Final Report

Invasive predator suppression on critical spawning reefs
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Prepared for:
USEPA-GLNPO
77 West Jackson Boulevard (G-17J)
Chicago, Illinois 60604-3590
Meonii Bristol, Project Officer
312-886-6399 (Phone); 312-692-2938 (Fax)
Bristol.Meonii@epamail.epa.gov

Prepared by:

Lindsay Chadderton and Andrew Tucker, The Nature Conservancy, 1400 E Angela Blvd, Unit #117, South Bend IN, 46617, 574-217-0262, lchadderton@tnc.org, atucker@tnc.org

Mathew Herbert, The Nature Conservancy, 101 East Grand River Avenue, Lansing, MI 48906, 517-316-0300 (Phone), 517-316-9886 (Fax) , mherbert@tnc.org

Randall Claramunt , Michigan Department of Natural Resources , Charlevoix Fisheries Research Station, 96 Grant Street, Charlevoix, MI 49720, 231-547-2914 ext. 224, claramuntr@michigan.gov

Tracy Galarowicz, Central Michigan University, Department of Biology , Brooks 217, Mt. Pleasant, MI 48859, 989-774-1336, galar1tl@cmich.edu

Jackson Gross, Smith-Root, Inc. 14014 NE Salmon Creek Ave. Vancouver, WA 98686, 360-573-0202 ext 124 (Phone), 360-573-2064 (Fax), jgross@smith-root.com

Cover photo: Baited underwater video image taken on shallow Lake Trout spawning reef habitat in Grand Traverse Bay, Lake Michigan, showing Round Goby and Rusty Crayfish congregating around a bait bag containing Lake Trout eggs. *Photo credit: Krista Robinson, Central Michigan University.*

Brief summary

Summary and overview

We set out to develop innovative and refine traditional methods to control the impacts of Round Goby and Rusty Crayfish, on spawning reefs in Northern Michigan. Our objective was to enhance natural spawning success of Lake Trout, Cisco and Lake Whitefish by minimizing the impacts of egg predation by these two key interstitial invasive species. Replicate reefs were treated using combinations of trapping, tangle nets and diver removal and trials undertaken to measure the efficacy of seismic guns prior to fall spawning. Extensive monitoring was undertaken to measure the effects of predator control and outreach activities undertaken to promote the need to restore spawning reef habitats.

Extensive control efforts removed 3,900 Rusty Crayfish and 8,600 Round Goby from the 4 treatment reefs in northern Lake Michigan during the fall of 2012 and 2013. In 2013, reef treatment with seismic gun technology was implemented on 2 reefs. However, no sustained reduction in abundance of either species was measured in either year for any reef and persistent recolonization of spawning reefs appears to have occurred in both treatment years. Hence, efforts to suppress abundance of Round Goby and Rusty Crayfish in order to enhance spawning success of Lake Trout, Lake Whitefish and Cisco (a.k.a. Lake Herring) on shallow spawning reefs in northern Lake Michigan were ultimately unsuccessful.

Nevertheless, valuable insights into Rusty Crayfish and Round Goby behaviors on native fish spawning habitat suggest the concept of Rusty Crayfish and Round Goby control on these spawning reefs is warranted and achievable. Indeed, these reef communities are dominated by Rusty Crayfish and Round Goby, which accounted for over 90% of fish and crayfish observed. We found no evidence to support the assumption that seasonal exodus into deeper waters is a dominant process for either species. Rather, our study results and observations indicate that both species remain on the reef and move into reef substrate overwinter. Densities of Rusty Crayfish (as measured by direct diver quadrat counts) are high and appear equivalent to what has been observed in inland lakes of the Great Lakes region (Hein et al. 2006). When Rusty Crayfish densities are considered alongside previous research that has documented feeding during winter months and our observations that Rusty Crayfish are the only active benthic species on these reefs in early spring (when Round Goby are still seemingly dormant under the substrate), it is evident that Rusty Crayfish may be a far more important benthic egg predator on these reefs than previously thought.

A crayfish movement study (Buckley in prep) undertaken in association with suppression efforts indicated Rusty Crayfish appear to maintain only transient home ranges and helps explain the persistent recolonization of our treatment areas, in combination with evidence that neither Rusty Crayfish nor Round Goby undertake seasonal migration into deeper water at the onset of winter.

We also found that traditional trapping using Gee minnow traps, which have proved successful at reducing Rusty Crayfish in inland lakes (Hein et al. 2006), were not as effective in the Great Lakes, where our catch per unit effort was just a fraction of that observed by Hein et al. (2006) despite similar levels of effort and comparable densities. Manual removal by divers proved to be a far more cost effective strategy and entanglement traps/netting also showed promise as both a control tool and barrier to recolonization. Our observations and data suggest that if barriers to recolonization could be developed, Rusty Crayfish populations could be successfully suppressed and maintained at low densities on these small spawning reef structures. Furthermore, declines in the average size of Round Goby over the course of removal indicate that our suppression efforts did impact Round Goby. Given egg predation is limited by gape size in Round Goby, we may have reduced predatory pressure on native fish eggs. Declining size frequency and high catch rates for Round Goby suggest that Round Goby might also be suppressed, if control is undertaken over a wider buffer area around the reefs to slow recolonization of larger fish. Combining trapping efforts with barriers or repellant technologies for Round Goby and/or increasing native predatory pressure might further enhance our ability to reduce Round Goby impacts and benefit native fish reproductive success.

Seismic technology was not an effective control method for these two invasive benthic species, but it did prove to be lethal to a range of native and introduced fishes with gas bladders (alewife, lake chub, rock bass), further emphasizing its potential as a control tool for a wide range of fish species. Experiments with two different sized seismic guns produced significant short term behavioral responses, including a reduction in density and feeding activity during seismic treatment for Round Goby. No mortality was observed for either Rusty Crayfish or Round Goby, and feeding rates and reef occupancy returned to levels observed prior to treatment within 30 minutes of cessation of treatment. An incidental outcome of the research effort was the experience gained by MDNR staff in deployment and use of the seismic gun — knowledge that may be beneficial if MDNR needs to respond to detections of Asian carp or other aquatic invasive species in its waters.

Finally, our egg monitoring and seeding experiments show that spawning success and egg survivorship is a function of not only biotic, but also abiotic factors, which is an additional challenge for monitoring native fish recruitment and demonstrating success of management efforts. In extreme storm years (as observed in 2012), egg loss as a result of wave turbulence will completely overwhelm any impact by predators. Whereas in years where storm patterns do not result in significant egg loss, predators account for up to 50% of all eggs lost. Clearly, restoration of Lake Trout, Lake Whitefish and Cisco will require both habitat improvement and predator control.

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The statements, findings, conclusions, and recommendations in this report are those of the authors and do not necessarily reflect the views of our organizations, nor of the Environmental Protection Agency.

Core project team members, Beaver Island, July 2013.

Management Recommendations

1. The concept of crayfish and goby control on these spawning reefs is warranted and appears to be achievable.
2. Crayfish are less conspicuous (than goby), but are abundant and crucially resident on these spawning reefs year round. Spring observations suggest they may also be more active than goby over winter. These observations suggest crayfish may be a far more important benthic predator than previously thought and efforts need to be made to reduce their impacts on key habitats for valued native species (e.g. spawning habitats).
3. Successful suppression of Rusty Crayfish will require the development of benthic barriers that can be used to reduce or prevent recolonization of high value habitats so removal efforts can be sustained and provide short term protection for vulnerable life stages of native species.
4. Alternative harvest methods for Rusty Crayfish are needed to replace the universal standard Gee minnow traps, and rates of capture per unit effort need to substantially improve in order to make control efforts possible and cost effective.
5. The potential to enhance control efforts by integrating biological control approaches (sensu Sparkling Lake — Hein et al. 2006) through increased native predator densities or through techniques to attract native predators to key habitat should also be investigated for both Rusty Crayfish and Round Goby.
6. Further research on basic biology of crayfish in Great Lakes is needed; especially a greater understanding of winter movement and activity patterns are required to better quantify relative impacts (to goby), and to identify behaviors that make crayfish vulnerable to control.
7. Since behavioral changes influence crayfish catchability across habitats seasonally, which causes the effectiveness of different gears to vary, integrated sampling methodologies that employ multiple gear types should be considered either in combination with intensive harvest or as a standalone project.
8. Quantitative counts by divers should be used more routinely to monitor crayfish in the Great Lakes.
9. The efficacy and feasibility of short term goby suppression using traditional fisheries methods needs to be tested. Declining size frequency and high catch rates for goby suggest that goby populations may be able to be successfully suppressed through intensive harvest — provided it is undertaken over a wider buffer area around the reefs (to slow recolonization) and immediately prior to spawning as water temperatures decline.
10. Efforts should be made to see if goby can be repelled or recolonization rates slowed by barrier technology.

11. Additional harvest methods for Round Goby should be developed to supplement standard Gee minnow traps, and increase capture per unit effort rates and cost effectiveness of control efforts.
12. There is a need to better understand goby winter movement and feeding patterns to quantify relative impacts and identify vulnerabilities.
13. Baited underwater video should be utilized for benthic fish monitoring in the Great Lakes, particularly in complex habitats that are difficult to sample with traditional gear.
14. A better understanding of the relationship between spawning reef habitat quality and the impacts of benthic predators is needed. Our understanding of egg predation rates once eggs are deep within the spawning reef is limited, and it is unclear whether eggs that settle deep are vulnerable to goby and Rusty Crayfish.
15. Habitat restoration through a combination of both invasive predator control and physical habitat rehabilitation should be considered for its potential to contribute to fisheries goals, in particular the recovery of Cisco in the Great Lakes.
16. Combined seeding of artificial eggs and early maturing Lake Trout eggs should routinely be used to monitor egg survivorship on spawning reefs.

Objectives and outputs

This GLRI project had six main objectives. Here we describe the main outputs and outcomes arising from this project arrange by each objective.

1: Deplete crayfish prior to spawning through intensive trapping on three spawning reefs to reduce densities of or crayfish during native reef fish egg deposition and egg development.

We undertook intensive Rusty Crayfish control efforts on four reefs in 2012 and three reefs in 2013, resulting in the removal of 1268 crayfish in 2012 and 2693 crayfish in 2013. In addition, incidental bycatch of Round Goby also resulted in the removal of 8600 Round Goby; the majority of which were removed from the four treatment reefs in the fall of 2012. However, no sustained reduction in abundance of either species was measured, and rapid recolonization of spawning reefs appeared to occur in both treatment years. We did find a significant decrease in size structuring, for Round Goby indicating we were having some impact on resident populations. Hence, efforts to suppress abundance of Round Goby and Rusty Crayfish in order to enhance spawning success of Lake Trout, Lake Whitefish and Cisco on shallow spawning reefs in northern lake Michigan were ultimately unsuccessful.

However, over the course of control efforts and associated monitoring, major gains in our understanding of the biology and behavior of Rusty Crayfish on Great Lakes spawning reefs were gained. First, we found that in contrast to Hein et al. (2007), modified Gee minnow traps were not as effective as a control tool in the Great Lakes. Furthermore, manual removal by divers was a far more cost effective removal technique, resulting in the removal of a larger number and more representative size classes of Rusty Crayfish. However, removal of Rusty Crayfish by divers results in no incidental bycatch of goby.

We also found that Rusty Crayfish were not undergoing a seasonal offshore migration and were staying on the reefs over winter, capable of persistent recolonization of the treatment area during warmer months of summer and fall. Given this, control efforts should be focused on the time immediately prior to and if possible during spawning (provided it was undertaken without interfering with spawning activity), when water temperatures were declining and presumably rates or recolonization slowed as crayfish activity slows (Fitzsimmons et al. 2006). However, as we found in 2013, when the "gales of November come early" (Lightfoot 1976), weather conditions can keep control teams off the water during this critical period. Long term suppression will require the development of a benthic crayfish barrier and more effective harvesting methods, so Rusty Crayfish suppression efforts can occur prior to the fall storms but be sustained during spawning by barriers that are set in deeper water and immune to wave disturbance.

2. Experimentally quantify the lethality, effective control range (radius and interstitial depth) and suppression duration of seismic guns on goby and Rusty Crayfish.

Two series of experiments were conducted between July 2013 and October 2013 to evaluate the potential lethal effects of a small 410-cm^3 and larger 1966-cm^3 pulse pressure water guns. We successfully deployed and operated a new hydraulic pump firing system that dramatically increasing user safety and increased of manually operated rate of firing. Repeated dose experiments using caged animals held at set distances from each gun were conducted on sand and rock substrate. Lethality and non-lethal impacts (e.g., barotrauma, effects on Round Goby hearing cells and ear bones) were quantified for Round Goby, Rusty Crayfish and rock bass (*Ambloplites rupestris*) across a wide range of exposure distances and number of doses. No measurable impacts were observed for Round Goby or Rusty Crayfish whereas high mortality was observed for rock bass. We also found no significant hair cell loss or otolith damage, suggesting that a higher peak pressure level or longer duration of discharges are needed to cause significant damage to goby hearing anatomy (Wagner et al., in review).

3. Use seismic guns to treat up to four spawning reefs to reduce the abundance of all size classes of goby immediately prior to native reef fish egg deposition.

We experimentally treated two reefs with the seismic gun in the fall 2013. In the absence of any evidence for lethal or chronic impacts on Round Goby(or Rusty Crayfish – Objective 2 above), we set out to determine if seismic treatment could be used to repel goby and reduce densities over the spawning reefs and whether any decrease was sustained. We treated the northern Elk Rapids reef (ER North) by towing and repeatedly firing a 1966-cm^3 water gun across the spawning reef and a surrounding buffer area, so that each part of the reef received between 3 and 5 pulses from the gun. Examination of underwater videos suggested that there was both a reduction in density and feeding behavior, but the rate of recolonization or recovery was inconclusive owing to weather shortened period of post treatment monitoring. Subsequently, we intensively treated the Crib reef by anchoring the treatment boat over the reef and using the motor and varying the anchor ropes in order to move the 1966-cm^3 water gun across the reef structure. Once again, underwater video monitoring measured a significant reduction in goby density and feeding rates during treatment but both recovered to pretreatment levels within 30 minutes of the cessation of treatment. No mortality was observed for caged or wild Round Goby on the reefs, but a number of caged rock bass died and a small number of dead rock bass, alewife and lake chub were observed on or floating above the reef. Because we found no evidence that seismic treatment was reducing Round Goby or Rusty Crayfish or their impacts on the reef but was impacting other non-target fishes, treatment of the remaining two reefs was cancelled.

While seismic treatment operations were unsuccessful, the feasibility and towing methods needed to enable repeated firing and treatment of a reef or river with a seismic gun was determined and demonstrated; hence, we increased MDNR aquatic invasive species response capabilities.

4. Measure changes in abundance and distribution and interstitial densities of target and non target invasive species (e.g., goby, Rusty Crayfish, dreissenid mussels, *Hemimysis anomala*) and native egg predators.

A large number (8600) of Round Goby were removed as incidental bycatch during Rusty Crayfish control efforts in 2012. However, we found no evidence that this or the removal of 3900 Rusty Crayfish impacted non-target species found on the spawning reefs. Indeed, Round Goby and Rusty Crayfish account for over 90% of all crayfish and fish observed. Despite the large numbers of Round Goby removed, there was no significant decrease in Round Goby abundance. However, we did find a significant decline in Round Goby length, suggesting that removal efforts were successful in removing larger Round Goby that are likely to be the most important egg predators, and that the fish that recolonized the reef tended to be smaller, reflecting perhaps that neighboring soft bottom habitats are poorer Round Goby habitat. We did not find any evidence that Round Goby(or Rusty Crayfish) undergo a seasonal offshore migration as has been previously inferred. As water temperatures drop below 5^0C, both species move into the substrate and are not detected using monitoring methods that rely on traps or video. However, diver observations on these reefs at the onset of winter and in early spring show both species are resident on the reefs beneath the rocky substrate, suggesting that egg predation likely continues on these reefs after spawning when eggs settle into the reefs. Taken together, these observations suggest that suppression of Round Goby is feasible if control is undertaken over a large enough buffer area and effective would likely be enhanced if this is coupled with either barriers to recolonization, and or repellant technologies or enhance native fish predation.

Finally, extensive monitoring of Round Goby as part of this project has provide additional evidence of the importance of Round Goby in Lake Michigan food web and highlighted the inadequacies of current goby monitoring efforts at a Great Lakes scale. One outcome of this work has been that MDNR fisheries staff (PI Claramunt) have proposed to the Lake Michigan technical committee that there is a need for a renewed focus on Round Goby to incorporate this important prey fish into fisheries monitoring efforts and assess basin wide biomass.

5. Quantify changes in Lake Trout, Cisco and Lake Whitefish egg deposition and survival.

We completed gill net monitoring of adult spawners (Lake Trout, Lake Whitefish and Cisco) on all six reefs in 2012 and 2013; numbers of mature fish was higher in 2013. However, this increase did not correspond to an increase in eggs collected on the reefs in 2013. Measurements of egg deposition and retention rates for Lake Trout, Cisco and Lake Whitefish were undertaken on all six reefs in 2012 and 2013, although fewer sampling periods were completed in the latter because of inclement weather conditions. We used a combination of methods including seeding experiments that used both artificial eggs and eyed Lake Trout eggs to differentiate between loss from physical forces (wave energy, currents) and egg predators. We found that physical forces accounted for between 33 and 50% of egg loss on these reefs, and losses were most pronounced on disturbed reefs with poor reef habitat quality. Furthermore, predation rates averaged near or greater than 50% and are comparable across treated and untreated reefs and provided no evidence that egg predation levels were suppressed on the most intensively controlled reefs (the Crib and ER North).

We successfully trialed a combined egg seeding monitoring method that used artificial eggs and early maturing eyed Lake Trout egg (to differentiate these eggs from natural spawn) to separate the relative impacts of physical forces and predation. This approach will be adopted by MDNR as standard (best) practice going forward.

6. Cause integrated pest management paradigm shift by communicating successful restoration efforts, promoting the Grand Traverse Bays reef complexes as a demonstration site, providing standard operation procedures, operational costs, and recommendations to fisheries managers and stakeholder communities on how these methods can be adopted at other shallow and deep spawning reefs in the Great Lakes basin.

During the course of the project our team has completed numerous presentations to a broad range of audiences including technical fisheries meetings, science conferences, tribal, and community stakeholder groups. However, because we were unsuccessful in our efforts to suppress Rusty Crayfish and Round Goby on these spawning reefs, we were not in a positon to affect a paradigm shift among fisheries managers and community stakeholders. Nevertheless, we have successfully raised awareness of the importance of these spawning reef structures, the need for both control methods and an improved understanding of the biology of Round Goby and Rusty Crayfish in the Great Lakes. In addition, we have been able to show that efforts to control these benthic predators need to be integrated with habitat restoration on degraded reefs. Evidence of our success is demonstrated by an award by the Great Lakes Basin Fish Habitat Partnership (administrated by the USFWS) to MDNR to restore the degraded reef habitat on the northern Elk Rapids reef (ER North, Figure 1) and a large five year private award/gift to The Nature Conservancy from a major Michigan foundation to provide ongoing support for our adaptive management efforts on these reefs, to restore native fisheries through benthic predator suppression and habitat restoration efforts on these spawning reefs. The latter funds have been used to contract a material engineering firm to design a more effective tangle trap and potential Rusty Crayfish barriers that will tested on the spawning reefs this summer. In addition, our efforts have helped stimulate renewed focus on the importance of goby in Lake Michigan and recent efforts to calculate basin wide biomass.

This project has supported 2 masters project (Robinson 2014, Buckley, in prep) and spawned a third that is focused on Elk reef restoration and interaction between habitat improvement and benthic predator impacts. Three articles have been submitted to scientific journals for publication, and our expectation is that at least a further three scientific paper will be produced over the next 12 months from this work. We have published one popular article in the MDNR Fisheries newsletter and our work was featured on a Detroit public TV documentary/program.

Final report

Objectives results and narrative

Study area:

Restoration efforts focused on six shallow spawning reefs in Grand Traverse and Little Grand Traverse Bays (Figure 1). We use paired treatment (1, 2, 3, 6) and control reefs (4, 5) to assess the effectiveness of Rusty Crayfish trapping and seismic gun treatment (Figure 1). The three spawning reefs in Grand Traverse Bay near Elk Rapids represent the only Lake Michigan reefs where Lake Trout, Cisco and Lake Whitefish are known to spawn were paired with a no treatment control site at Ingalls Point, whereas one of the two spawning reefs in Little Traverse Bay was treated (6) with the other at Bay Harbor (5) acting as the control (Figure 1).

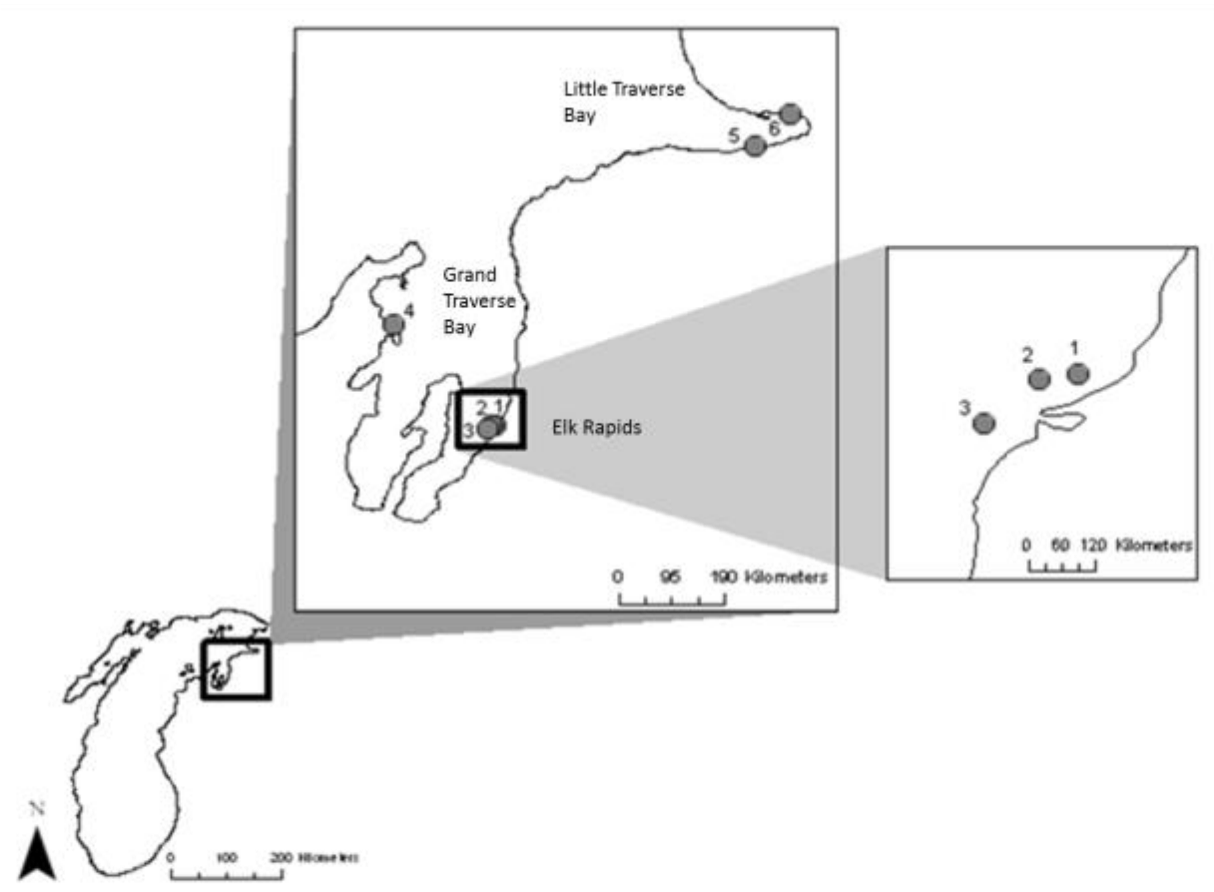


Figure 1. Location of the six spawning reefs in Grand Traverse and Little Traverse Bays, Lake Michigan. Site key: 1 = ER North, 2 = ER Central, 3 = ER South, 4 = Ingalls Point, 5 = Bay Harbor, and 6 = Crib

<u>Objective 1</u>: Deplete crayfish prior to spawning through intensive trapping on three spawning reefs to reduce densities of or crayfish during native reef fish egg deposition and egg development.

Rusty Crayfish are present and active over native fish spawning reefs throughout the spawning and egg development period in late fall and early winter, and they are the first active predators present on the reefs in spring. We proposed to remove Rusty Crayfish on multiple spawning reefs in an effort to minimize predation on native reef fish eggs and limit negative effects of invasive crayfish on native fish recruitment. To reduce Rusty Crayfish abundance on the reefs, we proposed to undertake intensive trapping (in combination with seismic gun treatments per Objective 2 and 3) during summer and fall, just prior to Lake Trout, Cisco , and Lake Whitefish spawning (i.e., July through November). Previous Rusty Crayfish control at a whole-lake scale showed that intensive trapping with Gee minnow traps effectively reduced Rusty Crayfish numbers and biomass (Hein et al. 2006; Hein et al. 2007). While we did not expect to eradicate Rusty Crayfish on the spawning reefs, we hypothesized that our intensive removal efforts throughout the summer, coupled with reduced crayfish activity on the reef as water temperatures fall and a potential offshore migration into deeper water would generate substantial reductions in Rusty Crayfish densities that would be sustained through the onset of native fish spawning due to the limited mobility of individual crayfish. Our data quality objective for this objective was to measure temporal changes in Rusty Crayfish densities and size frequency and sex ratios across treatment and control reefs in order to quantify the effect of intensive trapping/removal with adequate resolution to distinguish treatment effects on crayfish abundance from any potential seasonal changes in abundance arising from a winter movement off of or into the spawning reefs. Our aim was to reduce crayfish densities by 80-90% during the spawning period (Mid October-mid December). Here we describe our trapping and monitoring activities on four treatment and two control reefs and related results for the project period (2012-2013).

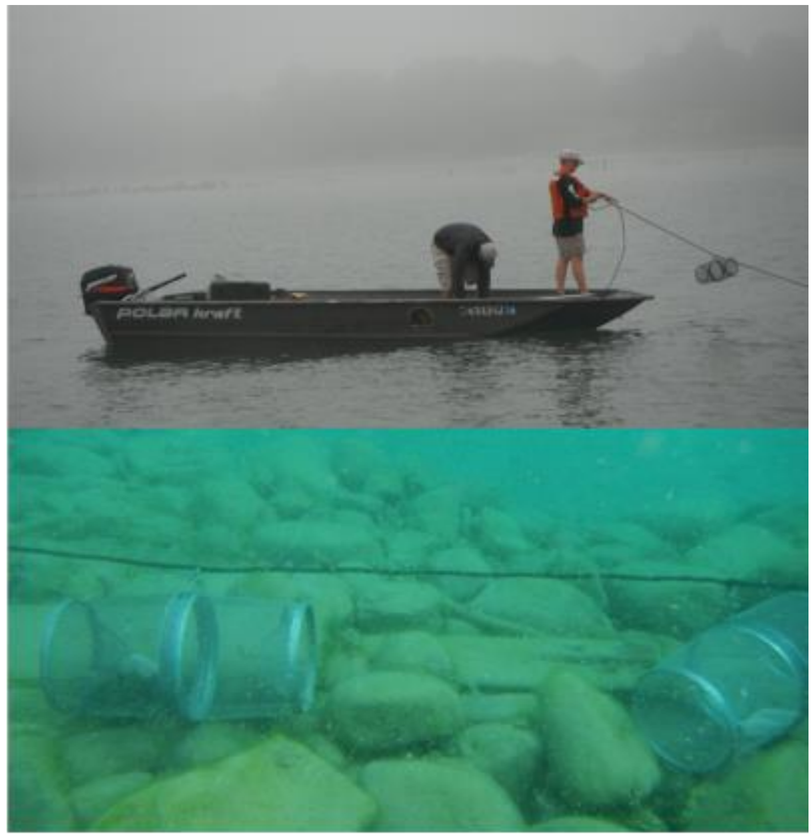
METHODS

Intensive removal efforts

Intensive trapping using baited Gee minnow traps, modified tangle nets, and hand removal by divers was implemented to deplete Rusty Crayfish across the four reefs where removal efforts were carried out in 2012 and 2013 (Table 1.1; Figure 1). In 2012, at each reef and over an approximately 10 m buffer area surrounding each reef, strings of traps were set out in an approximate 5m x 5m grid. We employed a rolling front of traps starting at one end of each reef and working along the reef to systematically trap the entire reef. Sets of 10-20 traps were tethered to a long line, with each trap attached at 5 m intervals along the line. The traps were standard Gee minnow traps (23 cm x 45 cm with 0.64 cm steel wire mesh) and trap lines consisted of alternating small (3 cm) and large (6 cm) entrance traps, each baited with a single piece of Chinook Salmon (*Oncorhynchus tshawytscha*), carcass or beef liver. In 2012, intensive trapping occurred at least two times per week from August through early September on each of the 4 treatment reefs (Appendix Table A1.1). At the Crib, trapping commenced in mid-July.

Trapping effort on each reef varied primarily as a function of reef size (Appendix Table A1.2). Set times for traps ranged from just 3 hours up to 144 hours (6 days), in rare instances when weather or lake conditions precluded recovery of traps. In general, traps were retrieved within 24 - 48 hours of deployment. The trapping regime described here resulted in fairly constant trapping pressure over each reef from 1 August to 9 September 2012. Minnow traps and tangle nets were also deployed in the first week of October 2012 (just prior to Lake Trout spawning) at both the Crib and ER North. Tangle nets were modified nylon gill nets of uniform mesh size from which floats were removed to enable deployment on the lake bottom and measured 25 to 100 meters in length. Tangle nets were baited with whole Lake Trout carcasses at approximately 5 m intervals, and effectively operated like a trap, with crayfish becoming entangled in the netting.

Seasonal trends in Rusty Crayfish abundance from index monitoring (see below) and observations from 2012 minnow trap and tangle net deployment and diver observations seemed to confirm suspicions that during the fall 2012 "trapping depletion effort" the low catch rates recorded during August and September were likely a function of crayfish trap avoidance and low activity by female Rusty Crayfish. As a consequence, in August an experimental removal by divers was initiated to deplete crayfish on the two smallest reefs (The Crib, and ER North) so we could get a better understand recolonization patterns. In addition, barrier tangle nets were deployed around these reefs to see if this approach could be used to confirm if — and from where — recolonization was occurring. Furthermore we adjusted the seasonal timing of trapping efforts to focus on the peak in abundance and catch rates to maximize depletion efforts at the time where declining temperatures might also limit crayfish movement onto the reefs.



Crayfish depletion trapping, ER Central, August 2012. MI DNR

Thus, Rusty Crayfish capture methods and the seasonal timing of trapping efforts were modified in 2013 (Appendix Table A1.1). Diver removal generally consisted of two (but up to four) divers swimming 5 to 50 meter transects and collecting all Rusty Crayfish visually observed on the surface or in the first layer of overturned cobble. A maximum of four tangle nets were deployed on a reef at any one time. Tangle nets were deployed for a minimum of three hours and for up to 40 days in one case. For all crayfish removal efforts crayfish were identified to species, measured, and sexed. Rusty Crayfish were sacrificed. Native crayfish, <5% of the crayfish captured, were returned to the reefs.

Table 1.1. Treatment and reference (i.e. no-treatment) classification for reef sites in 2012 and 2013. In cases where fewer than 10 animals were removed we consider the site a reference site.

	2012 Goby	2012 Rusty	2013 Goby	2013 Rusty
Crib	Treatment	Treatment	Reference	Treatment
ER North	Treatment	Treatment	Treatment	Treatment
ER Central	Treatment	Treatment	Reference	Treatment
ER South	Treatment	Treatment	Reference	Reference
Bay Harbor	Reference	Reference	Reference	Reference
Ingall's	Reference	Reference	Reference	Reference

Index monitoring

To track progress towards the 80% reduction goal and to provide a standardized measure of temporal changes in Rusty Crayfish densities, we employed index monitoring on all six reefs using egg bags, baited minnow traps, and underwater video cameras. Index monitoring for Rusty Crayfish with eggs bags occurred biweekly from mid-July to early December in 2012. Rusty Crayfish abundance as bycatch in egg bags deployed by Michigan DNR to monitor Lake Trout and Lake Whitefish egg deposition, was measured at two additional time points in 2012 (early November and early December) and again in 2013. Biweekly egg bag monitoring did not occur in 2013. Index monitoring with minnow traps occurred on a biweekly basis from early July to the end of November in 2012. In 2013, monitoring with both minnow traps and baited underwater video cameras occurred at each site once every three weeks from early September to the first week in December. Monitoring at each site occurred across three depths—at approximately 3m (on reef), 6m, and 9m. The 6 m and 9 m monitoring locations at each site were located on a straight line perpendicular to the shore and extending out from the reef. Sampling locations at all depths were georeferenced, remained fixed for the two-year monitoring period, and were chosen regardless of substrate type.

In 2012, crayfish were sampled using the standard Gee minnow traps on trap lines consisting of ten traps with alternating small and large entrance openings placed 1 m apart. Each trap was baited with previously collected, frozen, and thawed Lake Trout eggs. Lake Trout eggs (~30 g) were placed in 8 cm x 13 cm mesh bags and suspended in the middle of each trap. Trap lines were deployed at each depth and retrieved after 24 hours. All captured crayfish were immediately measured for carapace length (mm) and then euthanized. Water temperature (°C) was recorded at each depth. In 2013, baited minnow traps were individually buoyed and placed 10m apart to ensure sample independence. Traps were deployed at each of four depth strata and were retrieved after just 1.5 hours.

Underwater video cameras (GoPro HERO 3®) were used to monitor crayfish abundance at each depth in 2013. Each camera (total n=5) was mounted at the top of a steel camera frame with a quad-pod base (height = 60 cm; base = 0.45 m^2). The camera lens was aimed at the substrate and captured a field of view approximately 1m^2 (photoquadrat). Each camera frame was baited with previously collected, frozen, and thawed Lake Trout eggs (~30 g) contained in a mesh bag suspended approximately 5 cm from the substrate in the center of the photoquadrat. All five cameras were individually buoyed and positioned 10 m apart at each depth. The cameras recorded one photograph per minute for 20 minutes. In the laboratory, the number of crayfish in each image (at one minute intervals) was counted. Two technicians independently analyzed images in the laboratory and any discrepancies in crayfish counts were settled after review by a third technician. Crayfish were included in the total count when at least 50% of the crayfish body was visible in the image. The maximum number of crayfish recorded during the 20 minute period was used for analysis (Willis et al. 2000; Cappo et al. 2006).

Egg bags were intended primarily to quantify interstitial habitat use. Each egg bag was constructed of a 50 cm deep nylon mesh material (0.16 cm mesh size) attached to a PVC ring measuring 29.8 cm in diameter (similar to Barton et al. 2011 and Claramunt et al. 2005). For the biweekly egg bag monitoring in 2012, divers buried 10 egg bags at 1 m intervals along a single transect approximately 2 m deep at each site. For the Michigan DNR egg bags, a target of 30 egg bags were buried along a single transect approximately 1m apart crossing the primary suitable spawning habitat at each site. Egg bags were buried by scuba divers approximately 1 month before sampling to allow time for acclimation. Egg bags were retrieved by cinching the tops closed with cable ties to minimize crayfish escape. Any losses from the egg bags were noted by divers and included in abundance estimates.

In 2013, monthly diver counts with randomly placed quadrats occurred across four depths at each of four of our six sampling locations (Crib, ER North, ER Central, and Bay Harbor). The quadrat sampling functioned as an additional measure of crayfish movement and abundance on the spawning reefs in 2013. Each site was divided into four habitat types (shallow, reef, deep, off reef). SCUBA divers randomly placed 10-1m^2 quadrats within each of the four habitat types one time per month from May to December. Divers searched the first layer of substrate in each quadrat and recorded the total number of Rusty Crayfish.

Statistical analysis

We used simple linear regression models to estimate changes in catch rates over time during removal in 2012 and to estimate changes in M:F sex ratios and crayfish size during removal in both years. Limited time series for removal activities precluded analysis of changes in catch rates in 2013. Analysis of covariance (ANCOVA) was used to compare capture numbers from index monitoring (egg bags, traps, cameras, and quadrats) over time and among sites, to compare CPUE between treatment and reference sites, and to analyze mean crayfish size over time and across years.

RESULTS

Intensive removal efforts

We removed more than 3900 Rusty Crayfish from the four treatment reefs (Figure 1.2; Appendix Table A1.2). The majority (64%) of captured crayfish were taken at Crib, with 21%, 14%, and <2% of total capture occurring on ER North, ER Central, and ER South respectively. For reference, based on estimates from a mark-recapture study (J. Buckley, in prep), mean population size estimates for 225 m^{-2} sections of the ER Central reef (the largest of our treatment reefs) ranged from just under 1000 crayfish to about 3500 crayfish (Appendix Figure A1.1).

In 2012, minnow traps were the predominant gear used for removal efforts and captured more than 92% of all crayfish. There were no significant changes in Rusty Crayfish catch rates over time. Marginal declines in mean crayfish catch rates were observed at the ER North and ER Central sites, whereas catch rates increased marginally at the Crib and at ER South (Table 1.2). Removal efforts in 2013 relied primarily on hand removal by divers (>82 % of captured crayfish; Figure 1.2). Removal via minnow traps was reduced to just 0.5% of the total catch. Limited time series data for removal efforts (e.g., no more than two removal events per method per site) and/or unspecified values needed to calculate CPUE (e.g., dive time or transect length) precluded analysis of catch rate trends over time in 2013, except at the Crib site where a simple linear regression was calculated to predict CPUE based on removal date. Catch rates by hand removal increased over time but not significantly ($F_{1,5}$=0.50, P = 0.51, R^2 = 0.09). Removal efforts in 2013 generally occurred later than efforts in 2012, beginning no earlier than mid-September and extending into October and even late November at the ER North and ER South sites (Appendix Table A1.1).

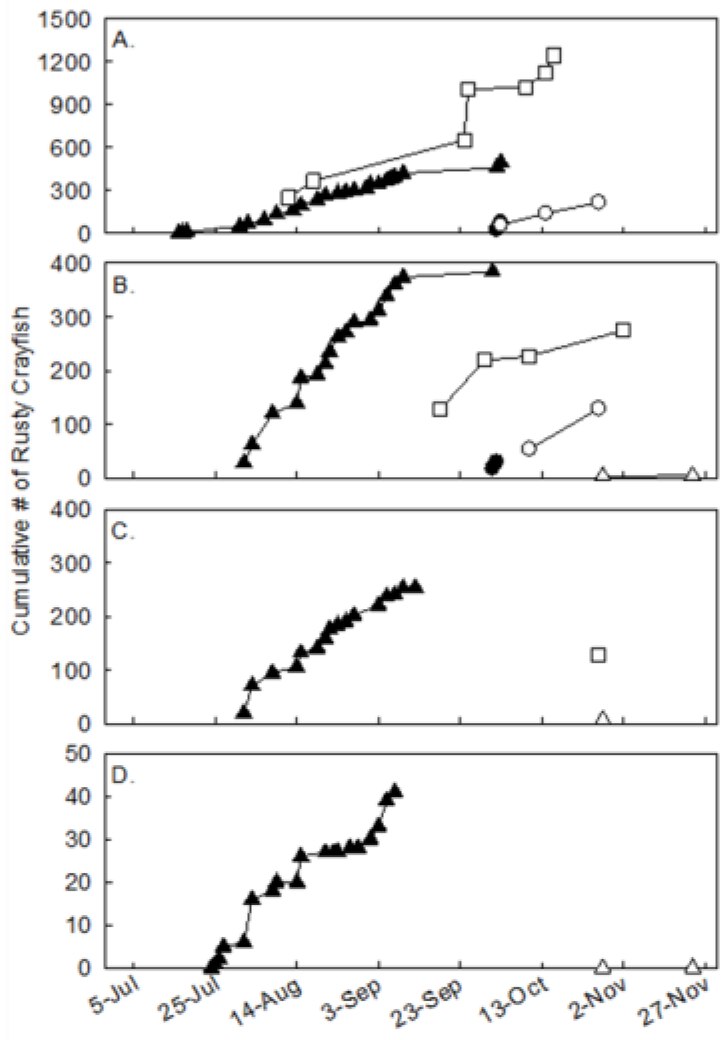


Figure 1.2. Cumulative number of rusty crayfish removed in 2012 (solid symbols) and 2013 (open symbols) minnow traps (▲), tangle nets (●), and hand removal via scuba diving (■) at A.) Crib, B.) ER North, C.) ER Central, and D.) ER South. See Figure 1 for site locations.

Table 1.2. Parameter estimates from simple linear regression models of CPUE over time for 2012 crayfish removal efforts.

Site	b_1	b_0	R^2	Lower 95% CI	Upper 95% CI	t	p
Crib	0.007	-271.98	0.12	-0.002	0.014	1.62	0.12
ER North	-0.003	130.64	0.07	-0.009	0.003	-1.06	0.30
ER Central	-0.003	116.27	0.15	-0.006	0.0009	-1.60	0.13
ER South	$5.15E^{-5}$	-2.14	0.001	-0.0007	0.0008	0.15	0.88

Index monitoring

In 2012, Rusty Crayfish CPUE from baited minnow traps increased significantly over time on average across all sites ($F_{1,536}$=12.99, *P*=0.0003; Figure 1.3). There was no main effect of treatment condition (i.e., reference versus treatment) on CPUE in 2012 ($F_{1,536}$= 0.09, *P*=0.76) and no differences in trends between treatment and reference sites ($F_{1,536}$= 1.43, *P*=0.23). Index monitoring with baited minnow traps in 2013 began approximately two months later than comparable monitoring in 2012. Rusty Crayfish CPUE from index monitoring with minnow traps in 2013 did not change over time at any site ($F_{1,195}$= 0.04, *P* = 0.84), and no main effect of treatment was observed ($F_{1,195}$= 0.94, *P* = 0.45, Figure 1.4). There was also no differences in trends between treatment and reference sites ($F_{1,195}$= 0.55, *P*=0.46). 2013 index monitoring with baited cameras showed a significant decline in crayfish density over time on average across all sites ($F_{1,110}$=13.67, *P* = 0.0003; Figure 1.5). However, there was no main effect of treatment condition (i.e., reference versus treatment) on the number of Rusty Crayfish observed by cameras in 2013 ($F_{1,110}$= 1.63, *P*=0.20) and no differences in trends between treatment and reference sites ($F_{1,110}$= 0.42, *P*=0.52). Based on quadrat sampling in 2013, Rusty Crayfish densities differed across sites ($F_{1,3}$=3.7, *P* = 0.01); densities at ER North and ER Central were significantly higher than at the Crib and Bay Harbor. Quadrat sampling indicates that Rusty Crayfish were still present on the reefs in late November at densities similar to those observed at the start of monitoring (Figure 1.6).

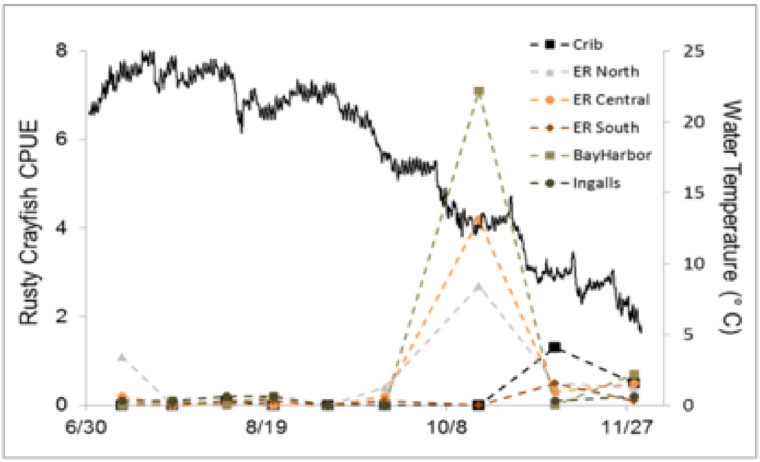


Figure 1.3. Mean rusty crayfish CPUE from bi-weekly index monitoring with baited minnow traps in 2012. Solid line is water temperature averaged across five of the six sites (Crib, ER Central, ER South, Bay Harbor, and Ingalls).

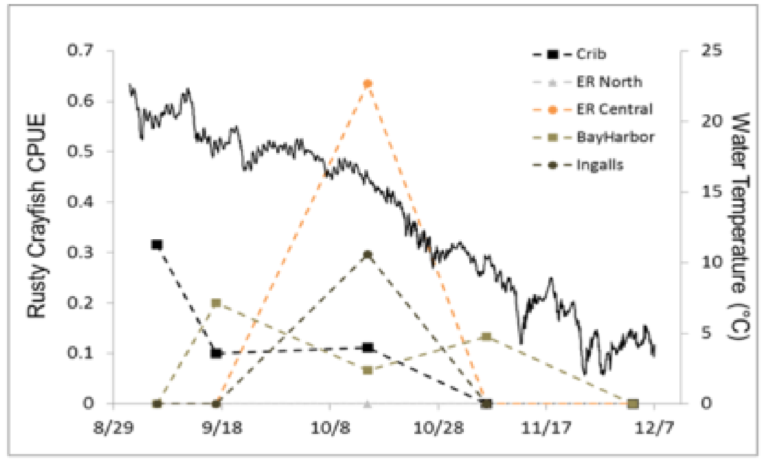


Figure 1.4. Mean Rusty crayfish CPUE from bi-weekly index monitoring with baited minnow traps in 2013. Solid line is water temperature averaged across four of the five sites (GTB North, GTB Central, GTB South, and Ingalls).

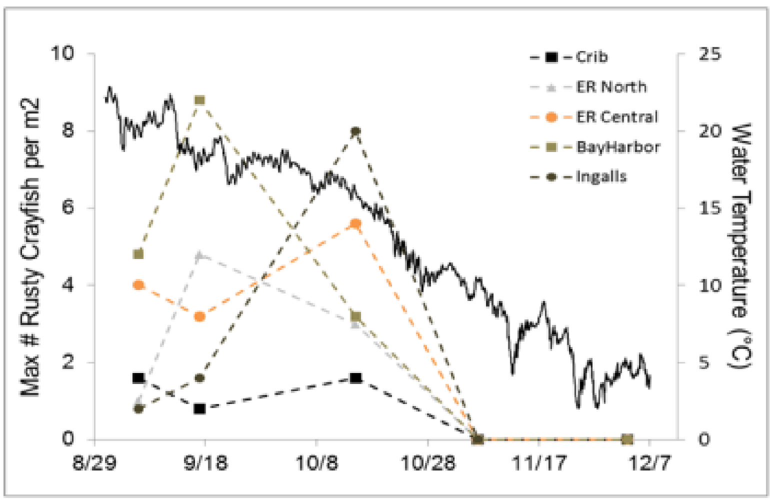


Figure 1.5. Maximum number of Rusty crayfish per square meter from index monitoring with baited camera frames in 2013. Solid line is water temperature averaged across four of the five sites (GTB North, GTB Central, GTB South, and Ingalls).

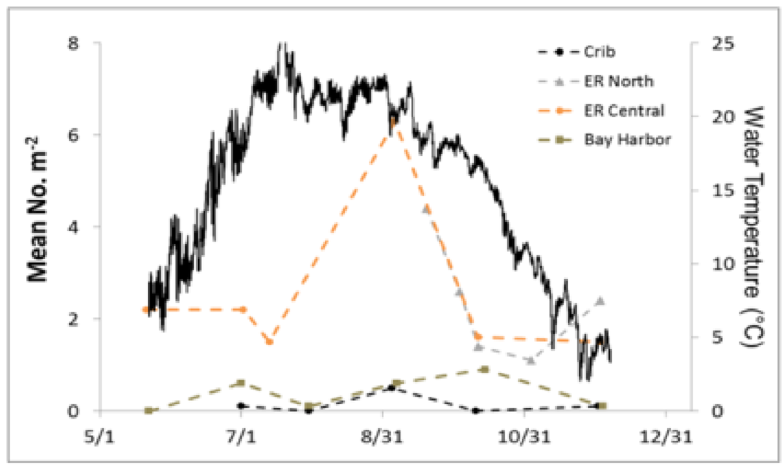


Figure 1.6. Mean number of rusty crayfish per square meter from index monitoring with quadrat sampling in 2013. ER South and Ingalls sites are not plotted as quadrat sampling at these locations was limited to just one (ER South) or two (Ingalls) events. Solid line is water temperature averaged across four of the five sites (GTB North, GTB Central, GTB South, and Ingalls).

Plots of CPUE across 3, 6, and 9m depths from minnow traps in 2012 and 2013 and baited cameras in 2013 show no evidence of a seasonal pattern of migration from shallow to deep or vice versa (Appendix Figures A1.2, A1.3, A1.4). When seasonal peaks in CPUE did occur, they tended to occur across all depths simultaneously (e.g., Appendix Figure A1.2).

Based on biweekly egg bag monitoring, Rusty Crayfish interstitial densities differed among sites ($F_{5,649}$= 10.99, P < 0.001), with ER North and Ingalls having higher densities than Bay Harbor, ER South, and the Crib. Densities declined significantly over time across all sites ($F_{1,649}$= 2.19, P <0.001. Average relative densities were lowest during the week of September 17 (0.06 crayfish· egg bag^{-1} ± 0.05 SE) and increased the next month (to 0.35 crayfish· egg bag^{-1} ± 0.17 SE by October 16) and declined again by the end of sampling in December (0.08 crayfish ·egg bag^{-1} ± 0.06 SE; Fig 1.7). Declining trends were not consistent across sites though, as there was a significant interaction between time and site ($F_{5,649}$= 4.75, P < 0.001): the slope over time for ER South was different (more increasing) than the slopes for Ingalls, ER Central, and ER North. Relative densities from MDNR deployed egg bags were greater than those from egg bags deployed for bi-weekly monitoring in 2012, (Fig. 1.8). Densities of Rusty Crayfish in MDNR egg bags were higher in November than in December for the majority of sites in 2012 and 2013.

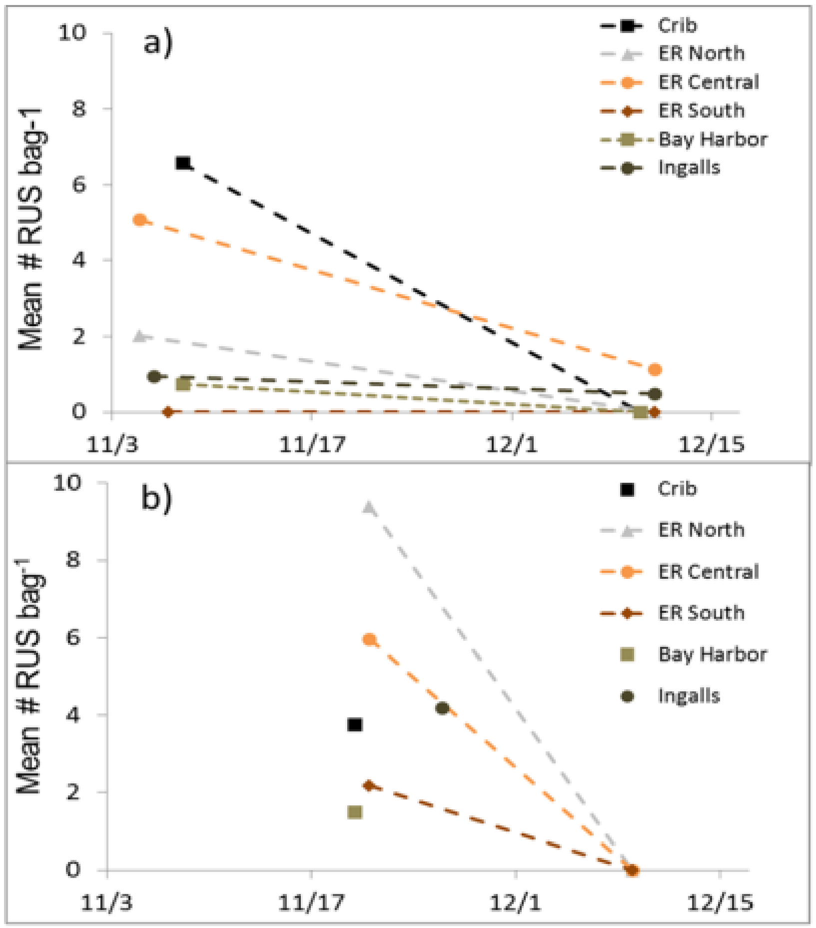


Figure 1.8. Mean Catch (#RUS bag-1) from egg bags deployed by MI DNR on each reef in 2012 (a) and 2013 (b). Adverse weather conditions precluded collection of egg bags in December 2013.

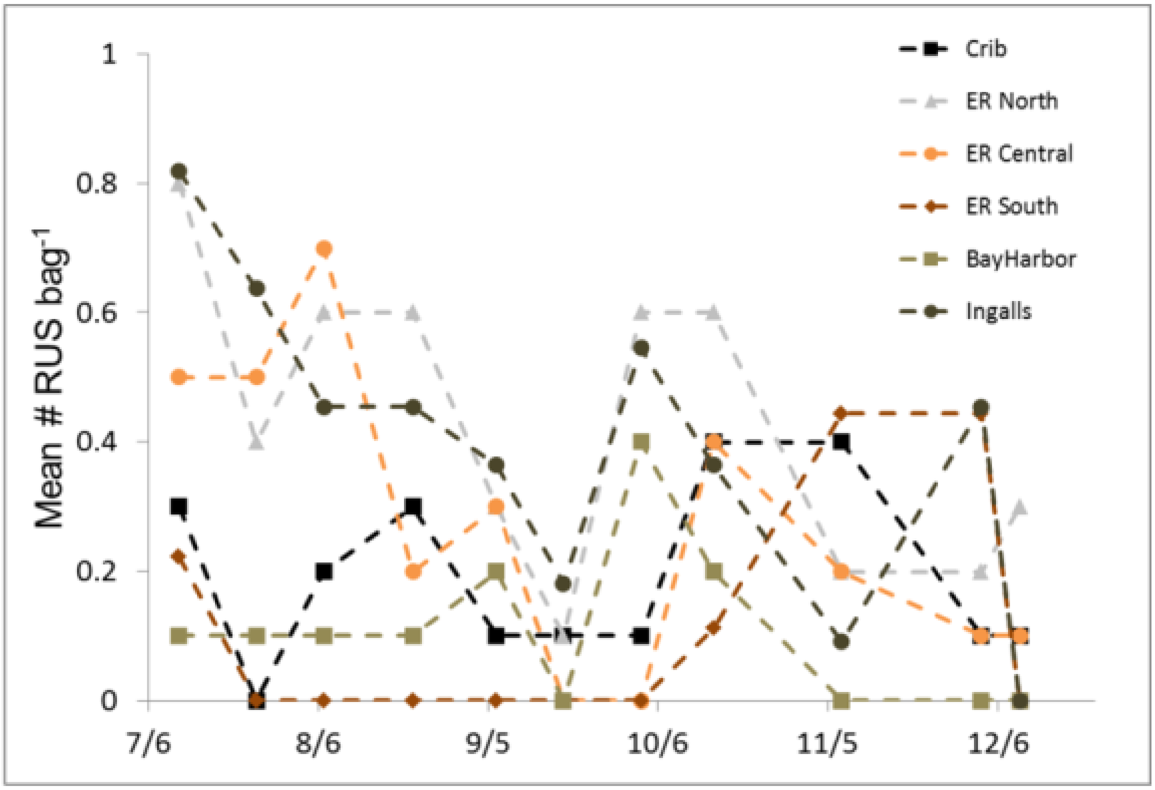


Figure 1.7. Mean catch (#RUS bag-1) from egg bags deployed for bi-weekly sampling on each reef in 2012.

Size Structure and Sex Ratios

Male: Female sex ratios declined significantly over the course of removal at three of the four removal sites in 2012 (Table 1.3, Figure 1.9). In 2013, no significant changes in sex ratios were observed. Mean length of captured Rusty Crayfish did not change significantly over time at any site in 2012, except ER North, where crayfish size decreased significantly (t= -3.43, $P = 0.0007$). In 2013, mean Rusty Crayfish length increased significantly at all sites over the course of removal (Table 1.4, Fig. 1.10). After blocking for sex, mean size of crayfish was significantly smaller in 2013 than in 2012 at all sites (Crib, $F_1 = 931.24$, $P < 0.0001$; ER North, $F_1 = 31.54$, $P < 0.0001$; and ER Central, $F_1 = 38.27$, $P < 0.0001$).

Table 1.3. Parameter estimates from simple linear regression models of M:F sex ratio over time for 2012 and 2013 crayfish removal efforts.

Year	Site	b_1	b_0	R^2	Lower 95% CI	Upper 95% CI	t	p
2012	Crib	-0.002	87.29	0.09	-0.007	0.003	-0.84	0.42
2012	ER North	-0.007	286.98	0.86	-0.010	-0.004	-6.05	0.00
2012	ER Central	-0.006	259.29	0.58	-0.012	-0.0002	-2.65	0.05
2012	ER South	-0.017	695.01	0.75	-0.027	-0.007	-4.28	0.01
2013	Crib	-0.000	30.31	0.16	-0.002	0.001	-1.06	0.33
2013	ER North	-0.007	283.15	0.40	-0.018	-0.005	-1.62	0.18
2013	ER Central	0.009	-373.98	0.50	-0.106	0.124	1.00	0.50

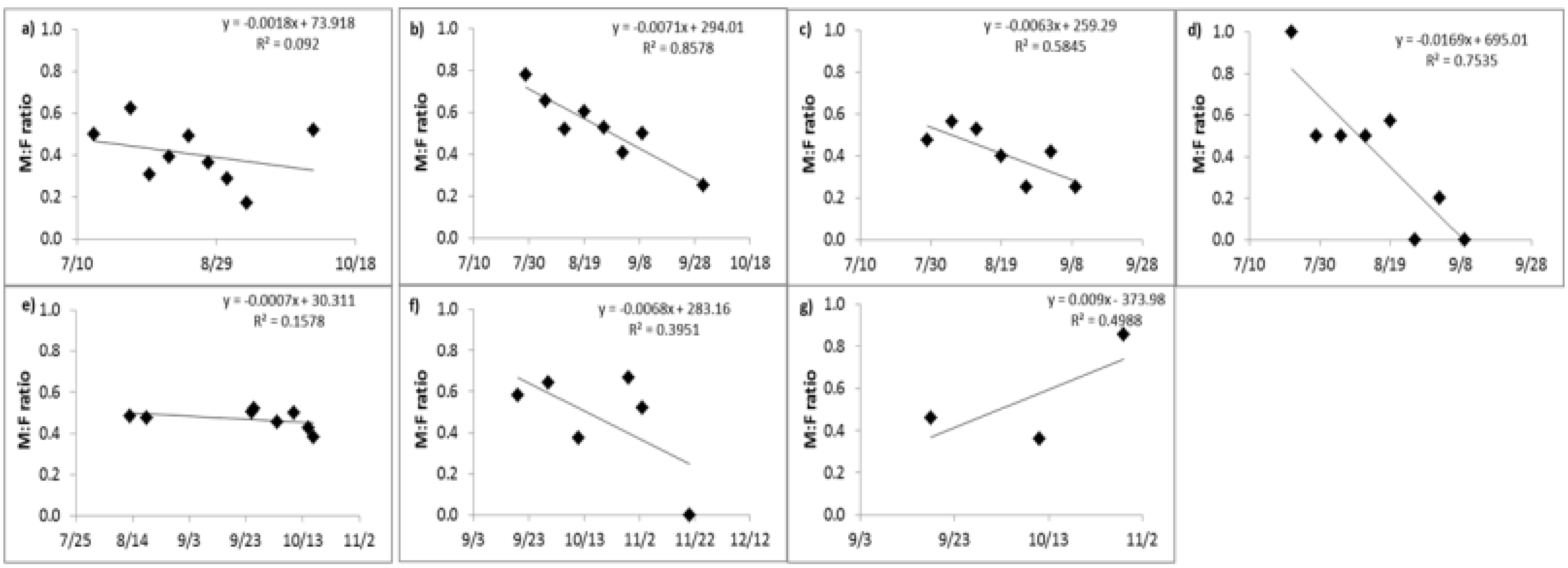


Figure 1.9. Regression models for sex ratios of captured Rusty crayfish over the course of removal efforts in 2012 (panels a-d) and 2013 (panels e-g). Crib (a,e), ER North (b,f), ER Central (c,g), and ER South (d).

Table 1.4. Parameter estimates from simple linear regression models of crayfish carapace length (mm) over time for 2012 & 2013 crayfish removal efforts.

Year	Site	b_1	b_0	R^2	Lower 95% CI	Upper 95% CI	t	p
2012	Crib	-0.01	552.6	0.002	-0.04	-0.01	-1.02	0.31
2012	ER North	-0.07	2938.5	0.03	-0.11	-0.03	-3.43	0.0007
2012	ER Central	-0.04	1466.1	0.009	-0.08	0.008	-1.59	0.11
2012	ER South	0.016	-633.2	0.0009	-0.16	0.19	0.19	0.85
2013	Crib	0.01	-428.5	0.002	-0.0008	0.02	1.81	0.07
2013	ER North	0.06	-2274.5	0.019	0.009	0.1	2.33	0.02
2013	ER Central	0.12	-4801.0	0.04	0.03	0.20	2.68	0.008

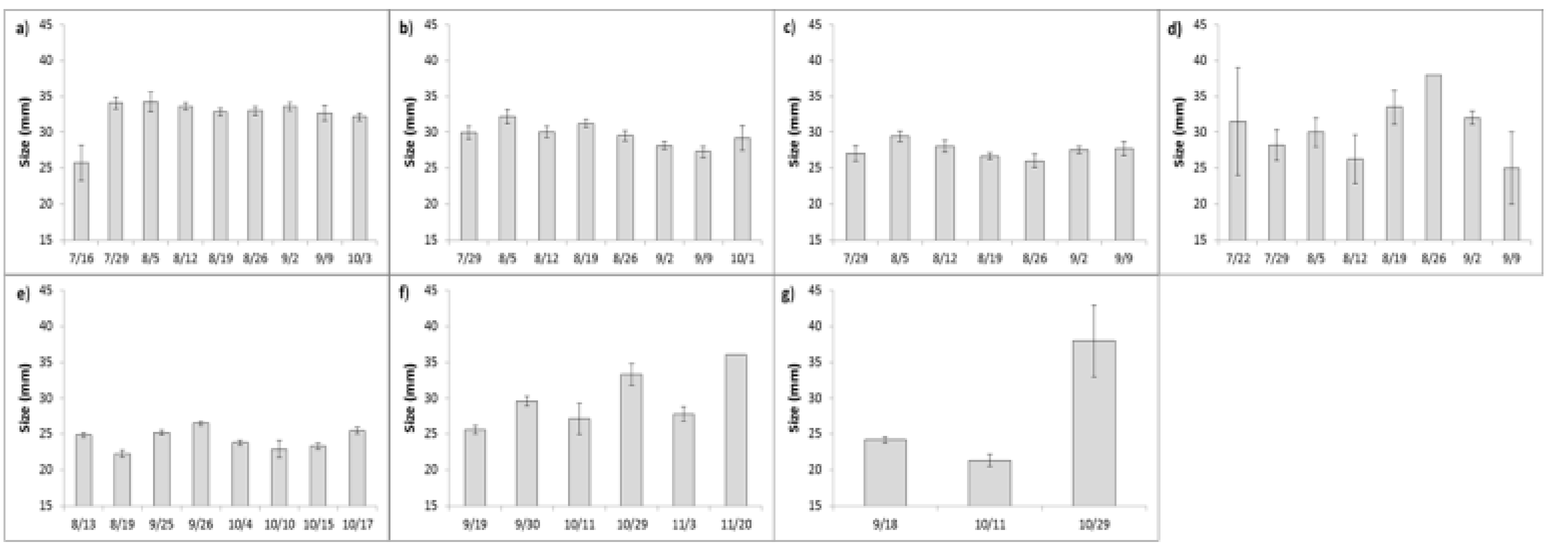


Figure 1.10. Mean crayfish size (± SE) for captured rusty crayfish over the course of removal efforts in 2012 (panels a-d) and 2013 (panels e-g). Crib (a,e), ER North (b,f), ER Central (c,g), and ER South (d).

DISCUSSION

Our efforts, spanning nearly six months in each year of the project period (2012 & 2013), resulted in removal of almost 4000 Rusty Crayfish from native fish spawning reefs. However, neither catch rate data from removal efforts nor CPUE data from repeated index monitoring suggest that we caused a significant reduction in crayfish densities during the critical native fish spawning period. Marginal declines in catch rates were observed at some locations over the course of our removal efforts and minor changes in CPUE for index monitoring were observed, but any differences in CPUE were not attributed to treatment. Our data suggest that sub-optimal capture of resident Rusty Crayfish on reefs and especially recolonization of reefs from neighboring habitats are the primary reasons that we did not successfully reduce and sustain low densities of crayfish. However, we feel confident that optimization of the timing of removal efforts and implementation of improved capture methods, in conjunction with barrier deployments to prevent reef recolonization could result in the successful removal of Rusty Crayfish from spawning reefs.

Based especially on the success of Hein et al. (2006) who reduced numbers, size frequency, and biomass of Rusty Crayfish in a Wisconsin inland lake through intensive trapping using baited gee minnow traps, we anticipated that we could induce temporary but substantial reductions in invasive crayfish abundance on our study reefs. However, despite a comparable level of trapping effort we were unable to achieve our targeted crayfish control goals. In 2012, the total number of trap days (where a trap day is equal to one trap fished for 24 hours) from our study was 5901, which exceeds trapping effort in the first two years of the Wisconsin study and is equal to about 70% of the mean trapping effort over the course of that study (Table 1.4). Nevertheless, our trapping efforts resulted in a catch per unit effort in 2012 that was less than 5% of the mean CPUE from the Wisconsin study (i.e., 0.20 vs. 4.43 CPUE). The discrepancy doesn't appear to be a result of differences in crayfish densities between the two systems. Rusty Crayfish density for Sparkling Lake was estimated at approximately 1 Rusty Crayfish m^{-2} in

Table 1.4. Comparison of crayfish capture with minnow traps from Hein et al. 2006 (Sparkling Lake WI) and our study (2012 only). In 2013 we primarily deployed tangle nets and divers to remove crayfish, which prevents a direct comparison to Hein et al. 2006 of trap days and CPUE. O.r. is non-native Orconectes rusticus, O.v. is native Orconectes virilis. A trap day is one trap fished for 24 hours.

	Hein et al. 2006					**Our study**
Year	2001	2002	2003	2004	2005	2012
Trap days	1584	3497	7432	13984	14011	5901
# female O.r.	3570	17567	10691	4120	2472	627
# male O.r.	7425	20983	11894	4978	4562	543
Total # O.r.	10995	38550	22585	9098	7034	1181
CPUE O.r	6.94	11.02	3.04	0.65	0.5	0.2
Per cent male	67.5	54.4	52.7	54.7	64.8	46
Total # O.v.	24	16	23	23	132	69
CPUE O.v.	0.0152	0.0046	0.0031	0.0016	0.0094	0.0117

2003, which is comparable to the mean density of Rusty Crayfish on our study reefs (see Fig 1.6; mean density across all sites = 1.3 crayfish m^{-2}).

The disparity in crayfish capture rates between the Wisconsin study and our study is likely a result of differences in crayfish ‘catchability’ (related primarily to timing of trapping efforts) and, differences in trap entrance sizes It is possible that some of the traps that we deployed were more prone to lose or exclude crayfish relative to traps used in the Wisconsin study. We deployed trap lines consisting of alternating small (3 cm) and large (6 cm) entrance traps, whereas Hein (et al. 2006) deployed wire minnow traps with the smaller entrance only (3.5 cm diameter). Two of the major criticisms that have been levied against wire minnow traps for crayfish capture are the high frequency of escapees and the tendency for captured crayfish to exclude uncaptured crayfish from baited traps (Kozak & Policar 2003, Ogle and Kret 2008). It is conceivable that our large opening traps were both easier to escape from and able to accommodate the larger crayfish that are most successful at excluding uncaptured crayfish. Importantly, the difference in Rusty Crayfish size that we observed at all sites across sample years (Fig. 1.10) may have been driven by our use of divers and tangle nets in 2013 instead of the more size selective minnow traps. Hein et al. (2007) fished traps for 1 to 4 days in the last two years of their study after noting a linear increase in numbers of fish per trap up to three days, whereas our catch rates from 48 hour sets in 2012 were much lower.

Variation in Rusty Crayfish catchability between the two systems, possibly arising from differences in timing of crayfish life history events between Great Lakes and inland lakes crayfish, is probably the more important driver of dissimilarity in overall CPUE between the two studies. Hein et al. (2006) generally deployed traps from late June to late August, which corresponded with the warm water temperatures during which Rusty Crayfish activity (and thus catch rates) were maximized. This trapping period also maximized female capture, because it occurred at a time when females had already released their young but had not yet molted and were thus active in the lake. Removal efforts in our study generally began in early to mid-July and continued into the second week of September in 2012, a period similar to that of Hein et al. (2006) and during which water temperature on the reefs was presumably optimal to maximize catch rates (i.e. 20-25 °C). However, index monitoring activities from our study in 2012 showed that peak Rusty Crayfish capture occurred later in the season in the Great Lakes reef habitat (e.g., Figure 1.3; early to mid-October at most sites). Rusty Crayfish in the Great Lakes likely mate and/or molt later in the season than in inland lakes (like Sparkling Lake), similar to many fish species (e.g., smallmouth bass) that spawn later in Great Lakes waters (Becker 1983). During removal in 2012, the male to female sex ratio declined at most sites (i.e., relative to the number of males captured, female capture increased over the course of removal; Fig. 1.9). This pattern would be consistent with a period of reduced female activity in early to mid-summer (when capture in Sparkling Lake was optimal), during which females may retreat into the cobble to molt. As a result, our removal efforts in 2012 probably ended before the peak in both male and female activity on the reef and thus under sampled females early in the season.

We modified our removal methods for 2013 with the aim of removing Rusty Crayfish during the period of peak Rusty Crayfish activity, immediately prior to spawning and when CPUE could be maximized. Thus in 2013, we planned to intensively trap the reefs during this period and prior to this we used tangle nets and hand removal by divers on two smaller reefs so we could measure recolonization, and because these methods should either be less affected by low crayfish activity, or in the case of tangle nets could be left set for extended periods. However, early storm events in October prevented trap teams getting onto the water and in the end our removal efforts were almost exclusively by divers and tangle nets. The use of capture methods other than minnow trapping precludes a direct comparison of catch efficiency between our efforts in 2012 vs. 2013 or between 2013 and the Hein et al. (2006) study. However, we captured substantially more Rusty Crayfish in 2013 than in 2012 over a shorter period of time and with fewer removal events, which suggests a substantial improvement in our catch per unit effort. For example, at the Crib site we captured just 559 crayfish during 23 sampling events over the course of almost three months in 2012, as compared with 2013 when we removed 2189 crayfish in just 8 removal events spanning approximately one month (Figure 1.11). Despite the improvement, we were still unable to induce significant declines in or sustain low levels of crayfish densities on the treatment reefs.

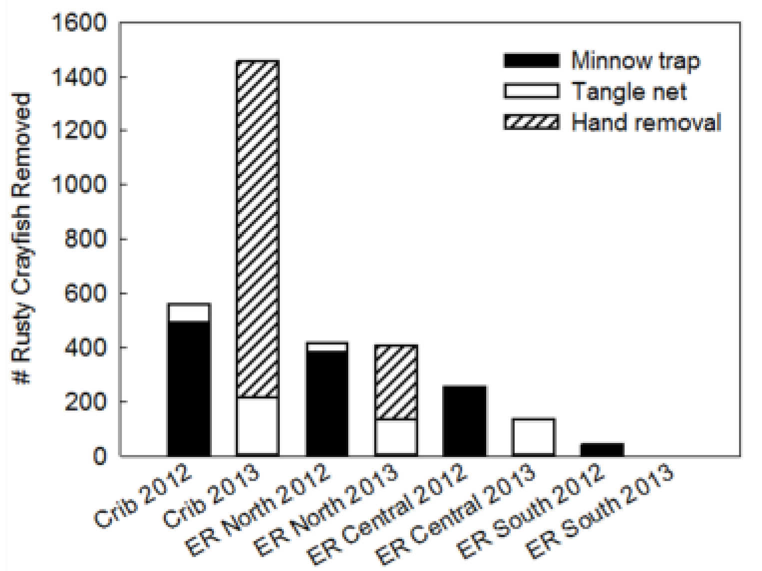


Figure 1.11. Number of rusty crayfish removed in 2012 and 2013 by minnow traps (solid bars), tangle nets (open bars), and hand removal via scuba diving (hatched bars) at the Crib, ER North, ER Central, and ER South.

We contend that sustained recolonization of the spawning reefs by crayfish from adjacent habitat is the most significant reason that we were unable to reduce crayfish densities on our treatment reefs. There does not appear to be evidence to suggest a significant migration of Rusty Crayfish onto or off of the spawning reef (e.g., Appendix Figures A1.2, A1.3, & A1.4). Instead, we suspect that Rusty Crayfish on these reefs are generally resident year-round, that they occupy transient home ranges (Buckley *in prep*) and immigration to the reef occurs in a density-dependent fashion, with Rusty Crayfish moving onto the reef from adjacent habitat to take advantage of available space and food resources, as a result of reduced density. It has been postulated that animals, including crayfish, that display intraspecific aggressive behaviors and territoriality will disperse in a density related fashion. Both laboratory and field studies of crayfish have demonstrated that the density of conspecifics can initiate dispersal, where crowding is a sub-optimal situation from which there is a shift (Bovbjerg 1959, Fast & Momot 1973). If our removal efforts were sufficient to alter the relative densities of

Rusty Crayfish in removal versus adjacent non-removal areas, these studies suggest that Rusty Crayfish at our study sites may have been immigrating into removal areas along a density gradient.

More recent studies have tested the specific hypothesis that manual removal of crayfish can facilitate ingress of individuals into the removal site. For example, removal of signal crayfish (*Pacifastacus leniusculus*) in a U.K. river did not increase the number of individuals immigrating into the removal area but it did result in an increase in the movement distances (up to 239 m) of large crayfish immigrating from non-removal areas (Moorehouse & MacDonald 2011a). The implication for control of crayfish in a non-enclosed habitat is that the extent of the population from which relatively large individuals are preferentially removed will extend at least 200 m out from the removal area. The finding that larger individuals actively recolonize removal areas may also explain why we were unable to affect any change in size distribution on most of our treatment reefs in 2012 and could perhaps explain why in 2013 crayfish size actually increased over the course of our removal efforts (Table 1.4). Thus the difference in Rusty Crayfish size among years was probably the result of our modified removal methods and the ability of divers to capture a full range of size classes whereas the minnow traps are known to select for larger individuals (Hein et al. 2007 refs- Momot & Gowing, 1977; Lodge, Beckel & Magnuson,1985; Rach & Bills, 1989; Momot, 1993). In a related study, signal crayfish movement decreased inside removal areas, presumably as either (or both) a direct response to decreasing population density (which the authors suggest sufficiently diminished the 'dispersal impulse') or as a result of an increased availability of food and shelter (Moorehouse & MacDonald 2011b). Changes in the availability of food and shelter have been postulated as factors that could alter movement distances of crayfish (Capelli & Hamilton 1984). For example, crayfish tend to be overrepresented in cobble habitat relative to its availability, presumably because it provides shelter from fish predation and increases food availability (Flint & Goldman 1977, Kershner & Lodge 1995). In turn, the availability of cobble habitat can directly affect crayfish dispersal rates (Perry et al. 2001). These studies suggest that if removal efforts successfully freed up space in the reef cobble, Rusty Crayfish may have actively emigrated away from outlying sand or compacted cobble habitat to take advantage of preferred cobble substrates on the reef.

There are at least two important implications of our findings. First, seasonal peaks in catchability (possibly related to life history traits) suggest that there is a relatively narrow window within which crayfish capture is most likely to be effective. Optimization of control methods for invasive species using life-history information (e.g., timing and duration of mating season) can increase the probability that techniques will be effective to reduce impacts of nuisance species (Rogowski et al. 2013). In our study, we were able to increase removal efficacy on the treatment reefs from year 1 to year 2 by focusing our efforts later in the season when both males and females are active on the reef (late September to late October). Future removal efforts should aim to intensively control Rusty Crayfish during this time period and employ a method that minimizes sampling bias for large male crayfish (e.g., hand removal by divers, or improved traps), but may also need to be initiated earlier as a contingency against early fall storm events that can drastically impede removal efforts as we saw in 2013.

The second implication is that the existence of a resident crayfish population on each spawning reef that is effectively subsidized by adjacent populations of Rusty Crayfish, means that removal efforts will be ineffective unless removal rates exceed rates of re-colonization (Myer et al 2000). Re-colonization rates could be slowed (or perhaps even stopped) in at least two ways, 1) by trapping a large buffer area surrounding the reef, or 2) by deploying a benthic barrier around the perimeter of the reef to block crayfish passage. The former option is not likely to be viable in a management context where crayfish control is desired at numerous locations but personnel and financial resources are limited. We employed three full time technicians to intensively capture and remove crayfish on just three reefs, each with an associated buffer area of only 5 to 10 meters, and our efforts were insufficient to overcome the effects of re-colonization. As noted previously, removal may induce immigration by crayfish at least 200 m beyond the extent of the removal area (Moorehouse & MacDonald 2011a), which suggests that removal efforts at the scale needed to create an effective buffer may be intractable. The latter option (i.e., a benthic barrier) could be a relatively low-cost solution to slow or prevent recolonization to the reef and allow for a temporary reduction in crayfish abundance, especially on smaller patch reefs like the Crib or ER North. Barrier design would require careful consideration of potential non-target impacts and escape or avoidance behaviors of Rusty Crayfish. But a barrier that adequately conforms to the lake bottom to prevent passage underneath and that presents an effective obstacle to impede passage over or around could be an effective deterrent to crayfish immigration. When combined with trapping efforts timed to coincide with periods of optimal Rusty Crayfish catchability, effective Rusty Crayfish control on native fish spawning reefs could be achieved.

Rusty crayfish trapped in a tangle net, Little Traverse Bay, October 2012. MI DNR

<u>Objective 2</u>. Experimentally quantify the lethality, effective control range (radius and interstitial depth) and suppression duration of seismic guns on goby and Rusty Crayfish.

A targeted and integrated approach to the control of invasive species has the potential to successfully reduce impacts on native species during critical life history stages (Myers et al. 2000). Underwater sound sources have been explored in recent years as a tool to control and eradicate aquatic nuisance species and can be deployed as part of an integrated approach. Underwater sounds have the potential to directly injure fish such as lethal effects seen with underwater explosives. Other research has evaluated responses of fish associated with seismic surveying for natural gas and oil resources. Endpoints include both lethal and sub-lethal effects of pulse pressure from dynamite, pile driving and air guns such as ruptured gas bladders and kidneys, soft tissue hemorrhaging and hematomas in kidneys, livers, muscle and other tissues, and behavioral responses such as altered swimming and schooling density (Stephenson et al 2010, Yelverton 1975, Casper 2013). Recently, sound technologies such as pulse pressure emitted from seismic water guns have been evaluated as a control tool for invasive Lake Trout, Bighead and Silver Carp, and Northern Pike (Gross et al. 2013)

There are two main mechanisms by which changes in pressure can cause barotrauma: mechanical and decompression (Carlson 2012). Both pathways are a result of changes in the state (free or within a solution) and the expansion of a volume of gas within structures (Carlson, 2012). The pathways are governed by two laws: Boyle's law, where injury results from the expansion of preexisting gas within the body (mechanical), and Henry's law, where gas comes out of blood and tissue solutions due to decompression, causing bubble formations (decompression) (Brown, 2012). Though Round Goby and Rusty Crayfish lack gas bladders, we hypothesized that physical sound energy emitted from a water gun would have both physical and behavioral effects caused by high pressure water expulsion and decompression by cavitation and fast moving sound waves. Furthermore, with repeated exposure to sound energy, we expected physical and behavioral responses would also increase. Avoidance behaviors in Rusty Crayfish and Round Goby constitute seeking cover within a cobble reef versus swimming away from an undesirable area. It was this shelter-seeking behavior (i.e., not swimming away) that gave promise for the use of pulse pressure from a water gun, as we could potentially use a towed gun to treat a large area on and around the spawning reefs and expose resident fish to multiple treatments.

METHODS

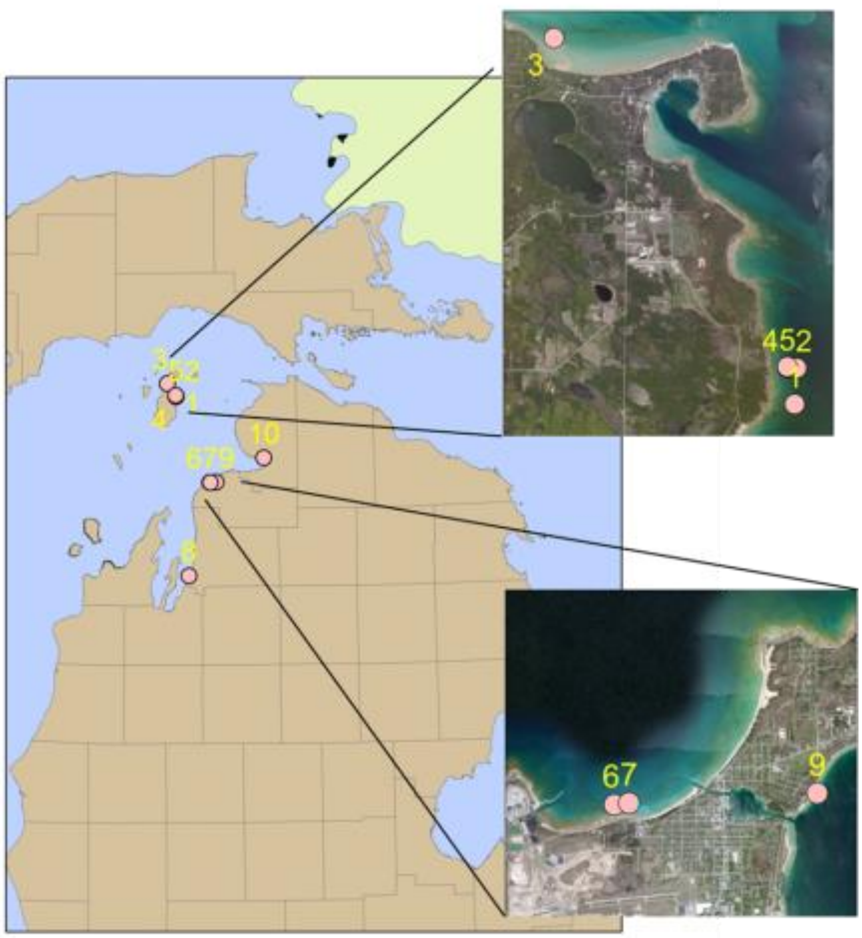
Mortality and Growth Experiments

A series of experiments was conducted between July and October 2013 to evaluate the potential lethal effects of pulse pressure sound emitted from a seismic water gun on Round Gobyand Rusty Crayfish. Two different sets of experiments were completed (July experiments and September/October experiments), examining the effect of pulse pressure on target organisms at different depths and distances from the sound source, over different substrates, and number of pulses. The experiments conducted in July (Table 1) used a 410-cm^3 water gun whereas the experiments in September/October (Table 2) used a 1966-cm^3 water gun. Experiments conducted in September/October included a native reef fish, the Rock Bass as a non-target control and evaluated the potential effects on Round Gobyhearing cells and ear bones (otoliths hereafter).

Study Sites

Experiments in July 2013 were conducted on sand and cobble habitats (sites 1-5) around Beaver Island, Lake Michigan (Fig. 2.1), whereas experiments in September/October were conducted over cobble reef habitats just offshore of Charlevoix, Michigan (sites 6-9).

Figure 2.1. Study sites utilized in Lake Michigan**.**



Study Animals

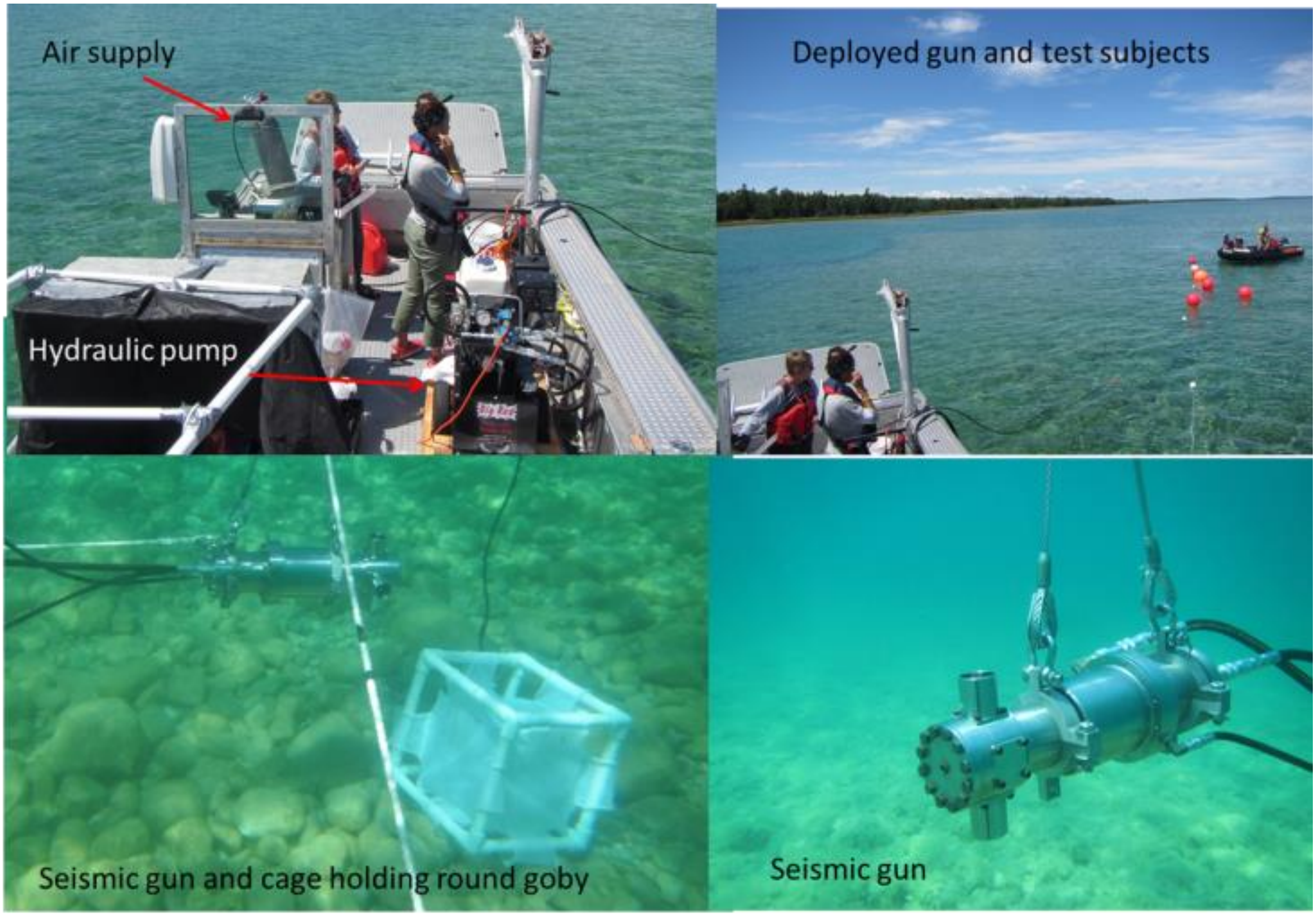
In July 2013, Rusty Crayfish and Round Gobywere caught using baited minnow traps placed on the lake bottom in water <5 meters deep around Little Traverse Bay. Traps were retrieved within 24 hours, and fish and crayfish were transferred to the United States Coast Guard Station, Charlevoix, Michigan, by boat under the authority of MDNR. To abate stress caused during transportation, animals were maintained in 0.48 m wide x 0.53 m long x 2 m deep fish troughs and transferred by boat to the Central Michigan University Biological Station on Beaver Island and held in (4) 1136 L flow-through circular tanks. Animals for the study in July were transported via the research vessel Steelhead and transferred to 4.3m^2 (1m deep) mesocosms that possessed a flow-through plumbing system that drew water from Lake Michigan.

In September 2013, Rusty Crayfish and Round Gobywere captured by the same method described above and held in (2) 378 L troughs with flow-through lake water at the US Coast Guard Station, Charlevoix, Michigan. Rock Bass were captured by fyke nets and held in the same manner as Round Gobyand Rusty Crayfish.

In both experiments, approximately ten days prior to treatment, study fish (Round Gobyin July, Round Gobyand Rock Bass in September/October) were anaesthetized with Tricaine methanesulfonate (MS-222) in groups of ten and monitored until stage-4 anesthesia was reached (i.e., total loss of gasping motion, weak opercular movement as defined by Yoshikawa et al., 1988). Fish were then removed one at a time, sized to the nearest millimeter, and all fish greater than 70 mm were tagged for individual identification with 8 mm Passive Integrated Transponder (PIT) (Biomark, Boise, Idaho) tags inserted sub-dermally in the ventral region above the anus. Fish were then placed in circular tanks and monitored during recovery. Rusty Crayfish were individually removed from the holding tank; the carapace was measured to the nearest millimeter and marked using either a waterproof marker or tail fin clips. Weight and sex of each Rusty Crayfish were also recorded. Only animals that appeared healthy with no obvious signs of external trauma or abnormal swimming behaviors were used in experiments. Unhealthy animals were euthanized with a lethal dose of MS-222 and disposed of according to MDNR protocols. All animals were handled equally including sedation, tagging and transportation to control. Animals were fed salmon pellets starting one week prior to testing.

Water gun deployment

The water guns were lowered into the water from a boat by davit and a rope line was set and anchored to maintain the position and horizontal orientation of the water gun throughout each experiment. The July experiments used the 410-cm^3 water gun and was operated manually using a hydraulic directional control valve and a 5 gallon per minute (gpm) hydraulic pump rated for 3000 PSI pressure. For experiments conducted in the fall, a 1966-cm^3 water gun was operated manually using a hydraulic directional control valve and a 28 gpm hydraulic pump rated for 2800 PSI pressure. Operating pressure was maintained at a constant pressure throughout each experiment by a 45L, 2,400 PSI nitrogen gas cylinder.



Seismic Gun deployment – Beaver Island July 2013

Animal exposures

Study animals were monitored and maintained in aerated coolers on an aluminum boat until time of exposure. While on the boat, coolers were filled with lake water, and water temperature and dissolved oxygen (DO) levels were monitored to ensure water quality remained consistent with lake water parameters. Prior to each exposure, animals were identified by PIT tags or markings, placed in cages deployed at different distances from the gun and exposed to different numbers of pulse pressure discharges. The lateral distance for each trap was determined by measuring the point at which the sound pressure was reduced by half, 6 dB sound pressure level (SPL_{peak}) from the previous point. Buoy markers were set along a static line to ensure the locations of cages remained consistent between trials. Exposure cages were 0.3 m^3 framed by 5 cm diameter polyvinyl chloride (PVC) pipe covered with 6 mm nylon mesh. Holes were drilled into the PVC frame to ensure cages sank to the desired depth. Exposure trials were conducted using a randomized block design. Each replicated block would have a randomized order for each treatment (i.e. distances 1-4 plus control) which were pre-assigned. At the completion of each block of treatments (a replicate), the new treatments were randomly re-ordered and exposed. For each experiment, an equal number of control animals (fish and crayfish) were held to evaluate any potential effects of transport stress.

Immediately following all exposures, animals were retrieved and transferred to a separate holding cooler. At the end of each day's trials, the exposed study fish from that trial were transferred into a single 1,136 L holding tank (mean temperature 16.7ºC in September/October trials, temperature data not available for July) at the CMU Biological Station (in July) or the USCG Station (in September/October) and monitored daily for 7-14 days depending on the trial. All post-exposure fish were fed ad libitum. In both experiments, all mortalities and molted Rusty Crayfish exoskeletons were collected. Fish were examined for barotrauma; size, physical damage and identifications recorded. At the completion of the holding period (listed in the last column of Table 2.1), all animals were euthanized and data collected as described above. All fish in the 28 and 30 September and 7 October experiments had heads removed for later otolith analysis and their tag number was recorded.

In the July experiments, three statistically distinct size classes of Rusty Crayfish were submerged at two different depths at varying distances from the 410-cm^3 gun and exposed to varying numbers of gun discharges. Rusty Crayfish were placed near rocky reef substrates at sites 1 and 5 (Figure 2.1). Three statistically distinct size classes of Round Goby were submerged at three different depths at varying distances from the 410-cm^3 gun and exposed to varying numbers of gun discharges. Round Goby were placed near both rocky and sandy substrates at sites 2, 3 and 4 (Figure 1). All animals were individually exposed (i.e., a single animal was placed in a cage, lowered and exposed to sound pressure at one depth or distance at a time).

In the September/October experiments, Rusty Crayfish, Round Goby and Rock Bass were submerged at slightly different depths than for the July experiments and at varying distances (refer to Table 2) from the 1966-cm^3 gun and exposed to varying numbers of gun discharges. Rusty Crayfish and Rock Bass were placed near rocky substrates, and Round Goby were placed near both rocky and sandy substrates. Each animal was individually exposed, with the exception of the 28 and 30 September experiments which had a single Round Goby and a single rock bass sharing an exposure cage. Details of the different variables tested throughout the duration of the July trials are shown in Table 2.1; details of the September/October experiments are shown in Table 2.2.

Table 2.1. Design for all July 2013 experiments conducted to evaluate the effects of pulse pressure on round goby and rusty crayfish.

Animals	# of pulses	Cage depth (m) from surface	Cage lateral dist. (m) from gun	(Site #)	# of reps (individuals per treatment)	Total # of animals treated	# of distinct size classes (mean length, mm)	Days held post-exp.
Rusty crayfish	0,3	3.7	1,3,6,12	Rocky (Site 1)	14 (n=6)	84	3 (21, 26, 29)	14
Round goby	0,3	3	1,3,6,12	Rocky (Site 2)	15 (n=6)	90	3 (82, 89, 98)	14
Round goby	0,2	4	3,6,12,24	Sandy (Site 3)	15 (n=6)	90	3 (81, 89, 99)	7
Round goby	0,6,12,24	1.7	0,2,5	Rocky (Site 4)	10 (n=11)	110	1 (N/A)	7
Rusty crayfish	0,3,6,12,24	2	3	Rocky (Site 5)	13 (n=6)	78	3 (18, 21, 26)	7

Table 2.2 Design for all September/October 2013 experiments beginning conducted to evaluate the effects of pulse pressure on round goby, rusty crayfish, and rock bass

Date of Exp.	Animals	# of pulses	Cage depth (m) from surface	Cage lateral dist. (m) from gun	Substrate type	# of reps (individuals per treatment)	Total # of animals treated	# Of distinct size classes	Days held post-exp.
27-Sep	Rusty crayfish	0,1,3	3	1,6	Rocky	7 (n=5)	35	1 (N/A)	10
28/30Sep	Round goby & Rock bass	0,1,3	3.3	1,3,6	Rocky	10 (n=7)	140, 70 goby and 70 bass	1 (goby N/A, bass 128)	12
7-Oct	Round goby	0,6	3.8	1	Sandy (near Charlevoix)	20 (n=20)	40	1 84	2.5

Morphological Analyses
Barotrauma assessment

Necropsies were performed to evaluate individual fish for signs of barotrauma. A cut was made parallel to the long axis of the body on the ventral surface extending anteriorly from the anus to the pericardium (Gross et al. 2013). Careful, shallow incisions were used to ensure that trauma was not induced by necropsy. Internal organs were assessed for hematomas, ruptures and any unusual appearance; presence or absence of injuries was documented for each fish. The necropsy and data recording were performed using a blind process. Necropsies were completed on all Round Goby during the July experiments only, because the necropsies demonstrated that Round Goby did not experience barotrauma as do fish with gas bladders. During the September/October experiments, there were no Round Goby mortalities, so necropsies were only performed on Rock Bass. Primary endpoints evaluated during all necropsies included body wall, liver, kidney, gas bladder (in Rock Bass) and in the September/October experiments, otoliths and hearing cells.

Otolith removal and preservation

For the experiments conducted on the 28 and 30 September, Round Goby and Rock Bass heads were removed and placed in individually-labeled zip lock bags and immediately placed in a freezer for preservation. These bags were packaged and shipped to Smith-Root in Vancouver, Washington, for analysis and placed in a freezer upon arrival.

For the 7 October Round Goby trials, fish heads were removed after necropsy and fixed in 4% paraformaldehyde for later processing. Fish heads were removed making an incision behind the operculum, and the ventral portion of the mandible was detached with parallel cuts made through the mouth towards the posterior region of the head. The prepared heads were placed in labeled Nalgene bottles with a 4% paraformaldehyde solution for adequate fixing of the hearing cells. The bottles were placed in the refrigerator and after 24 hours, the paraformaldehyde solution was removed, the tissues were rinsed once with a phosphate buffered saline (PBS) solution for one hour, and then placed in a second rinse of PBS solution. The bottles were placed on ice and shipped overnight to Smith-Root, Inc. headquarters in Vancouver, Washington, for analysis of the hearing cells. Bottles were placed in a 2.7^{o} C refrigerator upon arrival. Hearing cells were removed by surgical scissors to separate the nerve endings between the hearing cells and the brain. The tissue surrounding the saccular otoliths was removed, and otoliths were placed in individually labeled micro-centrifuge tubes with deionized (DI) water. These tubes were left for a minimum of 24 hours, and then the DI water was removed the tube was left unsealed until dry.

Otolith assessment

Saccular otolith condition was determined in blind assessments utilizing a label with a random non-repeating number. The pairs of saccular otoliths were submerged in deionized water in a petri dish and evaluated with a dissecting microscope. Otoliths were assessed for any abnormalities in shape, texture and condition. Left and right saccular otolith distinctions were only made in the experiment conducted on 7 October. For the remainder of the experiments, both otoliths were assessed but right and left ear distinctions were not recorded. Once all otoliths were assessed for an experiment, the random labels were removed and matched to the corresponding treatment.

Acoustic Exposure

A real-time monitoring system utilizing high-pressure underwater acoustic sensors was used to record output levels from the 410-cm^3 and 1,966-cm^3 hydraulic water guns. A PCB Piezotronics blast sensor was attached to fish exposure cages, and then connected to a 482C05 Signal Conditioner (PCB Piezotronics), delivering 10mA to the sensor to compensate for cable length. The signal conditioner was then connected to a 9234 CompactDAQ card and Chassis from National Instruments (signal input range of 5 to -5 Volts) with a BNC cable for signal conversion. A Panasonic laptop computer was used to log acoustic data through utilization of LabVIEW Signal Express software. Preliminary measurements were recorded from the water guns to assess output levels at different locations, and to confirm the equipment’s proper function prior to animal exposure. Testing of the equipment was conducted using a Sweep Function Generator and a PCB Piezotronics ICP Sensor Simulator.

Both the large and small water guns had output bandwidths with the majority of energy between 1 Hz to 5,000 Hz. The average peak sound pressure level (SPL_{peak}) for a single shot represents the highest pressure level the water gun emitted at the apex of the discharge. Table 3 presents the SPL_{peak} for the two water guns in relation to slant distance (z coordinate) at various lateral distances from the water gun. The power spectral density (PSD) graph was used to yield the dominant frequencies that comprised the pressure emitted. The $SPL_{peak,}$ and the PSD were derived post-experiment from the logged data using Aquatic Acoustic Metrics Interface (AAMI) software. For more detailed acoustic information on each water gun, including SPL_{peak}, PSD, and fundamental frequencies for each experiment, refer to Tables 3 and 4.

Table 2.3. Decibels measured at varying distances from the 410 cm^3 water gun for all round goby and rusty crayfish exposures for experiments conducted in July 2013. Major frequencies range between ~1-15,000Hz. Contributing ~1-4,000Hz, ~5,000-9,000Hz, ~12,000-15,000Hz .

Depth m (X)	Dist. m (Y)	Slant Dist. m (Z)	Avg. Peak SPL (dB)	PSD (dB)	Fundamental Freq. (Hz)
0	1.7	1.7	240	~167	0-250
2	1.7	2.6	237	~164	250-300
1	2.7	2.8	241	~162	50-100
1	3	3.2	233	~160	50-100
3	1.7	3.4	229	~157	0-450
3	2.7	4	230	~162	100
3	3	4.2	227	~157	0-100
2	3.8	4.3	233	~158	150-200
5	1.7	5.3	226	~157	100-350
6	2.7	6.6	223	~162	100-350
6	3	6.7	221	~155	0
12	2.7	12.3	215	~165	50-350
12	3	12.4	216	~150	100

Table 2.4. Decibels measured at varying distances the 1966 cm^3 water gun for all round goby and rusty crayfish exposures for experiments conducted in September/October 2013. Major frequency range between ~1-5,500Hz. Contributing ~1-500Hz, ~1,000-2,500Hz, ~3,200-4,100Hz.

Depth (m) (X)	Dist. (m) (Y)	Slant Dist. (m) (Z)	Avg. Peak SPL (dB)	PSD (dB)	Fundamental Freq. (Hz)
1	3	3.2	233	~163	50-100
1	3	3.2	234	~161	50-300
3	3.5	4.6	228	~157	100
6	3.5	6.9	224	~155	100
1	3.5	3.6	233	~160	50-100
1	3.8	3.9	228	~162	50-100
2	3.8	4.3	228	~160	100

RESULTS

Barotrauma and Mortality Assessment
Rusty Crayfish

No mortality was observed in Rusty Crayfish exposed to the pulse pressure. Molting was not initially identified as an endpoint, but test animals were monitored daily, and exoskeletons became a significant part of the tank debris. Rusty Crayfish were exposed to 3 pulses from the 410 cm^3 water gun at varying distances and the percentage of Rusty Crayfish that molted from each treatment group is shown in Table 2.5. In the second experiment conducted on July 24, Rusty Crayfish were exposed to varying number of pulses 6.1 m from the 410 cm^3 water gun. These animals were held for 7 days post exposure; no control animals molted during that time (Table 2.6) whereas molting was observed in control animals in experiment 1 (Table 2.5).

Table 2.5 The number of rusty crayfish that molted post exposure to three pulse pressure discharges at various distances on 7/17/13

Distance (m)	Size	Replicates	Molt	Missing
Transport Control	S	1	0	1
	M	13	2	5
Control	S	5	3	0
	M	5	1	1
	L	5	1	2
3.9	S	5	0	4
	M	5	2	0
	L	5	0	0
6.7	S	5	0	4
	M	5	1	3
	L	5	4	0
10.4	S	5	3	1
	M	5	1	3
	L	5	4	0
17.4	S	5	2	3
	M	5	3	0
	L	5	0	3

Table 2.6. Distribution of rusty crayfish molting by size and the number of pulse pressure received from a 410 cm^3 watergun at 6.1 m on 7/24/13

Number of pulses	Size	Replicates	Molted	Missing
0	Small	14	0	3
	Medium	12	0	6
	Large			
3	Small	3	0	1
	Medium	6	2	1
	Large	4	3	0
6	Small	6	1	1
	Medium	5	0	1
	Large	2	0	0
12	Small	3	1	2
	Medium	7	4	3
	Large	3	1	1
24	Small	6	0	1
	Medium	5	1	1
	Large	2	0	0

Round Goby

There was no significant effect of pulse pressure discharges on Round Goby survival in any of the trials. One Round Goby was found dead following exposure. In addition, no observable injury was recorded during necropsy evaluations. No effects on Round Goby growth were found 14d post exposure for the 3m depth over rocky substrate experiment (Figure 2.3) or 7d post exposure for the 4 m depth over sandy substrate experiments (Figure 2.4).

Figure 2.3. Effects of 2 pulse pressure exposures on growth in round goby exposed at 3 m depth over rocky substrate. C refers to treatment control while TC refers to non-exposed transport controls.

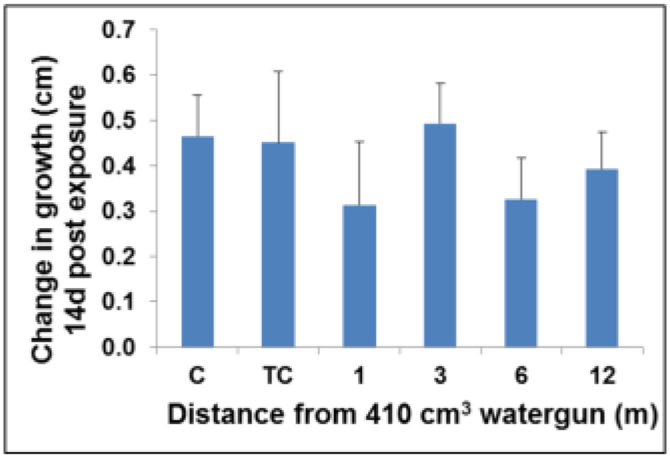
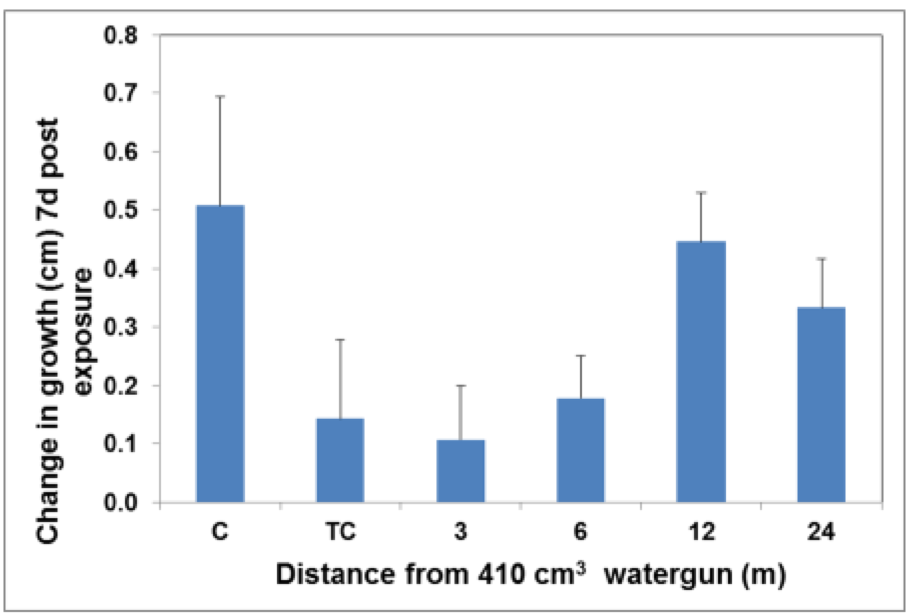


Figure 2.4. Effects of 2 pulse pressure exposures on growth in round goby exposed at 4 m depth over sandy substrate. C refers to treatment control while TC refers to non-exposed transport controls.



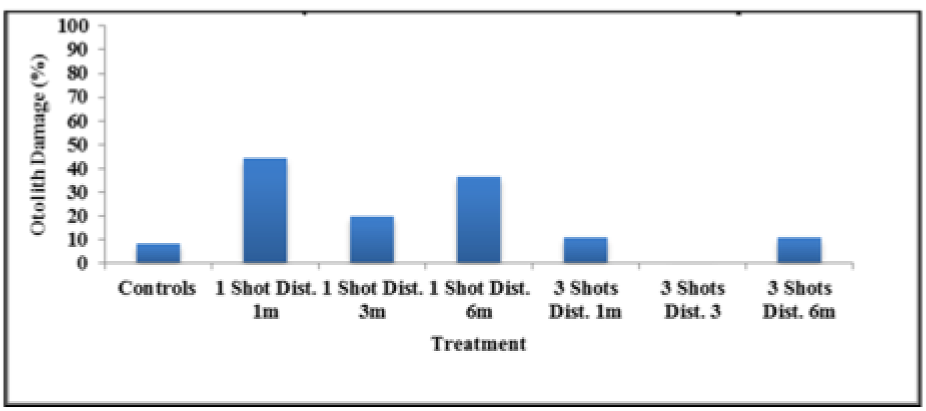
Rock Bass

Rock Bass were used as a positive control to the Round Goby in the September experiments. Though no internal barotrauma was observed in Round Goby, there were two forms of lethal injuries in Rock Bass exposed to the same treatment. Gas bladder damage was recorded in three of the six treatment groups. There was no incidence of gas bladder rupture in Rock Bass held 6.8 m from the gun for either number of pulse discharges. Injury to kidneys was recorded in identical treatments that experienced gas bladder rupture with the greatest percent injury in fish exposed to multiple pulses from the water gun. At the closest distance of 3.3 m, the Rock Bass exposed to 3 pulses displayed 38% kidney rupture, while there was no damage found in those that received only one shot. At the distance of 4.3 m, the group of Rock Bass that received 3 shots exhibited 22% kidney rupture and 9% for those that received one shot. There were no kidney injuries in the control group of either the treatment groups positioned at the 6.8 m distance (Table 2.6).

Table 2.6. Effects of pulse pressure on rock bass gas bladder and kidney rupture

Treatment	# of Pulses	n expected	n recorded	Gas bladder ruptures	Gas bladder rupture (%)	Kidney ruptures	Kidney rupture (%)
Control	0	10	10	0	0	0	0
3.3	1	10	10	0	0	0	0
	3	10	8	1	13	3	38
4.4	1	10	11	3	27	1	9
	3	10	9	2	22	2	22
6.8	1	10	5	0	0	0	0
	3	10	8	0	0	0	0

Figure 2.5. Round Goby otolith condition for the 28 and 30 September experiment.



Otolith Assessment

Round Goby located closest the sound source, 1 m away, experienced the greatest amount of otolith damage. Though the highest percentage of damaged otoliths was found in the closest treatment, there was an unexpected decrease in percent of damaged otiliths with the increased number of discharges from the water gun at the same distance. Round Goby that experienced a single discharge regardless of distance from the gun had higher percentages of damage than fish that experienced three shots (Figure 2.5). One of twelve control Round Goby also had otolith damage. Saccular otoliths from 20% of untreated control fish used in the 7 October experiment had damaged otoliths compared with 30%of the treated fish. No otolith damage was found in any exposed Rock Bass.

DISCUSSION

We attempted to test the ability of pulse pressure technology to induce baratrauma on Round Goby and Rusty Crayfish in order to develop control methodology for spawning reefs in the Great Lakes, but we found no evidence that this innovative approach has potential to suppress either species. Physical barotrauma is described as the rupturing of blood vessels, bruising, and severe physical damage to organs, including gas bladder rupture and occlusion of the circulatory system (Carlson 2012). These characteristics had previously been described in other studies evaluating pulse pressure from pile driving, dynamite, air guns and water gun (Popper and Hastings 2009). Other noted physical effects from these studies include damaged otoliths, scale removal, and cracked shells and loosened adductor muscles in bivalves (Schaefer et al. 2010, Paparella and Merton 1970). Few published studies have evaluated the effects of pulse pressure energy emitted by water guns on fish or crayfish or other aquatic taxonomic groups, Experimentation was necessary to ascertain the lethal response variables in Round Goby and Rusty Crayfish.

The first set of trials conducted in July 2013 sought to evaluate the potential for pulse pressure water gun technology to induce Round Goby and Rusty Crayfish tissue damage over various substrate types. We hypothesized that the pressure wave and the other physical disturbances such as cavitation and the rapid expulsion of water would induce barotrauma and that different substrates may minimize or amplify the effects of sound such as absorptive properties of sand versus reflective properties of rock respectively. Other studies using fish with gas bladders demonstrated that the majority of mortality from pulse pressure generated from water guns was not immediate but was delayed (Gross et al. 2013). Given such, it was expected that Rusty Crayfish and Round Goby would not experience acute mortality as neither species possess gas bladder-like organs. The July studies utilized a small water gun but one that produced significant energy levels that have induced gas bladder rupture in other fish (Gross et al. 2013), and 3 or 4 dead long nose suckers were observed at our treatment sites in July. Due to the lack a gas bladder, the Round Goby tested in this project displayed no physical barotrauma at $SPL_{peak,}$ even though they were exposed to 10X more pulses that induced mortality and gas bladder damage in Lake Trout and Northern Pike (Gross et al 2013).

Based on the inability to identify a lethal thresholds with the smaller gun, we hypothesized that a larger water gun might be able to induce lethal effect by producing a different sound energy (particularly frequency) and peak sound pressure levels. Peak sound pressure levels only indicate the highest sound level recorded at single frequency among all frequencies. After the July experiments, a larger hydraulically fired water gun was built that was equivalent in size to others gas fired water guns that had previously been tested (Gross et al. 2013). The new water gun was significantly more powerful and generated more low frequency energy than the watergun used in July, and achieved similar sound pressure level (SPL). When we compared the frequencies emitted at those SPL measured from both water guns, the spectral densities were very different as there was more low frequency sound accounting for the pressure wave from the larger water gun. Of the contributing sound energy, the smaller watergun's power spectral density PSD contained contributing frequencies as high as 15,000 Hz whereas the larger watergun's energy was 4,100 Hz and lower. During the fall trials, the larger watergun was capable of rupturing gas bladders and inducing kidney damage in Rock Bass but not for Round Goby.

Additional goals of this research were to evaluate other potential negative effects on Round Goby behavior such as damage to hearing organs and other stress responses such as reduced feeding activity and growth. Even though pulse pressure was not lethal, if feeding rates of Round Goby and Rusty Crayfish decreased, via injury or a stress response, these impacts may still reduce the levels of predation on Lake Trout Cisco and Lake Whitefish eggs. Other endpoints we evaluated were effects on growth and hearing structures. There is a suggestion in one of the experiments that exposure to pulse pressure had an impact on growth (Fig. 2.4) whereas no pattern was apparent in the second experiment and the data was highly variable across both exposure gradients and consequently we believe these results should be interpreted with care and are perhaps just an artifact of sampling.

To evaluate the effects of sound on Round Goby hearing and balance, otoliths were removed from fish from two experiments conducted in the fall using the larger watergun. We hypothesized that the sound energy would be so extreme or that the concussive effects of cavitation in the cobble spawning bed that Round Goby otoliths would be damaged, which might affect feeding success or increase Round Goby vulnerability to predators. The fundamental frequency where the majority of sound energy exists from the water guns is within Round Goby hearing ranges, which is believed to be 100 to 600 Hz (Belanger et al. 2010). While otolith damage might be one possible outcome from pulse pressure exposure, the low rates of incidence seen in fish exposed to three pulses in close proximity to the gun provides further evidence that this method probably has limited utility as a control tool to reduce the short term impacts of Round Goby (Wagner et al. in review). Furthermore, the data are conflicting, - we expected that an increased number of pulses would equate to more damage; instead, our results almost suggest that more pulses are protective.

Similar to Round Goby, Rusty Crayfish experienced no acute mortality during the July trials. Post exposure, Rusty Crayfish began molting during the July experiments, which was unexpected during our post treatment monitoring. Molting did affect our ability to follow some individuals, but nevertheless molting patterns were not consistent between experiments so these results should also be treated cautiously and again may be an artifact of sampling or the timing of experiments.

Based on the results of this study and observations made in Objective 3 (below), we concluded that pulse pressure sound energy was not a viable tool to control Round Goby or Rusty Crayfish on small patches of spawning reef. We were unable to cause lethal effects in either species at a number of pulses that would simulate a mobile water gun reef treatment.

Heading home after another day testing the effectiveness of the seismic gun – Beaver Island, July 2013

<u>Objective 3</u>. Use seismic guns to treat up to four spawning reefs to reduce the abundance of all size classes of goby immediately prior to native reef fish egg deposition.

In order to control wide spread established invasive species, like Round Goby, new technologies are needed that will increase the efficiency and effectiveness of control efforts. To control Round Goby for the benefit of spawning reef habitat, the ideal tool(s) would be implemented relatively rapidly (within a few days) and be highly effective at reducing Round Goby densities on the reefs (by >90%) at the onset of the fall spawning season. Seismic technology offers promise as an aquatic invasive species control tool: it can be used to treat large areas in a single day, and preliminary studies indicate that mortality rates for other invasive fish are high (Gross et al. 2013). Furthermore the technology may also be used to manipulate fish movement (Layhee et al. 2013). Given that Round Goby are a benthic fish that is more likely to hide under rocks in the immediate area when faced with a threat, rather than to flee a site entirely, we hypothesized that they may be particularly susceptible to this technology, because we should be able to repeatedly dose these fish, and if necessary we could treat large area around the reef and over multiple days.

We proposed to treat three spawning reefs using seismic technology in order to reduce the abundance of all size classes of Round Goby immediately prior to native reef fish egg deposition. Since the experiments conducted in Objective 2 failed to document impacts of the seismic treatment on Round Goby, a larger, more powerful pulse pressure gun was deployed for the fall treatment. We had proposed to utilize the experimentally quantified lethality results from Objective 2 to calculate an appropriate treatment design (i.e., number of shots and distance from shots applied to the reef using a grid). However, because we were unable to document lethal or chronic adverse effect (see Objective 2 above) we conducting two reef treatment experiments to determine whether there was any evidence on a behavioral response including avoidance of the treated area by Round Goby that could reduce egg predatory impacts on the spawning reefs. Additional attempts to treat the remaining reefs were abandoned after review of preliminary data from the two treatments described here indicated that no meaningful affect was being achieved.

METHODS

We conducted seismic treatments on two reefs, ER North and the Crib, in early October 2013, prior to the onset of Lake Trout spawning in late October (which precedes Cisco and Lake Whitefish spawning). We used a 1,966-cm^3 pulse pressure water gun operated manually using a hydraulic directional control valve and a 28 gpm hydraulic pump rated for 2,800 PSI pressure. Operating pressure was maintained at a constant pressure throughout each experiment by a 45L, 2,400 PSI nitrogen gas cylinder. To measure treatment effects, video-monitoring was conducted during both treatments to evaluate behavioral changes, and sentinel fish were used to evaluate mortality and post-treatment feeding rates for the second treatment on the Crib.

ER North Treatment

On 6 October 2013, seismic technology was used to treat the ER North reef in Grand Traverse Bay, Lake Michigan (Figure 1 above; Figure 3.1). The "pulse pressure gun" was deployed 3-m behind the boat using a triangular "A" frame. The gun was suspended horizontally in the water column at 1-m depth by two low drag floats to mitigate the mass of the gun while towed. The gun was towed at the minimum no wake speed required to maintain the boat position and direction. The boat moved back-and-forth across the reef and the adjacent area with the gun being fired approximately every 5 seconds. The treatment was administered for 19 minutes, when the treatment had to be cut short due to the gun becoming entangled in one of our underwater video bouy lines and a subsequent O-ring seal failure on the gun. A total of approximately 240 pulses were applied to the reef and adjacent area.

A GPS unit was used to track the boat movement during the treatment. To estimate the intensity of treatment across the reef area, the linear track of the boat was overlaid with a 10-meter grid across the treatment area using ArcMap 10.0. The total length of the linear boat track within each grid cell was summed to identify the relative intensity of the treatment for each cell (Figure 1B). The most intense treatment was administered on and around the primary spawning habitat. The seismic treatment was not administered within the portion of the reef that is inaccessible by boat due to the presence of bridge pilings from an old dock that emerges from the water surface over part of the reef and do not allow for boat access (Figure 3.1).

To evaluate Round Goby behavior during and immediately following treatment, baited underwater videos were deployed at four different locations within the primary spawning habitat. GoPro HERO 3® cameras were mounted onto steel rod frames and baited with Lake Trout eggs (described in detail in Robinson 2014, Robinson et al., *under revision*). The cameras were dropped from a boat onto the reef approximately five minutes prior to treatment and remained on the reef at least 20 minutes following the treatment, though video data were not collected toward the end of this time period due to disturbances that could have impacted goby behavior. Unfortunately, two of the cameras were knocked over (and in one case dragged) by the boat as it passed by during the treatment, after which no behavioral data is available.

The total number of Round Goby visible within the camera view was recorded at each one minute mark. Numbers of Round Goby observed feeding each minute were also recorded. The total number of Rusty Crayfish was also recorded at the same one-minute mark that Round Goby were recorded, to evaluate whether Rusty Crayfish might exhibit a behavior response to the treatment.

Results were analyzed for differences in the number of Round Goby visible and feeding, and Rusty Crayfish visible among pre-, during, and post-treatment periods using a repeated-measures analysis-of-variance, with Tukey's multiple comparison. In addition, changes in trends in the number of Round Goby visible or feeding, and Rusty Crayfish visible during and post-treatment were evaluated using regression, blocking for camera, to evaluate whether Round Goby behavior changed over time during or following the treatment.

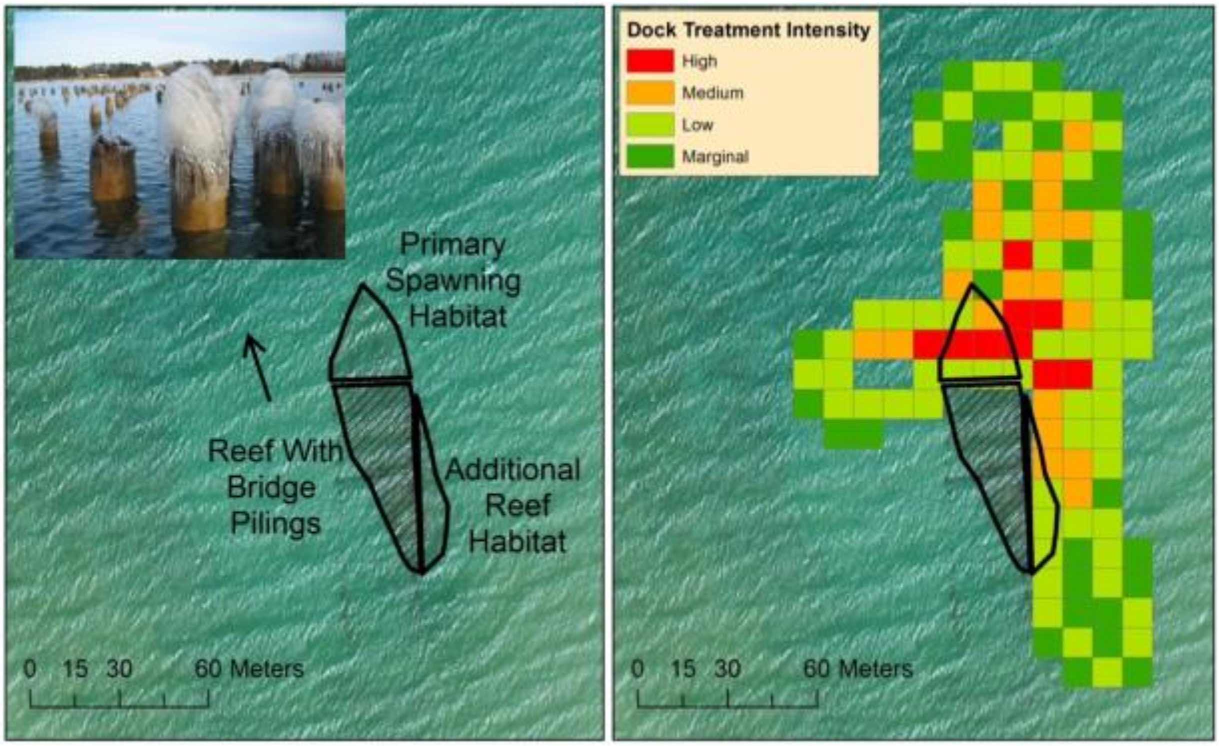


Figure 3.1. The ER North spawning reef (A), with the primary spawning habitat, where Cisco, Lake Trout, and Lake Whitefish spawn, and the rest of the reef habitat—much of which is inaccessible by boat due to pilings from a former dock that rise above the water surface (see photo). On 6 October 2013, a seismic treatment (B) was implemented with varying intensity on and adjacent to the ER North reef. See text for details on how intensity was determined. The spawning habitat and the immediately adjacent area received the most intense seismic treatment. Photo by R. Claramunt.

Crib Treatment

On 8 October 2013, seismic technology was used to treat the Crib reef in Little Traverse Bay, Lake Michigan (Figure 1; Figure 3.2). The pulse pressure gun was deployed 3-m off the starboard bow of the boat and suspended vertically below a large buoy at 1-m depth. The anchored boat was motored so that it swung back and forth around a core treatment area. The seismic gun was fired an estimated 230 times over an 18.5 minute period with the core area of the reef experiencing high impact (>50 pulses within 10m) and adjacent areas experiencing moderate (~25-50 pulses within 10 m) or low impact (<~25 pulses within 10 m). As such, these areas were qualitatively grouped into High, Medium, and Low treatment zones (Figure 3.2). It should be noted that given the larger pulse pressure gun and high density of pulses, the cumulative seismic exposure on the Crib exceeded the experiments described in Objective 2 and the ER North reef treatment.

Ten sentinel cages, each containing one Round Goby, Rusty Crayfish and Rock Bass, were distributed haphazardly across the reef (Figure 3.2). Rock Bass were among the more common native fish species on the Crib and other similar reefs (Robinson 2014). A PIT tag had been inserted into each cage fish at least 48 hours prior to the experiment, so that it could be identified and tracked throughout the experiment. A number was written on the carapace of each Rusty Crayfish, so that each individual Rusty Crayfish could be tracked. Cages were submerged from a boat 5-10 minutes prior to the treatment and were retrieved approximately 10 minutes following treatment.

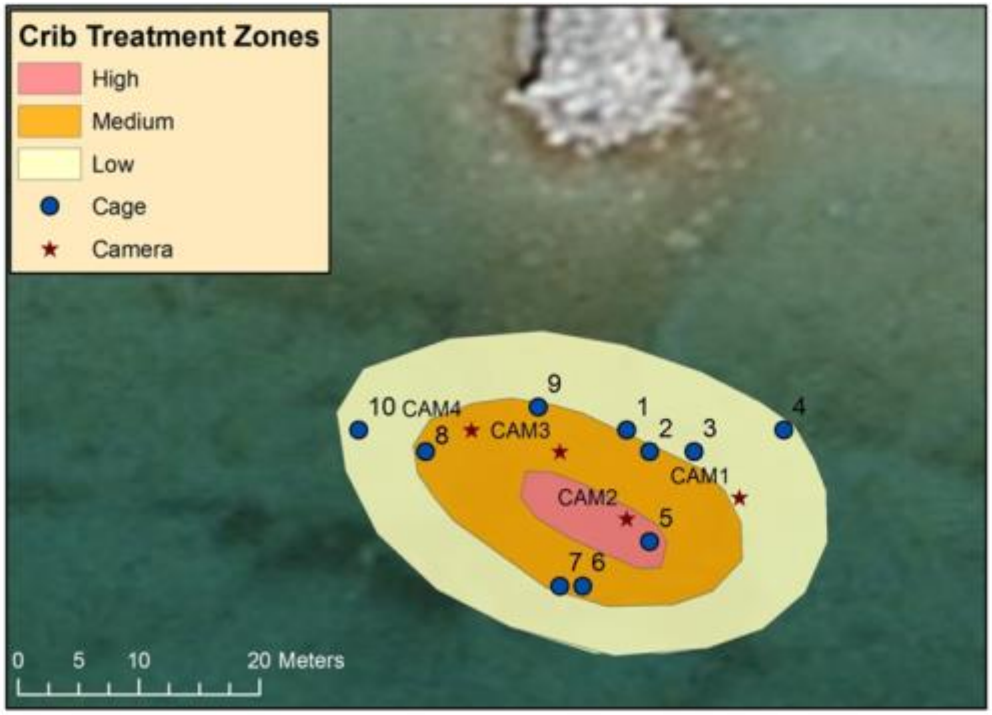


Figure 3.2. Crib treatment map showing a high treatment area (>50 shots within 10m) and adjacent areas experiencing medium (~25-50 shots within 10 m) or low (<~25 shots within 10 m) treatment intensity. Ten sentinel cages containing one Round Goby, Rusty Crayfish, and Rock Bass were placed haphazardly across the reef to evaluate mortality. Four cameras were also place across the reef to evaluate behavior during the treatment.

Three additional cages, with one individual of each species, were also submerged at the site immediately following the conclusion of the treatment, and these were retrieved after approximately 10 minutes. Treatment and control fish/crayfish were then placed in an aerated cooler—all Round Goby and Rock Bass (treatment and control) were held together in one cooler and transported to the Charlevoix Biological Station.

To evaluate lethality due to seismic treatment, all study organisms were observed periodically for 24 hours. Stress behaviors were observed and mortalities were removed and identified by their PIT tag code.

To evaluate Round Goby behavior during and immediately following treatment, baited underwater videos were taken at four different locations (Figure 3.2). GoPro HERO 3® cameras were mounted onto steel rod frames and baited with Lake Trout eggs (described in detail in

Robinson 2014, Robinson et al., *under revision*). The cameras were dropped from a boat onto the reef approximately five minutes prior to treatment and remained on the reef at least 20 minutes following the treatment, though video data were not collected toward the end of this time period due to disturbances that could have impacted Round Goby behavior. Camera 2, which was placed within the high treatment zone, collected video imagery up until it shattered approximately 15 minutes into the treatment due to the pressure from the seismic treatment.

The total number of Round Goby visible within the camera view was recorded at each one-minute mark. The numbers of Round Goby observed feeding each minute were also recorded. Since there were almost no observations of Rusty Crayfish in the Crib cameras, their observations were not evaluated. Results were analyzed for differences in the number of Round Goby visible and feeding among pre-, during, and post-treatment periods using repeated-measures analysis-of-variance, with Tukey's multiple comparison. In addition, changes in trends in the number of Round Goby visible or feeding during and post-treatment were evaluated using regression, blocking for camera, to evaluate whether Round Goby behavior changed over time during or following the treatment.

The morning following the seismic treatment, feeding trials were conducted on each Round Goby from the sentinel (and control) cages (18-21 hours post-treatment). Each fish was placed in a large (approximately 47 liter) cooler for ten minutes. Each cooler had three pellets and three Lake Trout eggs when the Round Goby was placed in the cooler. If either was depleted, three more were carefully added to the cooler. The observer sat approximately 3-meters away and watched, while remaining generally unnoticed by the Round Goby. Trials were conducted blind to whether gobies were treatment or control fish. At each feeding, the food item type and time it was eaten was recorded. At the end of the feeding trial for each fish, the PIT tag was scanned and recorded so that fish could be identified (control or treatment, and treatment position - cage number).

The number of food items consumed and the timing of consumption was compared between treatment and control fish. Since there was substantial variation in the intensity of exposure to the seismic gun (Figure 1), additional tests were run comparing the number of food items consumed for control Round Goby and separately for treatment fish based on treatment zone (combining high and medium treatment zone fish) and based on whether Round Goby were or were not in cages with Rock Bass mortality.

RESULTS

Dock Treatment

Video footage showed that many Round Goby exhibited alarm behavior during treatment, with most immediately jumping and appearing alert upon the initial firing of the seismic gun and many moving into the substrate—and therefore not visible in the videos (Figure 3.3). As a result, the number of Round Goby declined throughout the evaluation period, with differences among treatment periods ($F_{2,93}$=46.1, P < 0.0001) where the number of Round Goby pre-treatment was higher than during the treatment, and both pre and during treatment were higher than post-treatment (Figure 3A). Throughout the treatment, Round Goby increasingly entered the substrate; therefore fewer were visible over time ($F_{1,62}$=66.34, P < 0.0001; Figure 3.3A). The number of Round Goby visible did not change over time during the post-treatment period ($F_{1,18}$=0.17, P = 0.68; Figure 3A). Similarly, the number of goby feeding differed among treatment periods ($F_{2,93}$=25.89, P < 0.0001; Figure 3.3B), with the pre-treatment higher than during and post-treatment, and with during treatment higher than post-treatment. The number of Round Goby feeding decreased throughout the treatment period ($F_{1,62}$=4.16, P = 0.046) but did not change over time during the post-treatment period ($F_{1,18}$=46.1, P = 0.59).

Rusty Crayfish behavior exhibited trends opposite of Round Goby, where treatment periods again differed ($F_{2,93}$=10.76, P < 0.0001; Figure 4), but included post-treatment period Rusty Crayfish numbers higher than during and pre-treatment, and numbers during treatment higher than pre-treatment. Further, the number of Rusty Crayfish visible increased during the treatment ($F_{1,62}$=7.97, P = 0.006) but did not change during the post-treatment period ($F_{1,18}$=2.67, P = 0.12).

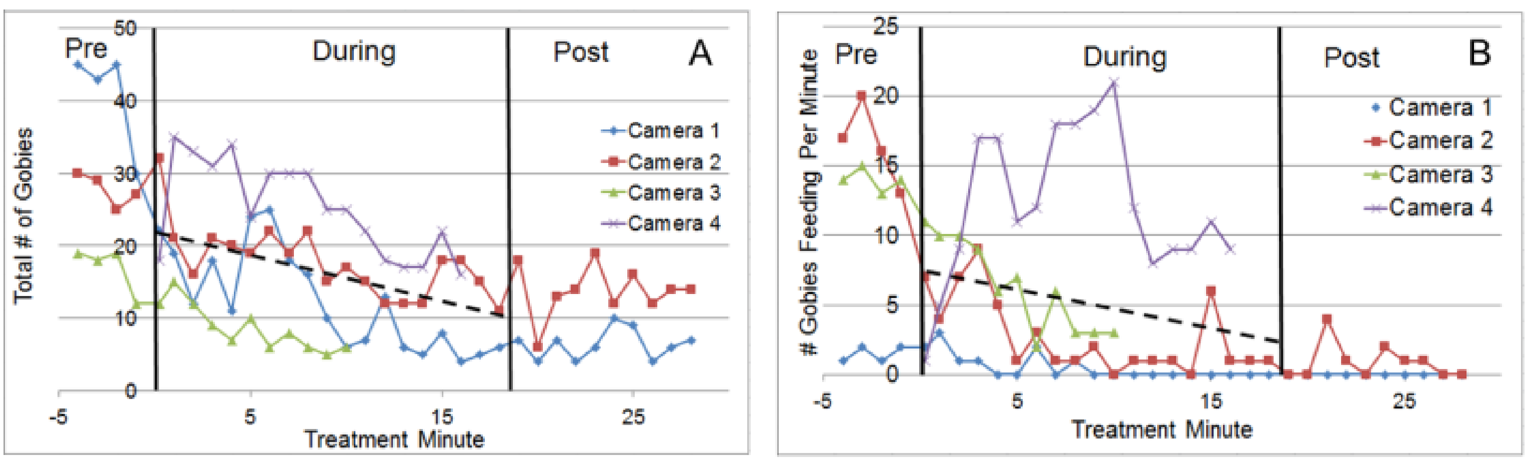


Figure 3.3. The (A) total number of Round Goby visible and the (B) number of Round Goby feeding on bait at one-minute intervals in baited underwater videos recorded prior to, during, and post seismic gun treatment of ER North. The total number of Round Goby and the number feeding differed among time periods (P < 0.0001), with the Pre period having significantly more than both the During and Post periods and the During period having more than the Post period. There was a decline in the total number of Round Goby (P < 0.0001) and the number of Round Goby feeding (P = 0.046) as the treatment progressed. Note that data for Cameras 3 and 4 end during the study because they were knocked over (and in one case dragged) by the boat.

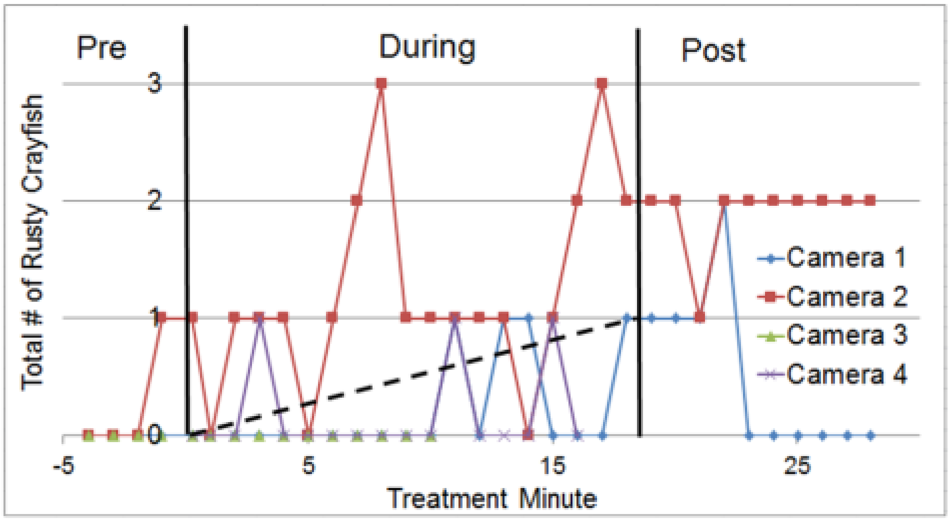


Figure 3.4. The total number of Rusty Crayfish visible at one-minute intervals in baited underwater videos recorded prior to, during, and post seismic gun treatment of ER North. There were differences among time periods ($P <$ 0.0001), with the Post period having significantly more Rusty Crayfish than both the Pre and During periods and the During period having more than the Pre period. There was an increase in the total number of Rusty Crayfish during the treatment ($P = 0.006$). Note that data for Cameras 3 and 4 end during the study because they were knocked over (and in one case dragged) by the boat.

Crib Treatment

During the experimental treatment on the Crib, large numbers (>50) of fish were observed floating to the surface. Impacts on fish appeared to be cumulative, as the number of fish floating from the reef increased as the treatment progressed. A subsample collected via dip net included dozens of Rock Bass, several small Smallmouth Bass, two small Alewife, and a Lake Chub. Divers searched the substrate within and around the high treatment zone following the treatment and found one Round Goby mortality, which may or may not have been a result of the treatment.

Immediately following removal from the sentinel cages, seven of the 10 treatment Rock Bass were lying on their side exhibiting stress behavior and had large pale patches on their lower side, behind their pectoral fin extending posteriorly past the beginning of the anal fin. Five of those fish died within 24 hours. There was substantial heterogeneity in Rock Bass mortality across the treatment area, but of the six cages within the more heavily treated areas, four had Rock Bass that died, whereas only one of four Rock Bass died in low treatment areas (Figure 3.5). All Round Goby and Rusty Crayfish looked healthy immediately following treatment and all survived through 24 hours. Video footage showed that many Round Goby exhibited alarm behavior during treatment, with most immediately jumping upon the initial firing of the seismic gun and many moving into the substrate—and therefore not visible in the videos (Figure 3.6). As a result, fewer Round Goby were visible per minute as the treatment progressed ($F_{2,129}$=9.03, P = 0.0002; Figure 3.3). Throughout the treatment Round Goby increasingly entered the substrate; therefore, fewer were visible over time ($F_{1,65}$=53.49, P < 0.0001; Figure 3.6). Upon conclusion of the treatment, Round Goby increasingly emerged from the substrate so that they were growing numbers were visible throughout the post-treatment period ($F_{1,48}$=10.27, P = 0.002; Figure 3.6). Some of those that remained visible during the treatment continued to exhibit alarm behavior, but some seemed to behave normally, in many cases they even continuing to feed (Figure 3.7). Though the total number of Round Goby feeding did not significantly differ among pre, during, and post-treatment periods ($F_{2,129}$=1.33, P = 0.27), the number feeding significantly decreased during the treatment ($F_{1,65}$=18.72, P < 0.0001; Figure 3.7) and significantly increased post-treatment ($F_{1,48}$=8.64, P = 0.005; Figure 3.7).

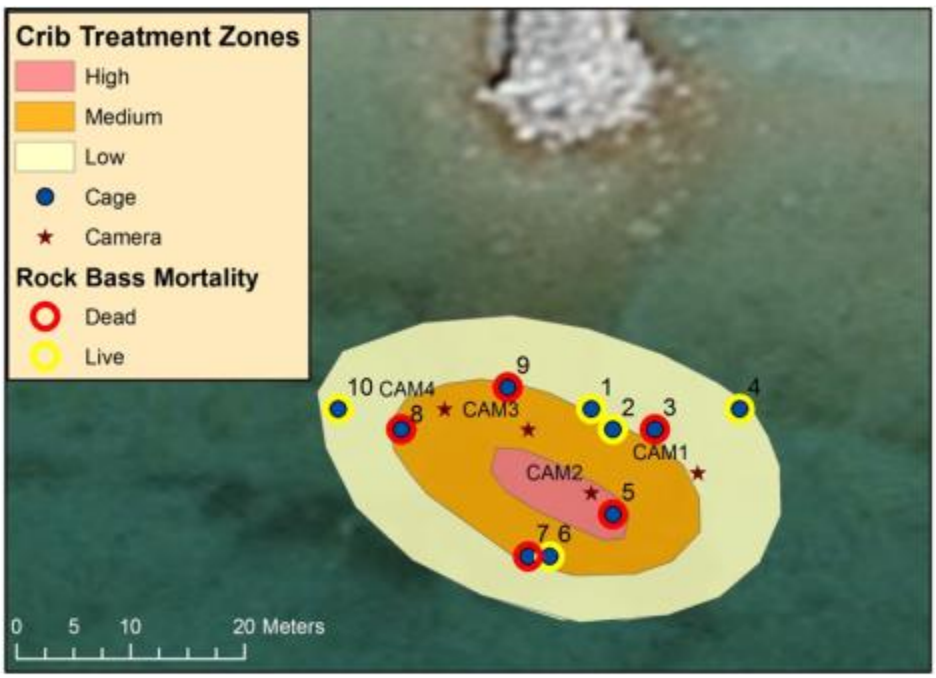


Figure 3.5. Treatment cages where Rock Bass had died within 24 hours of treatment are shown with red circles. Camera 2, which was placed within the high treatment zone, shattered approximately 15 minutes into the treatment due to the pressure from the seismic treatment.

Round Goby behavioral responses were strongest within the high treatment zone (i.e. camera 2; which shattered), with all Round Goby quickly entering the substrate and mostly staying there and not feeding throughout the treatment (Figure 3.6 and 3.7). Camera 3, which was in the medium treatment zone and relatively close to the high treatment zone (Figure 3.2), also showed most Round Goby quickly moving into the substrate. The two cameras furthest from the high treatment zone generally exhibited the same—but much weaker—trends, as Round Goby continued to be visible throughout the treatment, and feeding observed throughout this period (Figure 36 and 3.7). While it is possible that interstitial spaces within the rocks may or may not provide some relief from the seismic treatment, camera 2 video showed a stunned Rock Bass, which had been hidden in the substrate, emerging from the rocks after the sixth firing of the gun, lying on its side, and passively drifting out of camera view, and presumably then floating to the surface.

There were no differences in the number of items consumed ($F_{1,11}$=1.77, P = 0.21), though the average was more than twice as high for control fish (Figure 3.8A) and all control fish consumed food, while only 40% of the treatment fish did. There were also no significant differences based on treatment zone ($F_{2,10}$=1.87, P = 0.20; Figure 3.8B) or Rock Bass mortality ($F_{2,10}$=0.89, P = 0.44; Figure 3.8C), though this may be influenced by sample size, particularly for treatment zone, which did have trends that fit the expected pattern.

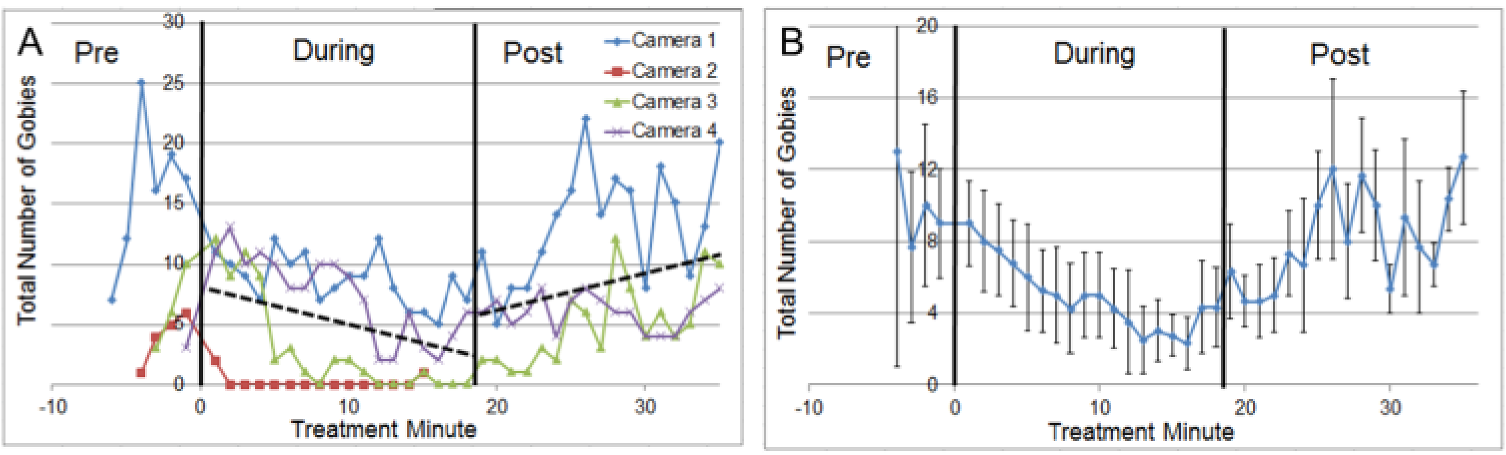


Figure 3.6. The total number of Round Goby visible at one-minute intervals in baited underwater videos recorded prior to, during, and post seismic gun treatment for (A) each camera separately and for (B) averages across cameras (± 1 S.E.). More Round Goby are visible during the Post-experiment period than there were during the experiment (P = 0.0002). Further, the number of Round Goby visible decreased throughout the experiment (P < 0.0001) and increased following the conclusion of the treatment (P = 0.002). Camera 2 data stops during the treatment at the 15 minute mark, because the camera case shattered at that point due to pressure build-up from the seismic gun.

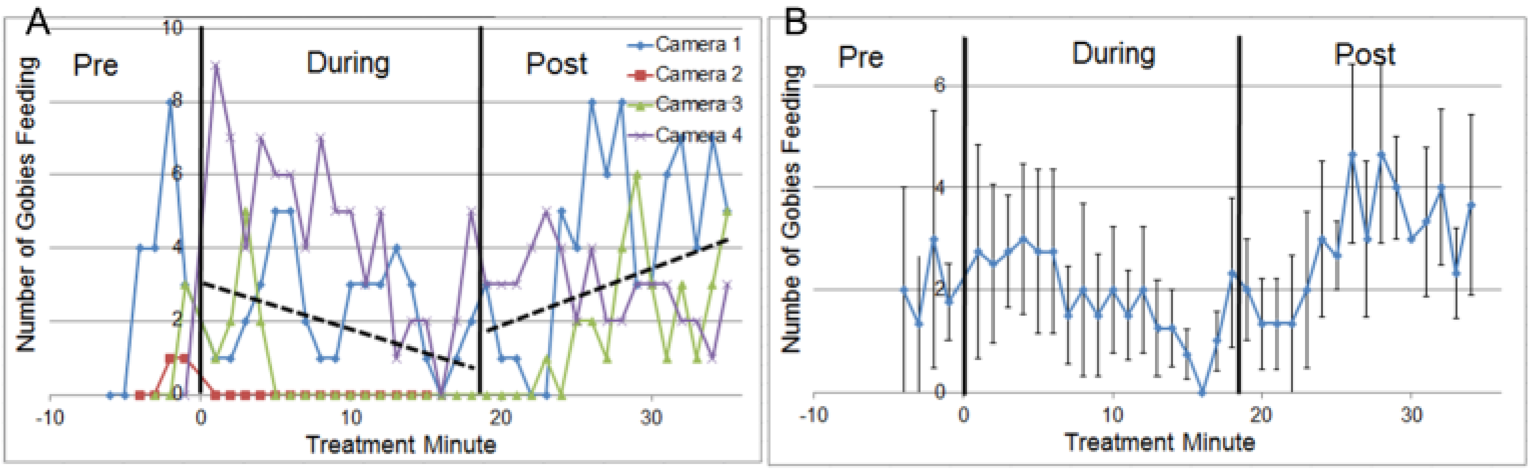
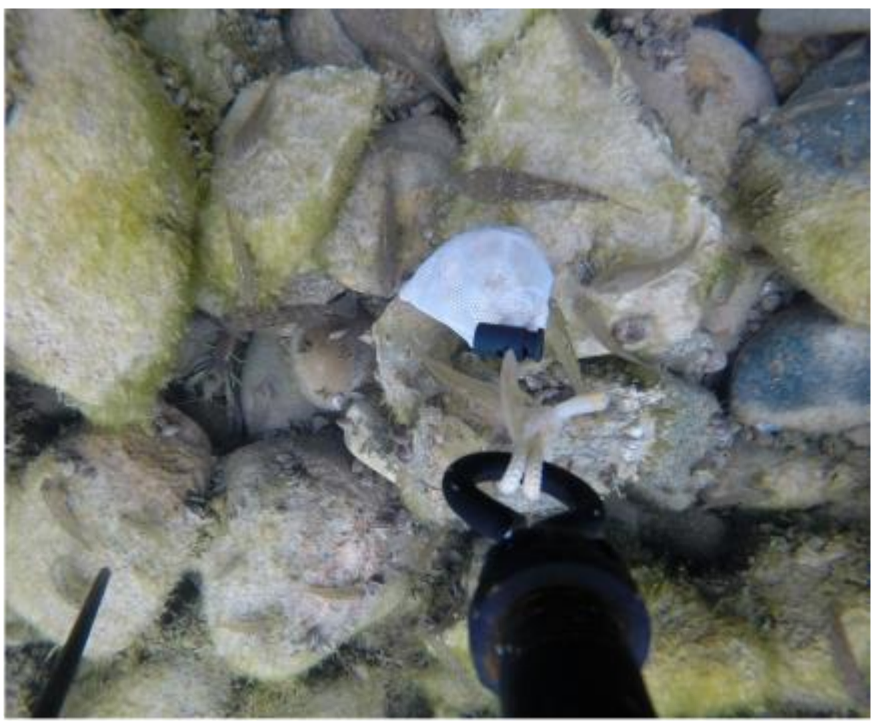


Figure 3.7. The number of Round Goby feeding on bait at one-minute intervals in underwater videos recorded prior to, during, and post seismic gun treatment for (A) each camera separately and for (B) averages across cameras (± 1 S.E.). The number of Round Goby feeding did not differ among pre, during and post-treatment periods ($P = 0.27$), but the number of Round Goby feeding decreased throughout the experiment ($P < 0.0001$) and increased following the conclusion of the treatment ($P = 0.005$). Camera 2 data stops during the treatment at the 15 minute mark, because the camera case shattered at that point due to pressure build-up from the seismic gun.



Typical drop camera image on Crib – showing Round Goby and Rusty Crayfish congregating around the bait bag.

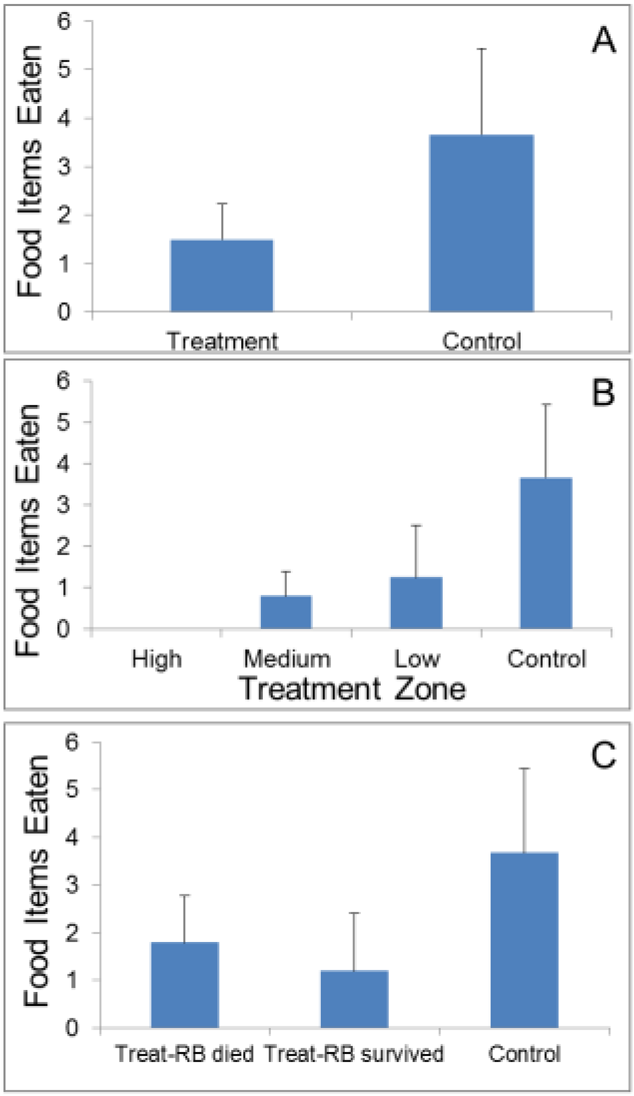


Figure 3.8. Comparisons of the number of food items eaten for A) Round Goby from treatment cages versus control Round Goby, B) Round Goby within each control zones and control Round Goby, and C) Round Goby from treatment cages with and without Rock Bass (RB) that died and control Round Goby.

DISCUSSION

As in the seismic experiments conducted during the summer with a smaller pulse pressure water gun (Objective 2), we found no evidence of Round Goby mortality. Their tendency to increasingly hide in the substrate and reduce feeding during the treatment indicates that Round Goby may at least be alarmed or stressed by the seismic treatment. However, our feeding results, suggest Round Goby begin to feed less within 24 hours after treatment even among the most intensively treated fish, although our results are not conclusive, because of the small sample size. Nevertheless the data suggests that actual or potential effects are short-lived. With the longer post-treatment period used for the Crib treatment, we were able to show that Round Goby quickly (within 15 minutes) emerge from the substrate following treatment and begin to feed in numbers comparable to pre-treatment (the post-treatment monitoring period for ER North was presumably not long enough to detect this recovery). Moreover, we found no evidence that the effects were substantial enough to have any meaningful impact on the Round Goby populations and hence reduce their impact on egg predation.

While impacts to Round Goby from the seismic treatments were trivial at best, the seismic treatment was clearly effective on other species on these reefs, as exhibited by 50% Rock Bass mortality in the cages and the large number of non-target fish that floated to the surface at the Crib. The greater fish mortality observed on the Crib is likely a function of the much smaller treatment area (approximately 1/3 the size of the ER North treatment), resulting in fish exposed to extreme cumulative levels of pulse pressure.

It is notable that, while Rock Bass in cages closer to the high treatment area were more likely to experience mortality, there was substantial spatial heterogeneity in mortality rate (Figure 3.5). This would indicate that many factors may influence pulse pressure exposure and or that individual fishes can have substantial variation in pressure tolerance. We hypothesized that rock crevices might provide a refugia from pulse pressure but the video observation of a moribund Rock Bass emerging from the substrate and passively drifting out of camera view floating on its side early in the treatment would suggest that rock crevices are not particularly effective refugia.

The behavioral pattern for Rusty Crayfish during the ER North treatment indicates that they were drawn into the camera field by the bait and were apparently unfazed by the seismic treatment. This higher behavioral tolerance of the seismic treatment (relative to Round Goby) indicates that the treatment causes minimal, if any, stress to Rusty Crayfish, which would indicate that the potential molting effect observed during the summer (Objective 2) may be spurious.

In the search for technological tools to use in controlling aquatic invasive species, seismic technology offers substantial promise (Gross et al. 2013). As previously documented for fishes in other systems (Gross et al. 2013), we did find lethal impacts on Great Lakes fish. However, we were unable to document lethal effects on Round Goby or Rusty Crayfish. We were also unable to document significant behavioral changes that would indicate the potential for reduced impacts of these predators on target fish eggs.

<u>Objective 4:</u> Measure changes in abundance and distribution and interstitial densities of target and non-target invasive species (e.g., Round Goby, Rusty Crayfish, dreissenid mussels, *Hemimysis anomala*) and native egg predators.

The Round Goby, like the Rusty Crayfish, are found in high densities on native fish spawning reefs during Lake Trout spawning and actively feeds on Lake Trout eggs. Round Goby were assumed to move to deeper water to overwinter by the time that Lake Whitefish spawn. However, uncertainty remains regarding Round Goby concurrence with Cisco spawning and the nature or extent of any offshore migration. We hypothesized that seismic gun treatments could deplete Round Goby on spawning reefs and reduce their impacts on native fish recruitment (see objectives 2 & 3). Intensive trapping, intended primarily for Rusty Crayfish control (see Objective 1) resulted in incidental bycatch of Round Goby. All Round Goby captured during Rusty Crayfish control efforts were also removed. As a result, our trapping efforts are considered here as part of our expanded definition of "Round Goby control." For Objective 4, we proposed to monitor Round Goby abundance, distribution, size frequencies, and interstitial densities before, during, and after control efforts so that we could: 1) assess efficacy of control (i.e., seismic treatments and trapping) to deplete Round Goby, and 2) distinguish any treatment effects from changes resulting from natural migrations over the course of the native fish spawning season. Our data quality objective for this component of our project was to quantify whether reductions in Round Goby occurred and were sustained throughout the native fish spawning season. Here we describe our trapping and monitoring activities and related results for Round Goby.

We anticipated that a reduction in Round Goby (and/or Rusty Crayfish) abundance could trigger cascading food web effects that might ultimately result in changes in the abundance of non-target native and invasive species (sensu competitive release and the predator release hypothesis). Thus, monitoring of reef fish communities was undertaken in association with our efforts to monitor the abundance, spatial and temporal distribution of goby. We also tracked abundance and temporal distribution of non-target invasive *Dreissenid* mussels and the crustacean *Hemimysis anomola* as well as other common benthic invertebrate taxa. Our data quality objective as it related to non-target taxa was to quantify whether control treatments resulted in an increase in non-target invasive species or other taxa. We describe here our monitoring activities and related results for *Dreissenid* mussels, *Hemimysis* and other common benthic invertebrates.

METHODS

Intensive removal efforts

Treatment with seismic guns to deter and/or kill Round Goby on the Crib and ER North reef in October 2013 is described in Objectives 3. Intensive trapping using baited minnow traps was implemented in 2012 across four reefs, primarily to deplete crayfish, but with the added benefit of capturing goby (Table 1.1, Fig. 1.1 objective 1 above). In 2013, baited minnow traps were deployed on just two dates (29 Oct and 20 Nov) and only at the Crib, ER North, and ER South. Trapping efforts at the Crib and ER South in 2013 resulted in very few captures and thus these sites were treated as reference sites for analysis purposes (see Table 1.1). Trapping methods are described in detail in Objective 1. Briefly, we deployed sets of 10-20 standard Gee minnow traps, tethered to a long line at 5 m intervals. We employed a rolling front of traps to allow us to trap over the entire reef and an approximately 10 m adjacent buffer area. In 2012, intensive trapping occurred at least two times per week from the start of August through early September on each of the four treatment reefs. The trapping regime we employed resulted in fairly constant trapping pressure over each reef from 1 August to 9 September 2012. All Round Goby were measured with calipers to the nearest millimeter and then euthanized. Native fish species (<5% of the catch) were noted and returned to the reefs.

Index monitoring

To provide a standardized measure of temporal changes in Round Goby densities, we employed index monitoring on all six reefs using baited minnow traps, underwater video cameras, egg bags, and counts from quadrat sampling. Index monitoring for Round Goby with minnow traps and baited underwater video cameras occurred on a biweekly basis from early July to end of November in 2012 and from early September to end of November in 2013. Monitoring with minnow traps and cameras at each site occurred across three depths—at approximately 3 m (on reef), 6 m, and 9 m. The 6 m and 9 m monitoring locations at each site were located on a straight line perpendicular to the shore and extending out from the reef. Sampling locations at all depths were georeferenced, remained fixed for the two-year monitoring period, and were chosen regardless of substrate type.

The steel rod photoquadrat frame used in Lake Michigan to assess benthic fish abundance.

In 2012, Round Goby were sampled using standard Gee minnow traps as described for Rusty Crayfish in Objective 1. For underwater video monitoring in 2012, Round Goby abundance was measured with a SeaViewer® Sea-Drop 950 underwater video camera (www.seaviewer.com). The unbaited video camera was attached to the top of a steel rod frame (height = 1.26 m; base = 0.25 m^2) facing downward so that the image encompassed the entire base. The video camera was suspended approximately 16 cm from the top of the frame. At each depth, the video camera was lowered to the bottom, and continuous video was recorded for one minute. Ten one-minute videos were recorded for each depth stratum. Each video camera drop was 1 m apart. All videos were processed by counting the number of Round Goby present in four time intervals (0–15, 15–30, 30–45 and 45–60 sec) as well as the total throughout the video. If a Round Goby left the field of view but returned, the individual was assumed to be a new fish. The 'mean maximum' number of Round Goby (i.e., the average of the maximum number of Round Goby observed in the field of view across each of 10 replicate camera drops at each site) was calculated for analysis.

In 2013, the video monitoring methodology was adapted when smaller more mobile underwater cameras (GoPro HERO 3®) became available and additional field trials indicated longer soak times provided a more robust measure of goby relative abundance (Robinson *under revision*). Each camera (total n = 5) was mounted at the top of a steel camera frame with a quad-pod base (height = 60 cm; base = 0.45 m^2). The camera lens was aimed at the substrate and captured a field of view approximately 1 m^2 (photoquadrat). Each camera frame was baited with previously collected, frozen, and thawed Lake Trout eggs (~30 g) contained in a mesh bag suspended approximately 5 cm from the substrate in the center of the photoquadrat (Figure 4.1). The cameras were deployed and goby counted as described for crayfish in Objective 1. Briefly, five cameras were individually buoyed, positioned 10 m apart at each depth, and recorded one photograph per minute for 20 minutes. In the laboratory, the number of Round Goby in each image (at one minute intervals) was counted. Two technicians independently analyzed images in the laboratory and any discrepancies in Round Goby counts were settled after review by a third technician. The maximum number of Round Goby recorded during the 20 minute period was used for analysis.

In 2012, index monitoring for Round Goby using egg bags occurred biweekly from mid-July to early December. In both 2012 and 2013, Round Goby abundance as bycatch in separate egg bags deployed by Michigan DNR (typically to monitor Lake Trout and Lake Whitefish egg deposition) was also measured but only on two dates (early November and early December). Egg bag deployment and recovery is described fully in Objective 1.

Dreissenid relative abundance was measured at the Crib, ER North, ER Central, and Bay Harbor on a monthly basis in 2013 only, from end of May to mid-December. Relative abundance was measured from still images taken by SCUBA divers at each location and was equal to the percent of substrate covered in randomly placed 1 m^2 quadrats. *Hemimysis* density (#/m^2) and density of other common benthic invertebrate taxa was measured at each location using egg funnels. Egg funnels were constructed of perforated stainless steel with a 58 x 58 cm base and a depth of 30 cm (as described in Barton et al. 2011). Egg funnels were deployed in June and placed 4 m apart in an array across each reef. Funnels were pumped to collect invertebrates one time per month from late June to December in both 2012 and 2013. Each funnel was pumped for a minimum of 90 s to ensure that the entire sample was suctioned from the egg funnel to the catch filter; pumping continued if material was observed in the hoses after the initial 90 s. After the entire sample was collected in the catch filter, the sample was washed in a collection bucket and transferred to a sample jar. All samples were kept in fresh lake water and processed within 24 h of retrieval. Samples were sorted by eye in a white tray and individual animals identified and assigned to an operational taxonomic unit under binocular microscope.

Statistical analysis

We used simple linear regression models to estimate changes in catch rates over time during removal in 2012 and to estimate changes in male:female (M:F) sex ratios during removal in both years. Limited time series for removal activities precluded analysis of changes in catch rates in 2013. Analysis of covariance (ANCOVA) was used to compare capture numbers from index monitoring (egg bags, traps, cameras, and quadrats) over time and among sites and to compare CPUE between treatment and reference sites (see Table 1.1). One-way ANOVA was used to compare mean size over time from removal efforts at each site.

RESULTS

Round Goby:
Intensive removal efforts

We removed more than 8,600 Round Goby from the 4 treatment reefs (Figure 4.1). Ninety-nine percent of the Round Goby removed from the reefs were taken in 2012 when minnow traps were deployed intensively. Of the goby captured in 2012, thirty-nine percent were taken at the Crib (n= 3,364), with 20%, 26%, and 15% of total capture occurring on ER North, ER Central, and ER South respectively. Removal efforts in 2013 focused largely on Rusty Crayfish, but a small number of Round Goby were captured and removed during minnow trap sets: ER North (n=82), ER Central (n= 4) and ER South (n= 7). Removal efforts in 2013 occurred later than efforts in 2012, and were limited to just two removal events with minnow traps (on 29 Oct and 20 Nov;).

Index monitoring

There was no effect of treatment (i.e., reference versus treatment) on Round Goby CPUE using baited minnow traps in 2012 ($F_{1,536} = 0.02$, $P = 0.88$), but Round Goby CPUE did generally increase over time across all sites ($F_{1,536} = 5.04$, $P = 0.03$;), driven by peak abundances at most sites in early October (Fig. 4.2). Trends in Round Goby CPUE over time did not differ between treatment and reference sites ($F_{1,536} = 1.52$, $P = 0.22$).Round Goby CPUE declined significantly over time on average across all sites in 2013 ($F_{4,189} = 12.57$, $P = 0.0005$; Figure 4.3). The decreasing CPUE was significantly correlated with declining temperatures over the course of 2013 index monitoring ($t = 3.09$, $P = 0.02$). The decline in CPUE was significantly greater in the lone treatment site (ER North) than at the reference sites ($F_{1,195} = 6.13$, $P = 0.01$;), but Round Goby CPUE at ER North was more than double that of any other location at the start of 2013 monitoring (Figure 4.3), which suggests that the difference between treatment (i.e., ER North) and reference site CPUE is likely an artifact of the large difference in initial CPUE, rather than a real treatment effect.

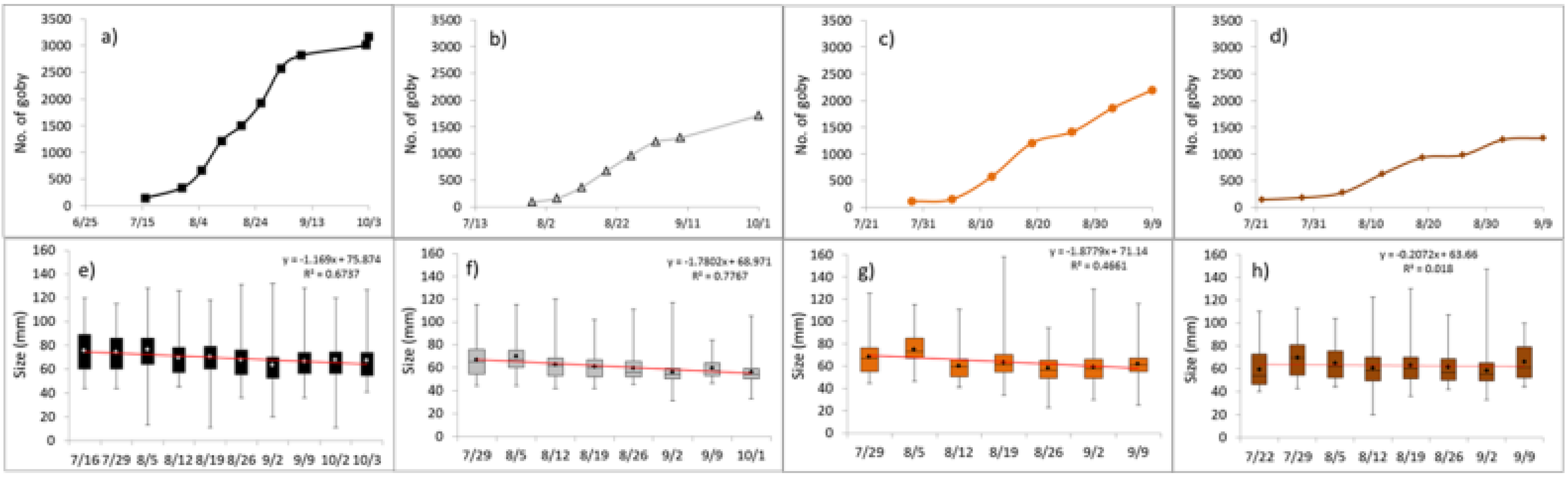


Figure 4.1. Cumulative goby capture at all removal sites in 2012 (panels a-d), and boxplots of goby size (panels e-h). Crib (a,e), ER North (b,f), ER Central (c,g), and ER South (d,h). Significant differences in mean size over time were observed at all sites.

Index monitoring with baited cameras in 2012 showed no effect of treatment on the mean maximum density of Round Goby ($F_{1,533}$ = 2.82, P = 0.09) (Fig. 4.4). There was a significant decline in the mean maximum density of Round Goby across all sites ($F_{1,533}$ = 44.83, P < 0.0001;), and the trend over time did not differ among sites ($F_{1,533}$ = 0.44, P = 0.50). The decreasing CPUE was significantly correlated with declining temperatures over the course of 2012 index monitoring (t = 4.55, P = 0.0001). Maximum Round Goby density also declined significantly on average across all sites in 2013 ($F_{1,110}$= 25.96, P < 0.0001;). Again, the decreasing CPUE was significantly correlated with declining temperatures over the course of 2013 index monitoring (t = 2.71, P = 0.03) (Fig. 4.5). As in 2012, there was no effect of treatment condition on CPUE in 2013 ($F_{1,110}$= 0.60, P =0 .44) .

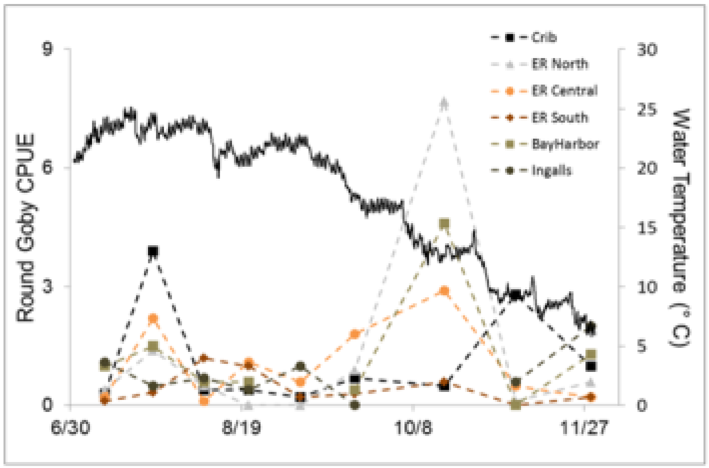


Figure 4.2. Mean Round Goby CPUE from bi-weekly index monitoring with baited minnow traps in 2012. Solid line is water temperature averaged across five of the six sites (Crib, ER Central, ER South, Bay Harbor, and Ingalls)

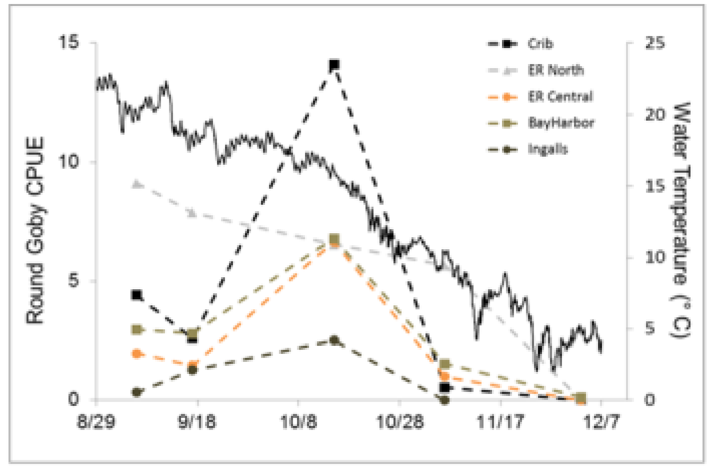


Figure 4.3. Mean Round Goby CPUE from bi-weekly index monitoring with baited minnow traps in 2013. Solid line is water temperature averaged across four of the five sites (ER North, ER Central, ER South, and Ingalls).

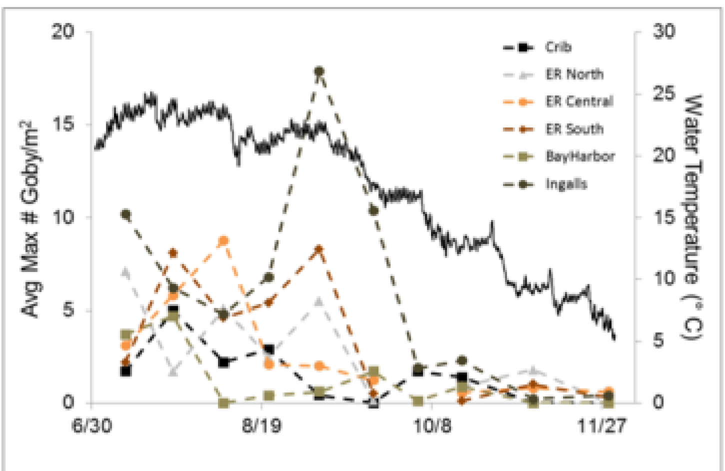


Figure 4.4. Mean maximum number of Round Goby per square meter from index monitoring with baited camera frames in 2012. 'Mean maximum' is calculated as the average of the maximum number of crayfish observed in the field of view across each of 10 replicate camera drops at each site. Solid line is water temperature averaged across five of the six sites (Crib, ER Central, ER South, Bay Harbor, and Ingalls)

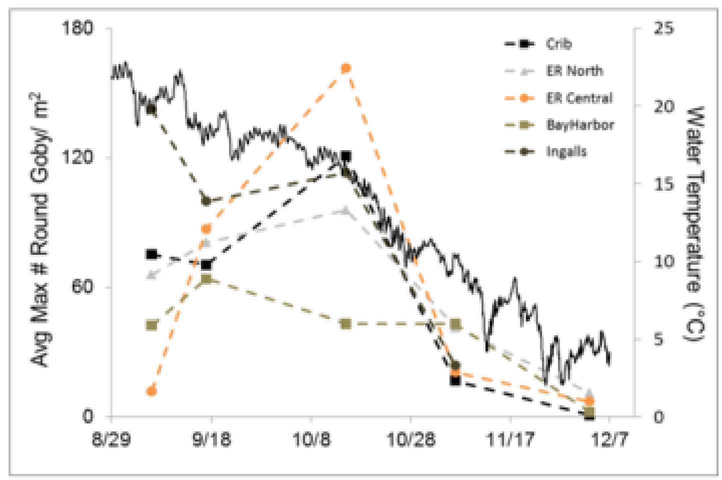


Figure 4.5. Mean Maximum number of Round Goby per square meter from index monitoring with baited camera frames in 2013. Mean maximum' is calculated as the average of the maximum number of crayfish observed in the field of view across each of 5 replicate camera drops at each site. Solid line is water temperature averaged across four of the five sites (ER North, ER Central, ER South, and Ingalls).

Plots of CPUE and maximum Round Goby density (#/m^2) across 3, 6, and 9 m depths from minnow traps and baited cameras in both 2012 and 2013 show no evidence of seasonal migrations of Round Goby from shallow to deep water or vice versa (Appendix Figs. A4.1,A4.2, A4.3, & A4.4). Seasonal peaks in Round Goby densities were either not observed (e.g., Appendix Fig. A4.1, ER South) or occurred across all depths simultaneously (e.g. Fig. A4.2, ER Central). In 2013, when water temperatures reached about 5^{0}C (approximately December), we were unable to catch Round Goby in minnow traps at any depth and very few Round Goby were observed with baited underwater videos. However, diver observations and quantitative counts undertaken in association with Rusty Crayfish monitoring in December 2013 indicate that Round Goby were still resident on the reefs (Table 4.1). Round Goby were generally under rocks, immobile, and easily captured by hand.

Table 4.1. Abundance of Round Goby on rocky habitats as measured by dives from 10 1m^2 quadrats on December 3, 2013

Site	Total #	Goby/m^2	SE
ER North (spawning reef)	16	1.6	0.5
ER Central (1-2m)	5	0.5	0.2
ER Central (3m)	7	0.7	0.4
ER Central (15 m)	16	1.6	1.0

Biweekly egg bag monitoring suggests that interstitial densities of Round Goby did not exhibit a significant trend over time on average across all sites ($F_{5,649} = 0.61$, $P = 0.44$; Fig 4.6), but average relative densities were lowest during the week of September 19 (0.05 Round Goby· egg bag^{-1} ± 0.02 SE) and highest during the week of December 3 (0.29 Round Goby ·egg bag^{-1} ± 0.13). The lack of a significant trend across sites can be attributed to significant differences in trends among sites ($F_{5,649} = 6.56$, $P < 0.001$), with Ingalls having a significant upward trend that differs from the downward trend exhibited by ER Central. Round Goby density also increased in DNR egg bags from November to December in 2012 (except at Bay Harbor and ER North where no Round Goby were captured; Fig. 4.7). This trend did not hold for 2013, when December egg bag captures were lower than November captures for all sites where egg bags were surveyed on both sampling dates. However, adverse weather conditions precluded collection of egg bags at all sites in 2013.

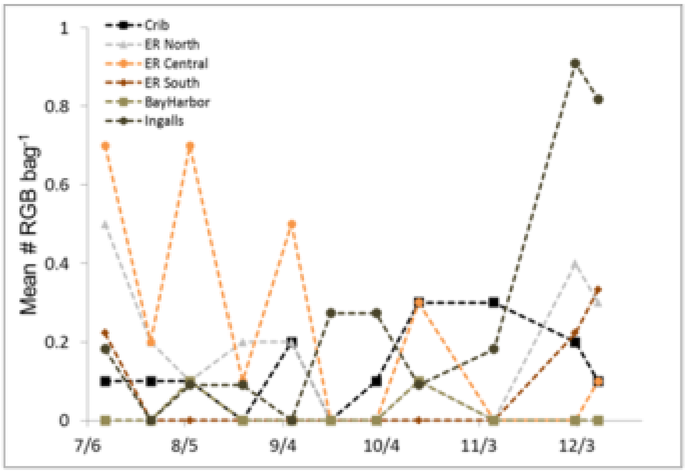


Figure 4.6. Mean catch (#RGB bag-1) from egg bags deployed for bi-weekly sampling on each reef in 2012.

Table 4.2. Quartiles for goby size at the beginning and end dates of removal efforts in 2012. Removal dates were as follows: Crib- July 16 to Oct 3, ER North- July 29 to Oct 1, ER Central- July 29 to Sept 9 & ER South- July 22 to Sept 9.

Site	Min	Q1	Median	Q3
Crib	44/41	60/54.5	75/62	89/74
ER North	44/33	54/50	65/54	76/59
ER Central	44/25	55/55	67/61	76/67
ER South	40/44	46/52	54/61	73/79

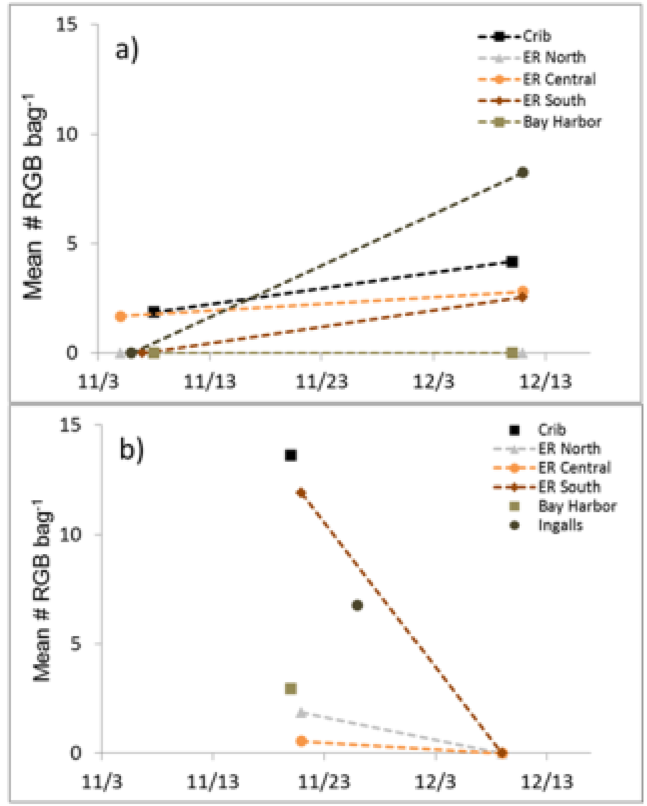


Figure 4.7. Mean catch (#RGB bag^{-1}) from egg bags deployed by MI DNR on each reef in 2012 (a) and 2013 (b).

Size Frequency

Mean length of Round Goby varied significantly over time at all four removal sites and generally declined as cumulative number of goby captured increased (Fig 4.1). At the end of removal efforts, a substantial proportion of Round Goby present on each reef were smaller than 54 mm (total length), a threshold below which Round Goby may be unable to consume Lake Trout eggs (Chotkowski & Marsden 1999). For example, 50% of goby at the ER North site were ≤ 54 mm in total length, and at three other sites (Crib, ER Central, and ER South), approximately 25% of captured Round Goby were ≤ 54 mm (Table 4.2). The smallest Round Goby at ER Central was 25 mm, which is smaller than the threshold below which Round Goby are unable to feed on Lake Whitefish eggs (i.e., 32 mm; MDNR unpublished data).

Non-target fish and crayfish

A total of ten fish and crayfish taxa were observed by one or more of the three goby/crayfish index monitoring methods (Table 4.3). Across both years, non-target species were rarely observed and collectively they accounted for less than 10% of all crayfish and fish recorded. We found no evidence that goby or crayfish removal efforts resulted in a positive or negative impact on non-target fish and crayfish taxa.

Table 4.3. Bycatch abundance by gear type from July-December 2012 and September-December 2013 in Grand Traverse and Little Traverse bays, Lake Michigan. Abundance measures for each species reflect gear set times: 2012 minnow traps (24 hour soak times), egg bags (2 weeks), underwater video (1 minute counts); 2013 minnow traps 1.5 hour soak times, baited underwater video (20 minutes).

Common Name	Scientific Name	2012 Minnow Traps	2012 Eggs Bags	2012 Underwater Video	2013 Minnow Traps	2013 Baited Photo Quadrats
Sampling effort		x	y			
Northern Clearwater Crayfish	*Orconectes propinquus*	3	2	—	—	—
Virile Crayfish	*Orconectes virilis*	18	—	—	—	—
Lake Chub	*Couesius plumbeus*	23	—	—	—	—
Emerald Shiner	*Notropis atherinoides*	—	2	—	—	—
Largemouth Bass	*Micropterus salmoides*	2	—	—	—	—
Rock Bass	*Ambloplites rupestris*	62	1	—	3	16
Smallmouth Bass	*Micropterus dolomieu*	11	2	37	1	37
Bluegill	*Lepomis macrochirus*	—	—	—	—	1
Spottail Shiner	*Notropis hudsonius*	17	—	—	1	—
Cyprinid spp.		8				
Subtotal non target taxa		**144**	**7**	**37**	**5**	**54**
Round goby	*Neogobius melanostomus*	1674	102	485	2805	88887
Rusty crayfish	*Orconectes rusticus*	325	169	1	65	
Total		**2143**	**278**	**523**	**2875**	**88941**

Non target invertebrates

Macroinvertebrates collected from egg funnels were dominated by seven taxonomic groups (Table 4.4). We documented considerable seasonal variation both within and among sites in the abundance of all common taxa collected (see Appendix Figures A4.5 – A4.11) but observed no evidence that the removal of Rusty Crayfish or Round Goby at any site had an effect on abundance of these taxa (Figure 4.8) or on *Dreissenid* abundance (Figure 4.9).

Table 4.4. density (#/m²) of A) *Hemimysis anomala,* B) Amphipoda, C) Isopoda, D) Ephemeroptera, E) Annelida, F) Chironomidae, and G) *Bythotrephes longimanus* captured in egg funnels in 2012 and 2013 at the Crib, ER North, ER Central, ER South, Bay Harbor and Ingalls during pre- and post treatment periods. The number of samples is represented by n.

Site	Year	Pre/Post Treatment	n	*Hemimysis* (#/m²)	Amphipoda (#/m²)	Isopoda (#/m²)	Ephemeroptera (#/m²)	Annelida (#/m²)	Chironomidae (#/m²)	*Bythotrephes* (#/m²)
Crib	2012	Pre	10	2.97	0	0.3	0	0	3.86	0
		Post	15	8.72	5.55	0	0	0.2	0.59	0
	2013	Pre	10	0	0	4.76	0	0	0.59	0
		Post	15	0.59	0.2	0	0	0	0.2	0
ER North	2012	Pre	11	1.35	0.54	0	0.27	0	0.81	0
		Post	19	3.29	2.03	0.47	0.16	1.72	11.58	0.16
	2013	Pre	5	0	0	0	0	0	1.78	0
		Post	25	0.48	0.48	0.59	0.36	0.12	8.32	0
ER Central	2012	Pre	10	13.38	0.3	0.3	0	0	1.78	0
		Post	20	14.57	1.63	0.45	0	0.45	1.04	0
	2013	Pre	5	0.59	0	0	0	0	2.38	0
		Post	25	9.87	0.95	0	0	0.36	2.85	0
ER South	2012	Pre	10	2.97	5.65	1.49	0	2.08	5.95	0
		Post	20	1.19	6.09	2.82	0.3	2.53	25.86	0
	2013	Pre	5	0	0	0	0	0	0	0
		Post	25	12.25	1.66	0.59	0.12	10.82	20.81	0
Bay Harbor	2012	Pre	10	74.02	1.49	5.65	0	0	1.49	0
		Post	25	7.37	7.61	0.36	0.24	0	0.12	0
	2013	Pre	10	4.46	3.27	4.76	0	0	1.19	0
		Post	20	3.12	7.73	0	0.15	0.15	0.45	0
Ingalls	2012	Pre	10	0	0.59	2.68	0	0	0.3	0
		Post	25	4.4	1.43	2.38	0	0	0	0
	2013	Pre	5	0	0	0	0	0	0	0
		Post	25	0	0.12	2.73	0	0	0	0

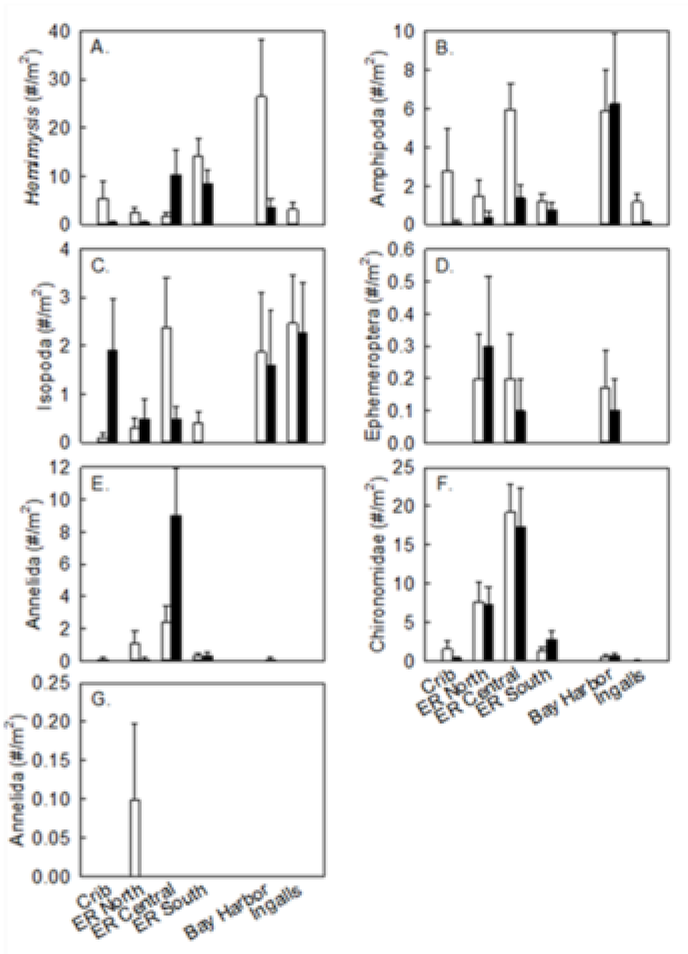


Figure 4.8. Density (mean ± SE) of A) Hemimysis anomala, B) Amphipoda, C) Isopoda, D) Ephemeroptera, E) Annelida, F) Chironomidae, and G) Bythotrephes longimanus captured in egg funnels in 2012 (solid bars) and 2013 (open bars) at Crib, ER North, ER Central, ER South, Bay Harbor, and Ingalls.

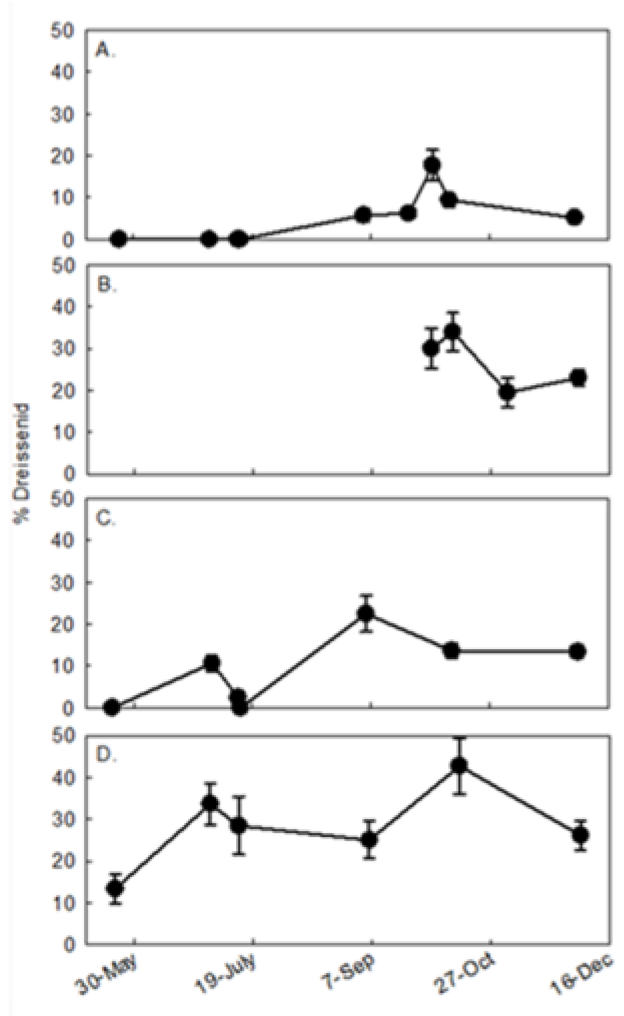


Figure 4.9. Mean (±SE) % of substrate covered by dreissenid mussels found in 1m2 quadrats sampled by scuba diving at A.) Crib, B.) ER North, C.) ER Central, and D.) Bay Harbor.

DISCUSSION

Over the course of just two months of intensive removal efforts in 2012, we removed more than 8,000 Round Goby from across 4 treatment reefs. Despite the substantial number of Round Goby captured in 2012, there is no evidence to suggest that our removal efforts reduced Round Goby densities on any of the four treatment reefs. However, the average size of Round Goby (i.e., total length) declined significantly over the course of removal in 2012, suggesting that our removal efforts may have been sufficient to induce a change in Round Goby population demographics on the reefs, perhaps as a result of reef colonization by juvenile Round Goby immigrating from outlying suboptimal habitat (see below for further discussion). Our study results from 2012 imply that sustained intensive trapping, while potentially unable reliably reduce Round Goby densities in the short term, may be sufficient to alter their size structure on spawning reefs, with important implications for consumption of native fish eggs.

Round Goby densities vary widely in the Great Lakes (Table 4.5). Chotkowski & Marsden (1999) reported densities in Calumet Harbor, Lake Michigan, as high as 133 m^{-2} on sand (where only juveniles were found) and of adult fish on cobble at 3.35 m^{-2} (on average). In our study, Round Goby mean density estimates on the spawning reefs ranged 1.0 - 60.1 fish m^{-2} (Table 4.5). We suspect that the variability in our density estimates primarily reflects the varying sensitivity of our survey methods (see Robinson 2014). While the accuracy of our estimates is unknown, it is likely that the range we report encompasses the true density of Round Goby on the reefs. Minnow traps and underwater video have been used to estimate fish abundance elsewhere (He & Lodge 1990, Bryant 2000, Cappo et al. 2006), and numerous studies have recommended the use of multiple methods concurrently to obtain more accurate estimates of overall abundance (e.g., Connel et al. 1998, Bacheler et al. 2013). Furthermore, density estimates from three of our four survey methods were within the range reported from the above studies, which implemented underwater visual census methods using SCUBA or remotely operated video surveys.

Assuming that our Round Goby density estimates are within the range of those reported for similar substrates elsewhere in the basin, it is possible that we removed from the reef as many Round Goby as were present at the start of our trapping efforts. In 2012, for example, we removed 3,364 Round Goby from the Crib, which is almost twice the number of Round Goby that may have initially been resident on that reef (area of Crib reef = 375 m^2, mean density= 4.5 fish m^{-2}, estimated abundance = 1,725 fish). This is significant because previous research suggests that larger Round Goby may exclude smaller fish from preferred rock habitats (Ray & Corkum 2001).

Table 4.5. Round goby mean density estimates on rock habitat from our study and other published studies in the Great Lakes. The range reported for Ray & Corkum reflects the density range for small (< 5cm) to large (>5cm) fish, while the reported range from our study is a result of the various sampling methods employed during monitoring (e.g. minnow traps vs. baited underwater video). Estimated abundance is based on a reef area of 375 m^2, which is the approximate surface area of the Crib reef which measures 25 m long by 15 m wide.

	Chatkowski & Marsden 1999	Ray & Corkum 2001 (St. Clair)	Ray & Corkum 2001 (Detroit R.)	Johnson et al. 2005	Our study
Density (no. m^{-2})	3.35	5.0 – 9.0	0.3 – 3.0	6.94	1.0 – 60.1
Estimated abundance (375 m^2)	1256	1875 – 3375	113 – 1125	2603	375 – 22500

In fact, numerous studies have documented that mean Round Goby size is highest on rock and till substrates and lower on mud or sand (Charlebois et al. 1997, Chotkowski & Marsden 1999, Ray & Corkum 2001, and Johnson et al. 2005), which may explain why we observed a decline in mean Round Goby size on all of our treatment reefs over the course of removal efforts in 2012. As large Round Goby are extracted from the reef, the smaller Round Goby occupying outlying sand or soft bottom habitats would have been able to colonize newly available rock substrate on the reef. Mean Round Goby size from the first three removal events compared to the last three removal events declined by more than 8mm at the Crib (75.0 to 66.7 mm), by 9.4 mm at ER North (66.0 to 56.6 mm), by 8 mm at ER Central (67.7 to 59.7), and by 2.6 mm at ER South (64.4 to 61.8 mm).

Because only Round Goby of a certain size are able to consume Lake Trout and/or Lake Whitefish eggs, the change in mean Round Goby size that we report may have reduced predation pressure on native fish eggs in 2012. Chotkowski & Marsden (1999) concluded that the ‘critical’ size below which Round Goby cannot ingest a Lake Trout egg was between 50 mm and 56 mm (total length). Based on the relationship between Round Goby total length, standard length, and gape height (derived from Marsden et al. 1997 and Ray & Corkum 1997) and our own observations of Lake Trout and Lake Whitefish egg diameter (unpublished data), we estimate that only fish above 54 mm and 32mm total length can consume Lake Trout and Lake Whitefish eggs, respectively. Using the latter criteria, this means that 50% of Round Goby at the ER North site could not feed on Lake Trout eggs, compared with just 25% of the population that were too small at the start of removal efforts (Table 4.2). Shifts of that magnitude are not evident at the three other treatment sites, but total lengths of the 25th percentile at each of the Crib, ER Central, and ER South were close to the 54 mm Lake Trout threshold at the conclusion of removal efforts. It is also worth noting that our diver observations, both in December at the onset of winter and then again in early May at the end of winter, suggest resident Round Goby present on the reef are not active and indeed can be caught by hand.

Unfortunately, minnow traps were used sparingly for removal efforts in 2013, in part due to increased emphasis on testing the seismic technology (described in Objectives 2 and 3) but also because inclement weather prevented our Rusty Crayfish removal teams from getting out on the water immediately prior to and during spawning (mid-October through November) when we had aimed to trap the reefs intensively. Consequently, we deployed minnow traps on only two occasions, and only a small number of Round Goby were removed from the spawning reefs in 2013. Additional research is needed to determine whether the shift towards smaller Round Goby on the reefs can be sustained over successive years of intensive trapping and to assess whether trapping might ultimately reduce goby population size through the selective removal of larger, more fecund adult fish (Corkum et al. 1998). In addition, on discrete reefs like the Crib and ER North, it may be possible to augment trap removal by using seines or bottom trawls on sand habitats around the reef.

Despite their status as a prolific invader and increasing evidence of their importance as prey for a variety of Great Lakes fish, it is clear that there is still much to learn about Round Goby biology and the potential for management. To date, management efforts have largely focused on extensive prevention campaigns and early detection efforts. Various control options (mostly for repelling Round Goby) have been proposed or tested experimentally, including piscicides, electric barriers, acoustic devices, and pheromone strategies (Savino et al. 2001, Corkum 2004, and Rollo 2007, Schrier et al. 2008). More recently, evidence for predator control of Round Goby has been documented in Lake Erie, suggesting the potential for application of a biological control agent (Madenjian et al. 2011). However, the only other attempt that we are aware of to actively manage Round Goby in the Great Lakes basin occurred in 2005 when the Ontario Ministry of Natural Resources (OMNR) applied the chemical piscicide rotenone to a 5 km stretch of Pefferlaw Brook as part of an ad hoc rapid response effort intended to eliminate Round Goby from the waterway and prevent spread to nearby Lake Simcoe (Dimond et al. 2010). The effort was not successful.

The general dearth of information on Round Goby control is part of what makes the results of our study compelling, since we show that intensive control has the potential to induce changes at the population level that could ultimately reduce the impact of Round Goby on native fish spawning reefs. Sustained, baited trapping was implemented successfully to control the invasive Rusty Crayfish in a small temperate lake (Hein et al. 2006). Since Round Goby are susceptible to the same trapping method (this study; see also Diana et al. 2006 and Kornis & Vander Zanden 2010), it has been suggested that a sustained trapping effort could also be effective to control Round Goby in a small, bounded ecosystem (Kornis et al. 2012). While the Great Lakes are hardly a ‘small, bounded ecosystem’, the spawning reefs in Grand and Little Traverse Bays may represent ideal areas for refining and demonstrating the efficacy of physical removal methods to control Round Goby. Round Goby are typically sedentary with small home ranges (generally <10 m and likely not more than 67 m; Ray & Corkum 2001, Wolfe & Marsden 1998). Round

Goby also have a high tendency towards site fidelity in rock habitats (Bjorkland & Almqvist 2010; Ray & Corkum 2001). While others (e.g., Walsh et al 2007) have noted (or inferred) a seasonal offshore migration of goby to overwinter, we observed no evidence of this behavior during our index monitoring (e.g., see Appendix Figures A4.1-A4.4 and Robinson 2014). In fact, our egg bag surveys and diver counts and observations indicate that Round Goby are still present on the reef beneath rocks in November, December, and also in early May when water temperatures are still below 5^0 C. Taken together, these observations indicate that, while not bounded, the spawning reefs and an outlying buffer area may be composed of largely resident Round Goby, which suggests that active trapping on the reef and over an outlying buffer area of not more than 200 m (approximately three times the maximum home range) could significantly reduce Round Goby densities on the spawning reef. The chances of successful reduction would be further enhanced if control efforts could be coupled with a repellant or barrier that reduced recolonization rates, and or if predation by native fish predators could be enhanced.

Scuba diver undertaking 1m^2 crayfish count on spawning reef habitat, Elk Rapids, 2013. Photo Eric Calabro CMU

<u>Objective 5</u>. Quantify changes in Lake Trout, Cisco and Lake Whitefish egg deposition and survival.

Native fish stocks of the Great Lakes experienced substantial declines during the 1940s through the1960s from anthropogenic stressors, invasive species, and unregulated fishing. Progress in fish population recovery, in general, can often be limited to a single life history stage or trait. In the case of native fish in the Great Lakes, including Lake Trout and Coregonid spp. (Cisco and Lake Whitefish *)*, rehabilitation is often linked to failed recruitment processes during the early life stage. The potential reasons for limitations in the early life stage can be attributed to minimal egg production levels because the adult biomass is too low, and native fish in the Great Lakes are relatively slower growing with delayed maturation. For example, Lake Trout approach 100% maturation by age 9 in Lake Michigan. Slow maturation rates are also coupled with a long egg incubation period for the aforementioned native fishes. Lake Trout eggs are typically deposited in late October or early November, Coregonid spp. eggs are deposited after Lake Trout in mid-November to early December and the eggs must overwinter in the harsh Great Lakes environment until fry emergence after ice out in late March to early April.

There has been substantial effort in the restoration of Lake Trout spawning stocks via wide-scale stocking efforts. In addition, spawning stocks of Lake Whitefish have improved, indirectly, through activities by management agencies to mitigate for or limit anthropogenic stressors and directly through the regulation of the fishery through catch quotas and control of Sea Lamprey. However, the recruitment of these native fish is still limiting recovery (i.e., Lake Trout) or recruitment success is highly variable (i.e., Lake Whitefish) causing management agencies to be concerned about the stability of stocks. The early life stage dynamics become problematic to native fishes not only when egg deposition rates are too low but also when other factors influencing egg mortality increase. Although progress has been made in native fish recovery in the Great Lakes, new stressors may reverse the progress made by negatively impacting egg survival rates.

Egg mortality can be classified as either abiotic, caused by physical-force stress (e.g., currents, wave energy), or biotic through direct predation of eggs post egg deposition. The introduction of several invasive species in the past decade in the Great Lakes may be impacting both the abiotic and biotic stress on native fish eggs. Abiotic stress will impact eggs by dislodging them from spawning habitats or reefs. Quality spawning habitat will consist of rock with interstitial spaces that can protect eggs and is produced with optimal substrate size (8–10 cm diameter) and shape (more uniform or rounded). These characteristics will provide uniform and less variable sizes of interstitial space thereby potentially limiting access by interstitial predators (e.g., Rusty Crayfish, Round Goby, and sculpins *Cottus* spp.), which are often abundant on shallow, rocky habitats (Claramunt et al., 2005; Jonas et al., 2005; Fitzsimons et al., 2007). With the introduction of Dresseinid mussels, interstitial spaces are being altered by the colonization of mussels which could change egg vulnerability to physical forces or predation, and interstitial water quality.

In addition to habitat changes from Dresseinid mussels, spawning reef habitats in Lake Michigan are colonized by a new suite of egg predators. The Round Goby and the Rusty Crayfish dominate spawning habitats and have infiltrated interstitial spaces on reef complexes. Several studies have suggested that their predation rates on native eggs can deplete egg levels within weeks of spawning; especially when egg deposition rates are low. Recognizing this limitation, Lake Trout rehabilitation plans have set targets for egg deposition rates to overcome such challenges. The Lake Michigan rehabilitation plan has a target of detecting a minimum density of 500 viable eggs/m^2 in rehabilitation areas by 2015 and in non-rehabilitation areas in 2025. Although not specified in rehabilitation plans, recovery of Coregonid spp. stocks is also dependent on the recruitment success beginning with egg survival post the deposition period.

The goal of this objective was to use egg densities as a metric to evaluate the survival bottleneck in the egg stage of native fish in Lake Michigan. As part of a study, but described in Objectives 1 and 4, control efforts were established to reduce the impacts of invasive species on a subset of spawning reefs in northern Lake Michigan. Herein, we compare the natural egg deposition rates on those reefs compared to a set of control (non-treated spawning reefs) to determine the influence of predator reduction.

METHODS

Reef Locations

Sampling was conducted on active fish spawning reefs in Grand Traverse and Little Traverse Bays in northeastern Lake Michigan (Figure 1). Six study sites were evaluated for changes in natural egg deposition rates and survival of native fish eggs during the spawning period. Three study sites (ER North, ER Central, and ER South) were located on a reef complex (44°54’N, 85°25’W) in the eastern arm of Grand Traverse Bay near Elk Rapids, Michigan, and one study site (Ingalls Point) was located in the western arm of Grand Traverse Bay near Ingalls Bay (45°04'N, 85°34'W; Figure 1). Of the two study sites located in Little Traverse Bay, one site (Crib) was located in northern Little Traverse Bay near Harbor Springs, Michigan (45°25'N, 84°56'W), and the other site (Bay Harbor) was located in southern Little Traverse Bay near Bay Harbor, Michigan (45°22'N, 84°59'W; Figure 1). Predator removal and control versus treatment sites are described in Objectives 1 and 4. Rusty Crayfish and Round Goby control efforts ranged from intensive removal on the Crib and ER North sites and reduce levels of suppression occurring on ER Central and low levels of ER South with Ingalls and Bay Harbor sites acting as our non-treatment control sites (Table 1.1, Objective 1 above).

Spawning Fish Assessment

Graded-mesh experimental gill nets were used on each of the 6 reefs, and set every other week in 2012 and 2013 starting in the first week of October and ending in mid-December for a total of 5 gill net sets per reef site. Gill nets were constructed of five 30.5 m panels with monofilament mesh graded from 6.4 to 11.4 cm in 1.3-cm increments; all nets were set for 24 h at the same location during each sampling event in 2012 and 2013. Fish were identified to species and spawning condition was recorded. Gill nets were used to estimate relative catch-per-effort (CPE; number per 305-m) for Cisco, Lake Trout, and Lake Whitefish in the general location of the spawning reefs. To estimate species specific and total CPE, we estimated the mean (±SE) CPE for each reef and year and then we compared CPE estimates with a two-way ANOVA to evaluate differences in the relative abundance of spawning fish.

Natural Egg Levels

Natural egg levels were assessed with standard egg bags at each of the six spawning reefs in 2012 and 2013 to measure egg deposition as per Perkins and Krueger (1994). These assessments built on previous monitoring at these sites that began in 2008, results of which are included here. A target of 30 egg bags were buried along a single transect approximately 1m apart crossing the primary suitable spawning habitat at each site. Egg bags were buried by scuba divers approximately 1 month prior to peak spawning to allow time for acclimation. Bags were retrieved post-spawning and eggs were identified to either Lake Trout or Coregonid spp. and counted. Because of the similarity of Coregonid spp. eggs, differentiation between Cisco and Lake Whitefish eggs based on size and color was not possible. Instead, we identified these eggs collectively as Coregonid spp. for this study. Egg bags measure production (deposition minus egg loss) because the nets are open to the environment and eggs will experience both physical displacement and predation mortality. Natural egg levels at the end of the spawning season (indexed by egg bag retrieval) will be quantified as eggs per m^2 based on the surface area of the egg bag.

Seeding Study

Because low natural egg levels were expected, we seeded a sub-set of the spawning reefs (Crib and all ER sites) with artificial and live Lake Trout eggs to estimate sources of egg mortality. In addition to the egg nets deployed on the reefs to estimate natural egg levels, we deployed five egg funnel collection devices on each reef. An egg funnel is a new sampling gear that combines a passive trap with a suction sampler to collect eggs from a known area of substrate via diaphragm pump (Barton et al. 2011). Each funnel has a 6 m reinforced hose running from the base of each egg funnel and is placed near the base of the reef drop-off. The hose is weighted, but an attached line and buoy allowed retrieval of the hose for pumping egg funnels to collect samples. Each funnel was pumped for a minimum of 90 s to ensure that the entire sample was suctioned from the egg funnel to the catch filter; pumping continued if material was observed in the hoses after the initial 90 s. After the entire sample was collected in the catch filter, the sample was washed in a collection bucket and transferred to a sample jar. All samples were kept in fresh lake water and processed within 24 h of retrieval.

Egg funnels and egg bags were seeded with artificial Lake Trout eggs (6 mm - diameter, orange-plastic beads) to estimate loss from physical forces and with live egg to test for predation from interstitial egg predators during spawning. Divers seeded the sampling gear by opening vials of live and artificial eggs approximately 5 cm above the substrate centered on each egg funnel or egg bag to mimic natural egg deposition into the substrate contained in each gear. Settling velocity of artificial Lake Trout eggs (~7.2 cm/s) was similar to that of natural Lake Trout eggs (~7.8 cm/s; Barton et al. 2011). All egg funnels and 15 egg bags from each site were seeded with 20 artificial Lake Trout eggs to test for loss from physical forces and live eggs to test for predation loss. For the live eggs, we maintained an equal seeding density (mean = 288.38 eggs/m^2; Barton et al. 2011) of 100 Lake Trout eggs per funnel and 20 Lake Trout eggs per egg net. Because seeding should mimic natural egg deposition patterns, we used the egg funnels to estimate peak Lake Trout spawning because the funnels can be sampled periodically during the spawning period. We targeted every other week to estimate peak spawning and seeded the live eggs when funnel collections indicated spawning was occurring. To differentiate seeded eggs from natural eggs, we obtained Lake Trout eggs from the U.S. Fish and Wildlife Service Sullivan Creek National Fish Hatchery, Brimley, Michigan. The eggs were collected from Seneca Lake strain of Lake Trout that were spawned on October 2, 2013. The eggs were approximately 45% developed at the time of seeding and had already developed to the eyed-egg stage resulting in a seeded egg that was distinguishable from wild eggs based on their advanced development.

For both estimates of natural egg deposition and seeding recovery from egg bags, the egg bags were recovered after peak spawning occurred, as estimated based on gill-net catches of spawning fish and egg funnel collections. Scuba divers carefully removed the substrate from the egg bags and then closed the egg bags with cable ties to prevent loss of eggs (Perkins and Krueger 1994; Fitzsimons et al. 2007). Any detected losses were noted by divers and recorded at the surface. Similar to the egg funnels, all egg bag samples were processed within 24 h of collection. Artificial and naturally deposited eggs were counted, and natural eggs were identified as Lake Trout eggs or Coregonid eggs based on coloration and size (Becker 1983).

To partition sources of egg mortality, we first estimated the proportion of artificial (A) eggs lost to physical forces F as the number of artificial eggs recovered / total number of artificial eggs seeded per gear = AF. Next, we estimated the proportion of live (L) eggs recovered as the number of live eggs recovered / total (T) number of live eggs seeded per gear = TT. Because the live eggs represent the mortality from both physical force and predation, we estimated the proportion of live eggs lost to physical forces by using the artificial egg loss as follows:

- Equation 1: (1-AF)*LT = Live Eggs Lost to Physical Forces (LF)

To estimate predation (P), we partitioned out the loss live eggs from physical forces (LF; Equation 1) and removed them from the total number of live eggs seeded (T) so that:

- Equation 2 P = (T – LF) / T

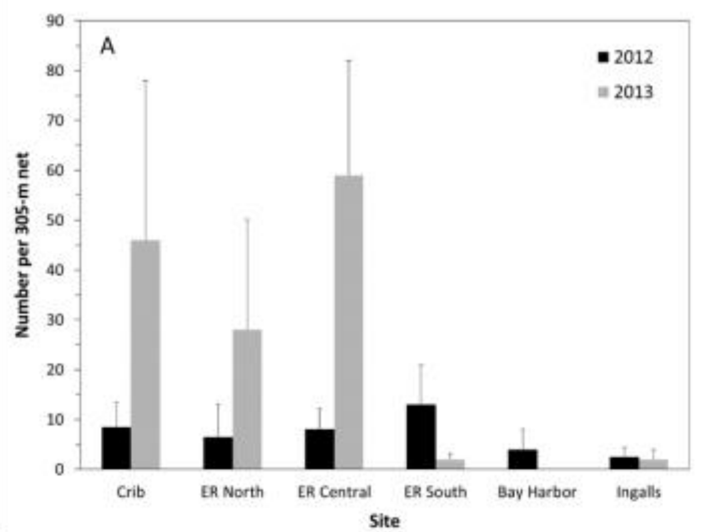
We compared the average proportion of artificial eggs recovered (AF), the total proportion of live eggs recovered (L), and the proportion consumed by predators (P) across the four sites to evaluate potential differences in egg predation rates across sites with the egg nets. We also compared these results to seeding of the funnels, which protect eggs from predators to ground truth the methods for partitioning out mortality using artificial and live eggs.

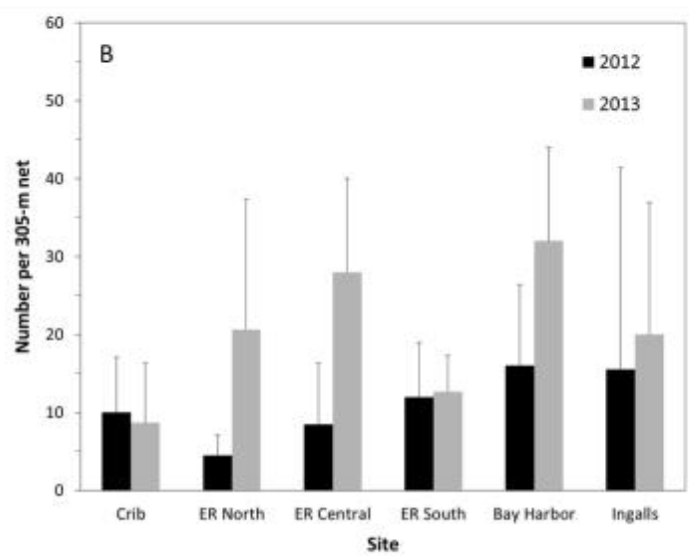
RESULTS

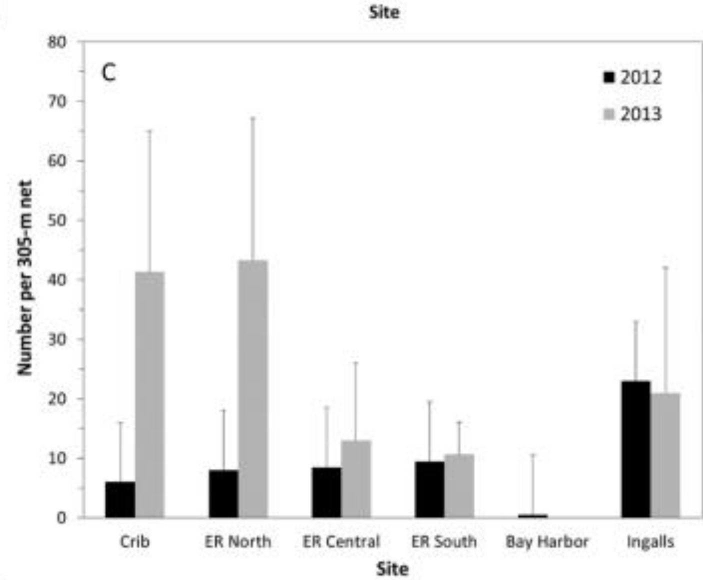
Gill nets

Overall gill net CPE (fish per 305-m) was higher in 2013 (68.9 ± 9.6) compared to 2012 (51.9 ± 7.4), although variation in the catch rates was high and differences were not significant in either year ($F_{1,39} = 2.0$, $P = 0.17$) or site ($F_{5,39} = 1.6$, $P = 0.19$). For the individual species, Cisco CPE was significantly different by year ($F_{1,39} = 5.0$, $P = 0.03$) as CPE was higher in 2013 (22.8 ± 11.4) compared to 2012 (7.1 ± 4.3; Figure 5.1A). There were no significant differences between site ($F_{5,39} = 2.5$, $P = 0.$) and the site by year interaction ($F_{5,39} = 1.7$, $P = 0.16$) for Cisco . Similar to the overall abundance pattern, there were no differences observed with Lake Trout CPE by year ($F_{1,39} = 2.5$, $P = 0.13$) and site ($F_{5,39} = 0.8$, $P = 0.53$; Figure 2B) and with Lake Whitefish CPE by year ($F_{1,39} = 2.6$, $P = 0.121$) and site ($F_{5,39} = 1.2$, $P = 0.330$; Figure 5.1C).

Figure 5.1 (A-C). Mean relative abundance of Cisco (A), Lake Trout (B), and Lake Whitefish (C) caught in gill nets set during the spawning season on each of the six spawning reefs.







Natural Egg Levels

Natural Coregonid egg deposition levels were low and variable across years and sites. For Coregonid egg deposition, no eggs were collected at Bay Harbor and the Crib only had one year (2012) when Coregonid eggs were collected (density<1 eggs/m²). The average across the time series (2008-2013) indicated that Coregonid egg deposition was variable with natural egg levels ranging from 13.6±10.0, 117.3±53.9, and 241.9±102.6 at the ER North, ER Central, and ER South sites, respectively. Relative to those sites, Ingalls had low natural Coregonid egg levels at 32.3 ± 8.9 eggs/m² (Figure 5.2). Across all sites, natural Coregonid egg levels showed a decreasing trend over time with egg levels highest in the first year of monitoring (2008; 101.6 ± 59.4 eggs/m²) and lowest in the last year (2013; 33.5±24.2 eggs/m²; Figure 3).

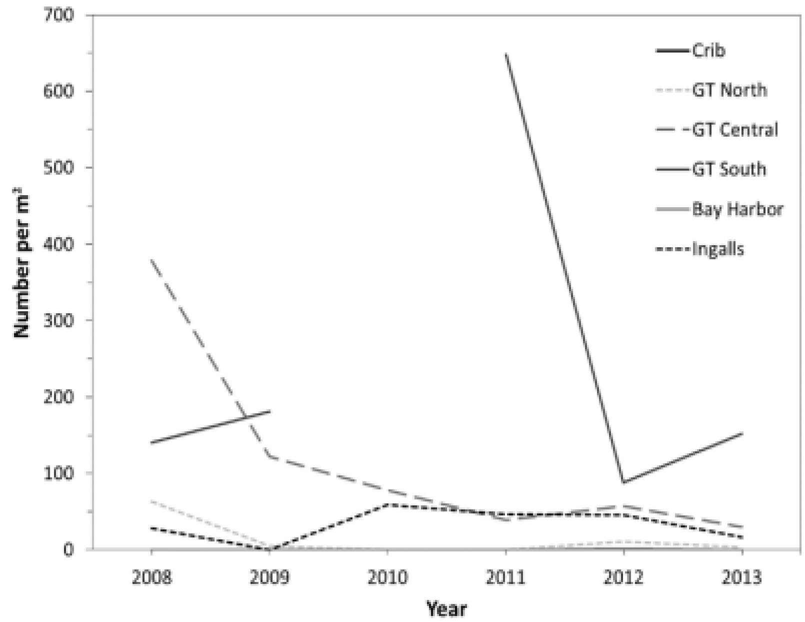


Figure 5.2. Natural egg levels (eggs/m²) for Coregonid spp. as measured from egg nets

Natural Lake Trout egg levels were detected at all sites but they were very low and highly variable. Two sites had several years with no Lake Trout eggs detected including 2009 – 2012 at GT North and 2008, 2010, and 2012 at GT South. The average (± SE) across the time series (2008-2013) indicated that Lake Trout egg deposition was low and variable with natural egg levels ranging from 17.3 ± 4.8, 0.6 ± 0.5, 7. 9 ± 3.2, 3.9 ± 2.8, 6.9 ± 1.9 and

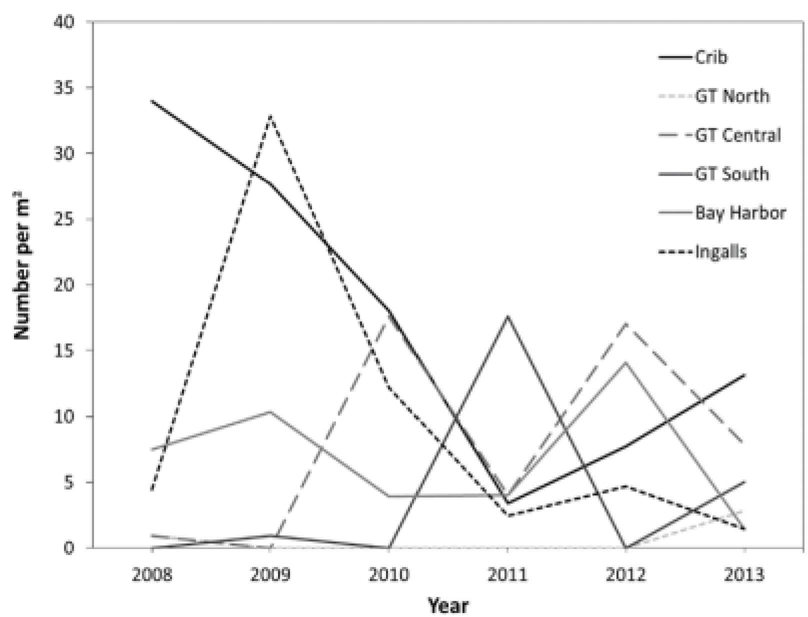


Figure 5.3. Natural egg levels (eggs/m²) for Lake Trout as measured from egg nets.

9.7 ± 4.96 at the Crib, ER North, ER Central, ER South, Bay Harbor, and Ingalls sites, respectively (Figure 5.3). Similar to Coregonid eggs, natural Lake Trout egg levels tended to trend downward over time across sites with egg levels highest in 2009 (12.0 ± 6.0) and lowest in 2011 (5.3 ± 2.5) and 2013 (5.3 ± 1.9 eggs/m²; Figure 4).

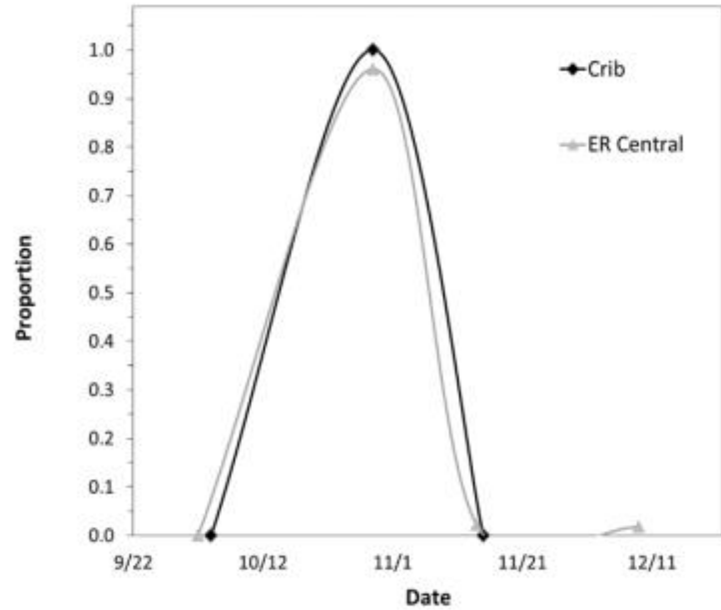


Figure 5.4. Patterns of natural lake trout egg deposition patterns as measured by proportion of eggs deposited in the funnels at the Crib and ER Central sites in 2013

Seeding Study

Using the funnel collections, we estimated peak Lake Trout spawning in 2013 to be occurring on 29 October at both the Crib and ER Central sites. Funnel collections at the other sites were too low for estimating peak egg deposition rates. Lake Trout egg deposition was not detected during the early October funnel sampling (4 October for the Crib, 2 October for Elk Rapids). After peak deposition, Lake Trout eggs were collected on 14 November and 9 December at the ER Central site, but at very low levels. For both the Crib and ER Central site, over 96% of the eggs collected were deposited between 4 October and 29 October (Figure 5). Live and artificial eggs were on seeded on 5 November 2013 and retrieved on 20 November 2013 at the Crib site and seeded on 6 November 2013 and retrieved on 21 November 2013 at the ER North, ER Central, and ER South sites. Bay Harbor and Ingalls egg nets were not fully seeded because of inclement weather conditions preventing diver access to the reefs or limiting divers to successfully seed the egg bags or funnels.

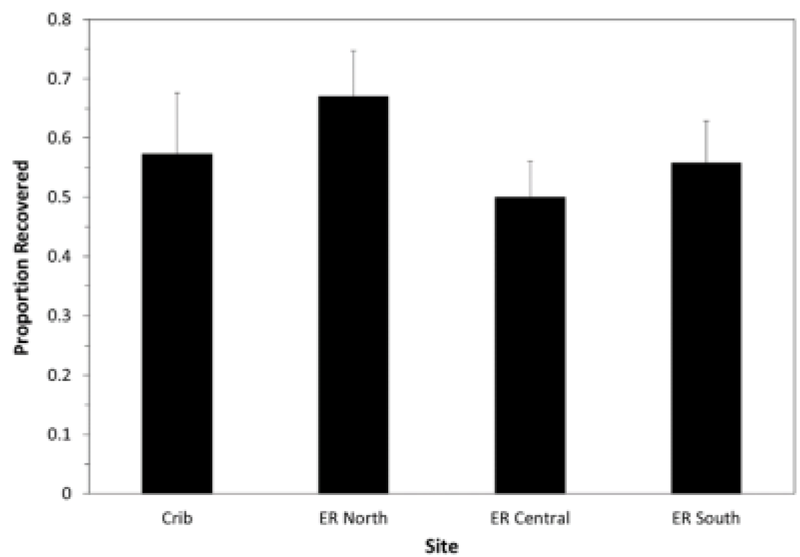


Figure 5.5. Bead recovery (proportion) in egg bags as an index of abiotic loss.

The proportion, expressed as a percentage (±SE), of artificial eggs recovered was 57.3 ± 10.2, 67.0 ± 7.7, 50.0 ± 6.0, and 55.8 ± 7.1

for the Crib, ER North, ER Central, and ER South sites, respectively (Figure 6). Because live eggs experience loss from both physical forces and predation, the recovery rates for live eggs were lower at 6.0 ± 2.4, 24.4 ± 5.2, 5.6 ± 1.5, 6.9 ± 2.2 for the Crib, ER North, ER Central, and ER South sites, respectively (Figure 7).

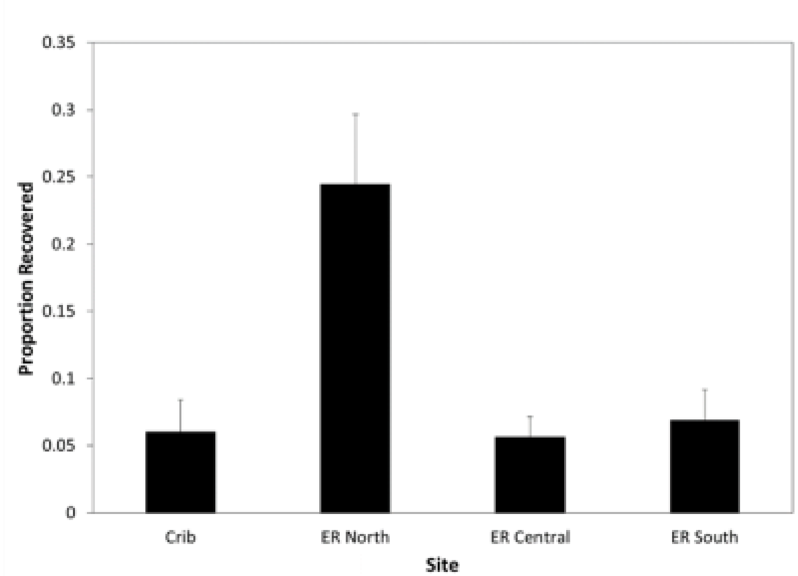


Figure 5.6. Live egg recovery (proportion) in egg bags as an index of total (abiotic and biotic) loss.

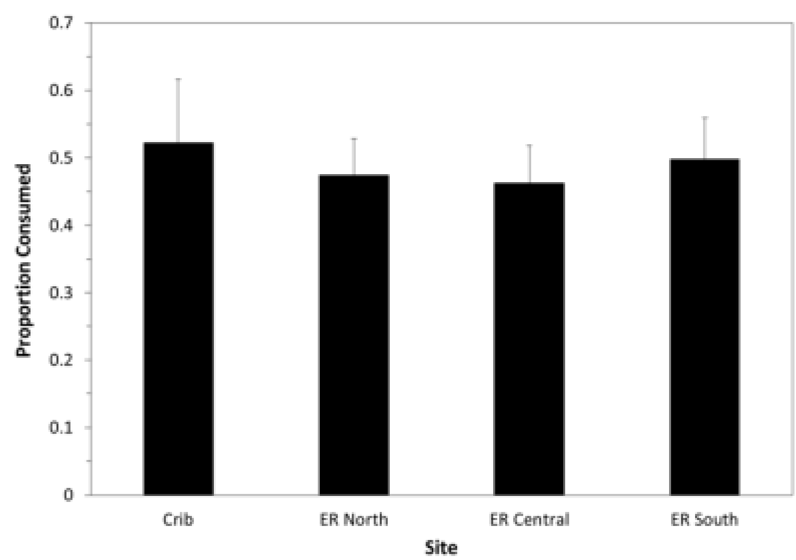


Figure 5.7. Estimation of consumption (proportion of live eggs lost in egg nets) by egg predators.

Based on the estimates of egg loss from physical forces from the artificial egg recovery rates, we estimated the egg mortality from both physical forces and predation on live eggs. Based on the assumption that a live egg has the same exposure to physical force loss, we estimated egg consumption to be 52.3 ± 9.3, 47.5 ± 5.4, 46.3 ± 5.5, and 49.8 ± 6.1 for the Crib, ER North, ER Central, and ER South sites, respectively (Figure 8).

Egg recovery rates from the funnels supported the finding that predation rates were high across the seeding sites given that the eggs seeded in the funnels will be exposed to lower predation rates (Barton et al. 2011). The eggs seeded in the funnels will be initially exposed to both physical loss and predation. However, as the eggs approach the collection tube, access from predators and loss from physical forces will substantially decrease. We found that recovery rates were much higher in the funnels for both physical loss (9.2 ± 6.1, 64.8 ± 24.8, 34.4 ± 21.4, 31.8 ± 15.7 for the Crib, ER North, ER Central, and ER South sites, respectively) and predation (6.0 ± 2.9, 71.0 ± 23.5, 46.0 ± 28.6, 68.0 ± 18.1 for the Crib, ER North, ER Central, and ER South sites, respectively).

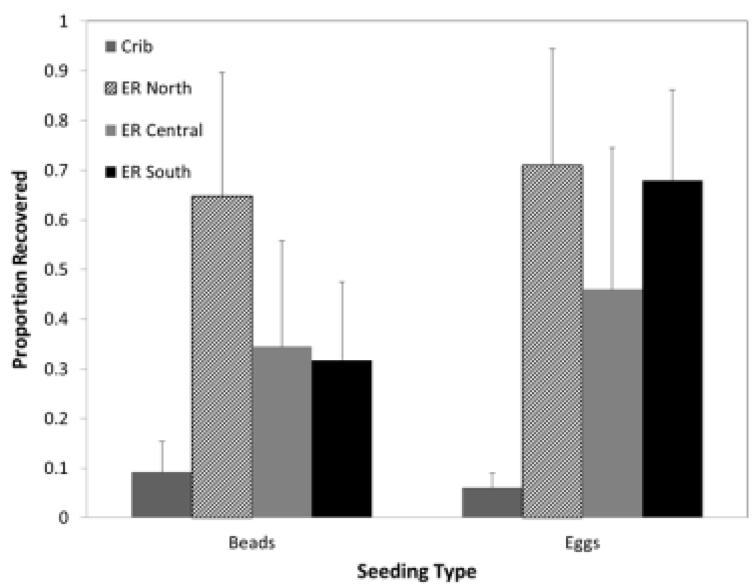


Figure 5.8. Funnel recoveries (proportion) of artificial and live egg recoveries from seeding

DISCUSSION

We found no evidence that egg predation levels were suppressed on the most intensively controlled reefs, the Crib and ER North. Both sites had predation rates that average near or greater than 50% and were comparable to the low treatment ER Central and reference site, ER Point. As anticipated based on results in Objective 1-4, predator densities will need to be reduce much more substantially to provide meaningful reductions in egg loss due to predation.

A precursor to successful natural reproduction in Great Lakes fish stocks is the presence of mature spawners on good spawning habitat. In the case of Cisco, Lake Trout, and Lake Whitefish in this study, we found sufficient numbers of spawning individuals with our experimental gill net sets. Although recognized as a crude index of relative abundance because it is a passive gear with low replicates due to the lethal nature of the gear, the estimates of relative abundance were similar across sites and years (2012 and 2013) for Lake Trout and Lake Whitefish. Cisco CPE, however, appeared to increase between 2012 and 2013 at some of the sites (Crib, ER North, and ER Central). If the relative abundance of spawners is indicative of reproductive capacity, then we should have expected similar egg deposition levels for Lake Trout and Lake Whitefish between 2012 and 2013, and potentially higher egg deposition levels for Cisco in 2013.

From this study, however, we found that natural egg levels for all species appeared to be decreasing over time and were not influenced by the relative estimates of spawner abundance. In addition, the longer time series of natural egg levels shows a decreasing trend in egg densities across all sites suggesting that sources of egg mortality are increasing (habitat loss/degradation or predation). At current egg levels, egg densities are much lower than rehabilitation targets for Lake Trout (500 eggs per m^2) in rehabilitation areas by 2015. Although not identified as a rehabilitation target, areas of Lake Michigan with stable Coregonid stocks have produced estimates of natural Coregonid egg levels around 1,000 eggs per m^2 (Hog Island Shoal, 2001, MDNR unpublished data).

Because of the declining and low natural egg levels, seeding experiments provide a critical approach to evaluating potential sources of egg mortality. Egg mortality, post successful fertilization, is likely to be caused by physical force stress or predation. Seeding only live eggs, however, will provide only estimates of total egg mortality rates. The approach used in this study was unique because we seeded both artificial and live Lake Trout eggs simultaneously to partition mortality sources. The artificial eggs were recovered at relatively consistent rates indicating that 33-50% of deposited eggs were lost from physical forces during the spawning period. It is notable that there were several severe storms throughout the 2013 spawning season, with above average high energy wave action throughout the season. These loss rates suggest that the better the quality of spawning habitats, the better the chance that the eggs can be protected. The egg seeding in the funnels also suggest that once the eggs, which are negatively buoyant, move deeper into the reef interstitial spaces then they are likely protected from sources of physical loss such as wave energy and currents. It is unclear as to whether or not Dresseinids have altered the quality of the interstitial spaces thereby limiting or reducing the rate of eggs from moving deep into the reef, though Dressenid densities on these reefs are relatively low compared with reefs that have been reported with Dressenid issues in the Great Lakes.

Recovery rates for live eggs were very low because live eggs are exposed to both physical loss and predation. Based on the recovery rates, egg survival during the two week seeding period averaged 10.8% in total. Based on the mortality rates of live eggs (almost 90% eggs lost in two weeks), it is highly unlikely that any Lake Trout eggs would survive the incubation period. In addition, we seeded at an egg density level of 288.38 eggs per m², but natural egg deposition levels were much lower (most sites were less than 10 eggs per m²). Using the artificial eggs, we were able to partition physical loss and consumption by egg predators. Approximately 50% of the live eggs were lost to egg predators during the two week seeding experiments. If egg predator levels could be reduced, the egg survival rates would likely increase substantially. As natural egg levels remain low and predation rates from invasive species including the Round Goby and the Rusty Crayfish are high, there is reason for concern that native fish rehabilitation efforts will be challenging. Monitoring of natural egg levels and threats to native fish spawning reefs should be a critical consideration in future rehabilitation efforts.

Finally, the Elk Rapid reef complex is the only documented active spawning location for Cisco in Lake Michigan and this Cisco population has been increasing rapidly over the last decade (MDNR, unpublished data). This upward population trend is particularly impressive given the high egg mortality observed here, due to both invasive egg predators and losses to physical-force stress. If these stresses could be minimized through a combination of invasive predator control and physical habitat rehabilitation, the Elk Rapids reef complex could provide substantial numbers of Cisco to northern Lake Michigan. If these techniques could then be expanded to other potential Cisco spawning habitat, it could contribute to the recovery of Cisco in Lake Michigan, as well as other Great Lakes.

MDNR fisheries staff setting gill nets off the research vessel – SV Steelhead to monitor adult Lake Trout, Lake Whitefish and Cisco spawner numbers.

<u>Objective 6</u>. Cause integrated pest management paradigm shift by communicating successful restoration efforts, promoting the Grand Traverse Bays reef complexes as a demonstration site, providing standard operation procedures, operational costs, and recommendations to fisheries managers and stakeholder communities on how these methods can be adopted at other shallow and deep spawning reefs in the Great Lakes basin.

During the course of the project, our team has completed over 20 oral presentations and 4 posters were presented to a broad range of audiences including technical fisheries meetings, science conferences, tribal, and community stakeholder groups. In addition, this project has supported 2 masters project (Robinson 2014, Buckley *in prep*) and spawned a third that is focused on Elk reef restoration and interaction between habitat improvement and benthic predator impacts. Three articles have been submitted to scientific journals for publication and our expectation is that at least a further three scientific paper will be produced over the next 12 months from this work. We have published one popular article in the MDNR Fisheries newsletter and our work was featured on a Detroit public TV documentary/program.

We were unsuccessful in our efforts to suppress Rusty Crayfish and Round Goby on these spawning reefs and hence did not affect a paradigm shift among fisheries managers and community stakeholders. Nevertheless, we have successfully raised awareness of the importance of these spawning reef structures, the need for both control methods and an improved understanding of the biology of Round Goby and Rusty Crayfish in the Great Lakes. In addition, we have been able to show that efforts to control these benthic predators need to be integrated with habitat restoration on degraded reefs. Evidence of our success is demonstrated by the award of a Great Lakes Basin Fish Habitat Partnership grant administrated by the USFWS and awarded to MDNR to restore the degraded reef habitat on the northern Elk Rapids reef, (ER North, Figure 1) and a large five year private award/gift to The Nature Conservancy from a major Michigan foundation to provide ongoing support for our adaptive management efforts on these reefs, to restore native fisheries through benthic predator suppression and habitat restoration efforts on these spawning reefs. The latter funds have been used to contract a material engineering firm to design a more effective tangle trap and potential crayfish barriers that will tested on the spawning reefs this summer. In addition, our efforts have helped stimulate renewed focus on the importance of goby in Lake Michigan and recent efforts to calculate basin wide biomass.

ORAL PRESENTATIONS

- Claramunt, R.M. and M.E. Herbert. Restoration of Critical Fish Spawning Reefs through Invasive Predator Suppression and Habitat Restoration. October 2012. Traverse City, Michigan. Freshwater Summit: Our Changing Great Lakes.
- Claramunt, R.M. Impacts of invasive species on native fish in the Great Lakes Traverse City Rotary. December 2013. Traverse City, Michigan.

- Chadderton, W.L. Yanking Our Chain. March 2013. Detroit, Michigan. Public Lecture Series by the Cranbrook Institute of Science.
- Galarowicz, T.L., K.M. Cole, R. Moser, J.T. Buckley, M. Herbert, W.L. Chadderton, R.M. Claramunt, P.J. O'Neill, and J. Gross. Restoration of critical fish spawning reefs through invasive predator suppression and habitat restoration. February 2013. Gaylord, Michigan. Teachers Environmental School Conference.
- Buckley, J.T., R., T. Moser, T.L. Galarowicz, R.M. Claramunt, W.L. Chadderton, M.E. Herbert, and A.J. Tucker. Crayfish suppression on critical spawning reefs by intensive trapping. February 2013. Gaylord, Michigan. Annual Meeting of the Michigan chapter of the American Fisheries Society.
- Cole, K.M., T.L. Galarowicz, R.M. Claramunt, W.L. Chadderton, M.E. Herbert, and P.J. O'Neill. Offshore movement of the Invasive Round Goby in Northern Michigan. February 2013. Gaylord, Michigan. Annual Meeting of the Michigan chapter of the American Fisheries Society.
- Claramunt, R.M. Project update and preliminary results of invasive species control. January 2014. Chicago, Illinois. Lake Michigan Technical Committee.
- Herbert, M.E. Native Fish Restoration in the Great Lakes (included substantial emphasis on the reef restoration work and EPA grant). January 2014. Bloomfield Hills, Michigan. Science Talks: a lecture series featuring The Nature Conservancy scientists at the Cranbrook Institute of Science.
- Claramunt, R.M. Impacts of invasive species on native fish in the Great Lakes. February 2014. Grayling, Michigan. Retired Teachers Association.
- Cole, K.M., T.L. Galarowicz, R.M. Claramunt, W.L. Chadderton, M.E. Herbert, and A.J. Tucker. Spatial and temporal distributions of the invasive Round Goby(*Neogobius melanostomus*) and Rusty Crayfish (*Orconectes rusticus*) on critical spawning reefs. February 2014. Holland, Michigan. Michigan Chapter of the American Fisheries Society.
- Claramunt, R.M. Research activities in Lake Michigan. March 2014. Charlevoix, Michigan. Meeting with local anglers.
- Claramunt, R.M. Managing Lake Michigan fisheries with invasive species interactions. May 2014. Lansing, Michigan. Lake Michigan Fisheries Advisors Meeting.
- Claramunt, R.M. Managing Lake Michigan fisheries with invasive species interactions. May 2014. Lansing, Michigan. Lake Michigan Fisheries Advisors Meeting.
- Herbert, M.E. Native Fish Restoration in the Great Lakes. May 2014. Ann Arbor, Michigan. Conservation Café presented by The Nature Conservancy.
- Herbert, M.E. Native Fish Restoration in the Great Lakes. May 2014. Grand Rapids, Michigan. Conservation Café presented by The Nature Conservancy.
- Buckley, J.T., T.L. Galarowicz, W.L. Chadderton, R.M. Claramunt, and M.E. Herbert. Monitoring Invasive Rusty Crayfish on Critical Lake Michigan Spawning Reefs**.** May 2014. Hamilton, Ontario. International Association for Great Lakes Research (IAGLR) conference.
- Cole, K.M., T.L. Galarowicz, R.M. Claramunt, W.L. Chadderton, M.E. Herbert, A.J. Tucker, and J.A. Gross. Spatial and temporal distributions of the invasive Round

Goby(*Neogobius melanostomus*) and Rusty Crayfish (*Orconectes rusticus*) on critical spawning reefs. May 2014. Hamilton, Ontario. International Association for Great Lakes Research (IAGLR) conference.

- Claramunt, R.M., M.E. Herbert, T.L. Galarowicz, W.L. Chadderton, and J.A. Gross. The effects of pulse pressure water gun technology on Rusty Crayfish (*Orconectes rusticus*) and Round Goby(*Neogobius melanostomus*) non-native egg predators in Lake Michigan. August 2014. Quebec City, Quebec. American Fisheries Society's Annual Meeting.
- Wagner, T., A. Coffin, J.A. and Gross. The effects of seismic technology on the inner ear of the invasive Round Goby. August 2014. Quebec City, Quebec. American Fisheries Society Annual Meeting,
- Chadderton, L.W. Challenges and solutions to managing aquatic invasive species in Great Lakes. September 2014. Grand Rapids, Michigan. Presentation to invited Michigan Business Leaders.
- Claramunt, R.M., M.E. Herbert, T.L. Galarowicz, and W.L. Chadderton. Native Fish: Spawning Reef Studies. November 2014. Harbor Springs, Michigan. Natural Resource Committee meeting of the Little Traverse Bay Bands of Odawa Indians.

POSTERS

- Buckley, J.T., T.L. Galarowicz, W.L. Chadderton, R.M. Claramunt, and M.E. Herbert. **Monitoring Invasive Rusty Crayfish on Critical Lake Michigan Spawning Reefs.** February 2014. Holland, Michigan. Michigan Chapter of the American Fisheries Society.
- Buckley, J.T., T.L. Galarowicz, W.L. Chadderton, R.M. Claramunt, and M.E. Herbert. **Monitoring Invasive Rusty Crayfish on Critical Lake Michigan Spawning Reefs.** April 2014. Central Michigan University, Mount Pleasant, Michigan. Institute for Great Lakes Research — Science in Action Symposium: An Interdisciplinary and Multi-Institutional Approach.
- Calabro, E.J., T.L. Galarowicz, M.E. Herbert, R.M. Claramunt, and W.L. Chadderton. Effectiveness of critical Lake Trout and Coregonid reef spawning habitat restoration in northern Lake Michigan: Mitigating for environmental and invasive egg predator impacts. April 2014. Central Michigan University, Mount Pleasant, Michigan. Institute for Great Lakes Research — Science in Action Symposium: An Interdisciplinary and Multi-Institutional Approach.
- Cole, K.M., T.L. Galarowicz, R.M. Claramunt, W.L. Chadderton, M.E. Herbert, and A.J. Tucker. Spatial and temporal distributions of the invasive Round Goby(*Neogobius melanostomus*) and Rusty Crayfish (*Orconectes rusticus*) on critical spawning reefs. April 2014. Central Michigan University, Mount Pleasant, Michigan. Institute for Great Lakes Research — Science in Action Symposium: An Interdisciplinary and Multi-Institutional Approach.

ARTICLES AND PUBLICATIONS

- Anon: Hopes for a Sustainable fishery. The Nature Conservancy. Michigan Newsletter, Spring 2012. http://www.nature.org/cs/groups/webcontent/@web/@michigan/documents/document/prd_053137.pdf
- Skop, N.J. and P.J. O'Neill. The trials and tribulation of a water cannon to suppress invasive species. May 2014. Department of Natural Resources' Charlevoix Fisheries Research Station (CFRS) Fall Newsletter, p.1–3.
- Robinson, K.M., T.L. Galarowicz, W.L. Chadderton, R.M. Claramunt, M.E. Herbert, and S.L. Eagan. Under revision. Monitoring shallow benthic fish assemblages in the Laurentian Great Lakes using baited photoquadrats: augmenting traditional fisheries monitoring methods. Journal of Great Lakes Research.
- Wagner, T.L., C.D. Cooper, J.A. Gross, and A.B. Coffin. The effect of seismic waterguns on the inner ears of Round Goby. In review. Journal of Great Lakes Research.
- Claramunt, R.M., T.L. Galarowicz, M.E. Herbert, A.J. Tucker, and W.L. Chadderton. In review. Managing invasive fish in the Laurentian Great Lakes; "Darwin's Dreampond" or Nightmare? "Lake Restoration Handbook: A New Zealand Perspective." Springer.
- Robinson, KM. 2014. Spatial and seasonal distribution of the invasive Round Goby(*Neogobius melanostomus*) and Rusty Crayfish (*Orconectes rusticus*) on critical nearshore spawning reefs in Northern Lake Michigan. MSc thesis Central Michigan University.

  Buckley, J.T. In prep. Spatial and Temporal Distribution of Invasive Rusty Crayfish (Orconectes rusticus) on Crucial Nearshore Spawning Reefs in Northern Lake Michigan. MSc thesis thesis Central Michigan University

EXAMPLES OF GENERAL MEDIA ARTICLES

- The Nature Conservancy. Grant Award for Innovative Technology May Help Native Fish in Grand Traverse Bay. January 6, 2012. Press release. http://www.nature.org/ourinitiatives/regions/northamerica/areas/greatlakes/newsroom/traverse-bay-invasives-1.xml.
- Flesher, J. Underwater cannon may help protect Great Lakes. December 30, 2011. Associated Press story published in several news outlets (sometimes under varying titles), including *Detroit News*, *The New York Times*, *Huffington Post*, and many others.
- http://www.goerie.com/article/20120404/NEWS02/304049959/Great-Lakes-biologists-to-fire-underwater-cannon-to-combat-goby#
- http://www.syracuse.com/news/index.ssf/2011/12/underwater_cannon_may_help_pro.html
- http://news.jrn.msu.edu/capitalnewsservice/2012/01/20/underwater-guns-traps-aim-to-shake-up-great-lakes-invaders/

WEB CONTENT

- http://www.nature.org/cs/groups/webcontent/@web/@michigan/documents/document/prd_239333.pdf
- http://www.nature.org/ourinitiatives/regions/northamerica/areas/greatlakes/explore/grand-traverse-bay-reef-restoration.xml
- http://www.smith-root.com/services/ansd/
- http://www.nrmsc.usgs.gov/research/spawning_reefs

TELEVISION PROGRAM

- Great Lakes Now Connect: Fisheries. Detroit Public Television. November 12, 2014. http://www.greatlakesnow.org/native-fisheries/ (see "Restoring Reef Habitats" under "Features" tab).

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Appendices

APPENDIX 1.

Objective 1. Crayfish Control

Table A1.1. Summary of Rusty Crayfish removal methods (minnow traps, tangle nets, and hand removal via scuba diving) in 2012 and 2013 at GTB North, GTB Central, GTB South, and LTB Crib. For each site and date, the number of minnow traps and the set times (hrs) are provided for the minnow trap removal method. The total length of surveyed transects and total hours of diver removal are given for the hand removal methods. The total length of nets (m) and total hours deployed are provided for the tangle net method. Dashes (-) indicate unspecified value (e.g. transect length not recorded). See Figure X for site locations.

			Minnow traps		Hand removal		Tangle nets	
Site	Year	Date	# traps	hrs	Total transect length (m)	hrs	Total net length (m)	hrs
GTB North	2012	1-Aug	24	48				
		3-Aug	24	120				
		8-Aug	24	144				
		14-Aug	24	24				
		15-Aug	24	96				
		19-Aug	24	48				
		21-Aug	24	48				
		23-Aug	24	48				
		25-Aug	24	48				
		27-Aug	24	48				
		29-Aug	24	72				
		1-Sep	24	48				
		3-Sep	24	48				
		5-Sep	24	48				
		7-Sep	24	48				
		9-Sep	24	72				
		1-Oct	27	3			75	3
		1-Oct	73	24			75	24

		2-Oct					75	24
	2013	19-Sep			-	-		
		30-Sep			-	4.83		
		11-Oct			-	-	200	264
		28-Oct					200	408
		29-Oct	30	1				
		3-Nov			-	2.6		
		20-Nov	30	3				
GTB Central	2012	1-Aug	40	48				
		3-Aug	40	120				
		8-Aug	40	144				
		14-Aug	40	24				
		15-Aug	40	96				
		19-Aug	40	48				
		21-Aug	40	48				
		23-Aug	40	48				
		25-Aug	40	48				
		27-Aug	40	48				
		29-Aug	40	120				
		3-Sep	40	48				
		5-Sep	40	48				
		7-Sep	40	48				
		9-Sep	40	72				
		12-Sep	20	24				
	2013	18-Sep			-	2.5		
		11-Oct			-	-		
		28-Oct					230	960
		29-Oct	30	1				
GTB South	2012	24-Jul	30	24				
		25-Jul	30	24				
		26-Jul	30	24				
		27-Jul	30	72				
		1-Aug	40	48				
		3-Aug	40	120				
		8-Aug	20	144				
		9-Aug	20	120				

		14-Aug	40	24		
		15-Aug	40	144		
		21-Aug	40	48		
		23-Aug	40	24		
		24-Aug	40	72		
		25-Aug	38	48		
		27-Aug	40	48		
		29-Aug	40	72		
		1-Sep	40	48		
		3-Sep	40	48		
		5-Sep	40	48		
		7-Sep	40	120		
	2013	29-Oct	30	1		
		20-Nov	61	3		
LTB Crib	2012	16-Jul	60	24		
		17-Jul	50	24		
		18-Jul	50	24		
		31-Jul	24	48		
		2-Aug	24	96		
		6-Aug	24	72		
		9-Aug	24	96		
		13-Aug	24	48		
		15-Aug	24	96		
		19-Aug	24	48		
		21-Aug	24	72		
		24-Aug	24	48		
		26-Aug	24	48		
		28-Aug	24	72		
		31-Aug	24	24		
		1-Sep	24	48		
		3-Sep	6	48		
		5-Sep	18	24		
		6-Sep	24	24		
		7-Sep	24	48		
		9-Sep	24	96		
		2-Oct	41	24	75	24
		3-Oct	57	24	75	24

2013	13-Aug	-	-		
	19-Aug	32	-		
	23-Sep	80	2.88		
	24-Sep	150	6.97		
	25-Sep	100	8.42		
	26-Sep	250	11.63		
	4-Oct	60	2.98	550	384
	10-Oct	-	-		
	15-Oct	90	2.85	550	264
	17-Oct	70	3.3		
	28-Oct			550	312

Table A1.2. Total number and mean (SE) catch-per-unit-effort (CPUE; #/24hr) of Rusty Crayfish and Round Goby by date removed via minnow traps and tangle nets and hand removal via scuba diving in 2012 and 2013 at GTB North, GTB Central, GTB South, and LTB Crib. The number of *O. virilis* and native fish bycatch that was captured and returned to the site is also provided. CPUE is: #RUS*trap^{-1}*day^{-1} (for traps), #RUS*meters of net^{-1}*day^{-1}(for tangle nets), and #RUS*meters of transect^{-1}*day^{-1} (for hand removal). See Figure X for site locations.

				Rusty Crayfish		Round Goby		*O. virilis*	Fish spp.
Site	Year	Gear	Date	# removed	CPUE Mean (SE)	# removed	CPUE Mean (SE)	# captured	# captured
GTB North	2012	Minnow trap	1-Aug	28	0.61 (0.12)	88	1.91 (0.48)	0	12
			3-Aug	34	0.30 (0.05)	69	0.60 (0.24)	0	5
			8-Aug	58	0.44 (0.08)	68	0.52 (0.12)	0	6
			14-Aug	19	0.83 (0.21)	140	6.09 (1.38)	0	0
			15-Aug	48	0.50 (0.09)	91	0.95 (0.23)	0	1
			19-Aug	5	0.10 (0.04)	39	0.81 (0.22)	0	5
			21-Aug	20	0.43 (0.11)	74	1.61 (0.40)	0	2
			23-Aug	23	0.48 (0.13)	101	2.10 (0.62)	0	4
			25-Aug	27	0.56 (0.12)	123	2.56 (0.70)	0	3
			27-Aug	10	0.21 (0.07)	111	2.31 (0.67)	0	1
			29-Aug	18	0.26 (0.08)	61	0.88 (0.27)	0	3
			1-Sep	4	0.08 (0.05)	25	0.52 (0.15)	0	1
			3-Sep	18	0.38 (0.11)	125	2.60 (0.47)	0	3
			5-Sep	27	0.56 (0.17)	108	2.25 (0.62)	0	2
			7-Sep	21	0.44 (0.09)	23	0.48 (0.12)	0	1
			9-Sep	14	0.19 (0.04)	52	0.72 (0.26)	0	0
			1-Oct	2	0.59 (-)	66	19.56 (-)	1	3
			1-Oct	8	0.11 (-)	351	4.81 (-)		
		Tangle net	1-Oct	11	1.17 (-)	1	0.50 (0.50)	0	0
			1-Oct	6	0.08 (-)				
			2-Oct	13	0.17(-)	1	1.00 (-)	0	0
	2013	Minnow trap	29-Oct	3	2.38 (-)	74	58.7 (-)	0	0
			20-Nov	1	0.27 (-)	7	2.13 (-)	0	0
		Hand removal	19-Sep	129	-	-	-	-	-
			30-Sep	92	-	-	-	-	-
			11-Oct	8	-	-	-	-	-
			3-Nov	48	-	-	-	-	-
		Tangle net	11-Oct	54	0.02	-	-	-	-
			28-Oct	75	0.02	-	-	-	-
GTB Centra	2012	Minnow trap	1-Aug	20	0.25 (0.04)	113	1.41 (0.25)	0	4

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			3-Aug	51	0.26 (0.05)	37	0.19 (0.05)	0	2
			8-Aug	24	0.10 (0.03)	166	0.71 (0.16)	0	0
			14-Aug	11	0.28 (0.12)	262	6.55 (1.44)	0	0
			15-Aug	26	0.18 (0.05)	88	0.61 (0.13)	0	1
			19-Aug	9	0.12 (0.04)	94	1.21 (0.41)	0	1
			21-Aug	18	0.23 (0.05)	274	3.43 (0.66)	0	0
			23-Aug	17	0.21 (0.05)	175	2.19 (0.73)	0	0
			25-Aug	8	0.10 (0.04)	152	1.90 (0.42)	0	3
			27-Aug	6	0.08 (0.03)	58	0.73 (0.31)	0	0
			29-Aug	12	0.06 (0.02)	23	0.12 (0.03)	0	0
			3-Sep	19	0.24 (0.05)	237	2.96 (0.49)	0	0
			5-Sep	16	0.33 (0.07)	182	2.33 (0.46)	0	0
			7-Sep	4	0.06 (0.04)	28	0.45 (0.09)	0	1
			9-Sep	12	0.10 (0.04	254	2.12 (0.47)	0	0
			12-Sep	1	0.05 (0.05)	60	3.00 (1.36)	0	0
	2013	Minnow trap	29-Oct	7	5.56 (-)	4	3.17 (-)	0	0
		Hand Removal	18-Sep	117	-				
			11-Oct	36	-				
		Tangle net	28-Oct	127	0.014	-	-	-	-
GTB South	2012	Minnow trap	24-Jul	0	0 (0)	7	0.23 (0.12)	0	2
			25-Jul	1	0.03 (0.03)	26	0.87 (0.22)	0	0
			26-Jul	1	0.03 (0.03)	41	1.41 (0.38)	0	0
			27-Jul	3	0.04 (0.03)	41	0.49 (0.16)	0	1
			1-Aug	1	0.01 (0.01)	65	0.81 (0.17)	0	2
			3-Aug	10	0.05 (0.02)	92	0.46 (0.11)	0	2
			8-Aug	2	0.02 (0.01)	46	0.38 (0.13)	0	1
			9-Aug	2	0.02 (0.01)	55	0.58 (0.16)	0	0
			14-Aug	0	0 (0)	252	6.30 (1.45)	0	0
			15-Aug	6	0.03 (0.01)	92	0.38 (0.07)	0	1
			21-Aug	1	0.01 (0.01)	147	1.84 (0.37)	0	0
			23-Aug	0	0 (0)	69	1.73 (0.37)	0	1
			24-Aug	0	0 (0)	26	0.22 (0.07)	0	0
			27-Aug	1	0.01 (0.01)	9	0.11 (0.06)	0	0
			29-Aug	0	0 (0)	13	0.11 (0.03)	0	0
			1-Sep	2	0.03 (0.02)	93	1.16 (0.28)	0	3
			3-Sep	3	0.04 (0.02)	94	1.18 (0.21)	0	1
			5-Sep	6	0.08 (0.03)	104	1.30 (0.26)	0	1
			7-Sep	2	0.01 (0.01)	28	0.15 (0.04)	0	0
	2013	Minnow trap	29-Oct	2	1.63 (-)	7	5.56 (-)	0	0
			20-Nov	0	0 (-)	0	0 (-)	0	0

LTB Crib	2012	Minnow trap	16-Jul	1	0.02 (0.02)	119	2.02 (0.67)	0	29
			17-Jul	3	0.06 (0.04)	9	0.19 (0.11)	0	30
			18-Jul	4	0.08 (0.04)	16	0.32 (0.12)	2	55
			31-Jul	40	0.83 (0.18)	193	4.02 (1.15)	8	28
			2-Aug	19	0.20 (0.06)	77	0.80 (0.28)	8	41
			6-Aug	23	0.32 (0.13)	246	3.42 (0.81)	2	21
			9-Aug	44	0.48 (0.11)	328	3.56 (0.83)	7	26
			13-Aug	25	0.52 (0.18)	230	4.79 (1.00)	3	12
			15-Aug	37	0.39 (0.08)	49	0.51 (0.17)	11	11
			19-Aug	37	0.77 (0.14)	112	2.33 (0.68)	6	17
			21-Aug	28	0.39 (0.11)	123	1.71 (0.53)	5	18
			24-Aug	18	0.38 (0.21)	71	1.48 (0.54)	0	13
			26-Aug	12	0.26 (0.14)	37	0.80 (0.27)	0	5
			28-Aug	9	0.13 (0.05)	55	0.76 (0.24)	0	10
			31-Aug	13	0.54 (0.19)	266	11.08 (3.02)	4	14
			1-Sep	27	0.56 (0.14)	263	5.48 (0.99)	3	20
			3-Sep	1	0.08 (0.08)	35	2.92 (1.75)	0	0
			5-Sep	26	1.44 (0.44)	177	9.83 (2.58)	4	12
			6-Sep	16	0.67 (0.25)	166	6.92 (1.74)	1	4
			7-Sep	11	0.23 (0.08)	148	3.08 (1.00)	1	29
			9-Sep	24	0.25 (0.10)	98	1.02 (0.32)	2	9
			2-Oct	50	0.96 (-)	195	3.75 (-)	0	19
			3-Oct	26	0.58 (-)	351	7.8 (-)	2	15
		Tangle net	2-Oct	23	0.31 (-)	0	0 (-)	0	0
			3-Oct	42	0.56 (-)	0	0 (-)	0	0
	2013	Hand removal	13-Aug	250	-	-	-	-	-
			19-Aug	116	-	-	-	-	-
			23-Sep	86	8.96				
			24-Sep	224	5.15				
			25-Sep	286	8.17	-	-	-	-
			26-Sep	352	2.93	-	-	-	-
			4-Oct	226	31.38				
			10-Oct	18	-	-	-	-	-
			15-Oct	99	9.17	-	-	-	-
			17-Oct	123	12.55	-	-	-	-
		Tangle net	4-Oct	56	0.006	-	-	-	-
			15-Oct	83	0.014	-	-	-	-
			28-Oct	75	0.010	-	-	-	-

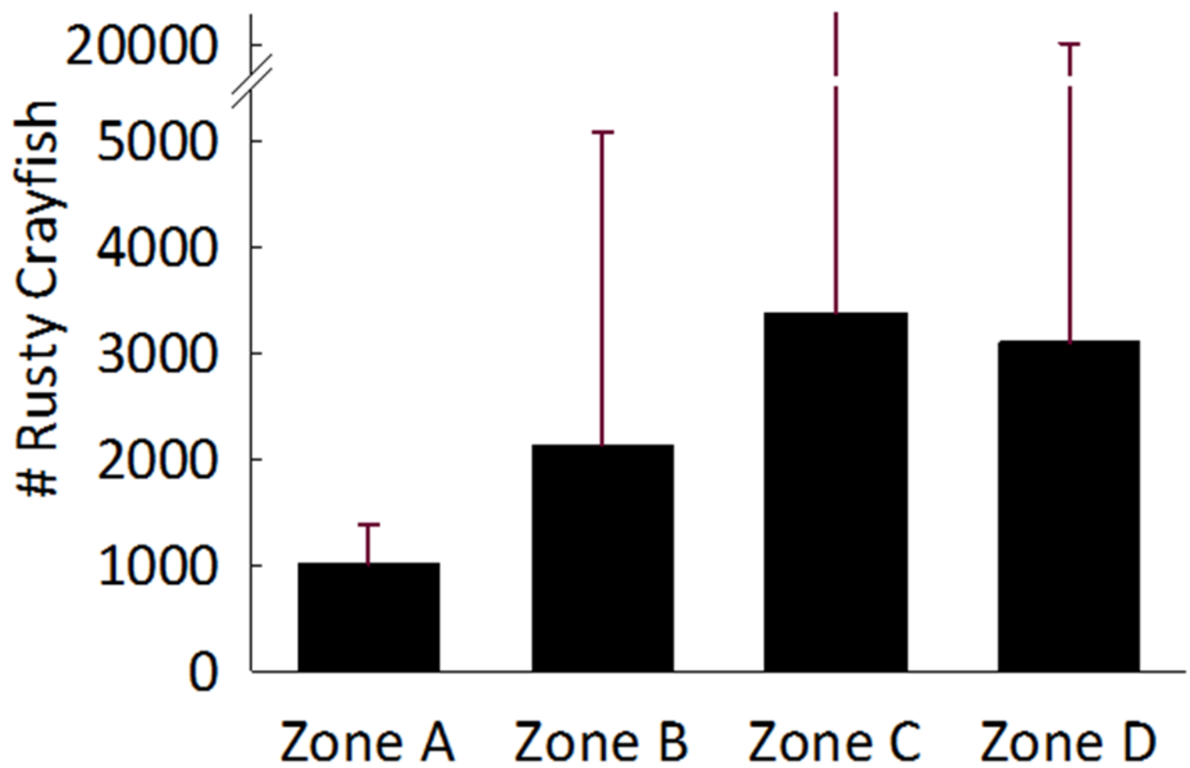

Figure A1.1. Population estimates (+95% CI) of Rusty Crayfish in 225 m2 zones at the ER Central site. See J. Buckley (in prep) for details.

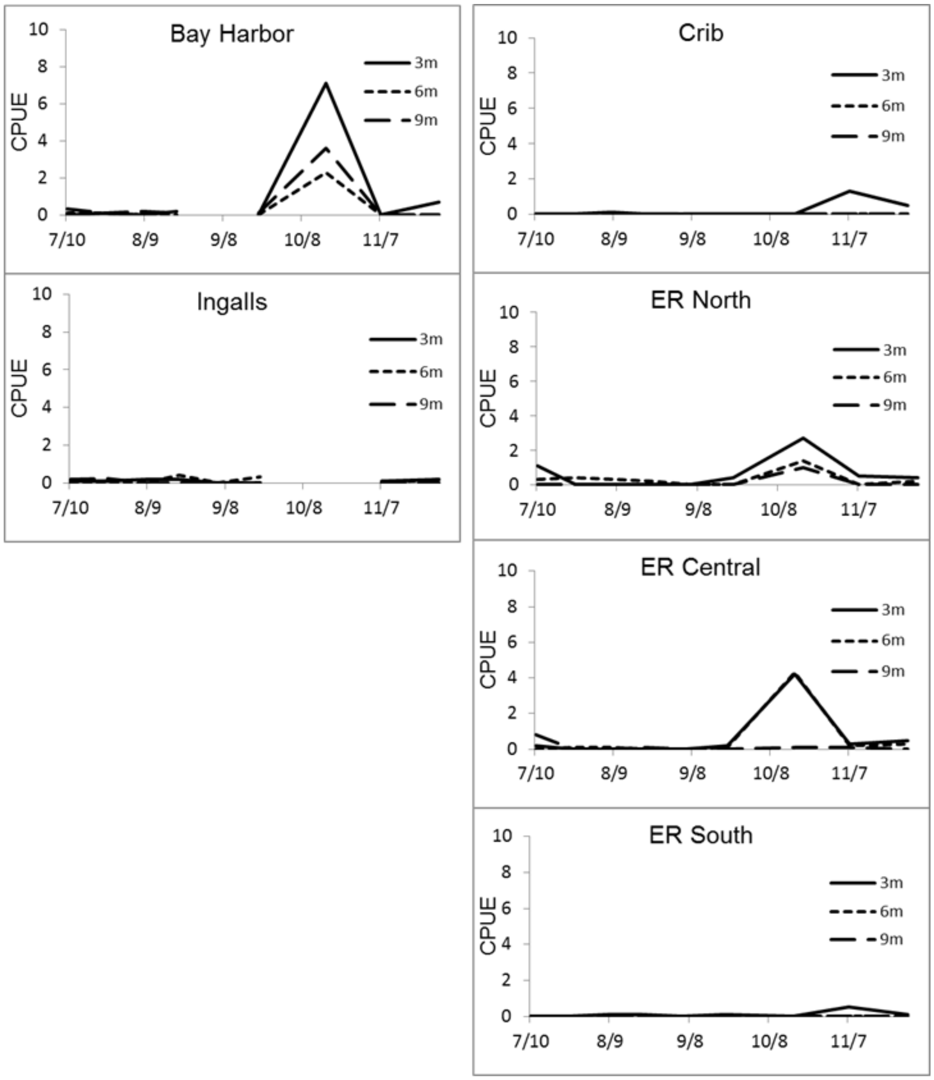

Fig A1.2. Rusty crayfish CPUE from minnow trap index monitoring all depths/all sites 2012. No treatment control sites (Bay Harbor and Ingalls) on left.

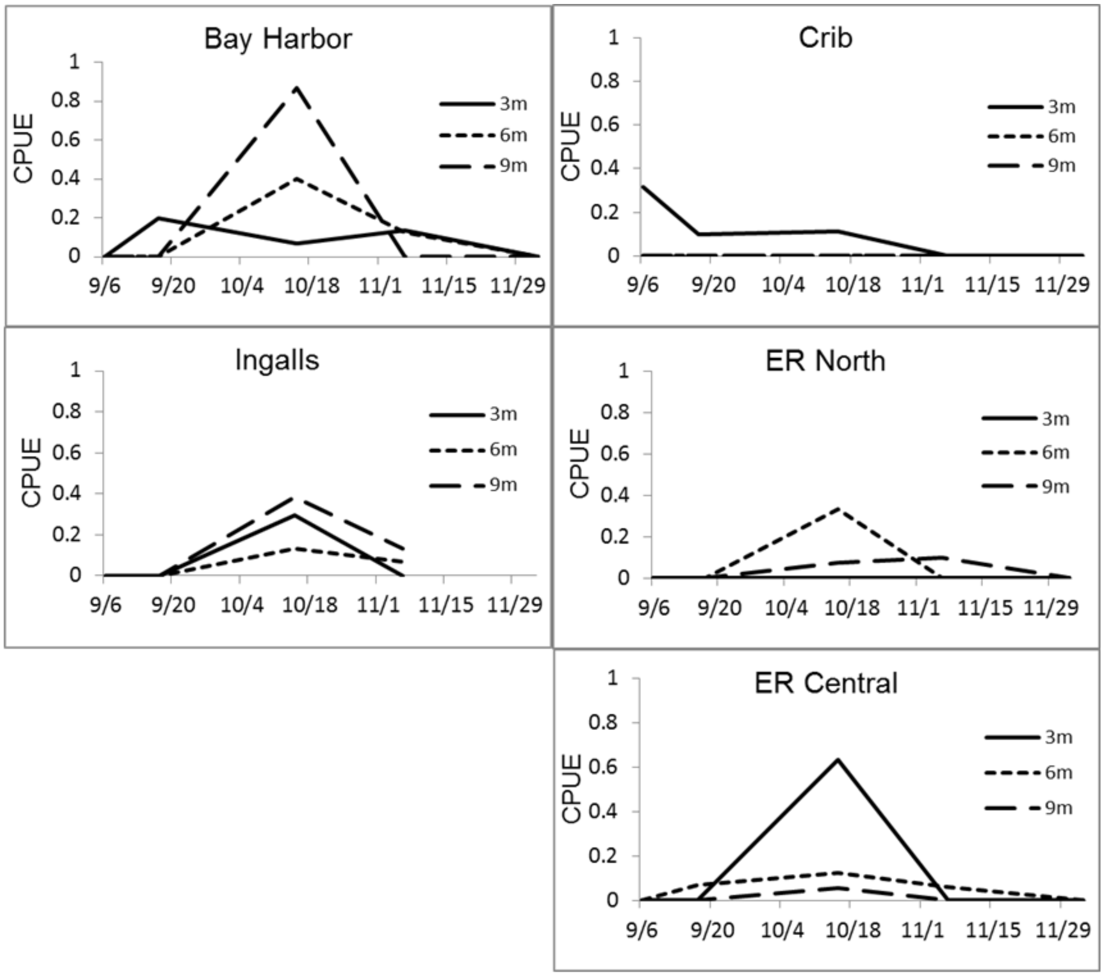

Fig A1.3. Rusty crayfish CPUE from minnow trap index monitoring all depths/all sites 2013. No treatment control sites (Bay Harbor and Ingalls) on left.

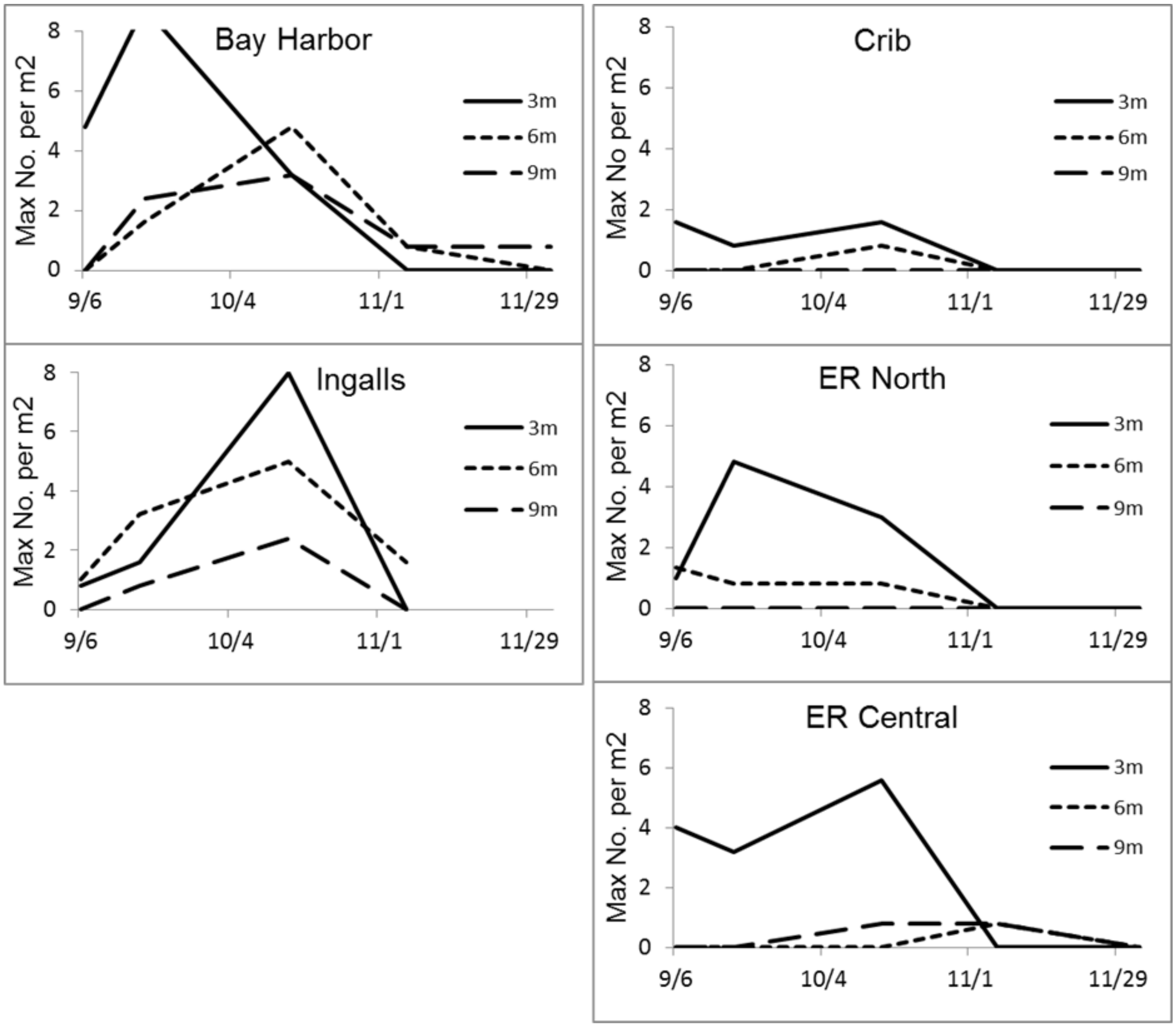

Fig A1.4. Rusty crayfish CPUE from camera index monitoring all depths/all sites 2013. No treatment control sites (Bay Harbor and Ingalls) on left.

APPENDIX 2. Objective 4. Non Target Monitoring.

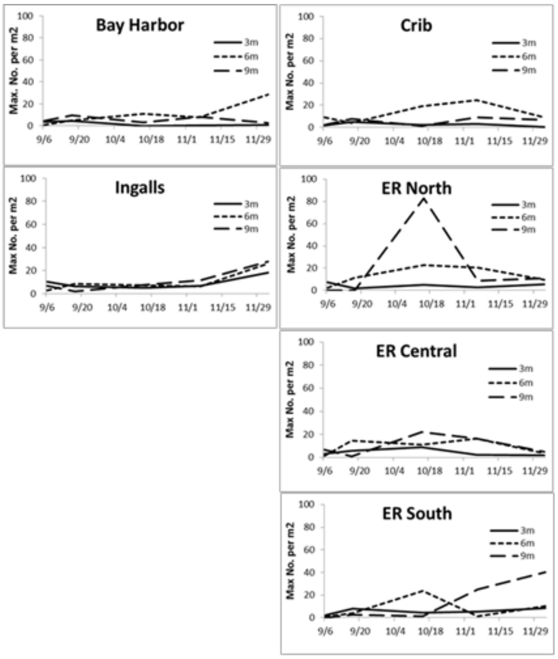

Fig A4.3. Maximum number of goby m-2 from camera index monitoring all depths/all sites 2012.

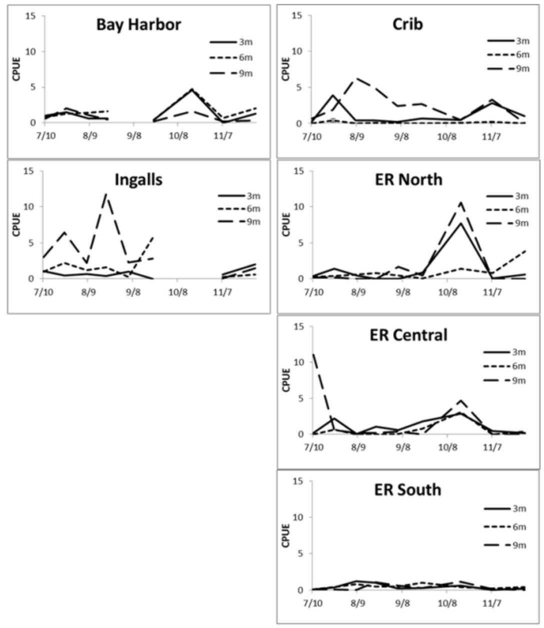

Fig A4.1. Goby CPUE from minnow trap index monitoring all depths/all sites 2012.

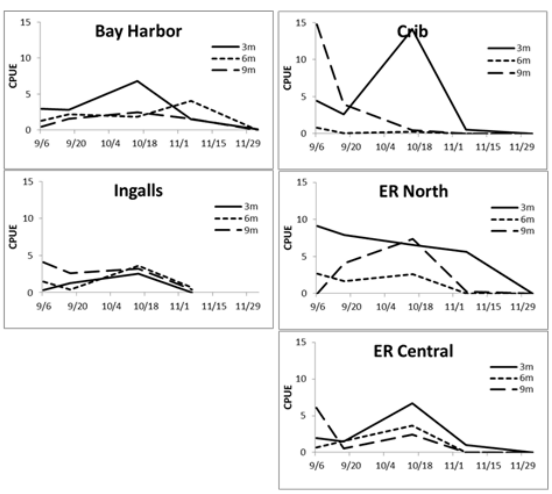

Fig A4.2. Goby CPUE from minnow trap index monitoring all depths/all sites 2013.

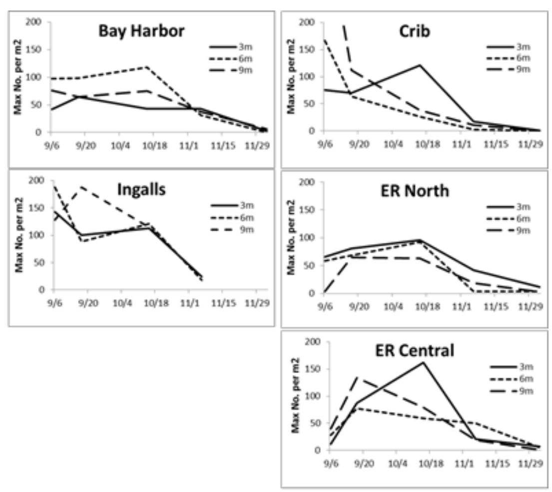

Fig A4.4. Maximum number of goby m-2 from camera index monitoring all depths/all sites 2013.

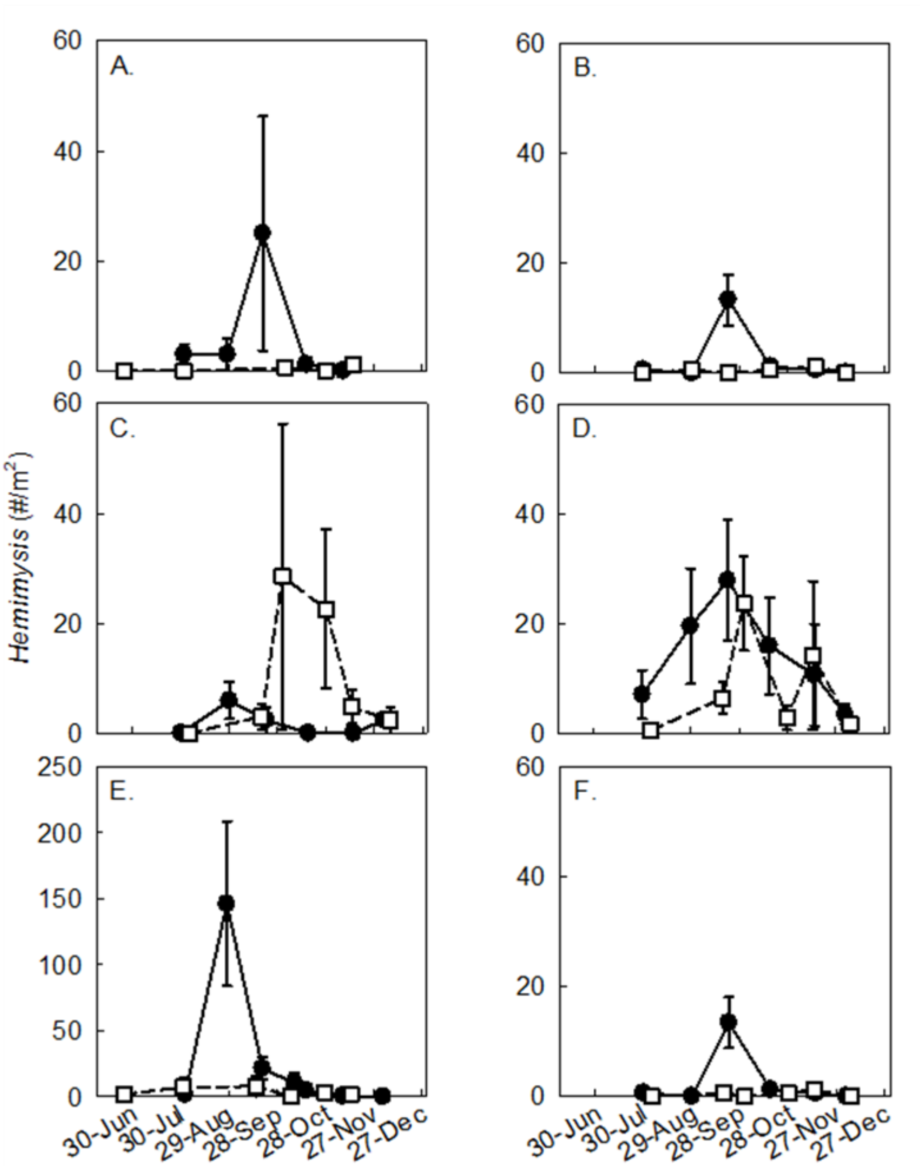

Figure A4.5. Density of Hemimysis anomala (#/m2) captured in egg funnels in 2012 (solid symbols) and 2013 (open symbols) at A.) ER North, B.) ER Central, C.) ER South, D.) Ingalls, E.) Bay Harbor, and F.) Crib..

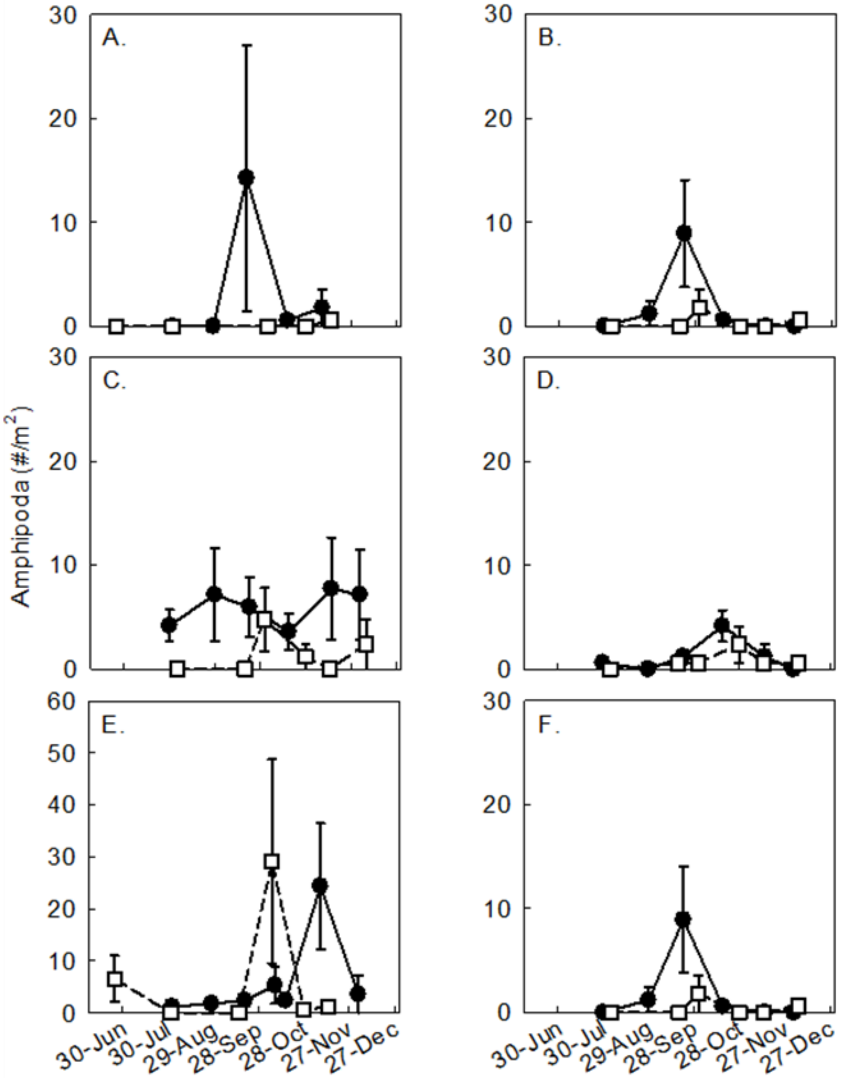

Figure A4.6. Density of Amphipoda (#/m2) captured in egg funnels in 2012 (solid symbols) and 2013 (open symbols) at A.) ER North, B.) ER Central, C.) ER South, D.) Ingalls, E.) Bay Harbor, and F.) Crib.

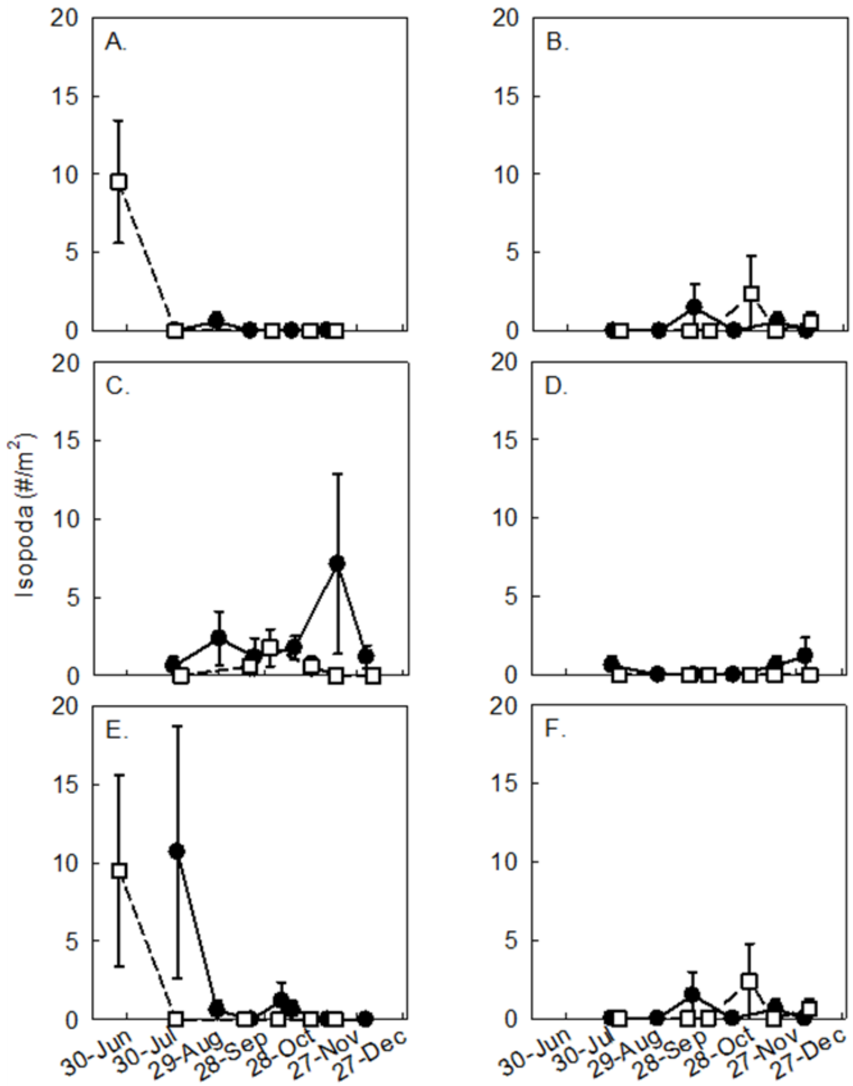

Figure A4.7. Density of Isopoda (#/m2) captured in egg funnels in 2012 (solid symbols) and 2013 (open symbols) at A.) Crib, B.) ER North, C.) ER Central, D.) ER South, E.) Bay Harbor, and F.) Ingalls.

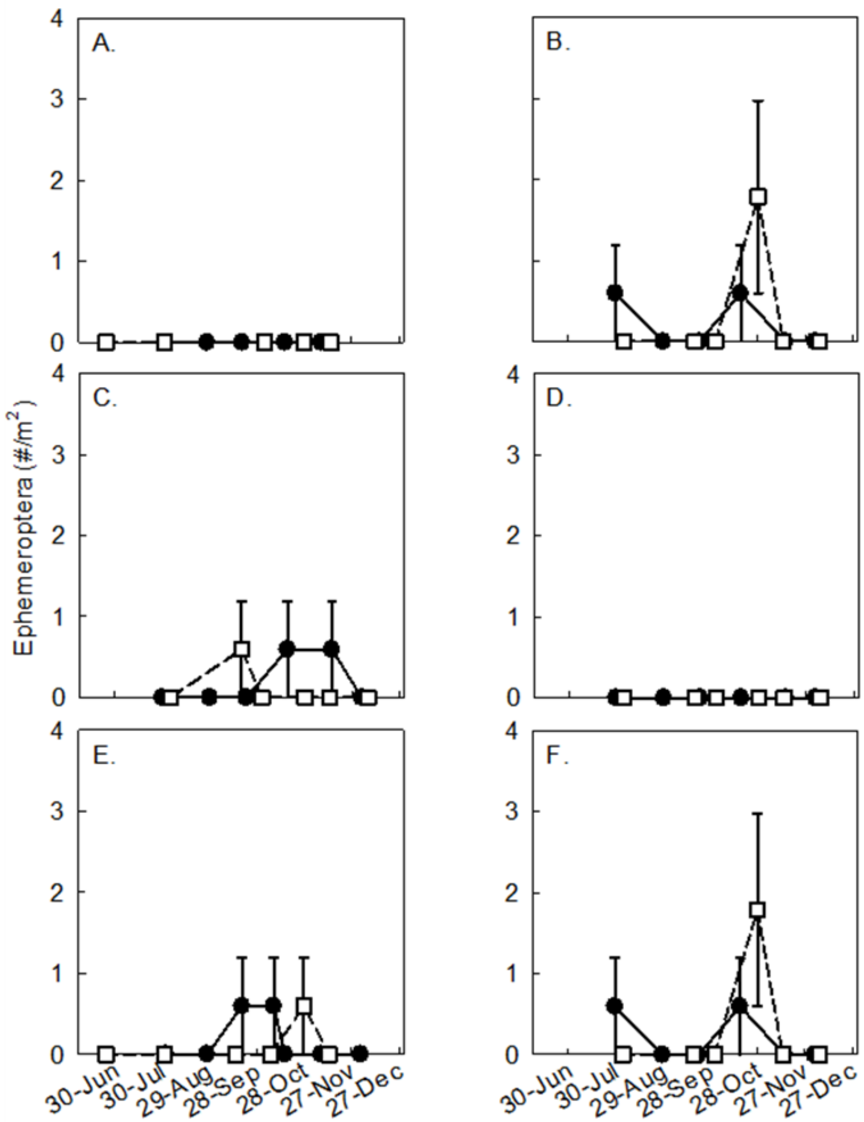

Figure A4.8. Density of Ephemeroptera (#/m2) captured in egg funnels in 2012 (solid symbols) and 2013 (open symbols) at A.) Crib, B.) ER North, C.) ER Central, D.) ER South, E.) LTB Bay Harbor, and F.) Ingalls.

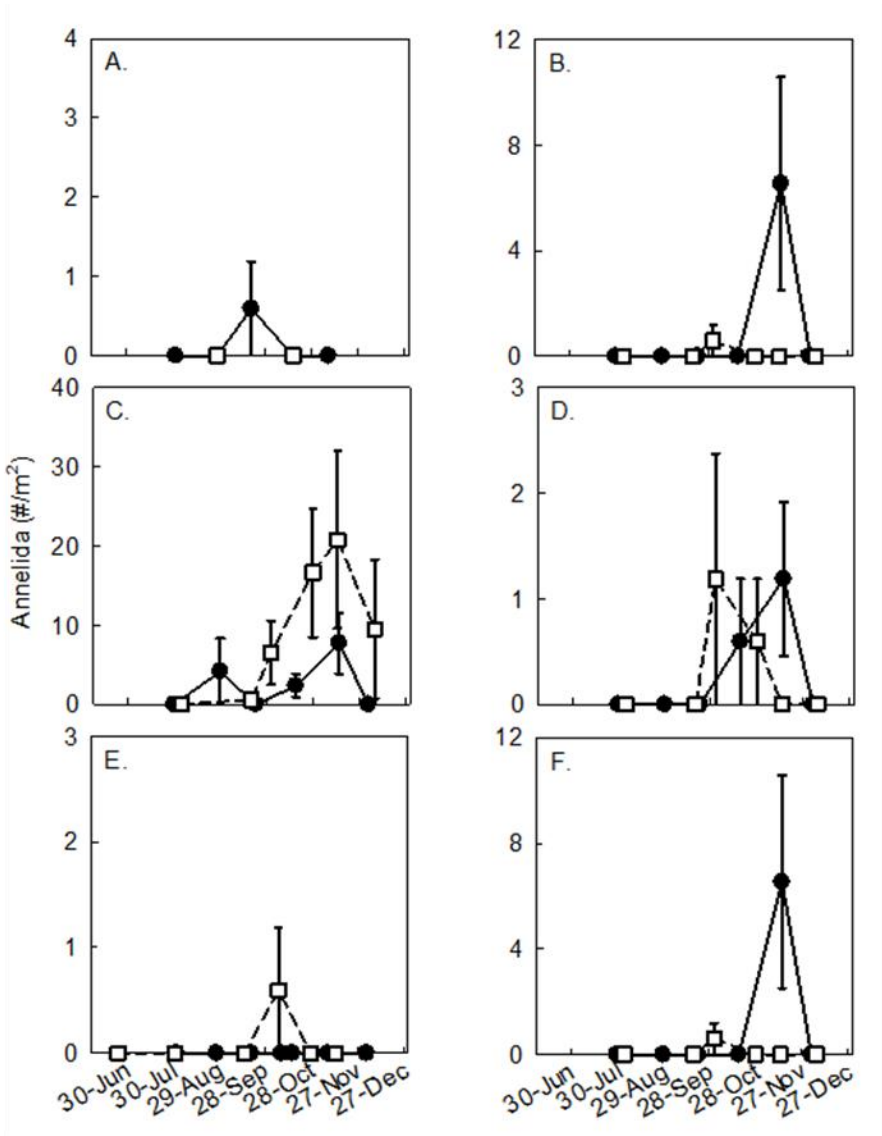

Figure A4.9. Density of Annelida (#/m2) captured in egg funnels in 2012 (solid symbols) and 2013 (open symbols) at A.) Crib, B.) ER North, C.) ER Central, D.) ER South, E.) LTB Bay Harbor, and F.) Ingalls.

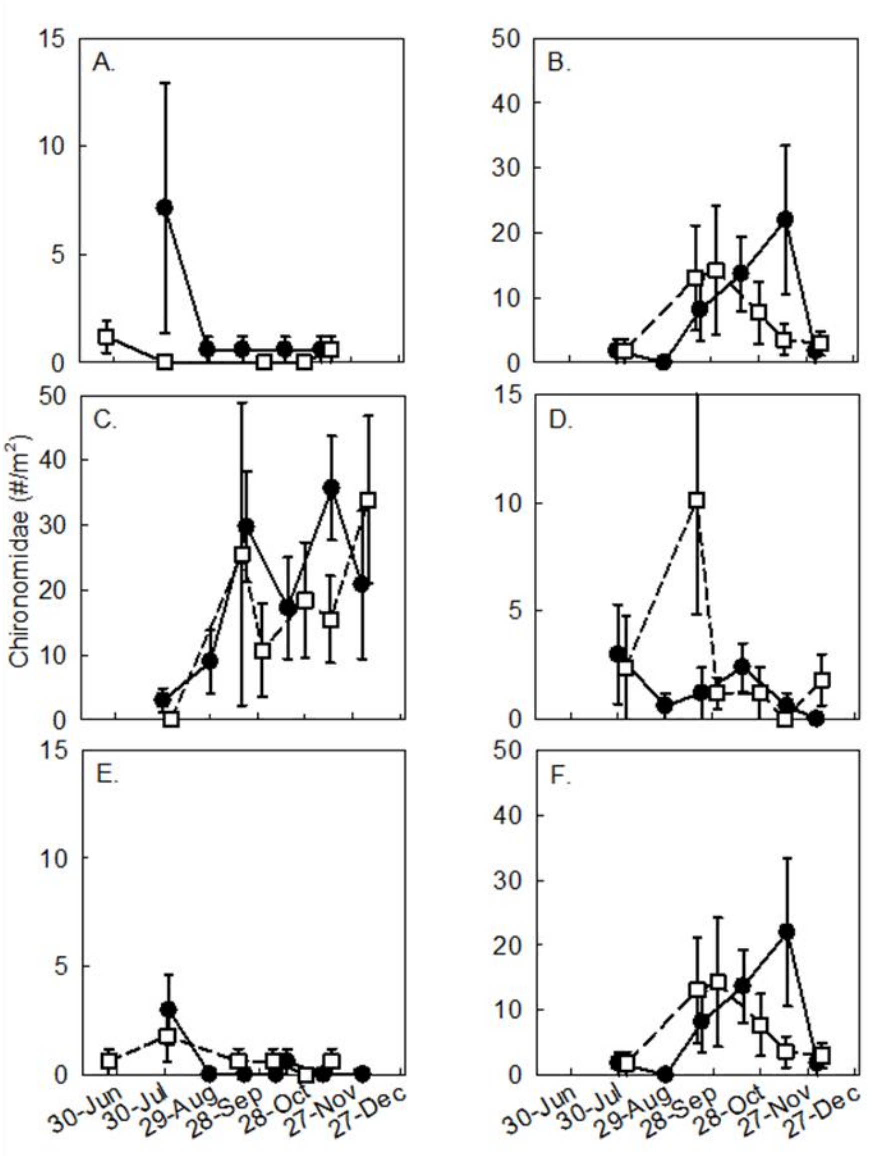

Figure A4.10. Density of Chironomidae (#/m2) captured in egg funnels in 2012 (solid symbols) and 2013 (open symbols) at A.) Crib, B.) ER North, C.) ER Central, D.) ER South, E.) LTB Bay Harbor, and F.) Ingalls.

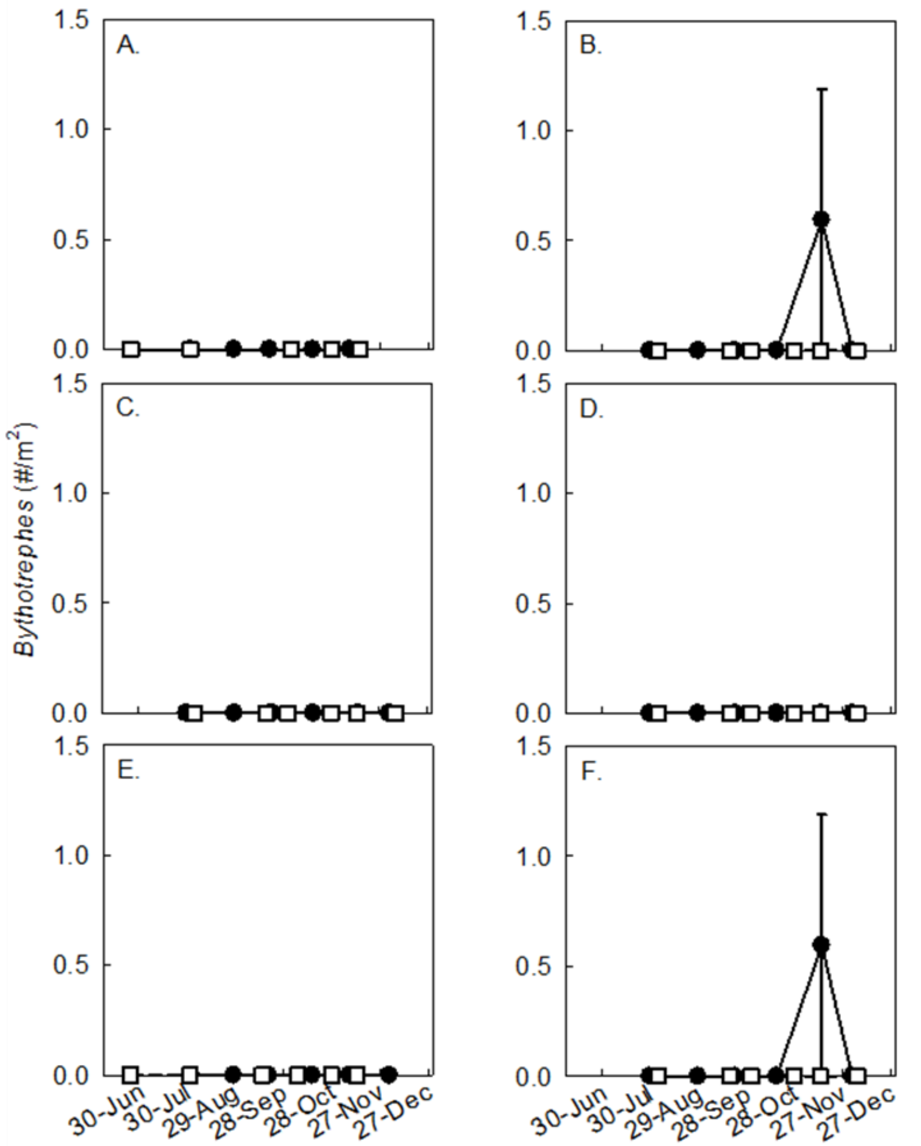

Figure A4.11. Density of Bythotrephes longimanus (#/m2) captured in egg funnels in 2012 (solid symbols) and 2013 (open symbols) at A.) Crib, B.) ER North, C.) ER Central, D.) ER South, E.) LTB Bay Harbor, and F.) Ingalls.