

**NEGATIVE EFFECTS OF SEDIMENTATION ON LITHOPHILIC
SPAWNING FISH EMBRYOS AND METHODS TO POTENTIALLY
MITIGATE THESE EFFECTS**

by

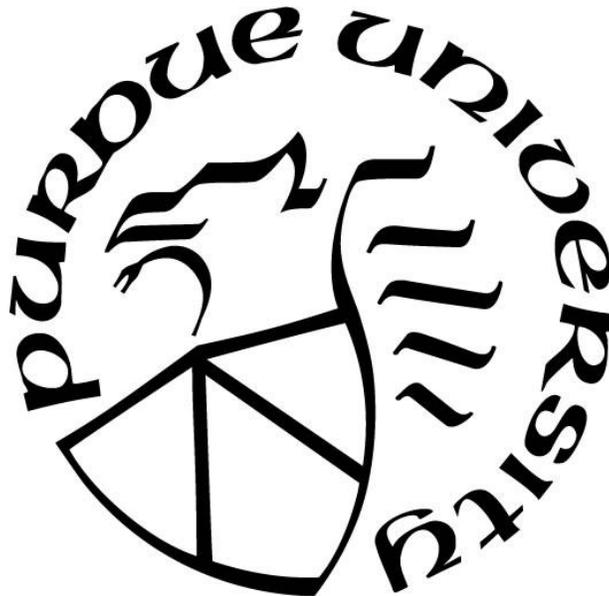
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A Thesis

Submitted to the Faculty of Purdue University

In Partial Fulfillment of the Requirements for the degree of

Master of Science



Department of Forestry and Natural Resources

West Lafayette, Indiana

December 2019

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*Dedicated to my parents, James and Melissa Gatch, sister, Amanda Robertson, and Fiancée,
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ACKNOWLEDGMENTS

I would like to thank my advisor Tomas Höök for guidance, project development and support throughout my master's thesis. I would also like to thank my committee members Mitch Zischke and Ed Roseman for project development and thesis revisions. Thanks also goes to our reef restoration working group Ellen Marsden, Dave Fielder, Jason Fisher, and Andrew Muir for advising the development of cleaning devices. I also thank members of the Höök lab including Scott Koenigbauer, Ben Leonhardt, Taylor Senegal, Patricia Nease, Suse Lagory, Annie Schofield, Chris Malinowski, Josh Tellier, Marissa Cubbage, Jay Beugly and Matt Hamilton for support in the field and the office. I would like to thank Jay Beugly, Scott Koenigbauer, and Ben Leonhardt for help developing, building and testing reef cleaning devices. Thank you to fisheries biologist Jeff Malwitz and Andy Richards at the Indiana Department of Natural Resources for walleye egg collection and thanks to Robert Rode for the upkeep and observation of all study tanks at the Purdue University Aquaculture Research Laboratory (ARL). I would also like to thank all of those who funded this research including the Great Lakes Restoration Initiative, U.S. Geological Survey (USGS) and the Department of Forestry and Natural Resources at Purdue University.

TABLE OF CONTENTS

LIST OF FIGURES	7
ABSTRACT	9
CHAPTER 1. INTRODUCTION	11
CHAPTER 2. THE EFFECT OF SEDIMENT COVER AND FEMALE CHARACTERISTICS ON THE HATCHING SUCCESS OF WALLEYE	16
2.1 Introduction.....	16
2.2 Methods	20
2.2.1 Egg collection.....	20
2.2.2 Egg incubators.....	20
2.2.3 Sediment experiment 1	22
2.2.4 Sediment and maternal effect experiment 2.....	23
2.2.5 Statistical Analyses.....	24
2.3 Results.....	25
2.3.1 Sedimentation Experiment 1	25
2.3.2 Sedimentation and Maternal Effects Experiment 2.....	27
2.4 Discussion	30
2.4.1 Sediment cover and Hatching Success	31
2.4.2 Maternal Effects and Hatching Success.....	34
2.4.3 Conclusions	36
2.5 Acknowledgments	36
2.6 References.....	37
CHAPTER 3. THE POTENTIAL FOR RESTORATION OF ROCKY REEF SPAWNING HABITAT WITH CUSTODIAL MAINTENANCE.....	42
3.1 Introduction.....	42
3.2 Methods	45
3.2.1 Development of Maintenance Devices	45
3.2.2 Spawning Reef Maintenance.....	46
3.2.3 Relative Hardness Measurements.....	48
3.2.4 Egg Deposition Sampling	49

3.2.5	Statistical Analyses.....	49
3.3	Results.....	51
3.3.1	Relative Hardness.....	51
3.3.2	Egg deposition.....	53
3.4	Discussion.....	54
3.4.1	Sediment Removal.....	55
3.4.2	Practical Application of cleaning Devices.....	57
3.4.3	Egg Deposition.....	58
3.4.4	Conclusion.....	59
3.5	Acknowledgements.....	59
3.6	References.....	60

LIST OF FIGURES

- Figure 2-1** Complete PVC Walleye egg incubator with coupler body containing insert, ventilation holes, and nylon mesh. In sediment treatments, bottom sediment was located from the bottom of the coupler to the base of the eggs and cover sediment was inside of the insert covering eggs. Modified from Kock et al. (2006).....21
- Figure 2-2** Hatching success of Walleye eggs during 2018 experiment 1. The middle horizontal line in each box indicates the treatment median response, top and bottom of each box indicates the first and third quartiles and whiskers extend to maximum and minimum observations. Box plots with different lettering represent statistically significant differences Tukey’s HSD; $P < 0.05$26
- Figure 2-3** Hatching success of Walleye eggs during 2019 experiment 2. The middle horizontal line in each box indicates the treatment median response, top and bottom of each box indicates the first and third quartiles and whiskers extend to maximum and minimum observations. Note that control in 2019 experiment includes 25 mm of silt beneath eggs with no sediment cover over eggs. Box plots with different lettering represent statistically significant differences Tukey’s HSD; $P < 0.05$28
- Figure 2-4** The relationship between 2019 female Walleye ($n = 15$) length and mean egg diameter. Data points represent mean female egg size (± 1 standard error). No statistically significant relationship was found between egg diameter and female length ($P = 0.464$).....29
- Figure 2-5** Relationship between egg diameter [A], female length [B] and mean number of Walleye eggs hatched in 2019 Walleye experiment. No statistically significant relationships were found between mean Walleye eggs hatched and mean egg diameter (ANCOVA; $F_{1,39} = 3.541$, $P = 0.067$) and mean Walleye length (ANCOVA; $F_{1,39} = 3.853$, $P = 0.057$).30
- Figure 3-1** Images of the propulsion and hydro-jet benthic sleds. Image A shows the front of the propulsion sled with buoys connected to the top of the device to prevent the sled from inverting. Image B shows the two KASCO marine hydro-fans attached to the sled at a 30° angle. Image C shows the entire hydro-jet sled with hose attached from water pump to sprayer jet piping. Image D shows the sprayer jet piping (view from the bottom of the sled looking up), containing 16 separate sprayer heads.46
- Figure 3-2** Location of Coreyon (average depth: 5 m) and North Island (average depth: 3 m) Reefs within Saginaw Bay, Lake Huron.47
- Figure 3-3** Percent change in relative hardness between pre and post-cleaning of study plots. In 2018, points indicate single mean, percent change for each treatment (a) Coreyon Reef; b) North Island Reef). In 2019 replicates of treatments were added thus, points indicate the group mean, percent change of all treatment plots on Coreyon Reef ($n = 3$; c) and group mean percent change of all treatment plots on North Island Reef ($n = 2$; d). Error bars indicate one standard error from the mean for 2019 sample plots.52

Figure 3-4 Egg deposition rates over study plots on Coreyon and North Island Reefs during the fall of 2018 (lake whitefish) and spring of 2019 (walleye). The middle horizontal line in each box indicates the treatment median response, top and bottom of each box indicates the first and third quartiles and whiskers extend to maximum and minimum observations. Egg deposition rates were significantly different among study plots in 2018 on both Coreyon and North Island Reef. In 2019, egg deposition rates were significantly different among study plots on North Island Reef but not on Coreyon Reef. Letters above box plots represent significant differences, Tukey's HSD post-hoc test $P < 0.05$54

ABSTRACT

Natural and constructed rocky reef habitats constitute important areas for lithophilic spawning fishes and their embryonic and larval offspring. Interstitial spaces created by the structure of rocky reefs create micro-environments where incubating embryos and juvenile fishes are potentially protected from predators. However, if interstitial spaces are filled or blocked by sediment deposition or biofouling, the reef structure may lose the protective benefits for embryonic and larval fish survival. Lake whitefish (*Coregonus clupeaformis*) and walleye (*Sander vitreus*) are native Great Lake lithophilic broadcast spawning fish that use rocky spawning habitats that are vulnerable to degradation caused by deposition of suspended sediments. To restore degraded rocky reef habitat, common practices include addition of material to existing reef structures or construction of new reefs, but both of these practices can be costly and time intensive. In this study, we measured the effect of different types and amounts of sediment cover on hatching success of walleye eggs and assessed if differences in female walleye (female length and egg size) account for tolerance to sediment cover. Additionally, we explored an alternative approach for reef restoration, custodial maintenance, in which we created two novel devices to potentially clean rocky reef habitat. We carried out two laboratory experiments in 2018 and 2019 to test the effect of sediment cover on hatching success of walleye eggs (2018) and to test how female identity and female length or egg size may interact with sediment cover to influence hatching success (2019). We exposed walleye eggs to instantaneous sediment cover (0 mm – 7mm) of either sand (course) or silt (fine) sediments from fertilization until day 15 of incubation. Our results indicated that walleye eggs were sensitive to silt cover (71% mortality- 2 mm cover silt) but not sand (47% mortality- 7mm cover sand). While there was an indication that hatching success was marginally related to female length and egg size, we concluded that sediment cover seemed to have similar

effects on eggs, regardless of female length or egg size. The susceptibility of walleye eggs to mortality caused by sediment cover underscores the need for non-degraded spawning habitat. Our two cleaning devices used either propulsion or pressurized water jets to clean sediments from the rocky structure as they were towed behind a small vessel (i.e., did not require the use of SCUBA divers). We used devices to clean two natural rocky reefs in Saginaw Bay, Lake Huron in 2018 and 2019. We measured relative hardness before and after use of devices on cleaned and uncleaned study plots to determine effectiveness of devices. In addition, we measured egg deposition by fall (lake whitefish) and spring (walleye) lithophilic spawners on study plots to determine potential differences in fish usage of cleaned and uncleaned areas. We found that cleaning devices contributed to changes in relative hardness among study plots. Egg deposition was also variable on study plots but in general, egg deposition was consistently highest on treatment plots cleaned by our device that used propulsion. The practicality of cleaning devices was seemingly related to the magnitude of degradation of rocky reefs, nevertheless, our results show that the use of these or similar devices may potentially increase egg deposition by creating areas of higher-quality habitat. While more testing is necessary to fully understand the potential of our reef cleaning devices, this two-year study suggests that these devices may be capable of restoring degraded rocky spawning habitat which could potentially minimize the negative effects associated with sediment degradation on lithophilic spawning fish.

CHAPTER 1. INTRODUCTION

Lithophilic spawning fish rely on rocky substrate as habitat for spawning, embryonic incubation, and larval development (Tupper and Boutillier 1995; Roseman et al. 2007,2011; Bouckaert et al. 2014). In lakes, rocky substrate such as rocky reefs can be created through natural events (i.e., glacial till) or constructed by fisheries managers (McLean et al 2015). In the Laurentian Great Lakes region, there are over 34 constructed reefs and unknown number of natural reefs (McLean et al 2015). While widespread throughout the Great Lakes region, individual rocky reefs are often quite small (e.g., 95 m²; Marsden et al. 1995), but these small areas of spawning habitat may have disproportionately large impacts on early life survival and subsequent recruitment for lithophilic spawning fishes. The interstitial spaces created by the overlapping of rocks within the reef structure, create potentially important microhabitats for embryonic and juvenile fish. (Biga et al. 1998; Chotkowski and Marsden 1999; Claramunt et al. 2005). Both embryonic and larval stages of some fish species use these interstitial spaces as protective cover from predators (Chotkowski and Marsden 1999). When free of degradation, the interstitial spaces of rocky reef habitat also allow water to flow through the reef structure, providing fish embryos with potentially oxygen-rich water (Jensen et al. 2009; Franssen et al. 2012). When rocky reef habitats lose interstitial spaces or have reductions in interstitial space depth, the functionality of reef structures may also be reduced.

Degradation of rocky reef spawning habitat in the Great Lakes region can be caused by both sediment deposition and biofouling from organisms such as benthic algae or dreissenid mussels (e.g., *Dreissenid polymorpha* and *D. bugensis*). Sediment deposition is a natural process in which sediment particles deposit over benthic substrate. Sediment suspension through wind/wave action and subsequent transportation, can carry sediments long distances in aquatic systems whereby

sediments can redeposit in areas where sediment cover was previously limited. Sediments can also be introduced to aquatic systems through runoff from terrestrial environments (Harms et al. 1974). Though sediment suspension and introduction through runoff are natural events, these processes can be amplified through anthropogenic influences. For example, dredging to deepen or widen river channels can resuspend sediments and cause burial of downstream habitat (Nichols et al. 1990). Deforestation and removal of buffer zones around agricultural fields can increase the amount of sediment loading through terrestrial runoff into aquatic systems (Harms et al. 1974; Meyers 2006; Smith and Friedrichs 2011). The combination of natural sedimentation and anthropogenic additions can have negative effects on rocky reef habitat by filling interstitial spaces and blocking the flow of water through the reef structure. Similarly, biofouling (i.e., the attachment of benthic algae and invasive mussel mussels to the reef structure) can limit the functionality of the reef by blocking interstitial spaces and limiting oxygen availability, but also potentially damaging incubating eggs (Marsden and Chotkowski 2001).

Current strategies to remediate or restore rocky reef habitat include construction of new reefs or addition of material to historic reef locations (McLean et al. 2015). Constructed reefs are assembled with a variety of stone sizes ranging from cobble to boulders and it is not uncommon to construct reef habitat with a variety of stone sizes (Marsden et al. 1995; Roseman et al. 2011; McLean et al. 2015). Materials used for reef construction are generally chosen to fit the requirements or preferred spawning substrate of target spawning fish (McLean et al. 2015). Similarly, the size of the materials used to construct reef habitat have implications for interstitial space size and thus, egg predation. For example, smaller, more complex interstitial spaces provide more protection for incubating fish eggs compared to larger, more uniform, interstitial spaces (Biga et al. 1998). Smaller interstitial spaces may be more favorable for egg protection from predators;

however, smaller interstitial spaces could potentially be filled with sediments at greater rates compared to larger interstitial spaces. Therefore, choosing appropriately sized rock material for rocky reef habitat can be a tradeoff between the functionality and the longevity of the reef structure. Much like natural reefs the longevity of constructed reefs is also dependent on hydrological processes like suspension and redistribution of sediments and biological factors such as biofouling (Marsden and Chotkowski 2001; McLean et al. 2015). Due to the susceptibility of constructed reefs to degradation, it may be useful to develop methodology to preform custodial maintenance on rocky reef habitat potentially extending the longevity of these spawning structures. Custodial maintenance of rocky spawning habitat may be particularly attractive as it would likely be less costly in terms of time, effort, and funding, compared to new reef construction.

Due to the negative effects that sediment degradation can have on potentially important spawning habitat for lithophilic spawning fishes, we conducted a two-year study. First, we aimed to determine the negative impacts of sediment cover (a proxy for sediment degradation) on the hatching success of incubating walleye eggs. Through controlled laboratory experiments we found that sediment cover reduces hatching success of walleye eggs with fine particle silts having the greatest negative effect on hatching success. We also examined how maternal traits such as female length and egg size, may influence hatching success when walleye eggs were covered with sediments. We explored such potential effects because there may be a relationship between female walleye length and the size of their eggs (Feiner et al. 2016) and egg size may have implications for hatching success in low oxygen environments (Sargent et al 1987; Van den Berghe and Gross 1989; Einum et al. 2002). While we found that individual females influenced hatching success, the negative effects of sediment cover on hatching success were not significantly related to maternal traits (egg size, and female length).. These results provided evidence that sediment degradation

may have negative effects on lithophilic spawning fishes, and further validated the utility of restoration of degraded rocky reef habitats.

With the goal of developing novel methodology for rocky reef restoration, we next, developed two cleaning devices that were tested in Saginaw Bay, Lake Huron. Test locations included two natural rocky spawning reefs, Coreyon Reef and North Island Reef, which differed in levels of degradation. Over a two-year period, we cleaned experimental treatment plots with both cleaning devices and recorded percent change in relative hardness (i.e., an indicator of soft and hard benthic substrate) before and after cleaning took place. We also determined fish usage of cleaned and uncleaned treatment plots by collecting both walleye and lake whitefish eggs in the spring and fall respectively, using passive egg collection gear. Our results varied between years and reefs, however, several patterns were apparent. On Coreyon Reef (the heavily degraded reef), in general, the cleaning devices did not create obvious differences in relative hardness after cleaning. Similarly, egg deposition seemed to be similar among treatment and control study plots, indicating that the cleaning devices likely did not uncover reef structure which potentially would have led to higher egg deposition in treatment plots. These trends of relatively low changes in relative hardness before and after cleaning, and no significant difference in egg deposition among treatment plots may be explained by the severe degradation (e.g., likely up to a meter of sediment cover in some areas, personal observation by Alex Gatch) on Coreyon Reef. Our cleaning devices thus, may not have been capable of removing enough sedimentation to uncover the reef structure and remove sediments from interstitial spaces.

Results on North Island Reef, were more encouraging for the development of our reef cleaning devices. In particular, we found that one of our cleaning devices (using propulsion fans) consistently increased relative hardness after cleaning and that treatments cleaned by this same

device consistently had greater egg deposition by both spring and fall lithophilic spawners compared to uncleaned control plots. These results potentially indicate that one of our reef cleaning devices was capable of consistently removing sediments from the reef structure, creating higher quality spawning habitat which may have attracted spawning lithophilic fish.

Although our cleaning devices had varied success in removing sediments from the reef structure and affecting egg deposition in treatment areas, the devices show potential as an option for rocky reef maintenance. We conclude that the practicality of our cleaning devices likely depends on magnitude of degradation present on a reef and physical characteristics of the reef and its location. Nevertheless, we suggest that more development and testing of these or similar devices be continued, as custodial maintenance of degraded rocky reef habitat is a potentially viable option for reef restoration.

CHAPTER 2. THE EFFECT OF SEDIMENT COVER AND FEMALE CHARACTERISTICS ON THE HATCHING SUCCESS OF WALLEYE

2.1 Introduction

Introduction and resuspension of sediments are natural processes in aquatic systems. However, anthropogenic contributions to sedimentation such as dredging, deforestation, and agricultural runoff, have increased the rate at which these processes occur (Harms et al. 1974; Meyers 2006; Smith and Friedrichs 2011). When fine particulate sediments enter an aquatic system or existing sediments are resuspended, they have the potential to deposit on important spawning and nursery habitat used by demersal spawning fishes. Rocky spawning habitat is an example of a habitat type that is negatively affected by sedimentation. The interstitial spaces created by rocky spawning habitat provide protection for incubating fish eggs and larva, (particularly non-guarding lithophilic spawning fishes; Claramunt et al. 2005) and as sediments are deposited over these habitats, the protective interstitial spaces can be lost due to infilling (McLean et al 2015). Fine particulate sediments such as sands and silts, have been found to have stronger effects on incubating eggs compared to coarse-sized sediments such as gravels (Reiser and White 1988). When deposited over incubating eggs, fine particulate sediments (i.e., sands and silts) clog interstitial spaces potentially depriving incubating eggs of oxygen-rich water (Bennett et al. 2003; Greig et al. 2005; Kemp et al. 2011) and contribute to egg mortality.

Incubating fish eggs can be negatively affected by sediment cover in multiple species of lithophilic spawning fishes including Pacific salmonids *Oncorhynchus sp.* Brook Trout *Salvelinus fontinalis*, White Sturgeon *Acipenser transmontanus*, and Lake Whitefish *Coregonus clupeaformis* (Turnpenny and Williams 1980; Fudge and Bodaly 1984; Argent and Flebbe 1999; Kock et al. 2006). Additionally, species differ in their relative susceptibility to sediment-induced egg mortality.

For example, white sturgeon eggs have a 50% survival rate when covered with 5 mm of sediments (Kock et al. 2006) whereas Lake Whitefish eggs have a <18% survival rate when covered in 1-4 mm of sediments (Fudge and Bodaly 1984). Another species of non-guarding demersal spawning fish that may be negatively affected by sediment cover on incubating eggs is Walleye *Sander vitreus*. Due to their selection of lithophilic spawning habitats in both rivers and nearshore areas of lakes (Strange and Stepien 2007), Walleye are potentially susceptible to unintentional burial of their eggs. In their native ranges, Walleye spawn in early spring when abiotic factors such as sediment loading and river discharges (Mion et al. 1998), water pH (Hulsman et al. 1983) and water temperature (Ivan et al. 2010) are highly variable which can have large negative impacts on embryo survival. With a relatively short incubation period (e.g., 2-3 weeks) compared to other lithophilic spawning fish (e.g., Lake Whitefish 3-4 months), Walleye eggs may still be susceptible to burial by sediments due to highly variable environmental factors. Spring storm events during incubation periods can produce large waves and strong currents that temporarily suspend sediments which later deposit over incubating eggs and can transport Walleye eggs from rocky substrates into less suitable habitats (Roseman et al. 2001; Kelder and Farrell 2009). Lentic and lotic spawning habitats may experience different magnitudes of sediment degradation, resuspension and redistribution over spawning habitats. For example, large lotic systems can be influenced by channelization and the movement of large vessels. Dredging events used to increase the size of shipping channels, can resuspend large amounts of sediments (e.g., 500 ml/L; Suedel et al. 2014) and deposit these sediments downstream potentially depositing over spawning habitat (e.g., 5 – 19 cm sediment cover; Nichols et al. 1990). Similarly, the physical forces associated with movement of vessels through lotic systems can both resuspend sediments and increase bank erosion, thus introducing new sediments to the system (Parchure et al. 2001). In lentic systems,

the main source of sedimentation over spawning habitat is likely due to resuspension of sediments by waves and storm events. Anthropogenic land uses may exacerbate introduction of sediments from runoff (e.g., Harms et al. 1974) thereby increasing sediment loads and potentially negatively affecting spawning habitats in lentic systems. In general, lentic systems have lower water current velocities compared to lotic systems and this may affect the distribution of sediments over spawning habitat and the length of time which the sediments are present on the habitat. Walleye can spawn on rocky habitat in both lotic and lentic systems and though the sources and magnitudes of sediment degradation can be different, Walleye embryos are potentially susceptible to sediment cover in a variety of aquatic systems. While other studies have focused on the influence of suspended sediments on incubating Walleye eggs (Leis and Fox 1994; Mion et al. 1998; Suedel et al. 2012, 2014), there is a knowledge gap on the effect of sediment cover on incubating Walleye eggs.

Similar to species differences in egg survival under sediment cover, individual Walleye may differ in their susceptibility to sediment-induced egg mortality. Walleye populations differ in mean egg sizes (Johnston and Leggett 2002; Wang et al. 2012), and within populations there is evidence of weak maternal effects, whereby egg size increases with maternal size and age (Feiner et al. 2016). In other species, such as Sockeye Salmon *Oncorhynchus nerka*, egg size appears to be related to substrate size and composition, however, this seems to be a function of selection for females that produce eggs of a more suitable size depending on the substrate in a given habitat (Quinn et al. 1995). The size of an egg has implications for oxygen requirements. Larger eggs potentially require more total oxygen, but less oxygen per unit mass, compared to smaller eggs and therefore, may have differential hatching success in low oxygen environments (e.g., sediment cover) (Sargent et al 1987; Van den Berghe and Gross 1989). On the other hand, because smaller

eggs have a larger surface area to volume ratio compared to larger eggs, smaller eggs have a greater ability to acquire oxygen relative to their size (Sargent et al. 1987). This idea is inconsistent, however, with Einum et al. (2002) who determined that when subjected to low concentrations of dissolved oxygen, Brown Trout *Salmo trutta* eggs that were larger in diameter, had higher survival rates compared to smaller diameter eggs. Einum et al. (2002) suggested that the increased survivability of larger eggs was likely due to the non-proportional relationship between oxygen requirement and egg mass. Similarly, larger eggs have larger energy reserves which are likely favored in stressed (i.e., low oxygen) environments (Einum et al. 2002). Finally, due simply to their greater size, larger eggs are less likely to become completely covered by settling particles. This study attempted to further elucidate the relationship between egg size and hatching success in situations where incubating eggs may be covered with fine particulate sediments.

The purpose of this study was to determine the effect of sediment cover on the hatching success of incubating Walleye eggs. Due to the negative effect sediment cover has on other demersal spawning fish, we hypothesized that Walleye hatching success would decrease as sediment particle size decreased and as sediment cover depth increased. Furthermore, this study examined if eggs of different females varied in their susceptibility to sediment cover and whether female size and egg size influence the effect of sediment cover on hatching success of Walleye eggs. Because maternal size has been correlated with egg size in past studies and because larger eggs have been shown to have higher survival in low oxygen conditions, we hypothesized that larger eggs from larger females would have better hatching success.

2.2 Methods

2.2.1 Egg collection.

Walleye eggs were collected by the Indiana Department of Natural Resources from mature females at Brookville Reservoir in Brookville, Indiana (April 1, 2018 $n = 7$ females, April 2019 $n = 15$ females). The Brookville Walleye population represents a lentic spawning population that utilizes near shore rocky reef spawning habitats. During 2018 and 2019, eggs from individual females were fertilized by a mixture of milt from three randomly selected males (also sourced from Brookville Reservoir, Indiana) to ensure successful fertilization and control for paternal effects. To limit mortality during transport, Walleye eggs were water hardened for four hours before transport to Purdue University's Aquaculture Research Laboratory (ARL) in West Lafayette, Indiana. While collecting eggs during 2019, sub-samples of unfertilized eggs from each female were taken and preserved in separate 20 mL glass scintillation vials of 10% formalin to be measured for egg diameter.

2.2.2 Egg incubators

Walleye egg hatching success was evaluated in polyvinyl chloride (PVC) egg incubators (Figure 1). The 2018 sedimentation experiments used PVC egg incubators similar to those used in Kock et al. (2006; Figure 2-1). The incubators were built using a 101 mm inside diameter PVC coupler body with six 9.52 mm ventilation holes evenly spaced, a 101 mm outside diameter PVC insert that was 127 mm long, and 500 micron nylon mesh. The nylon mesh was used to hold bottom sediments in the coupler body and to prevent sediments from moving through ventilation holes. The mesh was also located on the bottom of the PVC insert to hold incubating eggs and on the top of the PVC insert to ensure larval Walleye did not escape incubators once they had hatched. The

mesh at the top of the PVC insert was removable, such that Walleye eggs could be inserted into the incubator and then the mesh could be reattached. The 2019 sedimentation and maternal effects experiment used a similar incubator design, but incubators were approximately half the size of the 2018 experiment (i.e., 50.8 mm diameter coupler body and 50.8 mm diameter insert). The smaller sized incubators in 2019 were used to increase the number of incubators that could fit into the experimental tanks.

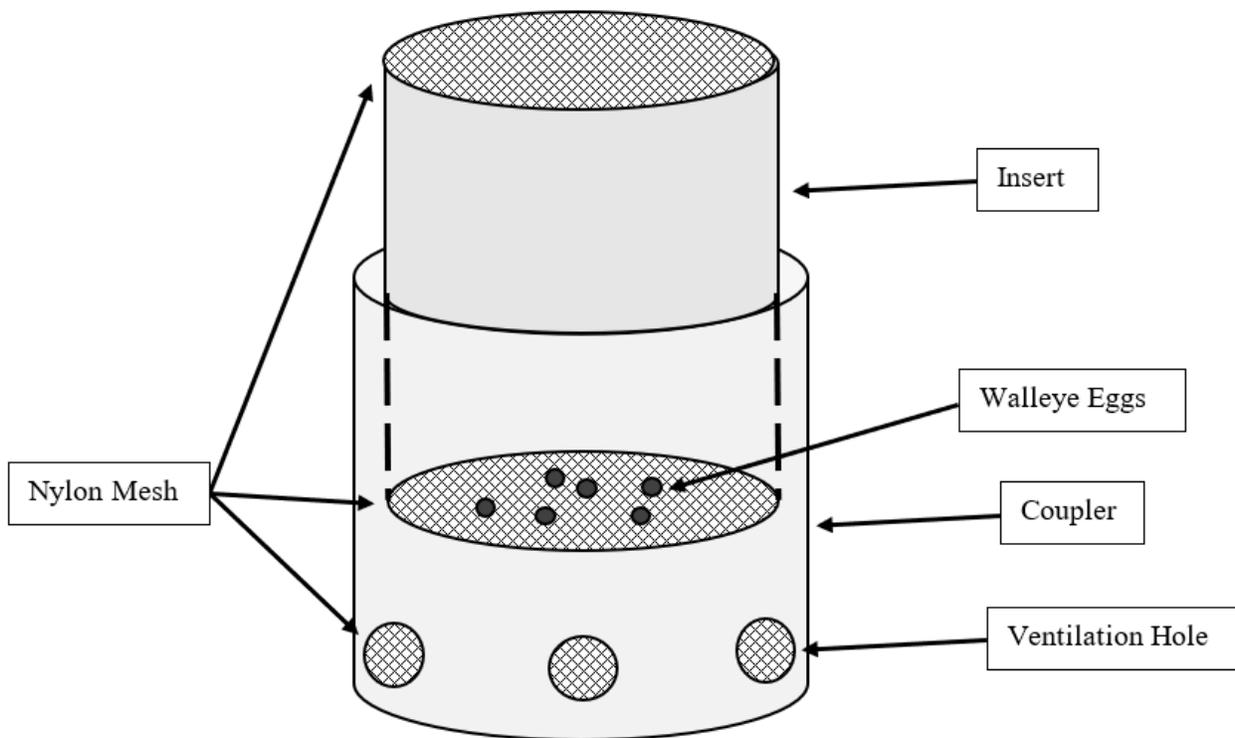


Figure 2-1 Complete PVC Walleye egg incubator with coupler body containing insert, ventilation holes, and nylon mesh. In sediment treatments, bottom sediment was located from the bottom of the coupler to the base of the eggs and cover sediment was inside of the insert covering eggs. Modified from Kock et al. (2006).

2.2.3 Sediment experiment 1

To test the effect of sediment cover on hatching success of Walleye eggs, sand (0.5mm-2mm diameter) and silt (< 0.425 mm diameter) were used in varying sediment height treatments. Treatments of sand and silt cover consisted of 1) control – no sediment beneath or covering eggs 2) 50 mm of sediment (either sand or silt) under eggs with no cover over eggs 3) 50 mm of sediment under eggs with 2 mm (low) of cover over eggs and 4) 50 mm of sediment under eggs with 7 mm (high) of cover over eggs. All treatments with sediment (no cover or cover) included sediment underneath the incubating eggs to simulate natural conditions in which walleye eggs may be incubating over surfaces that contain sediments (i.e., sediments within interstitial spaces). Sediment cover treatments (2 mm or 7 mm) represented sediment depth above the base layer of sediments, (i.e., sediment cover represents height of sediment from base of egg not top of egg). The sediment cover of 2 mm and 7 mm were chosen for this experiment in part because of the conclusions of Kock et al. (2006) who found reductions in White Sturgeon (a fish with similar lithophilic spawning behaviors) hatching success with similar sediment cover depths. The 2 mm depth was also the approximate minimum sediment depth needed to completely cover walleye eggs. Each tank contained three treatments of sand cover, three treatments of silt cover, and one control, totaling seven treatments per tank. Sand was sourced from a hardware store and silts were sourced from a pond in West Lafayette, Indiana and sieved with a 0.425 mm sieve to remove larger particles. Sand and silt sediments were sterilized in an autoclave at 110°C, 24 hours before being used. All incubators received 25 fertilized Walleye eggs (fertilized 6 hours before start of experiment) before cover sediments were added to egg incubators.

Eggs from individual females ($n=7$) were grouped into seven 190 L tanks in a flow through system, such that each tank contained eggs from only one female and included all seven treatments. Egg incubators containing different treatments were positioned randomly in the 190 L tanks with

the inflow of water coming from the center of the top of the tank and draining from the base of the tank (flow rate 3.79 L/sec). Ground water sourced from the ARL well system was used for the experiment. Dissolved oxygen (8.9 mg/L mean, \pm 0.5 SE), photoperiod (12 hours) and water temperature (14.4°C mean, \pm 0.7 SE) were monitored and maintained within a small range among the seven tanks. To limit egg mortality due to fungus, hydrogen peroxide (30%) was added to tanks every other day. At 14.4°C Walleye eggs start hatching in approximately 10 days (Smith and Koenst 1975); therefore, Walleye eggs were incubated for 15 days to allow proper hatch time. Egg incubators were monitored daily but dead eggs were not removed from treatments because they were not visible under silt and sand treatment. After 15 days of incubation, hatched larvae (live or dead) were counted from each treatment incubator and stored in 95% ethanol.

2.2.4 Sediment and maternal effect experiment 2.

To test the interactive effect of sediment cover and maternal effects on hatching success, silt cover treatments and eggs from different females (n= 15 females) were used. Due to the strong negative effect of silt cover on hatching success that was found in the 2018 experiment (see Results), only silt treatments and a control were used in the 2019 experiment. Treatments consisted of 1) 25 mm of silt under eggs with no cover over eggs (here used as the control), 2) 25 mm of silt under eggs with 2 mm (low) of silt over eggs, and 3) 25 mm of silt under eggs with 4 mm (high) of silt over eggs. Silts in experiment 2 were sourced from the same pond as experiment 1 and sieved with a 0.425 mm sieve. Silt was sterilized in an autoclave at 110°C, 24 hours before being used. Egg incubators each contained 25 eggs from a single female. Egg incubators containing different treatments and separate female eggs were placed randomly in three 190 L tanks in a flow through system, such that each tank contained three treatments from each female (i.e., 45 total egg incubators per tank, 135 egg incubators in total). Ground water sourced from the ARL well system

was used for the experiment and flow rate was 3.79 L/sec. Dissolved oxygen (7.2 mg/L mean, \pm 0.2 SE), photoperiod (12 hours) and water temperature (14.3°C, \pm 0.3 SE) were maintained fairly consistent among the seven tanks. To limit egg mortality due to egg fungus, hydrogen peroxide (30%) was added to tanks every other day. After 15 days of incubation, hatched larvae (live or dead) were counted from each treatment incubator and stored in 95% ethanol.

Mean egg diameter was calculated for individual females to test for variation in egg size among females and to test for interactions with egg diameter and hatching success. For each female, 10 preserved unfertilized eggs were photographed under a dissecting microscope. To standardize for potential shrinkage due to preservation, all eggs were photographed on the same day (April 19, 2019). Using ImageJ software, two perpendicular diameters of each egg were measured (Wang et al. 2012; Feiner et al. 2016). Mean egg diameters for individual females were calculated using both measurements from each of 10 eggs per female.

2.2.5 Statistical Analyses

Experiment 1. — A blocked one-way analysis of variance (ANOVA) was used to test the effect of sediment cover on hatching success of Walleye eggs. Total number of eggs hatched per incubator was used as the dependent factor, with treatment as the independent factor and tank as the blocking factor. Note that due to the design of one female to each tank, blocking encapsulated both tank and female effects. A Tukey's HSD post hoc test was used to determine which treatments were significantly different from each other.

Experiment 2. — A blocked ANOVA was used to test for the effect of sediment cover and individual female identity on hatching success of Walleye. Total number of eggs hatched per incubator was used as the dependent factor ($n = 135$ incubators) with treatment and individual

female as independent factors, and tank as the blocking factor. A Tukey's HSD post hoc test was used to determine statistical significance among treatments and among tanks.

After determining that female identity significantly affected hatching success (see Results), subsequent analyses were performed to evaluate the potential for female size and egg diameter to explain hatching success. A linear regression was used to determine the relationship between egg size and female length. Analysis of covariance (ANCOVA) were used to test for either the effect of female length or mean egg diameter (covariates), as well as sediment cover and the interaction of sediment cover with either female length or egg diameter. To account for dependence of eggs from the same female among tanks, a single mean hatching success across tanks in each treatment and control was used per female as the dependent variable in ANCOVAs (i.e., $n = 45$). All statistical tests were analyzed with JMP statistical software version 13 and tested at $\alpha = 0.05$ level of significance.

2.3 Results

2.3.1 Sedimentation Experiment 1

Sediment cover had a statistically significant effect on hatching success of Walleye eggs (ANOVA; $F_{6,36} = 8.637$, $P < 0.001$; Figure 2-2). Specifically, silt treatments of low (2 mm) and high (7 mm) cover had significantly lower hatching success compared to the control (Tukey's HSD; $P = 0.001$ and $P < 0.001$, respectively). In contrast, there was no statistically significant difference between sand treatments and the control. Additionally, silt treatments of high cover had significantly lower hatching success compared to sand treatments with no cover, low cover and high cover (Tukey's HSD; $P = 0.007$, $P = 0.008$ and $P = 0.01$, respectively). Treatments that included silt as a bottom sediment but no cover, were not significantly different from sand

treatments or the control (Tukey's HSD; $P > 0.05$ for all comparisons). A tank effect was detected among the seven tanks (ANOVA; $F_{6,36} = 3.585$, $P = 0.007$) however, it is unclear if this was due to the tank environment or a female identity effect because the experimental design did not allow us to differentiate female effects from tanks effects. Similarly, the tank effect that was observed indicated that only one of the seven tanks had significantly less hatching success compared to the other six tanks. Importantly, the negative effects of silt cover on hatching success (i.e., 2 mm and 7 mm silt cover treatments significantly reduced hatching success compared to the control) were found in this tank as well.

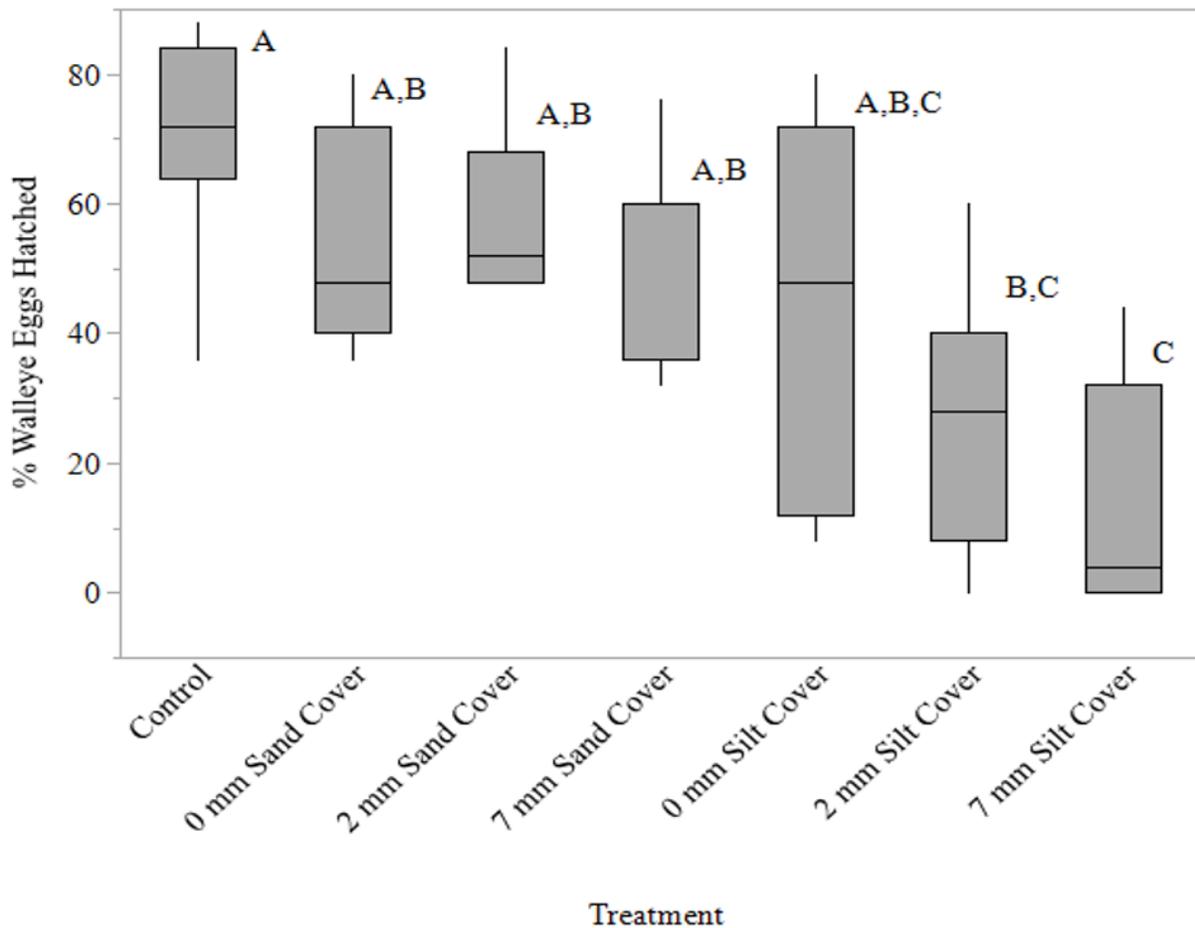


Figure 2-2 Hatching success of Walleye eggs during 2018 experiment 1. The middle horizontal line in each box indicates the treatment median response, top and bottom of each box indicates the

first and third quartiles and whiskers extend to maximum and minimum observations. Box plots with different lettering represent statistically significant differences Tukey's HSD; $P < 0.05$.

2.3.2 Sedimentation and Maternal Effects Experiment 2

Sediment cover (ANOVA; $F_{2,116} = 27.95$, $P < 0.001$), individual female identity (ANOVA; $F_{14,116} = 2.867$, $P = 0.001$) and tank had a significant effect on hatching success of Walleye eggs (overall model ANOVA; $F_{18,116} = 6.025$, $P < 0.001$). Low and high treatments of silt cover had significantly lower hatching success compared to the control (Tukey's HSD; $P < 0.0001$ and $P < 0.001$ respectively; Figure 2-3) but were not significantly different from each other.

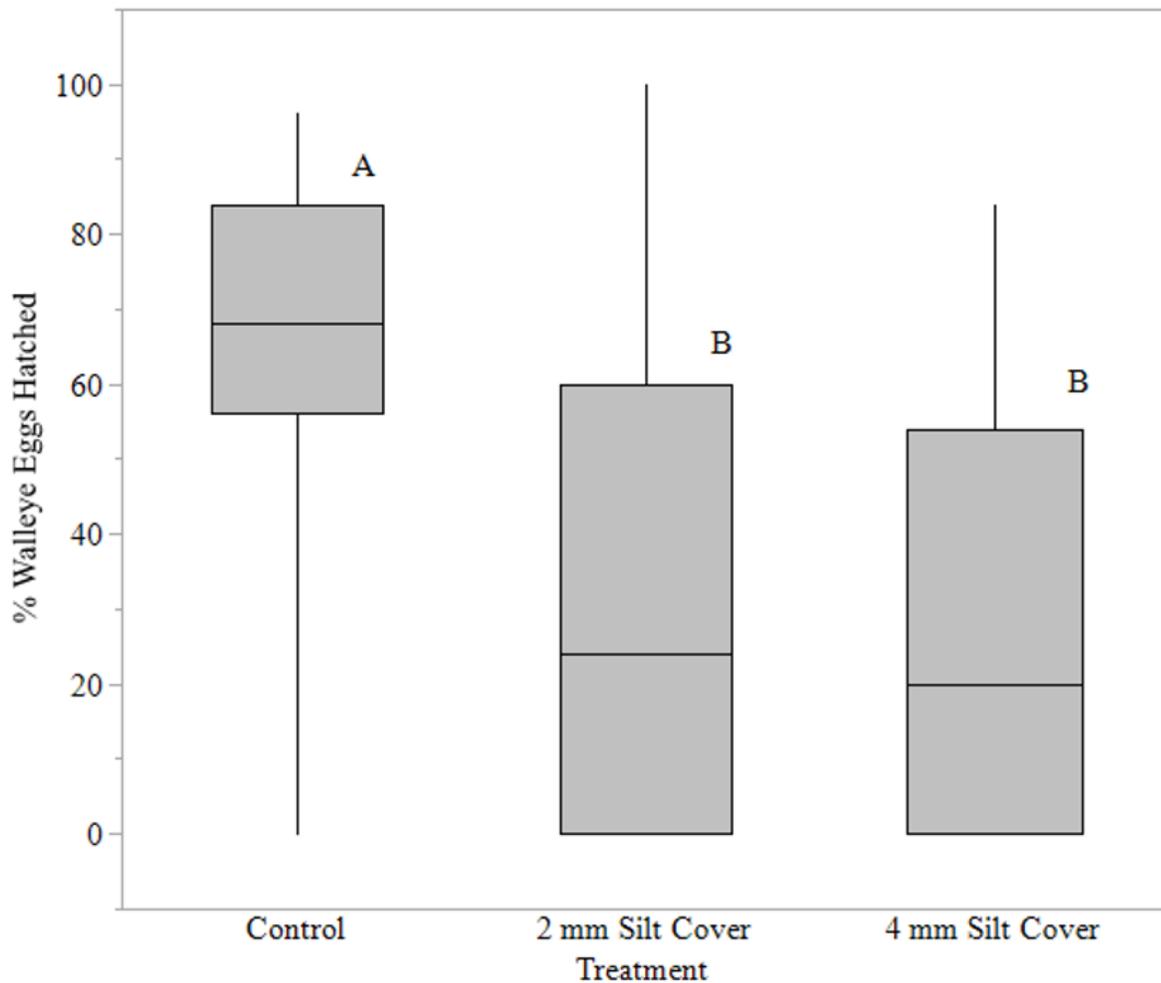


Figure 2-3 Hatching success of Walleye eggs during 2019 experiment 2. The middle horizontal line in each box indicates the treatment median response, top and bottom of each box indicates the first and third quartiles and whiskers extend to maximum and minimum observations. Note that control in 2019 experiment includes 25 mm of silt beneath eggs with no sediment cover over eggs. Box plots with different lettering represent statistically significant differences Tukey’s HSD; $P < 0.05$.

To evaluate the potential effects of specific maternal attributes, the influence of female length and mean egg diameter on hatching success were also tested. There was no relationship between egg diameter and female length ($r^2 = 0.004$, $P < 0.464$; Figure 2-4).

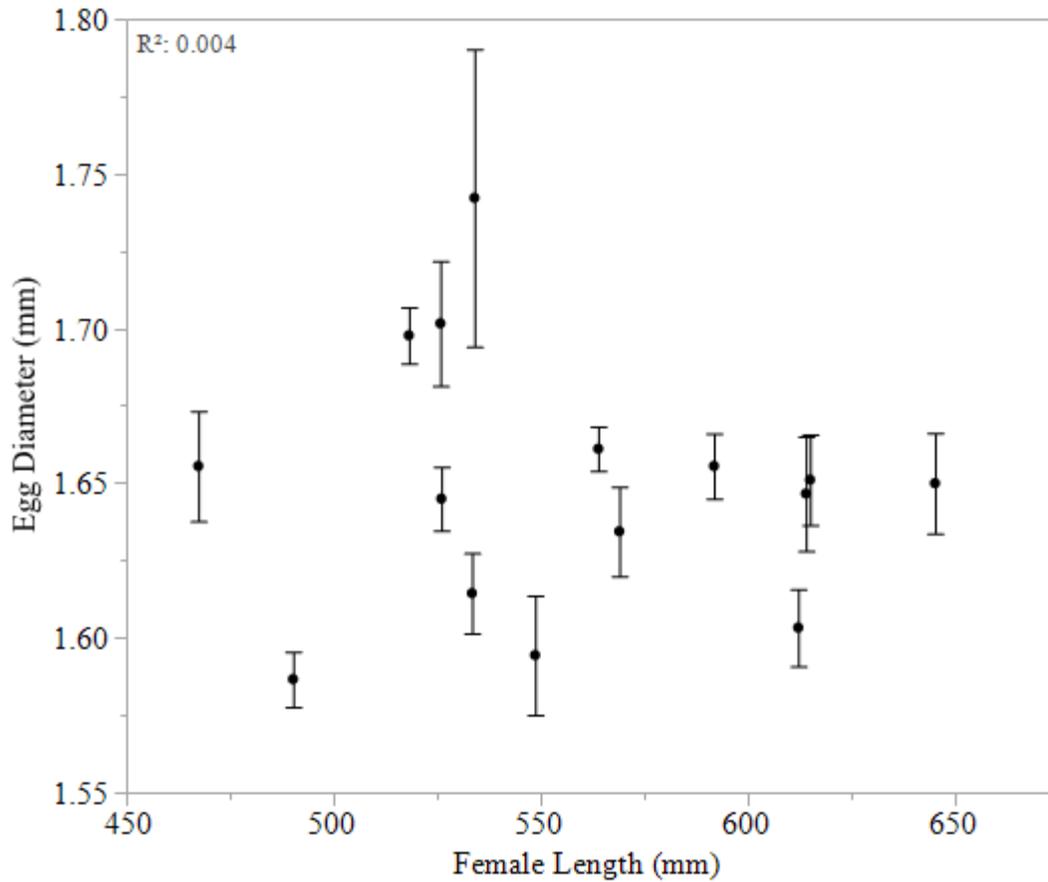


Figure 2-4 The relationship between 2019 female Walleye ($n = 15$) length and mean egg diameter. Data points represent mean female egg size (± 1 standard error). No statistically significant relationship was found between egg diameter and female length ($P = 0.464$).

Additionally, both egg diameter (ANCOVA; $F_{1,39} = 3.541$, $P = 0.067$; Figure 2-5) and the interaction between egg diameter and treatment (ANCOVA; $F_{2,39} = 0.136$, $P = 0.873$) were not significantly related to hatching success, overall model (ANCOVA; $F_{5,41} = 7.31$, $P < 0.001$). Similarly, neither female length (ANCOVA; $F_{1,39} = 3.853$, $P = 0.057$; Figure 2-5) nor the interaction between female length and treatment (ANCOVA; $F_{2,39} = 0.258$, $P = 0.774$) were significantly related to hatching success, overall model (ANCOVA; $F_{5,41} = 7.50$, $P < 0.001$). Note, that while the effects of egg diameter and female length were marginally insignificant, in general, hatching success increased with egg size and decreased with female length. However, the clear

lack of interaction of these attributes with sediment cover suggests that hatching success responded similarly to sediment cover, regardless of egg diameter and female length.

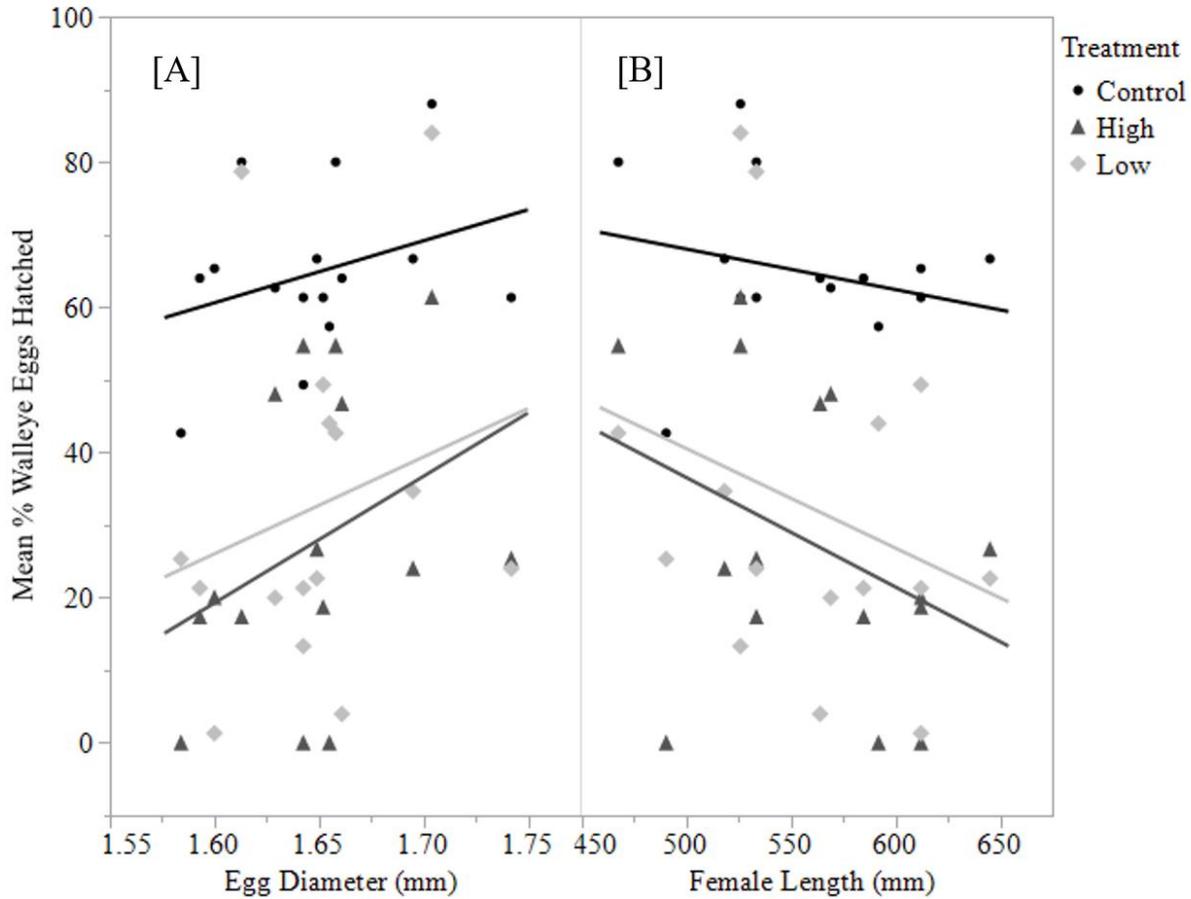


Figure 2-5 Relationship between egg diameter [A], female length [B] and mean number of Walleye eggs hatched in 2019 Walleye experiment. No statistically significant relationships were found between mean Walleye eggs hatched and mean egg diameter (ANCOVA; $F_{1,39} = 3.541$, $P = 0.067$) and mean Walleye length (ANCOVA; $F_{1,39} = 3.853$, $P = 0.057$).

2.4 Discussion

Experimental results support the hypothesis that sediment cover, particularly silt cover, has a negative effect on hatching success of incubating Walleye eggs. While it is known that eggs from other demersal reef spawning fish such as White Sturgeon (Kock et al. 2006) are negatively affected by sediment cover, this is to our knowledge the first account of incubating Walleye eggs

showing similar negative trends in hatching success due to silt cover. Moreover, we hypothesized that larger maternal size and larger egg size would increase hatching success, thus somewhat compensating for mortality caused by sediment cover. Interestingly, we found that individual females differed in hatching success, and that maternal length and mean egg diameter had a marginally insignificant effect (negative and positive, respectively) on hatching. However, the negative effect of sediment cover on hatching success appeared to be similar regardless of maternal length or mean egg size.

2.4.1 Sediment cover and Hatching Success

Sediment resuspension events caused by storms or dredging can lead to sediment cover over spawning habitat (e.g., 0.5 cm – 19 cm after a dredging event; Nichols et al. 1990), which was simulated in this study. Results indicated that fine particle silts (which are more easily suspended and deposited) negatively affect Walleye hatching success at 2 mm of cover. This suggests that even relatively small amounts of silts that are resuspended and deposited over incubating Walleye eggs have the potential to influence egg survival and potential year class strength starting at the embryonic stage. Sediment cover produced by dredging events at the scale found by Nichols et al. (1990), was not simulated in this study, however, negative effects were found at as low as 2 mm of silt cover, suggesting that if similar levels of sediment cover found in Nichols et al. (1990) were used in this study (i.e., 0.5 cm – 19 cm) 100% mortality may have occurred. Resuspension of fine silts and sands are common in dredging events, however, Suedel et al. (2014) determined that the suspension of sediments (500 mg/L) had no significant effect on hatching success of Walleye. While Suedel et al. (2014) demonstrated that suspended sediments may have no significant effect on embryo survival, they did not take into account the settling of suspended sediments covering incubating eggs nor other negative effects associated with

suspended sediments. For example, Mion et al. (1998) determined that river discharges, which contribute directly to suspended sediments in the water column, negatively affect the survival of larval Walleye. Mion et al. (1998) hypothesized that the large influx of suspended sediments during increased river discharges led to larval death immediately after hatch due to suffocation, gill damage and abrasion associated with suspended sediments and strong currents. Additionally, Crane and Farrell (2013) showed that Walleye eggs had zero retention (i.e., were removed from substrate by water currents) when deposited over rocky substrate that was covered in fine sediments. This loss of egg adhesion caused by fine sediment cover has the potential to increase transport of incubating Walleye eggs which can be vulnerable to removal from rocky habitat by perturbation events (Roseman et al. 2001; Kedler and Farrell 2009). Further, Walleye eggs are commonly preyed upon during incubation (Roseman et al. 2006) and loss or shrinkage of interstitial spaces on rocky habitat due to infilling from deposited sediments, potentially increases predation by egg predators (Chotkowski and Marsden 1999). Lastly, locations of walleye spawning habitats can influence the type and magnitude of sedimentation. For example, spawning habitat in some lotic systems may be susceptible to sedimentation due to vessel-induced sedimentation, as waves created by the movement of vessels has the potential to erode banks and suspend sediments which can later deposit over spawning habitats (Parchure et al. 2001). Spawning habitats both natural and constructed in lotic systems are also potentially vulnerable to degradation due to the influence of shipping channels. Shipping channels in large lotic systems are frequently dredged, and these dredging events combined with other anthropogenic stressors (i.e., vessel waves and prop-wash) may increase sedimentation over Walleye spawning habitat and negatively affect incubating embryos. Spawning habitat in lentic systems may be susceptible to degradation through sediment loading from runoff, nearshore dredging, and resuspension of

sediments within the system. While the sediment load per unit area may generally be lower in lentic systems than lotic systems, there are nonetheless, potential negative effects on spawning habitats and incubating embryos.

Fine sediments as large as 0.84 mm have been shown to negatively affect hatching success in other fish species such as Pacific salmonids and Brook Trout (Resier and White 1988; Argent and Flebbe 1999). Results from this study suggest that Walleye eggs are moderately tolerant to cover by larger sized (0.5mm – 2mm) sediment as 7 mm of sand cover had no significant effect on the hatching success of Walleye eggs. White sturgeon eggs from Kock et al. (2006) had a significant reduction of 80% in hatching success when exposed to 5 mm of sediment (>0.85 mm diameter particles) cover over a duration of 9 days. Walleye eggs in this study incurred no significant reduction in hatching success when covered by sediments > 0.85 mm diameter particle size, for the entirety of the incubation process (15 days). These results suggest that Walleye eggs may be more tolerant to cover by larger sized sediment particles, compared to fish species with similar spawning habitat selection and strategies.

Treatments containing high levels (≥ 4 mm) of silt or sand were commonly found with egg fungus by the end of the experiments. The egg fungus may have been a result of fungal growth on already dead Walleye eggs (though tanks were treated with hydrogen peroxide), but it is possible that the stagnant water under the sediments facilitated the growth of fungus causing egg mortality from a combination of oxygen deprivation and fungal growth. If incubating eggs had gone untreated with hydrogen peroxide, it is possible that mortality in treatment incubators would be higher, further supporting the negative effects of sediment cover on incubating Walleye eggs.

A tank effect was also found in both experiment 1 and experiment 2. Though a tank effect was present, only one tank in both experiment 1 and experiment 2 was significantly different

(lower hatching success) from other tanks. Environmental conditions (i.e., dissolved oxygen, temperature, photoperiod) were nearly identical in all tanks since the tanks were connected in a flow through system. In experiment 1, the female effect and tank effect could not be separated due to the experimental design, therefore, it cannot be determined if the observed differences in hatching success among the one significantly different tank, could be attributed to a female effect or environmental tank effects. Regardless of the tank effect in both experiments, the negative effects of silt cover on hatching success were still present in the tank that had significantly less hatching success.

2.4.2 Maternal Effects and Hatching Success

Eggs from individual females differed in hatching success. However, this effect could not be definitively related to specific maternal traits. Walleye egg diameter was not correlated to female length in the 2019 experiment. This was contrary to the results of past studies which found a weak positive relationship between Walleye egg diameter and female length (Wang et al. 2012; Feiner et al 2016). A sample size of 15 females in the 2019 experiment may not have been large enough to detect a similar relationship. Although egg size and female length were not statistically significantly related to hatching success ($P < 0.067$ and $P < 0.057$, respectively), it is noteworthy that larger eggs and eggs from smaller females tended to have better hatching success. Several other studies have demonstrated a positive intra-specific relationship between egg size and offspring performance in fish (Ojanguren et al. 1996; Einum and Fleming 1999; Burton et al. 2013), including positive effects on hatching success (Einum et al. 2002). Negative relationships between female size and offspring performance are less common. However, Andree et al. (2015) demonstrated that for confamilial Yellow Perch *Perca flavescens* offspring from larger females experienced relatively poor survival during the first few days post hatch. It was hypothesized in

this study that larger eggs may have greater hatching success in sediment cover treatments. Larger eggs may require more oxygen compared to smaller eggs, but this relationship is not linear and larger eggs have greater energy reserves which may be favored in stressed environments (Einum et al. 2002). Further, larger eggs are less likely to be completely buried compared to smaller eggs and when buried, larger eggs potentially have less cover between the surface of the egg and the sediment-water interface compared to smaller eggs. These benefits of larger eggs may partially explain why larger eggs had greater (albeit marginally insignificant) hatching success.

Potential benefits of having a larger egg may not outweigh the negative consequences of sediment cover, however. While there was a female identity effect on hatching success (i.e., some females consistently had higher hatching rates in all treatments compared to other females), there was no interaction between egg size and hatching success (i.e., larger eggs were similar to small eggs in sediment cover treatments). An anoxic environment would negate any benefit of a smaller or larger egg, and this may be why an interaction between egg size and sediment cover was not observed. Einum et al. (2002) determined that larger eggs had significantly higher survival rates in low oxygen environments (2.3 mg/L) but if dissolved oxygen concentrations are low enough, it is possible that neither small or large eggs would have an advantage. Previous studies have determined that Rainbow Smelt *Osmerus mordax* embryos covered by sediments have an increased oxygen demand compared to Rainbow Smelt eggs without sediment cover, regardless of the size of the egg (Wyatt et al. 2010). Wyatt et al. (2010) also determined that the increased oxygen demand by the rainbow smelt eggs caused the environment in the sediment to become anoxic and contributed to mortality.

2.4.3 Conclusions

The results of this study have confirmed that sediment cover over incubating eggs is detrimental to the hatching success of Walleye. Our results show that Walleye eggs are sensitive to sediment cover as low as 2 mm, reducing hatching success by an average of 71%. Female length and egg size did not significantly influence hatching success caused by sediment cover. While there was a marginal positive effect of egg size and negative effect of female length on hatching success, the interactions of egg size or female length with sediment cover were statistically insignificant. That is, eggs of different sizes and from females of different sizes tended to respond in a similar manner to sedimentation. The strong effect that sediment has on incubating eggs further supports the need to better manage sediment loading, suspension, and settlement in aquatic systems to mitigate events that can drive reductions in embryonic survival.

2.5 Acknowledgments

This research was funded by the Great Lakes Restoration Initiative, U.S. Geological Survey (USGS) and the Department of Forestry and Natural Resources at Purdue University. Walleye eggs were supplied by fisheries biologist Jeff Malwitz and Andy Richards at the Indiana Department of Natural Resources. Robert Rode was responsible for the upkeep and observation of all study tanks at the Purdue University Aquaculture Research Laboratory (ARL). Handling of fishes followed the guidelines provided in the “Guidelines for the use of fishes in research” published by the American Fisheries Society, Bethesda, MD. Use of trade names is for descriptive purposes and does not imply endorsement by the U.S. Geological Survey.

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CHAPTER 3. THE POTENTIAL FOR RESTORATION OF ROCKY REEF SPAWNING HABITAT WITH CUSTODIAL MAINTENANCE

3.1 Introduction

Rocky reef habitats are potentially important areas for many freshwater fishes. These rocky structures are used as spawning sites that provide protection for incubating eggs and serve as nursery habitat for developing larval fish (Tupper and Boutillier 1995; Bouckaert et al. 2014). In particular, the interstitial spaces created by reef structure are potentially important for protecting incubating eggs and larvae. However, these spaces are susceptible to infilling from sediments (Bush et al. 1975; Schneider and Leach 1977) and biofouling from organisms such as *Dreissenid polymorpha* and *D. bugensis* (henceforth dreissenid mussels; Neary and Leach 1992) and benthic algae (Edsall et al. 1991). Once rocky reef structures become degraded (i.e., shrinkage or loss of interstitial spaces) survival of incubating eggs and larval fish can be negatively affected. With decreased interstitial spaces, predators may more readily detect or access incubating eggs (Chotkowski and Marsden 1999). Sediment cover over rocky habitats also changes egg adhesion to the substrate such that egg retention within the rocky substrate decreases when the substrate is covered with fine sediments (Crane and Farrell 2013). Furthermore, clogging of interstitial spaces with sediments restricts the flow of water through rocky substrate which has the potential to limit oxygen availability for incubating eggs (Jensen et al. 2009; Franssen et al. 2012). Biofouling from dreissenid mussels and benthic algae can also congest interstitial spaces impeding the flow of water. Moreover, fouling from sharp, jagged dreissenid mussel shells can damage incubating eggs and potentially cause mortality (Marsden and Chotkowski 2001). Increases in mortality at the embryonic and larval stages can have negative effects on year class strength and recruitment

(Bannister et al. 1974; Forney 1976). Therefore, maintenance of high-quality spawning and nursery habitat (e.g., rocky reefs) can potentially have positive population-level effects.

When rocky reef structures become heavily degraded, typical restoration approaches have included construction of new reef habitat on top of or near the existing rocky reef location. In the Laurentian Great Lakes system, there are more than 34 constructed reefs with many of the reefs having been built in the 1990's and 2000's (McLean et al. 2015). Construction of reefs creates high quality spawning habitat; however, these structures are still vulnerable to degradation (McLean et al. 2015). Constructed reefs can be quickly covered by dressenid mussels (Marsden and Chotkowski 2001) benthic algae (Johnson et al. 2006) or sediments (Johnson et al. 2006; McLean et al. 2015). Proper planning and site location can increase the longevity of high-quality constructed spawning reefs (Marsden et al. 2016), but processes contributing to degradation are variable and still not fully understood. Both natural and constructed rocky reefs are prone to infilling of intestinal spaces and we propose that reef maintenance, through the removal of sediments and materials contributing to biofouling, may be a lower-cost option to repair and maintain high-quality rocky reef habitat.

Custodial maintenance of rocky reefs is uncommon and there is no standard methodology for reef maintenance. While peer reviewed evidence of reef maintenance is largely absent, Johnson et al. (2006) attempted to clean small sections of artificial reef (9 m by 18 m) with SCUBA divers that used a combination of hand tools and pressurized water although the success of this methodology was not documented. Reef maintenance at this scale could be possible using teams of SCUBA divers, though on larger reefs, this methodology may not be practical. Sediment removal can also be achieved using methods developed for marine archeology. Sediment removal in this field is typically small in scale where SCUBA divers use hand-held devices such as air lifts

and suction dredges to remove sedimentation (Goggin 1960; Cook 2018). While these methods may be useful on smaller scales, hand-held suction devices may not be practical to rapidly clean large reef areas. As another alternative, “mailbox” devices and similar designs are used by marine archeologist to redirect or deflect propulsion created by the vessels engine to uncover materials below sediments (Bass 1985). This method allows the vessel to suspend over an area and direct pressurized water onto a selected area, but this requires a large vessel to produce sufficient propulsion to reach the substrate at depth, and the vessel has limited mobility while the device is being used (Bass 1985). Outside of archeological research, large scale sediment removal is commonly achieved using hopper dredges, suction cutter head dredges, and bucket dredges (Nichols et al. 1990; Williams 2001). These methods may not be practical for reef maintenance applications; however, because they can potentially damage underlying structure. To successfully and practicably clean rocky reef habitat, novel methods are likely necessary to cover large areas of reef habitat, with limited damage to underlying structure.

In this study we aimed to restore rocky reef habitat that had been degraded by sedimentation and other biofouling using novel cleaning devices and methods. In so doing, our objectives were to 1) develop devices and methodology to clean rocky reef habitat, 2) determine if the cleaning devices could remove sediment to the extent that substrate hardness was affected and 3) determine if egg deposition from fall and spring lithophilic spawners increased on areas of cleaned rocky reef.

3.2 Methods

3.2.1 Development of Maintenance Devices

We created two benthic sled cleaning devices. The first sled design, from here on referred to as the propulsion sled, measured 1.35 m long by 1.0 m wide by 0.75 m tall and weighed approximately 90 kg (Figure 3-1A). Attached to the sled were two Kasco $\frac{3}{4}$ hp marine de-icer fans directed down at a 30° angle (Figure 3-1B). We chose to direct the fans downward at a 30° angle in an attempt to direct sediments in one direction away from the cleaned area to prevent immediate settling over treatment areas. The propulsion sled was powered by a 6500-W portable generator (Powermate PC0146500) and we transferred power through two 30 meter, 120 Volt extension cords from the deck of the towing vessel to the sled.

The second sled design, from here on referred to as the hydro-jet sled, measured 1.35 m long by 1.0 wide by 0.5 m tall and weighed approximately 35 kg (Figure 3-1C). Attached to the sled was a spout system that was constructed with 38 mm galvanized plumber piping fitted with 16 separate sprayer jets (Figure 3-1D). We included a pressure relief valve on the right side of the hydro-jet sled in order to direct suspended sediment in one direction once suspended from the benthic surface. The hydro-jet sled was powered by a 5 hp transfer water pump (Honda GC160) and we transferred water to the sled through a 30 meter discharge hose from the deck of the towing vessel to the sled. We towed both sleds behind a 6.4 m aluminum research vessel with a 150 hp outboard engine using a kinetic energy towing rope to prevent the sleds from catching the reef structure and stopping the boat. See *Supplemental Material* for video of cleaning devices in use.

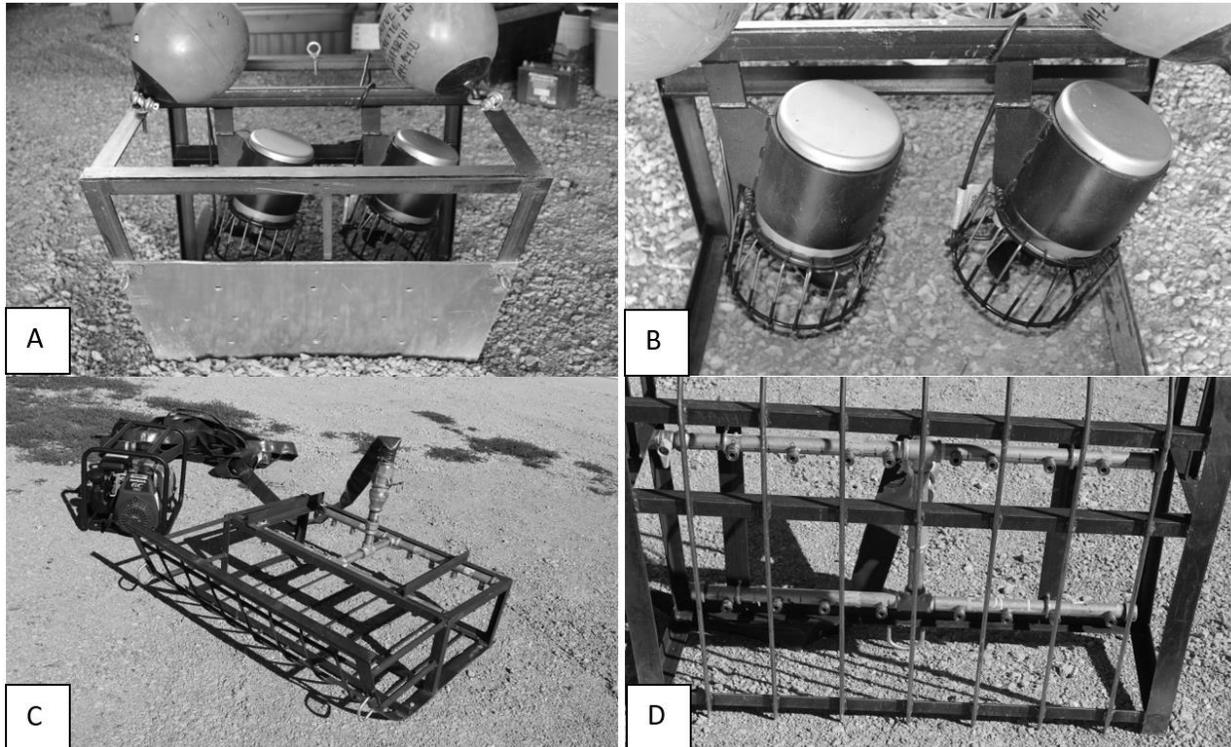


Figure 3-1 Images of the propulsion and hydro-jet benthic sleds. Image A shows the front of the propulsion sled with buoys connected to the top of the device to prevent the sled from inverting. Image B shows the two KASCO marine hydro-fans attached to the sled at a 30° angle. Image C shows the entire hydro-jet sled with hose attached from water pump to sprayer jet piping. Image D shows the sprayer jet piping (view from the bottom of the sled looking up), containing 16 separate sprayer heads.

3.2.2 Spawning Reef Maintenance

In October of 2018 and March of 2019, we performed reef cleaning of circular (50 m diameter) plots on Coreyon Reef and North Island Reef in Saginaw Bay, Lake Huron (Figure 3-2).

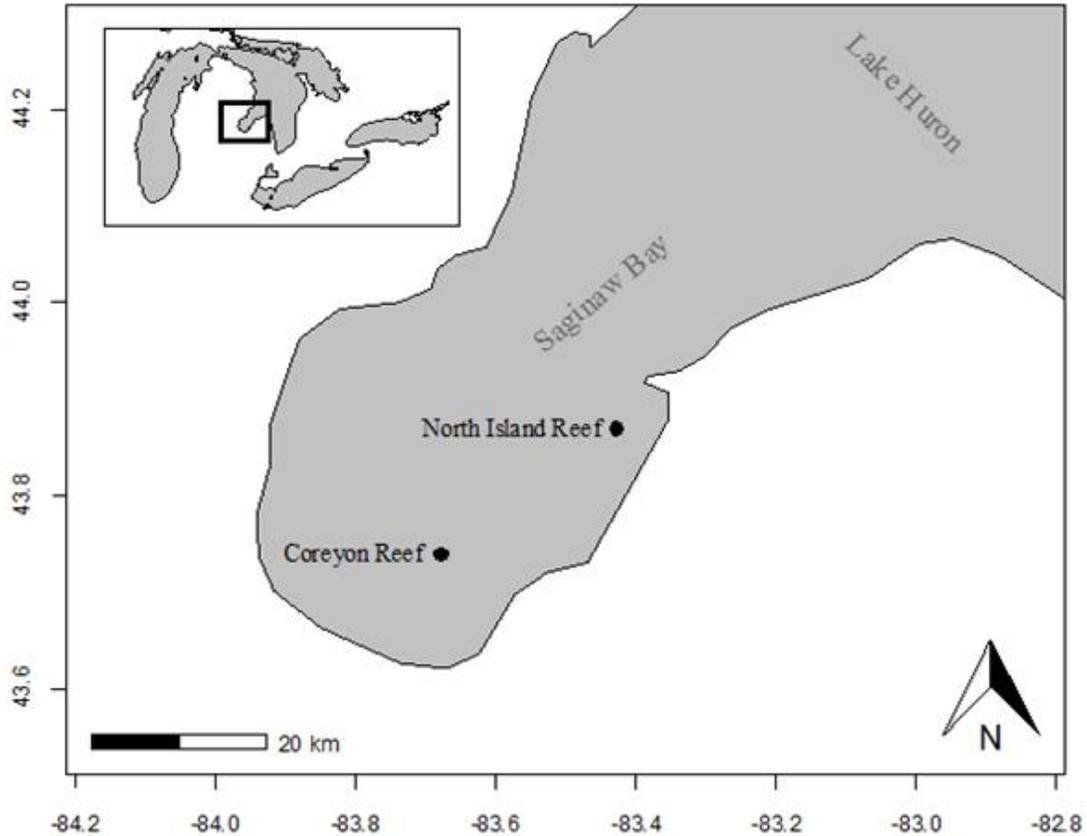


Figure 3-2 Location of Coreyon (average depth: 5 m) and North Island (average depth: 3 m) Reefs within Saginaw Bay, Lake Huron.

Coreyon Reef is a natural rocky structure (5 m mean depth) that has severe degradation from sediment deposition and biofouling. North Island Reef is also a natural rocky structure (3 m mean depth) but has less degradation from sediment deposition and biofouling than Coreyon Reef. In 2018, we defined three plots per reef: one treatment plot cleaned with the propulsion sled, one treatment plot cleaned with the hydro-jet sled, and one uncleaned control. To increase sample size of treatments in 2019, we included triplicates of each treatment and control on Coreyon Reef (i.e., nine total plots) and duplicates of each treatment and control on North Island Reef (i.e., six total

plots). We randomly assigned treatment and control plots within the reef boundaries. We cleaned circular treatment plots for a standardized time of 30 minutes in which the cleaning sleds were towed (~ 1.5 kts) from the inside of treatment plots in a counter-clockwise direction until the sled reached the outer boundaries of the plot. We towed the sleds in a circular counter-clockwise path due to the direction of the 30° angled fans on the propulsion sled and the pressure relief valve on the hydro-jet sled, which we designed to direct suspended sediments to the outside perimeters of the treatment plots as the sleds were initially towed from the inside of each plot. We demarcated the study plots a minimum of 100 m between each other's perimeters and we cleaned plots according to water current direction (e.g., we cleaned plots up current first) as to limit suspended sediments from one study plot settling on nearby study plots after they had already been cleaned. See *Supplemental Video* (<https://youtu.be/3qEvSiIiWWo>) for video of reef cleaning devices in use.

3.2.3 Relative Hardness Measurements

To determine if cleaning devices removed a measurable amount of sediments from the reef structure, we collected relative hardness measurements from each study plot. We took relative hardness measurements using a side scan sonar sounder (Lowrance HDS 7 Carbon) which was scanned over each study plot before cleaning and 24 hours after cleaning. We scanned each study plot in a grid pattern with three equally distanced straight grid lines extending north to south and three equally distanced straight grid lines extending east to west. We also took relative hardness measurements outside of the perimeter of each study plot as an assumed unchanging baseline hardness which was used as a correction factor (see below) when comparing relative hardness of study plots before and after cleaning. We processed and analyzed relative hardness data using Reefmaster software (Version 2.0, Reefmaster Software Ltd. 2017).

3.2.4 Egg Deposition Sampling

We used fish egg deposition rate as an index of reproductive usage of study plots. We used egg mats (Roseman et al. 2011) to quantify lake whitefish egg deposition on Coreyon and North Island Reefs from November 1 – December 2, 2018 and walleye egg deposition sampled from April 4 – May 11, 2019. Egg mat frames were constructed from steel (38 x 24 x 0.5 cm) and wrapped in furnace filter (38 x 50 x 2.5 cm) (Roseman et al. 2011). To create ganglines of egg mats, we connected three separate egg mats with 3 m of line between each egg mat. Each study plot received three ganglines (i.e., nine egg mats per study plot). During fall 2018, we deployed egg mats for 32 days. Given lake whitefish incubation time, we assumed no eggs hatched over this duration. However, due to shorter incubation of walleye eggs, during spring of 2019, we retrieved egg mats two weeks into the sampling period, we picked eggs from egg mats and we re-set egg mats on study plots. During the first egg mat retrieval in the spring of 2019, we picked walleye eggs from egg mats on the research vessel, counted eggs and then returned the egg mats to study plots once all visible eggs had been removed (Roseman et al. 2011). When we retrieved egg mats at the end of the sampling period, we removed furnace filter material from each steel frame and then individually bagged each filter, placed filter on ice and transported to Purdue University. We then picked lake whitefish and walleye eggs from furnace filter material and enumerated and stored eggs in 95% ethanol. We identified eggs to species by size (diameter) of egg, color and spawning season.

3.2.5 Statistical Analyses

Relative Hardness

Sonar measurements of substrate hardness are influenced by a variety of factors such as water temperature, water clarity, water depth, sediment loading, and wave action (Buscombe 2017).

Since individual hardness data points are calculated relative to each other, hardness is not an absolute value. Furthermore, the interdependence among relative hardness data points within a single study plot violates the assumption of data independence. Due to myriad factors that can potentially affect substrate hardness measurements, Reefmaster software calculates relative hardness by comparing sonar measurements collected during a single sampling period or pass (i.e., data points in a single sampling period are relative to each other and not relative to separate sampling periods). Thus, to compare relative hardness of each study plot before and after cleaning, we calculated the percent change of relative hardness by using the equation

$$\left[\text{Post Cleaning} \left(\frac{\mu_i}{\mu_o} \right) \right] - \left[\text{Pre Cleaning} \left(\frac{\mu_i}{\mu_o} \right) \right] \times 100$$

where we calculated mean relative hardness on the inside of the treatment plots (μ_i) and compared this to the mean relative hardness outside of the plot (μ_o) before and after cleaning. Note that relative hardness measurements of the inside and the outside of a given treatment plot, were recorded during the same sampling period, thus they are relative to each other. For 2019, when we added replicate treatment plots, we calculated mean percent change in relative hardness by treatment (i.e., $n = 3$ on Coreyon Reef and $n = 2$ on North Island Reef).

Egg deposition

We used nested analyses of variance (ANOVA) to test for differences in egg deposition (eggs m^2/day) among study plots. Due to large variation in egg deposition among individual traps, egg deposition data were natural log transformed $\ln(x + 1)$ to more closely match a parametric distribution. We compared deposition rates among treatments with egg deposition of individual egg mats nested within ganglines. If ANOVA indicated significant difference in egg deposition rates among treatments, we used Tukey's post hoc test to evaluate differences among treatments.

All statistical tests were analyzed with JMP statistical software version 13 and tested at $\alpha = 0.05$ level of significance.

3.3 Results

3.3.1 Relative Hardness

Changes in relative hardness were variable between seasons and among study plots. In fall 2018 on Coreyon Reef, we found that percent change in relative hardness was negligible after cleaning both the propulsion (+ 3.4%) and hydro-jet (+ 3.0%) study plots, while the control remained marginally unchanged (+ 1.2%; Figure 3). In fall 2018 on North Island Reef, we found that the percent change in relative hardness was positive for the propulsion treatment (+ 67.0%), negative after cleaning in the hydro-jet study plot (- 10.1%) and stayed relatively unchanged in the control (+ 4.0 %; Figure 3-3).

In 2019, we increased the number of replicates at each study location and found variation in relative hardness among treatment plots. In spring 2019 on Coreyon Reef, mean percent change of relative hardness was marginal on both treatments (- 4.8 % propulsion; + 0.01% hydro-jet) and control plots (- 4.1 %; Figure 3). In spring 2019 on North Island Reef, we found that mean percent change in relative hardness was positive with the propulsion (+ 20.0 %) and hydro-jet (+ 19.7 %) devices, and that control plots remained relatively unchanged (+ 2.9%; Figure 3-3).

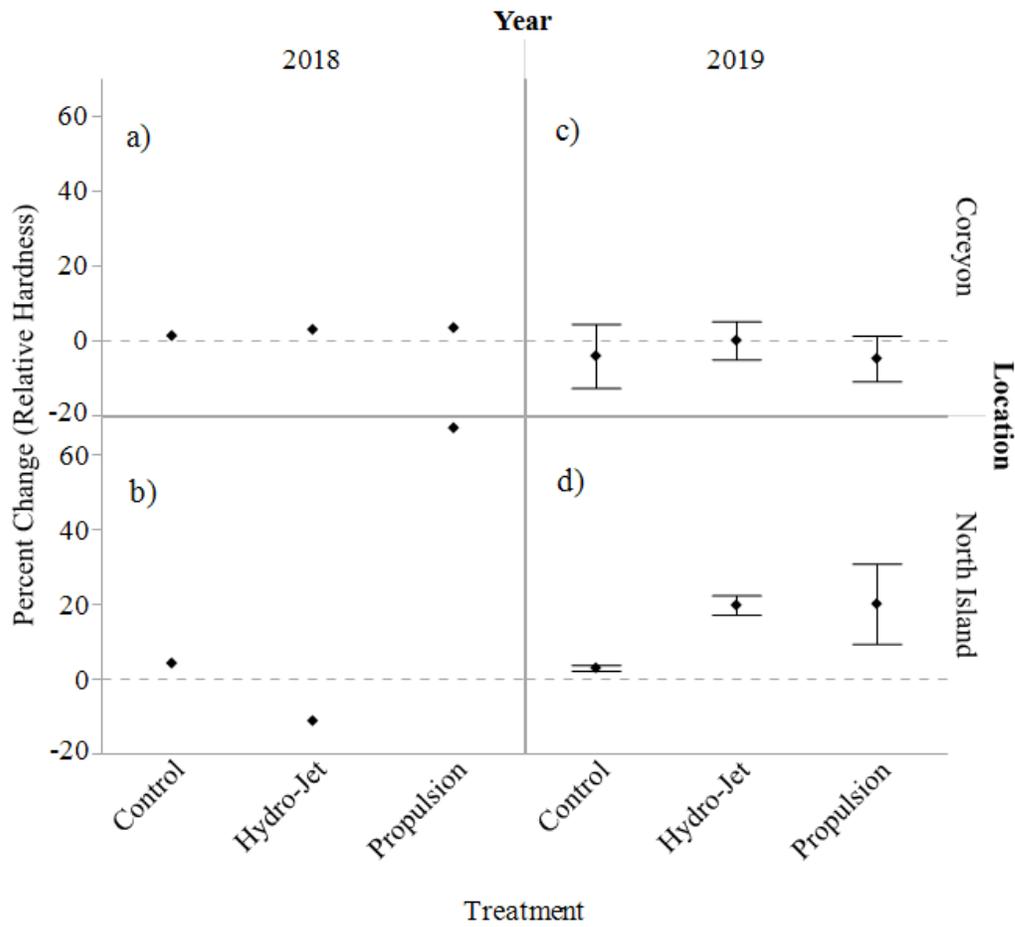


Figure 3-3 Percent change in relative hardness between pre and post-cleaning of study plots. In 2018, points indicate single mean percent change for each treatment (a) Coreyon Reef; b) North Island Reef). In 2019 replicates of treatments were added thus, points indicate the group mean, percent change of all treatment plots on Coreyon Reef (n = 3; c) and group mean percent change of all treatment plots on North Island Reef (n= 2; d). Error bars indicate one standard error from the mean for 2019 sample plots.

3.3.2 Egg deposition

Egg deposition by lake whitefish and walleye was variable between seasons and among study plots. On Coreyon Reef in fall 2018, there was a statistically significant difference in lake whitefish egg deposition among study plots (ANOVA; $F_{2,24} = 9.267$, $P = 0.001$; Figure 3-4). Specifically, we recorded higher lake whitefish egg deposition on the propulsion study plot compared to the control study plot (Tukey's HSD; $P = 0.003$) but we found no difference in egg deposition between the hydro-jet and control study plots (Tukey's HSD; $P = 0.999$). Similarly, on North Island Reef in fall 2018, there was a statistically significant difference in lake whitefish egg deposition among treatment plots (ANOVA; $F_{2,24} = 7.994$, $P = 0.002$; Figure 3-4). Specifically, lake whitefish egg deposition was higher on the propulsion treatment plot (Tukey's HSD; $P = 0.002$) and the hydro-jet study plot (Tukey's HSD; $P = 0.037$) compared to the control study plot.

In 2019, we increased replicates of treatment plots and again found evidence of variable trends in egg deposition. On Coreyon Reef in spring 2019, we found no significant difference in walleye egg deposition among treatment plots (ANOVA; $F_{2,78} = 1.40$, $P = 0.252$; Figure 4). However, On North Island Reef in spring 2019, there were significantly different walleye egg deposition rates among treatments (ANOVA; $F_{2,51} = 9.121$, $P < 0.001$; Figure 3-4). Specifically, walleye egg deposition rate was higher on the propulsion study plots (Tukey's HSD; $P < 0.001$) compared to the hydro-jet and control study plots (Tukey's HSD; $P = 0.072$).

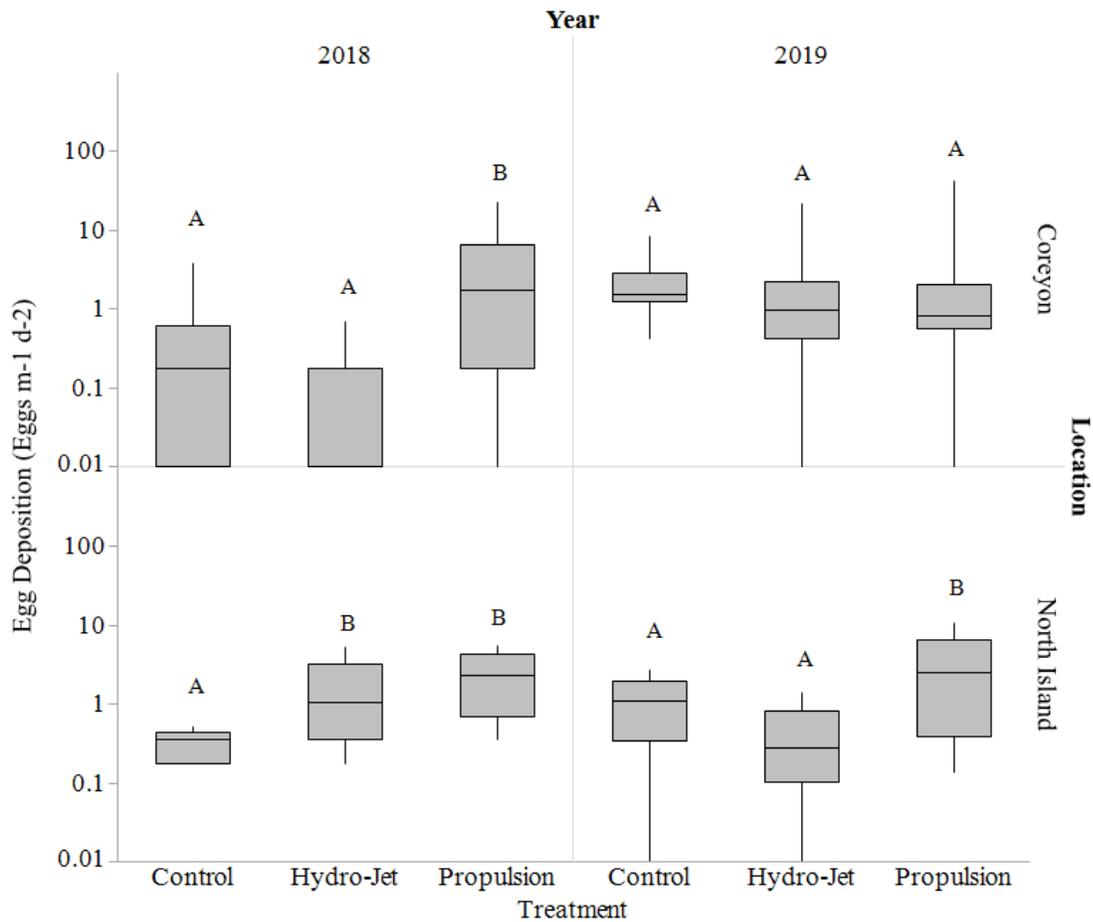


Figure 3-4 Egg deposition rates over study plots on Coreyon and North Island Reefs during the fall of 2018 (lake whitefish) and spring of 2019 (walleye). The middle horizontal line in each box indicates the treatment median response, top and bottom of each box indicates the first and third quartiles and whiskers extend to maximum and minimum observations. Egg deposition rates were significantly different among study plots in 2018 on both Coreyon and North Island Reef. In 2019, egg deposition rates were significantly different among study plots on North Island Reef but not on Coreyon Reef. Letters above box plots represent significant differences within each season and reef location, Tukey’s HSD post-hoc tests $P < 0.05$.

3.4 Discussion

Our results suggest that custodial maintenance of degraded spawning reefs is possible and has the potential to increase egg deposition in areas that have been treated with cleaning devices. Changes in relative hardness measurements before and after cleaning were variable between cleaning devices, however, the propulsion sled generally, created treatment plots that displayed

increased relative hardness measurements after cleaning. Lake whitefish and walleye egg deposition was also generally greater in areas cleaned by the propulsion sled compared to the untreated control, in both years except spring 2019 on Coreyon Reef. These results support the hypothesis that cleaned areas on degraded spawning reefs potentially create higher quality habitat compared to the surrounding degraded reef, and that such areas are selected for by lake whitefish and walleye during spawning events. Reef structure and magnitude of degradation influence the extent to which cleaning devices can remove detectable amounts of sediments from the reef structure.

3.4.1 Sediment Removal

According to relative hardness measurements, the propulsion device appeared to be more successful at removing sediments from the reef structure compared to the hydro-jet device. The hydro-jet device may not have had high enough water pressure to consistently remove detectable amounts of sediments from the reef structure. To increase the water pressure, we may have needed to use a larger transfer pump that could supply pressurized water at a higher rate. The decrease in relative hardness observed with the hydro-jet treatments in 2018 on North Island Reef, may also indicate that the device suspended sediments from the reef structure, but these sediments did not settle away from the treatment. In contrast, the two marine underwater fans used by the propulsion device seemed to create the thrust necessary to remove noticeable amounts of sediments from the reef structure. Further, it appears that the propulsion device was somewhat successful in directing these sediments away from the cleaned area of the treatment plots. We did observe an increase in relative hardness post-cleaning for all of the propulsion treatments from North Island reef in 2018 and 2019, suggesting that sediments were both removed from the plots and transported elsewhere.

Relative hardness between pre and post-cleaned treatment plots appeared to change less on Coreyon Reef compared to North Island Reef. One explanation for the difference observed between the two reefs, is that Coreyon Reef has severe degradation (likely up to a meter of sediment cover in some areas, personal observation Alex Gatch) compared to North Island Reef. The severity of degradation on Coreyon Reef could explain the lack of changes in relative hardness pre and post-clean, because our cleaning devices could not remove enough sediments to uncover hard rocky substrate. Similarly, if sediments were removed from treatment plots on Coreyon Reef, it is possible that infilling of cleaned areas may occur rapidly (especially at the fringes of the treatment plots) due to the amount of sediment on the outside perimeter of the treatment plots and the surrounding reef structure. To determine the rate at which infilling occurs post-cleaning, we suggest that continued monitoring of the treatment areas be performed.

One limitation to our study was the low number of treatment plots which impacted our statistical power. The size of our treatment plots (i.e., 50 m diameter) and the spacing between plots (i.e., 100 m between plots) limited the total number of treatment plots that could fit on North Island Reef. With an increased sample size for treatment plots, we may be able to better quantify the variation in changes in relative hardness that we observed among treatments and seasons. However, increasing the number of replicates of treatment plots on a smaller reef such as North Island Reef would have required us to create smaller diameter plots (i.e., < 50 m). Smaller treatment plots would require fine scale maneuverability with cleaning devices that would be a challenge while pulling a benthic sled. Additionally, treatment plots smaller than 50 m in diameter may not be large enough to attract lithophilic spawning.

Finally, since substrate hardness is expressed on a relative basis, it is difficult to determine the exact scale of hardness change from pre and post-cleaning events and thus, the amount of

sediment removed by cleaning devices is unknown. Side scan sonar has been extensively used to map and classify benthic substrates (Reut et al. 1985; Intelmann and Cochrane 2006; Kaeser et al. 2013; Manny et al. 2015; Walker and Alford 2016) but is less commonly used to measure the scale of sediment removal. Ground truthing (e.g., visual observation) is a common practice when using side scan sonar to map benthic substrates (Degraer et al. 2008). Ground truthing treatment plots in our study was attempted by SCUBA divers, however, highly turbid waters in Saginaw Bay, did not allow for clear visual assessment. Nevertheless, we were able to detect noticeable changes in relative hardness pre and post-cleaning in treatment plots using side scan sonar, that allowed us to make inferences about the relative success of the cleaning devices.

3.4.2 Practical Application of cleaning Devices

The study aimed to create two versatile cleaning devices that could be used on multiple rocky reef structures. We found that these devices were easily towable in shallow areas where reef substrate was relatively uniform, and rocks were of fairly small size (<30 cm). Only on a single occasion did the hydro-jet sled turn over under tow, and neither sled caught the reef structure in such a way that brought the vessel to a complete stop. The two cleaning devices were manageable from a small research vessel (6.5 m powered by a 150 hp outboard engine) with 2-3 crew members and did not compromise the safety of the vessel while under tow. We were also able to clean 50 m diameter plots in 30-minute durations. While size and shape of natural and constructed spawning reefs are widely variable, these devices are potentially capable of covering considerable areas in a feasible amount of time. Sediment resuspension during and after cleaning was substantial. Resuspended sediments have the potential to re-settle over cleaned areas, thus, it was important to use devices only on days when currents directed sediments away from existing reef structure. Strong currents in large lotic systems could be ideal to carry re-suspended sediments off reef

structures that are being cleaned, however, our devices (benthic sleds) could present safety concerns in high current areas while under tow. It was our intent to create portable and affordable cleaning devices that could be used on small vessels, however, these cleaning devices could potentially be scaled up in size and used with larger vessels. Increasing the size of either sled would expand the amount of reef area the sleds could cover. Similarly, increasing thrust by using more or larger marine fans, or increasing water pressure, could potentially have a greater positive effect on substrate removal.

3.4.3 Egg Deposition

In general, lake whitefish and walleye egg deposition were higher on treatment plots compared to uncleaned control plots. Our results suggest that the treatment plots were areas of higher quality spawning habitat (potentially deeper interstitial spaces) and therefore, spawning lake whitefish and walleye likely selected for these areas for spawning. Selection of higher quality reef spawning habitat across a local area has been shown in lake trout (*Salvelinus namaycush*) (Marsden and Chotkowski 2001; Marsden et al. 2016). Specifically, Marsden and Chotkowski (2001) determined that lake trout (a native lithophilic spawning fish) had higher egg deposition on portions of an artificial reef that were free of dreissenid mussels compared to an artificial reef in close proximity that was degraded by dreissenid mussels. Similarly, Marsden et al. (2016) found that lake trout egg deposition rates were higher on high quality, non-degraded artificial and natural reefs compared to nearby low-quality, degraded natural reef. High quality spawning habitat that is surrounded by lower quality spawning habitat has also been shown to increase egg deposition by lake whitefish (Roseman et al. 2007), walleye (Raabe and Bozek 2012) and lake sturgeon (Caswell et al. 2004; Johnson et al. 2006; Roseman et al. 2011; Bouckaert et al. 2014). We suggest that our

cleaning devices created higher quality spawning substrate compared to uncleaned control areas and this likely contributed to increased egg deposition in these treatment areas.

3.4.4 Conclusion

We developed and evaluated the utility of two portable and affordable devices that were used to clean sediments and other fouling from spawning habitat. Our results indicate that these devices can increase relative hardness of reef habitat and potentially increase the quality of spawning habitat. Increased egg deposition by lake whitefish and walleye in treatment plots further supports this conclusion. The practicality of using these cleaning devices likely depends on spawning reef location, depth, and magnitude of degradation. We propose that custodial maintenance of spawning reefs is a potentially useful approach for increasing the quality of degraded rocky reef spawning habitat. Development of this approach should be continued as a viable alternative to expensive reef construction projects.

3.5 Acknowledgements

This research was funded by the Great Lakes Restoration Initiative, U.S. Geological Survey (USGS) and the Department of Forestry and Natural Resources at Purdue University. Thanks to Scott Koenigbauer, Ben Leonhardt, and Jay Beugly for help with development and testing of cleaning devices. We would also like to thank Ellen Marsden, Dave Fielder, Jason Fisher, and Andrew Muir for advising the development of cleaning devices. Handling of fishes followed the guidelines provided in the “Guidelines for the use of fishes in research” published by the American Fisheries Society, Bethesda, MD. Use of trade names is for descriptive purposes and does not imply endorsement by the U.S. Geological Survey.

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