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by

## Gayathri S. Sreedharan

## A Thesis Submitted to the Faculty of Graduate Studies and Research Through the Department of Biological Sciences

in Partial Fulfillment of the Requirements for the Degree of Master of Science at the University of Windsor.

## Windsor, Ontario, Canada

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### Abstract

The well-established, invasive round goby (*Neogobius melanostomus*) is known to negatively impact native fish populations in the Great Lakes. My research examined whether food-based traps could capture round gobies which use chemical stimuli to find food and mates.

My first experiment examined the response of round gobies in the laboratory and field to traps baited with lake whitefish, dreissenids, rainbow trout eggs and a control. Results showed that lake whitefish and dreissenids were preferred over other treatments in the field. Digestive tract analysis of captured gobies revealed that dreissenids were the dominant prey type. Subsequently, a laboratory experiment showed that round goby swam faster and spent more time near the odour source when exposed to soaked lake whitefish compared to other treatments, suggesting that lake whitefish could be used to capture non-reproductive fish. However, further studies are required to create a food baited trap to control the round goby.

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## **Statement of Originality**

I certify that this thesis, and the research to which it refers, are the product of my own work and that any ideas or quotations from the work of other people, published or otherwise, are fully acknowledged in accordance with the standard referencing practices of the discipline. I acknowledge the helpful guidance and support of both my supervisors, Drs. Lynda Corkum and Tim Johnson.

I hereby certify that the work embodied in this thesis is the result of original research and has not been submitted for a higher degree to any other University or Institution. Chapter 2 of my thesis has been submitted on August 23, 2007, for publication in Verhandlungen Internationale Vereiningung fur Theorische und Angewandte Limnologie (Proceedings of the International Association of Theoretical and Applied Limnology).

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### **Chapter 1: Introduction**

Chemoreception is defined as the ability to sense and respond to a local concentration change of a particular chemical compound (Atema *et al.*, 1988). In fish, chemosensory systems (olfactory and gustatory) are extremely well developed and mediate important behaviours such as food-finding, recognition/location of familiar habitat, predator avoidance, and intraspecific communication (Sorensen and Caprio, 1998).

Chemical stimuli present in the aquatic environment are often detected by the olfactory system of fish. Chemical odourants are detected by the G-protein coupled receptors on the dendrites of bipolar neurons. These neurons are usually found on the apical surface of olfactory sensory neurons in the olfactory epithelium of the nares. Once binding has occurred with an odourant an action potential is initiated and propagated along nerves where the sensory information is integrated in higher order centers of the brain that may induce a behavioural or physiological output in the animal (Firestein, 2001; van der Goes van Naters and Carlson, 2006).

Chemical stimuli can be defined as either cues or signals. A cue is a chemical released into the surrounding environment by an originator and has no intended receiver. If detected, the receiver would benefit from the information gathered. A signal is a chemical released by a signaller, carrying information that is detected by a receiver which then responds, allowing for the exchange of information or communication which will benefit both signaller and receiver (Wisenden and Stacey, 2005).

Food odours are kairomones, chemicals released by an originator e.g., prey into

the surrounding environment that is detected by receivers such as predators, which may benefit by following odour trails that may lead to potential prey (Wisenden and Stacey, 2005). Specifically, kairomones are odours that are released by individuals of one species and detected by a second species to the benefit of the receiver (Corkum and Belanger, 2007).

## Food Search Behaviour in Fish

Fish exposed to food odours are known to exhibit behaviours which improve their chances of locating and consuming prey (Bateson, 1890). Patterns exhibited by fish that search for prey differ among species (Wunder, 1927). Search patterns include 1) an initial arousal or excitement period, when the fish initially detects the chemical stimulus; 2) a subsequent search or exploratory phase, where the excited fish tries to locate the source of the odour; and, 3) a consummatory phase during which the fish attempts to ingest the food item (Wunder, 1927).

Food odours stimulate a response by organisms once the odours attain sufficiently high concentrations to exceed an individual's threshold for perception (Jones, 1992). The first response to odour detection may be subtle; however, with continued low-level stimulation, more obvious behaviours are exhibited. Sedentary, benthic species such as ictalurid catfish, display these patterns well. Upon detecting a stimulus, they increase their gill ventilation rates, twitch their maxillary barbels, sway their heads back and forth in an exaggerated fashion, take one or more large gulps and finally initiate their search (Jones, 1992).

In other fishes, arousal behaviours include extensions and flickings of fins,

body jerks, twitches and quivers, rapid eye shifting and exaggerated lateral movements of the head and tail. Continued stimulation will eventually lead to locomotor activity, which occurs in the second phase of food search behaviour (Jones, 1992).

Initiation of locomotor activity marks the beginning of the search phase, during which the fish attempts to locate the food source. Fish usually have to swim through odour clouds, plumes or trails released into the surrounding medium as patches shaped by currents and turbulence (Jones, 1992). The search phase is the most variable of the three phases involved in feeding behaviour. Under low stimulation, black bullheads (*Ictalurus melas*), explore substrates and objects along the bottom using their fin and barbel extensions. Exposure to higher concentrations, results in body quivers, digging, body swipes across the substrate and snout-pushing of potential food items (Hodgson and Matthewson, 1978).

Some benthic species also exhibit tactile behaviours when exposed to chemical stimuli. For example, chemically excited eels (*Anguilla anguilla*), partially buried in the sand, feel for prey using their snouts and have been known to circle around an odour source (Kleerekoper, 1969).

Other species such as cod (*Gadus morhua*) and goatfish, (*Parupeneus porphyreus*) typically search the substrate for prey by trailing their barbels over the ground. When a chemical source is detected by taste receptors, the prey item is taken into the mouth (Atema, 1982). Some species exhibit what is known as 'benthic food search' behaviour. Red hake (*Urophycis chuss*), a marine fish will immediately move to the bottom and begin searching for food when exposed to chemical stimuli. Chemically aroused cod also swim to the bottom and exhibit stereotypical benthic search behaviour. Cod often swim

backwards with their heads lowered, trailing their sensitive chin barbels and fins along the bottom, following the odour trail to the potential prey item buried in the substrate (Ellingsen and Doving, 1986). The northern searobin (*Prionotus carolinus*) and striped searobin (*Prionotus evolans*) use their first three extended fin rays to walk over the substrate and, when food odours were detected, exhibit digging behaviours (Bardach and Case, 1965).

Once an odour source is located, the final, consummation phase of search behaviour begins. Here, the fish takes the food item into its mouth and assesses its palatability since chemical stimuli alone give no indication of the quality of food (Jones, 2002). This ensures that inedible or harmful substances, such as toxins are not ingested mistakenly. Therefore, the final decision of ingestion is determined based on responses of taste buds and other tactile receptors within the oral cavity and other parts of the body. Only food items that pass both chemical and tactile tests are swallowed (Jones, 1992).

### Chemosensory Systems in Fish

Two different channels of chemoreception are used to detect chemical stimuli associated with food, olfaction (smell) and gustation (taste). Chemical information that is detected and transmitted directly to the central nervous system by neurons of the cranial nerve I is termed olfaction, while stimuli detected by specialized epithelial cells transmitted by the cranial nerves VII (facial), IX (glossopharyngeal), or X (vagus) are termed gustation (Hara, 1994). Olfaction is considered to be a 'distance' sense, which enables fish to search for and locate food. In contrast, gustation is used more for final approval or rejection of the food source (Hansen and Reutter, 2004). Fishes have highly diverse olfactory systems reflecting the degree of development and ecological habitats they occupy. Typically, olfactory organs are paired structures situated in the snout of fish. Each consists of an olfactory chamber which is connected to the external environment through one or two openings called nares. The peripheral olfactory organ is usually lined with the olfactory epithelium, which contains olfactory sensory neurons (OSN) which detect odour molecules present in the water (Kleerekoper, 1969).

The main component of the gustatory system is the taste bud. It is the structural basis of the 'taste' system and can be found not only within the oral cavity, pharynx, oesophagus and gills but also on the lips, mouth barbels, fins and in some species the entire body surface (Hara, 2006). Taste buds are more abundant in fish than in any other vertebrate. Densities depend on the species of fish and their location on the body surface. For example, bottom feeding catfishes have their entire bodies and fins covered in taste buds while surface-feeding cyprinid fishes have fewer taste buds on their body surfaces (Kasumyan and Doving, 2003).

The taste bud is pear-shaped and slender, standing upright within the stratified squamous epithelium of the skin (Kasumyan and Doving, 2003). The base of the taste bud is situated on top of a small ascending papilla of the dermis. Taste buds are located either on a dome, slightly elevated or sunken in the epidermis. Marginal cells are found between the taste bud and the squamous epithelium. Oral and extraoral taste buds are made up of gustatory receptors, supporting and basal cells. The gustatory receptor and supporting cells are elongated and run parallel to the long axis of the taste bud. They reach the surface of the epithelium by a pore and terminate at large conical receptor microvilli

which detect odours (Kasumyan and Doving, 2003).

## **Chemical Stimuli**

Chemical substances can be divided into several categories, depending on their effects on the feeding behaviour of fishes. Principal stimulants are low molecular weight (500-1000) metabolites such as amino acids, quaternary ammonium compounds, nucleosides, nucleotides and organic acids (Hara and MacDonald, 1976; Carr and Derby, 1986). Feeding is often elicited by specific mixtures of these compounds that are present in prey. Water-soluble amino acids can be detected by fish in both seawater and freshwater at concentrations as low as 10<sup>-7</sup> and 10<sup>-9</sup> M. Free amino acids are dissolved in the cytoplasm and leak from living organisms and carrion and are then detected by predatory fish (Valentincic, 2004)

Both field and laboratory studies have found that whole natural extracts are more effective at eliciting a response than mixtures of amino acids which are in turn more effective than single compounds (Atema, 1980). Johannes and Webb (1970) found that different predator fish species tested were attracted to different mixtures of compounds. A study by Konosu *et al.* (1968) examined the response of eels (*Anguilla japonica*) to clam extracts (glycine, taurine, glutamic acid, serine and threonine) and found that the most pronounced response was to the complete extract not single components or synthetic mixtures (Kasumyan and Doving, 2003). Groups of these soluble substances which usually escape from organisms by leakage, excretion, tissue damage and decomposition (Carr, 1988) are often detected by conspecifics and/or predators which utilize them to find the source (Atema, 1980).

#### Factors Affecting Natural Bait

The practice of chumming, baiting and trap fishing have taken advantage of the well developed chemosensory systems in fish (Atema, 1980). Often natural bait is used depending on its availability, low cost, appropriate consistency and ability to maintain its chemical potency throughout its soak time (Sutterlin *et al.*, 1982). Catch in baited traps is known to be influenced by turbulence and chemical composition of the bait which in turn impacts the foraging behaviours of organisms responding to those odours (Carr and Derby, 1986; Zimmer-Faust, 1993).

Odour-mediated search in aquatic organisms is influenced by three factors: 1) chemical composition of the odour, 2) release rate of the odour, and 3) the fluid dynamic conditions in the habitat. The chemical composition of the odours reveals what the source may be for e.g., food or a mate, and also indicates the quality of the odour source. Amino acids often released from carrion for example are a general indicator of food. Over time, the quality of the odour source may change and this is often reflected in the chemicals released. Adenosine triphosphate (ATP), which is attractive to foragers, degrades to adenosine monophosphate (AMP). The freshness of the carrion is determined by the relative proportion of ATP to AMP (Zimmer-Faust, 1993; Finelli *et al.*, 2000). Another factor impacting odour-mediated foraging is the release rate of odour (molecules/time) from bait. This is often influenced by hydrodynamic conditions. Turbulence and other physical factors such as wind and water currents influence odour release rate and dispersal over time (Murlis and Jones, 1981).

Hence, the amount of time bait remains soaked in water will influence its attractiveness, i.e. the number of individuals captured in baited gear. Soak time is defined

as the time bait remains soaked in water between successive lifts of traps (Bennett, 1974; Miller and Rodger, 1996). Hence, the impact soak time has on bait in terms of attractiveness would need to be determined before a novel bait can be used to capture target species.

### Round goby (Neogobius melanostomus)

Invasive species have invaded and expanded through the Great Lakes and other water bodies for centuries (Mills et al., 1994). The range expansion of some of these species such as zebra mussels (Dreissena polymorpha) and sea lamprey (Petromyzon *marinus*) has been explosive. Both invaders have led to losses of some species of invertebrates and local populations of fish in the case of sea lamprey, leading to great economic and ecological impacts (Hall and Mills, 2000). A number of invasive teleost fishes pose a threat to Great Lakes communities including the Eurasian ruffe (Gymnophelaus cernuus) (Ogle et al., 1995), goldfish (Carassius auratus) the common carp (Cyprinus carpio) (Roberts and Tilzey, 1997) and several species of Asian carp (grass carp (Ctenopharyngodon idella), silver carp (Hypophthalmichthys molitrix), bighead carp (Aristichthys nobilis), and black carp (Mylopharyngodon piceus) (Chick and Pegg, 2001). Ponto-Caspian invaders include the round and tubenose gobies (Jude et al. 1992). The tubenose goby (*Proterorhinus marmoratus*) has not spread as successfully and is limited to the St. Clair River, Lake St. Clair, the Detroit River, and the St. Louis River Estuary in western Lake Superior. The round goby, on the other hand, is distributed throughout the Great Lakes and into connecting waters (Cox, 1999).

The round goby is a bottom-dwelling fish, introduced into the Great Lakes

presumably by ballast water from transoceanic vessels in 1990 (Jude *et al.*, 1997). It is a small, soft-bodied fish with a distinct black spot on its dorsal fin and fused pelvic fin (characteristic of all species within the family Gobiidae). The goby uses the fused pelvic fin (suctorial disc) to anchor itself to the rocky or cobble substrate in fast-flowing water and to assist in releasing gametes on nest surfaces.

Round gobies were initially discovered on the U.S. side of the St. Clair River in April 1990 (Jude *et al.*, 1992) and were later found in Canadian waters in June 1990 (Crossman *et al.*, 1992). They have since spread rapidly through all five Great Lakes and it has been reported that there are about 9.9 billion gobies present in western Lake Erie alone (Johnson *et al.*, 2005). Proliferation is due to many factors including its broad diet. The round goby feeds mainly on bivalves and amphipods (Diggins *et al.*, 2002), but also consumes polychaetes, cladocerans, crayfish, dragonflies, isopods, mayflies, fish larvae and fish eggs (Jude *et al.*, 1992; Corkum *et al.*, 2004). Round goby males are aggressive nest defenders (Wickett and Corkum, 1998). Parental males provide sole parental care, fanning eggs to keep them well oxygenated and defending them against predators (Wickett and Corkum, 1998). In addition, most gobiids are iteroparous, with an extended reproductive season (Corkum *et al.*, 1998; MacInnis and Corkum, 2000).

The round goby has had many adverse effects on native populations, including altering ecological function by changing energy and contaminant pathways (Morrison *et al.*, 2000), feeding on native fishes, perhaps spreading botulism to migratory birds (Corkum *et al.*, 2004) and predation on the eggs and larvae of native fishes, leading to a decrease in recruitment (Steinhart *et al.*, 2004). Dubs and Corkum (1996) suggested that the aggressive behaviour of round gobies may have forced mottled sculpins to deeper

waters where they had fewer spawning sites, less food and were more susceptible to large predators. Janssen and Jude (2001) also documented the extinction of a local population of mottled sculpins in southern Lake Michigan, due to recruitment failure, mainly brought about by round goby interference with spawning. It has also been predicted that gobies will negatively affect the reproduction and hence, rehabilitation of lake trout (*Salvelinus namaycush*) (Chotkowski and Marsden, 1999). Round gobies also feed on benthic invertebrates such as zebra mussels which are exposed to contaminated sediments. When gobies are in turn consumed by piscivores such as burbot (*Lota lota*) and yellow perch (*Perca flavescens*), human health is at risk (Corkum *et al.*, 2004). In the Bass Islands in Lake Erie, video footage has shown that removal of nest-guarding smallmouth bass (*Micropterus dolomieu*) greatly increases predation on embryos and young (Steinhart *et al.*, 2004).

Fishes have very diverse olfactory systems, which vary based on the ecological habitats they occupy. In the round goby characteristics such as the presence of accessory sacs, OSN extending from the anterior nostril to the accessory nasals sacs and the narrow, tubular opening of the naris all help this sedentary, benthic fish use its sense of smell in order to feed and reproduce (Hara, 1992). Other fish species have multilamellar olfactory rosettes in their nasal cavities, with a smaller olfactory chamber, densely packed with OSN. Some fish lack accessory nasal sacs which are usually present in stationary, bottom-dwelling fish (Burne, 1909; Kapoor and Ojha, 1972). Even when present, fish may have one, two or fused nasal sacs.

Like other fish that live in murky or turbid waters, round gobies have evolved highly developed chemosensory systems. This enables them to detect chemical stimuli in

the environment. Their olfactory systems consist of a peripheral olfactory organ with accessory nasal sacs and a tube-shaped unilamellar olfactory chamber with microvillar and ciliated OSN covering the dorsal, ventral and lateral surfaces of the olfactory chamber (Belanger *et al.*, 2002; 2003).

The peripheral olfactory organ has a continuous surface of olfactory epithelium with two small anterior depressions along the floor of the nasal cavity. The two accessory nasal sacs called the lateral lachrymal sac and the medial ethmoidal sac regulate the flow of water over the OSN by compression and decompression of the surrounding buccal muscles and movement of the maxillary bones (Belanger *et al.*, 2003). The presence of the accessory sacs designates the round goby as a cyclosomate. The presence of cilia together with the compression of the accessory sacs creates directional waterflow in through the anterior naris and out through the posterior naris. Odours are probably detected by a combination of 'sniffing' (Nevitt, 1991) and ciliary beats (Belanger *et al.*, 2003). A tendon connecting the accessory sacs to the gills may control the expansion and contraction of the sac (Belanger *et al.*, 2002). Hence, greater gill movement would mean more water could be 'sniffed' for samples of potential chemical stimuli emanating from food. Benthic round goby typically perch on substrates and "sample" water (Belanger *et al.*, 2003).

In terms of sensitivity to odours, round goby respond electrophysiologically to various free and conjugated steroids but not to prostaglandins (Murphy *et al.*, 2001). Except for the use of alanine before steroid testing, I was unable to find studies where

round goby sensitivity to food odours has been tested. A better understanding of how this fish responds both electrophysiologically and behaviourally to food odours is needed.

Although round goby behavioural experiments have measured variables such as time spent near the odour source, swimming velocity, orientation to conspecific washings and gill ventilation to steroids (Murphy, et al., 2001; Gammon et al., 2005; Marentette and Corkum 2007), no studies I am aware of have examined round goby behavioural response to food odours. Other behavioural studies have examined goby response to various food types in laboratory experiments. Benthic goby species such as the tidewater goby (Eucyclogobius newberryi) and the bay goby (Lepidogobius lepidus) have exhibited different behaviours when fed different prey types. Two methods of prey capture have been observed, these include: midwater capture and sideways bites (Swenson and McCray, 1996) also referred to as 'substrate biting' by Grossman et al. (1980). Midwater capture occurred when live or frozen prey was added to the tank. During this time fish would swim towards the surface and intercept the prey item usually midway in the water column. When food was on the bottom substrate, a fish would turn its head sideways and attack the prey item. Fish were also observed taking mouthfuls of sand, churning it, ejecting sediments through the opercula and sifting small invertebrates into the mouth. This behaviour was also referred to as 'substrate biting' by Swenson and McCray (1996). Additionally, Grossman et al. (1980) found that bay gobies were territorial feeders, if one individual exhibited substrate biting, conspecifics in close proximity exhibited the same behaviour.

Odours detected by round goby may be chemical stimuli released by potential prey, or by reproductive conspecifics to coordinate spawning at nesting sites. With

respect to reproduction, sex pheromones are released by males and detected by females which respond by searching for and arriving at the nests. In the round goby, males provide sole parental care, maintaining and defending eggs without feeding (MacInnis and Corkum, 2000). Since males usually occupy nests on complex substrates with low visibility, communication between the males and females likely occurs through chemoreception.

Development of an integrated pest management strategy to control the invasive round goby is on-going (Corkum, 2004). Once developed, multiple co-ordinated strategies would be utilized to increase mortality of round gobies much more effectively than if any one method was used alone (Sorensen and Stacey, 2004). Utilizing chemical stimuli such as food attractants and sex pheromones may be an 'environmentally friendly' way to trap and remove this species. Pheromone and/or food-baited traps have been successfully used to remove insect pests (Corkum and Belanger, 2007). The combination of using pheromone-traps to target reproductive fish during spawning season and foodbased traps to capture non-reproductive fish, post-spawning should be considered as tools to remove gobies. In this manner, larger numbers of round goby would be removed when they arrive near shore and are most vulnerable to capture.

The development of pheromone traps is being investigated by our research group. However, the goal of my research was to determine whether round gobies responded to food-baited traps, whether they exhibited preference (in the field) for any one bait type and if they reacted behaviourally to prepared bait in a laboratory flume. If successful, food-baited traps could be used as part of an integrated management strategy to control and prevent the round goby from spreading further inland and causing harm to

commercially important fisheries.

## **Objectives**

The objectives of my research were to first determine if round goby would enter food baited traps in field and laboratory experiments and if gobies would exhibit preference in the field for any one bait type, when exposed to odours from various natural baits such as lake whitefish, dreissenids, rainbow trout eggs and a control (no odour). Secondly, I wanted to determine whether odours from frozen, thawed and soaked (1h and 24 h) lake whitefish bait varied in their attractiveness to round goby. My thesis investigated the response of round goby to food odours and determined their attraction to food baits that could be used in the development of food-based traps to capture this invasive species.

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# Chapter 2: Round Goby Response to Food Odours in Laboratory and Field Experiments

## **Synopsis**

Round gobies have well developed chemosensory systems which allow them to detect chemical stimuli released from food and conspecifics. This study examined whether round goby differed in their response when exposed to traps baited with lake whitefish, dreissenids, rainbow trout eggs and a control (no odour) in laboratory and field experiments. In the lab experiment, number of fish entering traps did not differ significantly for any of the four treatments ( $\chi^{2}_{0.05, 3} = 0.6$ ; n = 20, p > 0.05), however, there were slightly more round gobies in traps baited with lake whitefish. In the field experiment, greater numbers of round goby entered dreissenid and lake whitefish traps compared to egg-baited ones ( $F_{1, 64} = 4.132$ ; p = 0.045). Field results suggest that lake whitefish which is more readily available than dreissenids could potentially be used as a natural bait to trap round gobies. However, further trapping studies need to be conducted to confirm this.

### Introduction

Fishes detect changes in the chemical composition of their surroundings using highly developed chemosensory systems (olfactory and gustatory) (Jones, 2002). Chemical stimuli are used by teleost fishes in feeding, predator avoidance, reproduction and migration. Chemicals released from living organisms have three distinct properties: they are specific, last beyond the moment of production, and are usually non-directional (Jones, 1992).

Studies have shown that various types of common low molecular weight metabolites, acting either alone or as components of mixtures serve as stimulants of feeding behaviour. These substances include free amino acids, quaternary ammonium compounds, nucleotides, nucleosides and organic acids (Carr *et al.*, 1996; Zimmer-Faust *et al.*, 1988; Jones, 1992). Fish exposed to chemical stimuli emanating from food sources exhibit stereotypical behavioural responses (Jones, 1992). This food search behaviour is characterized by three distinct phases: arousal, search and consumption. Fishing practices have taken advantage of this chemosensory response of fish to food odours and several studies have examined the efficiency of food-baited traps to capture fish such as Atlantic cod (*Gadus morhua*), and torsk (*Brosme brosme*) (Furevik and Lokkeborg, 1994) or longline gear to capture sablefish (*Anoplopoma fimbria*) (Sigler, 2000). In freshwater systems, anglers fishing for yellow perch (*Perca flavescens*) often use minnows as bait (Freshwater Angler, 2001). Benthic traps baited with fish native to the area are used to catch freshwater crayfish (Skurdal *et al.*, 1992; Taugbøl *et al.*, 1997). Chumming (dropping food into water, usually cattle corn) is used to draw carp (*Cyprinus carpio*) into

an area of water after which a hook and line is used to capture individual fish (Pyzer, 2006).

Using naturally odourous materials as chemical lures has been common practice in commercial and sport fisheries for centuries. Chemical lures used in commercial or recreational fishing posses some of the same advantages inherent in chemical cues. They last beyond the moment of production, can disperse over a substantial area and have the potential to be species-specific (Jones, 1992). Often, success with a type of bait depends on its chemical nature because odours from natural organisms have broad appeal and are attractive to a variety of predatory fish. Fish species exhibit a preference order for different natural odours and this would determine which prey are most attractive to a given predator (Jones, 1992). Understanding these fish preferences would enable managers to determine which bait types are most effective in capturing a target species.

The round goby is a bottom-dwelling fish, introduced into the Laurentian Great Lakes presumably by ballast water from transoceanic vessels (Jude *et al.*, 1992). They have spread rapidly through all five Great Lakes due to their broad diet, extended reproductive season, aggressive interactions with other fishes and male parental care (Corkum *et al.*, 1998; MacInnis and Corkum, 2000). Round gobies have had detrimental effects on native fishes by out-competing benthic species such as the mottled sculpin (*Cottus bairdii*) (Dubs and Corkum, 1996; Janssen and Jude, 2001) and logperch (*Percina caprodes*) (Balshine *et al.*, 2005) for food and nest sites.

In addition, round gobies feed on the eggs of smallmouth bass (*Micropterus dolomieu*), lake trout (*Salvelinus namaycush*), and lake sturgeon (*Acipenser fluvescens*) (Steinhart *et al.*, 2004; Chotkowski and Marsden, 1999; Nichols *et al.*, 2003). Steps need

to be taken to prevent the expansion of this invasive species further inland where they may negatively impact native fish populations.

The round goby, like many fish use chemoreception to detect odours (food and sex pheromones) in their surrounding environment. During the spawning season reproductive males (RM) release sex pheromones to attract reproductive females (RF) (Gammon *et al.*, 2005). Because round gobies use sex pheromones for communication, there is a potential to control this invader using pheromone-based traps that are analogous to insect traps used to manage pest species (Wyatt, 2003; Corkum and Belanger, 2007). Because the round goby pheromone lure is in its initial stages of development (Zielinski *et al.*, University of Windsor, in progress) and because bait has been used as an attractant for other species (references above), I wanted to test whether or not the round goby could be captured effectively using food as an attractant. If successful, this could be more effective (stronger attraction, higher catch rates) and/or cost-effective than pheromone traps and could be part of a management strategy to attract non-reproductive individuals. The purpose of this study was to examine the response of round gobies to odours from natural bait such as lake whitefish (*Coregonus clupeaformis*), dreissenids and rainbow trout (*Oncorhynchus mykiss*) eggs in laboratory and field experiments.

### **Materials and Methods**

To determine the response of the round goby to food odours, we conducted laboratory and field studies in which traps were seeded with bait to lure the fish. In both experiments, 15 g of frozen lake whitefish filet (LW), crushed dreissenids (DR) or rainbow trout eggs (E) were each placed in mesh bags (10 cm L x 10 cm W consisting of 0.5 mm square mesh netting) secured inside, rectangular, collapsible minnow traps with two open ends (Fish Farm Supply Ltd., Elmira, Ontario. Model FTA: 61 cm L x 46 cm W x 20 cm H with 1.2 cm L x 1.2 cm W mesh size). An empty trap was used as the control (C). Male and female round gobies were collected by angling at Erieau (46° 16' N, 81°, 27' W) on the Canadian north shore of the central basin of Lake Erie between May and July, 2006. After each laboratory and field trial, fish were euthanized and their gonads dissected and weighed to determine gonadosomatic index or GSI (gonad weight/total body weight x 100). Unfortunately, GSI, our only means to confirm reproductive status, is determined post-mortem. Other morphological traits (puffy cheeks and grey/black colouration) are characteristic of many reproductive males but are unreliable indicators of reproductive status, causing non-reproductive males (NRM) to be incorrectly identified as reproductive males at the beginning of experiments. Since only NRM were used in this experiment I did not encounter problems faced by other researchers working with reproductive males. Dissected fish revealed extremely small gonads, confirming the nonreproductive status of the male round gobies.

## Laboratory Study

Studies have shown that fish (Jones, 1992) and crustaceans (Ristvey and Rebach, 1990) respond more favourably to familiar food types than unfamiliar ones. Fish held in the laboratory were fed Nutrafin® fish flakes. None of the three bait types (treatments) were used to feed captured fish held in the lab. This ensured that results were not confounded by the enhanced sensitivity of round gobies to a particular bait type.

The response of 80 NRM round gobies was conducted in a 6 m, 2-channel wooden flume from August 21<sup>st</sup> to October 6<sup>th</sup>, 2006. Raw Lake Erie water (temperature

18-24 °C) was pumped (flow rate: 1.4 cm/s) through the flume using FSI Filters (Filter Specialists Inc.) to remove sediment. During each trial, a single round goby (total length (TL) >8 cm), deprived of food for 72 h, was isolated in a clear shelter (26 cm x L x 10.8 cm x W x 4.4. cm H) placed 1 m (downstream) from the minnow trap positioned at the upstream end of the 3 m channel. Since the flume had two 3 m channels at its upstream end, this set-up was repeated in the second channel so that two trials could be conducted simultaneously. Every effort was made to minimize disturbance of the focal fish during handling, when food was added and the shelter gate was lifted.

Each 60 min trial consisted of an acclimation period (20 min), when no odour was added to the trap and the experimental fish was enclosed in the shelter behind a wire gate. At the end of the acclimation period, a randomly selected treatment (LW, DR, E, C) was assigned to the trap and the odour allowed to disperse (based on dye tests) for 10 min. During the following 30 min stimulus period (time picked arbitrarily to create a 1 h trial), the fish was released from the shelter and allowed to move freely within the channel. Afterwards, each trap (20 replicates per treatment) was examined to determine the presence or absence of fish.

## Field Study

Four minnow traps with randomly assigned treatments (LW, DR, E, C) and 22 replicates per treatment were deployed from the Erieau pier (18-24 °C) for 24 h from August 24<sup>th</sup> to September 30<sup>th</sup>, 2006. Each of the four traps (separated by 3 m) were secured to 9-m long wire cable affixed to the harbour breakwall. Once traps were retrieved, all fish were removed and identified to species. Fish other than round gobies

were released following identification and measured for total length, weight, head length and head width. Captured gobies were sexed, euthanized using MS-222 and preserved in 70 % ethanol and returned to the lab for digestive tract analysis. The digestive tracts of round gobies were dissected and their contents identified. Of the gut contents recovered, the frequency of occurrence of each prey type was determined.

# Statistical Analysis

Chi squared analysis was used to compare observed and expected frequency of round goby that entered the traps for each of four (LW, DR, E, C) treatments in the laboratory flume. Given that one odour was presented at a time, the expectation was that of the four treatments and 20 replicates, the gobies would respond equally to all treatments.

A one-way Analysis of Variance (ANOVA) was used to determine if there was a significant difference among mean numbers of round gobies that entered the traps deployed from the Erieau pier. The experiment was designed as a randomized block, i.e., traps representing each set of treatments were deployed at one time. Owing to high variability in the numbers of round goby caught among treatments, a Winsoring technique (Zar, 1999) was used (on 5 trials) in which the highest and lowest numbers of round goby caught in a trap were replaced with the next highest and lowest value, respectively, from the replicates within each treatment. Planned comparison tests among treatments were performed to determine 1) if the control traps differed in numbers of round gobies captured from the three treatments baited with food; 2) if traps seeded with lake whitefish and

dreissenid tissue; and, 3) if traps seeded with dreissenids differed in numbers of round gobies captured compared with traps seeded with lake whitefish.

Planned comparisons, which compare chosen means, based on experimental design or interests of the researcher (Day and Quinn, 1989) were used because I expected traps baited with food odours to be more attractive to round gobies than empty traps. I also expected (based on the literature) that round gobies would enter traps baited with dreissenids (Ray and Corkum, 1997) and eggs (Chotkowski and Marsden, 1999; Nichols *et al.*, 2003; Steinhart *et al.*, 2004). Based on laboratory studies conducted in the summer of 2005 (Appendix I), I expected more round gobies to enter lake whitefish baited traps. However, I was uncertain of how these bait types (lake whitefish, dreissenids and eggs) would fare relative to one another.

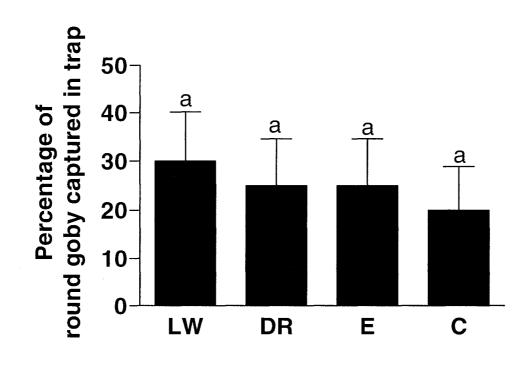
The ANOVA test was followed by the planned comparison tests. In contrast to unplanned comparisons (post-hoc tests), it is acceptable to conduct planned comparisons despite an insignificant ANOVA F-test. Although ANOVA was used in analysis, it is not necessary to conduct such a test first, before proceeding with planned comparisons (Rutherford, 2001).

#### Results

#### Laboratory Study

There was no significant difference in the response of round gobies to lake whitefish, dreissenids, fish eggs and the control (Figure 2.1;  $\chi^{2}_{0.05, 3} = 0.6$ ; n = 20, p > 0.05), indicating that gobies exhibited no difference in response when exposed to any one food type. However, more round gobies entered traps baited with lake whitefish (6/20

replicates) relative to other treatments. Equal numbers of round goby entered dreissenid (5/20 replicates) and egg-baited traps (5/20 replicates). The control trap had the fewest number of fish (4/20 replicates).



# **Odour Source**

Figure 2.1: Response of round goby to four food odour treatments in the lab. Odour sources: LW (lake whitefish), DR (dreissenids), E (rainbow trout eggs) and C (control, an empty net).

### Field Study

The number of round gobies captured in seeded traps deployed in Lake Erie showed no statistically significant difference among treatments (Figure 2.2; 1-way ANOVA;  $F_{3,84} = 1.879$ ; p = 0.139). However, mean number of round goby captured was highest in lake whitefish and dreissenid traps, followed by control traps. Egg-baited traps had the fewest number of gobies. Results from planned comparison analysis showed that there was no significant difference in numbers of round gobies caught in control traps vs. traps with food odours ( $F_{1,64} = 1.162$ ; p = 0.284). However, there were significantly more round gobies caught in traps seeded with lake whitefish or dreissenids than traps seeded with fish eggs ( $F_{1,64} = 4.132$ ; p = 0.045). There were no significant differences in numbers of round gobies captured in dreissenid and lake whitefish traps ( $F_{1,64} = 0.344$ ; p = 0.558).

Of the total number of fish captured (n = 106), round gobies comprised 93 % (n = 96) of the individuals (TL of 95 gobies were > 8 cm; the other goby was 5.7 cm). The remaining 7 % of fishes caught in traps were brown bullhead (*Ameiurus nebulosus*), bluegill (*Lepomis macrochirus*), and rock bass (*Ambloplites rupestris*). Of the 96 round gobies captured, 91 were NRM, 3 were non-reproductive females, 1 was a RM, and 1 was a RF.

Dreissenids were the major prey type (36 %) found in the digestive tracts of captured round gobies. Other prey included Chironomidae (6 %), Gastropoda (7 %), Amphipoda (4 %), unidentified insect larvae (6 %), other unidentified invertebrates (4 %), Hydracarina (2 %), Culicidae (1 %) and eggs (1 %). Partially digested, unidentifiable prey were 33 % and 12 % of all captured round gobies had empty digestive tracts.

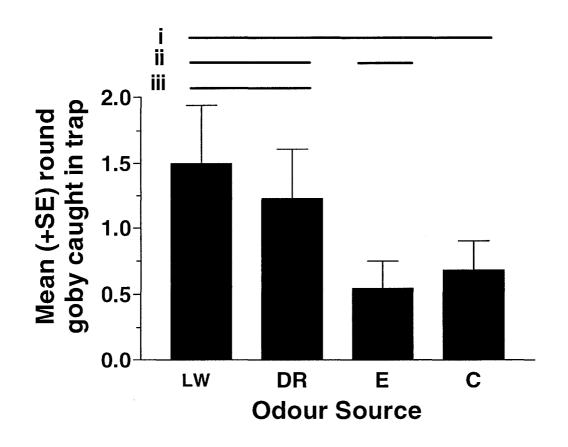


Figure 2.2: Mean round goby caught in traps in the field. Odour sources: LW (lake whitefish), DR (dreissenids), E (rainbow trout eggs) and C (control, an empty net).

## Discussion

Fish exhibit behavioural patterns when exposed to chemical stimuli associated with food, further improving their chances of locating and consuming the food source (Jones, 1992). The round goby is a generalist feeder with a varied diet (Diggins, 2002), one of the factors which has enabled its rapid spread into newly invaded environments. Hence, we expect that, like other generalist fishes (Jones, 1992), gobies would be responsive to chemical stimuli from various food sources.

The lab experiments were not as successful as the field experiments (Figure 1.1). The sudden relocation of fish from a tank with conspecifics into a new confined space (clear shelter) and handling may have caused stress which inhibited a natural tendency to move toward odours. A similar laboratory trapping study conducted in 2005 and 2006 in a circular, above ground, static pool showed that round goby exposed to odours for 24 h (2005) and 72-96 h (2006) exhibited no significant preference for any of the four treatments (Johnson and Lee, OMNR, unpublished data). Since this study did provide sufficient time (24 h in 2005 and 72-96 h in 2006) for the experimental fish to respond, it is unlikely that a lack of response from fish (absence in trap) was due to a short stimulus period. Other laboratory studies that examined the response of round gobies to fish washings have reported directed responses by conspecifics (Gammon et al., 2005; Marentette and Corkum, 2007). However, these studies were conducted in 1-m flumes, isolating holding quarters where disturbances were minimized and only the researcher had access to the room during trials. This controlled environment ensured that experimental fish were likely unaffected by outside influences. In contrast, the flume and

pool were located in a warehouse beside a harbour used constantly by fishing vessels. This background noise, which I was unable to control, may have affected fish response; i.e., the fish may have been stressed. Despite the lack of significant results in the laboratory studies, field trapping studies with round gobies have a greater likelihood of success. Baited traps deployed into rivers and lakes where fish are often less influenced by human disturbance (Taugbøl *et al.*, 1997) may result in a more dramatic response by fish to food odours.

In the field study, significantly more round goby were captured in lake whitefish and dreissenid traps than traps that were seeded with rainbow trout eggs (p < 0.05). Round gobies appear to respond well to lake whitefish which is not part of their regular diet, but was a readily available odour source to use in trials. It is common practice in both commercial and experimental trapping studies to use native bait that is readily available (Taugbøl *et al.*, 1997). The high response of round gobies to the dreissenid treatment was expected. Ray and Corkum (1997) showed that dreissenids represented 58 % of round goby diets. In addition, Kovtun *et al.* (1974), who examined round goby diets within their native range (Sea of Azov), reported that 78 % of goby diets consisted of molluscs.

Digestive tract analysis conducted on all gobies captured during field trials revealed that dreissenids formed the major component of goby diets. Previous studies by Ray and Corkum (1997) have found that round gobies over 7 cm (standard length) fed predominantly on zebra mussels. SCUBA surveys conducted at the field site where traps were deployed found the most abundant round gobies were over 8 cm in length. Other invertebrates such as chironomids, amphipods and gastropods have also been found in

round goby digestive tracts (Miller, 1986; Ray and Corkum, 1997; Ghedotti *et al.*, 1995). The large number of round gobies captured in traps is likely due to the effectiveness of minnow traps at capturing most benthic fish. Based on angling success, round gobies seem to be the dominant species at this particular site. Being a live capture gear, if minnow traps were used as part of a control program, non-target species could be released unharmed.

Round gobies also feed on the eggs of native fishes such as smallmouth bass, (Steinhart *et al.*, 2004), salmonids (Fitzsimons *et al.*, 2006) and lake sturgeon (Nichols *et al.*, 2003). Thus, the low mean number of round goby captured in the rainbow trout egg treatment in our study was unexpected. It suggests that round gobies may not be as effective egg consumers as previously thought. In lab experiments, Chotkowski and Marsden (1999) found that although gobies readily preyed on lake trout eggs and fry, only individuals over 50 mm in length consumed eggs. However, mottled sculpins of the same size were able to feed on more eggs than the gobies. Recently, Roseman *et al.* (2006) found white perch (*Morone americana*), not round gobies to be the most important consumer of walleye (*Sander vitreus*) eggs in Lake Erie.

One explanation for why round gobies were not attracted to egg-baited traps may be because the eggs used were not releasing chemical cues. Salmonid eggs released into the environment are known to undergo a process called water-hardening where the external components of the eggs become insoluble in water and impervious to most compounds (White, 1930; Hemming and Buddington, 1983). Clary (1972) seeded artificial redds with water-hardened brown trout (*Salmo trutta*) eggs and reported that slimy sculpins (*Cottus cognatus*) were not attracted to them. Dittman *et al.*, (1998)

conducted lab studies where sculpins were not attracted to eggs water-hardened for 12-24 h. Chivers and Mirza (2002) also found that water-hardened eggs were less attractive to sculpins than fresh eggs. Sculpins were, however more attracted to odours from injured eggs than water-hardened ones, suggesting that when injured, eggs may be releasing chorionic fluid and various components of the yolk sac which act as chemical cues (Hemming and Buddington, 1983). Apparently, water-hardening limits the period during which chemosensory cues are released from eggs, possibly decreasing the time available for predators to locate buried eggs (Dittman *et al.*, 1998).

The large numbers of round gobies captured in field traps reflects the dominance of this invasive fish in near shore areas of Lake Erie. For the past decade, round gobies have consistently ranked in the top three most abundant species captured in trawls, and the gear and habitats sampled are known to bias low the catch rate of round gobies (Lake Erie Forage Task group, 2007). Bunnell *et al.* (2005) reported that in 1999 round goby numbers peaked at 350 million individuals in the central basin of Lake Erie.

Our study site, Erieau, is located in the central basin and thus it was no surprise that round gobies comprised a majority (93 %) of the fish species captured in traps. Interestingly, 94 % of all captured gobies were NRM. A likely explanation for this is that NRM exhibit greater behavioural activity than RM. Flume studies by Marentette and Corkum (2007) found that NRM exposed to odours from conspecifics, exhibited greater activity levels and spent the least amount of time inside shelters. Nesting round goby males aggregate at spawning sites where RM establish territories and tend to eggs (Charlebois *et al.*, 1997). Video footage of gobies nesting on a shipwreck showed that when nests were left unguarded, neighbouring conspecifics immediately moved in and

fed on the eggs (Wickett and Corkum, 1998). This suggests that although NRM do not engage in spawning activity, they benefit from traveling to nesting sites because it may give them access to unguarded eggs, a lipid-rich high energy food source (Foote and Brown, 1998). NRM making the journey from deeper waters may also learn routes and locations of spawning sites from conspecifics (Warner, 1990). Gammon *et al.* (2005) suggested that NRF round gobies may be responding to odours from RF in flume studies because they may be using odours to follow them to spawning sites in the field.

Developing an effective control strategy for round gobies is on-going and will likely utilize multiple strategies similar to those used to control the sea lamprey (Sorensen and Vrieze, 2003; Stebbing *et al.*, 2004; Gammon *et al.*, 2005). The round goby management program is in its preliminary stages of development, hence it was important to determine whether food baited traps could be used as part of a management strategy to control this invasive fish.

Results from the field component of this study suggests that bait such as lake whitefish could be used to successfully capture round gobies. On average whitefish baited traps captured ~1-2 round gobies/day, although this may be too small a number to be very effective in eliminating them from established sites. In Inland tributaries, where round gobies are recent arrivals and have yet to establish populations, trapping could prove to be effective. In addition, using natural food as bait is relatively less expensive than alternatives such as pheromones which would require the development of tabletted steroids. It is essential to repeat these trapping studies in inland waterways where round gobies co-occur with native spawning fishes to determine whether this method could be effective in controlling the further spread of this fish.

It is hypothesized that when developed, pheromones could be used to target reproductive fish during spawning season and post spawning season, food could be used to target non-reproductive individuals. This way, additional pressure could be applied on round goby populations by targeting reproductive as well as non-reproductive individuals.

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# Chapter 3: Round goby response to lake whitefish bait odours

# **Synopsis**

Historically, fishers have used baited traps to capture desired species. Traps seeded with various food baits such as lake whitefish and dreissenids have been shown to lure non-reproductive male round gobies (Chapter 2). I examined the effectiveness of variously prepared lake whitefish bait (tissue that was frozen, thawed, soaked for 1 and 24 h) and a control in attracting round gobies in the lab. Fish reacted differently to control and bait treatments. Round goby spent more time ( $F_{1,20} = 17.492$ , p = 0.004) and swam faster ( $F_{1,20} = 7.608$ , p = 0.012) when exposed to bait odours compared to a control. Fish also swam faster when exposed to soaked baits (1 h and 24 h) compared to frozen and thawed baits ( $F_{1,20} = 6.846$ , p = 0.016). This study identifies properties of the bait that will make the food-baited traps more effective at capturing round gobies.

# Introduction

Natural baits are known to release chemicals that are attractive to fish and hence have been incorporated into baited longlines, pots, and other kinds of gear to capture commercial fish species (Sutterlin *et al.*, 1982). The primary stimulants of prey extracts are common metabolites of low molecular weight that include amino acids, quaternary ammonium compounds, nucleosides, nucleotides and organic acids (Carr and Derby, 1986; Zimmer-Faust *et al.*, 1988; Jones, 1992). These stimulants are most attractive when presented as mixtures of compounds rather than as single substances. The attractiveness of these baits in the field is often impacted by their soak time i.e., the amount of time they spend in the water between successive lifts of traps (Bennett, 1974; Miller and Rodger, 1996). Longer soak time reduces the attractiveness of bait due to the release or leaching out of feeding attractants into the surrounding environment (Moore and Wong, 1995). Attractant release rates are initially rapid, but decline over time (Mackie *et al.*, 1980). It is therefore important to determine how long novel bait can remain attractive to fish before it can effectively be used to lure and trap a target species.

The round goby is a bottom-dwelling invasive fish, introduced into the Laurentian Great Lakes presumably by ballast water of transoceanic vessels (Jude *et al.* 1992). They have spread rapidly through all five Great Lakes owing largely to their broad diet, extended reproductive season, aggressive interactions with other fishes, and parental care (MacInnis and Corkum 2000). The round goby, like many fish, use chemoreception to detect odours in their surrounding environment (Corkum and Belanger, 2007). This makes the species an ideal candidate to control using traps baited with chemical cues.

Field studies conducted for 24 h showed that more round gobies entered traps baited with lake whitefish (*Coregonus clupeaformis*) and dreissenid mussels [(*Dreissena polymorpha*) and (*Dreissena bugensis*)] than fish eggs (Chapter 1). Since lake whitefish is readily available, it could be used as bait to trap round goby in the field. Despite fish being caught during the 24 h trials, it is not known whether the bait remains attractive throughout this period or if its attractants leach out within a couple of hours. Once we determine when baits become less attractive, traps could be rebaited at an appropriate interval to maximise capture efficiency.

I sought to determine if bait preparation (thawed vs. frozen) and soak times affected the response of gobies to odours. The goal of this study was to examine how round goby responded when exposed to odours from lake whitefish bait that was frozen, thawed and soaked for 1 h and 24 h.

## **Materials and Methods**

Round goby were collected by angling from the Canadian shore of the Detroit River at Windsor, ON (42°20'N, 82°56'W) and the northwestern shore of Lake Erie at Leamington, ON (42°03'N, 82°36'W) between May and July, 2007. Fish were housed at the University of Windsor Animal Quarters in accordance with Animal Care Guidelines.

All fish were maintained in the laboratory at temperatures of  $19 \pm 2^{\circ}$  C with a photoperiod of 16 L:8 D, and fed daily with Nutrafin® flakes. To induce a response of round goby to food odours, experimental fish were deprived of food for 72 h before

experiments began. The experiments were conducted within a 1-m long (70.5 L) flowthrough fibreglass flume.

Treatments (different preparations of whitefish) were prepared ahead of time and frozen until needed for experiments. Fresh lake whitefish filets were divided into 15 g portions and frozen at -80° C. To prepare treatments, frozen pre-weighed portions were placed in 0.5 mm mesh bags (10 cm L x 10 cm W), stapled closed and allowed to sit in dechlorinated tapwater (9.0 L) in glass aquaria (30 cm L x 20 cm W x 15 cm H) for various periods of time depending on the treatment. Water in the holding tanks was discarded before a new bag was added. External filters created currents to draw odours out of the lake whitefish tissue faster than simple passive diffusion, mimicking conditions in the field, where odour would be 'lost' to the surroundings. The five prepared treatments included: 1) 15 g of frozen lake whitefish soaked in dechlorinated water for 24 h; 2) frozen lake whitefish soaked for 1 h; 3) frozen lake whitefish thawed in a plastic bag in air for 0.5 h; 4) frozen lake whitefish; and, 5) control, i.e., an empty mesh bag. All mesh bags were attached to a 162 g lead weight prior to introducing them into the flume.

Each experimental trial (1 h) was divided into three sequential 20-min periods: acclimation (no additional water was added to the flume), control (dechlorinated tap water was added to the flume at a rate of 1 cm/s) and stimulus (bait treatments were added). The bait was placed at the upstream end of the flume in front of an airstone so that the inflowing water passed over the mesh bag, dispersing the odour. Fish were secured behind a clear, perforated, plexiglas gate during the acclimation and control periods and released only after the treatment was added at the upstream end during the stimulus period. Each fish was used only once and the gonadosomatic index (GSI) was

determined to confirm that the fish were not reproductive. The reproductive status of round gobies is often difficult to determine using external morphological characteristics and can only be confirmed by their GSI. Here, dissection often revealed fish with extremely small testes and no seminal vesicles which often gave a reading of 0 on the weigh scale. All trials were videotaped using a Hitachi-Kokusai CCD colour camera (model KP-D20A) and recorded with a DVD Sony RDR-GX 300 player. The images were analysed using a software program Fishtracker 2.0 (Shen, 2005).

# Statistical Analysis

Dependent variables measured were time spent near (within 25 cm) the odour source (s), swimming velocity (cm/s), reaction time (time at which fish crossed the start line, (s)) and distance ratio (Spears, 2007) (the actual distance (cm) the fish travelled during the 20 min stimulus period/ hypothetical most direct distance = 75 cm) was collected for each trial. With a nest length of 25 cm, hypothetically, once released the most direct distance (a straight line) a fish can travel would be 75 cm (100-25).

Swimming pathway revealed the path followed by the fish every second for the 20-min stimulus period. This variable was not quantifiable as the program I used Fishtracker 2.0 was not designed to measure swimming pathway. It produced a final figure depicting the path followed by the fish to determine what regions of the flume it had been to. The height of the camera mounted above the flume prevented any detailed behavioural responses such as head/fin movements, gill ventilation, biting/snapping and orientation to be observed.

I would have expected round goby to spend more time near the odour source, swim faster and react quicker when exposed to odours from frozen and thawed whitefish

compared to stale bait that was soaked in water for 1 h or 24 h. Distance ratio was expected to be closest to 1 (75/75), i.e. a direct, straight path to the odour source for thawed bait. A value greater than 1, i.e. a meandering path was expected for the frozen and/or stale bait (soaked for 1 h and 24 h). Swimming pathway, another variable was also expected to be a more or less straight path towards the upstream end of the flume when exposed to odours from thawed bait which would have leached out readily and dispersed throughout the tank. Fish were expected to meander much more when exposed to frozen and/or stale bait (1 h and 24 h). This was likely due to fish having to 'sniff' trace odours and eventually make it to the source at the upstream end of the tank.

Data were analyzed using a 1-way analysis of variance (ANOVA) to determine whether there was a significant difference among the means of each variable measured. Planned comparisons were used to determine if fish reacted differently to: 1) the bait treatments compared to the control; 2) tissue that was soaked for 1 h and 24 h compared to frozen and thawed tissue; 3) tissue that was soaked for 24 h compared to 1 h; and 4) frozen tissue compared to thawed tissue. Distance ratio data were transformed to normalize the variance. However, data were not normally distributed and a Kruskal-Wallis test was used in analysis.

I did devise a rule to ensure that variables measured could be used in statistical analysis and to minimize the experimenter's influence on fish response. I ignored all trials in which fish did not leave the shelter or darted out in response to the gate being lifted (i.e. a response to the operator).

## Results

There was no significant difference in mean reaction time of round goby among any of the five treatments ( $F_{4,20} = 2.151$ , p = 0.118) (Figure 3.1). There was however, a trend suggesting reaction time was lowest for soaked baits compared to other treatments. Bait soaked for 24 h and 1 h had fish reacting within 412 and 436 s respectively. Fish response to frozen bait occurred after about 728 s of exposure. Nevertheless, round goby spent significantly more time near (i.e., within 25 cm) the mesh bag for all four odour treatments compared with the empty (control) bag ( $F_{4,20} = 5.451$ , p = 0.004) (Figure 3.2). There was no difference in the mean time spent among the four odour types.

There were significant differences in mean swimming speed of round goby among the five treatments ( $F_{4,20} = 3.809$ , p = 0.018) (Figure 3.3). Round goby swam faster towards odours that were soaked for one hour or more than to odours released from lake whitefish tissue that was recently thawed or still frozen. The slowest mean swimming speeds were recorded when no bait was added to the mesh bags. Although the swimming speed towards the control (i.e., inflowing water) was the slowest, it does indicate the tendency for round goby to move upstream. It is important to point out that fish remaining in the shelter throughout a trial or darting out when the gate was lifted, which led to trials being repeated was not associated with any one treatment and was completely random. The control treatment had the least number of repeated trials (8), followed by the lake whitefish soaked for 1 h (9), the lake whitefish soaked for 24 h and frozen lake whitefish (10) and lastly thawed whitefish (12). With five replicates per treatment, results from five fish which left the shelter and moved to the upstream end of the flume for each treatment were used in analysis. A total of 25 fish were used in the entire experiment.

There were no significant differences in distance ratio among the five treatments  $(H_{4,24} = 6.73, p = 0.151)$  (Figure 3.4). Distance ratio was slightly lower for thawed bait compared to other treatments. All treatments had values higher than 1 (1.75 – 1.85), suggesting that irrespective of treatment, fish did not meander much, deviating from a straight line by only a factor of 2 before finally reaching the farthest upstream end of the flume.

Swimming pathways revealed that round gobies exhibited one of two swimming patterns. Fish moved directly from the shelter to the upstream end of the flume and in most cases remained within 25 cm of the stimulus until the end of the trial (Figure 3.5) or as in other cases moved back and forth between the downstream (shelter) and upstream (treatment) end of the flume (Figure 3.6). Once at the upstream end, fish usually swam keeping their bodies perpendicular to the far wall where the treatment is added.

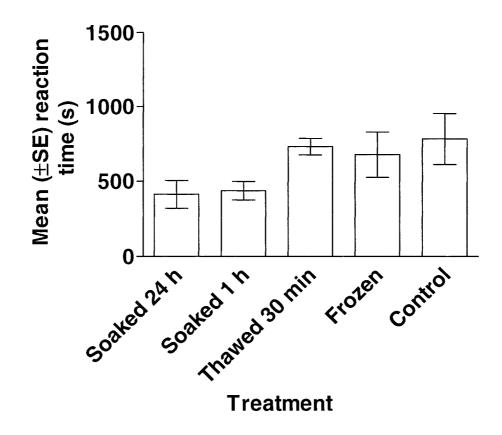


Figure 3.1: Mean (standard error, SE) reaction time (seconds) at which male non-reproductive round goby responded to odours. Odour sources: lake whitefish tissue soaked for 24 h, 1h, thawed for 30 min, or frozen and a control (no odour).

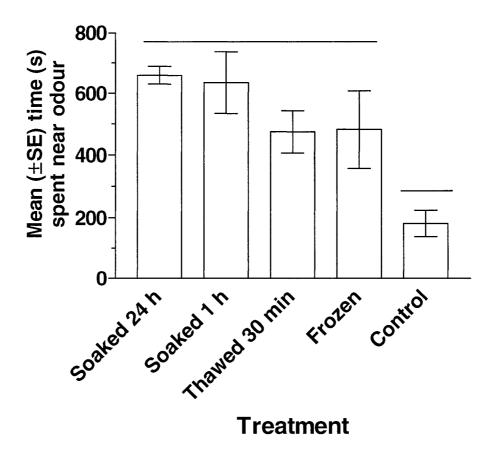


Figure 3.2: Mean (standard error, SE) time (seconds) that male non-reproductive round goby spent within 25 cm of an odour source. Odour sources: lake whitefish tissue soaked for 24 h, 1h, thawed for 30 min, or frozen and a control (no odour).

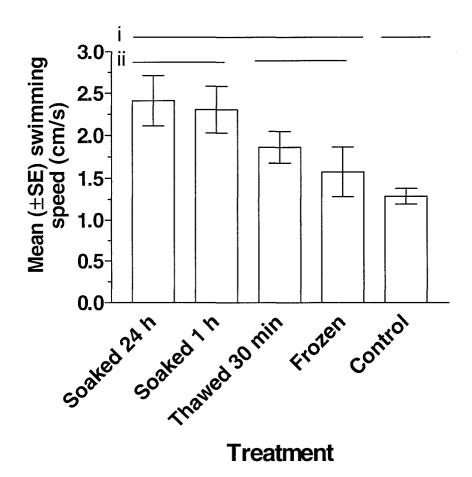


Figure 3.3: Mean swimming speed (standard error, SE) that non-reproductive round goby exhibited toward the 5 treatments. Odour sources: lake whitefish tissue soaked for 24 h, 1h, thawed for 30 min, or frozen and a control (no odour).

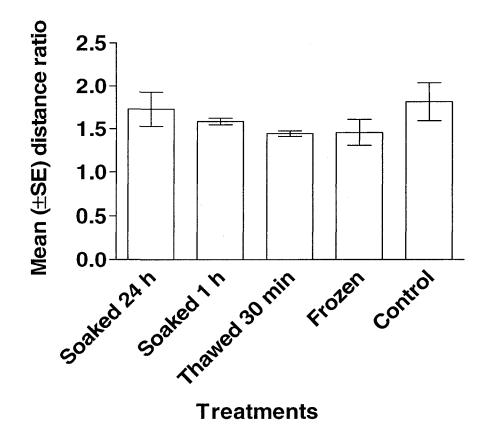


Figure 3.4: Mean (standard error, SE) distance ratio that non-reproductive round goby exhibited towards the five treatments. Odour sources: lake whitefish tissue soaked for 24 h, 1 h, thawed for 30 min, or frozen and a control (no odour).

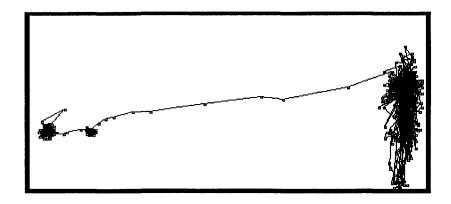


Figure 3.5: One of two swimming pathways exhibited by round goby exposed to treatments. Fish swam directly from the shelter to the upstream end of the flume.

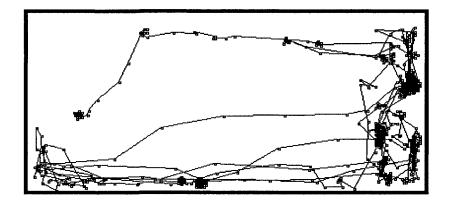


Figure 3.6: Fish swam along corner walls of the flume and did not remain in any one area for long.

# Discussion

Round goby spent more time near mesh bags that contained bait compared to a control, indicating that chemical cues are being released by the bait, invoking a response in experimental fish. Fish were clearly able to distinguish between the control and bait, and went on to further discriminate soaked baits from frozen and thawed ones. The increased swimming velocity to soaked baits is important because round gobies are negatively buoyant, slow-swimming fish, which may incur high energy losses when trying to stabilize their swimming trajectories (Webb, 2002; Gammon *et al.*, 2005). Hence, despite the energy required, swimming towards an odour source could increase their likelihood of encountering and consuming potential prey.

The lack of significant differences in distance ratio among treatments was unexpected and suggests that fish may have travelled similar distances towards the odour source. Typically, fish exposed to thawed bait should have had the lowest distance ratio since thawing causes cell rupture leading to a greater initial odour release rate from bait (Daniel and Bayer, 1987; Fahy, 2001), causing fish to meander less and follow odours to the source at the upstream end. Reaction time is linked to distance ratio because the distance travelled by the fish is calculated only after the fish reacts or crosses the 25 cm mark. Despite the fact that fish exposed to soaked baits reacted about 300s quicker than other treatments, on average all fish, irrespective of treatment, exhibited no significant difference in reaction time when exposed to different treatments.

Although control trials where fish did not leave the shelter were discarded, the fact that fish were leaving the shelter in trials that were used in analysis, when no food odours

were present does raise questions about whether fish were responding to odours from contaminated mesh bags which were re-used due to their limited supply. It is important to point out that every effort was made to ensure that bags were washed thoroughly and reused in the same treatment. This way mesh bags used in control trials were never used to carry any of the bait treatments, hence the presence of residual odours in the mesh bags could not have lead to fish leaving their shelters. Experimental fish could have been responding to current or sound (from inflow, where water flowed into the tank) at the upstream end. However, it should be noted that fish did vary their responses when other variables (time spent and swimming velocity) were measured among treatments.

The greater swimming velocity towards stale bait was unexpected. Studies have shown that fresh bait is far more attractive to target species than stale bait because soaking bait over time causes its attractants to leach out, rendering it unattractive to fish (Løkkeborg and Pina, 1997). Moore and Wong (1995) found that traps baited with green crab (*Carcinus maenus*) leached for 6 days (144 h) were less attractive to amphipods (*Orchomene nanus*) than freshly killed bait. California spiny lobster (*Panulirus interruptus*) fed on significantly fewer mussels (*Mytilus edulis*) that had been soaked for 24 and 48 h compared to freshly killed mussels (Zimmer-Faust, 1993).

In contrast, European lobsters (*Homarus gammarus*) were caught in traps containing stale bait that had been soaked for several days (Bennett, 1974). Scavenging caenogastropods typically prefer fresh over decayed food (Britton and Morton, 1994). However, species of *Bullia* (Nassariidae) prefer slightly decayed tunicates over fresh ones (Morton and Jones, 2003).

Stale bait may contain the products of tissue decomposition including biogenic amines such as putrescine, cadaverine and histamine (Rawles, 1996). In addition, bacteria break down trimethylamine oxide into trimethylamine (TMA) after death. TMA is a component of fish odour and is often used together with biogenic amines as indicators of freshness (Fraser and Sumar, 1998). Round gobies are known to be opportunistic feeders (Charlebois et al., 1997) and have been observed feeding on dead conspecifics in aquaria (personal observation). This suggests that they might not be repulsed by the odours released from decomposing tissue. Hoese and Hoese (1967) examined the behavioural response of the naked goby (Gobiosoma bosci) to oyster extracts and found that decomposition by-products putrescine and TMA, among others, initiated a feeding response. These substances together with ammonium acetate have also been used in food-based terrestrial traps to capture Mediterranean fruit flies (Diptera: Tephritidae) (Midgarden et al., 2004). An alternate explanation for why round gobies responded more to soaked baits could simply be that the release of amino acids from the bait was much slower over time and hence may have still been attractive despite being soaked for 24 h.

A study by Montgomery (2005) examined the impact that soak time (and other factors) had on the numbers of rock lobster (*Jasus verreauxi*) captured in traps. Despite traps being soaked for up to 3 days, there were no significant differences in the number of lobsters captured. However, the greatest numbers of lobsters were caught within 48 h, followed by a drop in catch at 72 h. In our study, lake whitefish bait remained attractive after a 24 h soak period, however, we did not assess the effects of longer soak times.

Swimming pathways of the round goby in this study were similar to those observed by Gammon *et al.* (2005), who found that round goby swam along the far wall

of the flume near the odour source with its body in a perpendicular position. Gammon *et al.* (2005) also suggested that round gobies may be using chemotropotaxis i.e., directed turns based on the simultaneous comparison of chemical stimuli on either side of the body (cf. Wyatt, 2003) to detect odours. Round gobies also moved to the upstream end of the flume even under control (no odour) treatments, suggesting that, with their well-developed lateral line systems, gobies may have been moving in the direction of the current (Jude *et al.*, 1995).

Previous field experiments (Sreedharan *et al.*, 2007) have shown that round goby can be captured using lake whitefish baited traps. However, we did not determine how long the bait remained attractive. This study shows that frozen lake whitefish would likely remain attractive during most, if not the entire, 24 h soak period.

Despite fish swimming a little faster towards soaked bait (1 h and 24 h) in the laboratory, where temperature is constant, environmental variables such as temperature and water currents would cause frozen fish to thaw quickly in the field, hence it would be unnecessary to thaw out bait before securing them in traps. More field experiments are required before actual trapping programs can begin in earnest. However, based on results from my field (Sreedharan *et al.*, 2007) and laboratory studies, I would recommend that managers use frozen whitefish in benthic minnow traps preferably in areas where round goby co-occur with native fishes. Additional field experiments using food baited traps could determine if there are any impacts on native fishes. If other benthic fishes are captured in traps, all but round gobies could be returned unharmed. Food baited traps should also be evaluated in tributaries to assess the effectiveness of this control strategy under varying flow regimes.

Trapping programs should probably be conducted during the spring and summer months because low temperature is known to make fish sluggish (Fry, 1971), which in turn could alter their swimming activity and trappability (Stoner, 2004). When water temperatures are high, ectothermic round gobies like other fishes are likely to swim towards and enter baited traps more readily. This could help restrict round goby numbers to where they can be controlled so that their chances of spreading are diminished. Additionally, high numbers of round goby could be captured if traps are deployed on complex substrate such as rock or cobble which round goby are known to inhabit. Also, the addition of rock or objects that could increase the complexity of soft substrates could draw more round gobies to an area making them more vulnerable to capture.

Ultimately an integrated control strategy employing a variety of methods targeting different life stages and habitats, similar to that used for sea lamprey (*Petromyzon marinus*) (Jones, 2003) and rusty crayfish (*Orconectes rusticus*) (Hein *et al.* 2007), will prove to be more effective than any single control. In Sparkling Lake, Wisconsin, Hein *et al.* (2007) demonstrated that the combination of intensive trapping and restricted harvest of predators led to the successful removal of rusty crayfish. Current research is exploring the potential of food, pheromone (Corkum, Zielinski and colleagues, U Windsor), and sound traps (D. Higgs, U Windsor) to limit the spread of round gobies. When developed, pheromone traps could be used effectively during spawning season to target only reproductive fish, while food and/or sound traps could be an effective lure for other life stages and during other times of the year.

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### **Chapter 4: Conclusions and Future Work**

## **Conclusions**

When exposed to various natural bait types in the field for 24 h, round goby exhibited preference for lake whitefish and dreissenids, with the greatest number of fish being captured in these traps. From this initial field study, we know that round goby do respond to bait odours by entering benthic minnow traps which can be used to effectively capture this fish in the field. Chemical attractants found in bait are known to initially have rapid release rates which slow down over time (Mackie *et al.*, 1978). Hence, it is important to determine if round goby attraction to bait was affected by how long it was soaked in water and its mode of preparation (frozen vs. thawed). Results showed that round gobies swam faster when bait was soaked in water (1 h and 24 h) compared to other treatments.

Apart from actual odour type and concentration, turbulence and odour release rates are two factors that determine attractiveness of bait. The field study showed that cut up pieces of lake whitefish are attractive to round gobies, however, my experimental design did not allow me to determine any temporal variation during the 24 h soak period. My design focussed on soak time and sample preparation (frozen vs. thawed). Results showed that round goby swam faster to the bait the longer it was soaked (24 h vs. 1 h). This suggests that traps deployed for 24 hours can efficiently capture round gobies because odours continue to be released up to and beyond 24 h.

Although food baited trap odours do not capture more than 1-2 round gobies/day, in novel habitats where round goby numbers are low, this method of trapping could become an important part of an integrated pest management system. Other round goby

control methodologies currently under development include sound and pheromone controls. While pheromone traps will target reproductive females, food and sound-based traps could target other life stages and could be used at other times of the year. The combination of methods targeting multiple ages throughout the year may exert sufficient pressure on round goby populations to slow or even prevent their spread into waterbodies connected to infected waters such as tributaries and lakes surrounding the Great Lakes.

#### Future Work

Invasive species, such as the round goby are a leading threat to biodiversity (Sala *et al.*, 2001; Vander Zanden *et al.*, 2004). Hence, it is imperative that we rapidly develop methods to slow down and eventually control the further spread of this species in order to limit their colonization in recently invaded waters. It is important that we try to prevent the further spread of organisms that have become established and are negatively impacting native populations.

There are several examples of successful controls of invasive species. In a subalpine lake in Sierra Nevada, California, introduced trout species (*Oncorhynchus* sp and *Salvelinus* sp.) were successfully removed using gill netting with minimal impact on non-target species (Knapp and Matthews, 1998). In 2000, Culver and Kuris reported the first successful eradication of a locally well-established marine polychaete (*Terebrasabella heterouncinata*). This marine pest was eradicated by first removing its preferred host, the black turban snail (*Tegula funebralis*), and also preventing the release of additional infested material from an abalone mariculture facility which was the source

of the established population. This shows that early detection and pro-active aggressive action can help eradicate even well-established invaders (Culver and Kuris, 2000).

In the Great Lakes, efforts are being made to prevent the introduction of invasive species, however there are relatively few practices in place to control invaders once established (Vásárhelyi and Thomas, 2003). One of the few exceptions is the integrated sea lamprey control program, which has limited further spread of the sea lamprey without complete eradication of the species (Jones *et al.* 2003; Sorensen *et al.* 2005). This integrated management strategy includes barriers to block upstream migration of spawning lampreys, the use of a lampricide, 3-trifluoromethyl-4-nitrophenol (TFM) to kill larvae with few impacts on native fauna, and a sterile male release program to reduce reproductive success in hard to chemically treat areas such as the St. Mary's River (Jones *et al.*, 2003).

Recently, it has been shown that sea lamprey use migratory (Sorensen and Vrieze, 2003) and sex pheromones (Li *et al.*, 2002) to attract conspecifics. Migrating adults are attracted into streams by a 'migratory' pheromone released by stream-dwelling larvae (Sorensen and Vrieze, 2003). Wagner *et al.* (2006) found that 90 % of migrating sea lamprey swam into streams and barrier-integrated traps containing the migratory pheromone. Once in the streams, males construct nests and release sex pheromones to attract ovulating females (Li *et al.*, 2002). Wagner *et al.* (2006) also found that traps baited with spermiating males were highly attractive to females. This strongly suggests that pheromone traps can be added to the integrated pest management system used to control sea lamprey populations in the Great Lakes (Wagner *et al.*, 2006).

Similar research is underway to develop pheromone-based traps to exploit the use of sex pheromones by round gobies during the spawning season. Reproductive males (RM) establish nests and release sex pheromones to attract reproductive females (RF) (Gammon *et al.*, 2005). Our research group is currently working to identify the specific pheromone blend used by males to attract females so that they can be used to bait pheromone traps. Sex steroids identified in the testes of round goby (Arbuckle et al., 2005) are believed to be released in the urine to attract RF. A preliminary study by Yavno and Corkum (2007) showed that artificial RM models in the presence of RM urine were able to lure RF regardless of urine concentration. This finding reinforces the role of multiple cues (in this case chemical and visual) in attracting conspecifics (Heath et al. 1995). If combined with olfactory (food) cues, an even more successful integrated control may result. Faleiro et al. (2003) demonstrated that while food baited traps alone did capture the target pest weevils (Rhynchophorus ferrugineus) they were more effective and less influenced by environmental conditions when used simultaneously with the maleproduced aggregation steroid ferrugineol.

Round gobies also appear to use the auditory sense to communicate with conspecifics. Laboratory studies by Rollo *et al.* (2007) showed that round gobies responded to playback calls from male conpsecifics. Work is on-going to develop sound traps which could potentially be used to lure round goby. This could be one of the strategies of an integrated program to curb further harm caused by this species.

### Towards an Integrated Pest Management System

Round gobies are a very successful invading species owing to their broad diet, extended reproductive season, aggressive interactions with other fishes, and parental care (MacInnis and Corkum 2000). This aquatic invader has spread to new waterways and has now invaded Lake Simcoe (first report August 2006). As such an aggressive control strategy is needed to limit their success in occupied habitats and prevent (or slow) their spread into uninfected waters. Pheromone and sound based controls are currently in development, and food-baited traps while showing promise, require additional refinement to ensure maximum capture success. Combining highly specific pheromone controls that target reproductive females with food and sound controls that should be effective year round and on all life stages should provide a comprehensive control program.

Refinements to the food-based control strategy would include exploration of alternate natural baits, optimal mass of bait, and more detailed analysis of soak time effects. I chose a suite of natural baits that represented different types (fish eggs, fish flesh, preferred prey) and found fish flesh (lake whitefish) and preferred prey (dreissenids) evoked the strongest response. Lake whitefish flesh is also readily available compared to dreissenids making it a cost-effective bait. Subsequent work on characteristics of the lake whitefish used a constant mass of 15 g, an amount in excess of the daily ration of round gobies (Lee and Johnson, 2005). Studies have revealed increased behavioural and electrophysiological response to increasing concentrations of food odour (Fuzessery and Childress 1975). Additional work should investigate the ideal amount of food under field conditions that yields the highest round goby catch rates. Finally, a more detailed analysis of soak time effects to determine the duration of effectiveness of the

bait, will inform managers of how frequently the traps need to be serviced. Depending on the soak time result, research into the development of artificial bait that produces ideal traits (sustained attractiveness over an extended period) may be warranted (Miller and Heukelem, 1988; Fahy, 2001). Such refinements will improve the quality of a food-based trap, and therefore its effective contribution to any control strategy.

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## **Appendix I**

# A Preliminary Laboratory Food Odour and Home Range Study on Round Goby Response to Food

### Introduction

The round goby (*Neogobius melanostomus*) is a bottom-dwelling fish, introduced into the Great Lakes presumably by ballast water of transoceanic vessels (Jude, 1997). They have since spread rapidly through all five Great Lakes and it has been reported that there are about 9.9 billion gobies present in western Lake Erie alone (Johnson *et al.*, 2005). Proliferation of this species is due to its broad diet, extended reproductive season, aggressive interactions with other fish and male parental care (Corkum *et al.*, 1998; MacInnis and Corkum, 2000). In addition, the diel vertical migration of larval round goby has likely led to them being taken up with ballast water collected from near the water surface during the day and dispersed to other regions of the Great Lakes (Hensler and Jude, 2007). Their abundance in rocky habitats (Ray and Corkum, 2001), and predation on the eggs of lake trout (*Salvelinus namaychush*) (Chotkowski and Marsden, 2001), lake sturgeon (*Acipenser fluvescens*) (Nichols *et al.*, 2003), and smallmouth bass (*Micropterus dolomieu*) (Steinhart *et al.*, 2004) may cause reduced recruitment in populations of these native species.

Steps need to be taken to prevent the further spread of this species. Although complete eradication of the round goby from the Great Lakes is unrealistic, it is reasonable to try and stop the spread of this fish into inland waters by removing round gobies that have moved into an area but have yet to establish populations there.

Similarly it would be fruitful to decrease the abundance of round goby where they co-occur with native fishes that are spawning so that round goby predation on native fish eggs would be reduced. In addition, round goby recruitment could also decrease if sex pheromones are used to attract gravid females into traps or if other odours (food) are used to trap conspecifics. Due to their well-developed olfactory systems (Belanger *et al.*, 2003), it would be beneficial to use olfactory cues to trap round gobies. Work is on-going to try and develop odour-based traps as attractants in luring round gobies that move to shallow waters to spawn during the spring and summer months (Chapter 1 and 2).

Round gobies exhibit high site fidelity, having a home range of  $5 \pm 1.2$  m<sup>2</sup> (Ray and Corkum, 2001). Home range, defined as "the area over which an animal normally travels" (Gerking, 1953) is an important factor that could be used to effectively capture this invasive species. Although we expect round gobies to respond to baited traps, their actual movement into traps may depend on how far away traps are deployed from spawning sites. Despite traps releasing attractive food odours, it is possible that sitespecific round gobies, even the non-reproductive males that do not guard nests, may not venture far from established spawning sites. Therefore, it is useful to determine what distance would be ideal to deploy traps to effectively capture round gobies. If results showed that round gobies enter traps regardless of whether they are set close to or farther away from spawning sites, then less attention needs to be paid to this aspect of trap deployment. However, if we find that these fish only enter traps that are within their home ranges, then perhaps traps will need to be placed closer to spawning sites where round gobies may be more likely to enter them.

Experiments were conducted as a background study on the home range of non-

reproductive adult round goby. As an attractive scent, bait odours were used to lure round goby to traps. I conducted two pilot experiments; 1. Which food bait type (dreissenids, dew worms, lake whitefish) is most attractive to round gobies. 2. Given the preferred bait (exp. 1), determine how far round gobies travel to reach food baited traps.

## **Materials and Methods**

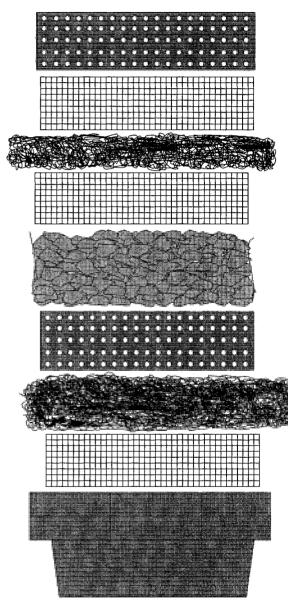
### Flume Design and Filters

The entire flume was built using plywood. Lake Erie water was pumped into the flume to create a more natural setting than if dechlorinated tap water was used. Water was pumped into a head tank (100 cm wide) which when full, overflowed into two channels (each 50 cm wide). Each channel extended for 300 cm and opened into a 100 cm wide common channel (~300 cm long). During the second experiment which utilized the wider common channel, a considerable amount of time was needed for food odours to mix evenly. To allow for rapid and even dispersal of odours, two baffles together with an airstone were placed in the common channel. Bubbles from the airstone (placed at the 300 cm mark in the common channel) created currents which dispersed odours while the baffles, which narrowed the channel to a width of 50 cm ensured that dispersal would be rapid and even. This was confirmed by dye tests. The baffle adjustments and addition of the airstone ensured that the sedentary round goby could remain motionless in any corner of the channel and still encounter the odour.

Background water from the harbour reduced visibility in the flume and so several attempts were made to reduce turbidity using filters designed with input from the Ontario

Ministry of Natural Resources (OMNR) staff. The initial filter was a plastic tote (77 cm L x 44 cm W x 29 cm H) filled with layers of aquarium (5 cm) and quarry gravel (5 cm) covered in window screening (15 mm L x 15 mm W). However, neither sand nor gravel improved visibility.

A second filter was designed by Steve Budinsky and colleagues at the University of Windsor Tech Services Department (Figure 1). Using the same tote, different materials and additional layers were used. Perforated plastic screening (1.3 cm deep) was placed at the bottom of the tote to allow water to flow through the filter. About 8 cm of Aquapure® filter fibre was placed on top of the plastic and a stainless steel perforated sheet to contain the fibre filter. Wire netting containing quarry gravel and plastic screening was added followed by 5 cm of filter fibre, plastic screening and lastly (top layer) stainless steel perforated sheets. With this new design, visibility was further improved, however, the water was still not clear enough to see fish and hence trials could not be videotaped. Also, visibility was dependent on weather conditions, if conditions were clear with no wind and rain, visibility was high however, in choppy weather, visibility was poor.



**Perforated stainless** steel (0.32 cm) **Plastic screening** (1.27 cm) Aquarium filter (5.08 cm) **Plastic screening** (1.25 cm) Window screening with quarry gravel (8.25 cm) **Perforated stainless** steel (0.32 cm) Aquarium filter (7.62 cm) **Plastic grading** (1.25 cm)

Flume tote

Figure 1: Tote filter with various layers of perforated stainless steel, plastic screening, quarry gravel and aquarium filter.

All experiments were conducted from August 27<sup>th</sup> – September 12<sup>th</sup>, 2005 in a 6 m, 2-channel wooden flume at the OMNR in Wheatley, Ontario. Round gobies were collected by angling from the Canadian waters of the Detroit River, Learnington and Wheatley. Fish were stored in holding tanks (205 L) in the animal quarters facility at OMNR, following animal care guidelines. All round gobies were fed with Nutrafin® fish flakes, however experimental fish were deprived of food for a 24-h period before being used in experiments. In all trials only 15 g of frozen bait (Lee and Johnson, 2005) enclosed in mesh netting (10 cm L x 10 cm W; mesh size 0.5 mm L x 0.5 mm W) was used. After use, all fish were disposed of as per OMNR guidelines.

### Food Odour Study

This study was conducted from August 27<sup>th</sup> to September 1<sup>st</sup>, 2005. In each channel, experimental fish were placed in a clear shelter (16 cm L x 11 cm W x 5 cm H) behind a wire gate at the downstream end of the flume. At the opposite end, one of three bait types (frozen lake whitefish, dreissenids and dew worms) were placed in a minnow trap (Fish Farm Ltd., Model FTA: 61cm L x 46 cm W x 20 cm H) located 100 cm away at the upstream end of the flume. Due to time constraints, each channel (300 cm long) was used to run 2 trials simultaneously (Figure 2). It was necessary to use fish with a total length of at least 8 cm in all trials. One reason for this was that only fish over 7 cm in length feed on dreissenids (Ray and Corkum, 2001), hence using fish under this length could confound results. In addition, a total body length of at least 8 cm was needed to ensure the fish would be visible in DVD recordings

Each trial (n = 12) was 1 h and 30 min long. During this time an experimental fish

was placed in the clear shelter and secured behind a wire gate. The trial commenced with a 30-min control period in which Lake Erie water flowed through the channel; flow rate was ~ 0.80 cm/s. After this, I randomly selected one of three baits and positioned the bait within a designated trap. On the basis of dye tests, odours from bait were allowed to diffuse for 15 min. At the end of this period, the gate, which restricted the fish to the downstream area of the channel, was lifted. The subsequent stimulus period was 45 minutes. Temperature readings were measured before every trial and only non reproductive males (NRM) were used.

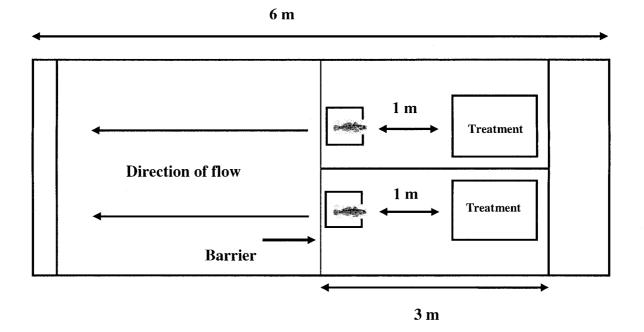


Figure 2: Sketch of food odour study. Experimental fish placed 1 m downstream from the treatments placed in each 3 m channel.

#### Home Range Study

The goal of this study was to determine if round gobies would travel outside their home range (~ 5 m<sup>2</sup>) to enter food baited traps. To determine what number would best represent distances inside and outside their home range, we assumed 5 m<sup>2</sup> to be a circle and calculated the radius as 1.26 m ( $\pi$ r<sup>2</sup> = 5; r =  $\sqrt{5}/\pi = \sqrt{5}/3.142 = 1.26$ ). For convenience 1.25 m was considered to be the distance inside and 2 m as the distance outside the home range.

The home range study was essentially a preference study which utilized the entire length of the 6-m flume (Figure 3). Each trial (n = 12) was divided into periods similar to the food odour study, with a designated control (30 min), time for the odour to travel the length of the channel (15 min) and stimulus period (45 min). Experimental fish were placed in the clear shelter (16 cm L x 11 cm W x 5 cm H) downstream from the odour source at 1.25 m or 2 m. Lake whitefish bait was placed inside one of two minnow traps, one located 0.25 m inside each channel. During each trial, a NRM round goby was secured inside a shelter at a distance of either 2 m or 1.25 m downstream from the two traps. Frozen lake whitefish bait (15 g) was randomly added to one of the traps; the second trap was empty and designated as the control. After the 15 min time to diffuse, the experimental fish was released and preference was determined by its presence or absence in the trap at the end of each trial.

These trials were not used in analysis. Although behavioural trials had been recorded, none could be used in analysis because high turbidity of Lake Erie water made it impossible to follow fish movement in the channels. Accordingly, only fish entry into traps was recorded.

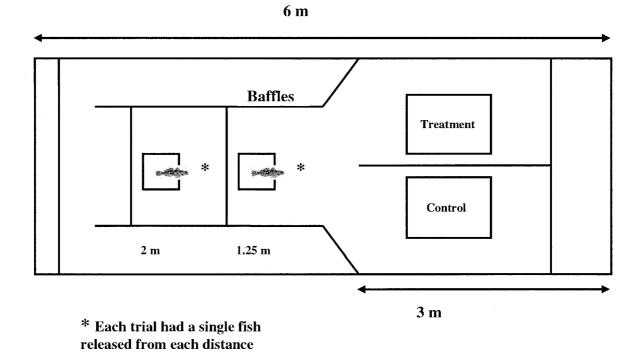
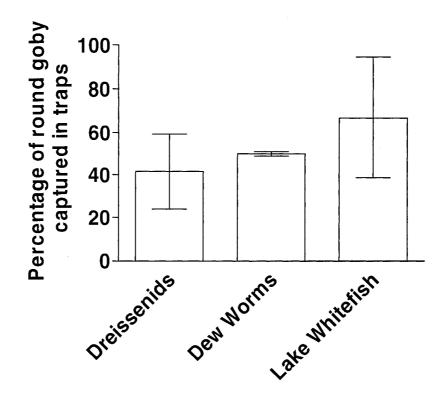


Figure 3: Sketch of home range study with the lake whitefish treatment and control traps in each of the 2 channels and the round goby placed released from a distance of either 1.25 m (inside home range) or 2 m (outside home range) from the traps. The location of the treatment trap in the left or right channel was determined by a coin toss.

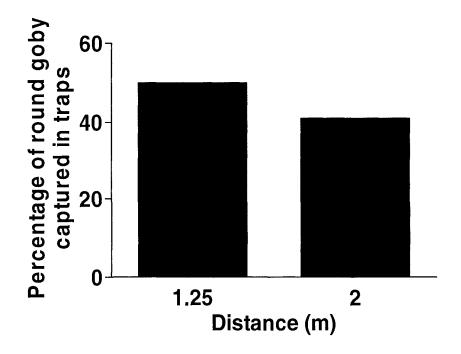
### Results

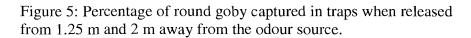
The results of the food odour experiment showed that there was no significant difference ( $X^2_{0.05, 2} = 5.25$ , NS; n = 12) in the number of round goby caught in traps baited with any of the three food types (lake whitefish, dew worms and dreissenids). However, the mean number of round goby caught in lake whitefish traps was greater than fish caught in the other traps (Figure 4). Therefore, lake whitefish was used as a food attractant in the home range study. Analysis of results using a Fisher's Exact test (p > 0.05, NS; n = 12) showed that there was no difference in response of fish to food odour (lake whitefish) from inside (1.25 m) and outside (2 m) their home range (Figure 5). Six round goby moved 1.25 m (i.e. within their home range) into an upstream trap; this does not include the 4 individuals which entered the wrong trap (control). Five round goby released from the 2 m mark (outside their home range) entered traps. From this distance only one fish entered the control trap.



Treatments

Figure 4: Percentage of round goby captured in traps in a 6-m flume.





#### Conclusions

Living in dark, turbid environments has led round gobies to evolve highly developed olfactory systems (Belanger *et al.*, 2003). Round goby feed on a wide variety of benthic prey items such as dreissenids, trichopterans, dipterans, amphipods, gastropods and softshelled crayfish (Kovtun *et al.*, 1976; Ray and Corkum, 1997; French and Jude, 2001). Having to rely on chemical stimuli to detect food, it is likely that round goby would be responsive to various food odours which could lead them to potential prey in their natural environment. This study showed that round gobies did not differ significantly in how they responded (entered traps) to the different bait types despite a slightly higher number of fish entering traps with lake whitefish in the lab. Low replication could have been a factor influencing the lack of significance in statistical tests. The slightly higher number of round goby in lake whitefish baited traps and easy availability of this native fish were factors that led to this bait type being used as an odour source in the second study.

This study also examined whether high site fidelity (Ray and Corkum, 2001) could be a factor influencing trappability of round goby. A home range study conducted off Peche Island using SCUBA to follow individual fish for an hour found that round goby had a mean home range of  $5 \pm 1.2 \text{ m}^2$  (Ray and Corkum, 2001). Evidence for this was provided by Wolfe and Marsden (1998) and Ray and Corkum (2001) who conducted mark-recapture studies, which showed that round goby are highly site specific, with a large proportion fish being recaptured in and around the area where they were tagged.

Results from this home range study showed that round gobies did not differ in their movement into traps from inside and outside their home range.

It is important to mention that here too low replication (n = 12) could have been a factor influencing statistical significance of results. Despite round gobies being readily available, at the time this study was conducted, the summer season was winding down and time was a limiting factor. This restricted how long experiments could be conducted for and the number of replicates had to be restricted to 12. It would be beneficial to repeat the lab study on home range with greater replication to confirm if this was a factor influencing results. However, the present results suggest that despite being a sedentary benthic fish with a small home range round gobies could travel distances in search of food when exposed to attractive odours.

Although there were no differences in the response of round goby to traps baited with odours from three food types, a control trap (trap without bait) was not examined. This made it difficult to determine if round gobies entered traps because of attractive odours released by the bait, their preference for structured surroundings which the traps would have provided or because of currents created by water flowing in from the upstream end of the channel.

Conducting these lab studies were important in determining how round gobies respond to food odours in a more controlled environment without the influences of biotic and abiotic factors that could have confounded results in the field. This study was the initial step in determining whether round gobies would enter baited traps with varying food odours and finally whether they would move outside their home range when

exposed to attractive odours. The home range experiment needs to be tested in the field to determine how far round gobies would travel to reach food baited traps.

Variables such as temperature, turbidity and prey availability could influence trappability of fish (Stoner, 2004). Temperature is known to be one of the most important abiotic factors influencing fish metabolism (Fry, 1971) and it is generally understood that fish become sluggish at lower temperatures. He (1991) found cod swimming ability significantly decreased at lower temperatures, mostly because muscle contraction time increases which determines how quickly fish can swim (Wardle, 1975). Several researchers have examined the impact temperature has on spontaneous locomotor activity of economically important species such as Atlantic cod (Gadus morhua) (Castonguay and Cyr, 1998) and sablefish (Anoplopoma fimbria) (Stoner and Sturm, 2004). The decrease in swimming activity decreases chances of encountering odour plumes and locating potential prey (Stoner, 2004). Turbidity which suspends sediments and other materials scatters and reduces light levels which in turn influence feeding ability by reducing reactive distances (Sweka and Hartman, 2001). High turbidity levels have been found to decrease feeding ability in fishes (Rowe and Dean, 1998), which could in turn impact their ability to react to and find bait.

The presence or absence of prey does impact how target species respond to baited traps, Løkkeborg *et al.* (1995) found that sablefish responses to bait odours was closely tied to the hunger level of fish. Field studies by Engås and Løkkeborg (1994) revealed that few cod were captured on longlines when capelin (*Mallotus vollusus*) were abundant, suggesting that feeding on capelin may have kept cod satiated and hence not motivated to follow odour trails to bait.

Future experiments could be modeled after the food preference study conducted in the field (chapter 2) where food baited traps could be deployed from a breakwall on a pier in an area with an established population of round gobies. Fish could be tagged and released within 5 m of the breakwall with one colour and fish beyond 5 m with another colour and so on. Then traps could be deployed for 24 h from the breakwall using airline cable of a known length. Based on the different coloured tags on captured fish we may be able to determine whether fish captured were only those from within the 5 m distance or whether they included fish from farther away. This information could then be used to deploy traps from appropriate distances which would ensure capture of the greatest number of round gobies and hence prevent the establishment of populations in inland waterways.

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# **Analysis of Variance Tables**

Table 1: One-way analysis of variance for number of round goby captured in traps baited with lake whitefish, dreissenids, eggs and control in the field

| Source of Variation | df | SS      | MS      | F     | р    |
|---------------------|----|---------|---------|-------|------|
| Treatment           | 3  | 13.3977 | 4.46591 | 1.879 | 1.39 |
| Error               | 84 | 199.591 | 2.376   |       |      |
| Total               | 87 | 212.989 |         |       |      |

Table 2: One-way analysis of variance for reaction time of round goby exposed to prepared baits and a control

| Source of Variation | df | SS      | MS     | F      | p       |
|---------------------|----|---------|--------|--------|---------|
| Treatment           | 4  | 582463  | 145616 | 2.1513 | 0.11889 |
| Error               | 20 | 1353722 | 67686  |        |         |
| Total               | 24 | 650149  |        |        |         |

Table 3: One-way analysis of variance for swimming velocity of round goby exposed to prepared baits and a control

| Source of Variation | df | SS      | MS      | F      | р       |
|---------------------|----|---------|---------|--------|---------|
| Treatment           | 4  | 4.53869 | 1.13467 | 3.8086 | 0.01848 |
| Error               | 20 | 5.95796 | 0.2979  |        |         |
| Total               | 24 | 10.4967 |         |        |         |

Table 4: One-way analysis of variance for time spent near the odour source for round goby exposed to prepared baits and a control

| Source of Variation | df | SS      | MS     | F     | р        |
|---------------------|----|---------|--------|-------|----------|
| Treatment           | 4  | 724920  | 181230 | 5.451 | 0.003905 |
| Error               | 20 | 664942  | 33247  |       |          |
| Total               | 24 | 1389862 |        |       |          |

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