Investigating the Effects of Anthropogenic Stressors on an Aquatic Top Predator

by

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ABSTRACT

INVESTIGATING THE EFFECTS OF ANTHROPOGENIC STRESS ON AN AQUATIC TOP PREDATOR

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University of Guelph, 2018

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Multiple anthropogenic stressors are simultaneously impacting ecosystems and interacting with each other at multiple scales. Marine and aquatic systems are especially susceptible to cumulative effects as they accumulate and distribute contaminants and nutrients and are used for a variety of human purposes. In this study, lake trout were sampled from differentially stressed locations in Georgian Bay, Ontario based on mapping data of anthropogenic stressors. First, I show that the relative weight, hepatosomatic index, relative brain weight, degree of littoral coupling and trophic position of lake trout are significantly different in the site with the lowest CAS score. Second, I used a canonical correlation analysis to show that specific human activities and stressors had different proportional effects on the biologic indicators used in this system. The results of this study can be used to inform environmental managers how to mitigate CAS in Georgian Bay.
I'd like to thank all the volunteers who helped me in the lab and field through my years at the University of Guelph. It is a great community that I am very happy to be a part of it. I'd also like to thank the project students who worked with me: Ashleigh Ooi, Alytta Teuber, Reilly O'Connor, Sean Yardley and Jamie Bain. I'd like to thank some colleagues Marie Gutgesell, Carling Bieg and Tim Bartley. I'd also like to acknowledge some of the invaluable help from Brandon Graham, Laura Johnson, Ashleigh Ooi and Emelia Myles-Gonzalez who always went the extra distance.

I would like to thank Tony Desgasperis for sharing his knowledge of salmon and lake trout in Georgian Bay and for the many insightful conversations. I'd like to thank all the Harbour Attendants in Collingwood for connecting us with anglers. I'd like to thank Kana Upton for help us with everything in Parry Sound from benthic samplers to rescue missions. I'd like to thank Gord Cole for his depth of knowledge and spirited discussion about aquaculture, fish biology and water quality. I would also like to thank all of the anglers who donated lake trout tissue samples as well as the Owen Sound Salmon Spectacular and the Collingwood fishing derby for letting me obtain samples and talk to local anglers about this research.

I'd like to thank Paul Sibley and Frederick Laberge for their help developing, reviewing, and polishing my scientific work. Kevin McCann, I felt like a student of yours and appreciate all the advice; field, research and life. Lastly, Neil Rooney thanks for a great two years. You have been advising me for longer than that and I am very happy with where I am today so I think that says it all; thank you.

Finally, I would like to thank my family for always supporting me in everything I do.
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**Figure 2.6** A biplot resulting from a CCA using lake trout biologic indicators. CCA1, the main axis explaining variation in a cumulative measure of indicators, is illustrated. The combined biologic indicator is based on relative body weight, hepatosomatic index (HSI), relative brain weight, littoral coupling and trophic position. Each dot represents the ordination location of each lake trout based on their respective combined biologic indicator CCA value. The ellipsoids represent the 95% confidence interval for site (colour differences). Grey vector arrows denote the stressors which significantly (p < 0.05) described variation in the lake trout combined biologic indicator. The shape of each dot indicates sampling month, which had a significant relationship with lake trout biologic indicators.

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<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>CAS</td>
<td>Cumulative anthropogenic stress</td>
</tr>
<tr>
<td>CEA</td>
<td>Cumulative effects assessment</td>
</tr>
<tr>
<td>CW</td>
<td>Collingwood, Ontario</td>
</tr>
<tr>
<td>DB</td>
<td>Dyers Bay, Ontario</td>
</tr>
<tr>
<td>GLEAM</td>
<td>Great Lakes Environmental Assessment and Mapping</td>
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<tr>
<td>HSI</td>
<td>Hepatosomatic Index</td>
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<tr>
<td>LGL</td>
<td>Laurentian Great Lakes</td>
</tr>
<tr>
<td>OWS</td>
<td>Owen Sound, Ontario</td>
</tr>
<tr>
<td>PS</td>
<td>Parry Sound, Ontario</td>
</tr>
<tr>
<td>TB</td>
<td>Tobermory, Ontario</td>
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</table>
1  Prelude

1.1  Cumulative Anthropogenic Stress Affects Ecosystems

Cumulative anthropogenic stress (CAS) is a global concern, and regions once considered isolated and pristine, have clear and considerable human footprints. For example, remote arctic regions of the world, once untouched by anthropogenic impacts, are being influenced by multiple stressors, like increasing global temperature\(^3,^4\) and the atmospheric deposition of heavy metals\(^5^-^7\). These anthropogenic stressors are simultaneously impacting ecosystems and interacting with each other at multiple scales\(^8^-^10\). Rising temperature operates on a global scale and interacts with stressors at a local scale such as species invasions or nutrification\(^8,^9,^11\). The timing of the interaction between environmental changes and anthropogenic stressors can also lead to synergistic effects. For example, the depth and duration of the summer thermocline in oceans and lakes is increased by warmer air temperatures, reducing the ability of cold-water adapted species to avoid hypoxic conditions which have been amplified by nutrification\(^12,^13\). These burgeoning effects of CAS present complex environmental problems requiring new and innovative tools to classify and comprehend ecosystem change\(^14\).

CAS is driven by human activities. In Canadian legislation, cumulative effects are defined as changes to the environment in response to an action in combination with another past, present and/or future human action\(^15\). While multiple definitions exist\(^16\), the common constituent is the alteration of the environment as the result of human activity\(^17^-^19\). Understanding that there is a relationship and a clear distinction between human activities, stressors and environmental effects is necessary for the analysis of CAS. Human activities, actions or deeds that people do or cause to happen\(^15\), are also referred to as a ‘drivers’ or ‘pressures’ in CAS literature\(^20,^21\). Clarifying this terminology is necessary as stressors have also
been referred to as ‘pressures’\textsuperscript{22–24}. For the purpose of this paper, stressors are defined as the physical, chemical or biologic components of a human activity, that interact with the environment and can lead to environmental effects\textsuperscript{25}. Environmental effects are biotic or abiotic factors that exceed the natural range of variation, based on historic or reference site conditions\textsuperscript{26–28}. Human activities may not directly exert stress on the environment, however they contribute to the magnitude and frequency of stressors. For example the production of greenhouse gases is driven by multiple human activities such as transportation, agriculture and energy production\textsuperscript{29}. Furthermore, these activities have other stressors associated with them such as oil and gas pollution, nutrient loading to water bodies and increased atmospheric sulfur and nitrogen oxides (causing acid rain). Together these stressors can cause unexpected ecological effects that are the result of stressors simultaneously affecting an ecosystem\textsuperscript{9,26}.

Devastating accounts of major environmental alterations like the bleaching of ocean corals from the combined effects of ocean acidification and increased water temperature\textsuperscript{30,31} are what drive researchers and managers to predict and mitigate the impacts of human activity before they contribute to environmental change.

Understanding CAS is important because it can affect the sustainability of ecosystem functions and food webs\textsuperscript{32–34}. Stressors directly affect biodiversity, for example invasive species can outcompete local indigenous biota\textsuperscript{35}. Stressors can also work cumulatively as habitat loss, climate change and pollution increase the likelihood of extinctions in the current Anthropocene\textsuperscript{36–38}. More importantly, the loss of biodiversity can reduce ecosystem functions and services that benefit society\textsuperscript{39}. Human activities have been shown to erode the stability of complex dynamic ecosystem structures. When multiple stressors exacerbate the resilient features of ecosystems, these systems lose some of their structure, potentially resulting in decreased stability\textsuperscript{40,41}. In this chapter I will discuss frameworks for multiple stressors and propose environmental indicators that can be used to assess the environmental effects of CAS.
1.2 A Framework for CAS Research

To understand the impacts of CAS, linkages between human activities and environmental effects must be established. There are pre-existing frameworks which help to understand pathways from human activity to environmental effects. In 2014, the World Wildlife Fund released a review of the science and application of cumulative effects research\textsuperscript{25}. Four major pathways were identified that map the connection between human activity, stressors and the resulting environmental effect (Figure 1.1). These pathways are: (1) the independent pathway, through which a single human activity affects a single stressor and produces an environmental effect, (2) the multiple activities pathway where a single stressor is affected by multiple human activities, (3) the multiple stressors pathway where a single human activity drives various stressors and (4) the multiple effects pathway were stressors can have more than one affect on the environment. Utilizing this and similar frameworks to organize CAS allows researchers to understand what environmental effects are being generated by multiple stressors and what human activities are driving the occurrence of those stressors.

Marine and aquatic ecosystems are affected by multiple stressors, both physical and chemical, and these stressors result in diverse effects. However, most research regarding human impacts on the environment address the independent pathway, focussing on the effect of a single stressor, or human activity\textsuperscript{42}. Experiments conducted in laboratory settings are designed to isolate a single variable. While these studies can provide critical background information, it has proven difficult to predict environmental effects when multiple stressors are affecting the environment\textsuperscript{26}. Canadian regulatory policy for chemicals, estimates the toxicity of chemical mixtures, by assuming chemicals with the same toxicological effects will act additively\textsuperscript{43}. This assumption can lead to underestimating risk from chemical mixtures that act synergistically. A call for the evaluation of multiple stressors requires that new methods be developed to understand the more complex interactions\textsuperscript{26,44}. However before designing a study
to understand cumulative stressors and effects, a model ecosystem is required\textsuperscript{14}. A model ecosystem must accumulate multiple stressors and have measurable environmental effects as a result of these stressor induced changes. A lot of work on cumulative effects is based in marine and freshwater environments as these ecosystems provide a sink for contaminants and are often used for a variety of human purposes\textsuperscript{11,45}. More importantly changes to these ecosystems have been attributed to the stressors affecting them.

1.3 Framework for CAS in the Laurentian Great Lakes

1.3.1 Great Lakes Assessment and Mapping of Stressors

The Laurentian Great Lakes (LGL) are highly susceptible and responsive to CAS and provide a model system for studying cumulative effects\textsuperscript{44}. Large bi-national programs, such as the Great Lakes Restoration Initiative and the Great Lakes Fishery Commission, denote the biologic and socioeconomic value of protecting and restoring the LGL\textsuperscript{46,47}. These vast freshwater ecosystems have undergone significant changes in nutrient regimes, native species persistence, toxicological status, exotic species invasions and temperature fluctuations. All of these changes have been influenced by human actions if not solely driven by them. Managing to mitigate these perturbations while attempting to move forward with the restoration of these valuable systems presents an opportunity to develop tools to measure effects.

The LGL are also an ideal study system because a relatively recent comprehensive assessment of CAS has been completed\textsuperscript{1}. The Great Lakes Environmental Assessment and Mapping project (GLEAM) assessed 34 anthropogenic stressors in 1 km\textsuperscript{2} grids across all the LGL (available at https://portal.glos.us/#). These spatially explicit evaluations of anthropogenic stress are ranked on a scale from 0 to 1 to reflect their relative impact on ecosystem condition\textsuperscript{1,46}. This mapping process showed that while many areas of the LGL had relatively low cumulative impact scores (less than 10\% of pixels had a cumulative impact score over 0.3) most
regions were affected by 10-15 different stressors. Thus, as mentioned earlier, a focus on one or a few stressors will often overlook other important anthropogenic stressors contributing to observed effects. To further utilize this assessment of CAS in the LGL, we can use the pathways framework to hypothesize the links between human activities, stressors and environmental effects.

1.3.2 Categorizing Stressors

The anthropogenic stressors identified by GLEAM can be applied to the pathways of CAS, discussed in Section 1.2 (Table 1.1, Figure 1.1). Considering the definitions of human activities, stressors and environmental effects I presented in Section 1.1, 16 of the stressors identified by GLEAM more accurately fit the definition of human activity, 13 accurately fit the definition of a stressor and I propose that 4 of the GLEAM stressors are more appropriately considered environmental effects (Table 1.1). Putting these human activities and stressors in a pathways framework can help formulate predictions as to which stressors are linked to multiple human activities, and which environmental effects are exposed to the greatest number of stressors. All of the stressors from the GLEAM mapping project are included in the pathways framework under the proposed revised classification (Figure 1.2). When links in the human activity, stressor and environmental effect pathways were missing I provide examples to complete them. In this conceptual diagram, stressors such as sediments and nutrients, PCBs and heavy metals are linked to the most human activities (Figure 1.2). The environmental effects which are influenced by the greatest number of stressors, are most likely to be affected by cumulative stress (Figure 1.2).

The exercise of drawing connections between human activities, stressors and environmental effects accentuates the importance of grouping types of stressors or human activities. Shifting to a multiple stressors perspective can lead managers to consider the
mitigation of human activity in a more integrative manner; one that might better resolve the environmental effect of current concern. For example, if the environmental effect of concern is hypoxia, mitigating the human activities that contribute to sedimentation, nitrogen and phosphorus will be most effective based on the conceptual diagram (Figure 1.2). While different groups of stressors can interact, like nutrients and invasive species\(^9\), it is establishing the link to human activities that allows environmental managers to actively combat the problem. This framework parallels Canadian policy for the protection of fisheries, through the Fisheries Act\(^{23}\). Referred to as pathways of effect this is a process currently used in Canada to mitigate the anthropogenic effects of construction and development projects by eliminating the greatest number of alterations to a water body\(^{23}\). Bringing this framework to large ecosystems like LGL could greatly improve the allocation of restoration efforts by focussing on the human activities and stressors that are having the greatest collective impact on the environment.

### 1.4 Selecting Indicators for CAS

While an estimation of the intensity of stressors across the LGL has been made, the cumulative effect of these stressors remains unquantified. It is important to map the distribution of anthropogenic influence on our ecosystems, but whether these distributions are reflecting a biologically relevant gradient of environmental stress requires empirical investigation. When multiple stressors act upon an ecosystem, many responses to these stressors can manifest in that system\(^{19}\). In large dynamic systems like the LGL, different stressors will act upon different levels of biologic organization, for example the individual health of an organism, the population viability of a species or the overarching structure of the food web\(^{48}\). Measuring the effects of disturbance at only one of these biologic scales will overlook some of the environmental effects. By using a single index, the cumulative effect of stressors is dampened by not accounting for variation outside of that measure. Adding indices has been shown to increase the consistency in
detecting environmental gradients\textsuperscript{49}, however selectively adding indicators that correspond to different levels of organization may be more beneficial\textsuperscript{48}. This selective addition could reduce redundancy in indicators, while effectively monitoring a wide-range of environmental effects. Capturing these effects without measuring every component of the environment requires an understanding of the levels of organization within a given environment and the deployment of multiple indicators to trace them.

CAS affects different levels of biologic organization such as individual health, population viability, community structure, food web attributes and ecosystem effects\textsuperscript{48}. An effective indicator of CAS must not only integrate stressors over space and time, but also respond to changes at different levels of biologic organization. Top predators in aquatic systems are ideal study species to assess CAS because they integrate a breadth of anthropogenic stressors, they are mobile and integrate stress over long periods of time\textsuperscript{50}. Moreover, indicators of individual and population health of these top predators can be readily assessed\textsuperscript{51}. Estimates of reproduction, recruitment and survival can inform population estimates and is currently used in the LGL to monitor fisheries\textsuperscript{52–54}. These top predators can also provide information about community structure through stomach content analysis\textsuperscript{55}. Top predator stable isotopes values can identify changes in the flow of carbon between pelagic and littoral habitats and nitrogen values can be used as indicators for the length of food chains, which are important ecosystem level attributes\textsuperscript{56,57}. With this information we can make predictions as to how different human activities and stressors will affect these predators (Table 1.1). The advantage to using indicators of stress from top predators is that they comprise a wealth of environmental information. This method of using multiple indicators from a sentinel organism can provide insight to the effects of CAS efficiently and effectively.
1.5 Conclusion

To inform environmental managers how to mitigate CAS, studies of top predators might be useful to capture the wide-ranging effects of stressors. The GLEAM analysis of stressors on the LGL has introduced researchers to the multidimensional problems managers face when dealing with large complex ecosystems. The next step is to investigate whether biologically relevant indicators respond to these gradients of anthropogenic stressors. Identifying which stressors and human activities are having the largest effect on ecosystems would prompt managers to act upon this information. Informing restoration objectives based upon indicators that respond to anthropogenic stress is the cornerstone of preventative conservation. Mitigating environmental degradation starts with an understanding of the consequences of different human activities and mitigating the stressors associated with these activities. By adopting an integrative approach, which combines empirical data and mapping, it may be possible to improve predictions, refine methods for monitoring harmful human impacts, and provide a stronger foundation for environmental managers’ decision-making in lessening the impact of cumulative effects.
### 1.6 Tables and Figures

Table 1.1 Description of GLEAM stressor layers and their potential effects on top predators. GLEAM stressors were classified as human activities, stressors or environmental effects based on the definitions provided in section 1.1.

<table>
<thead>
<tr>
<th><strong>GLEAM Layer</strong></th>
<th>Description</th>
<th>Effect on Top Predators</th>
<th>Effect Type</th>
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<tbody>
<tr>
<td><strong>Mercury</strong></td>
<td>Mercury contamination affects human health and although direct deposits of mercury to freshwater systems from spills and industry has been reduced, atmospheric deposition of mercury still poses a serious threat to organisms.</td>
<td>Mercury can have a strong effect on top predators and on long lived species like lake trout that will accumulate mercury throughout their lifetime. Biotransformation of methyl mercury is performed by the liver, causing enlargement.</td>
<td>Stress, Mercury is a chemical and is influenced by multiple human activities. Burning fossil fuels releases mercury into the atmosphere which is deposited into lakes and oceans.</td>
</tr>
<tr>
<td><strong>Copper</strong></td>
<td>Copper is an essential compound for vertebrate respiration and enzyme synthesis.</td>
<td>Copper does not readily bioaccumulate, however dietary and aqueous Cu(^{2+}) can affect the liver and gills. It can cause acute toxicity at low concentrations to zooplankton reducing available resources.</td>
<td>Stress, Copper is a chemical component and is influenced by multiple human activities. Elevated levels of copper can be found in mining effluents.</td>
</tr>
<tr>
<td><strong>PCBs</strong></td>
<td>Polychlorinated biphenyls are resistant to degradation, bioaccumulate and biomagnify in the environment.</td>
<td>PCBs drive most of the fish consumption guidelines in Canada and USA, and long-lived large species obtain especially high concentrations of these compounds. Lake trout are susceptible to PCBs because they bioaccumulate. PCBs affect the liver and brain function salmonids.</td>
<td>Stress, PCBs are chemical components. PCBs are synthetically manufactured for multiple purposes.</td>
</tr>
<tr>
<td><strong>Suspended Sediments</strong></td>
<td>Suspended sediments alter the physical characteristics of water by changing the turbidity or lack of water clarity. Sediments also carry organic and inorganic chemicals, and introduce biologic components such as microbes that interact with the receiving ecosystem.</td>
<td>Sustained sedimentation, can affect a predator’s ability to forage, the survival of bottom settling eggs (salmonids) and reduce biologic productivity.</td>
<td>Stress, Sediment is a physical, chemical and biological component. Sediment loads to aquatic systems are altered by multiple human activities.</td>
</tr>
<tr>
<td><strong>Phosphorus</strong></td>
<td>Phosphorous is a limiting nutrient which stimulates phytoplankton and algae growth. Excess amounts of phosphorous cause significant disruption to lakes via eutrophication.</td>
<td>Increased phosphorus increases food resources however it can also produce low oxygen conditions, which may affect population viability over time. Lake trout populations in Ontario are</td>
<td>Stress, Phosphorus is a biologically important chemical component that is altered by human activity. Agricultural nutrients</td>
</tr>
<tr>
<td><strong>Nitrogen</strong></td>
<td>Nitrogen is an essential nutrient for aquatic productivity. Often not a limiting nutrient, algae and phytoplankton require the presence of nitrogen to propagate.</td>
<td>The link between top predators and nitrogen is not consistent, however nitrogen plays a pivotal role in nutrient dynamics, interacts with other stressors and has been associated with biodiversity loss(^71). <strong>Stressor.</strong> Nitrogen is a chemical component of the environment that is altered by human activity. Agricultural fertilizer contains high amounts of biologically available nitrogen.</td>
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<tr>
<td><strong>Zebra and Quagga Mussels</strong></td>
<td>Zebra and quagga mussels are pervasive invaders of the LGL, altering ecosystem nutrient dynamics and species abundances(^72,73).</td>
<td>Zebra and quagga mussels compromise the food resources available to pelagic top predators. They shift significant amounts of biomass out of the pelagic-profundal zone, into the littoral benthic energy pathways(^74). <strong>Stressor.</strong> Zebra and Quagga mussels are biologic components that alter habitats and have been introduced through human activity. Zebra mussels were transported by the shipping industry from the Caspian Sea.</td>
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<tr>
<td><strong>Round Gobies</strong></td>
<td>Round gobies are an invasive species in the LGL that have significant impacts on native biota, from top predators through the consumption of eggs, to benthic invertebrates through foraging(^75,76).</td>
<td>Round gobies affect top predators by potentially hindering recruitment(^75) altering food web structure(^77) and providing a conduit for contaminant uptake which would affect the liver and growth of individuals(^78). <strong>Stressor.</strong> Round gobies are biologic components that alter food web structure and have been introduced through Human activity. Round gobies were transported by the shipping industry from the Caspian Sea.</td>
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<tr>
<td><strong>Sea Lamprey</strong></td>
<td>Sea lamprey first invaded the upper Great Lakes in the 1940s. Following their introduction a decline in lake trout populations was attributed to their parasitism(^79).</td>
<td>Sea lamprey directly cause mortality to top predators through parasitism and indirectly through subsequent infection. Individuals that survive following parasitism from the sea lamprey will likely have some loss to their energetic reserves. This can manifest in changes to liver weight, body weight or fat reserves(^80). <strong>Stressor.</strong> Sea lamprey are biologic components that negatively affect native species and have been introduced to Lake Erie, Michigan, Huron and Superior through Human activity. Sea lamprey invaded the upper Great Lakes following the construction of the Welland Canal.</td>
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<tr>
<td><strong>Phragmites</strong></td>
<td>Phragmites is an invasive common reed that alters wetlands that feed into the Great Lakes.</td>
<td>Phragmites effect on top predators is not clearly linked, however dense aggregations of phragmites can crowd out native plants, inhibit animal movement and serve as poor quality food for animals.</td>
<td><strong>Stressor</strong> Phragmites is a biologic component that alters nearshore habitats and has been introduced through human activity.</td>
</tr>
<tr>
<td>---------------</td>
<td>---------------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td><strong>Commercial Fishing</strong></td>
<td>Commercial fishing or netting large proportions of fish has detrimental effects to population sizes and overfishing can extirpate species.</td>
<td>Lake trout and salmon are not directly fished commercially in the Great Lakes, however there is still significant harvest of these species while fishing for pelagic forage fish like lake herring and bloopers.</td>
<td><strong>Human activity</strong> Commercial fishing is a human activity that drives associated stressors which may cause change to physical, chemical or biologic components of the environment.</td>
</tr>
<tr>
<td><strong>Charter Fishing</strong></td>
<td>Charter fishing can increase the fishing pressure on specific stocks of fish.</td>
<td>Charter fishing has a smaller impact than commercial fishing however the cumulative effect of all fishing pressure drives the severity of impact to fish stocks.</td>
<td><strong>Human activity</strong> Charter fishing is a human activity that drives associated stressors which may cause changes to physical, chemical or biologic components of the environment.</td>
</tr>
<tr>
<td><strong>Stocking Natives</strong></td>
<td>Native fish stocking, the addition of extirpated individuals to rehabilitate populations is a conservation tool used by environmental managers.</td>
<td>Lake trout stocking has positive and negative impacts on native naturally reproducing lake trout. Stocked fish compete for resources and spawning sites.</td>
<td><strong>Stressor</strong> Stocked fish are biologic components of the environment, that impact food web structure. Fish stocking is caused by human activity.</td>
</tr>
<tr>
<td><strong>Stocking Non-Natives</strong></td>
<td>Non-native stocking for improving the fishery through improving populations of desirable fish species such as salmon is a tool used by environmental managers.</td>
<td>Salmon compete with native species such as lake trout and walleye for resources, and compete with river species like brook trout for spawning habitat.</td>
<td><strong>Stressor</strong> Non-native stocked fish are biologic components that compete with native species and impact food web structure. Fish stocking is caused by human activity.</td>
</tr>
<tr>
<td><strong>Aquaculture</strong></td>
<td>Aquaculture and specifically open water net pen aquaculture can affect water quality, introduce fecal waste and resources from feed pellets into the surrounding environment.</td>
<td>Aquaculture can have positive and negative effects on top predators. Eutrophication can occur, which will impact the top predators. Resources from aquaculture facilities can also assimilate into food webs improving food resources for top predators.</td>
<td><strong>Human activity</strong> There are multiple stressors associated with the process of farming fish.</td>
</tr>
<tr>
<td><strong>Roads</strong></td>
<td>Roads near water bodies have various effects from increased run-off, altered</td>
<td>Roads contribute to multiple stressors that have various effects on top predators. For</td>
<td><strong>Human activity</strong> Roads near waterbodies drive</td>
</tr>
<tr>
<td><strong>Developments</strong></td>
<td>Hydrologic regimes, restriction to fish passage, increased access for fishing and the deposition of gasoline products from road traffic.</td>
<td>Example see sediments, lights, recreational fishing.</td>
<td>Associated stressors which may cause change to physical, chemical or biologic components of the environment.</td>
</tr>
<tr>
<td>------------------</td>
<td>-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td><strong>Mining</strong></td>
<td>Developments, specifically urban centers, alter natural habitats, disrupt hydrologic flow and run-off, and modify energy flow and nutrient cycles.</td>
<td>Development contributes to multiple stressors that have differential effects on top predators. See lights, sediments, PCBs, warming.</td>
<td><strong>Human activity.</strong> Developments drive associated stressors which may cause change to physical, chemical or biologic components of the environment.</td>
</tr>
<tr>
<td><strong>Power Plants</strong></td>
<td>Mining effects the environment through the generation of greenhouse gases, sediment additions to waterbodies and contamination of water.</td>
<td>Mining for metals such as copper and gold use mercury during the extraction process, refer to Copper, Mercury. Sedimentation during digging and transport can also have effects on top predators see sediments.</td>
<td><strong>Human activity.</strong> Mining drives associated stressors which may cause change to physical, chemical or biologic components of the environment.</td>
</tr>
<tr>
<td><strong>Shipping</strong></td>
<td>Power plants, and in the context of the Great Lakes specifically nuclear power plants, have an effect on the thermal properties of nearby water as well as direct mortality due to the impingement of fish.</td>
<td>Impingement is a concern for top predators as the intake pipes for water will cause direct mortality to fish swimming near power plants. For thermal effects see warming.</td>
<td><strong>Human activity.</strong> Power plants drive associated stressors which may cause change to physical, chemical or biologic components of the environment.</td>
</tr>
<tr>
<td><strong>Marinas, Ports and Harbours</strong></td>
<td>Lake trout are indirectly affected by wave action. Waves can increase sedimentation, nutrients and chemical suspension. Lake trout are also affected by gasoline products.</td>
<td>Boat traffic and maintenance activities can increase sedimentation and gasoline pollution.</td>
<td><strong>Human activity.</strong> Marinas, ports and harbours drive associated stressors which may cause change to physical, chemical or biologic components of the environment.</td>
</tr>
<tr>
<td><strong>Hardening</strong></td>
<td>Shoreline hardening can alter sediment dynamics and accelerate erosion.</td>
<td>For shoreline hardening effects see sediments.</td>
<td><strong>Human activity.</strong> Hardening of nearshore surface drives stressors which may cause change to physical and chemical components of the environment.</td>
</tr>
<tr>
<td>Recreation</td>
<td>Beach usage can increase potential pollution from waste.</td>
<td>Garbage from recreational beach usage can have an effect on fish in the area. Also can provide access to recreation fishing.</td>
<td>Human activity</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Lights</td>
<td>Artificial light alters the natural illumination periods in ecosystems.</td>
<td>Many fish are attracted to lights which can alter their behaviour at night. Artificial lights may also disrupt predator-prey relationships.</td>
<td>Stressor</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>Hypoxia or low dissolved oxygen concentration in water is caused by the decomposition of organic matter. While hypoxic zones occur naturally, anthropogenic nutrients increase the occurrence and distribution of hypoxic events.</td>
<td>Hypoxic conditions effectively reduce the habitable zone for fish. Large hypoxic events can cause substantial fish mortality.</td>
<td>Environmental effect</td>
</tr>
<tr>
<td>Warming</td>
<td>Climate change drives increase surface water temperatures beyond natural variation. This can cause an early onset of summer thermocline, increased thermocline depth and duration.</td>
<td>Temperature constraints of fish limit their ability to navigate warm waters for long periods of time. Increased temperature reduces the habitat available to cold, cool and in extreme cases warm water fish.</td>
<td>Environmental effect</td>
</tr>
<tr>
<td>Reduced Winter Ice Cover</td>
<td>Reduced winter ice cover is another effect of climate change. Increased temperatures can reduce the number of ice cover days.</td>
<td>Ice phenology in the Great Lakes is changing. The interaction of warming, ice cover, evaporation and water level drive temperature conditions for fish that modify behaviour, growth and survival.</td>
<td>Environmental effect</td>
</tr>
<tr>
<td>Changing Water Levels</td>
<td>Water levels are controlled by evaporation, precipitation, inflow and discharge of water to the water body. Changes to precipitation patterns and evaporation rates can greatly affect water levels of large lake systems.</td>
<td>Reductions in water levels can reduce habitat availability for top predators, however in the Great Lakes the size of these waterbodies will likely mean that small changes in water level will not significantly reduce habitat.</td>
<td>Environmental effect</td>
</tr>
</tbody>
</table>
Figure 1.1 Theoretical relationships between human activities (HA), stressors (St) and environmental effects (EE) adapted from Clarke Murray et al. (2014). Human activity can act independently on a stressor which produces an environmental effect. Multiple activities can contribute to a stressor. A human activity can produce multiple stressors. A single stressor can have multiple effects on the environment.
Figure 1.2 Application of the pathways between human activities (white boxes), stressors (light grey boxes) and environmental effects (dark grey boxes). Cumulative anthropogenic stressors from Allan et al. (2013) applied to in this study are bolded and underlined. The line colours correspond to the pathways from human activity to environmental effect.
2 Investigating the Effects of Anthropogenic Stressors on an Aquatic Top Predator, Lake Trout (*Salvelinus namaycush*)

2.2 Introduction

Environments worldwide are being altered by human activities.\(^{45,95,96}\) The aggregation of the individual and interactive effects of multiple stressors produces a cumulative effect, which can be potentially greater than the sum of individual impacts.\(^9\) To mitigate the effects of cumulative anthropogenic stress (CAS), assessments of specific ecosystem components are proposed to capture past, present and future human impacts.\(^{97}\) This makes cumulative effects assessment (CEA) inherently complicated,\(^{14}\) as it aims to address all of the uncertainty that is not embraced in current environmental assessment approaches.\(^{25}\) To aid in the assessment of CAS it is important to establish multifaceted assessments that incorporate and compare a breadth of impacts.

Understanding and monitoring the cumulative effects of anthropogenic stress is difficult for environmental managers to effectively measure.\(^{16,98,99}\) CEA highlights the idea that the effect of an individual influence may be minor, but collectively multiple stressors can become significant over space and time.\(^{16}\) So while the mitigation of global problems like climate change and biodiversity loss are important,\(^{29,100}\) combating these issues should not be carried out at the expense of more regional effects from human activities. To manage these effects, it has been proposed that more robust evaluations should include regional environmental assessments that are based on ecological knowledge.\(^{101,102}\) While a regional approach to environmental management is considered most effective,\(^{98,103}\) the refinement of regional assessment techniques for CEA has been slow.\(^{104}\) The reason for this is two-fold. First, the quantification of human activities and stressors requires significant effort.\(^{26}\) Second, there is no consensus regarding a standard set of environmental components that should be protected.\(^{105}\)
To observe how environments respond to CAS, information on the distribution and intensity of human activities and stressors is necessary. Human activities are defined as actions or deeds that people do or cause to happen, and their associated stressors are the physical, chemical or biologic components that interact with the environment. Alterations in the environment are considered significant if they exceed the natural range of variation, based on historic or well-defined reference site conditions. Modelling approaches to CAS have increased and there are some notable examples of mapping techniques in marine and freshwater studies of anthropogenic stress. Mapping models attempt to quantify the intensity of different human influences and do so over global and regional scales. Aquatic systems are especially susceptible to cumulative effects as they naturally accumulate and distribute contaminants, toxins, heavy metals and other compounds, and are used for a variety of human purposes. Allan et al. (2013) produced a CAS map for all the Laurentian Great Lakes (LGL). Contained in the map are 34 human activities and stressors that affect the LGL, ranked on a impact scale from 0 to 1. This provides the information required to assess biologic effects of CAS based on the distribution and intensity of stressors presented by Allan et al. (2013). The biologic and socioeconomic value of protecting and restoring the LGL is denoted by large bi-national programs, such as the Great Lakes Water Quality Agreement and the Great Lakes Fishery Commission. This makes the LGL a suitable system for developing indicators of cumulative effects.

Within the LGL, Georgian Bay of Lake Huron, has a gradient of CAS from low to high (Figure 2.1). Lake Huron and Georgian Bay are also susceptible to substantial alterations that can be exacerbated by anthropogenic stressors. It has been proposed that an ecological regime shift is occurring in Lake Huron and that changes to the prey base of top predators is affecting the structure and stability of fish populations. In order to capture these effects of CAS, effective indicators should integrate stressors over space and time, like the bioaccumulation of
contaminants, whilst responding to prevalent changes in the ecosystem like shifts in food web structure.

To develop assessments of the effects associated with anthropogenic stress gradients, indicators of the environmental characteristics being affected are required. In the LGL, top predators, such as lake trout (*Salvelinus namaycush*), are large, mobile and are under the influence of multiple stressors over an extended lifetime\textsuperscript{110,111}. By consuming a diversity of prey items and responding to all elements of a lake ecosystem\textsuperscript{112}, lake trout are affected by gross changes in food web structure and prey species condition. The historical ubiquity and abundance of lake trout in the LGL, indicates that it is a highly successful native top predator. Currently lake trout populations are supported by supplemental stocking\textsuperscript{113}. Restoring the historical population status of lake trout relies on the reversal or reduction of anthropogenic alterations to the natural habitat\textsuperscript{113}. While lake trout are reported to be responding to anthropogenic stress at a population level, they may be responding at an individual level as well. For example, bioaccumulation and biomagnification of contaminants such as mercury and polychlorinated biphenyls (PCBs) are well documented in lake trout\textsuperscript{65,114}, and have adverse effects on their liver and brain\textsuperscript{60,67}. The human consumption guidelines for lake trout are updated every two years which shows how responsive these organisms are to changes in contaminant concentrations. Lake trout have also been shown to respond to environmental gradients such as temperature, lake size, lake depth and nutrient profiles\textsuperscript{69,115,116}. The sensitivity of lake trout to changes in the environment are important attributes of an effective model organism to study CAS in the LGL.

Freshwater fish condition and physiology are broadly accepted as indicators of contamination and can potentially serve as powerful measures of ecosystem health and cumulative stress\textsuperscript{51}. Fishes, especially top predators, integrate direct contaminant effects, indirect bottom-up effects on the biota and gross changes to habitat quality or availability\textsuperscript{51}.
These indicators can effectively monitor the detrimental effects of human activities on the environment. Fish surveys in Canada have been used to effectively monitor the effects of pulp and paper as well as mining operations. Significant changes in the liver weight, gonad weight and growth of fish across effluent gradients are examples of how these biologic indicators can respond to stressors. By using a suite of indicators like relative weight, hepatosomatic index (HSI), relative brain size and stable isotope analysis, multiple effects of anthropogenic stress can be monitored simultaneously. Decreases in the relative weight of fish are indicative of a lack of resources to sustain optimal somatic growth or significant levels of contamination leading to growth impairment. Increased values of the HSI have been shown in response to contamination from mercury, copper, polycyclic aromatic hydrocarbons and PCBs. In response to CAS a combination of these compounds would also likely increase the HSI of lake trout. Relative brain size of individuals has been linked to habitat complexity and energetic constraints. A shift in energy availability and habitat complexity in the LGLs would reduce the capacity of lake trout to maintain large relative brain sizes. Smaller brain sizes thus being a potential indicator of energetic impairment and reduced habitat or food web complexity. Stable isotope analysis of top predator tissues can provide evidence for the impact of invasive species and determine changes to energy availability of specific habitats within lakes. Together all of these indicators can respond to a breadth of different anthropogenic stressors and potentially act as a multi-meter for multiple effects.

Utilizing the CAS map by Allan et al. (2013) I investigate if there are consequences of exposure to cumulative stressors in lake trout (relative weight, HSI, relative brain weight, degree of littoral coupling and trophic position) of Georgian Bay. Georgian Bay has a gradient of CAS, and has lake trout populations with different restoration statuses. First, I establish if there are differences in the lake trout biologic indicators among sites of Georgian Bay. I predict that the CAS scores of each site will associate with the magnitude of effects measured in biologic
indicators. To test this prediction, I will examine differences in each biologic indicator with respect to site, which represents CAS scores. Second, I hypothesize that specific human activities and stressors will have proportionally different effects on the lake trout biologic indicators. I predict that some human activities and stressors will have a greater proportional effect on biologic indicators than others. To test this prediction, I will examine the relationships between specific human activities, stressors and lake trout biologic indicators of stress across sites using a Canonical Correlation Analysis (CCA). By investigating these relationships, findings can potentially inform restoration projects in the LGLs and improve the monitoring of CAS.

2.3 Methods

2.3.1 GLEAM stressor data

To establish a gradient of CAS, I utilized the GLEAM stressor map layers (https://portal.glos.us/#). CAS scores were reported in 1 km² pixels and ranged from values of 0 to 1 based on estimated CAS level. First an average from the closest 50 pixels to a sampling location were used to determine the CAS score of a site. This CAS score represents 34 different human activity and stressor layers. For the second part of the analysis I used the individual layers. Human activity and stressor layers were designated based on the classifications from the prelude (Table 1.1). Of the 34 layers, 32 were available online. To classify the scores for each layer, 50 pixels (1 km²) closest to the sampling site were used. Some layers like “Road Density” only contained pixels closest to the shoreline. For these layers 30 closest pixels (1 km²) were recorded.

2.3.2 Collection

Lake trout were collected in August of 2016 and 2017 in Owen Sound, Collingwood, and Parry Sound (Figure 1) under MNRF permits UGLMU2017-05 and UGLMU2018-03. In June of 2017, 5 sites were assessed which included Owen Sound, Collingwood, and Parry Sound as
well as Tobermory and Dyers Bay, Ontario (Figure 1). Lake trout were collected by gill netting where 50-meter multi-panel gill nets (mesh sizes 36, 51, 64, 76, 89, 102, 114, 127mm) were set for approximately 12-hour intervals. Lake trout were also collected by angling and donation from charter and recreational fishermen. The time between collections was minimized to reduce seasonal effects\textsuperscript{132}. *Ephemeroptera* (Mayflies), *Gastropoda* (Freshwater snails), *Dreissenids* and *Unionids* (invasive and native mussels) were also collected from each site to obtain baseline estimates of food web properties. Littoral invertebrates (mayflies and snails) were collected off nearshore rocks and vegetation. Mussels were collected by diving or using an Ekman grab for retrieving benthic substrate. All procedures for animal handling followed the University of Guelph Animal Utilization Protocol 3563.

### 2.3.3 Processing

For each lake trout the total length (mm) and weight (grams; 50 lbs Berkley Digital Scale, Pure Fishing Inc., Columbia, SC, USA) were recorded. Lake trout were then dissected to obtain dorsal caudal muscle tissue, liver, stomach and gonads. Muscle tissue was inspected to ensure no bone or skin remained in a sample. The liver was removed from the abdomen and the gall bladder was drained. To remove the stomach, two cuts were made, one at the oesophagus and one at the pyloric sphincter. Gonads were snipped at the anterior end of the abdomen and pulled to the posterior cloaca. All muscle and organ samples were frozen at -20 \textdegree C following dissection. The spinal cord was severed between the first and second vertebrae and a vertical cut through the braincase towards the medulla oblongata allowed brain fixation after immersion in 10\% buffered formalin. Invertebrate collections were kept on ice until frozen at -20 \textdegree C for transport back to the University of Guelph.

### 2.3.4 Laboratory

In the laboratory, gonads and livers were thawed and weighed on a Texas Instruments top-loading electronic scale (Texas Instruments, Dallas, TX, USA) to the nearest 0.1 g. To
obtain brain weight, brains were dissected from fixed lake trout heads and trimmed to remove cranial nerves before being placed back into formalin (10% buffered). Each brain was then blotted dry using Kimwipes (Kimberly-Clark, Roswell, GA, USA) before weighing on a Accu-124D scale (Fisher Scientific, Waltham, MA, USA) at a resolution of 0.001 g. Weights for total stomach contents and weights of individual prey categories were recorded to the nearest 0.01 g. Prey categories included rainbow smelt (*Osmerus mordax*), alewife (*Alosa pseudoharengus*), round goby (*Neogobius melanostomus*), lake trout, cisco (*Coregonus artedi*), zebra mussels (*Dreissena polymorpha*), tapeworms (*Platyhelminthes*), macroinvertebrates and unidentified digested fish.

Frozen muscle tissue and invertebrate samples were thawed and dried for 24-36 hours at 60˚C. Once fully dry, the samples were ground to a fine powder and sent to the Stable Isotope Laboratory at the University of Windsor Ontario for stable isotope analysis. Stable isotopes measurements are a ratio of the sample compared to a standard. This isotopic ratio, reported as δX, is calculated by:

\[
\delta X = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 10^3 \text{ }^{133}
\]

where X is the heavy stable isotope, and R is the ratio of the heavy isotope to the common isotope (\(^{13}C/^{12}C, ^{15}N/^{14}N\)).

### 2.3.5 Indicator Calculation

Lake trout biologic indicators used for this analysis included the relative weight, HSI, relative brain size, while foraging ecology indicators included trophic position and degree of littoral coupling. Relative weight for lake trout among sites was compared by first establishing the length-weight relationship for all fish from all sites. Total length (mm) and standard weight (g) were used for this relationship.
Standard Weight = Wet Weight (g) – Gonad Weight (g) – Stomach Contents (g)

Residuals from the linear relationship were then calculated for each individual. Positive residuals indicating that an individual lake trout weighs more compared the average lake trout collected, and negative values indicating an individual weighs less.

The hepatosomatic index (HSI) was calculated using:

\[
\frac{\text{Liver Weight (g)}}{\text{Wet Weight (g)} - \text{Liver Weight (g)} - \text{Gonad Weight (g)} - \text{Stomach Contents (g)}} \times 100\%
\]

Relative brain for lake trout among sites was also compared by first establishing the length-weight relationship for all fish from all sites. Residuals from log-log relationship between brain weight (g) and standard weight (g) were then calculated for each individual. Positive residuals indicating that an individual lake trout brain weighs more compared the average lake trout brain collected, and negative values indicating an individual’s brain is relatively lighter.

Stable carbon isotope ratios can be used to delineate the origin of prey in the diet because baselines in the littoral and pelagic zones of the lake differentiate based on their carbon isotope signatures and exhibit little (<1‰) δ¹³C enrichment with transfer along the food web. Stable isotopes of δ¹³C were used to acquire measures of proportion of littoral carbon in the diet for each individual lake trout¹¹⁶,¹³⁴. The calculation for proportion of littoral carbon for an individual is:

\[
\text{Proportion Littoral Carbon} = \frac{\delta^{13}C_{\text{predator}} - \delta^{13}C_{\text{pelagic baseline}}}{\delta^{13}C_{\text{littoral baseline}} - \delta^{13}C_{\text{pelagic baseline}}}
\]

Here δ¹³C_{\text{predator}}, δ¹³C_{\text{pelagic baseline}} and δ¹³C_{\text{littoral baseline}} are the carbon signatures of lake trout, zebra mussels and littoral macroinvertebrates, respectively. The scale of this equation ranges from 0 – 1, where values closer to 0 indicate greater use of pelagic carbon sources and values approaching 1 indicate greater use of littoral carbon sources¹³⁴. The
proportion of littoral carbon values were compared to determine if lake trout relied differently on nearshore zone prey between sites.

In contrast to carbon isotopes, nitrogen isotopes typically become enriched 3-4‰ relative to diet\textsuperscript{134}. Trophic position can be defined as the location of an individual organism relative to other species within a food web \textsuperscript{57}. Trophic position can be used to infer the total length of the food chain if assessing the top predator in that ecosystem \textsuperscript{57}. The calculation of trophic position for each individual is:

\[
\text{Trophic Position} = \frac{\delta^{15}N_{\text{predator}} - \delta^{15}N_{\text{pelagic baseline}}}{3.4} + 2
\]

Some exceptions to this process included the carbon and nitrogen values for zebra mussels and macroinvertebrates in Collingwood and Tobermory, the values of which were considerably more variable than all other sites and specifically enriched in nitrogen. This did not correspond with the signatures found in lake trout in Collingwood and Tobermory and produced non-normal values. Therefore, nitrogen values for zebra mussels and carbon values for macroinvertebrates from Owen Sound and Dyers Bay were used as these values corresponded closely with values from other studies\textsuperscript{135,136}.

Stomach contents of lake trout were expressed using frequency of occurrence (%F) to represent prey composition\textsuperscript{2}. The equation used for %F is defined as:

\[
\%F_i = \frac{F_i}{F_{ni}} \times 100\%
\]

where %F\textsubscript{i} is the percentage of occurrence of prey item \textit{i}, \textit{F}_i is number of guts containing prey item \textit{i}, and \textit{F}_{ni} is the total number of guts examined.
2.3.6 Statistical Analysis

A series of analysis of variance tests (ANOVAs) were performed in R (Version 3.4.3) to compare the five lake trout biologic indicators (Relative weight, HSI, relative brain weight, percent littoral coupling and trophic position; dependent variables) between sampling sites. Prior to performing the ANOVAs I looked for relationships between the biologic indicators and no significant relationships were found. The ANOVAs assessed relationships between indicators and sampling site (one-way ANOVA) as well as sex, month and year (independent variables; two-way ANOVAs). An interaction between independent variables was included in the model, if it was insignificant it was removed, and the model was re-run. If it was significant it was left in the model and plotted in an interaction-plot. If there was a significant ANOVA ($\alpha = 0.05$), a pairwise Tukey Honest Significance Test was run to see where the significance lies. Boxplots to show variation between groups were also plotted. Boxplots and interaction plots were used to show variation in significant explanatory variables.

Residuals from each analysis were reviewed via histograms and normal probability plots to ensure the assumptions of the ANOVAs were not violated. A Levene’s Test was used to verify heteroscedasticity, and a Shapiro-Wilk test was used to verify non-normality. If a model was violated, outliers were removed if they were beyond the 1.5x interquartile range of values within a site. No outliers resulted in changes to significance in models.

Canonical correlation analysis (CCA) was conducted to relate lake trout indicators to human activities and stressors. The CCA was done using the vegan package in R 3.4.3\(^{37}\). Lake trout biologic indicators and impact scores for each human activity or stressor were used as input data for the CCA ordination space. The distance between points is based on orthogonal axes generated from correlations between lake trout biologic indicators and impact scores. Each lake trout is a point in the ordination space defined by these two axes. An impact score from the GLEAM layers for each human activity or stressor from a specific site was randomly
assigned to each lake trout collected from that location. Vectors of each human activity and stressor were displayed in the ordination to understand the relationship between axes and the original stressor variables. The CCA was independently performed on the human activity vectors and stressor vectors. Conducting this analysis separately was necessary as human activities and stressors are often strongly correlated and therefore both groups should not be included in the same statistical modelling of effects. Significant vectors (p < 0.05) were determined by a Monte Carlo permutation test. Vectors which were significant were included in the plot, with the direction of the arrows indicating its correlation to the axes and the length of the arrow indicating the importance of the vectors to the ordination between the lake trout biologic indicators and the human activity/stressor variables. The output consists of (1) the total inertia between indicators and human activities or stressors, (2) the proportion of inertia constrained by the correlations between indicators and human activities or stressors, and (3) a CCA biplot of lake trout indicators with ellipses to help identify groups of lake trout by site and the human activities and stressors represented by vector arrows.

2.4 Results

2.4.1 GLEAM map layers

CAS varied between sites of Georgian Bay with Parry Sound have the lowest average CAS score of 0.56, followed by Tobermory, Dyers Bay, Owen Sound and Collingwood which had CAS scores of 0.60, 0.64, 0.89 and 0.95 respectively. As CAS is scaled from a value of 0 to 1, these sites represent a moderate gradient of CAS from 0.56 to 0.95. For the analysis of human activities and stressors, 20 of the 32 CAS layers showed variation across the sites of Georgian Bay included in this study. Drawing from the classification of human activities and stressors from Table 1.1, 10 of these CAS layers were classified as stressors and 10 were classified as human activities.
2.4.2 Relative weight

There was a strong linear relationship between log total length (mm) and standard weight (g) \((r^2 = 0.95, p<0.01)\) from which residuals were drawn to assess relative weight variation for lake trout of a given length. There was a significant effect of site on relative weight \((F = 3.32, p = 0.01)\), and trout of Parry Sound were significantly higher in relative weight than trout from Collingwood and Dyers Bay \((p<0.05, \text{ Figure 2.2})\). No significant interaction was identified between site and collection month, year, or sex for relative weight \((p>0.05)\). The non-significant interaction terms were removed from ANOVA models and no significant effect of month, year or sex was found \((p>0.05)\).

2.4.3 Hepatosomatic index

There was a significant effect of site on HSI \((F = 32.18, p < 0.01)\) and Parry Sound had lower HSI values than all other sites \((p < 0.01, \text{ Figure 2.2})\). Lake trout of Owen Sound had lower HSI values than that of Dyers Bay and Tobermory \((p < 0.05, \text{ Figure 2.2})\). There was no significant interaction between years and site \((p = 0.54)\), or sex and site \((p = 0.06)\), however there was a significant interaction effect between month sampled and site \((F = 4.26, p = 0.02, \text{ Figure 2.3})\). The non-significant interaction terms were removed from the ANOVA models and no significant effect of month or year was found \((p > 0.05)\). There was a significant relationship between sex and HSI values \((F = 12.00, p < 0.01)\). Males on average had HSI values 0.21\% lower than females and showed less variation \((\text{ Figure 2.4})\).

2.4.4 Relative brain weight

There was a strong linear relationship between brain weight \((g)\) and standard weight \((g)\) for lake trout of all sites \((r^2 = 0.72, p < 0.01)\). There was a significant effect of site on relative brain size \((F = 13.24, p < 0.01)\) and relative brain size of lake trout from Parry Sound was significantly greater than that of all other sites \((p < 0.01, \text{ Figure 2.2})\). No significant interaction was identified between site and collection month, year, or sex for relative brain size \((p>0.05)\).
The non-significant interaction terms were removed from ANOVA models and no significant effect of month or sex was found ($p > 0.05$). Year and relative brain size had a significant relationship, with relative brain sizes increasing slightly in 2017 ($F = 12.99, p < 0.01$, Figure 2.4).

### 2.4.5 Food web attributes

There was a significant effect of site on lake trout littoral coupling values ($F = 39.52, p < 0.01$). Owen Sound lake trout had littoral coupling values significantly less than that of Tobermory, Dyers Bay and Collingwood ($p < 0.01$), and Parry Sound was significantly less than all sites, including Owen Sound ($p < 0.01$, Figure 2.2). For littoral coupling values, sites sampled showed no significant interaction effects of month, year or sex ($p > 0.05$). The non-significant interaction terms were removed from ANOVA models and no significant effect of month, year or sex was found ($p > 0.05$). There was also a significant effect of site on trophic position ($F = 26.50, p < 0.01$) and Parry Sound lake trout had significantly greater trophic position than all other sites ($p < 0.01$, Figure 2.2). There was a significant interaction effect between site and month ($F = 13.03, p < 0.01$, Figure 2.3), and site and year ($F = 8.30, p < 0.01$, Figure 2.3). When non-significant interaction terms were removed from ANOVA models, there was no significant effect of month, year or sex ($p > 0.05$).

### 2.4.6 Canonical correlation analysis

The inertia of the CCA measures the proportion of total variation in lake trout biologic indicators explained by each axis which comprised of the stressors or human activities affecting each lake trout. For the CCA conducted with human activity scores the eigenvalues for axis 1 and 2 were $1.38 \times 10^{-2}$ and $7.49 \times 10^{-4}$, respectively, of a total inertia of $2.82 \times 10^{-2}$. Axis 1 explained $48.8\%$ of the variation. Axis 2 explained an additional $2.7\%$ of the variation in lake trout indicators. The biplot reveals that lake trout in sites with aquaculture differ from those in sites with commercial fishing and to a lesser extent recreational fishing and shoreline hardening (Figure 2.5). Ballast water invasion risk accounts for some of the variation constrained by axis 1.
Road density, shoreline extensions, dams, ports and marinas were not significantly correlated with lake trout biologic indicators ($p > 0.05$).

The CCA which included stressors described slightly more variation with eigenvalues for axis 1 and 2 being $1.41 \times 10^{-2}$ and $8.06 \times 10^{-4}$. Axis 1 explained 50.1% of the variation and axis 2 an additional 2.9%. The biplot shows that lake trout in sites with higher mercury and copper scores separate from sites with higher scores in lake trout stocking, non-native stocking and to a lesser extent sea lamprey, suspended sediment, PCBs and round gobies. Zebra and quagga mussels and density of nearshore lights did not have a significant effect in the model ($p > 0.05$). Both CCAs indicated a significant effect of the month in which the lake trout were sampled.

### 2.5 Discussion

The results of this study show that (1) there are significant differences in lake trout biologic indicators among sites in Georgian Bay, however these indicators did not directly associate to the magnitude of CAS. The pattern of effect on lake trout biotic indicators across sites did not support the first hypothesis, however it generates a new proposition that CAS potentially holds a threshold effect on biotic indicators. Parry Sound had the lowest CAS score of all sites and consistently separated from all other locations (Figure 2.2). To act upon this, adding sites with low CAS scores will provide the necessary information to address this question. The results also showed that (2) specific human activities and stressors have different proportional effects on the lake trout biologic indicators, which supported the second prediction. Both human activities and stressors separate along the first canonical axis of their respective CCAs, allowing the interpretation of their importance in regard to the lake trout biologic indicators. A particularly encouraging aspect of this analysis is that the results do not merely represent statistical relationships, but aid in ecological discussion of the potential cause-effect relationships between CAS and biotic indicators. This analysis is unique because its application
of multiple stressors or human activities in combination with biologic indicators demonstrating that these complex problems can be simplified to provide insight of CAS in Georgian Bay. Furthermore, this process can extend to lake trout in all of the LGLs, or be adapted to multiple indicator species.

Based on the lake trout biologic indicators and human activities used in this study, responses observed in lake trout were distinguished by the polarity of commercial fishing and aquaculture in the CCA (Figure 2.5). Given that the biologic indicators of lake trout in Parry Sound suggested healthier fish and feeding (Figure 2.2), the anthropogenic effects of aquaculture at its current intensity may not be detrimental to the persistence of lake trout populations in comparison to the effects of commercial fishing. Both aquaculture and commercial fishing serve to harvest fish biomass for the purpose of human consumption but do so in different ways. Aquaculture facilities, feed fish housed in net-pens and grow these individuals until they can be harvested and in Parry Sound these facilities raise rainbow trout (oncorhynchus mykiss). The negative impacts of this activity are related to the excess feed and animal waste that enters the surrounding water body, the potential for fish to escape, as well as operational contamination (operation of machinery and maintenance of cages). Investigations into the negative effects of aquaculture on freshwater river and lake ecosystems have generated various results. In small lakes, intensive aquaculture has been linked to eutrophication and reduced water quality\textsuperscript{83}. In rivers, native salmonid reproduction \textsuperscript{139} and local water quality \textsuperscript{82} were not found to be significantly affected. In small and large lakes, the excess feed and fecal waste was shown to assimilate into lake food webs and enhance the productivity of local wild biota, simultaneously reducing accumulation of sediment waste\textsuperscript{84,140}. Site specificity has been postulated to control these varied impacts of aquaculture and it has been suggested that deep (>25m) well circulated sites, do not exceed the benthic community capacity to decompose organic inputs\textsuperscript{141}. The Parry Sound aquaculture facility, for example is greater than
50 meters in depth and has well circulated water. The results from this study and others, therefore, suggests that management practices such as site selection and feeding regimes may determine the extent to which aquaculture operations have positive or negative effects on local biota.

In contrast to the effects of aquaculture, my results point to a negative effect of commercial fishing on lake trout in Lake Huron. Beyond the direct effects of commercial fishing, stressors associated with this activity such as the operation of fishing vessels and physical alterations caused by trawling or net setting have also been cited as having potential negative effects\textsuperscript{142,143}. The negative effects of overharvesting populations has been documented on global\textsuperscript{144,145} and regional scales \textsuperscript{146}. These effects of overharvesting are apparent in the LGL. In Lake Huron, prior to 1940, the annual commercial harvest of lake trout ranged from 1.8 to 2.7 million kg\textsuperscript{147}. In 2010, lake trout harvest was 0.28 million kg\textsuperscript{52} and recruitment has continued to fall below expected targets\textsuperscript{113}. Ongoing commercial harvests are detrimental to the restoration of lake trout populations and can be exemplified by the restoration of lake trout in Lake Superior. Following the closure of commercial fishing in Lake Superior in 1962, quotas for lake trout were not increased until 1971 and lake trout populations improved\textsuperscript{148}. A sharp increase in whitefish commercial fishing in the 70s, crashed the population again in the 80s due to bycatch of lake trout before restrictions were made in 1991\textsuperscript{148}. Today, restricted harvest of lake trout and multiple refuges maintain healthy self-sustaining populations with less than 120,000 lake trout stocked lake-wide\textsuperscript{53,149}. Commercial harvest of lake trout in Lake Huron have continued despite the requirement to stock over 2 million lake trout a year to support the populations\textsuperscript{149}. Only one population of lake trout is considered fully restored (Parry Sound) and it remains the only population with no commercial harvest\textsuperscript{113}. In order to allow this fishery to restore and commercial fishing to operate without detrimental impacts, supplementing current harvest with increased aquaculture could potentially alleviate stress on food webs in Georgian Bay.
The second ordination analysis represents the relationships between lake trout biologic indicators and stressors and was mainly characterized by differences in the presence of invasive species, as well as stocked non-native and native (lake trout) individuals (Figure 2.6). This suggests that invasive species, such as the round goby (*Neogobius melanostomus*) and sea lamprey (*Petromyzon marinus*) have marked effects on the entire food web. However, these effects are not solely caused by unintentionally introduced species. Stocked species (non-natives) such as chinook salmon (*Onchorhynchus tshawytscha*) and potentially even stocked lake trout (natives) could be having an effect on native lake trout populations in Georgian Bay (Figure 2.6). Stocked species are often overlooked as potential causes of stress, especially when stocking is intended to improve populations of sport fish. However, there are negative effects of competing predators, and the presence of too many salmonids has created a bioenergetic bottleneck to the success of lake trout and salmonids in Lake Huron. It has also been hypothesized that non-native salmonids are having a disproportionate effect on prey fish populations. Lake trout are a well adapted cold water predator that can maintain lower metabolic rates and consume prey at lower rates than other predators. Chinook salmon by comparison have been found to consume 4 times more prey than lake trout, and can dramatically reduce prey abundance and impact other predators. The cessation of stocking all salmonids can allow natural populations to reproduce and has been documented in some cases to not significantly change the population size. Low prey populations in Lake Huron and Georgian Bay may be linked to the overabundance of top predators. The findings of the present study support the reduction of stocking lake trout and other salmonids to help reduce CAS in Georgian Bay.

Invasive species play a vital role in the reshaping of food webs, and have drastic effects on biodiversity by degrading various ecosystem properties. Invasive species are prevalent in the Great Lakes, and the CCA results support the hypothesis that invasive
species are having negative effects on lake trout in Georgian Bay (Figure 2.6). Sea lamprey preferentially parasitize lake trout and are one of the major factors that contributed to the initial decline in Great Lakes lake trout populations\textsuperscript{79}. Efforts to control sea lamprey populations began in the 1950s and have been ongoing ever since\textsuperscript{53,160}. Another invader, zebra mussels, have significantly altered the biogeochemical cycling of nutrients in the Great Lakes\textsuperscript{74}. In Lake Huron this has been exemplified in Saginaw Bay where the offshore transport of phosphorous was reduced by 60% following their invasion\textsuperscript{73}. Species invasions have displaced many native benthic invertebrates\textsuperscript{162} and this loss of food resources has transferred through the food web\textsuperscript{72}. The round goby has become a integral part of this new food web, directly preying upon zebra mussels and the eggs of other fish, while at the same time becoming an important component of lake trout diets\textsuperscript{163}. The effects of invasive species have various implications on the Georgian Bay ecosystem and is discussed further below in the context of each set of lake trout biologic indicators.

The ability to discern effects of CAS, can be further informed by considering each lake trout biologic indicator individually. Relative brain size is considered to be a function of habitat complexity\textsuperscript{164}. Fish, unlike other vertebrates, are able to make adjustments to relative brain size because fish exhibit life-long neurogenesis\textsuperscript{165}. This plasticity in brain size is also not constrained by brain cavity size as most fish have considerably smaller brains than the space that is available\textsuperscript{166}. Lake trout of Parry Sound have been shown to have different relative brain sizes in response to their habitat use flexibility\textsuperscript{167}. This suggests that lake trout of Parry Sound have access to the energetic resources necessary to change their relative brain sizes if required. In this study, looking across sites in Georgian Bay, individuals in Parry Sound utilized littoral habitats less than individuals from southern Georgian Bay (all other sites) and maintained significantly larger brains (Figure 2.2). While the pelagic zone is generally not considered a complex environment compared to the littoral zone, this could be a response to the pelagic
forage fish community diversity. In Parry Sound, the pelagic community includes healthy populations of alewife, smelt, cisco and whitefish which have all been frequently captured in the region and some of this increased pelagic prey diversity can be gleaned from stomach contents (Appendix 1). In a recent review of prey fish of Lake Huron and Georgian Bay, excluding Parry Sound, rigorous surveys yielded no alewife, low smelt abundance and decreased cisco biomass\textsuperscript{155}. While lake trout of southern Georgian Bay may utilize the more complex littoral habitat, the number of prey choices in the pelagic habitat is lower. Lake trout are a cold water adapted species and while utilizing the littoral habitat is important, it is energetically costly when water temperatures exceed tolerance thresholds\textsuperscript{168}. An explanatory hypothesis for my results is that a lack of prey within the thermal preference range of lake trout in southern Georgian Bay limits their energetic resources and in turn, affects their capacity to maintain energetically expensive tissues such as large brain sizes. Furthermore, the pelagic forage base for lake trout in Parry Sound is defensibly more diverse, which creates a more complex pelagic environment.

Stable isotope results support the hypothesis that lake trout of southern Georgian Bay have less access to pelagic forage fish, as individuals have lower trophic position and increased littoral coupling values on average (Figure 2.2). Trophic position of an individual is linked to the length of the food chain\textsuperscript{57} and in aquatic systems the pelagic food chain is longer than the littoral one\textsuperscript{169}. Thus the increased trophic position of individuals in Parry Sound is linked to their use of food resources in the longer pelagic food chain\textsuperscript{169}. Littoral coupling values calculated from the carbon isotope signatures corroborate that lake trout of Parry Sound consistently derive less carbon from littoral sources than locations in southern Georgian Bay (Figure 2.2). A major underlying mechanism for the occurrence of increased littoral dependence in southern Georgian Bay is the presence of invasive species. Filter feeding invasive zebra mussels have significantly reduced the transport of these nutrients to offshore zones\textsuperscript{73} diverting the nutrients to benthic energy pathways nearshore\textsuperscript{74,170}. A second invader, the round goby, which feeds on benthic resources and directly on zebra mussels\textsuperscript{76}, provides the conduit for incorporating these
resources into the muscle tissue of lake trout. In Parry Sound, round goby was not found to be a constituent of lake trout diets, which contrasted the occurrence of round goby in lake trout diets from other sites of Georgian Bay (Appendix 1). Together the effects of these invasive species create nearshore resource subsidies for lake trout through a shorter food chain (Figure 2.7). This effect not only decreases the trophic position but would also explain the increased reliance on nearshore resources. The deposition of littoral nutrients to the benthic environment, makes the δ\textsuperscript{13}C of round goby resemble that of the nearshore organisms\textsuperscript{171}. While the round goby may be accessible to lake trout at greater depths than common nearshore fishes, the round goby is providing the same littoral resource. Similar shifts in response to the round goby have been documented in Lake Ontario\textsuperscript{172}, and recent estimates in Lake Huron show increased reliance of lake trout on round goby as a diet source and even suggest that other species like rainbow smelt have become supplemental\textsuperscript{163,171}. This aligns with findings of the ordination analysis, indicating that invasive species are a major stressor to lake trout.

Lake trout of southern Georgian Bay face constraints to growth and success due to energy availability, but also may be impaired by increased energy expenditure. Increased requirements for detoxification of food sources and potentially other contaminants not directly measured in this analysis (ex. PAHs, dioxins) encountered by lake trout tax the energy budget of individuals. In a study of lake trout from Isle Royal, Lake Superior, a relatively pristine location, HSI values for male lean lake trout (the same morphotype of lake trout in Georgian Bay) ranged between 0.75 and 1.5\textsuperscript{173} remarkably similar to that of lake trout from Parry Sound (Figure 2.2). This indicates that the HSI values of lake trout in southern Georgian Bay (sites other than Parry Sound) are outside the range of normal values for lean lake trout. The CCA results show that PCBs were significantly different across sites and lower in Parry Sound where lake trout were found to have smaller livers. The significant difference in liver sizes between sites could also be influenced by invasive species. The invasive species pathway mentioned earlier (Figure 2.7) potentially explains the increased PCB contamination of lake trout in
southern Georgian Bay. Zebra mussels introduce contaminants to food webs, by incorporating them into muscle tissue and shell as well as changing water chemistry around them\textsuperscript{174}. Zebra mussels can excrete some accumulated contaminants in pseudofeces around the shell margins\textsuperscript{175}. When round gobies consume zebra mussels they ingest accumulated and deposited contaminants, creating a new pathway for contaminants to enter the food web \textsuperscript{78}. The increased proportion of round goby in the diet of lake trout from southern Georgian Bay is potentially increasing rates of bioaccumulation and associated stress on lake trout. This invasive species pathway is a good example of how multiple stressors can act cumulatively: zebra mussels and round gobies act as a biologic stressor, displacing native biota\textsuperscript{172}, and mobilize chemicals that may have been sequestered in sediments before invasive species colonization. Together this group of stressors is changing the food resources available, the source of energy, and the quality and the contamination level in the food available. These effects are expressed in lake trout through their utilization of littoral resources, their reduction in relative body weight and brain weight, as well as their increase in relative liver weight.

In this study, I aimed to empirically test multiple indicators of stress and foraging behaviour in lake trout that could potentially respond to a gradient of CAS. I found that, lake trout biologic indicators responded more sensitively to stressors than to human activities (Figure 2.5, Figure 2.6). Of the 10 human activities used to model lake trout biologic indicators only 5 had statistically significant correlations with the indicators, in contrast to the stressors which had 8 of 10 significant correlations with the indicators. This makes sense from the pathway's framework presented in chapter one as stressors are the biologic entities that directly interact with the environment (Figure 1.1). The work highlighted in the prelude led to the assessment of proportional effects on biologic indictors. By investigating the specific human activities and stressors involved in this CAS framework, it provides vital knowledge on how to address these complex multi-stressor problems.
Improving the resolution of this study could be accomplished through the assessment of more sites with different groups of stressors, or by selecting additional indicators. Additional indicators could be the assessment of cortisol in the bloodstream to investigate if stress-hormone levels vary across a gradient of CAS, histology of liver tissues to elucidate why liver sizes are different in lake trout and the assessment of brain lobe sizes to further understand why brain sizes in lake trout varied across sites. Fatty acids have also been shown to be an effective diet indicator that can pinpoint specific food sources. The suite of indicators that was used in this study were able to detect some of the effects of CAS in populations of lake trout. These biologic indicators are likely sensitive enough to pick up environmental differences before the effects are extremely detrimental as this study shows that differences in lake trout can be detected along a moderate gradient of CAS. While the refinement of methods to assess CAS should be ongoing, presented here are effective indicators that can be utilized in future studies.

The main insight that this study affords environmental managers is that aquaculture and commercial fishing stand as opposing human activities. To sustain the commercial sale of Great Lakes fish, a balance between commercial harvest and aquaculture operations may reduce the CAS on Georgian Bay food webs. Furthermore, invasive species and stocked fish also pose significant risk to native lake trout populations, suggesting that biotic interactions are currently of greater concern than chemical contamination in Georgian Bay. This is a promising perspective as environmental managers have the power to directly control some of these issues (stocking, aquaculture and commercial fishing) in Georgian Bay.
### 2.6 Tables and Figures

Table 2.1 ANOVA output from models examining the relationship between lake trout biologic indicators and sampling site.

<table>
<thead>
<tr>
<th>Lake trout biologic indicators</th>
<th>N</th>
<th>Sum of Squares</th>
<th>Residuals</th>
<th>P</th>
</tr>
</thead>
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<tr>
<td>Relative Weight</td>
<td>147</td>
<td>$1.42 \times 10^{-2}$</td>
<td>$7.73 \times 10^{-2}$</td>
<td>$1.44 \times 10^{-4}$</td>
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<tr>
<td>Hepatosomatic Index</td>
<td>139</td>
<td>20.8</td>
<td>13.7</td>
<td>$2.00 \times 10^{-16}$</td>
</tr>
<tr>
<td>Relative Brain Weight</td>
<td>115</td>
<td>0.382</td>
<td>0.872</td>
<td>$3.67 \times 10^{-8}$</td>
</tr>
<tr>
<td>Littoral Coupling</td>
<td>146</td>
<td>2.58</td>
<td>2.28</td>
<td>$2.00 \times 10^{-16}$</td>
</tr>
<tr>
<td>Trophic Position</td>
<td>140</td>
<td>2.68</td>
<td>2.22</td>
<td>$2.00 \times 10^{-16}$</td>
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</tbody>
</table>
Figure 2.1 Location of sampling sites in Georgian Bay, Ontario, Canada. The bottom map is the output of the cumulative anthropogenic stressor map, from which each of the 34 layers were characterized for analysis. Regions in red indicate the highest cumulative stress and regions in blue represent the lowest cumulative stress.
Figure 2.2 Box and whisker plots showing differences between sites for relative weight residuals, hepatosomatic index (HSI), relative brain weight residuals, proportion littoral coupling and trophic position for lake trout of Georgian Bay, during 2016 and 2017. Lake trout are grouped by site: Parry Sound (PS), Tobermory (TB), Dyers Bay (DB), Owen Sound (OWS) and Collingwood (CW). Each box represents the 25th and 75th percentile and whiskers represent the highest and lowest values within the 1.5x interquartile range. Dots are outliers. Means are represented by the bolded center line and boxes labeled with different letters indicate they are statistically different.
Figure 2.3 Plots showing significant interactions identified in two-way ANOVA models. The top plot shows the interaction between month and sampling site for the hepatosomatic index (HSI). The middle plot shows the interaction between month and sampling site for trophic position. The bottom plot shows the interaction between sampling year and site for trophic position. Lake trout sampled from Collingwood (CW) are shown in red, Owen Sound (OWS) in orange and Parry Sound (PS) in blue.
Figure 2.4 Box and whisker plots showing significant differences between hepatosomatic index (HSI) values and the sex of the lake trout (top plot), and between relative brain size and the sampling year (bottom plot). Significance was identified in a two-way ANOVA. Results represent lake trout from all sampling sites across all sampling years. Whiskers represent the highest and lowest values within the 1.5× interquartile range of the 25th and 75th percentiles.
Figure 2.5 A biplot resulting from a CCA using lake trout biologic indicators. CCA1, the main axis explaining variation in a cumulative measure of indicators, is illustrated. The combined biologic indicator is based on relative body weight, hepatosomatic index (HSI), relative brain weight, littoral coupling and trophic position. Each dot represents the ordination location of each lake trout based on their respective combined biologic indicator CCA value. The ellipsoids represent the 95% confidence interval for site (colour differences). Grey vector arrows denote the human activities which significantly ($p < 0.05$) described variation in the lake trout combined biologic indicator. The shape of each dot indicates sampling month, which had a significant relationship with lake trout biologic indicators.
Figure 2.6 A biplot resulting from a CCA using lake trout biologic indicators. CCA1, the main axis explaining variation in a cumulative measure of indicators, is illustrated. The combined biologic indicator is based on relative body weight, hepatosomatic index (HSI), relative brain weight, littoral coupling and trophic position. Each dot represents the ordination location of each lake trout based on their respective combined biologic indicator CCA value. The ellipsoids represent the 95% confidence interval for site (colour differences). Grey vector arrows denote the stressors which significantly ($p < 0.05$) described variation in the lake trout combined biologic indicator. The shape of each dot indicates sampling month, which had a significant relationship with lake trout biologic indicators.
Figure 2.7 Schematic of simplified food chains with respect to the potential alterations by invasive species. On the left is the impacted food chain, where basal resources are filtered by zebra and quagga mussels, which are consumed by round goby and incorporated into the top predator. On the right is an unimpacted pelagic food chain that comprises multiple trophic steps from phytoplankton to zooplankton before being consumed by pelagic forage fish and incorporated into the top predator.
2.8 References


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2.7 Appendix

Appendix 1. Dietary composition by frequency of occurrence (F%) calculated for each prey category between sample sites and collection dates. Round goby is bolded to show presence in all sites with the exception of Parry Sound.

<table>
<thead>
<tr>
<th>Site</th>
<th>Collection Date</th>
<th>Number of Stomachs</th>
<th>Prey</th>
<th>%F</th>
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</thead>
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<tr>
<td>Collingwood</td>
<td>August 2016</td>
<td>10</td>
<td>Round Goby</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Tapeworm</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Unknown Fish</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Empty</td>
<td>50</td>
</tr>
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<td>August 2016</td>
<td>11</td>
<td>Cisco</td>
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<td></td>
<td></td>
<td></td>
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<tr>
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<td></td>
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