

Studies of the Round Goby (*Neogobius melanostomus*) Population of the St. Louis River
Estuary

A Thesis
SUBMITTED TO THE FACULTY OF
UNIVERSITY OF MINNESOTA
BY

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
MASTER OF SCIENCE

Allen F. Mensinger

December 2013

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Acknowledgements

This thesis represents a culmination of effort involving a large number of individuals and agencies. I would like to thank my advisor, Dr. Allen Mensinger, his confidence in me gave me the ability to work independently. However, he was always willing to aid in any aspect of both my research and graduate school decisions. My committee members, Dr. Thomas Hrabik and Dr. John Pastor, were always willing to answer questions and gave crucial advice on experimental design. The courses I took with my committee members inspired me to conduct sound hypothesis driven research.

My fellow lab mates and graduate students provided friendship, advice, and field assistance at a moments notice. Undergraduates Matt Venas, Matt Johnson, Matthew Santo, Kara Annoni, and Ann Thomas provided vital field assistance and lab time aging fish that undoubtedly allowed my research to progress in a timely manner.

Lori Evrard at the USGS, Greg Peterson at the USEPA, and Andrew Edwards at the 1854 Treaty Authority provided trawling data opportunities to obtain fish. Bill Blust of the Wisconsin Department of Natural Resources allowed me to participate in beach seining surveys to obtain research specimens. Gary Czypinski of the USFWS provided windermere traps to aid in capturing specimens. Without the help of these agencies, I would have never obtained the required fish specimens and data required to complete my research.

Above all, I would like to thank my family and friends, particularly my wife and son for motivating me to the end.

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Chapter 1

Introduction

One of the most prominent threats to natural aquatic ecosystems and biodiversity are aquatic invasive species. Through competition, predation, and habitat alteration aquatic invasive species can displace preferred native species. The United States spends 100s of millions of dollars annually to manage infestations and prevent their spread to new habitats (Lovell and Stone 2005).

Human induced alterations to natural systems have opened new pathways for invasive species transport (Vitousek *et al.* 1997). For example, the St. Lawrence Seaway was opened in 1959 to allow ships access to the Laurentian Great Lakes (Mills *et al.* 1993). Since then, many organisms have been introduced to the Great Lakes through the ballast water of transoceanic ships travelling the Seaway (Claudi and Ravishankar 2006).

One of these invasive species is the round goby, *Neogobius melanostomus* (formerly *Apollina melanostomus*) (Neilson and Stepien 2009), first discovered in the Great Lakes in 1990 (Jude *et al.* 1992). Through repeated invasions and dispersal, both natural and human aided, the population expanded to many portions of the Great Lakes and its tributary streams (Steingraeber and Thiel 2000). The round goby can alter its new environment by competition with native species and altering trophic level interactions (Bergstrom and Mensinger 2009; Almqvist *et al.* 2010).

Native Range

The round goby is native to the Ponto-Caspian region of Eurasia (Figure 1). They typically inhabit the littoral zone of the Black and Caspian Seas along with the major

tributaries (Ghedotti *et al.* 1995). They are also thought to be native to the Sea of Azov and the Sea of Marmara (Charlebois *et al.* 1997). Round gobies have also been historically found in several river systems including the River Don in Russia, and the Dniester River in the Ukraine (Simonovicã *et al.* 2001; Skora and Rzeznik 2001; Miller 1986).

Life History

The round goby has many life history strategies common to other successful invasive species. Round gobies can withstand a wide range of salinities and temperatures (Sk'ora *et al.* 1999; Cross and Rawding 2009). Although no round goby populations exist in the open ocean, they are found in highly saline portions of the Caspian Sea. However, the dominant salt in the Caspian Sea is CaSO_4 whereas in the open ocean NaCl is the dominant salt (Strayer and Smith 1993). Furthermore, in laboratory experiments round gobies failed to survive more than 48 hours in a salinity of 30 grams per liter (Ellis and MacIsaac 2009). Round gobies are capable of inhabiting waters ranging from -1°C to 30°C (Moskal'kova 1996). However, they certainly prefer warmer waters with a thermal growth optimum estimated at 26°C (Lee and Johnson 2005). These characteristics undoubtedly allowed the round goby to survive in ballast water tanks of transoceanic ships (Ricciardi and MacIsaac 2000).

Round gobies also have a highly efficient reproductive strategy. Once the water temperature reaches approximately 9°C , males establish nest sites on rocky substrate (MacInnis and Corkum 2000a). Their body turns jet black and they emit calls and releasing pheromones to attract females (Rollo *et al.* 2007; Laframboise *et al.* 2011). It is

not uncommon for multiple females to deposit eggs in the same male's nest (Meunier *et al.* 2009). Four to six females may deposit up to 10,000 eggs in a nest (Charlebois *et al.* 1997). The male then fertilizes, guards, and cleans the eggs by fanning them with his tail until they hatch (Meunier *et al.* 2009). Males sometimes expend so much energy during spawning that they die (Charlebois *et al.* 1997). However, females may repeat the spawning process as much as every 18-20 days depending on water temperature (MacInnis and Corkum 2000). Recently, an alternative male morphotype has been documented (Marentette *et al.* 2009). Aside from the natural large robust male that turns black during spawning, there exists a "sneaker male." This morphotype exhibits a size and color more similar to a female round goby. It also exhibits a large gonadosomatic index compared to non-sneaker males. This allows the sneaker male to enter nests guarded by larger males and fertilize eggs.

Round gobies occupy a variety of habitats in both native and introduced locations (Johnson *et al.* 2005b; Taraborelli *et al.* 2009). However, rocky substrate provides interstitial spaces for shelter and nest building and often supports higher population densities (Ray and Corkum 2001).

Round gobies are opportunistic feeders which can become specialists when needed because they possess specialized molariform pharyngeal teeth that allow them to consume sedentary bivalves (Ghedotti *et al.* 1995). This ability is generally limited to round gobies greater than 60 mm total length (Ray and Corkum 1997; Lederer 2006). Smaller round gobies consume a range of aquatic insects, chironomids, amphipods, and other benthos (Jude *et al.* 1995). Large round gobies will also consume these prey when

sedentary bivalves are in short supply (Jude *et al.* 1995).

Physical Characteristics

Although the round goby is small and soft bodied, its aggressive nature and ability to survive harsh conditions allow it to outcompete other fish for resources. Its coloration can be described as mottled brown or sometimes green with occasional dark blotches. One of the most distinguishing characteristics of the round goby is its fused pelvic fin, a cup-like structure on the fish's ventral surface capable of forming a weak suction (Charlebois *et al.* 1997). This allows the goby to cling to substrate in weak to moderate currents. Round gobies have both an anterior and posterior dorsal fins. A dark spot on the anterior dorsal fin is a defining characteristic of the round goby along with their homocercal tail. Round gobies lack swim bladders and utilize their large pectoral fins for short bursts of swimming activity. The sex of a round goby can be determined externally by examining the urogenital papilla. Male papillae are long, pointed, and often white or dark. Female papillae are shorter, rounded at the apex, and often yellowish orange in color (Charlebois *et al.* 1997). Along the lateral line of the round goby are superficial neuromasts, potentially giving them a sensory advantage over other species which have neuromasts enclosed within a canal (Jude *et al.* 1995). However, laboratory experiments suggest there is not a significant difference in feeding success (Bergstrom and Mensinger 2009).

Introduced Range

Round goby larvae are nocturnally pelagic and are thus capable of being taken into ballast water as they feed on zooplankton at the surface (Hensler and Jude 2007; Hayden

and Miner 2009). Presently, introduced round goby populations have been established throughout Eurasia including populations in the upper Danube, Volga, and Moscow Rivers, as well as the Baltic Sea's Gulf of Gdansk (Figure 1) (Wiesner 2005; Simonovicá *et al.* 2001; Sapota and Skóra 2005). Round gobies were likely introduced to these areas via ballast water or as bycatch in stocking programs for other species. Their eggs also are attached to the hulls of ships and barges (Moskal'kova 1996).

Genetic testing suggests that the original source populations came at least partially from the Dnieper River (Brown and Stepien 2009). Round gobies were first discovered in North America in 1990, in the St. Clair River, Ontario (Jude *et al.* 1992). Through a combination of subsequent invasions via ballast water, natural dispersal, and anthropomorphic assistance (bait bucket transfer), round gobies quickly spread throughout the Laurentian Great Lakes (Steingraeber and Thiel 2000). By 1995, they were present at the western arm of Lake Superior, in the Duluth-Superior Harbor. Round goby populations are now established in all five Laurentian Great Lakes (Figure 2). However, the degree of which each lake is infested varies greatly. In Lake Superior round goby populations are restricted to a few harbors, presumably because of the cold, harsh environment of the deep main basin (Grigorovich *et al.* 2003). Conversely, the round goby population of Lake Erie has taken hold of the entire basin (Johnson *et al.* 2005a). In all Laurentian Great Lakes, gobies are much more abundant in nearshore areas, but have been caught in bottom trawls up to 130 meters (Walsh *et al.* 2007).

Round gobies are primarily sedentary with limited home ranges (Bjorklund and Almquist 2010). However, tagging studies suggest have documented substantial

movements of some adult individuals (Wolfe and Marsden 1998; Lynch and Mensinger 2011). It is thought that as a local population grows, preferred nesting and shelter sites become saturated, forcing non-dominant individuals to migrate to alternative sites (Charlebois *et al.* 1997; Ray and Corkum 2001). Additionally, currents are capable of spreading their nocturnally pelagic larvae. This dispersal pattern has led to the establishment of populations in estuarine habitats and tributaries of the Great Lakes (Phillips *et al.* 2003). Man-made dams inhibit further expansion in many systems. However, bait bucket transfer by humans has likely aided in the movement of round gobies past dams.

Growth and Aging

In their native range, round gobies can live upwards of five years and grow up to 25 cm (Berg 1949). However, in introduced areas such as the Great Lakes they seem to exhibit faster growth and shorter life spans (MacInnis and Corkum 2000*b*). Faster growth allows them to reach sexual maturity one year earlier than in their native range but they may reach a much smaller maximum size (mean total length around 100 mm). Substantial variation in growth and length at age relationships exists across native and introduced ranges. Marine populations seem to consistently grow larger than freshwater populations (Corkum *et al.* 2004; Sokołowska and Fey 2011).

Ecological Impacts

Due to its widespread abundance, the round goby has altered the ecology of many environments where it has been introduced. Some native fish populations have declined after round goby establishment and subsequent competition for food and habitat. Many

other species may compete with the round goby because of similarities in habitat preference or diet overlap, but a detectable affect at the population level has not yet been observed. Some of these fishes include *Etheostoma* sp., *Cottus* sp., and juvenile *Perca flavescens* (Jude *et al.* 1995; Dubs and Corkum 1996; Jansen and Jude 2001; Laurer *et al.* 2004; Bergstrom and Mensinger 2009; Barton *et al.* 2005; Carman *et al.* 2006; Corkum *et al.* 1998; French and Jude 2001; Balshine *et al.* 2005; Duncan *et al.* 2011).

Aside from competition, the round goby preys on egg and fry of native fishes. Some of these species include lake trout (*Salvelinus namaycush*), smallmouth bass (*Micropterus dolomieu*), and lake sturgeon (*Acipenser fulvescens*) (Chotkowski and Marsden 1999; Steinhart *et al.* 2004; Thomas and Haas 2002). Although the round goby preys on the eggs and fry of these species, the adults of these native species may heavily consume round gobies. In the Laurentian Great Lakes, round gobies have been found to contribute to the diets of brown trout (*Salmo trutta*), burbot (*Lota lota*), double-crested cormorants (*Phalacrocorax auritus*), Ictaluridae sp., the Lake Erie watersnake (*Nerodia sipedon insularum*), rock bass (*Ambloplites rupestris*), smallmouth bass (*Micropterus dolomieu*), and several others (Campbell *et al.* 2009; Johnson *et al.* 2005; Somers *et al.* 2003; King *et al.* 2006; Jude *et al.* 1995; Dietrich *et al.* 2006).

Few species rely so heavily on bivalves as a food source as the round goby. Round goby predation on sedentary bivalves releases energy stores in the bivalves and makes it available to the rest of the food web in novel energetic pathways (Almqvist *et al.* 2010). This new trophic link may have released contaminants previously confined to mussels and other benthos to higher trophic levels (Hogan *et al.* 2007). However, several other

studies have refuted the idea of round gobies facilitating bioaccumulation (Hanari *et al.* 2004; Fernie *et al.* 2008).

Although population dynamics and ecological interactions of round goby populations have been studied elsewhere, less is known specifically about the population of the Saint Louis River Estuary and Duluth-Superior Harbor.

In other areas of the Great Lakes, native fish species showed declines in abundance after the establishment of the round goby. Since species composition in these areas such as the St. Clair River is similar to that of the St. Louis River, we sought to determine whether or not these species were also exhibiting population declines in the St. Louis River. Long term trawling data was collected from the USGS, US EPA, and 1854 Treaty Authority. From this data we were able to observe changes in the benthic community, possibly as a result of round goby establishment.

Species abundances naturally fluctuate over time. Population dynamics, changes in abiotic conditions, or changes in predator and prey abundance may determine the density of any given species. However, natural fluctuations can be altered by interspecific competition with introduced species. In order to determine whether changes in logperch (*Percina caprodes*) abundance are a result of interspecific competition with the round goby, a field experiment using enclosures was conducted in the Duluth-Superior Harbor. Interspecific, intraspecific, and single fish treatments of round gobies and logperch were placed in enclosures for 28 days. Relative growth was then used to determine the “winner.” This study is significant because the enclosures allow resource exchange with the fish’s natural habitat, thus allowing for a more natural food supply and abiotic

conditions that are difficult to replicate in a laboratory setting.

Growth rates and length at various ages vary greatly among round goby populations. For instance, age-1 males from the central basin of Lake Erie are about 30 mm longer than age-1 males in Lake Huron (Johnson *et al.* 2005; French and Black 2009). Although growth rates of recaptured round gobies have been studied in the St. Louis River estuary (Lynch and Mensinger 2012), length at age distributions are largely unknown. Otoliths were removed from round gobies collected in August 2011 via bottom trawl. From this, length at age distributions could be constructed for male and female round gobies in the SLRE.

Figures

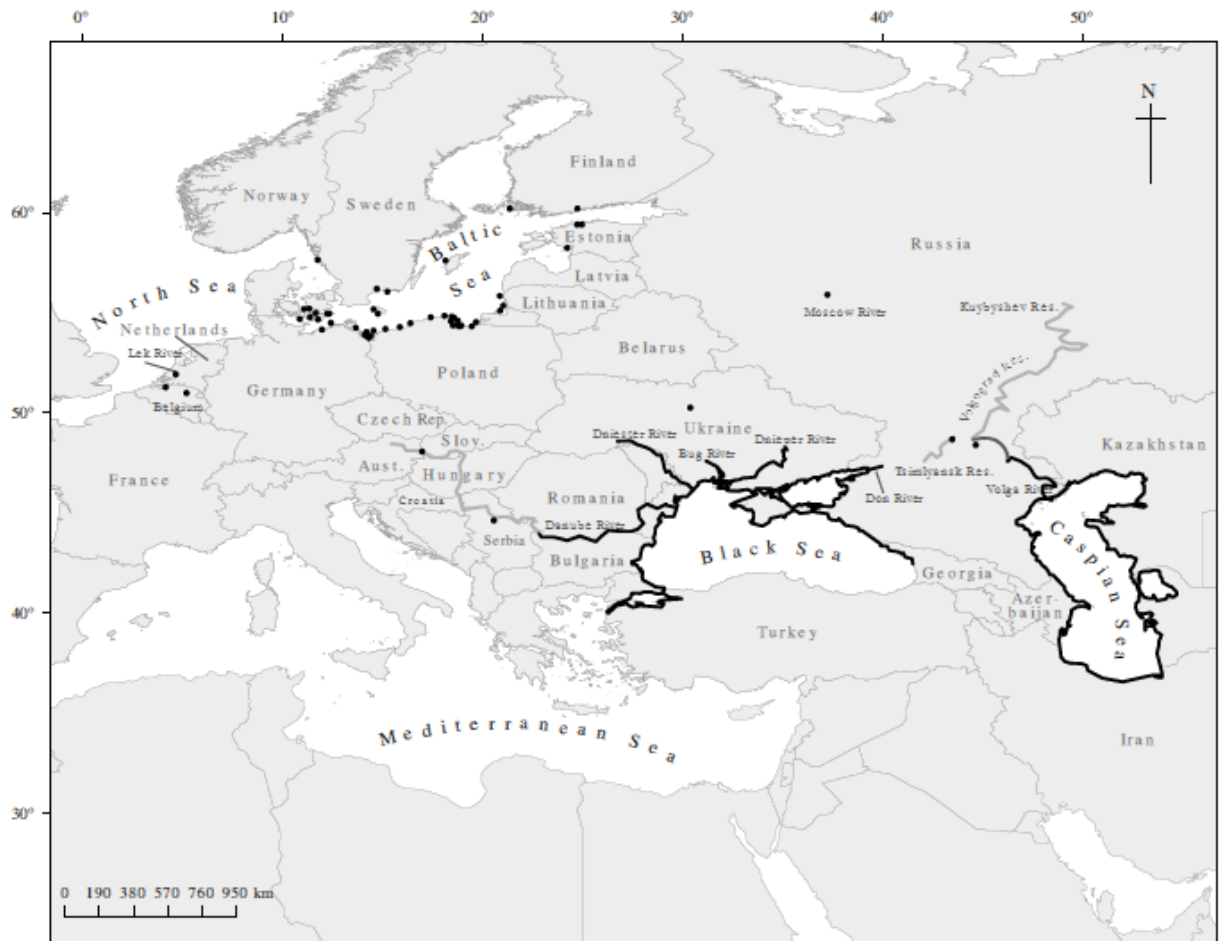


Figure 1. Round goby distribution in Europe and Asia. Black lines indicate the historic native range, black dots indicate invasive populations (Kornis *et al.* 2012).

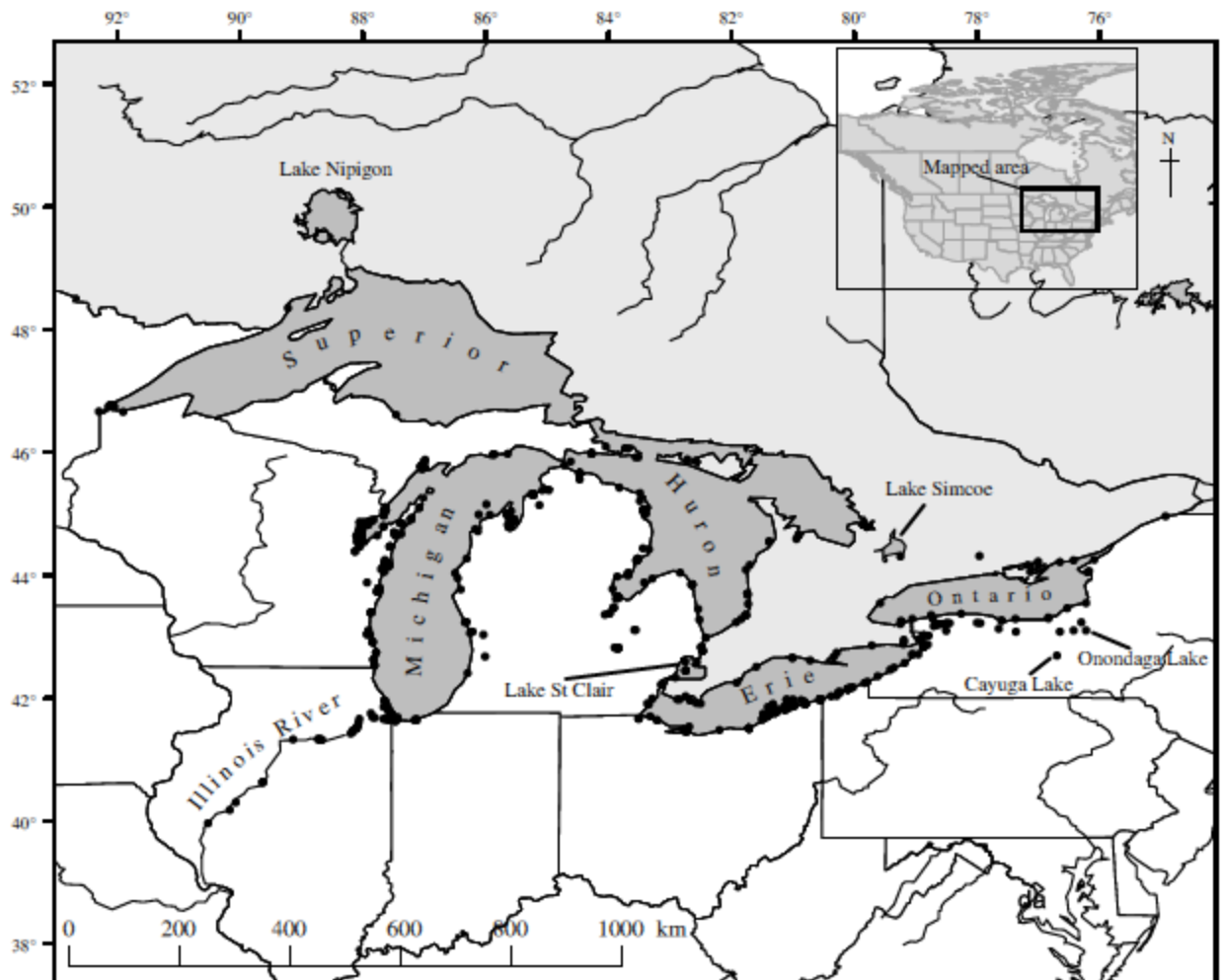


Figure 2. Current distribution of round gobies in North America (Kornis *et al.* 2012).

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Chapter 2

Interspecific competition between the round goby, *Neogobius melanostomus*, and the logperch, *Percina caprodes*, in the Duluth-Superior Harbor

Overview

The food webs of the Laurentian Great Lakes have been altered by aquatic invasive species such as the sea lamprey, zebra mussel and round goby. To further the understanding of invasive and native species interactions in situ, competitive interactions between the native logperch (*Percina caprodes*) and invasive round goby (*Neogobius melanostomus*) were examined using submerged enclosures in the Duluth Superior Harbor. Experiments were conducted on rock or sand substrate throughout the spring and summer months. Trials consisted of single fish, paired conspecifics and round goby/logperch pairs with weight changes determined after 28 days in situ. This study confirms that round gobies can outcompete and/or negatively effect logperch on rocky substrate as a direct result of round goby/logperch interactions. Logperch fared well when round gobies were excluded from the enclosures. This study shows that the round goby can negatively affect logperch on soft substrates. Despite these negative interactions, the logperch population remains at pre-round goby levels on the soft bottom in the harbor. The continued coexistence of the logperch with the round goby may have to do both with the vastly greater area of soft bottom in the harbor and that the round gobies in these areas tended to be younger and smaller than those in the rocky substrate. Recent evidence suggests that the round goby population is declining on the soft substrates indicting that the two populations will continue to coexist in the harbor.

Introduction

The round goby, *Neogobius melanostomus*, is an invasive fish species to the Laurentian Great Lakes that were first discovered in the St. Clair River in 1990 (Jude *et al.* 1992). The round goby spread throughout all five Laurentian Great Lakes within five years of this initial discovery (Jude 1997). Contaminated ballast water from transoceanic vessels appears to be responsible for the invasions as the fish originated in the Ponto-Caspian region of Eurasia (Ghedotti *et al.* 1995). Secondary invasions into the Great Lakes watersheds have been noted (Kornis *et al.* 2013; Kornis and Vander Zanden 2010) although the vector(s) facilitating this movement remain undetermined (Kornis *et al.* 2012).

Aggressive behavior and high fecundity have been implicated as prevailing factors behind the explosive growth of the population and subsequent local extirpation of native benthic species (MacInnis and Corkum 2000). Competition with round goby for spawning habitat led to subsequent recruitment failure of mottled sculpin (*Cottus bairdii*) populations in Calumet Harbor of Lake Michigan (Janssen and Jude 2001), and mottled sculpin and johnny darter (*Etheostoma nigrum*) trawl catches declined in Lake Michigan (Lauer *et al.* 2004). Furthermore, the round goby is hypothesized to be at least partially responsible for the collapse of the deepwater demersal fish community in Lake Huron (Riley *et al.* 2008).

Although the detrimental effect of the round goby on native populations has been well documented, the exact mechanisms by which they outcompete native species

remains to be elucidated. Several laboratory studies have addressed interspecific interactions and indicated that round gobies can aggressively displace mottled sculpin or logperch from shelters (Balshine *et al.* 2005; Dubs and Corkum 1996) and out compete native fish for food resources (Bergstrom and Mensinger 2009).

However, in situ studies other than population surveys are limited. As the round goby prefers rocky habitat, it is difficult to assess the population with traditional sampling gear. The impact of the round goby on the overall abundance of native fishes in the Duluth-Superior Harbor remains largely unknown. The round goby was discovered in Duluth in 1995 and has slowly expanded its range to throughout the harbor, the St. Louis River Estuary and the St. Louis River upstream to the first barrier (Fond du Lac dam). A mark recapture study using fish traps in the harbor on rocky substrate from the 2009 through 2012 revealed that 98% of the catch (N= 15000+ fish) consisted of round gobies (Lynch and Mensinger 2012). However, off shore trawls continue to find round gobies and several species of native benthic fish, including logperch (*Percina caprodes*), occupying the soft bottom communities (Bergstrom *et al.* 2008). Previous studies indicate that the round goby has a competitive advantage over native logperch populations for optimal habitat and food, yet the native fish have not shown significant population changes on the soft bottom community since the round goby appearance (Leino and Mensinger 2013b). The objective of this study was to extend laboratory studies to the field to determine the competitive interactions between the round goby and logperch on both soft and hard substrates.

Methods

Two sites, each encompassing 100 meters of shoreline were selected for the competition studies in a shallow (depth < 1 m), near area at Rice's Point, Minnesota in the Duluth-Superior Harbor (Figure 1). The eastern site was primarily rocky bottom dominated by round gobies while the second area, located 500 m west, was soft bottom and contained both round gobies and logperch. Wolman pebble counts were used to determine the substrate composition at each site. The rocky site was characterized by 50% large cobble (64 to 256 mm diameter) interspersed with 30% gravel (2 to 64 mm) and 20% sand (< 2 mm). The sandy site consisted of 85% sand with intermittent cobble (10 %) and gravel (5%).

Fish enclosures were constructed from 2.5 cm diameter PVC tubing overlaid with black plastic mesh screening (4.0 mm opening) and measured 40 cm x 55 cm x 25 cm. The bottom of each enclosure was covered with the appropriate composition of cobble, sand and gravel for each site and the material mixed to ensure random distribution. The cages were submerged in approximately 1 m of water at each site and spaced at least 3 m from adjacent enclosures.

Logperch and round gobies were collected from the Duluth-Superior Harbor by trapping, netting, beach seining, or bottom trawling throughout the spring and summer. Fish were maintained with conspecifics in aerated coolers and transported to the experimental site within 24 hours of capture. Fish weight (g) and total length (TL) (mm) was determined and the round gobies sexed.

Each enclosure contained one of the following experimental groups: 1) single fish; 2) intraspecific pair; 3) interspecific pair. For intraspecific competition, fish were paired with a conspecific of similar size with fin clipping (pectoral) used to allow recognition of individual fish, and all round gobies were paired with members of the same sex. For interspecific competition, logperch were paired with a round goby of similar length (± 5 mm). Trials were conducted both on rocky and sandy substrate and the cages left undisturbed for four weeks. Fish then were removed from the enclosures, identified, measured and sacrificed with an overdose of 0.05% MS-222. All experiments and procedures conformed to institutional animal care protocols.

Analysis

Percent weight changed was determined by dividing end weight by the initial weight. In the paired trials, if one of the fish was missing, the data was discarded. As the majority of the data failed normality tests (Shapiro-Wilk), non parametric statistical analysis was used. Values are reported as the median with the 25% and 75% quartiles following the median values. All percentages were arcsine transformed prior to statistical analysis. Kruskal-Wallis with Dunn pairwise multiple comparison procedures were used to compare different treatments. All statistical analysis was performed with Sigmaplot Version 12.5.

Results

The enclosures proved effective in maintaining the fish with eighty-five percent of the fish surviving the four week trials. An equal number of cages containing single fish of both species were found empty. No carcasses were found so it is unclear if the

missing fish escaped or died. However, in the paired trials, the majority of the cages with missing fish were completely empty as opposed to containing a single fish, suggesting both fish escaped.

The logperch used in the study (N = 101) ranged from 61 mm to 112 mm TL (mean 78.2), with weight ranging from 1.8 to 9.7 g (mean 3.93). Round goby (N = 117) TL ranged from 63 to 110 mm (mean of 83.2) mm while mass ranged from 2.9 to 16.2 g (mean of 7.13 g).

Single fish trials

Twenty seven (15 rock, 12 sand) single fish trials were conducted with the round goby. Male fish were placed in 67% of the enclosures and there was no significance difference between male and female weight changes on either substrate. We therefore pooled the data for both sexes. Round gobies on the rocky substrate lost a median percentage of 11.3% (-14.7%, -6.1%) of body weight which was significantly greater (Mann Whitney, $P < 0.001$) than conspecifics on sand (-1.3%: -5.6%, 14.5%). Seventeen (9 rock, 8 sand) individual logperch trials were also conducted. Logperch gained weight at both locations with fish on sandy bottom gaining slightly more weight (12.3%: 4.5%, 18.6%) than those on rocky substrate (7.8%: 3.1%, 9.7%), however there was no statistical difference (Mann Whitney, $P = 0.180$) in logperch weight gain between the two sites (Figure 2). The logperch weight gain was significantly different than the round goby (Mann Whitney, $P < 0.001$) on the rocky site, but there was no significant difference (Mann Whitney, $P = 0.174$) between the species in weight gain at the sandy sites.

Conspecific pair trials

Twenty nine (14 rock, 15 sand) round gobies pairs were placed in the enclosures with 12 cages on each substrate containing male pairs and the rest enclosing female pairs. No significant difference was found in male and female weight change on either substrate, and the data was pooled. When round gobies were paired with conspecifics, both showed weight loss independent of habitat. On the rocky habitat, the median weight loss per fish was -11.3% (-13.7%, -8.6%) with only 6 of the 28 fish exhibiting weight gain. The median loss (-4.1%: -8.0%, 1.9%) on the sand was significantly less (Mann Whitney, $P < 0.001$), however only 8 of the 30 fish gained weight (Figure 3).

Twenty six intraspecific logperch trials were conducted with 20 cages placed on rocky substrate. Logperch lost weight at both sites with fish on the rocky site losing significantly less weight (-3.1%: -4.6%, -0.4%) (Mann Whitney, $P < 0.001$) than the sandy site (-6.0%: -10.2%, -0.7%), with less than 15% of the individuals at either site showing weight gain (Figure 3). Logperch lost significantly less weight than round gobies on the rocky substrate (Mann Whitney, $P < 0.001$), however there was no significant difference between the species at the sandy sites (Mann Whitney, $P = 0.510$).

Interspecific pair trials

Thirty-two (14 rock and 18 sand) trials were conducted to examine interspecific interactions. Approximately 67% of the traps contained male round gobies, however no difference was found in male and female growth rates and the data was combined for the analysis. The round gobies fared better than the logperch on both substrates. On the rocks, the round gobies lost significantly less body weight (-3.6%: -7.7%, 1.7%) than logperch (-8.5%: -13.0%, -5.2%) (Mann Whitney, $P = 0.029$). The round goby fared better in 8 of the 14 enclosures on rock substrate with 2 enclosures showing equal growth

(within 0.5% body weight change). On the sand, round gobies gained a median of 3.0% (-1.5%, 6.0%) body weight which represented a significant difference (Mann Whitney, $P = 0.026$) in comparison to logperch which lost a median of -5.1% (-11.4 %, 3.1%) body weight. The round goby out gained the logperch in 10 of the 18 sand enclosures with 4 additional enclosures showing equal growth (within 0.5% body weight change) (Figure 4). No significant difference was observed in weight change when conspecifics were compared on the different substrates (round goby, Mann Whitney, $P = 0.055$; logperch, Mann Whitney, $P = 0.095$).

Substrate

When the round gobies were paired with logperch, they had significantly less weight change (Kruskal Wallis, $P < 0.001$) than the round gobies on the rocky substrate. On the sand substrate, round gobies had significantly greater weight loss when paired with logperch (Kruskal Wallis, $P < 0.001$) than the other round goby trials. Only when round gobies were paired with log perch on the sand did they gain weight (Figure 5).

Single logperch both on the rocky and sand substrates gained significantly more weight than when paired with round goby (Kruskal Wallis, $P < 0.001$). On sandy substrates, single logperch gained significantly more weight than logperch in the other two groups (Kruskal Wallis, $P = 0.008$) (Figure 6).

Discussion

The enclosures provided an opportunity to examine the competitive interactions of an invasive and native benthic species in the field on two different types of substrates. The close proximity of the two sites (within 500 m) insured that the water conditions

were similar. The cages proved reliable in containing both species as greater than 85% of the fish were recovered after the 4 week trials. Due to the prolonged submergence and the desire not to disturb the inhabitants, it could not be determined if the missing fish died or escaped, because no carcasses were found. Many of the intra or interspecific cages that were missing fish, were missing both fish, suggesting that the fish were able to escape these cages and were not being killed by other inhabitant. The caging only exhibited modest fouling and the 4 mm mesh openings remained unimpeded throughout each trial. Amphipods, crayfish, chironomids, and juvenile tubenose (*Proterorhinus semilunaris*) and round gobies often were found in and on the cages.

Submerged enclosures allow for animals to be maintained under comparable water conditions while allowing retrieval of the subjects at the end of the study. However, caution needs to be applied to insure that the cage itself is not influencing animal physiology (Connell 1983). Cage fouling may limit food availability or water flow through the structure. Additionally, fish foraging area is restricted and if insufficient food resources enter the cage, negative effects can ensue. Fortunately the relatively brief submergence (28 days) and already sparse vegetation at the two sites, limited both invertebrate and plant fouling of the cages, and the mesh openings remained unobstructed.

Single fish interactions

Although the main focus of the study was to examine interspecific interactions, single fish were placed in the enclosures to determine the effects of the cages on fish growth. Three week laboratory experiments that were conducted in an artificial stream

that was partitioned into sections with the same bottom area of the enclosures indicated that logperch pairs would maintain weight while round gobies would gain weight (Bergstrom and Mensinger 2009) when fed daily with fish pellets. Single logperch gained weight both on sandy and rocky substrate, indicating that the enclosure was not inhibiting food availability or growth. Long term (5+ years) mark and recapture studies in the rocky substrate, near the rocky site in the current study, yielded over 98% round gobies (out of 15,000 fish) without capturing a single logperch. However, when protected from round goby interaction, the logperch grew well in this habitat, strongly suggesting that their absence is due to the round goby, and not abiotic or other biotic factors.

In contrast to the weight gains exhibited by the logperch, single round gobies lost a small amount of weight (1.3%) on the sand but significantly more on the rocky substrate. The lack of weight gain of the round goby on the soft substrate could be partially explained by different food resources available on the sand or constraints on the foraging ability of the round because of the cage. The weight loss of the round goby in the rocks was quite surprising as a mark and recapture field studies had documented continual growth throughout the spring and summer months on rocky substrate (Lynch and Mensinger 2013). One possibility is that the high density of round gobies consumed the available food before it had a chance to get into the cage. However, if food was limiting, it would have been expected that the mark/recapture study would have contained a percentage of round gobies that exhibited no growth which was not the case. Additionally, cage effects were probably minimal as round gobies on the sand fared

significantly better, and round gobies paired with logperch during the same time and at the same location fared better, indicating food alone was not the factor. However, the rocky area had high densities of sexually mature round gobies, and the male fish in the enclosures may have expended energy to attract mates or establish and/or defend territory from the small, immature round gobies that were often found in the enclosures. Female gobies may have spawned either in the enclosure or through the mesh contributing to their equivalent weight loss.

Intraspecific competition

Logperch pairs lost weight on both substrates which contrasted sharply with weight gains evidenced by single fish. In the laboratory, there are very few interactions between logperch pairs and therefore aggressive behavior or other types of negative interactions in the enclosures were unlikely (Bergstrom *et al.* 2008). As single logperch gained weight during the same time and locations, their weight loss may be a result of competition for a limited food supply. This would be consistent with the overall density of logperch in the harbor as trawling studies have indicated logperch exist at much lower densities ($< 0.01/\text{m}^2$) than in the current study.

The median weight loss for round gobies pairs exceeded 10% on the rocky substrates which contrasted sharply with the minimal weight loss observed on the sand flats. Round gobies are known for their aggressive interactions, and while weight loss may have been expected for one of the pair, it was surprising that every fish lost weight. The significantly greater weight loss on the rocky habitat may be the result of competition for shelter/substrate in the enclosure. Additionally competition for food or

interactions with the gobies outside the cage may have also been a factor. The enclosures on the sand contained minimal habitat that was further degraded by sand deposition during the experiments which may have reduced the aggressive interactions.

Additionally, the lower population of round gobies in this area reduced the chances of interactions through the enclosures.

Interspecific

Laboratory studies showed that round gobies would out compete the logperch for shelter and food (Balshine *et al.* 2005 and Bergstrom and Mensinger 2009); however these studies used fish pellets added once daily that proved relatively easy for the round goby to monopolize. The fish in the current experiments needed to adapt to quantitative and temporally variable food availability. As logperch have been shown to have greater reaction and strike distances for live prey than the round goby, their foraging for live food in the wild had the potential to offset the round gobies aggressive interactions (Bergstrom and Mensinger 2009). However, the interspecific trials showed the ability of the round goby to dominate resources and/or negatively impact logperch through interactions in the field. Although the round goby on the hard substrate had minor weight loss, the logperch lost significantly more weight than in other trials conducted on the rock substrate. As these round gobies fared better than single or pairs of round gobies, it is hypothesized that these fish focused on dominating the logperch and acquiring resources that partially mitigated interactions with conspecifics outside the cage. On the soft substrates, the round goby gained weight while logperch lost significantly more weight, again showing the ability of goby to out compete the logperch. The greater growth exhibited by the

round gobies on the sand was again attributed to minimal interactions with other round gobies due to lower populations on the sand flats.

The round goby has extirpated native benthic species in near shore rocky areas and maintains a substantial presence on the soft bottom community. However, a long term trawling survey (1989 to 2011), that included sampling prior to the introduction of the round goby, indicates the logperch population remains relatively stable on the soft bottom community. Previous laboratory studies had indicated that the round gobies dominated the logperch on rocky substrate, but had indicated that the logperch greater visual reaction and strike distances under daytime conditions. It was hypothesized that the continued presence of the logperch on the soft bottom communities was attributed to these sensory advantages and that the fish could be able to at least compete with the round gobies on the soft bottom. However, the present study indicates that round goby can have a detrimental effect on the logperch on sandy substrates. As the logperch is not active at night, it may be possible that any sensory advantage during the day is mitigated by the ability of the round goby to successfully forage throughout the day and night.

The question arises as to why the round gobies with their ability to dominate the logperch both on rocky and sandy substrates have not extirpated the logperch from the sand flats. The coexistence of the logperch on the soft bottom communities may be attributable to the greater area of soft substrate compared to man-made structures or rocky areas lining the shores. As the round gobies need hard substrate for reproduction, the greatest densities of round gobies are found in these three dimensional habitats, and consequently the largest and arguably fittest individuals are found nearshore. The round

goby community on the sand flats are comprised of smaller or younger fish than found on the rocky substrate (Bergstrom *et al.* 2008; Lynch and Mensinger 2013; Leino *et al.* 2013) which may have lessened the impact on the logperch. The effects of the virtual exclusion of the logperch from the rocky habitats have not been observed in the soft bottom populations. As the round goby population is in a slight decline since 2006, and the logperch continue to exist at pre round goby levels, at least on the soft bottom, it appears that the two populations will continue to coexist.

The study confirms that round gobies can outcompete and/or negatively affect logperch on rocky substrate. This appears to be a direct result of round goby/logperch interactions as logperch fared well during the same time and in the same habitats when round gobies were excluded from the enclosures. Additionally, for the first time, the study shows that round goby can negatively affect logperch on soft substrates. Despite these negative interactions, the logperch population continues to be at pre round goby levels on the soft bottom and continual monitoring will be needed to observe the long term effects of round goby introduction on the native benthic fish in the soft bottom communities.

Figures



Figure 1. Schematic of the experimental site. Picture spans approximately 700 m of shoreline.

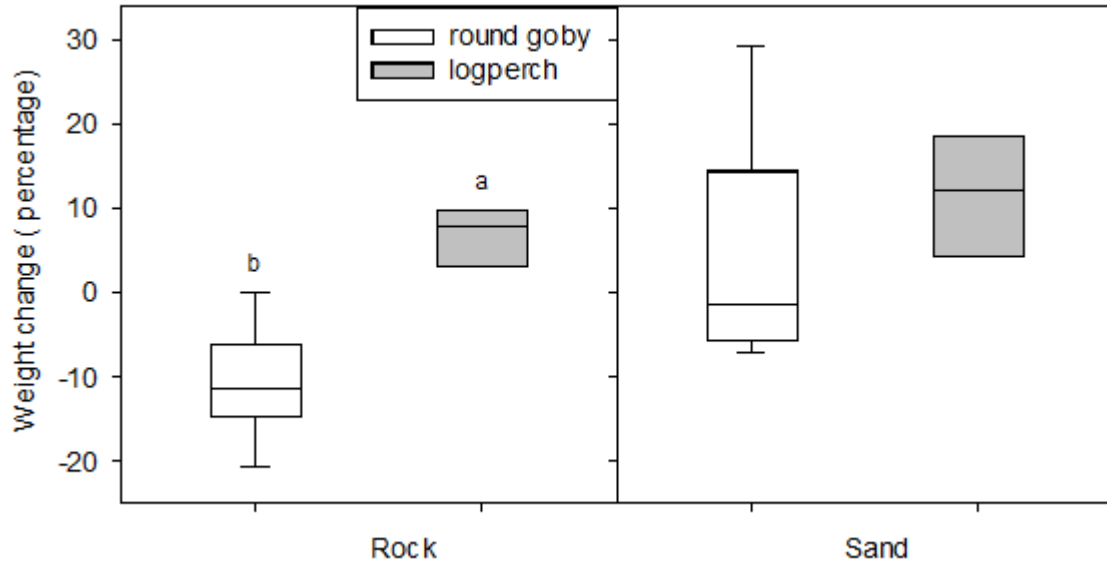


Figure 2. Single fish trials. The median weight change after 28 days is shown for single round gobies (open box) and logperch (gray box) on both rock (left panel) and sand substrate (right panel). The line within the box marks the median and the boundary of the boxes indicates the 25th percentile and 75th percentile. Whiskers below and above the box indicate the 10th and 90th percentiles. Letters indicate significantly different means (Mann Whitney, $P < 0.001$).

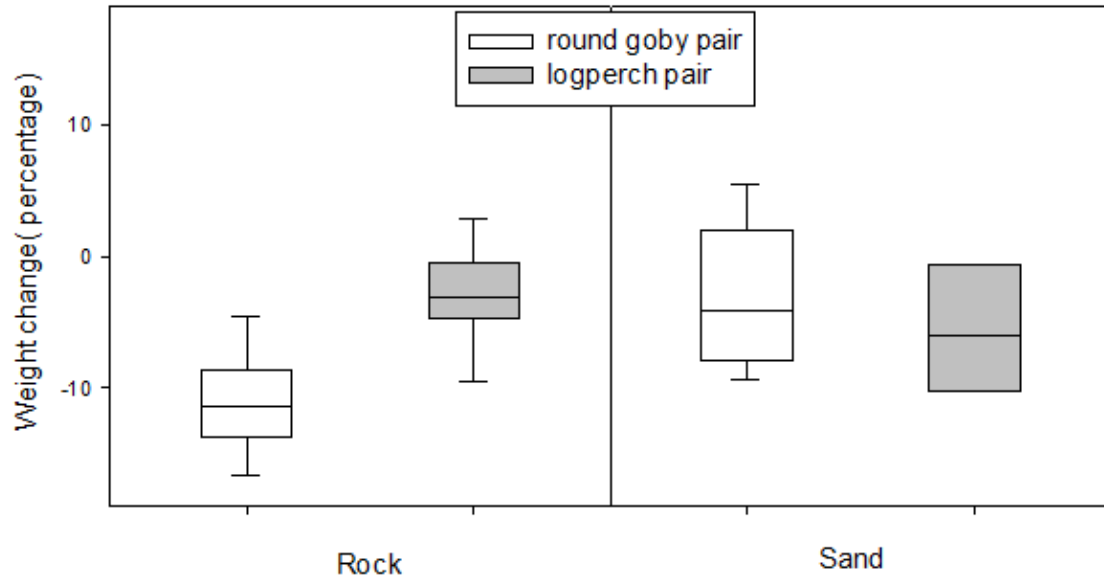


Figure 3. Conspecific pair trials. The median weight change after 28 days is shown for paired round gobies (open box) and paired log perch (gray box) on both rock (left panel) and sand (right panel) substrate. The line within the box marks the median and the boundary of the boxes indicates the 25th percentile and 75th percentile. Whiskers below and above the box indicate the 10th and 90th percentiles. Letters indicate significantly different means (Mann Whitney, $P < 0.001$).

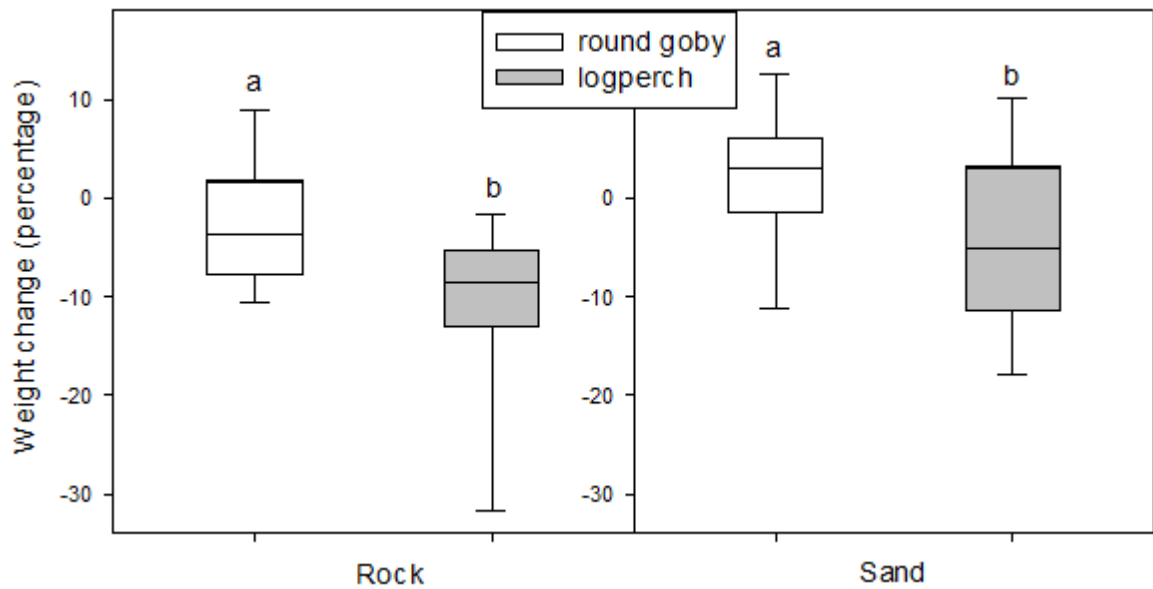


Figure 4. Interspecific pair trials. The median weight change after 28 days is shown for enclosures containing a round goby (open box) and logperch (gray box) on rocky (left panel) and sandy (right panel) substrate. The line within the box marks the median and the boundary of the boxes indicates the 25th percentile and 75th percentile. Whiskers below and above the box indicate the 10th and 90th percentiles. Letters indicate significantly different means (Mann Whitney, $P = 0.029$, rock; $= 0.026$ sand).

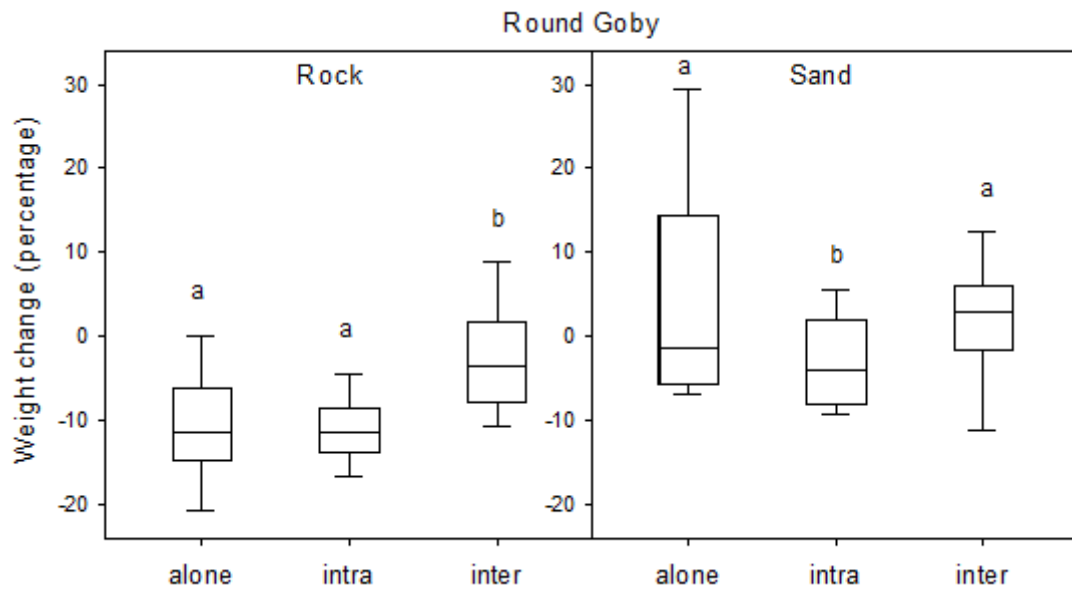


Figure 5. The median weight change is shown for all three round goby trials (single fish, intra and interspecific competition) on both rocky (left panel) and sandy (right panel) substrate. The line within the box marks the median and the boundary of the boxes indicates the 25th percentile and 75th percentile. Whiskers below and above the box indicate the 10th and 90th percentiles. Letters indicate significant difference medians in each panel (Kruskal-Wallis $P < 0.001$).

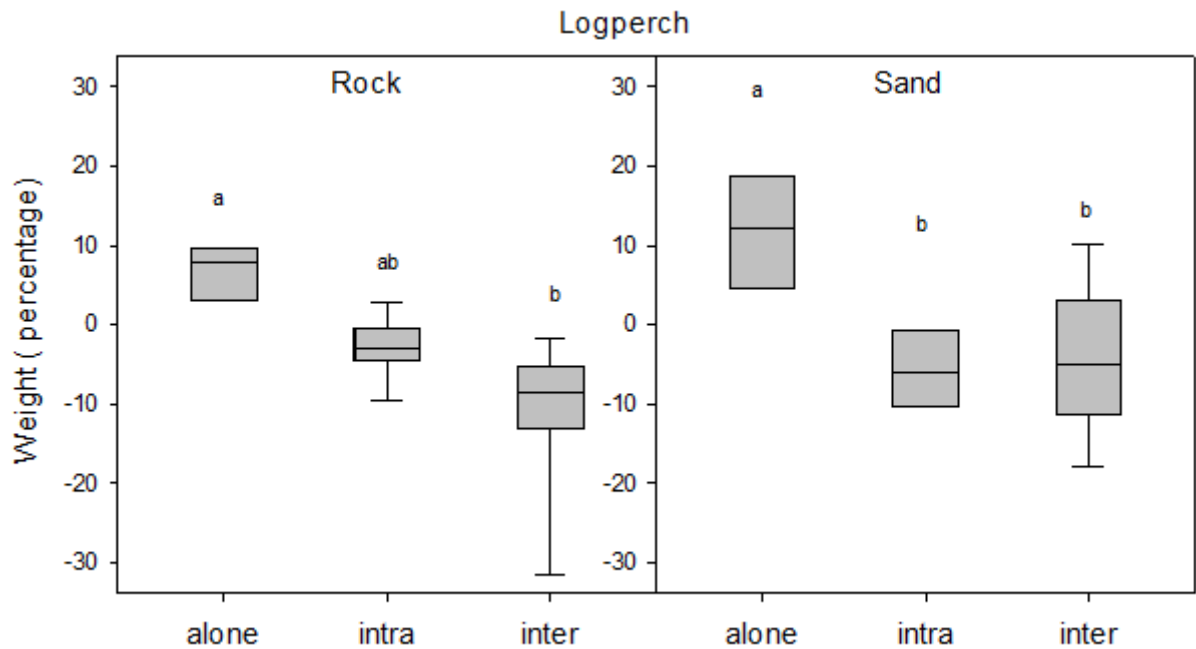


Figure 6. The median weight change is shown for all three round gobies trials (single fish, intra and interspecific competition) on both rocky (left panel) and sandy (right panel) substrate. The line within the box marks the median and the boundary of the boxes indicates the 25th percentile and 75th percentile. Whiskers below and above the box indicate the 10th and 90th percentiles. Letters indicate significant difference medians in each panel (Kruskal-Wallis $P < 0.001$, rock; $P = 0.008$ sand).

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Chapter 3

Age and size distribution of the round goby (*Neogobius melanostomus*) in the Duluth-Superior Harbor

Overview

The round goby *Neogobius melanostomus* is an invasive fish that has established populations in several large ecosystems including the Laurentian Great Lakes. Despite evidence of highly variable life spans and growth rates among spatially isolated populations, data on the structure of round goby populations in many ecosystems is limited. We examined the age and size structure of the round goby population in the St. Louis River Estuary. Fish were captured by bottom trawls in August 2011 and aged using sagittal otoliths. The median size of the round gobies captured was 61 mm TL with length ranging from 13 to 113 mm TL. Ninety-seven sexually mature fish were aged with approximately 90% of the fish determined to be age-1, and the remainder age-2. Females were smaller than males with age-1 females 73.0 mm TL and ranged between 59 to 94 mm TL, while age-2 females measured 94.0 mm TL with sizes ranging from 87 to 107 mm. Age-1 males measured 80.5 mm TL (range 59-114 mm), and age-2 males were 103.0 mm TL (range 70-136 mm). The large variability in size in similar year classes may be indicative of the extended breeding season of the round goby which exhibit multiple spawning over the later spring and summer.

Introduction

The round goby *Neogobius melanostomus* is a small, benthic fish native to the Ponto-Caspian region of the Eurasia (Ghedotti et al. 1995) that has spread into Europe and the Laurentian Great Lakes. The initial conduit for the North American invasion appears to be ballast water contamination of transoceanic ships (Kornis et al. 2012). Populations within each of the Laurentian Great Lakes vary in connectivity and thus population mixing. Lake Superior has several populations that are spatially isolated from each other and population mixing is highly unlikely.

The ability of the round goby to withstand wide variations in temperature and salinity has led to these populations colonizing diverse habitats resulting in variability in growth rate, age distributions and sizes. Growth rates, age of sexual maturity, and size appear to differ between Eurasian and North American populations (Kornis et al. 2012). Within their native habitat, round gobies can reach 250 mm (TL) and live up to six years (Berg 1962). However, in many Great Lakes populations, smaller sizes and shorter life spans have been observed (MacInnis and Corkum 2000a). In addition, there is great variability in growth rates of gobies as age-1 males from the central basin of Lake Erie are approximately 30 mm longer than Lake Huron age-1 males (French and Black 2009; Johnson et al. 2005a). The substantial variation in life span and growth rates makes it difficult to extrapolate results among isolated populations, highlighting the importance of documenting the age and size structure in each region.

Understanding a population's age structure and growth rates is critical to the management of the population and strengthens our understanding of the ecosystem as a

whole. Growth rates could be used to estimate consumption for round gobies which could have implications for native fauna. Round gobies also undergo a potential ontogenetic shift (at a specific size or age) to consuming sedentary bivalves. This additional trophic link is vital to our understanding of nutrients and contaminants flow through the system.

Round gobies were first detected in the Duluth Superior Harbor in 1995 (Jude 1997) and have colonized the St. Louis River upstream to the first barrier (Bergstrom et al. 2008). Trawling surveys of the soft bottom community from 1989 to 2011 (Leino, unpublished) combined with mark recapture studies from 2008 on rocky substrate have provided information on round goby abundance, population dynamics and growth rates in the harbor (Bergstrom et al. 2008; Lynch and Mensinger 2012). Within the Duluth-Superior Harbor, mark recapture studies indicated minimal movement from spring through fall, off shore winter migration and the ability of large males to successfully overwinter and return to similar locations. However, the age of these fish when initially tagged or the age distribution in the soft bottom community remained unknown. The objective of this study was to determine the length and age distribution of the sexually mature round goby population inhabiting the soft bottom community of the Duluth-Superior Harbor of the Saint Louis River Estuary.

Methods

Round gobies were collected from the Duluth-Superior Harbor via bottom trawl equipped with a 4.9 m headrope, 3.8 cm stretch-mesh body, and 31.8 mm stretch mesh cod end in August of 2011. Forty 5-minute trawls were conducted at randomly selected

quadrants with soft bottoms including flats and shipping channels (Bergstrom et al., 2008). Additionally, a beach seine (20 m in length with 6.4 mm mesh net and 3.2 mm mesh purse) was used to sample a sandy shoreline within the Duluth-Superior Harbor. All round gobies were frozen immediately after capture and transported to the laboratory for analysis. All experiments conformed to institutional animal care protocols.

Round gobies were thawed their total length was measured. Fish with a distinguishable external urogenital papilla were classified as sexually mature and their gender was then determined. A small craniotomy was made through the dorsal portion of the skull, and the saggital otoliths removed, cleaned, placed into individual well plates (Costar, 96 well, well volume 0.36 ml) and allowed to air dry for one week. Cytoseal 60 (Richard Allan Scientific) then was added to each well and allowed to set for a minimum of 48 hours.

Two double blind observers aged each otolith using dissecting microscopes (Nikon SMZ 1500). Semitransparent zones indicated periods of fast growth (summer), while white (opaque) zones of densely packed growth rings represented slow growth (winter growth). If the two observers disagreed on age, a third person attempted to resolve the discrepancy, and if unsuccessful, the sample was excluded from the analysis. All data were analyzed with SigmaStat version 3.5, and non parametric testing was used as most data failed the Shapiro-Wilk Normality Test. Data are reported as median and quartiles.

Results

487 round gobies were collected and a sub sample of 97 sexually mature fish were selected for determination of their age. Additionally, two larger round gobies (131 and 136 mm TL) that were captured with beach seines over an inshore sandy bottom were also included to determine if these fish represented a different age class.

The median size of the round gobies capture in the trawl was 61 mm TL (quartiles: 54 mm, 69 mm) with size ranging from 13 to 113 TL mm. The median size of the sexually mature fish examined for otolith analysis was 79 mm (68 mm, 87 mm) with size ranging from 59 to 136 mm (Figure 1).

Most otoliths had a distinct, central translucent zone indicative of fast summer growth surrounded by an opaque white band corresponding to slower, winter growth. In most cases, this opaque zone of slow winter growth was ringed by a translucent band of fast growth that extended to the outer edge of the otolith. The central translucent zone was highly variable in width with approximately 15% of fish lacking this initial band of fast growth. These fish were presumably born late in the year and had insufficient time to form a distinct band before cold temperatures inhibited growth.

Age-1+ fish dominated the sample at 89.6 % of the population. All of these fish were presumably sexually mature as indicated by a external urogenital papilla with 43.2 % of the age-1+ fish females. Age-1+ female fish were significantly smaller than males of similar age Mann-Whitney ($U=617.0$ $P = 0.007$), and although , age-2+ males were longer than females there was no significant difference Mann-Whitney ($U = 8.0$ $P = 0.67$) (Table 1).

Discussion

The soft bottom areas of the Duluth Superior Harbor contained round gobies of a wide range of sizes with the sexually mature fish determined to be age-1+ or age-2+. The majority were age-1+ fish with males of this year class being more numerous and exhibiting significantly larger size than age-1+ females.

A seven year survey of the soft bottom community from 1998 to 2004 showed that the mean round goby length varied yearly from 59.4 to 79.1 mm total length. Recent sampling using baited minnows traps over rocky bottom indicate that inshore areas contain larger fish (male 93.0 ± 0.2 mm TL, female 85.0 ± 0.2 mm TL) on average than fish trawled from the soft bottom community (Lynch and Mensinger 2012).

Despite the wide variability in size range, most fish were age-1+. The high variability in the width of translucent (fast growth) bands was consistent with the prolonged spawning season of the round gobies as fish hatched in spring would have a longer growing season than those spawned in late summer. The presence of fish < 20 mm total length in August is consistent with late season spawning and subsequent limited time for growth. The presence of otoliths without a central translucent zone, which is correlated growth before the first winter, also supports late season breeding. Differences in spawning time and growth kinetics resulted in considerable overlap in total length for age-1+ and age-2+ fish which makes it difficult to assign ages based on size alone.

Sexually dimorphic urogenital papillae were not developed in fish less than 50 mm indicating that this size class was not sexually mature. Although the ages of these fish were not determined, multiple lines of evidence suggest they are age-0 fish. Annual

growth rates, in the Laurentian Great Lakes, range from 4.6 mm SL (French and Black 2009) to 47 mm TL (Taraborelli et al. 2010) with rates varying by fish age (MacInnis and Corkum 2000a) and sex (Lynch and Mensinger 2013) with the fastest growth occurring in the first 2 years (MacInnis and Corkum 2000a; French and Black 2009; Sokołowska and Fey 2011). Additionally, age-0 fish in the central Basin of Lake Erie, ranged in size from 34.9 to 54.5 mm TL (Johnson et al. 2005b). Therefore, the evidence suggests that fish less than 50 mm in our study were age-0 fish and do not reach sexual maturity until age-1. Although reports suggesting alternate reproductive strategies with smaller sneaker males could complicate male aging studies, the smaller morphs were 70 mm (Marentette et al. 2009) indicating that fish with distinguishable urogenital papilla are at least age-1 in all populations examined.

The median size of age-1+ males and females and age-2+ females were slightly larger than previously reported which was initially surprising given that Lake Superior is colder than other Great Lakes (Bennington et al. 2010). However, the round goby population inhabits the St. Louis River Estuary and Duluth-Superior Harbor both of which have warmer temperatures than the main body of the lake (Johnson and Evans 1990). Additionally, only sexually mature fish from relatively late in the season were selected which may have artificially increased median lengths. As the Detroit River study pooled the data from earlier in the year and a longer sampling period, this may have reduced average size (MacInnis and Corkum 2000a; MacInnis and Corkum 2000b) and make comparison difficult. However, Duluth-Superior age-1+ sizes were comparable to the Lake Erie late season (Aug-Sept) values (Johnson et al. 2005b).

Although age-3 fish were not detected in the sample, this may have been a result of sampling method or location rather than absence of this year class from the population. As age-3 males and females from the Central Basin of Lake Erie averaged 148.0 and 129.8 mm TL respectively (Johnson et al. 2005a), it is possible that larger fish resided in rocky inshore areas, which were not accessible to the trawling or seining. Although larger and presumably older fish were routinely caught with baited minnow traps, these traps have an upper limit of approximately 140 mm TL, which means age-3 or above may need to be captured by different methods such as angling. However, mark recapture studies strongly suggest that age-3 fish exist. Several 120 + mm males were caught in late summer or early fall, and again the following spring (Lynch and Mensinger 2012). As these fish were outside the maximum size range of age-1+ fish when originally caught, it is probable they were age-2+ at time of capture and survived the winter to become age-3 fish.

The number of round gobies continues to increase in the soft bottom of the Duluth-Superior harbor and upstream into the St. Louis River. However, these fish are smaller than those captured in the rocky inshore area with most fish age-1+ or younger. Additionally, large males, which are readily found inshore, are almost completely absent. It appears that smaller, younger round gobies are the main residents of the soft bottom with a lower percentage of males than found inshore. Further study is needed to determine the age structure of the rocky inshore community and if the soft bottom residents eventually migrate inshore as they mature.

Figures

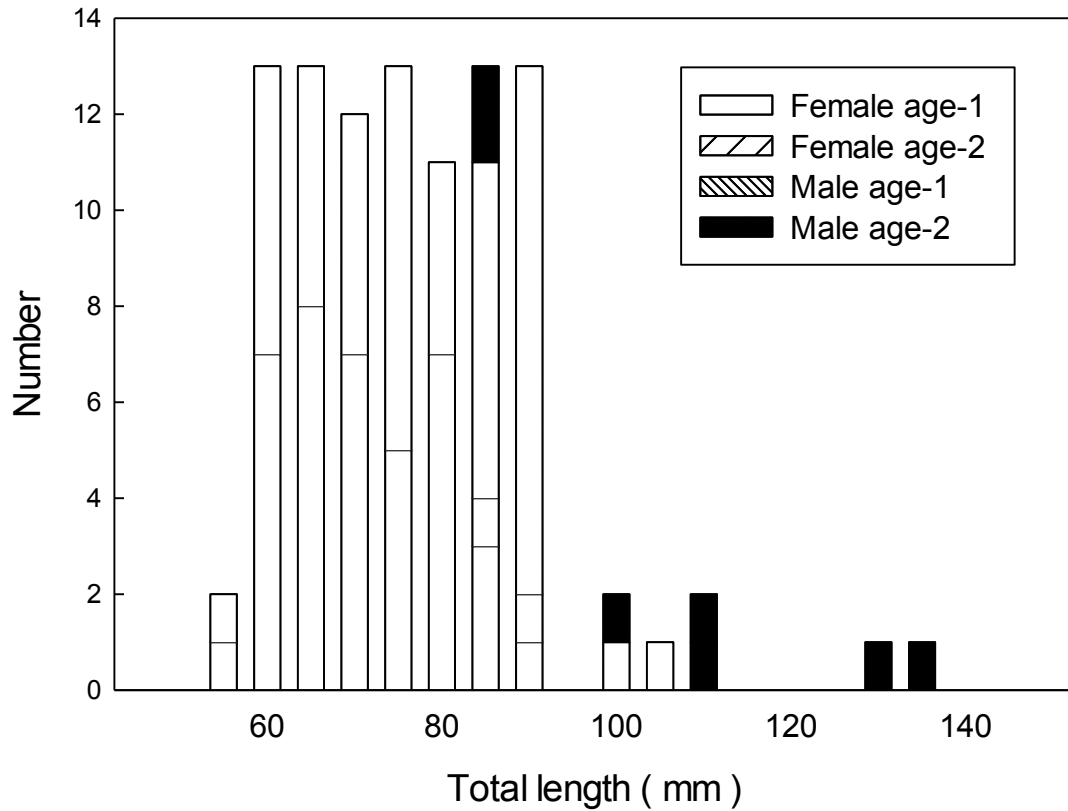


Figure 1. The number of round gobies analyzed for age class is plotted vs total length (mm) of the round goby. The gender and age of each fish was determined and is represented by different fills in each bar.

Table 1. Age, gender and size distribution of the round goby

Age	sex	N	Total Length (mm) (quartiles) range
1+	Female	39	73.0 (66.0, 82.0) 59.0 – 94.0
2+	Female	3	94.0 (87.0, 107.0) 87.0 – 107.0
1+	Male	48	80.5 (71.3, 91.8) 59.0 – 114.0
2+	Male	7	103.0 (88.0, 131.0) 70.0 – 136.0

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