Lake trout and their egg predators' ability to locate spawning substrate using olfaction

A thesis presented to
The Faculty of Graduate Studies
Of
Lakehead University
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In partial fulfillment of requirements
For the degree of
Master of Science in Biology

September 18, 2013

Abstract

Lake trout spawning sites, and the substrate on these sites, are structurally unique in many ways. These sites are generally at the end of the fetch of a lake, close to shore (1 m) and composed of small to medium sized rubble and cobble. Lake trout and their egg predators use the same site(s) annually. It was hypothesized that both lake trout and their egg predators utilize olfaction to locate the same sites annually. To test this hypothesis trap nets were baited with visually concealed lake trout spawning substrate or non-spawning substrate that was structurally similar to spawning substrate. Trap nets baited with lake trout spawning substrate captured significantly more lake trout and common white sucker (an egg predator) than trap nets that contained structurally similar non-spawning substrate. Spawning substrate-containing traps also caught more spawn-ready lake trout.

In a second experiment, significantly more egg predators were captured in unbaited minnow traps on lake trout spawning sites than structurally-similar non-spawning sites. To test whether smaller egg predators, such as sculpin or crayfish, were attracted to visually concealed spawning substrate, two standard minnows traps were placed on 8-10 sites on three lakes. One trap was baited with lake trout spawning substrate and the other with structurally similar non-spawning substrate. Traps with spawning substrate captured significantly more egg predators as well other opportunists than those with non-spawning substrate. These results demonstrate that both lake trout and their egg predators are able to locate visually concealed spawning substrate.

Lay Summary

Faculty and students in the Department of Biology are bound together by a common interest in explaining the diversity of life, the fit between form and function, and the distribution and abundance of organisms. This thesis helps to explain how lake trout mating systems and their egg predators use olfaction to help locate appropriate spawning substrate annually. The ability to locate this substrate using olfaction allows lake trout and their egg predators the ability to minimize the amount of time spent searching for the appropriate sites. This research helps to understand the process of lake trout utilizing certain areas for spawning even when there are structurally similar sites that are not utilized.

Acknowledgments

I would like to thank A. Azizi, A. J. Chapelsky, V. Danco, H. Forysth, L. Hrenchuk, L. Nelson, B. Sprules, A. Wall, and C. Weisbord for their contributions in the field. My projects were both funded by research grants from the Friends of ELA and a Natural Sciences and Engineering Research Council (NSERC) Discovery grant to Dr. Pyle, who is also supported by the Canada Research Chairs program. I would also like to thank my supporting and ever loving wife Britton for always believing in me; my family for always being there and supporting me along the way; and my supervisor Dr. Greg Pyle for giving me an opportunity to accomplish this.

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Chapter 1: Introduction

Within the aquatic environment, species' ability to interpret chemical information allows them to increase their chance of survival (Solomon, 1977; Liley, 1982; Brown & Smith, 1998; Bryer *et al*., 2001; Ache & Young, 2005). Chemical information found dissolved in water includes biochemical products released from conspecifics, amino acids signaling food, and alarm cues (Liley, 1982). The interpretation of these chemical cues mediate many fundamental ecological interactions across the taxonomic spectrum, including mate selection, foraging, predation, and establishing social hierarchies (Hara, 1994; Kats & Dill, 1998; Krieger & Breer, 1999; Hansen & Zielinski, 2005). The ability to use olfaction also allows many different species the ability to recognize and locate areas that are used annually as spawning sites as well as areas that have seasonal food sources.

Spawning areas are unique locations in nature as they are recognized in numerous ways as being attractive areas where reproducing members of a population congregate. Fish that are spawn-ready are prime examples of individuals being able to utilize their senses to coordinate and congregate annually on predetermined areas. The use of olfaction during spawning is beneficial as it aids in the ability to discriminate kin as well as helps to synchronize the reproductive efforts of the population (Brown & Brown, 1996; Olsen *et al*., 1998; Ward & Hart, 2003). The ability to discriminate kin allows the population to minimize the amount of inbreeding occurring and in turn increases the genetic diversity of the population as a whole. The ability of the reproducing population to synchronize their reproductive efforts at a certain location increases the reproductive success of the population (Knowlton, 1979; Blanchfield & Ridgway, 1997).

The role of olfaction in salmonid migration and spawning is well known (Hasler & Wisby, 1951; Banks, 1969; Hasler & Cooper, 1976; Dittman *et al*., 1996; Crossin *et al*., 2007). Once anadromous salmonids are ready to spawn, they will converge with other conspecifics at their natal river mouth (Royce *et al*., 1968; Burgner, 1980). Salmon will then make their way upriver to their natal sites relying primarily on olfactory discrimination of their home stream water (Hasler *et al*., 1978; Hasler & Scholz, 1983; Dittman & Quinn, 1996). Juvenile salmon are believed to imprint on characteristic natal river odours during their time there as young parr (Hasler & Wisby, 1951). One hypothesis on how salmon are able to imprint these olfactory cues is linked to different hormone levels at different stages in their development (Hasler & Scholz, 1983). During the parr-smolt transformation, levels of plasma thyroxine will be elevated (Dickhoff *et al*., 1989). This peak corresponds with a developmentally significant time, and may be associated with smolts imprinting key olfactory cues and relating them to landmarks. Thyroid hormones have also been associated with migration and juvenile migrating salmon have been found to have higher levels of plasma thyroxine than their non-migrating counterparts (Hoar, 1976). If thyroid hormones are associated with imprinting and migration then juvenile salmon may be able to imprint multiple waypoints on their journey to the ocean and later recall these waypoints on their ascension back upriver to natal spawning sites (Dittman & Quinn, 1996).

Orientation to suitable spawning habitat is equally important in non-anadromous salmonids. Lake trout (*Salvelinus namaycush*) are generally not anadromous, though there are arctic populations that will travel to salt water (Swanson *et al*., 2010). They spawn nocturnally, en masse, and at relatively few sites within a lake in mid-October (DeRoche, 1969; Martin & Olver, 1980; Gunn, 1995). Spawning sites may be influenced by numerous factors including olfactory cues, visual cues (recognition of substrate type), reef location with respect to the shoreline, water depth, proximity to deeper water, contour, substrate size and shape, depth of interstitial spaces, water temperature, water quality in interstitial spaces, and the presence or absence of other species (Marsden *et al*., 1995a). Ideal lake trout spawning beds are composed of cobble, rubble, or boulders with abundant interstitial spaces (Martin & Olver, 1980). During spawning, males congregate on spawning shoals 10-15 days before females (Gunn, 1995). While on the spawning shoals, males will exert "tail-whip" movement over spawning beds to clear interstitial spaces of organic debris (Martin & Olver, 1980). Once spawning occurs, negatively buoyant eggs will disperse into the interstitial spaces and incubate for a 5 – 7 month period (Gunn, 1995). Since lake trout do not construct redds (like other salmonids), the deposition of eggs into interstitial spaces is suspected to be an anti-predator strategy that is used to protect over-wintering eggs and fry from potential predators (Greeley, 1932; Martin & Olver, 1980; Edsall *et al*., 1992).

Within the Laurentian Great Lakes, lake trout populations have not been self-sustaining since over fishing, and sea lamprey (*Petromyzon marinus*) predation in the 1950s (Evans & Olver, 1995). Currently, Lake Superior is the only Great Lake that has a self-sustaining, reproducing lake trout population (Hansen *et al*., 1995). Since lake trout are a long-lived, slow maturing species, they are susceptible to any changes or effects to their annual recruitment. One of the factors affecting restoration of a self-sustaining lake trout population is egg predation (Krueger *et al*., 1995; Savino *et al*., 1999). At certain spawning sites in the Great Lakes, egg predation can have significant detrimental effects on the survivorship of lake trout embryos (with egg consumption rates reaching as high as 80% of available eggs) (Fitzsimons *et al*., 2002). Most egg predators are attracted to other salmonid eggs though olfaction, though this has not been tested with lake trout eggs directly (Dittman *et al*., 1998; Mirza & Chivers, 2002). Lake trout spawning takes place over a short period (approx. two weeks) every year, and egg predators would benefit from being at lake trout spawning sites during the onset of spawning activities in order to maximize the number of eggs they ingest. It is unknown how egg predators locate these spawning sites annually.

What allows lake trout and their egg predators to locate traditional perennial spawning sites? Spawning sites have certain physical attributes that make them attractive for spawning. However, searching for these areas annually would be an energy intensive process and would probably limit the amount of synchronization that occurs within the population. If olfaction was used to locate spawning sites then the amount of time spent by lake trout and egg predators searching for appropriate sites would be reduced. Though olfaction is suspected to play a role in lake trout and egg predators locating spawning locations, it has never been formally tested (Foster, 1985; Marsden *et al*., 1995b; Muir *et al*., 2012).

This thesis attempts to identify if lake trout and their egg predators use olfaction to locate spawning substrate annually. I hypothesized that lake trout and their egg predators use olfaction and would be attracted to visually concealed spawning substrate over visually concealed structurally similar non-spawning substrate. The objectives of this project were to determine if:

1) Lake trout were attracted to chemosensory cues associated with the substrate found at spawning sites;

2) Lake trout egg predators are more abundant on spawning sites when compared to structurally similar non-spawning sites; and if they are, then

3) Are lake trout egg predators attracted to visually concealed spawning substrate?

The objectives for this study were addressed over the course of two field seasons and have been compiled into two manuscripts.

In Chapter 2, trap nets were baited with visually concealed spawning substrate and structurally similar non-spawning substrate to observe whether lake trout and large egg predators were attracted to spawning substrate. This experiment was conducted adjacent to lake trout spawning shoal during the lake trout spawn.

In Chapter 3, the abundance of egg predators was compared on a lake trout spawning site and structurally similar non-spawning sites. Unbaited minnow traps were placed on spawning and non-spawning sites to compare the abundance of egg predators between the two types of sites. This experiment was conducted in two different lakes. The following field season, two baited minnow traps were placed away from a lake trout spawning site, with one trap containing lake trout spawning substrate and the other with structurally similar non-spawning substrate. Species abundance was compared between the treatments. This experiment was conducted on three different lakes and was conducted over the course of seven to ten days.

Chapter 2: Chemosensory cues attract lake trout *Salvelinus namaycush*, and an egg predator to spawning substrate [1]

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[1] Wasylenko, B. A., Blanchfield, P. J. & Pyle, G. G. (2013). Chemosensory cues attract lake trout *Salvelinus namaycush* and an egg predator to the spawning substratum. *Journal of Fish Biology* **82**, 1390-1397

2.1 Abstract

Lake trout *Salvelinus namaycush* return to the same spawning locations annually, despite the availability of local sites that are structurally similar. A field experiment was conducted to determine whether chemosensory cues emanating from lake trout spawning substrate attract breeding fish. Substrates from either a spawning site or a control site were randomly placed in trap nets around an isolated spawning shoal. Trap nets that contained spawning substrate caught significantly more lake trout, as well as a greater proportion of lake trout in breeding condition, than nets with control substrate. White sucker *Catostomus commersoni* were a predator of lake trout eggs and were also captured in greater numbers in nets with spawning substrate.

2.2 Introduction

Many fish species rely on chemosensory cues to locate perennial spawning sites (Horrall, 1981; Miller *et al*., 2001; Døving *et al*., 2006). Salmonids in particular have certain chemical characteristics or odourants emanating from their natal streams that are imprinted in their young and later recalled to guide the fish back to their natal spawning locations (Wisby & Hasler, 1954; Hasler & Cooper, 1976; Johnsen & Hasler, 1980).

Chemical cues associated with spawning sites may be especially important for long-lived salmonid species, such as lake trout, *Salvelinus namaycush* (Walbaum 1792). Lake trout spawn on rocky, windswept shoals in late fall, where fertilized eggs fall into the interstices of cobble substrate and remain there for several months before hatching (Gunn, 1995). Large-scale movements of lake trout to spawning sites and travel between spawning sites during the breeding season have been documented (MacLean *et al*., 1981), although factors that influence site selection are not well defined. Spawning sites are thought to contain chemical cues (odours) that promote perennial spawning site fidelity (McCrimmon, 1958; Martin, 1960; Foster, 1985; Muir *et al*., 2012). The identity of these chemical cues is currently unknown; however, they are likely renewed annually during spawning events. Foster (1985) suggested that lake trout are attracted to spawning sites due to the accumulation of juvenile feces on these sites. The olfactory systems of lake trout also contain multiple bile-acid receptors and lake trout have a preference for water containing bile acids (Zhang & Hara, 2009). In addition to spawning fish being attracted to these cues, egg predators may be attracted to similar cues, as they indicate the possibility of an available food source.

Although it has been hypothesized that lake trout find and congregate on spawning habitat using chemosensory cues, whether chemosensory cues play a role in the selection of natural, perennial spawning shoals by lake trout remains untested. This hypothesis predicts that lake trout use chemosensory cues to find spawning substrates previously used by conspecifics. Here, the experimental design used spawning substrate from a known lake trout spawning shoal and non-spawning substrate from a structurally similar non-spawning shoal (control) as a treatment in trap nets placed near an isolated lake trout spawning site during breeding season. The primary objective of this experiment was to test whether trap nets with concealed lake trout spawning substrate would catch more lake trout than those that contained concealed non-spawning substrate. A secondary objective was to test whether lake trout breeding condition differed between treatments. This experiment was also used to determine if egg predators were attracted to concealed lake trout spawning substrate.

2.3 Methods

Lake 468 (49º 40’ N, 94º 45’ W) is located within the Experimental Lakes Area (ELA), a pristine area set aside for aquatic research in northwestern Ontario, Canada (Fig. 2.1a). It is a medium-sized lake (292 ha) with a maximum depth of 29 m (Fig. 2.1b). Lake 468 is located within the Boreal Shield Ecozone and has a simple fish community that consists of lake trout, lake whitefish *Coregonus clupeaformis* (Mitchill, 1818), common white sucker *Catostomus commersoni* (Lacépède 1803), yellow perch *Perca flavescens* (Mitchill, 1814), Slimy sculpin *Cottus cognatus* (Richardson, 1836) and five cyprinid species (Sellers *et al*., 1998) (Table 3.1).

An isolated lake trout spawning site was selected that was located beside a small island, where historically lake trout were known to spawn (Fig. 2.1c). Use of the site for spawning was confirmed visually by the presence of lake trout and deposited eggs (October 13, 2011). Substrate was collected with a shovel, directly from the spawning shoal (spawning substrate), while the control substrate (non-spawning substrate) was taken from a structurally similar non-spawning site in Lake 468 with no known or observed spawning activity (approx. 600 m from spawning shoal). Substrate was collected during the lake trout spawn and was chosen based on cobble size typically selected by breeding lake trout (~4–10 cm diam.). Approximately 22 rocks were used in each substrate sample, for a mean sample weight of 5.2 kg (S.E. = ± 0.2 kg; n=4, 2 samples of each substrate type). Each substrate sample was wrapped in fine mesh netting to allow water to infiltrate the

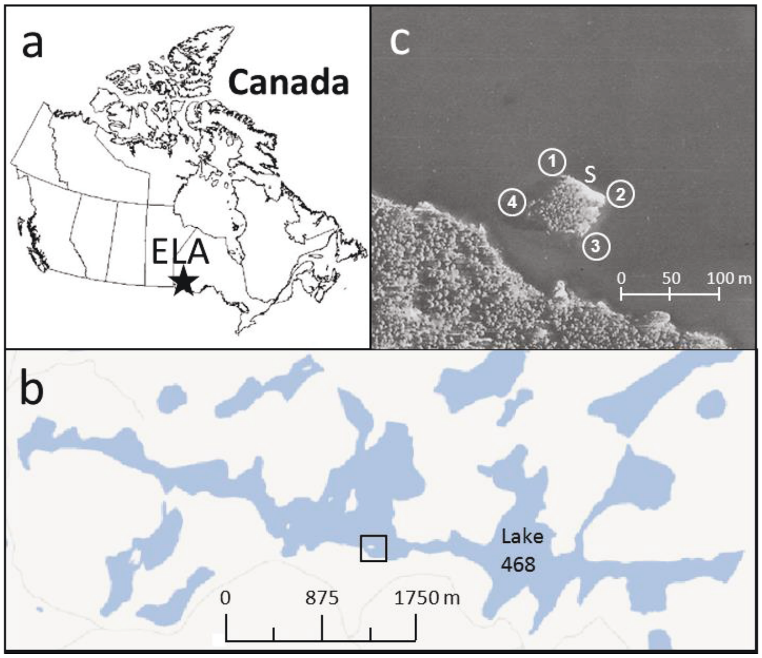

Figure 2.1 - Location of (a) the Experimental Lakes Area in northwestern Ontario, Canada (star), and (b) study site within Lake 468 (box). (c) Trap nets (1-4) were set around an island with a known lake trout spawning shoal (S).

sample, but visually conceal the substrate from the fish. All four substrate samples were used daily throughout the entire experiment (October 12-21, 2011). The central leads were removed from four trap nets (183 cm x 345 cm trap; 25 cm x 25 cm opening; 6.4 cm mesh size) and deployed them in approximately 3 m of water, each set at a different corner of the island (Fig. 2.1c). The habitat over which trap nets were deployed was similar and consisted of medium to large cobble mixed with small boulders. One wing of each net was tied to the shore and the other anchored to the lake bottom. Each net had either spawning substrate or non-spawning (control) substrate contained in the trap portion and each side of the island had one of each type of substrate throughout the experiment. All fish were removed from the trap nets daily (0900 and 1200 h) and from each net fish species, total and fork length (mm), mass (g) and sex were recorded from each fish when possible. All fish were returned to the lake on the opposite side of the bay, ~400 m away from the study site. After all nets were sampled, non-spawning substrate was exchanged with spawning substrate, and vice versa for all locations. Each substrate sample was placed in a different net following a sequential order (a sample would go into each net once before returning to the original net it was placed in).

2.4 Results

In total, 96 lake trout, 93 common white sucker and 17 lake whitefish and a single yellow perch were captured. The large mesh size did not permit the capture of small-bodied fish species present in the lake, such as cyprinids and sculpin. Catches were not distributed equally among the four nets. Trap nets 1 and 2 were located on either side of the spawning shoal (Fig. 2.1c) and captured the least (n=29), and the most (n=91) fish, respectively. Catches in the nets furthest away from the spawning shoal (net 3: n=55; net

4: n=32) were intermediate to nets in close proximity. A chi-square analysis of the median daily catch numbers for each trap suggests that there is no significant difference in catch among the four nets (χ^2=4.85, DF=3, $P > 0.05$). Further analysis was restricted to lake trout and common white sucker, as lake whitefish and yellow perch were caught in insufficient numbers for meaningful interpretation.

Overall, catches of lake trout were highly variable within and among the four traps (Table 2.1). Only net 2, when containing spawning substrate, consistently caught lake trout, all other nets had at least one instance of no fish capture. Similar catch variability was observed for white sucker and can be partially attributed to natural fluctuation in fish recruitment to spawning sites as well as the limited number of trials (n=3) for which treatments were placed in each net. Because of this variability, non-parametric analysis (Pearson Chi-square) was used to examine total catch. In total, a greater number of lake trout was attracted to the spawning substrate than to control substrate (χ^2=6.58, DF=1, P=0.01, Fig. 2.2). Similar results were observed with white

Table 2.1: Median daily catch numbers (and range) of lake trout and white sucker caught in individual trap nets (1-4; see Fig. 1c) placed near a lake trout spawning shoal when containing lake trout spawning substrate versus non-spawning substrate (control).

Trap	Lake Trout		White Sucker	
net	spawning	control	spawning	control
1	1 (0-5)	4 (0-4)	2 (1-3)	4 (0-7)
2	4 (4-8)	1.5 (2-13)	13 (5-23)	0.5 (1-4)
3	8.75 (0-29)	0(0-2)	4.25 (0-15)	1 (0-1)
4	2 (0-4)	2.75 (0-5)	0 (0-4)	2.5 (0-10)

sucker, which were also found in a higher number in the nets containing spawning substrate (χ^2=18.08, DF=1, *P*<0.0001, Fig. 2.2). For both lake trout and common white sucker, trap nets containing spawning substrate captured roughly twice the number of fish relative to nets with control substrate (Fig. 2.2).

The overall sex ratio of captured lake trout was similar to the ratio expected at most spawning sites, which is approximately 55–60% males to 40–45% females (spawning substrate nets: 58% males, 37% females, 5% unknown, n=60; non-spawning). Although there was no difference in sex ratios between treatments, a significantly greater proportion of lake trout in breeding condition (i.e. fish that could be sexed based on the expression of gametes) were captured in nets with spawning substrate (95%) relative to those captured in nets with control substrate (81%; one-tailed proportion test, $t_{(1, 94)}$=2.42, *P* = 0.01).

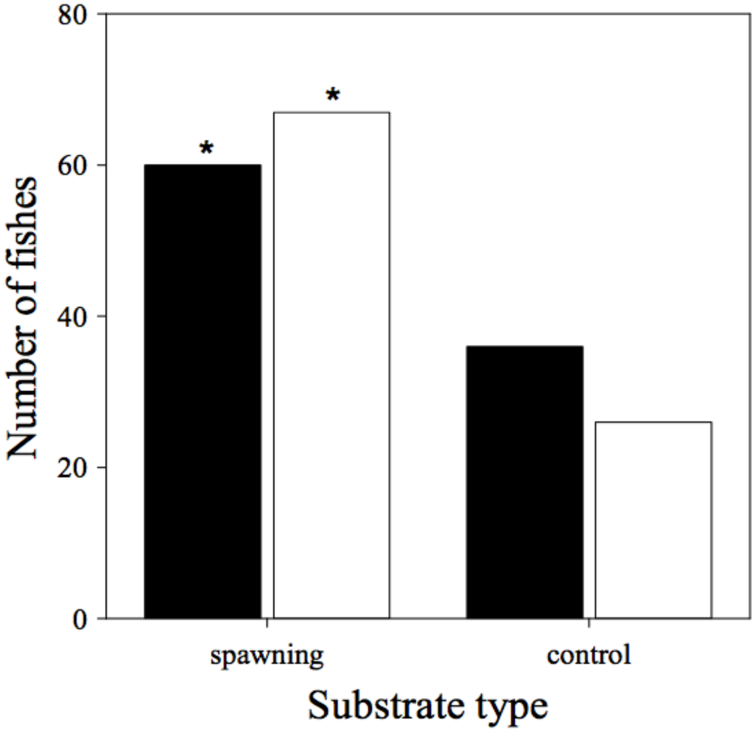

Figure 2.2 - Total number of lake trout (solid bars) and white sucker (open bars) captured in trap nets containing spawning substrate versus those with "control" (non-spawning) substrate. Asterisks indicate a significant difference between numbers of fish caught between treatments.

2.5 Discussion

This field experiment is the first to demonstrate that lake trout are attracted to natural spawning substrate when the substrate is visually concealed. The catches per net were highly variable, but overall there was significantly greater numbers of both lake trout and an egg predator, white sucker, in nets containing substrate taken directly from a known spawning site. Also, nets that contained spawning substrate had more lake trout in breeding condition than nets that contained control substrate, suggesting that spawning substrate attracts more lake trout that are ready to spawn. Traditionally, selection of spawning sites by lake trout has been considered in terms of physical habitat features;

specifically, the presence of appropriately-sized cobble located in areas of the lake receiving sufficient fetch (Flavelle *et al*., 2002). Our findings indicate that physical structure and location are not the only aspect of spawning site selection. The attraction of both species to the visually concealed spawning substrate confirms their ability to locate spawning substrate using olfaction.

The use of chemical cues to locate spawning sites may allow lake trout the ability to limit the amount of time searching for suitable spawning habitat annually (an energy intense process) (Goodenough *et al*., 2009). Odourants from conspecific juvenile salmonid feces may be the initial cue that reproducing fish recognize during spawning and allows them to return to successful spawning locations (Foster, 1985; Stabell, 1987). The intensity of chemical cues is predicted to be directly related to numbers of spawning fish (or eggs hatched), and therefore declines in lake trout populations would be expected to reduce the annual renewal of that cue. The missing olfactory cues may be partially responsible for the lack of success with stocking programs in the Laurentian Great Lakes, where lake trout populations have been declining (Krueger *et al*., 1995). For example, stocked lake trout are more likely to return to their release sites during spawning season in Lake Michigan rather than historical spawning sites (Bronte *et al*., 2007). Similar to other salmonids, the initial cue that lake trout recognize may be what is recalled during their earliest life stages, meaning that instead of imprinting on suitable spawning sites (i.e., where native fish would hatch), they instead imprint with the smell of the first site they encounter when stocked to a lake (Hasler & Cooper, 1976). If young lake trout follow a similar model to other salmonids, it can be expected that odour imprinting is occurring in hatching lake trout as well.

Egg predation can drastically reduce lake trout reproductive potential (Jones *et al*., 1995; Chotkowski & Marsden, 1999; Savino *et al*., 1999; Fitzsimons *et al*., 2007). Common white sucker has generally not been included in the interstitial egg predation guild on lake trout eggs; however, during this study, there was a significant response by common white sucker to trap nets that contained spawning substrate. Furthermore, visual observations confirmed common white sucker consumption of lake trout eggs in our study (P. Blanchfield, pers. obs.). Our experimental design did not allow us to test whether common white suckers were attracted to spawning site by olfaction or the presence of lake trout already in the trap nets. Nonetheless, common white suckers have been known to predate on other fish eggs, which is most likely via olfactory cues (Roseman *et al*., 2006). Consumption of lake trout eggs by a variety of predators is a major concern to the maintenance of lake trout populations in the Laurentian Great Lakes and elsewhere (Fitzsimons *et al*., 2002). An additional concern is that egg predators eliminate the potential for developing lake trout embryos to impart cues during the months of incubation within the interstices of the spawning shoal. Our findings suggest that egg predators may indirectly decrease lake trout spawning success by depressing chemical cues at spawning sites. At present, the extent to which white sucker contribute to reduced recruitment of lake trout through egg predation is unknown and requires further examination.

Lake trout spawning locations are inherently identified in some way. The ability of lake trout to select the same site annually is not an anomaly. These sites have unique characteristics that make them suitable and successful for viable reproduction: coarse cobble, well-aerated water through ample fetch that is relatively shallow but close to deeper areas (Gunn, 1995). Here is the first field evidence that olfaction may play a role in the choice of spawning location, in addition to these general physical and limnological features of lake trout spawning habitat. Currently, the lake trout reproductive model discusses olfaction's role in site fidelity but does not contain any evidence in its support (Esteve, 2005). This study is the first to demonstrate that lake trout are preferentially attracted to visually concealed spawning site substrate over non-spawning substrate, and that this is true for both the spawning fish, as well as its egg predators.

Chapter 3: Lake trout (*Salvelinus namaycush*) spawning substrate attracts egg predators and opportunists through chemosensory cues

3.1 Abstract

Lake trout egg predators are able to locate the same sites annually when lake trout spawning is occurring. Though these sites have certain structural characteristics that make them unique and ideal for egg incubation, these characteristics do not explain the ability of egg predators to return annually. To test whether egg predators were attracted to lake trout spawning sites, five unbaited minnow traps were placed on lake trout spawning sites and structurally similar non-spawning sites in two lakes. Species abundance was recorded for each site. Traps on spawning sites captured significantly more sculpin, shiners and crayfish than structurally similar non-spawning sites. To test whether egg predators were attracted to substrate located on these sites, a field experiment was conducted the following year during the lake trout spawn. Visually concealed substrates from either a lake trout spawning site or a structurally similar non-spawning site were placed in a minnow trap on 10 sites on three lakes in the Experimental Lakes Area. Each site, on each of the lakes, had two minnow traps; one with lake trout spawning substrate and one with non-spawning substrate. For all lakes, minnow traps that contained spawning substrate captured more fish over the course of the study, than those that contained non-spawning substrate as well as had higher average daily catches.

3.2 Introduction

In aquatic environments, chemical cues mediate many fundamental ecological interactions across taxa, including recognizing conspecifics, evaluating predation risk, finding food, and establishing social status (Hara, 1994; Kats & Dill, 1998; Krieger & Breer, 1999; Huertas *et al*., 2007). Chemosensory cues allow aquatic organisms to interpret their environment (Ache & Young, 2005). The olfactory system of fish responds to an array of diverse molecules including amino acids (Hara, 2006), bile acids (Døving & Stabell, 2003), peptides (Hara, 1992), and steroidal compounds (Sorensen *et al*., 2005). The interpretation these compounds can help coordinate different actions for different members of the aquatic community. Spawning shoals are unique locations that can contain chemical cues that can be interpreted as either spawning cues to conspecifics, or food cues to egg predators.

Organisms in the aquatic environment are able to distinguish, mark and recall areas that are significant to them (Odling-Smee & Braithwaite, 2003). In many species, spawning locations are recognized annually by olfactory cues (Johnsen & Hasler, 1980; Horrall, 1981; Miller *et al*., 2001; Døving *et al*., 2006). Salmon in particular, are able to migrate hundreds of kilometers to their natal streams using olfactory cues (Dittman & Quinn, 1996). In many instances, salmon that have had their olfactory systems occluded are unable to locate spawning sites (Wisby & Hasler, 1954; Hansen *et al*., 1987). The ability to locate these areas using olfaction, can help to limit the amount of time spent searching for suitable spawning locations and help to coordinate the reproducing population (Goodenough *et al*., 2009).

Lake trout, *Salvelinus namaycush* (Walbaum, 1792), specifically, use the same spawning sites annually even though there may be other structurally similar sites available (Gunn, 1995). A recent study demonstrating that lake trout are attracted to visually concealed substrate found on spawning sites suggests chemical cues may play a role in the selection of spawning sites in addition to physical features (Wasylenko *et al*., 2013). It is hypothesized that spawning lake trout are attracted to the accumulation of juvenile feces and discarded egg membranes that are found on successful reproductive sites (Foster, 1985). Typically these sites are on rocky, windswept shoals, where fertilized eggs fall into the interstices of cobble substrate where they remain for several months before hatching (Gunn, 1995). These locations are ideal for lake trout to successfully deploy their negatively buoyant eggs and keep them well oxygenated during incubation. Though these sites generally have characteristics that aid in the development of the embryos, they do not offer complete protection from egg predators.

Lake trout eggs are especially vulnerable to predation because, unlike all other salmonid species, the eggs are not buried in a redd for protection. As such, predators can consume up to 80% of eggs found on spawning sites (Fitzsimons *et al*., 2002). Lake trout eggs are a protein rich, highly abundant prey item for predators such as slimy sculpin *Cottus cognatus* (Richardson, 1836), crayfish (genus Orconectes (Cope, 1872)), and common white sucker (*Catostomus commersoni* (Lacépède 1803)) (Savino *et al*., 1999; Wasylenko *et al*., 2013). Predator densities on spawning sites increase as eggs become abundant on spawning sites (Fitzsimons *et al*., 2002). Some initial studies have shown that different sculpin species are attracted to salmonid eggs, but have not shown whether they are attracted directly to spawning sites (Dittman *et al*., 1998; Mirza & Chivers, 2002). Since the same spawning sites contain eggs annually and over a short period of time, it would be to the predator's advantage to locate these areas quickly.

This experiment was conducted through a series of replicated field trials to determine whether egg predators can locate visually concealed lake trout spawning substrate. The experimental design tested whether egg predators showed a preference for spawning substrate compared to structurally similar non-spawning (control) substrate. The initial objective was to determine if, in fact, egg predators were attracted to spawning locations, as determined by the abundance and diversity of species found on natural spawning shoals versus habitat-matched control sites. The second objective determined whether native species were preferentially attracted to lake trout spawning substrate by comparing the abundance of, and diversity of, species captured in traps with and without (control) spawning substrate.

3.3 Methods

3.3.1 Attraction to spawning shoals

My initial study examined whether egg predators were more abundant on spawning sites when compared to structurally similar non-spawning sites. The abundance of egg predators on the two types of sites was tested to determine whether structure of a spawning site played a role in the egg predator's decision to be on the site. If structure were the determining factor in their decision then we would see no significant difference in the abundance of egg predators on spawning sites and structurally similar non-spawning sites.

Egg predator attraction to spawning sites and structurally similar non-spawning sites was examined at two lakes; L020 (49° 07’ N, 92° 08' W) and L042 (49° 05’ N, 92° 09’ W) within the Coldwater Lakes Area (CLA) north of Atikokan, ON from October to November 2011 (Fig. 3.1). The lakes were chosen based on the known location of lake trout spawning sites from previous long-term research on these lakes related to deforestation (Steedman, 2000; Steedman & Kushneriuk, 2000).

Lakes 020 and 042 have similar fish species compositions, with lake trout and common white sucker, as the only large fish species present (Table 3.1). Three sites were chosen on each lake: one lake trout spawning site, and two structurally similar non-spawning sites. Structure of non-spawning sites was based on previous published criteria (Martin, 1955; Martin & Olver, 1980; Gunn, 1995). Structurally similar non-spawning sites were assessed based on cobble size and on the lack of spawning lake trout captured during previous netting programs. Each lake had one predominant lake trout spawning location, which was used for this study. The spawning site on Lake 042 was located approximately 6 - 8 m offshore on a shoal that was approximately 2 - 3 m in depth. All other sites in both lakes were located in the littoral zone in approximately 1 – 2 m of water and adjacent to shore.

Once sites were selected, five unbaited standard wire mesh minnow traps (6.4 mm mesh, 42 cm L x 23 cm W with a 22 mm opening) were placed on each of the three sites on each lake. Sampling was conducted on nine different occasions for each lake from October 3rd to November 23, 2011. During each sampling event, species abundance was recorded and traps were placed back on the site. All fish captured were released approximately 10 m from the sampling site.

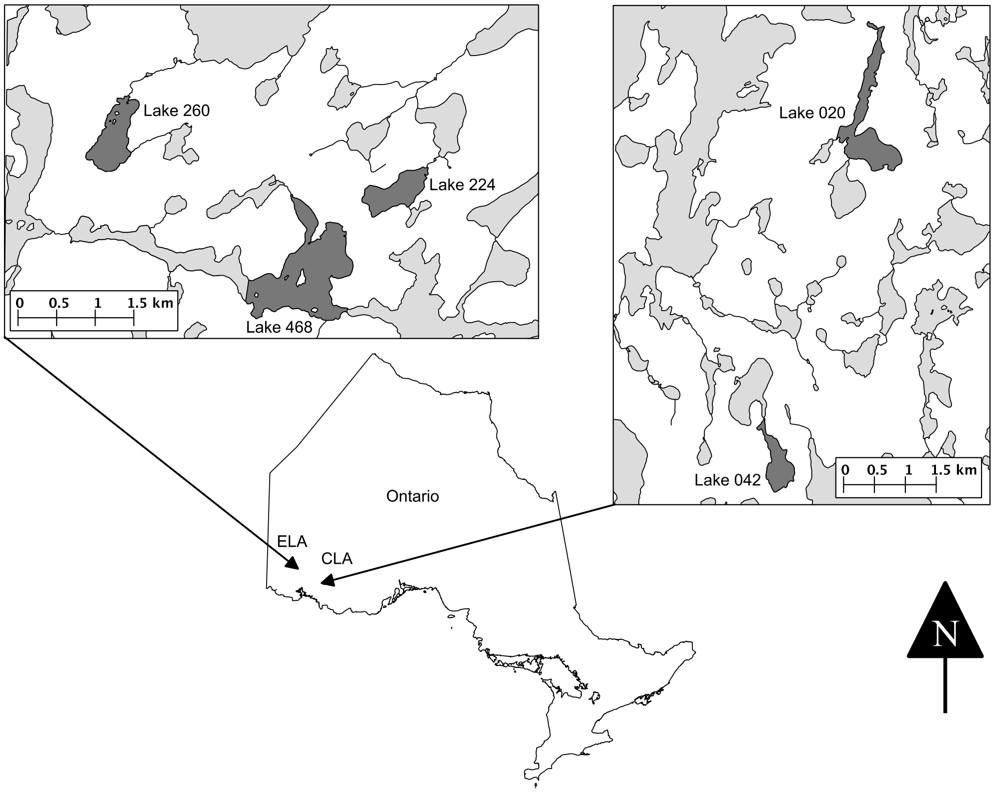

Figure 3.1 - Locations of study lakes used through out this experiment. Lakes 224, 260, and 468 are located in the Experimental Lakes Area (ELA). Lake 042 and lake 020 are located in the Coldwater Lakes Area (CLA).

3.3.2 Attraction to spawning substrate

The second part of this study was to determine whether egg predators were attracted to visually concealed spawning substrate. We examined whether native species were preferentially attracted to spawning substrate in three lakes at the Experimental

Table 3.1: Characteristics of study lakes.

Lake characteristics	Lake				
	020	042	224	260	468
Lake area (ha)	57	28	26	34	292(100[*])
Maximum lake depth (m)	32	19	27	14	29
Littoral fish species[1]	1-9	1,2,5-7,9	1,2,5-7,9	1,2,5,6,9,11	1,2,4,6,9,10,12, 13

[*] Size of basin used for study.

[1] Littoral fish species are coded as follows: 1) Common white sucker; 2) Northern redbelly dace *Phoxinus eos* (Cope 1862) and finescale dace *Phoxinus neogaeus* (Cope 1869); 3) Comnmon shiner *Luxilus cornutus* (Mitchell 1870); 4) Blacknose shiner *Notropis hetetolepis* (Eigenmann & Eigenmann 1893); 5) Fathead minnow *Pimephales promelas* (Rafinesque 1820); 6) Pearl dace *Marariscus margarita* (Cope 1869); 7) Brook stickleback *Culaea inconstans* (Kirtland 1841); 8) Iowa darter *Etheostoma exile* (Girard, 1860); 9) Slimy sculpin; 10) Yellow perch; 11) lake chub *Couesius plumbeus* (Agassiz 1850); 12) Bluntnose minnow *Pimephales notatus* (Rafinesque, 1820); 13) Longnose dace *Rhinichthys cataractae* (Valenciennes, 1842)

Lakes Area (ELA) 50 km east - southeast of Kenora, ON. The ELA is a pristine area that encompasses 58 research lakes that have been set aside for research purposes (Blanchfield *et al*., 2009). Lakes 260 (49° 41' N, 93° 46' W), 224 (49° 41' N, 93° 43' W) and the northeastern basin of 468 (49º 40’ N, 94º 45” W) (Fig. 2.1) were chosen based on the known locations of lake trout spawning sites as well as the accessibility of these sites. All lakes contained lake trout, and common white sucker as their predominant large fish species (Table 3.1).

Lake 468 and Lake 224 contained ten sampling sites per lake and Lake 260 contained eight sampling sites. All sites were located approximately 1 m from shore and were chosen based on their ability to accommodate two minnow traps, had a relatively gradual slope, and were not located close to a known lake trout spawning location. Sites were chosen to encompass as much of the lake as possible except in the case of Lake 468, we restricted our study area to the northeastern basin (Fig. 3.1).

Spawning substrate was collected from known lake trout spawning locations (during lake trout spawn) in each lake and separated into 0.25 kg units (approximately 5-8 pieces of substrate (cobble) per unit). Each substrate sample was wrapped in fine mesh netting to allow water to infiltrate the sample, but visually conceal the substrate. This method was repeated with substrate from a structurally similar non-spawning site. Spawning was confirmed by the presence of lake trout and eggs on the spawning location.

Two minnow traps (6.4 mm wire mesh, 42 cm L x 23 cm W with a 22 mm opening) were placed at each sampling site approximately 2 – 3 m apart with the open ends facing each other. Each trap contained a fine mesh bag of either spawning substrate or non-spawning substrate. Traps were checked daily between 0900 and 1200 hours. Species, quantity and total length (T_L) were recorded for each individual captured in each trap and returned to the lake approximately 15 m from the original capture location. Once each trap was sampled at a particular site, trap position was switched with the position of the paired treatment (i.e. if spawning substrate was on the right, the next night it would be on the left, and *vice versa*). The same substrate was used continuously throughout the experiment and remained within the same trap throughout. Sampling continued for 7 d, with the exception of Lake 468, which was sampled for 10 d due to low catch numbers.

3.4 Results

3.4.1 Attraction to spawning shoals

Over the course of the study, catches from unbaited traps on spawning and non-spawning shoals were highly variable resulting in data that failed to meet parametric statistical assumptions, despite data transformations intended to reclaim such assumptions. Consequently, non-parametric analysis (Pearson Chi-square) was used to examine the catch data. Northern redbelly dace and finescale dace were grouped as dace due to the common hybridization of the two species.

In Lake 042, there was significantly more slimy sculpin (SS: n = 27, NS1: n = 4, and NS2: n = 10) and northern crayfish *Orconectes virilis* (Hagen 1870) (SS: n = 19, NS1: n = 8, and NS2: n = 6) caught on the spawning site compared to the two non-spawning sites (slimy sculpin: $\chi^2 = 20.83$, $p < 0.0001$; northern crayfish: $\chi^2 = 8.91$, $p < 0.01$) (Fig. 3.2). The amount of brook stickleback captured at spawning sites (n=11) was not significantly different than the amount captured at non-spawning sites (NS1: n = 25, NS2: n = 21) ($\chi^2 = 5.47$, $p > 0.05$) (Fig. 3.2). However, the number of dace captured at non-spawning sites compared (NS1: n = 254, NS2: n = 120) was greater than the number captured at spawning sites (SS = 98) ($\chi^2 = 90.63$, $p < 0.0001$) (Fig 3.2).

In Lake 020, slimy sculpin (SS: n = 49, NS1: n = 6 , NS2: n = 13) , blacknose shiner (SS: n = 106 , NS1: n = 9, NS2: n = 39) and common shiner (SS: n = 117 , NS1: n = 5, NS2: n = 16) were more abundant on spawning sites than non-spawning sites (slimy sculpin: $\chi^2 = 46.97$, $p < 0.0001$; blacknose shiner: $\chi^2 = 96.09$, $p < 0.0001$; common shiner $\chi^2 = 165.7$, $p < 0.0001$) (Fig. 3.3). There was no difference in the amount of dace (SS: n =

23, NS: n = 17, NS2: n = 11) captured on spawning sites compared to the structurally similar non-spawning sites ($\chi^2 = 4.23$, $p > 0.05$)(Fig. 3.3).

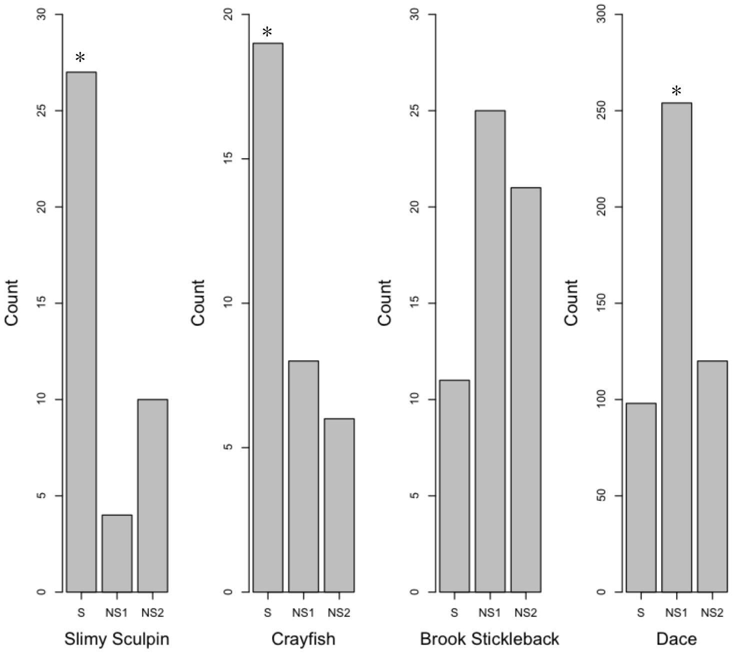

Figure 3.2 - Total catch from non-baited minnow traps for slimy sculpin, crayfish, brook stickleback, and dace in Lake 042 on three sites: S- Spawning site, NS1 – Structurally similar non-spawning site 1, and NS2 – Structurally similar non-spawning site 2. Asterisks denote a significant difference catch between sites.

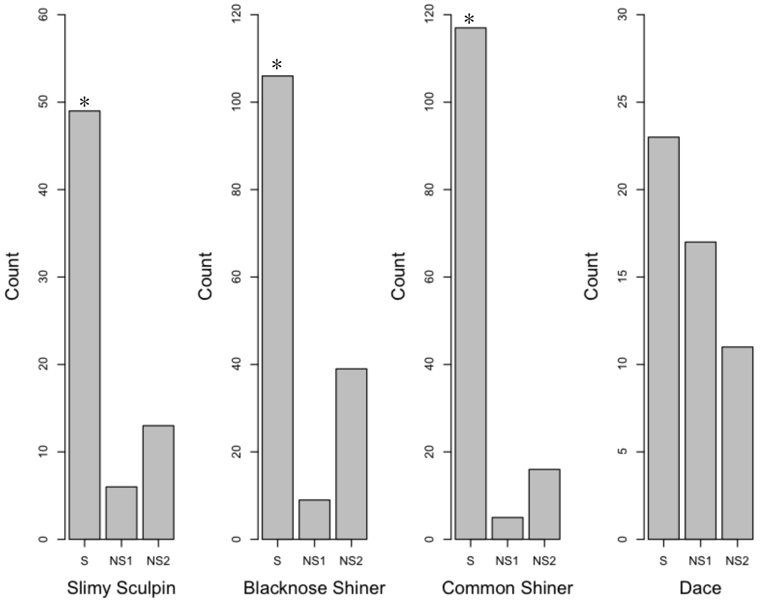

Figure 3.3 – Total catch from non-baited minnow traps for slimy sculpin, blacknose shiner, common shiner, and dace in Lake 042. Labeling conventions similar to Fig. 3.2

3.4.2 Attraction to spawning substrate

Over the course of the ELA study, catches among traps baited with either spawning substrate or non-spawning substrate were highly variable within each individual lake such that parametric statistical assumptions could not be met. Therefore, non-parametric analysis (Pearson Chi-square) was used to examine the data for each individual lake. Parametric assumptions were reclaimed from pooled-lake catch data using a $\log_{(x+1)}$ data transformation.

When comparing total catches for all lakes combined over the length of the study (7-10 days) there were 327 fish captured in spawning substrate containing traps and 206 fish captured in non-spawning site containing traps. The average catch per day in spawning substrate traps ($\bar{X} = 13.6$) compared to non-spawning substrate containing traps ($\bar{X} = 8.6$) was significantly different ($t = -2.12$, $p = 0.04$) (Fig. 3.4a). However, average catch per day between all lakes was significantly different (ANOVA, F-value = 29.72, $p<0.0001$) (Fig. 3.4b).

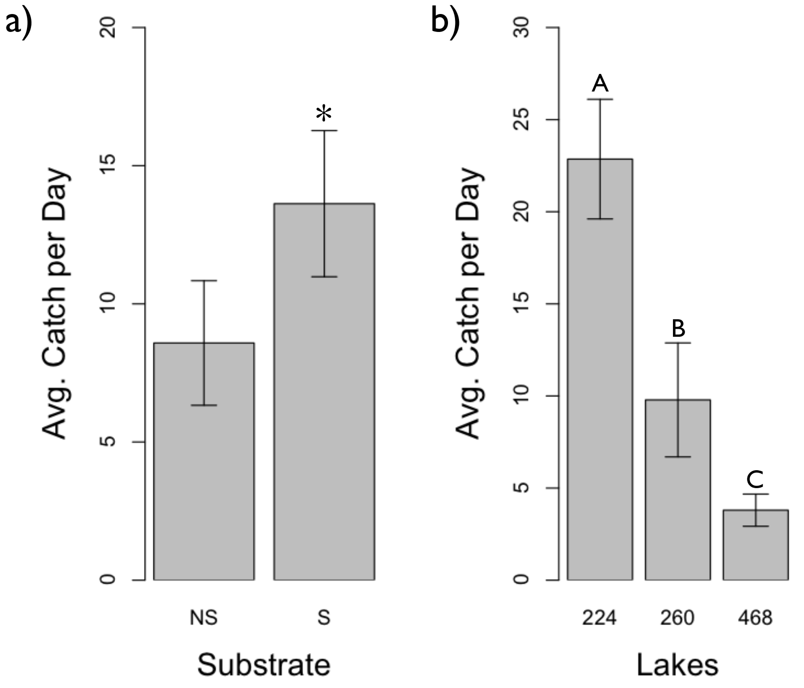

Figure 3.4 – (a) Average daily total catch in the ELA substrate experiment. (b) Average daily catch per lake. S- Spawning substrate, and NS – Structurally similar non-spawning substrate. Asterisks denote a significant difference catch between sites.

In Lake 468, traps that contained spawning substrate captured more yellow perch (n = 55) than non-spawning substrate containing traps (n = 21) ($\chi^2 = 15.21$, $p < 0.001$; Fig. 3.5). Slimy sculpin, blacknose shiner and pearl dace were caught in insufficient numbers for meaningful interpretation.

In Lake 260, slimy sculpin, fathead minnows, and pearl dace were captured in abundance. There were significantly more fathead minnows, pearl dace, and slimy sculpin caught in spawning substrate-containing traps (fathead: n = 70, pearl dace: n = 19, slimy sculpin: n = 17) than non-spawning substrate containing traps (fathead: n = 23, pearl dace: n = 6, slimy sculpin: n = 2) (fathead minnow: $\chi^2 = 23.75$, $p < 0.0001$; slimy sculpin $\chi^2 = 11.84$, $p<0.001$; pearl dace: $\chi^2 = 6.76$, $p<0.01$; Fig. 3.6).

In Lake 224, pearl dace was the only species that was caught in higher abundance in spawning substrate-containing traps (n = 70) than in non-spawning substrate containing traps (n = 35) (χ^2 = 11.67, p<0.001; Fig. 3.7). There was no difference in brook stickleback (χ^2 = 0.64, p > 0.05), and fathead minnow (χ^2 = 11.67, p > 0.05) catches between the spawning (stickleback: n =72, fathead: n = 24) and non-spawning substrate containing traps (stickleback: n =82, fathead: n = 37) (Fig. 3.7). Slimy sculpin were not captured in significant numbers for meaningful interpretation.

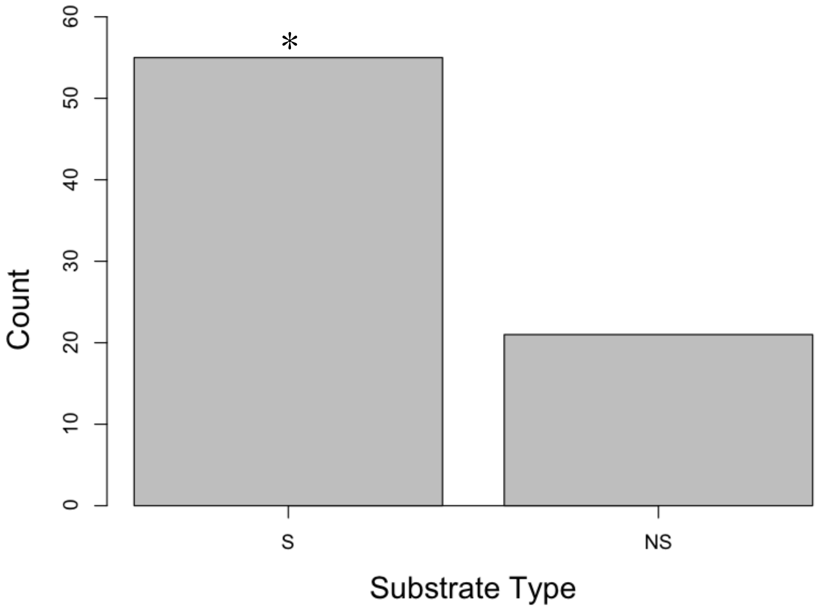

Figure 3.5 - Total catch for yellow perch in L 468. Labeling convention similar to Fig 3.4.

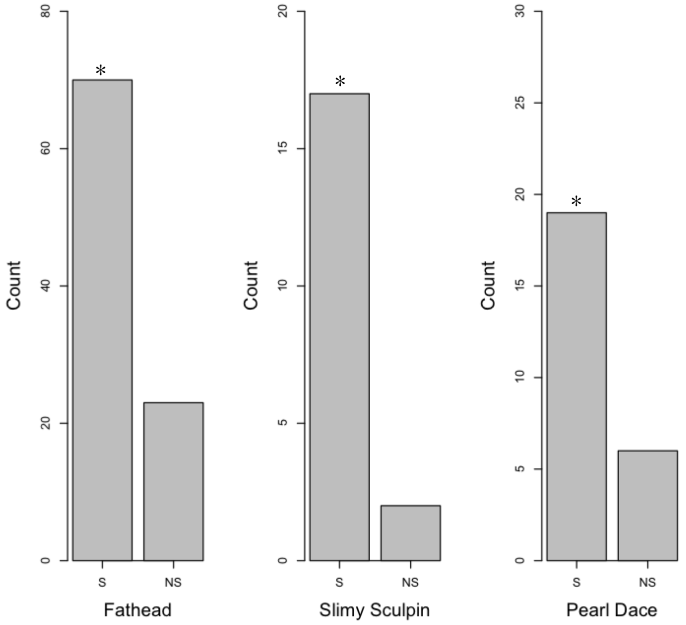

Figure 3.6 - Total catch for fathead minnows, slimy sculpin and pearl dace in L 260. Labeling convention similar to Fig 3.4.

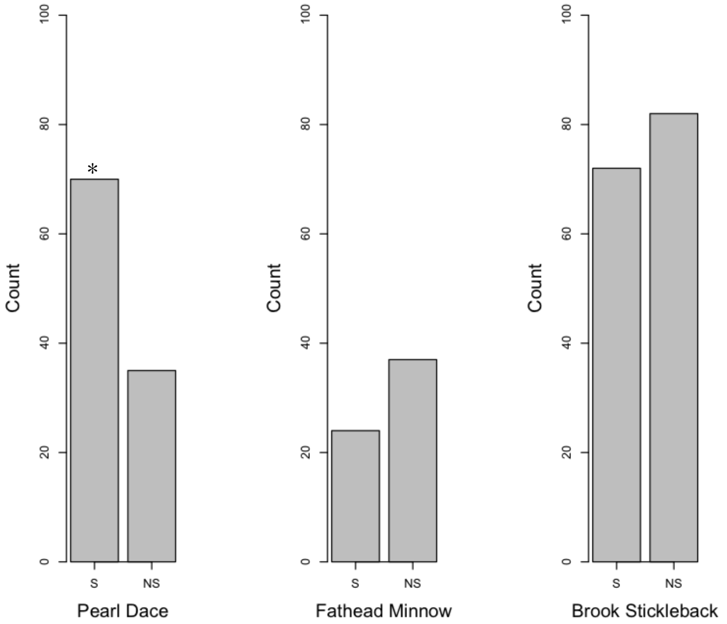

Figure 3.7 - Total catch for pearl dace, fathead minnow, and brook stickleback in L 224. Labeling convention similar to Fig 3.4.

3.5 Discussion

This is the first field study to document an attraction by several lake trout egg predators to lake trout spawning sites compared to structurally similar non-spawning sites. This study also documents the attraction of egg predators to visually concealed lake trout spawning substrate. This study demonstrates the attraction of several species to substrate from an area of ecological significance and displays that the cues associated with spawning sites are interpreted by many species. Known lake trout egg predators as well as other species (dace, fathead minnows) were attracted to the same spawning substrate. In this study, an attraction to spawning substrate has been observed but what kind of cue each species is associated the substrate with remains to be determined.

The initial results of the attraction to spawning shoals study shows that there were significantly more egg predators on spawning sites than structurally similar non-spawning sites. These results suggest that an egg predator is attracted to something other than structure on spawning sites. This initial observation led to the testing of whether the substrate from a spawning site had an effect on the choice of a spawning site to a structurally similar site. During the attraction to spawning substrate study, there were significantly more fish on average caught daily in traps that contained spawning substrate than those that contained non-spawning substrate. More individuals were attracted to substrate taken from a spawning site than substrate taken from a structurally similar non-spawning site. These results are significant due to the fact that there was no food source concealed within the substrate samples and the sites were located away from active spawning locations. Most studies to date have looked at an egg predator's attraction to eggs but not its attraction to the spawning substrate (Dittman *et al*., 1998; Mirza &

Chivers, 2002). Our study shows an attraction to spawning substrate even when the substrate is not located on, or near, a spawning shoal. This study suggests that egg predator species are not only attracted to lake trout eggs, they are also attracted to substrate that is associated with the annual presence of these eggs.

Slimy sculpin, a known lake trout egg predator (Stauffer & Wagner, 1979; Martin & Olver, 1980), was not only significantly more abundant on spawning sites when compared to structurally similar non-spawning sites, but was also attracted to lake trout spawning substrate in the absence of eggs. The highest number of slimy sculpin was caught in Lake 260 with the majority being captured in traps containing spawning substrate (89%, n=19). Though slimy sculpin have been shown to be attracted to olfactory cues released by salmon and brook trout *Salvelinus fontinalis* (Mitchill 1815) eggs (Dittman *et al*., 1998; Mirza & Chivers, 2002), the absence of eggs in this study illustrates that they can recognize chemically tagged spawning substrate. Understanding how slimy sculpin recognize lake trout spawning sites is ecologically significant. Fitzsimons *et al*. (2002) estimated that for a 30-d period post spawning (beginning from the peak spawning period), sculpins were able to consume 0-54% of the estimated egg abundance in Lake Ontario. In areas of low egg deposition, sculpin and crayfish were estimated to consume almost 100% of the eggs. This amount of predation can have negative effects on lake trout populations, especially those with declining populations. By understanding the way that sculpin are attracted to these sites, fisheries managers may be able to create alternative strategies to aid in recovery efforts.

Within Lake 468, yellow perch were also caught in significantly higher numbers in traps treated with spawning substrate than non-spawning substrate. Yellow perch are not typically associated with lake trout egg predation, but are known to predate on smaller walleye, *Sander vitreus* (Mitchill, 1818) eggs (Wolfert *et al*., 1975; Roseman *et al*., 2006). Since lake trout eggs are one of the larger salmonid eggs (5-6 mm), certain species would be limited by the size of their gape to being to ingest eggs whole. Juvenile yellow perch would not be gape-limited and could potentially be an efficient lake trout egg predator (Truemper & Lauer, 2005).

Several fish species that are not normally associated with egg predation were captured in significant numbers in spawning site traps at the CLA and in traps containing spawning substrate at the ELA. In Lake 020, blacknose and common shiners were more abundant on the spawning site as opposed to the non-spawning sites. In Lake 260, both fathead minnow and pearl dace were attracted to spawning substrate over non-spawning substrate but in Lake 224 only pearl dace was attracted to spawning substrate. Though not typically associated with egg predation (there are some instances of spottail shiners *Notropis hudsonius* (Clinton 1824) predating on eggs (Wolfert *et al*., 1975; Roseman *et al*., 2006)), these cyprinids may be attracted to these sites for other non-egg food opportunities. Blacknose and common shiners, fathead minnows and pearl dace generally feed on small invertebrates and detritus (Scott & Crossman, 1973). Spawning lake trout stir up detritus while cleaning and prepping spawning sites, which may attract invertebrates and cyprinids. So though these species may not be predating on lake trout eggs (they would be gape limited), they may associate spawning substrate with other types of food sources.

Lake 224 had the most fish caught captured (n=156) but the only species to show an attraction to spawning substrate was pearl dace. Though fathead minnows and brook sticklebacks were caught in Lake 224 we did not see a significant difference in catch numbers between the substrate types (opposite of the Lake 260 observations). In contrast to the other lakes studied at ELA, the spawning substrate in Lake 224 was scattered in small and patchy clumps located in two main areas. The dispersal of these sites may diminish the strength of a cue on the substrate for egg predators to sense or to locate.

One interesting tradeoff that has emerged from this study is the persistence of egg predators' attraction to spawning sites that have high predation risk. With lake trout being the top predator in the studied lakes, many baitfish species are prey. Spawning sites can be associated with high rewards with an abundance of food sources but can also be risky owing to an abundance of predators. Sculpins in particular, make up a large proportion of a juvenile lake trout diet (Owens & Bergstedt, 1994). Juvenile lake trout (though not sexually mature) are present on most spawning sites (Muir *et al*., 2012). The attraction to the spawning substrate suggests that the reward associated with entering spawning sites outweighs the risk of predation from juvenile lake trout.

My study demonstrates that several species are attracted to visually concealed spawning substrate during the lake trout spawn. Chemosensation allows an animal the ability to perceive and interpret the risk and reward of entering different habitats. In many instances, the ability to locate adequate food sources using olfaction limits the amount of energy expended on searching for food and maximizes the gain from the food source (Goodenough *et al*., 2009). The ability of an egg predator to locate seasonal spawning areas would allow predators to reduce the amount of energy spent searching for adequate food. Chemosensory "tags" inform potential egg predators of the location and value of a potential food source, thereby maximizing the reward while minimizing energy invested in foraging. The use and interpretation of chemical tags aids in the egg predator's ability to weigh risk and reward in areas that may high in food sources but also high in predators.

Chapter 4: Conclusion

Chemosensory cues play a vital role in locating spawning substrate for both lake trout and their egg predators. In both of these experiments, lake trout and their egg predators were caught in significantly higher numbers in treatments with spawning substrate than those with structurally similar non-spawning substrate. The ability to locate visually concealed spawning substrate allows both the spawner and egg predator the ability to locate spawning sites while minimizing the amount of time searching for them annually. Knowing that lake trout spawning substrate can be located using olfaction creates a number of management options and new research questions that can be developed and tested. The Laurentian Great Lakes are excellent candidates for research because of the lack of natural recruitment that is occurring in them.

Great Lakes lake trout populations, with the exception of Lake Superior, no longer have self-sustaining populations (Hansen *et al*., 1995). The populations in all of the Great Lakes have been depleted due to overfishing, and sea lamprey predation (Hansen *et al*., 1995). The restoration efforts to date have taken the approach of releasing hatchery-raised fish and releasing them into the Great Lakes with little success at regaining a self-sustaining population (Eshenroder *et al*., 1995). My research adds a new perspective to understanding the lack of recruitment from hatchery-reared lake trout and how egg predators are targeting spawning sites. A problem that has not been adequately addressed in studies regarding lake trout recruitment in the Great Lakes is whether introduced populations are able to be attracted to chemosensory cues that emanate from spawning substrate found on traditional spawning sites. My research has shown that lake trout are able to locate visually concealed spawning substrate however it does not indicate when lake trout learn to recognize this scent or if they innately recognize it. Research has indicated in other salmonids that this information is imprinted at a young age (Hasler, 1978). If hatchery-reared lake trout are released into lakes without imprinting of spawning substrate then the population may not adequately synchronize their reproductive efforts or choice of location. Furthermore, if reproduction has been low for decades then the chemical cue from the substrate may be deteriorating due to lack of annual renewal from reproducing fish. A future project could be rearing young lake trout in the presence of spawning substrate from the lake that they will be introduced into. If they are able to recognize spawning substrate after being exposed to it as a juvenile, they may be able to locate these areas more accurately once they reach sexual maturity.

Another study that could be completed from this work is to determine what the attractant is that is found on spawning substrate and how it is able to persist annually. If these chemical(s) were able to be isolated, the management implications that could result would be ground breaking. The ability to determine the chemicals responsible for the attraction to spawning substrate would aid in locating traditional spawning sites as well as aiding in deterring egg predators. Similar chemical isolation has been completed on sea lamprey chemoattractants to spawning sites and the methods to do so with lake trout may be similar (Sorensen *et al*., 2003; Sorensen *et al*., 2005). The ability to isolate these chemicals could allow managers or researchers the ability to “tag” appropriate areas that would be ideal spawning sites or man made structures that would be structurally sound for lake trout spawning. Once this type of information is available to fisheries managers, the way of understanding, manipulating, and helping lake trout fisheries will be on a totally different level than what is used now. Although we have known in the past that lake trout spawn on structurally understood areas within a lake, we are now able to see evidence for another aspect of site selection: olfaction.

Recent eco-toxicological work has centered on the impairment of olfaction in fish and how that can relate to specific ecological functions (Tierney *et al*., 2010). Different metals can induce temporary chemosensory impairment and hence affect ecologically-relevant, olfactory-mediated behaviours, such as mate selection, predator avoidance, or foraging. This work demonstrates the importance of how olfaction plays an essential ecological activities for lake trout and egg predators. Olfactory impairment from metal and chemical contamination could result in the loss of some the basic ecological activities seen in this thesis. Measuring the effect of metal impairment is always difficult because many ecological interactions (i.e. locating spawning sites annually) do not have benchmark behaviours in the wild. This study demonstrates basic uses of olfaction *in situ* in non-metal contaminated lakes.

In both experiments presented in this thesis, lake trout and their egg predators are attracted to visually concealed spawning substrate. Lake trout spawning sites have been historically recognized, and managed based on the visual aspects but my research has shown that there is an olfactory component that needs to be addressed further. Though the structural aspects of spawning sites aids in the development of embryos by protecting them, the chemical information that is recognized by numerous species aids in the location of these historically successful areas. The reactions of different fish species to different chemical cues has been observed in the laboratory, but no data have been collected to show that fish species are able to recognize substrate from a site of ecological significance. This study adds a new perspective to how fish use olfaction in nature as the data suggests that fish are able to differentiate structurally similar sites from one another. The ability of lake trout and numerous egg predators to discriminate substrate based on chemical cues adds a new perspective to how managers and biologists view spawning sites characteristics. Though structure and temperature play important roles in defining spawning sites characteristics and timing, olfaction and chemicals may play the role in locating perennial spawning sites.

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