. simultaneously via gentle pressure to the syringes over a predetermined delivery period. Stimulus delivery period should be sufficient to allow the s. and c. to diffuse down the arms of the arena to the acclimation barrier. Determine this using preliminary dye trials whereby an aqueous dye mixture is introduced into the end of either arm of the arena and mean latency to acclimation barrier is obtained by observing several repetitions of this process.

5.5. Following the stimulus delivery period, gently lift the acclimation chamber gate, ensuring not to disturb the test animal, and begin the observation period. The observation period will vary and should be based on published methods of relevance (please see cumulative works of Hazlett and Hazlett *et al.,* 1985-2007).

5.6. Behavioural endpoints include (but should not be limited to): first choice (s. or c.), last position following the observation period (s., c., or a.), time (sec.) in the stimulus *v.* control arms, latency (sec.) to first arm choice (Adams and Moore, 2003). Preliminary trials using known chemoattractants are essential to helping choose behavioural endpoints and establishing a baseline characterization of an attraction response in a given experimental population. Unique behaviours should be expected and may be highly relevant.

5.7. All Y-mazes should be thoroughly washed using a mild detergent solution (Sparkleen, Fisher Brand), and rinsed with dechlorinated water between each trial to ensure no stimulus contamination confounds subsequent trials. Syringes and airline tubing should also be washed in the same way if they are to be reused as well.

5.8. **Note:** Even the most meticulously implemented behavioural trial will not prevent a crayfish from behaving as it sees fit. They may hide, scale the sides of

the Y-maze, or spend the entire observation period chewing on something. This must be anticipated and *a priori* rules must be established regarding what constitutes a valid trial and what should be omitted from analysis. All behaviour is valid and careful observations should be made to inform on behavioural endpoint selection and methodology fine-tuning. Instances that may merit omission of a given trial may include when the animal does not make a choice over the duration of the observation period, fails to move at all, or takes more than a predetermined cut-off for latency to choice (e.g., takes 4 minutes or longer to enter an arm).

6. References:

6.1. Adams, J.A. and Moore, P.A. 2003. Discrimination of conspecific male molt odor signals by male crayfish, *Orconectes rusticus*. J. Crust. Biol. **23**(1): 7-14. doi:10.1651/0278-0372(2003)023[0007:DOCMMO]2.0.CO;2.

6.2. Hazlett, B.A. 1985. Chemical detection of sex and condition in the crayfish *Orconectes virilis*. Journal of Chemical Ecology. **11**(2): 181-189.

6.3. Hazlett, B.A. 1994a. Crayfish feeding responses to zebra mussels depend on microorganisms and learning. Journal of Chemical Ecology. **20**(10): 2623-2630.

6.4. Hazlett, B.A. 1994b. Alarm response in the crayfish *Orconectes virilis* and *Orconectes propinquus*. Journal of Chemical Ecology. **20**(7): 1525-1535.

6.5. Hazlett, B.A. 1996. Organization of hermit crab behaviour: Responses to multiple chemical inputs. Behaviour. **133**: 619-642.

6.6. Hazlett, B.A. and Schoolmaster, D.R. 1998. Responses of cambarid crayfish to predator odor. Journal of Chemical Ecology. **24**(11): 1757-1770.

6.7. Hazlett, B.A. 1999. Responses to multiple chemical cues by the crayfish *Orconectes virilis*. Behaviour. **136**(2): 161-177.

6.8. Hazlett, B.A. 2000. Information use by an invading species: do invaders respond more to alarm odors than native species? Biological Invasions. **2**(4): 289-294.

6.9. Hazlett, B.A., Acquistapace, P., and Gherardi, F. 2002. Differences in memory capabilities in invasive and native crayfish. Journal of Crustacean Biology. **22**(2): 439-448.

6.10. Hazlett, B.A. 2003. Predator recognition and learned irrelevance in the crayfish *Orconectes virilis*. Ethology. **109**(9): 765-780.

6.11. Hazlett, B.A., Burba, A., Gherardi, F. and Acquistapace, P. 2003. Invasive species of crayfish use a broader range of predation-risk cues than native species. Biological Invasions. **5**(3): 223-228.

6.12. Hazlett, B.A. 2007. Conditioned reinforcement in the crayfish *Orconectes rusticus*. Behaviour. **144**(7): 847-859.

Review/Approval:

Author: Cassidy Weisbord

Veterinarian: _____

Date of review: _____

ACC Chair: _____

Date of approval: _____

Appendix A.

e.g., Behavioural assay bench sheet

Species:

Stimulus:

Observer:

Trials #	Date/ Time	First Arm Choice (s. or c.)	Last position (s., c. or a.)	Time right arm (s.)	Time left arm (s.)	Latency to choice (s.)	Stimulus side (L or R)
1							
2							
3							
4							
5							

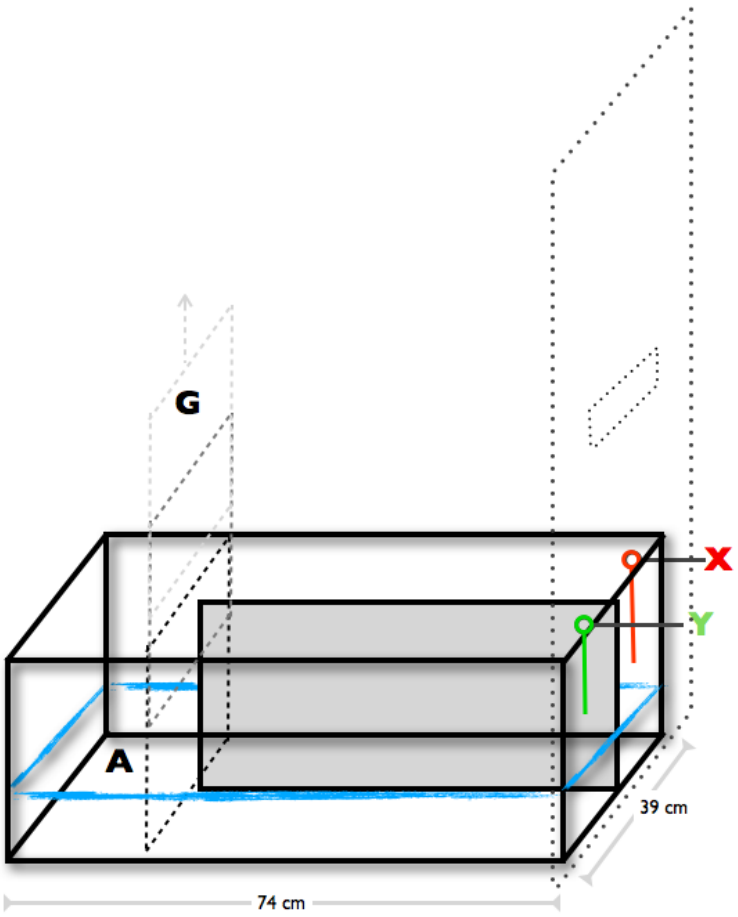


Figure 1. Y-maze schematic depicting the acclimation chamber (**A**), removable gate (**G**), as well as stimulus and control delivery points (**X** and **Y**). Approximate waterline (10 L) for arena of these dimensions is illustrated in blue. Visual barrier is shown in the dotted line along right margin.