Evaluating the current aquatic invasive species (AIS) treatment methods and exploring different restoration tools that could aid in ecosystem recovery in freshwater ecosystems of Nova Scotia

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Abstract

Invasive fish species in Atlantic Canada present threats to freshwater ecosystems by outcompeting and predating native organisms, resulting in a shift in biodiversity. This could also lead to social impacts, especially for recreational fishers and other users that benefit from the natural resources that are now altered by aquatic invasive species (AIS). As a result of the damage caused by invasive species, some organizations (government and private) have used different types of invasive species management strategies for maximizing the removal of these unwanted fish populations such as scientific angling, electrofishing, and the installation of fish barriers and traps. In practise the methods above are effective in suppressing AIS populations, but almost never succeed in full eradication. This is where managers sometimes turn to rotenone, a piscicide that has proven to be effective in AIS eradication but is more damaging to nontargeted organisms. The objectives of this study are to evaluate common treatment methods used to suppress or eradicate AIS species in Nova Scotian lakes, by analysing its primary function, operational costs, time to implement, and disadvantages (treatment limitation and/or ecosystem impacts). In addition, different restoration frameworks will also be reviewed for its potential in recovering the ecosystem from any damages caused from treatment application. Other existing management tools that do not directly assist in AIS removal or ecosystem restoration but help facilitate management options will also be explored. Lastly a management guide will be constructed, based on the previous objectives, to assist managers in decision making for dealing with future AIS invasions.

Key words: aquatic invasive species, control, eradication, species at risk, restoration, ecosystem recovery, biological indicator species, framework

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Chapter 1: Introduction

1.1 Context of Nova Scotian freshwater ecosystems

Lakes, rivers, streams, wetlands, and underground aquifers are all examples of different types of waterbodies that share a common, yet fundamental, feature among each other in that they are freshwater ecosystems. Freshwater ecosystems only make up approximately 0.01% of Earth's entire surface area, yet they contribute significantly to both biodiversity and to society (UNEP-WCMC, 2022). Specifically, freshwater ecosystems provide critical habitat for close to a third of all known vertebrate species on this planet and provide habitat for about 40% of known fish species (UNEP-WCMC, 2022). From a human resource perspective, these ecosystems provide various services that includes freshwater supply, energy, cultural identity, tourism and recreational activities, and food resources as well.

In Nova Scotia there are 6,700 lakes and other fresh waterbodies (streams, rivers, wetlands...etc.) in the province, and the Halifax Regional Municipality (HRM) county making up a sixth of the province's lake composition, with approximately 1000 lakes in the area (Lakes and Rivers, 2021; A survey of the sportfishing industry in Nova Scotia, 2010). As briefly described above, freshwater ecosystems play an important role in maintaining both biodiversity and human needs and Nova Scotia is no exception. Nova Scotia's freshwater ecosystems are rich in biodiversity where these waterbodies contain many sportfish species such as speckled trout, rainbow trout, brown trout, brook trout, landlocked salmon, yellow perch, chain pickerel, and smallmouth bass that is managed by the government of Nova Scotia. Other sport species includes striped bass, shad, smelt, American eel, and Atlantic salmon (not landlocked) that is managed federally due to diadromous nature of these species (A survey of the sportfishing industry in Nova Scotia, 2010). In a social significance standpoint, Nova Scotian lakes provide many human valued services which includes swimming, snorkeling, canoeing (both recreational and competitive races found in Banook Lake) and kayaking, boating (unique boating activities includes the competitive Pumpkin Regatta Festival-Windsor, N.S) (Banook Canoe Club, n.d.; Johnson, 2022). Other common activities that occur near lakes includes hiking/backpacking, camping, and other recreational activities on beaches (not all lakes have sandy shores) (Halifax Regional Municipality, 2021). However, the largest social activity that is also dependent on the aquatic biodiversity in Nova Scotia's freshwater ecosystems is recreational fishing/angling. Specifically, in 2021, over 79,000 Fishing Licences were sold, the highest it has ever been in decades (the high number of licences maybe due to the pandemic restrictions) (Craig, 2022). Overall, Nova Scotia's recreational fishing industry generates over $66 million per year that goes towards the province's economy (Craig, 2022). It is an inclusive recreational activity where beginners and experienced sport anglers are welcome alike which gives those an opportunity to explore new sites and challenge themselves with catches of various species (Craig, 2022). The Nova Scotia Department of Fisheries and Aquaculture had also acknowledged this local passion for this outdoors activity in the recreational fishing communities and have legally introduced a new highly active species of fish called smallmouth bass into several freshwater lakes in the past. However, the last point has also proven to be quite problematic for many decades as some recreational fishers who are looking for additional "thrills/challenges" in new locations have been known to illegally introduce smallmouth bass as well as chain pickerel into ecosystems (Nova Scotia Invasive Species Council, 2022; Fisheries and Oceans Canada, 2021). As a result of the introductions with these aquatic invasive species (AIS), numerous ecosystems have changed significantly, from loss of native fish biodiversity and abundance, due to the predatory dominance and competitiveness of both these fish species (Nova Scotia Invasive Species Council, 2022; Fisheries and Oceans Canada, 2021).

1.2 Biological synopsis and history of AIS fish species

Esox niger (chain pickerel) is a solitary freshwater fish species, distinguished by its chain-like pattern and slender body with an average adult size of up to three feet (smallest member of the Esox genus) (NS Invasive Species Council, 2022). Chain pickerel and other members of the Esox genus (ex: northern pike and muskellunge) are natural ambush predators, where they prefer habitats with slow/motionless aquatic ecosystems with underwater foliage (ex: lily pads, twigs, leaves…etc.) to act as temporary camouflage until they are ready to strike (Discover boating, 2022). Chain pickerel is native to the Atlantic and Gulf Coast tributaries that was first introduced illegally in Nova Scotia by anglers in 1945 who were attracted to this species as a sportfish due to its energetic behaviour once caught on-line (NS Invasive Species Council, 2022). Since its first introduction, chain pickerel has been introduced to 166 confirmed locations across Nova Scotia (last confirmed introduction at Nixtaux River in 2020) (Lowles, 2021).

Micropterus dolomieu (smallmouth bass) is a freshwater fish species belonging to the sunfish family. They are generally physically characterized for having a robust dark greenish brown to a bronze-coloured body, black vertical bars on the side, and a white ventral side (Brown et al., 2009). Smallmouth bass also have retractable spiny dorsal fins, like other species under the Perciformes order and their average size ranges between 23-38cm (NS Invasive Species Council, 2022). Smallmouth bass can often be confused with largemouth bass due to both species belonging to the same genus, however the jaw of smallmouth bass ends directly underneath their eye, whereas largemouth jaws extend past the eye (Fisheries and Oceans Canada, 2021). Smallmouth bass, in comparison to chain pickerel, prefer cool shallow areas of freshwater ecosystems with sandy and rocky bottoms which are ideal nesting areas for this species of fish and where adult males can sometimes be found guarding their nests. (NS Invasive Species Council, 2022; Coastal Action, 2020). Smallmouth bass' original distribution (native) in Canada was confined to the St Lawrence River system and most of the Great Lakes (Lake Eerie, Lake Huron, Lake Michigan, and Lake Ontario) except for Lake Superior. First authorized introductions of this fish species occurred in 1942 at Bunkers Lake (Yarmouth County), in which there were 12 more legal stockings in the province with the last in 1984, currently there are 327 confirmed lakes that have this fish species (last confirmed introduction in 2020) (NS Invasive Species Council, 2022; Lowles, 2021). The rest of lakes that had no official stocking record were most likely introduced due to illegal transfers by recreational fishers due to the general popularity of smallmouth bass, and accidental usage of live bait (Fisheries and Oceans Canada, 2021).

1.3 Biological and social impacts from the introduction of these fish species

Both chain pickerel and smallmouth bass are viewed as problematic (invasive) species due to both species becoming top predators once introduced into a previously undisturbed ecosystem and having a non-selective diet (Brown et al., 2009; NS Invasive Species Council, 2022). Their prey includes but are not limited to other fish species (including smaller members of the same species), crayfish, frogs, newts, turtle hatchlings, aquatic insects and other macroinvertebrates (NS Invasive Species Council, 2022). They also feed on species that are of social importance and at risk that includes salmon smolts, and Atlantic Whitefish (endemic to Nova Scotia). As a part of their life cycle, salmon smolts are being targeted by pickerel as they are migrating to the open ocean (passage through LaHave River) in which one pickerel was reported to have digested four smolts (Withers, 2017). Atlantic whitefish is endemic to only a few lakes (all in the Petite Riviere watershed) in Nova Scotia and is limited in numbers due to infrastructure development, acid rain, and predation from top predators such as chain pickerel and smallmouth bass (Withers, 2018). Chain pickerel currently have been recorded to be present in two (Hebb and Milipsigate) and smallmouth bass in three out of the four known lakes to host Atlantic whitefish (Hebb, Milipsigate and Minamkeak), which are all part of the Petite Rivere watershed (Fisheries and Oceans Canada, 2018).

Another aquatic species that has also been affected by the presence/introduction of these species is *Lampsilis cariosa* (yellow lampmussel). This bivalve species is endemic to a few areas in North America and only a couple of waterbodies in Nova Scotia, where they currently reside in the Sydney River of Cape Breton County (Environment and Climate Change Canada, 2017). Like many other bivalve species, the yellow lampmussel filters water from various debris and potential toxins (natural nutrient sinks), which helped improve the water quality for local communities near the Sydney River. The other significance of these mussels is that they developed a dependent parasitic life cycle with the native host fish (I.e., white perch) species in which these bivalves would spread its larvae by using its mantle as a fish lure to attract the fish and the larvae would attach to fish host (Environment and Climate Change Canada, 2017; Nova Scotia Department of Natural Resources and Renewables, 2022). However, the introduction of chain pickerel and smallmouth bass in these waterbodies pose an indirect threat to the survival of these remaining mussel population due to the predation and competition on fish host species from these AIS (Environment and Climate Change Canada, 2017).

In summary, aquatic ecosystems that have chain pickerel and/or smallmouth bass present, typically have less species richness and overall trophic stability compared to other aquatic ecosystems due to intense competition and predation (Mitchell, 2012).

1.4 Treatment methods currently employed to manage fish species

Due to the social and biological impacts that chain pickerel and smallmouth bass have on Nova Scotia's freshwater ecosystems, several organizations (both government and NGOs) have introduced different measures to treat watersheds invaded with these problematic fish species. Essentially, Nova Scotia practices two main types of AIS treatment methods that each have distinct management goals, where one treatment objective is to reduce/supress the undesired fish population. This AIS treatment goal aims to ensure that remaining numbers are in a level that does not significantly impact the rest of the ecosystem and its native biodiversity. Specifically, Coastal Action's (an environmental NGO that operates in Mahone Bay, N.S) main conservation projects focus on sustaining the remaining Atlantic whitefish populations that are endemic to the Petite Riviere watershed (Russell et al., 2022). In order to achieve this objective, Coastal Action's primary goal is to control/supress invasive species that are predating and outcompeting Atlantic whitefish through use of scientific-angling, electrofishing (both backpack and boat), deployment of larval light traps, and a rotary screw trap (RST) (Russell et al., 2022).

The second AIS management objective that has been accepted and practised in this province is the complete eradication of the invasive species population. However, this treatment method often leads to "non-desirable" impacts to rest of the ecosystem due to its nonspecific target of species in the habitat. Restoration follow-up procedures may need to be established to aid in ecosystem recovery if manager decide to use this type of treatment. An example of this practised treatment method was by the Department of Nova Scotia Fisheries and Aquaculture in which they aimed to completely eradicate all traces of smallmouth bass in Piper Lake in 2020. Smallmouth bass were first detected in this lake by biologists in 2019, where they suspected that this was a result of illegal translocation by recreational anglers (Withers, 2020). There was a high incentive to remove all the smallmouth bass that was located there due to the potential damages it could cause to the residing American eel population which is a culturally important species for the First Nation community and other native fish species (yellow perch, brown bullhead, creek chub, banded killifish, white sucker, and shiner species) (Lowles, 2020). The other reason for this ambitious management objective was to prevent further smallmouth bass spread into the Saint Mary's River watershed (Piper Lake directly flows into this watershed), which is an important habitat for salmonids such as trout species and Atlantic salmon (population is already at risk from water acidifications) (Lung, 2021; Withers, 2020). Originally, the AIS treatment methods were comparable to those practised by Coastal Action which included: angling, netting, boat electrofishing and dewatering the lake (before the winter freeze) (Withers, 2020). Despite the removal efforts, there were still small numbers of smallmouth bass being caught/detected, which pushed the final management decision to apply Noxfish II, a piscicide with active rotenone, to eradicate the remaining population (Leblanc & Lowles, 2021). In order to minimize the ecological impacts from this treatment, as much native fish as possible (emphasis on American eels) were removed and transferred in live boxes before and after the piscicide application (the boxes were located upstream to the lake) (Lowles, 2021). All retained fish were released into the lake once it was determined that rotenone concentration was below 2.0 ppb (concentration levels in which the most sensitive fish can survive) (Leblanc & Lowles, 2021). The decrease of rotenone concentration in Piper Lake was determined by placing experimental fish in sentinel cages for 48 hours to assess survivability (Lowles, 2021). Since this lake is inhabited by different fish species with varying levels of sensitivity to this piscicide, several test fish species were used from the least to most sensitive (I.e., yellow perch-LC50 of 4.6 ppb, brook trout-LC50 of 2.35 ppb, and Atlantic salmon-LC50 of 1.75 ppb for 24 hours) (Lowles, 2021). After a species was able to survive for 48 hours, the next highest tolerant species was placed in the cage. During this time, stop logs were added to the lake's outlets to prevent traces of rotenone entering the nearby watersheds such as the St. Mary's River and were removed once concentration levels were below 2.0 ppb (Lowles, 2021). Additional monitoring continued from May-June by the inland fisheries of 2021 to ensure there was no remaining smallmouth bass populations that survived the treatment (Lowles, 2021). All fish removal methods turned up no smallmouth bass, thus the rotenone application was a success (Leblanc & Lowles, 2021).

1.5 Current problems and knowledge gaps

Despite reaching the objectives in Piper Lake, in which rotenone was able to eradicate all of the AIS in the ecosystem and thus preventing further smallmouth bass spread into new and potentially more sensitive habitats, there is still missing links to this management project. One of the first problems of this study was that there was limited information on the initial removal methods of the smallmouth bass populations. Specific examples include the number of AIS removed with each method, exact size distributions of AIS caught from each treatment type, the time it took to remove the number of fish with each treatment type, and the number of bycatches with each treatment method (Lowles, 2023). However, values such as the total smallmouth bass caught with recorded size classes such as adult and young of year (YOY), and total time spent (hours and seconds) for each removal method was highlighted in the reports prior to rotenone treatment (Lowles, 2023). In addition, none of the physical removal methods used (I.e., minnow traps, scientific angling, boat and backpack electrofishing) inflicted any mortality nor significant physical for the species removed (Lowles, 2023). Furthermore, Coastal Action had limited data on the potential damages their removal methods from the types of AIS treatment used as well.

The second main problem with the Piper Lake report is that any of the restoration tools (for supporting/facilitating ecosystem rehabilitation after rotenone treatment) were either not well developed or still lacking data due to the recent occurrence of this event. Specifically, the research and management team acknowledge that Piper Lake is well connected with other bodies of water, where connectivity was confirmed to already restored in 2022 after the stoplogs were removed and expect recolonization of all surveyed fish species to occur naturally as a result. However, the research and management team of this project is still developing a backup plan for reintroducing the species lost during the treatment, in case the organisms do not recolonize the lake with the connectivity of neighbouring ecosystems and during the projected time (Leblanc & Lowles, 2021; Lowles, 2023). This could change the overall ecosystem dynamics of Piper Lake if the aquatic organisms do not return to the area whether it be from natural recolonization and/or physical reintroductions from conservation efforts.

In addition, the pre- and post-treatment values for invertebrate and planktonic communities were stated to be collected by McCallum Environmental Ltd to determine the recovery process of indicator species of the treated ecosystem (Lowles, 2021). Again, there was no mention as to how long the monitoring process took place after the application of rotenone. However, Enviosphere Consultants Ltd & Water Testing also conducted sampling procedures for both planktonic and invertebrate communities before and after 1 year of treatment and published the results of the monitoring surveys. To summarize the report, the lake was divided in 4 groups/monitoring areas, where group A had 54.85%, group B had 76.42%, group C 61.44%, and group D had a 50.92% average similarity between 2020 (pre-treatment assessment) and 2021 (post treatment assessment) invertebrate sampling. Enviosphere Consultants Ltd proposed that from these sampling results there was at least some acute similarity in invertebrate taxonomic diversity and abundancy levels between before and after 1 year of rotenone treatment in Piper Lake. However, it is imperative to also look at other studies that used rotenone and measured the same communities to determine if the one-year post treatment monitoring assessment is consistent throughout all studies, which could be applied to future rotenone management projects in other Nova Scotian lakes. Furthermore, Piper Lake is the only recently tested and completed project in Nova Scotia that involved rotenone treatment, and therefore there is nothing to compare it to in this province so other international studies will be used as comparison as well. In addition, other international studies will also be analyzed for monitoring sessions that took place earlier and/or more than 1 year after post rotenone treatment. The data collected could be used to determine whether future post treatment surveys can take place in a shorter time period or if the time after monitoring should be extended to get more significant results for biodiversity recovery.

Both studies (Coastal Action and Piper Lake) did not state any measures that they would employ in the future to better monitor and prevent the spread/establishment of AIS. Such examples include adopting either the Canadian Marine Invasive Screening Tool (CMIST) or the environmental DNA early detection monitoring method. The application of these methods/tools in this research paper will be used to form the steps for a management guideline for managers to use in ecosystems that are high risk and/or have already been invaded by AIS in Nova Scotia. Specifically, CMIST would be used as a means of focusing/prioritizing ecosystems that have no observations of invasive species at present but are at a high risk of being invaded and established by an AIS fish population. Ecosystems that have a higher potential for successful AIS invasions in the future would then be recommended to have periodic monitoring protocols with environmental DNA sampling to detect potential early stages of AIS invasion. This would allow managers to deal with the confirmed threat in a relatively short time period before the population can further increase and expand to other ecosystems than if these tools/methods were not used.

Lastly, there is no management guideline that currently exists in Nova Scotia to inform managers as to which management method, AIS control/suppression or eradication, that should be applied based on the circumstances of the ecosystem compared to other existing guidelines (RIPARIAS, n.d.; Invasive Species Council of Metro Vancouver, 2021).

1.6 Research objectives

In order to address the problems/knowledge gaps highlighted above, this research project will focus on three objectives to determine a possible solution. The first objective will be conducting literature review research and evaluating currently used methods for either controlling and/or eradicating AIS populations in Nova Scotia. The second objective of this project will be exploring “logical” ecosystem restoration concepts that could be used in aiding biodiversity recovery from any potential unintended damages/impacts as a result of the usage of AIS treatment. The last objective will be to develop a rough framework, based on the findings from the previous objectives, that could provide managers with a step-by-step guide for dealing with future fish AIS invasions in Nova Scotia.

Chapter 2: Methods

2.1 Literature search

2.1.1 Desktop research

In this research project, various search engines were used to get access and retrieve different types of information depending on the section of this paper, in which Google, Google Scholar, and Dalhousie University's online Novanet research data base were used as the main form of desktop research. General Google searches were used to get an idea of a certain topic in which more sophisticated search engines such as Google Scholar or Novanet online search were later used for further results. Additionally, any topics that did not require much research or was not a focal point for this research used Google search as the primary method for information extraction. Grey literature was used for this situation and this type of source was used extensively at the beginning of the introduction, especially for emphasizing themes such as the general recreational activities practised in Nova Scotian lakes and the general ecological/biological importance of lakes as well. Grey literature was also used where peer-reviewed literature was missing and/or limited for a certain topic in which online news articles were used that had extensive quoted dialogue of the interviewees. News article references were also used in explaining some of the events that occurred in the Piper Lake invasive species eradication project and the aquatic invasive species management project in Kejimkujik Park to restrict the expansion of both chain pickerel and smallmouth bass movement into critical habitats. The other scenario in which Google search was used exclusively in this research paper was to get access to publicly accessible project reports that were primarily sourced from Coastal Action, in which methods of trap and AIS treatment procedures were implemented in the targeted area.

As mentioned above, the other types of search engines used in acquiring sources and data was through both Google Scholar and Novanet online research database used in this research project in which peer reviewed literature was exclusively used from these data bases. Examples of key terms that were used in these databases to get access to the relevant literature for this study were: “aquatic invasive species management in Nova Scotia”, “chain pickerel and Kejimkujik Park”, “smallmouth bass and Kejimkujik Park”, “environmental DNA and aquatic invasive species”, “freshwater ecosystem restoration”, “fish reintroduction in Nova Scotia”, “rotenone application and ecological impacts”, “rotenone impacts on invertebrates”, “rotenone and ecological indicator species”, and “CMIST tool application in freshwater ecosystems”. Peer reviewed sources were primarily used in the results section of this paper, especially sources that had data which was necessary for conducting both my statistical analysis and literature review of the recovery rate of ecological indicator species after rotenone treatment in freshwater ecosystems. A more in-depth explanation of the statistical analysis and literature review process for the recovery of indicator species after rotenone treatment can be found under Methods in sub-section 2.2 *Data collection.*

2.1.2 Email inquiries

Despite the variety of sources and data used in this research, there were still some aspects of this study in which specific information was lacking. Specific examples included the financial costs of some of the traps and treatment methods as well as the deployment specifications from non-governmental organizations such as Coastal Action and the governmental Department of Nova Scotia Fisheries and Aquaculture. As such, inquiry emails were sent to both groups and all questions were ensured to be objective only and not related to personal opinions as this project did not get an ethics approval for interviews. Individuals from Coastal Action that had relevant knowledge and expertise that could provide support in my research were contacted via email in which their contact information was posted on the main Coastal Action website under *Species at Risk & Biodiversity Team.* Contact was made possible with the Department of Nova Scotia Fisheries and Aquaculture with the aid of my supervisor who had connections with an individual who worked there. Some of the questions asked to these groups and individuals did not have the exact answers and instead referred to another contact who had the relevant information and data, in which the same questions were sent to these new contacts. Each of contacts responded with a direct text answer and occasionally attached a PDF of a report/document with the information originally requested. All of the sent emails to the contacts have their associated subject titles (all email titles below are italicized) as well as the questions posted below with quotations. Due to privacy reasons, none of my contacts have their names listed and are instead mentioned with their pronouns.

Contact from the Department of Nova Scotia Fisheries and Aquaculture

1. *Fish species found in different lakes of Nova Scotia*

"… I am struggling to find different fish species found in selected areas of Nova Scotia. Essentially, I am looking for three lakes that have been introduced to AIS fish species (smallmouth, chain pickerel or both) and three "control" lakes (where the all the fish species are native and there are no records of AIS activity/presence) to do my study. If this is possible/achievable, could you please let me know where to find this or if I am allowed to get access to this type of information?"

*The next question was part of the same email thread that was sent as a reply after my contact answered the first inquiry:

"… I was wondering if I can get access to the data on Piper Lake, specifically for all the AIS management treatments and possible data of the lake biodiversity before and after each treatment.

2. *Rotenone inquiry*

"… I was wondering if know how much the cost is for rotenone (Noxfish ii) and who the supplier was for the Piper Lake treatment?"

*My contact did not have the exact answer to this question and suggested to contact Central Life Science who was the supplier of rotenone for this project. Refer to sub-section 2.1.2.2 *Zoecon Professional Products- Central Life Sciences* for the direct question sent to this contact.

3. *Last two questions about piper lake study*

*The second question was omitted as the information was provided in an attached document of the Piper Lake project that my contact sent me in the *Fish species found in different lakes of Nova Scotia* email:

"… I was wondering how long it took to prepare the rotenone solution and to disperse it throughout the lake…"

4. *Last bit of edits before library submission*

*The last series of questions pertained to the edits/confirmation that had to make under the *knowledge gap* section of this paper. Specifically, this was to validate that there was specific missing/incomplete data in the Piper Lake report. If incorrect assumptions were made based on my contact's responses, edits were made to ensure that this study was not discrediting/misinterpreting his work. Below is the copied email text that was sent to for additional clarification:

"Just wondering if Piper Lake study did this before rotenone treatment (I know that some of the data is still being made publicly available:

i. … Number of AIS removed with each capture method, size distributions of AIS caught from each treatment type, the time it took to remove the number of fish with each treatment type, and the number of bycatches with each treatment method as well as any noticeable damages to the ecosystem and technological disadvantages..."
ii. … I know that there is a plan to restock the lake with American eels (mentioned in both paper you sent me, thanks!) I was wondering what was the plan for other native fish species that were reportedly euthanized by rotenone treatment, is there a plan by NSDFA to reintroduce these species of fish?"
iii. …. there was also no publicly available data published as to what the pre-treatment and final post-treatment values were for the invertebrate and planktonic communities to make any assessment whether the ecosystem had made a natural recovery or not within the monitoring timeframe...

…I wanted to make sure that the above statement is true and I do not want to undermine you nor NSDFA. I know that a particular organization was hired to conduct invertebrate sampling before and after treatment, but there were no specifics on the quantitative and/or qualitative differences from before and after rotenone application nor time it took for the invertebrates to recover to pre-rotenone levels.

iv. Lastly, my supervisor mentioned that approximately 2 years was spent removing larger smallmouth bass individuals from Piper Lake before Rotenone application? Just confirming to make sure that is accurate."

Contact from Zoecon Professional Products- Central Life Sciences

1. *Price inquiry of rotenone*

"… I have been in contact with a fisheries biologist who works for the Nova Scotia Fisheries and Aquaculture department-(name of contact deleted to protect his identity). He purchased the rotenone solution from the company and suggested that I reach to you about the pricing. This is for my Masters project, where I'm looking at various AIS treatments used for controlling unwanted fish populations in Nova Scotia..."

First Contact from Coastal Action

1. *Passive AIS trap inquiry*

"… for the AIS traps that Coastal Action uses, rotary screw traps, I was wondering has to how much it costed to purchase and maintain if possible? I looked up the costs to purchase this type of trap however, I got no results from this."

*In addition to an in-text response, the first contact from Coastal Action also attached a brochure document from the manufacturer (E.G Solutions Inc.) that highlighted the specifications of the trap. This brochure also had the email address to directly contact the company, of which the next email inquiry was directed to E.G Solutions for more details on purchase costs back in 2009 and the cost to purchase one today. Refer to sub-section 2.1.2.5 *E.G Solutions Inc.* for the direct question sent to this contact.

Second contact from Coastal Action

1. *Light trap question*

"I was wondering if you know the approximate time it takes to deploy the larval light traps that Coastal Action owns, and how long it typically takes to go through the bottom petri-dish?"

*Continuing on with the email thread after my second contact's first response I followed up with other questions that came up which was related to the number of light traps deployed and how long the trap would be left out before they were collected:

"… how many of these traps were/are deployed (I'm assuming it depends on location and # of bass nest sites found), and how long would a trap be deployed before it was checked (I thought I read somewhere that it was 2 days but not sure) …"

2. *Some more questions about light trap and other AIS removal strategies*

i. "Has Coastal Action have/considered anchoring the light traps so that less time was spent looking for the traps if they floated away by chance?

ii. Has Coastal Action accidentally captured Atlantic Whitefish with these traps? If so, is there any risk of the invasive larval fish consuming the whitefish?

iii. if an Atlantic Whitefish is captured while using this AIS treatment, Coastal Action will cease all electrofishing activities and consult with DFO before resuming this type of AIS management method to prevent further potential injuries to native SAR

The above point I got this from the DFO registry, has an incident like this occurred where Atlantic Whitefish was accidently stunned from electrofishing? if so, when and which lake(s)?

iv. Are only vertebrates affected by electrofishing or other organisms such as invertebrates affected as well- has there been observational evidence of this occurring?"

E.G Solutions Inc.

1. *Rotary screw trap price*

"… I have been getting access to N.S Coastal Action's data on the effectiveness of their various traps that they have deployed so far in the Le Petite Rivere watershed. With this in mind, I was wondering if you could let me know what was the price of the entire RST when it was purchased, how many were purchased by Coastal Action…"

2.2 Data collections

One of the primary objectives of this research project was to determine the impacts (if any) on ecological indicator species that are commonly present in freshwater ecosystems that were treated with rotenone due to presence of chain pickerel and/or smallmouth bass. As there was limited time to conduct research for this project, only one ecological indicator group was used to measure ecosystem recovery which was done by analysing pre-treatment levels of EPT (*Ephemeroptera*-mayflies, *Plecoptera*-stoneflies, and *Trichoptera*-caddisflies) taxonomic diversity and abundance prior to rotenone and monitoring successive years for the same data. This was done in order to determine the rate of natural recovery/succession of these taxonomic groups after the implementation of rotenone. Members of the EPT taxa were chosen for this study as they are invertebrate species that are commonly used in scientific research as biological indicators to assess the relative health of the ecosystem, where they are often referred to being the most sensitive of the invertebrate taxa towards environmental changes/fluctuations (Pautasso & Fontaneto, 2008). Furthermore, macroinvertebrates in general are known to be fundamental in ecosystem and tropic stability as they are often classified as primary consumers, of which provide a significant portion of fish diet (Gnohossou et al., 2009).

In order to assess natural successive freshwater ecosystem recovery relative to time elapsed after the initial treatment, all compiled data was organized into four time-period ranges. This would aid in determining if there was a certain time range after treatment that EPT communities would be recovered to an extent that there would be no significant difference between pre- and post treatment levels of this ecological indicator group. The first and earliest post-treatment monitoring time range covered EPT community evaluations that took place before and until 1 year had elapsed of the initial rotenone treatment, the second time range covered more than 1 year and to a maximum of 3 years, the third spanned between 4 and 7 years, and the last time range covered EPT monitoring samples that took place after 8 years relative to the initial rotenone treatment of the ecosystem study site. EPT taxonomic diversity and abundancies were displayed separately to facilitate comparisons to any differences in the rate of recovery between both biological indicators (this led to a total of eight graphs being produced for the combined site time comparison). Each time range data was displayed with a bar graph that depicted the percent difference between pre-treatment levels and the lapsed time of post-treatment monitoring. The percent difference was calculated by dividing the post treatment numerical value with pre-treatment value of the same site and multiplying by 100 to get the percentage difference. Essentially, if the post treatment percentage values were less than 100%, it means that the taxa diversity or abundance values are less than the pre-treatment values in that particular year of monitoring. However, a value that is 100% means that post-treatment values are equivalent to pre-rotenone EPT values, and if the value is greater than 100% the post treatment values are greater than pre-treatment values. All bar graphs were created using Microsoft Excel version 2016 under the *Charts* category in which the histogram without the "curved line" was used. Additionally, an ANOVA test was used to determine the statistical significance between pre-and post-treatment EPT taxa and abundancy levels with respect to the time lapsed after the initial application of rotenone. Since the purpose of monitoring was to determine if EPT communities could eventually reach pre-treatment levels, accepting the null hypothesis would state that there is no significant difference between pre- and post-treatment and can be assumed the community has been restored. The acceptance of the alternative hypothesis could mean two things, where the community has not made a recovery that is close to pre-treatment levels or the number of EPT taxa and/or diversity has significantly increased past pre-treatment levels. A P-value of 0.05 was used to determine the significance of the results, in which values less than this value would be considered significant and the null hypothesis is rejected. Values that are greater than 0.05 would be considered insignificant and the null hypothesis would be accepted. All statistical tests in this research project were performed in Microsoft Excel version 2016, in which the ANOVA tests were completed in the *Data Analysis* heading under *Data*. Once in the *Data Analysis* heading, various statistical tools would appear in which *ANOVA: Single Factor* was selected for this study. It is also important to note that data groups were organized by columns **not** rows.

In order to obtain less biased results from potential unforeseen variables from specific study regions, extensive desktop research was conducted to obtain some studies that involved monitoring natural ecosystem restoration after rotenone treatment. A total of six international studies based in Montana, New Zealand, Norway, and Utah freshwater sites. Studies were only selected if they recorded the taxonomic diversity and/or abundancies of macroinvertebrates belonging to the EPT taxa group after rotenone application for the purposes of unwanted/invasive fish introductions. Also, it was essential that the studies recorded pre-treatment levels to get a sense of a baseline/threshold to evaluate the restoration success over time for a particular site. However, it is important to note that not all research study sites measured both taxonomic diversity and abundance levels of macroinvertebrates (and EPT taxa groups), in which the study site at Strawberry River-Utah (*Rotenone Effects on Aquatic Macroinvertebrates of the Strawberry River, Utah: A Five-Year Summary*) only analyzed changes in taxonomic diversity and study sites in New Zealand (*Rotenone treatment has a short-term effect on New Zealand stream macroinvertebrate communities*), and Norway (*Impacts of Piscicide-Induced Fish removal on Resource use and Trophic Diversity of Lake Invertebrates*) only analyzed changes in abundancies before and after rotenone application (Mangum & Madrigal, 1999; Pham et al., 2017; Eloranta et al., 2021). The research studies that monitored both EPT taxonomic diversity and abundancies were: Southwest Montana (*Piscicide impact extends beyond targets and toxicity*), Norway (*Effects of Three Consecutive Rotenone*

Treatments on the Benthic Macroinvertebrate Fauna of the River Ogna, Central Norway), and Montana (*Recovery of Freshwater Invertebrates in Alpine Lakes and Streams following Eradication of non-native Trout with Rotenone*) (Donnelly, 2018; Kjaerstad et al., 2015; Schnee et al., 2021). Due to 13 lakes and streams being analyzed in the Montana research, only four were chosen as it could have possibly created a study bias towards one research if all sites were used, which would have been disproportionate relative to other studies that focused only on one site. Additionally, the four sites, in contrast to the other nine, had monitoring stages that extended until 8 years which gave a larger monitoring time scale overall (Schnee et al., 2021). The study sites collectively had nonsynchronous post-treatment monitoring times with some studies having more frequent monitoring intervals and some of the graphs only showing data for a couple or even one site (ex: *Recovery of Freshwater Invertebrates in Alpine Lakes and Streams following Eradication of non-native Trout with Rotenone*) due to these studies conducting extended monitoring procedures (up to eight years) compared to other sites (Schnee et al., 2021).

2.3 Method description, time estimates, general cost assessments and potential limitations

Description

A general description of what the AIS treatment is, how it functions, and examples of real-life application of the treatment method applied from either NGOs (Coastal Action) or government sectors (Nova Scotia Fisheries and Aquaculture, and Kejimkujik National Park) from Nova Scotia. The number of AIS fish caught from the treatment by a particular organization was provided at the end of this section from. A brief summary of the treatment's application in Nova Scotia is added in the final paragraph of this sub-section.

Estimated time

This section aims to identify how long it takes to implement the specific treatment by the organization and how long the trap is left until contents are collected from the researcher. The average time to process the aquatic organisms caught in the trap is also considered in this part as well. Some organizations did not keep record for the length of time for some of the time related situations highlighted above, and some organizations instead used generalized terms to describe the length of time (i.e., processing stunned fish from electrofishing would be "instantaneous").

Cost assessments

The costs for most AIS treatment devices were determined by contacting suppliers of the traps through email and asking about the price for specific models that closely matches the trap description used by the referenced organization. There was a case in which a specific trap (i.e., rotary screw trap) was sold by a company a few years ago to the conservation group (Coastal Action), so the cost was adjusted for today's inflation in order to get the most up-to-date purchase price. In addition, there were some products that were sold outside of Canada (i.e., United States) so the original price was converted to Canadian currency. Lastly, only the price of the product is displayed in this section and does not consider shipping fees, taxes...etc.

Potential limitations

In this subsection, the disadvantages of the treatment methods are highlighted based on limitations of its capabilities (technical aspects), and ecological damages/impacts to native species that maybe bycaught in the treatment/trap. For eradication methods the same structure was followed as the control/suppression techniques except the potential damages towards commonly found freshwater ecosystem features was further analyzed. This is due to the nature of how this treatment works, where there is no species specificity (will impact AIS and non-targeted aquatic organisms that rely on gill respiration) for applying this management method. Various peer-reviewed studies were used that highlighted the impacts of rotenone treatment in the study sites. Organisms includes fish species from Piper Lake, invertebrate communities, amphibian life stages, planktonic species, and submerged plant species.

2.4 Literature review of CMIST, fish repatriation, eDNA, and CABIN ecosystem tools

These restoration concepts (Canadian Marine Invasive Screening Tool-CMIST, fish repatriation, environmental DNA-eDNA, and Canadian Aquatic Biomonitoring Network-CABIN) were chosen to be reviewed in this project for its previous and current usage in Canadian ecosystems. Due to the lack of data on these management tools, they were only described based on the guiding principles on how its functionality.

Chapter 3: Results

3.1 Control/suppression treatment methods

3.1.1 Physical barriers

Description

There are many types of physical barriers that can be used to prevent/reduce aquatic invasive species movement such as chain pickerel and smallmouth bass. Such barriers include weirs, culverts, rock gabions, velocity barriers, and exclusion screens with each barrier type hindering a specific fish movement (Jones et al., 2021). The design of weirs and culverts are designed to prevent the jumping/swimming/climbing ability of aquatic invasive species to limit upstream movement (Jones et al., 2021). Lipped weirs in Laurentian Great Lakes for example were able to stop sea lampreys from jumping over while jumping salmonids were able to overcome the barrier, but it was found that many native non-jumping species were blocked on the other side (Jones et al., 2021). Rock gabions function similarly to weirs and culverts where they prevent species with poor jumping capabilities from accessing the other side but instead have spaces in between the rocks that may facilitate access for smaller organisms (Loeza-Quintana., 2021). This could be considered as an advantage and disadvantage since it would allow for smaller native fish to access the other side that cannot jump high enough; however this would also facilitate access for smaller chain pickerel and smallmouth bass individuals especially larval stages. Velocity barriers are sloped weirs that create high velocity water currents in a concentrated area that could be useful against invasive species that are not able to effectively move against the current (Jones et al., 2021). The final barrier that will be discussed that has also been used extensively in the Kejimkujik National Park as part of the $797,000 CAD (funded in 2017) invasive control program is the usage of exclusion screens (comprised of mesh) and nets that are designed to stop large fish entry but may allow access for earlier life stages due to its permeable material (Withers, 2019; Loeza-Quintana et al., 2021). Parks Canada had recent success in preventing aquatic invasive species entry into the Peskowesk sub-watershed (comprises a third of Kejimkujik's wetland and is optimal habitat for native trout) such as pickerel and small mouth bass entry with the mesh barrier establishment (the establishment was determined by initial eDNA detections) (Withers, 2017; Loeza-Quintana et al., 2021).

Estimated time

The Kejimkujik barriers were originally implemented in 2018, but there is no mention of how long it took to fully set up (Withers, 2019). The length of time it takes managers to implement a mesh barrier depends on the size, depth, and any potential obstacles in the water passage. For example, a passage that is relatively narrow with shallow water depth should take less time to setup a mesh screen barrier as opposed to a water passage that is wider, deeper, and has more “obstacles”/debris in the way. Similar variables would also factor in the length of time it could take to conduct maintenance on the barrier if damaged as well as the extent of damage itself (I.E., a small tear in the mesh would take less time to fix than if an entire frame support collapsed).

Cost assessments

The cost of Parks Canada entire 2017 proposed Kejimkujik aquatic invasive management project, including the implementation of the mesh screen barriers, would have costed ~$932,880 CAD today ($797,000 in 2017) (Withers, 2017), yet no cost was found for the just the installation of the barrier. However, a few international AIS management studies that used small-scaled physical barriers as a means of containing/preventing the movement of AIS referenced the costs of implementing the barrier. Specifically in New Zealand, an exclusion barrier was installed to prevent carp entry, in which the implementation would have costed ~$4,953 CAD in the current year. Another study in Tumbling Creek (Missouri, U.S) created a physical barrier to prevent the movement of crayfish, which costed CAD $10,154 to implement in the waterbody including labour fees (Mouser et al., 2018). Although physical barriers tend to last for a relatively long time in the environment, regular maintenance (I.e., clearing of debris, checking structural integrity from the current pressure from upstream flow...etc.) is often required to ensure that this type of infrastructure is performing optimally (Daniel et al., 2014). In order to achieve this, consistent funding for maintenance and repairs must be achieved to prevent future failures of the barrier that could lead to unintended costs to both the screen and the ecosystem (full access for AIS with the barrier now damaged) (Clarkson, 2004; Jones et al., 2021).

Potential limitations

Since the main purpose of barriers is to prevent entry/escape of aquatic organisms, this could temporarily or permanently (depending on management decisions and goals) block invasive species movement into previously noninvaded areas with sensitive ecosystems. However, if managers have not taken account of all native aquatic species in the area or have bigger priorities (preventing AIS movement over native fish movement), this could also block access for anadromous or migratory aquatic species and potentially lead to ecosystem off-balance in the long term (COSEWIC, 2010).

In addition, depending on the gap size of the screen, the barrier will promote effective blockage for large-bodied fish, however smaller fish species or younger life stages may have a higher potential of bypassing the screen barrier if the mesh holes are big enough (Jones et al., 2021). This could be an issue as young life stages (I.e., young-of-year) of chain pickerel and smallmouth bass or other AIS that could pass through the screen and lead to an eventual invasion of the protected ecosystem. However, this type of barrier could also provide an opposite "positive" effect for juvenile native fish species, where the barriers could provide a sanctuary against adult AIS that cannot pass the mesh screen and enter the protected ecosystem.

3.1.2 Electrofishing (boat and backpack)

Description

Electrofishing is a type of fishing/extraction method that involves introducing a current into a particular area in the aquatic ecosystem that temporarily stuns all organisms within range of the electrical charge (Smith, 2019). Researchers then can quickly identify all the stunned fish floating on the surface and collect the targeted species for research by using a non-conductive gill-net and placing the AIS into built-in aerated live wells to keep them alive. The other fish that were not targeted for extraction typically recover in a relative short amount of time (Smith, 2019). The electrical currents released from the electrode arrays can be adjusted depending on the variables of the water quality and species targeted (Rytwinski et al., 2019). Specifically, aquatic ecosystems that are considered fresh or have low salinity concentrations would require less electrical intensity than compared to waterbodies that are brackish or salt water that are poor conductors of electricity and thus offer less coverage (Smith, 2019). In addition, the maximum current that most electrofishing boats use can only reach a water depth of approximately six feet (ideal for pond or shallow lake operations), where any fish below this range will be less likely to get stunned by the electrodes (Rytwinski et al., 2019). However, this does not seem to be an issue as most Nova Scotian lakes (660 measured) have an average depth of 2.8 ± 2.1 meters based on a mass survey conducted between 1964 and 1981 (Alexander et al., 1986). As such, these depth values are ideal parameters for electrofishing surveys as it allows for complete coverage of the water column. All lake data was collected from the Department of Fisheries and Oceans Canada, Nova Scotia Lands and Forests and the Canadian Wildlife Research Service in which 660 of the 781 lakes surveyed had average depths measured from various counties across Nova Scotia (Alexander et al., 1986). Parks Canada recently purchased an electric powered electrofishing craft that has been primarily used to remove invasive fish species such chain pickerel and smallmouth bass from ecologically important watersheds (Withers, 2019). Although Parks Canada has yet to publish the number of fish extracted from the watersheds, they intend to use the boat 40 times a year in both Mountain and Cobreille Lake (labelled as sanctuaries) to specifically target and permanently remove chain pickerel populations currently residing at those locations (Withers, 2019). Coastal Action actively uses both boat and backpack electrofishing (one of many removal methods they employ) to stun and remove these AIS fish species from the Petite Riviere watershed (Creaser, 2022).

Estimated time

Depends if there are any fish in the area and if there are any variables that may limit the potential of electrofishing capture such as presence of foliage and underwater vegetation (harder to stun fish in this type of habitat, depth of the water (electrical pulses offer less coverage compared to shallower water columns), and conductivity of the water (water with higher salt concentrations require a higher voltage of electricity to reach similar levels of range in freshwater environments) (Snyder, 2003). However, the capture time of fish species is immediate, where all the fish in range of the electrical current are stunned and float to the surface, in which researchers can identify which of the stunned fish species are invasive and remove them quickly with nets.

Cost assessments

The costs for renting an 18-foot heavy-duty Smith-Root electrofishing boat, including other gear/equipment and operator fees, would be ~$1,997.15 CAD ($1,500 USD) per day, if renting from FISHBIO. FISHBIO also provides Smith-Root LR-42 electrofisher backpacks for rent that cost ~$1,997.15 ($1,500 USD) per week (FISHBIO, 2022).

Potential limitations

Non-targeted aquatic organisms, as with fish AIS, that are within range of the electrofishing current have a potential chance of being temporarily stunned, but also may experience varying levels of damage to its body depending on the intensity of the electrical current. However, during Coastal Action’s electrofishing surveys, there has been no observations of aquatic organisms other than fish species such as invertebrates that have been affected from the activity (Russell, 2023). In terms of possible injuries that could occur to fish species within range of the electrodes, spinal injuries and hemorrhages were found in some studies to be present in more than half of the fish collected, however there are less occurrences of these types of injuries when using a low current (<30 Hz) (Snyder, 2003). Other potential injuries include gill bleeding, excessive physiological stress, and mortality. In addition to fish injuries, the electrical currents can also damage fish embryos if near spawning grounds (Snyder, 2003). Lastly, injuries to the boat crew and operator are possible if safety measures are being ignored or if the individuals lack training and certification (Smith, 2019). However, local conservation groups such as Coastal Action have taken precautions to minimise and/or prevent aquatic SAR bycatch from this AIS removal technique. Specifically, Coastal Action will cease all electrofishing activities and consult with DFO before resuming this type of AIS management method to prevent further potential injuries to native SAR (Government of Canada, 2018). The chances for Atlantic Whitefish to be stunned from electrofishing surveys are low since the *Species at Risk and Biodiversity* team under Coastal Action only conducts this activity in the summer and early fall near shallow areas along the coasts of the targeted lakes (Russell, 2023). During this time, Atlantic Whitefish tend to migrate to the deeper areas of the lake, where it is cooler in comparison to the shallow regions of the lake, thus reducing the possibility of stunning this fish species. As of now, no Atlantic Whitefish have been caught from electrofishing activities from the joint Coastal Action and DFO AIS removal effort (Russell, 2023).

3.1.3 Quadrafoil light traps

Description

Quadrafoil light traps are designed for passive sampling of both macroinvertebrates as well as larval fish species (Aquatic Research Instruments, 2021). This device lures and captures phototactic aquatic organisms by using a light source (modular design which allows for a variety of light sources to be used that includes chemical light sticks, squid fishing jigs, waterproof flashlights, LED pool lights…etc.) that is inserted in the light tube which is located on the top of the trap (Aquatic Research Instruments, 2021). The organisms that are attracted to the light enter the trap through the 5mm slits from the four tubed array and are suspended in the bottom collection tray until extraction by the researchers (Forestry Supplier, 2022). Coastal Action has been recently testing the effectiveness of these light traps by capturing invasive fish larva such as smallmouth bass and chain pickerel in freshwater bodies from the South Shore such as Millipisigate lake (Coastal Action, 2020). The conservation group currently deploys these traps twice per year based on prior research of chain pickerel spawning season times which occurs in early spring and in late summer, of which the traps are set for two days in the water before collection (Coastal Action, 2020). This organization uses a maximum of six traps (provided by DFO) in which they are positioned in areas confirmed to have smallmouth bass and chain pickerel nests present (from nest surveys) (Russell, 2022). However, if there are no larval fish caught after deployment then the trap(s) would be set in another confirmed nesting area (Russell, 2022).

Estimated time

Coastal Action typically set their light traps for two days before its retrieval and the contents are collected (Coastal Action, 2020). Deployment of these traps near the nesting sites are instantaneous (they are assembled on shore), where it only takes approximately between 2-5 seconds for the trap to fill with water (Russell, 2022). When checking the contents of the trap, if there are no fish present it takes ~2-3 minutes which includes the time it was taken out of the water, removal of the bottom tray, checking and reattaching, and placing the trap in the water. However, the longest it took for the team to process the fish from the trap was ~15 minutes due to the amount fish captured in the tray. Sometimes the retrieved light traps would be transferred into a new location that would take between 5 minutes to an hour, which the time depended on where it was relocated and if other traps were checked along the way (Russell, 2022). In order to reduce the time and effort in relocating/recovering, all traps are anchored to their designated position with a single brick with their GPS coordinates recorded as well. There were a couple of instances where the traps were slightly displaced due to high winds on the water but were still just as quick to relocate as their change in location was insignificant (Russell, 2023).

Cost assessments

The Aquatic Research Instruments website sells quadrafoil light traps for `~$771 CAD ($579 USD) per trap (not including the light source type chosen for the device). Other online shops sell this trap at a similar price range (Aquatic Research Instruments, 2021). There are no reports for maintenance costs, however the light source used, such as flashlights, will need their batteries to be replaced or charged for these traps to function optimally.

Potential limitations

In terms of potentially bycatching SAR such as Atlantic Whitefish along with predation from larval invasive fish species also caught, Coastal Action has assured that there is no risk of any of these situations from happening. Firstly, Atlantic Whitefish have been recorded to only spawn and hatch in the winter, which is much earlier than other species of fish residing in this lake and the light trap deployment is limited to the beginning of summer (June) until early fall where they are too big to enter the trap (Russell, 2023). Furthermore, when the traps are deployed, only juvenile AIS that are 6mm or less can enter the trap in which they are too small to predate on juvenile Atlantic Whitefish that spawned earlier in the year that are likely bigger than the catchable larval AIS (Russell, 2023). Secondly, Atlantic Whitefish in general (including larval stages) prefer to reside in areas of the waterbody that are both relatively cool and deep. In contrast, larval light traps are deployed in areas that are relatively shallow (close to the shorelines) and are relatively warm compared to other sections of the lake (summer), which both are ideal conditions for smallmouth bass spawning and hatching (Russell, 2023).

Despite the advantages of this trap having a relatively low bycatch probability compared to other AIS removal strategies, it is also one of the more limited/inefficient means of capturing AIS fish populations. As highlighted above, this trap only has the potential to catch larval individuals that are 6mm or less in diameter due to the size of the entry slits of the trap, which means it can only catch an insignificant portion relative to the entire AIS population. However, since this trap is designed to focus on larval capture compared to other traps/activities, it does have the potential to control/slow the rate of new generations of AIS fish populations from developing if captured during the early stages of their life cycle.

3.1.4 Conventional angling

Description

Conventional angling refers to an AIS treatment method in which fishing with a rod and line is used to catch and remove the AIS species from the ecosystem in focus. Depending on the study, researchers have the option to use different lures to attract specific fish species.

Coastal Action has been actively conducting scientific angling surveys since 2012 for its ability to exclude many native species due to the wide bait selectivity from both chain pickerel and smallmouth bass (Feener, 2018). In order to maximize the number of AIS catches and minimize the number of native species (i.e., Atlantic whitefish), stomach content analysis of both chain pickerel and smallmouth bass occurred to obtain bait that mimicked their dietary preferences (Feener, 2017). Angling sites were also chosen based on habitat preference and observed sighting of both fish species, which included areas with gravel/pebble sediments and dense vegetation (Feener & MacLeod, 2019).

Estimated time

The length of time for fish removal depends on the number of fish in the area and other variables (no determinant factor that fish will be caught). Coastal Action species at risk team would conduct angling surveys in the location for up to a maximum of three hours or until an AIS was caught (Feener, 2017).

Cost assessments

Neither costs nor type of angling gear that Coastal Action was using for scientific angling surveys were found. However, the general price depends on the equipment bought and used to conduct the operation of AIS extraction as well as the overall cost to permit the group to conduct angling surveys.

Potential limitations

With scientific angling of AIS, there is no damage to the ecosystem, unless the user is not being careful/following guidelines such as: improper handling of non-targeted fish species that could lead to either varying levels of injuries or even to a lesser extent-mortality, leaving/disregarding gear in the watershed, could lead to organism entanglement and/or digestion of the gear (Lyle et al., 2007). Additionally, the misidentification of fish species caught, which could either lead to the researcher returning an AIS back into the water or keeping a non-targeted fish species due to mistaking the native fish as an AIS. In general, all potential damages that could occur with type of treatment, would be entirely dependent on the angler. In an efficiency perspective, angling by itself would not be able to remove all the invasive species in the area and can potentially take a longer time to catch the fish overtime as more species are removed per year to where, it is longer practical/nor feasible to do so.

3.1.5 Rotary screw traps

Description

Rotary screw trap (RST) is a passive fish trap that functions based on the current/flow of the water from the ecosystem. RSTs main components includes a rotating cone (rotary screw) made up of aluminum mesh plating with a “propeller-like” front of the cone in order to utilize the hydrodynamics of the current to rotate the drum (Volkhardt et al., n.d.). The front “propeller” of the cone is also designed to ensure that fish cannot escape once it enters the trap. Connected directly behind the cone is the live box where all the funneled fish are kept until personnel physically remove them. The last main component of this trap is the pontoon barge which holds the above components together, while having enough room on the sides for researchers to operate the trap and remove specimens out from the live box (Volkhardt et al., n.d.). Coastal Action has primarily used these traps (in partnership with DFO) since 2012 to monitor fish life cycles particularly Atlantic White and have also been using them to capture AIS species such smallmouth bass and chain pickerel in the Petite Riviere watershed (Creaser, 2022).

Estimated time

The frequency of checking/monitoring the traps depends on the number of fish in the trap and the time it takes for the live box to get full. If the watershed is known by researchers to be concentrated with fish, there may have to be multiple inspections per day to ensure optimal health of the fish (so that they are not getting too crammed or preyed upon). However, according to the DFO species at risk public registry, the Rotary Screw Trap is checked at least once per day and may be checked more often if warranted (Government of Canada, 2018).

In terms of the time it will take to capture AIS species, there is no set time as there are multiple factors that will determine time capture. If there are anadromous prey species such as the Atlantic whitefish, which are found in the Petite Rivière watershed (emphasis on Hebb's lake), this may encourage predatory pursuit by both chain pickerel and smallmouth bass.

There is incentive to frequently check the traps especially in watersheds that have AIS species with native species and especially if the watershed in focus also contains species at risk or even endemic species. Assuming that the RST is dissembled and transported to the site, it would typically take less than an hour to remove the trap from the transport vehicle and have it fully assembled with 2-3 people and that the trap is either the 5 foot or 8-foot RST model (Creaser, 2022).

Cost assessments

Coastal Action purchased one 5-foot diameter rotary screw trap in 2009 from EG Solutions Incorporation with an initial cost of ~$19,000 CAD (EG Solutions, 2022). However, the cost of purchasing an RST with equivalent specifications and dimensions today has increased to about ~$47,000 CAD (EG Solutions, 2022). The RST is also subject to maintenance and repairs when necessary and the cost of fixing the trap depends on scale of damage/upgrade. Specifically, in the latest season (2022) a significant repair was needed on the trap that included welding patches. On average, the annual maintenance/repair costs for Coastal Action's RST are approximately ~$150 per year (Creaser, 2022).

Potential limitations

In comparison to electrofishing surveys, RSTs are not as physically damaging to aquatic organisms as they only function to restrain fish in a live box until the operators remove them out of the trap (Feener, 2018). However, this may lead to a high risk of predation especially if there are top predators such as smallmouth bass and chain pickerel contained in the box along with native species. There is more risk of predation as well since this trap does not stun species and are fully functional/active in the confined space until researchers filter out the species collected in the live box.

Some of technical liabilities of the RST is that it is water column specific, where it mostly filters out the middle and the surface of the water column in which fish can still pass by underneath (Volkhardt et al., n.d.). It has also been reported that this trap is relatively loud, where fish will often avoid it as a result so its placement must be selected in areas where the current is strong enough to "mask" the noise produced by the screw trap when operating (Volkhardt et al., n.d.).

3.1.6 Eradication treatment methods (rotenone)

Description

Rotenone ($C_{23}H_{22}O_6$) is a naturally occurring ketone compound most found in certain tropical and subtropical plants in bean family (ex: *D. involuta and L. utilis*) predominantly in the roots, stems and leaves (Fried et al., 2018). Prior to its usage in recent times as a piscicide to eradicate aquatic invasive species (AIS) from a targeted ecosystem (formerly used as both a pesticide and piscicide, though now only used a piscicide due to the harmful properties of rotenone as a spray), humans have been using this compound for centuries to harvest fish and alter fish communities (Dalu, et al., 2015). Specifically, indigenous communities in both North and South America in the past discovered that the roots from these family of plants were toxic to fish and developed different mechanisms to apply the roots to kill and harvest the fish present (Anderson, 1970). Rotenone has low water solubility and a relatively high lipid solubility making it ideal to diffuse into organic membranes of aquatic organisms, especially those that use gills for oxygen uptake via respiration (Dalu, et al., 2015). As a result, rotenone as a piscicide, functions by blocking the respiration of these specific organisms by means preventing the function of the mitochondria, thereby hindering the energy production due to less oxygen intake and later causing mass cell mortality, leading to imminent death of the organism (Fried et al., 2018). At similar applied concentration levels, reptiles, mammals and birds are not as impacted, where their skin blocks the absorption of this compound and is also broken down by enzymes found in the digestive system (Wu et al., 2020).

Estimated time

Dependent on the size of the waterbody, aquatic ecosystems with larger volumetric areas will take longer to implement rotenone concentrations effective enough to toxify fish species throughout the water column compared to ecosystems with smaller volumetric areas. In the case of the Piper Lake project treatment, it took approximately six hours in applying the necessary amount of Noxfish II (compound with active rotenone) with a vessel (canoe) and crew, in order to effectively kill off all remaining individuals of the smallmouth bass population (Lowles, 2022).

Cost assessments

Noxfish II is currently sold by Zoecon Professional Products at approximately $3,850 CAD ($2,865 USD) per ~114 L (30-gallon) drum coming from Dallas Texas (this does not include the cost of Customs Broker such as taxes, shipping fees.... etc.) (Ross, 2022).

Potential limitations

In the Piper Lake invasive species eradication project, efforts were made to remove the greatest number of native fish species (placed into temporary sanctuaries near the site) before rotenone was added in the area (Lowles, 2021). As soon as smallmouth bass were first detected in this lake during July of 2020 physical removals were the only method of extracting these AIS which took place until summer 2020 (approximately a year of physical removal efforts) (Lowles, 2023). The types of removal activities that were used during this time were the implementation of minnow traps (435 hours of deployment), scientific angling efforts (37.75 hours spent casting in the water), and both backpack and boat electrofishing in the shallow regions of the lake (4781 seconds of shocking) (Lowles, 2023). After all physical removal efforts were completed, a total of 17 adults and 42 young-of-year (YOY) smallmouth bass individuals were caught before the application of rotenone (Lowles, 2023). However, when rotenone was applied a total of 391.7kg of fish were collected after application of rotenone, where most poisoned fish were native species: white sucker (110kg), yellow perch (98kg), shiner species (55kg), American eel (54kg), brown bullhead (40kg), banded killifish (18kg), and creek chub (2kg). In comparison, 170 of mostly young-of-year smallmouth bass (14kg) were removed after the treatment (LeBlanc & Lowles, 2021).

Not only are fish species impacted by the application of this naturally occurring piscicide, but many other aquatic organisms that depend on gill-oxygen uptake as a means of respiration have a potential of being impacted as well albeit less than fish. Below is a list of common freshwater organisms, other than fish, that make up the majority of freshwater ecosystem biodiversity and their effects (if any) from rotenone treatment based on various peer-reviewed studies.

Invertebrates

Previous studies tested the rates of mortality with regards to various invertebrate species and found that crustaceans (i.e.: crabs) were not affected even after exposed to maximum rotenone concentration of up to 100ug L^{-1} (Dalu, et al., 2015). Yet, certain fly species (*D. pulex* and *P. lanellatus*) died in the lowest concentration. In fact, it was found in other studies that both crabs and crayfish species were mostly insensitive to rotenone presence due to their open circulatory system in contrast to flies being reliant on gill-based respiration (Dalu, et al., 2015).

Amphibians

Research conducted in northwest Montana wanted to determine the potential impacts of rotenone on four species of amphibians in 10 alpine lakes each with trout (non-native species in this region) present (Fried et al., 2018). Prior to the treatment, 2–4-year surveys were conducted to get an approximation of the population size in the lakes (Fried et al., 2018). However, efforts varied from lakes mainly due to aquatic vegetative coverage being denser than others making it difficult to detect these species (Fried et al., 2018). Monitoring surveys shortly after the post-treatment of the piscicide found that were no apparent changes in amphibian presence. Thus, the researchers suggested that this was either due to resiliency towards the compound, rotenone was applied after the gilled life stages, or adult amphibians from nearby ecosystems not treated by rotenone migrated to the lake (Fried et al., 2018).

Planktonic species

In Jasper National Park, Alberta, a study measuring the impacts of rotenone on zooplanktonic species were conducted in two lakes (Anderson, 1970). Crustacean planktonic species were absent until after six months of the treatment, however other species such as rotifers and net phytoplankton did not disappear completely (Anderson, 1970). It was speculated that any new species variation in both composition and abundance was due to changes in competition and predation rather than environmental changes as a product from the rotenone treatment (Anderson, 1970).

Submerged plant species

A recent study tested the possible impacts of rotenone exposure on common aquatic plant species (*V. natans, M. spicatum, and P. maackianus*) (Wu, et al., 2020). The researchers analyzed the growth and metabolism of these plant species from rotenone application, where the results displayed a negative impact towards shoot height, and both shoot and root dry weight (Wu, et al., 2020). The water was also affected in which there was a significant reduction in light transmission as well as slight increase in pH. It was suggested by the researchers that the change in the water led to the decrease of plant mass (Wu, et al., 2020).

3.2 Ecosystem restoration methods from the impacts of eradication treatment

3.2.1 Statistical analysis of EPT taxonomic diversity and abundancy recovery

The ANOVA statistical analysis of the four different time ranges revealed that there were no significant differences of EPT taxonomic diversity among freshwater sites for any of the elapsed post-treatment monitoring time ranges. Despite all the P-values not being significant (P-value>0.05) there were distinct differences with these values in relation to post-treatment monitoring time. Specifically for the taxonomic diversity recovery time, the </=1-year range had a P-value of 0.16, the second range of 1</=3 years had a value of 0.32, 4-7 years had a much higher value of 0.95, and the last time period of 8 years monitoring after treatment value dropped to a P-value of 0.32.

In addition, the ANOVA statistical analysis performed in excel for the change in EPT abundancies relative to the four elapsed post-treatment monitoring time periods determined that were no significant statistical differences (P-value>0.05) for all time ranges as well. Even though none of the post treatment monitoring time periods had no significant difference with their pre-treatment abundancy levels, there was an apparent decrease in P-value as the elapsed time increased. Specifically, after </=1-year elapsed monitoring time had a P-value of 0.74, the second time range of 1</=3 years had a value of 0.57, 4-7 years had a value of 0.22. After 8 years of elapsed post-treatment monitoring, the abundancy levels were the most distinguished compared to the earlier monitoring time ranges relative to the corresponding pre-treatment abundancies with a P-value of 0.078.

**Refer to the Appendix section for more details on the ANOVA statistical analysis used and the test values.*

EPT taxa diversity percentage of recovery after post rotenone treatment summary

Post-treatment monitoring </= 1 year:

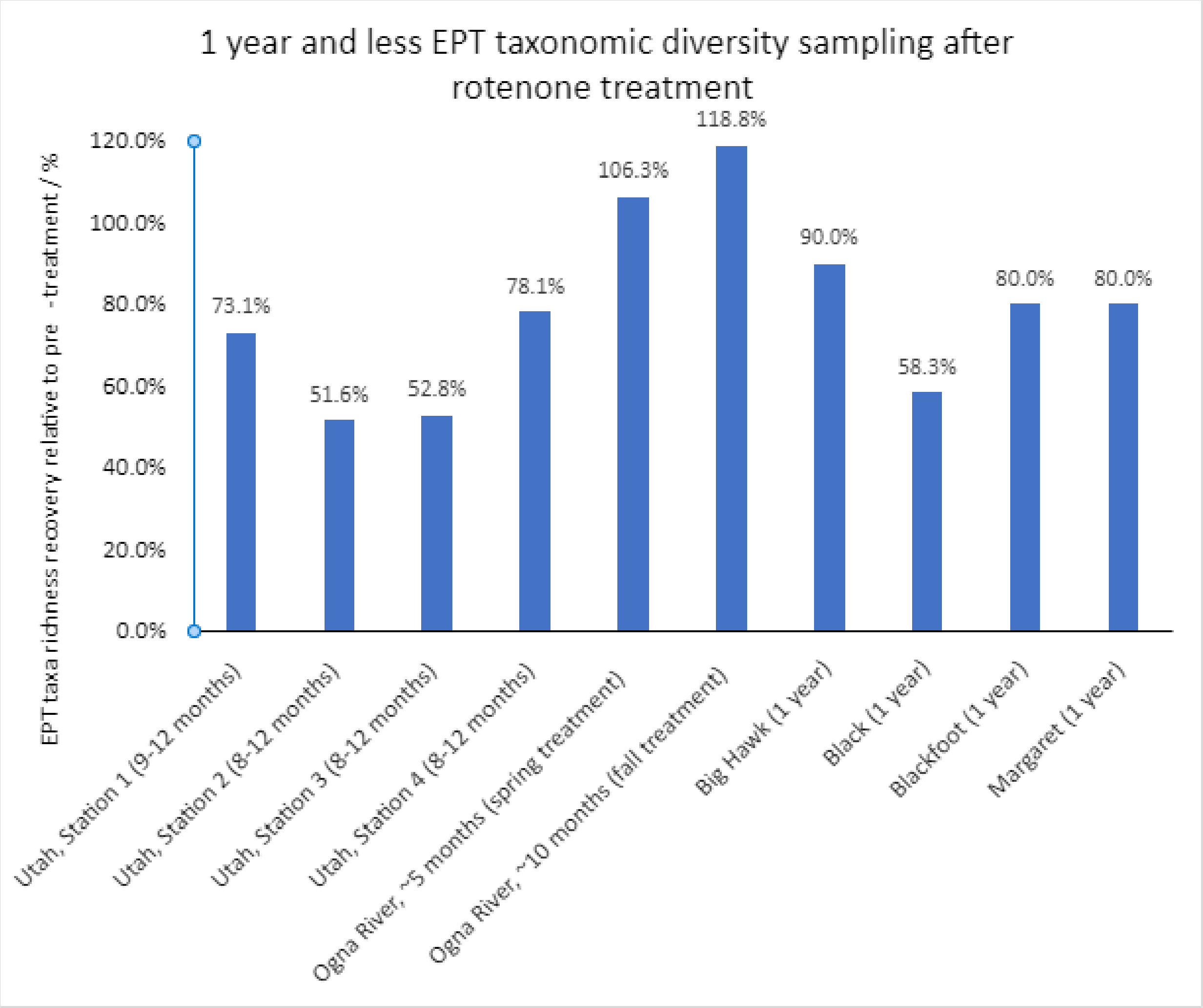

Fig 1. After less than and/or up until a maximum of one year monitoring from rotenone application in the ten freshwater locations depicted above, three of the ten locations had less than 60% of the original remaining EPT taxonomic diversity after post treatment relative to pre-rotenone treatment values. 4/10 locations had approximately </= 80% of EPT taxonomic diversity relative of the pre-treatment monitoring levels. Lastly, one site had more than 80% of the original EPT taxonomic diversity, and two sites had achieved greater EPT diversity in comparison to pre-treatment levels.

Post-treatment monitoring 1</=3 years:

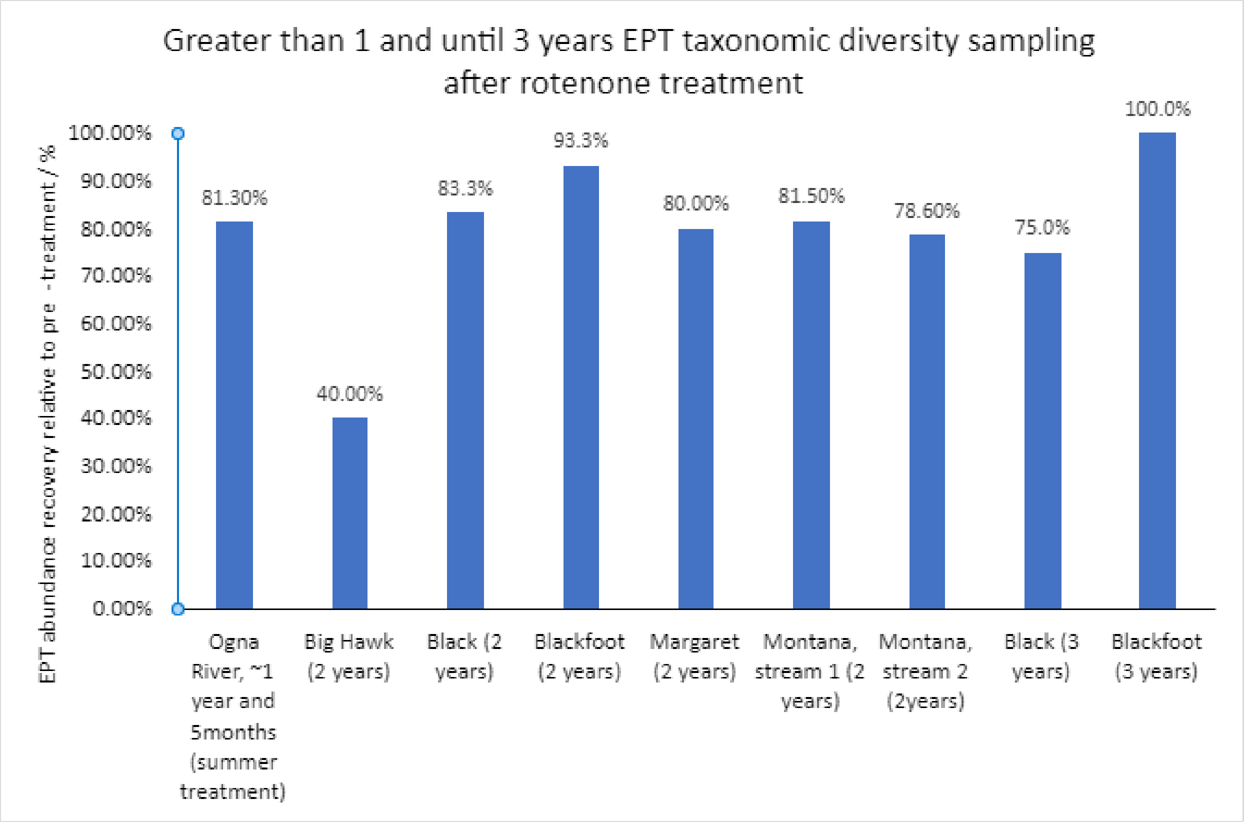

Fig 2. After more than one and/or until a maximum of three years monitoring from rotenone application in the nine freshwater locations depicted above, one location had approximately 40% of the original remaining EPT taxonomic diversity after post treatment relative to pre-rotenone treatment values. Two locations had <80% of the of EPT taxonomic diversity relative of the pre-treatment monitoring levels. 3/9 locations had recovered approximately </= 80% of the pre-treatment EPT diversity levels. Lastly, two locations recovered up to approximately </=100% of the pre-treatment diversity.

Post-treatment monitoring 4-7 years:

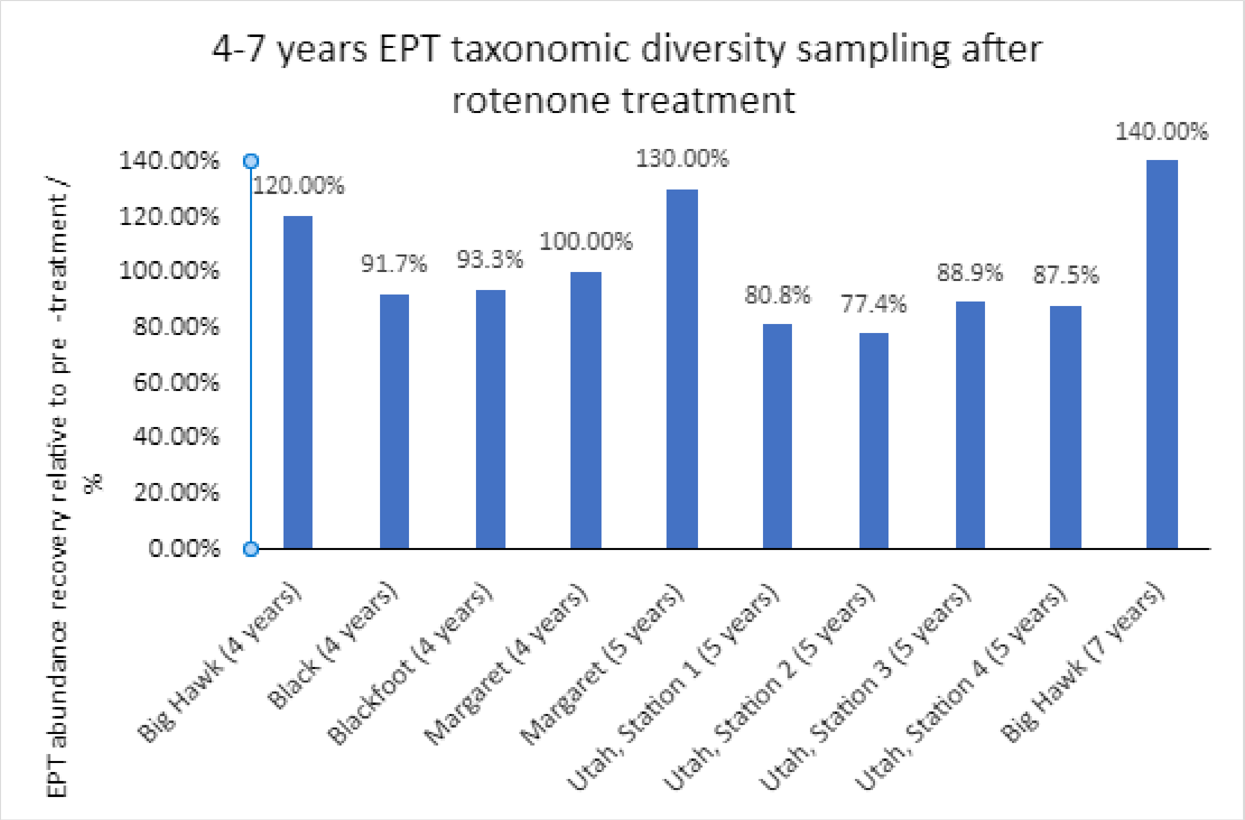

Fig 3. After 4-7 years of monitoring from rotenone application in the ten freshwater locations depicted above, two locations had approximately </= 80% of the original remaining EPT taxonomic diversity after post treatment relative to pre-rotenone treatment values. 4/10 locations had <80% of the of EPT taxonomic diversity relative of the pre-treatment monitoring levels. One location had recovered approximately 100% of the pre-treatment EPT diversity levels. Lastly, three locations had achieved greater EPT taxonomic diversity in comparison to pre-treatment levels.

Post-treatment monitoring 8 years:

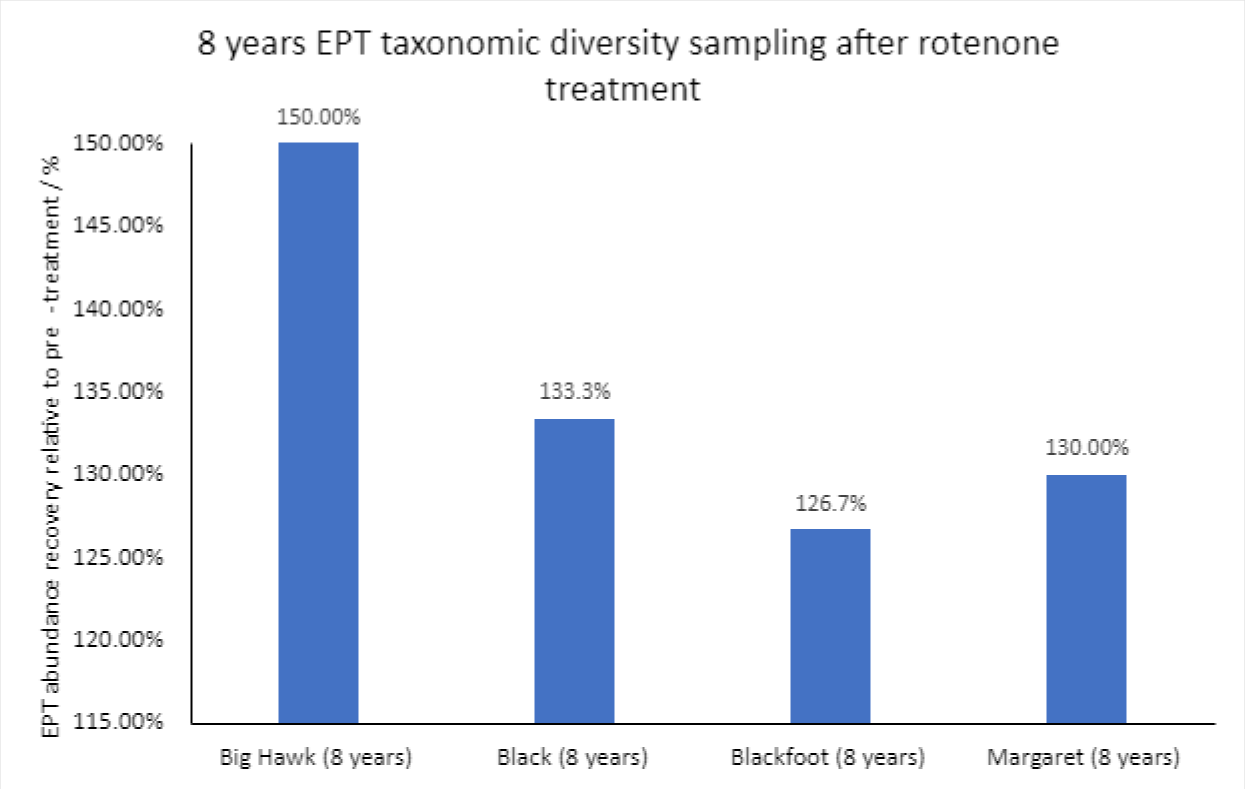

Fig 4. After eight years of rotenone treatment, all four sites depicted above had achieved a greater taxonomic diversity of the EPT groups, compared to their respective pre-treatment values. *All locations in this graph were conducted by a single research study (*Recovery of Freshwater Invertebrates in Alpine Lakes and Streams following Eradication of non-native Trout with Rotenone*) due to none of the other studies conducting recovery monitoring up towards eight years.

EPT abundancies percentage of recovery after post-rotenone treatment summary

Post-treatment monitoring </= 1 year:

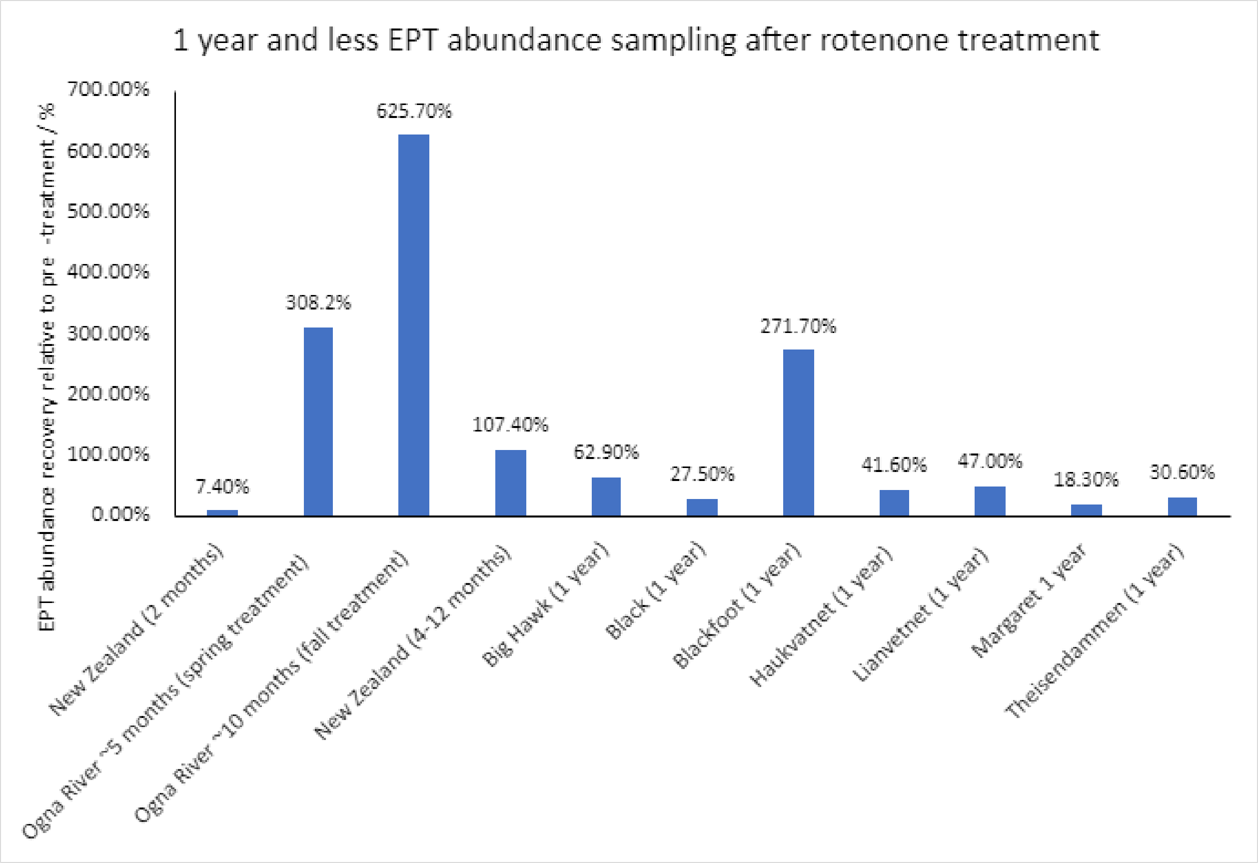

Fig 5. After less than and/or up until a maximum of one year monitoring from rotenone application in the ten freshwater locations depicted above, one location had less than 20% of the original remaining EPT density after post treatment relative to pre-rotenone treatment values. Two locations had approximately 30% EPT abundancy relative to pre-treatment levels. Two locations had >40% EPT abundancy relative to pre-treatment levels. One location had approximately 60% EPT abundancy levels. Lastly, one site had recovered approximately 100% of the original EPT abundancy levels, and three sites had achieved significantly greater EPT abundancies in comparison to pre-treatment levels.

Post-treatment monitoring 1</=3 years:

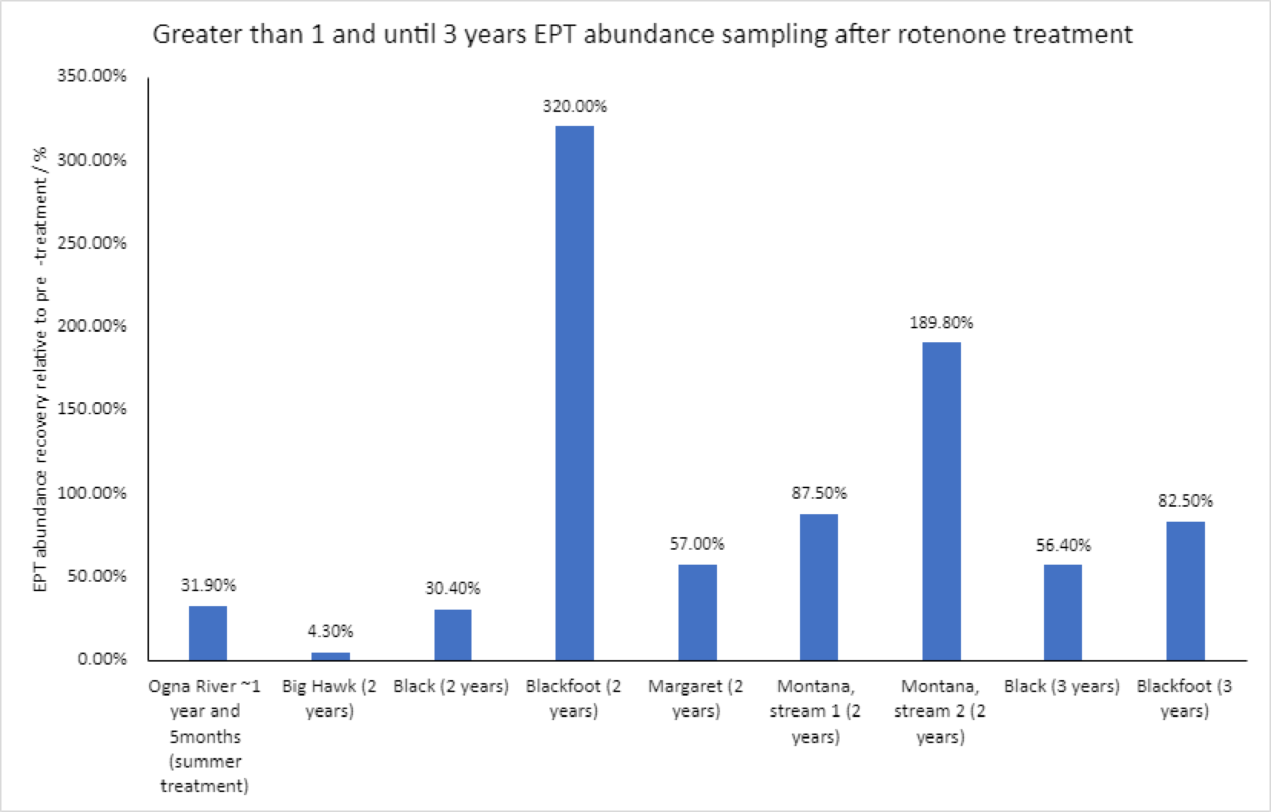

Fig 6. After more than one and/or until a maximum of three years monitoring from rotenone application in the nine freshwater locations depicted above, one location had <5% of the original remaining EPT abundancy levels after post treatment relative to pre-rotenone treatment values. Two locations had approximately 30% of the EPT density relative to the pre-treatment monitoring levels. Two locations had approximately 60% of the EPT abundancies relative to pre-treatment. Two locations had recovered >80% of the pre-treatment EPT abundancy levels. Lastly, two sites had achieved significantly greater EPT abundancy in comparison to pre-treatment levels.

Post-treatment monitoring 4-7 years:

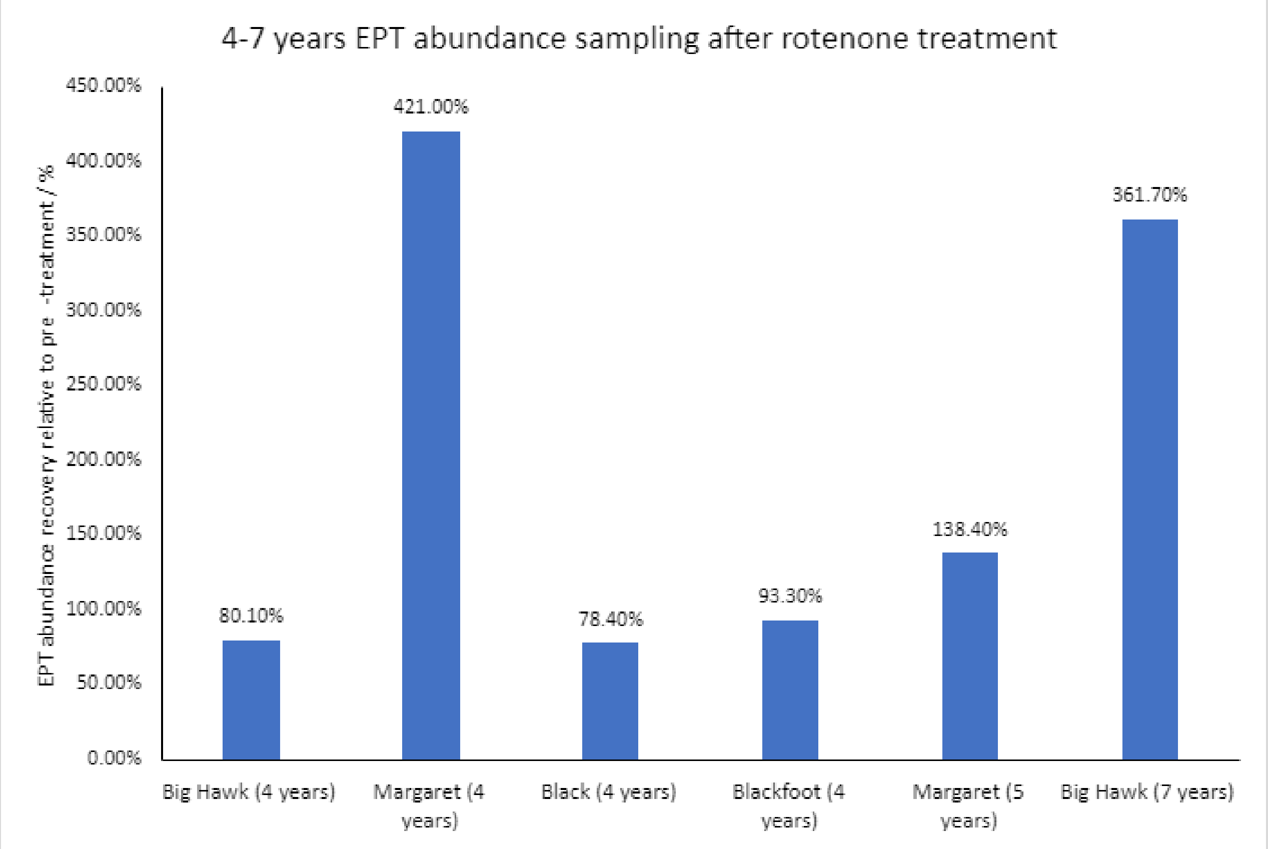

Fig 7. 4-7 years after rotenone application in the ten freshwater locations depicted above, it was found that two locations had approximately 80% of the original remaining EPT abundancy relative to pre-rotenone treatment values. One location had recovered approximately 100% of the pre-treatment EPT diversity levels. Lastly, one location had achieved >100% EPT density, and two other locations had achieved significantly greater levels of EPT abundancies in comparison to pre-treatment values.

Post-treatment monitoring 8 years:

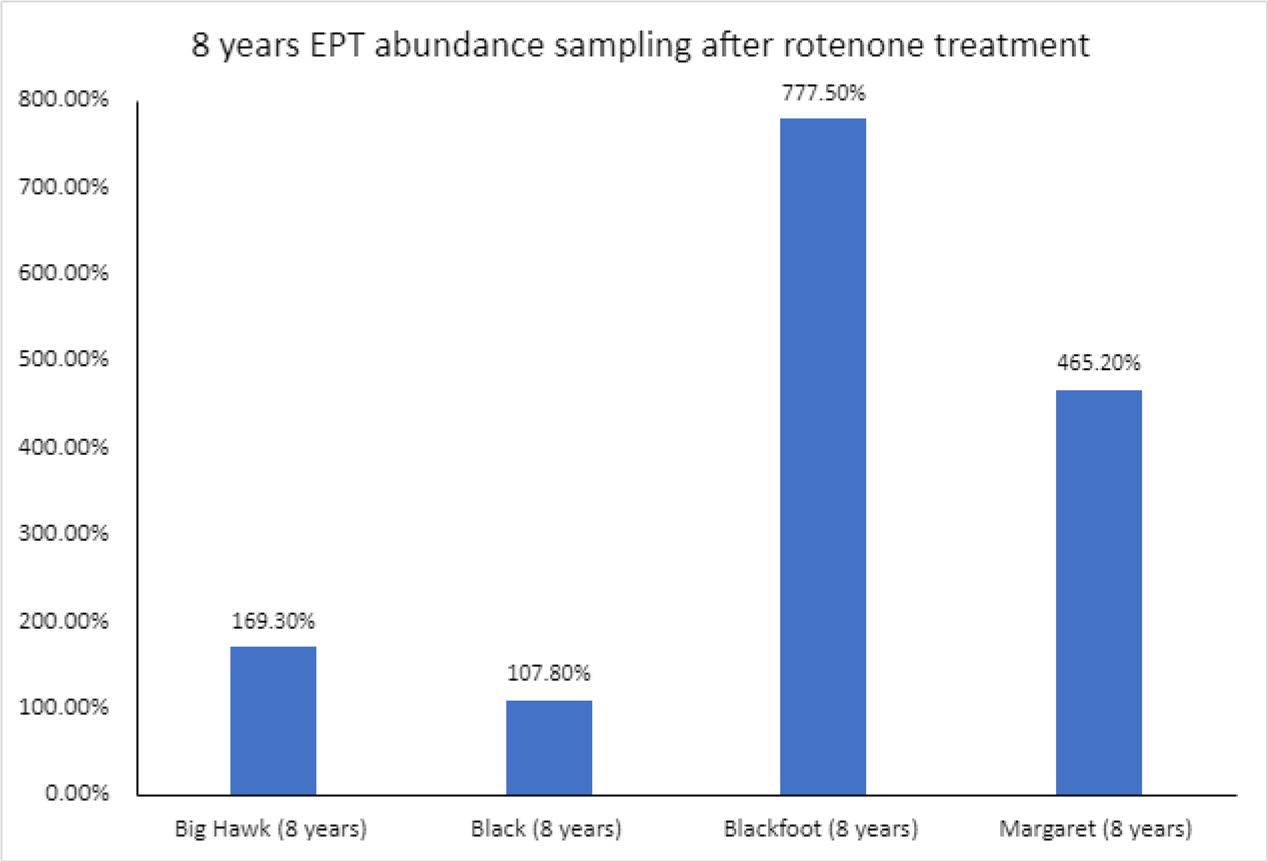

Fig 8. Eight years after the original rotenone treatment, one location had approximately 100% of the pre-treatment EPT abundancy levels, while 3/4 sites had achieved a significantly greater EPT density compared to their respective pre-treatment values. *All locations in this graph were conducted by a single research study (*Recovery of Freshwater Invertebrates in Alpine Lakes and Streams following Eradication of non-native Trout with Rotenone*) due to none of the other studies conducting recovery monitoring up towards eight years.

3.3 CMIST tool for freshwater AIS management

The Canadian Marine Invasive Screening Tool (CMIST) is a screening-level risk assessment tool that was originally designed to assess the risk of marine invasive invertebrates in uninvaded areas (Government of Canada, 2018). The tool is made up of a short list of questions that uses number scores to help evaluate each theoretical scenario asked to better aid assessors using this tool and avoids qualitative answers to maximize simplicity in evaluations (1-lowest risk score, and 3-is the highest risk score for each question) (Government of Canada, 2018). The scores for all questions answered are then averaged to assess the overall risk of the potential AIS for managers to decide on the next course of action. Specifically, an average CMIST score between 1.0-1.7 means that the AIS in question has both a low probability and impact of invasion in the area, whereas a score between 2.3-3.0 has a high probability and impact level of invasion (Fisheries and Oceans Canada, 2015). There are a total of 17 questions that are part of the CMIST with the first eight about the likelihood of invasion: questions one and two pertain to the arrival of AIS in the area, questions three and four on AIS survival in the invaded area, five and six on establishment, seven and eight on AIS spread (Fisheries and Oceans Canada, 2015). The second half of the questionnaire (9-17) asks questions regarding the impact of the AIS in focus on the invaded ecosystem and its native inhabitants (Fisheries and Oceans Canada, 2015). However, to best answer the questions provided in this screening tool, it is recommended that the assessors develop/have sufficient knowledge on the AIS in concern (based on the questions) prior to conducting the test in order to give an accurate evaluation/score (Government of Canada, 2018). Initially, CMIST (as the name implies) was designated to assess the risk of invasive marine invertebrates such as various types of mollusks, tunicates, crustaceans, and polychaetes introduced or at risk of introduction to three Canadian marine ecoregions (Government of Canada, 2018). However, as the questions are non-specific, the tool can be applied to species other than invertebrates and can also be used for potential freshwater invasions if some of the questions are adjusted to make it relevant for the situation in question (Government of Canada, 2018). An example of this altered application was done in British Colombia (B.C), where CMIST was used to evaluate the likelihood and impacts of various freshwater fish species known for successful invasions in other parts of North America (Fisheries and Oceans Canada, 2015). The evaluation was able to determine the potential and impact of invasive fish invasion like the marine invertebrate threats (Fisheries and Oceans Canada, 2015). This significant change in species and water conditions, while still being able to perform the assessment tool highlights the adaptability of the CMIST and could be used to determine freshwater AIS fish species on the Canadian east coast (ex: Nova Scotia) as this project is focused on.

Evaluation

CMIST tool is not designated to be a means/method to remove, eradicate, or act as a restoration method after AIS treatment is conducted in an area, rather it provides managers an overall evaluated risk assessment score of the likelihood and impact of a potential AIS invasion in the area of concern. As a result, this screening tool would be best used at areas that are of concern for future invasions, and based on the overall averaged score, managers can be better prepared/organized of what AIS treatment methods can be used in the location before the AIS become established or spread to further locations.

3.4 Repatriation of fish populations

The primary objective for species repatriation is the improvement of the overall status of wild populations by establishing self-sustaining, breeding populations in areas where they historically resided but have been eradicated/or are currently scarce (Lamothe & Drake, 2019). Despite the focus of this restoration concept being directed towards aquatic species at risk, it can also be applied to common occurring fish populations that may have been depleted from factors including but not limited to AIS treatment. Before managers can go forward with species repatriation/stockings in the ecosystem, there must be a well understood reason as to why the species was originally extirpated/in small numbers to begin with. This is to ensure repatriation of fish populations will be successful, rather than failing due to not fully understanding the underlying problems/factors that led to the near eradication of the imperilled fish species in the first place (less wastage of resources by understanding and predicting what led to the decline in the population) (Lamothe & Drake, 2019). If was found that there are five major themes that would support fish repatriation in the best viable way which are: understanding habitat characteristics, species characteristics with emphasis on life history, biotic interactions with other neighbouring species, and options for stocking approaches in the form of either hatcheries or translocation (Lamothe & Drake, 2019).

Habitat characteristics

A key success to repatriation of fish species is with the understanding of the species past and potentially current habitat quality and characteristics. Each life cycle of a certain fish species may require specific ecosystem qualities to deem the habitat sustainable (Lamothe & Drake, 2019). However, some fish species designated under the Species at Risk Act (SARA) may have limited research/data on their life history and suitable habitat parameters due to their scarce presence and overall low population abundance in the ecosystem to begin with (COSEWIC, 2010). Therefore, rigorous monitoring surveys using various methodologies such as snorkel surveys to track movement patterns as well as to observe/record reoccurring habitat features and conventional angling to determine the overall quantity of invasive fish caught in an area would be the best approach to studying habitat preferences. Depending on the status of the environment that was once “ideal” habitat for the species of concern, it may be difficult to restore if AIS is not the only factor limiting the population size (COSEWIC, 2010). For example, if an obstruction is blocking critical fish passage, it may be difficult or even impractical to repatriate/reintroduce the species as it would cost substantial amounts of money/funding to remove the obstruction and may even lead to intense local opposition as a result of potential socioeconomic losses (i.e., job loss) (COSEWIC, 2010). Another factor that would render or reduce the benefits of reintroduction of fish species into its original habitat is a shift in water quality as a result of climate change. This could pressure fish species to migrate and establish in areas that better meet the needs of the population which would require even more research, such as models for predicting future watershed quality differences as a result of climate change (Lamothe & Drake, 2019).

Species characteristics (life history)

Knowledge of species life history traits can help improve the repatriation success of the species in question. Specifications include survival rates when introducing the reared fish into their new habitat are essential to ensure stocking densities are optimized (Lamothe & Drake, 2019). Other characteristics includes age and size at maturity, duration and periodicity of spawning, extent (if any) of parental care, life cycle stage specific growth rates, average number of offspring per breeding season, and species lifespan (COSEWIC, 2010). The information of the species life characteristics ultimately aides in deciding when and how often (if necessary) repatriation should occur as well as provides population reference points during pre- and post-monitoring after introductions (Lamothe & Drake, 2019). As an example, white sturgeon would need higher levels of repatriation efforts than smaller fish species, due to more requirements such as larger habitat ranges, maturity reached later than other smaller fish species which would require fewer introductions to establish sustainable populations (COSEWIC, 2010). Consequently, size is a factor in determining the intensity of repatriation procedures. Understanding life history characteristics also aides in determining specific habitat types needed for each life stage as well (Lamothe & Drake, 2019). An example of this could be that a certain species would require a flow of water for a part of its life cycle so that larval stages are naturally dispersed. Through comparing historical or geographically distinct populations, information of species-specific life history traits can provide baselines for assessing the success rate of repatriations/introductions (COSEWIC, 2010).

Biotic interactions

As highlighted throughout this research it is apparent that species to species interactions plays a pivotal role in determining the stability of the ecosystem. AIS can often outcompete for resources and/or prey on native species resulting in a shift in the ecosystem that could lead to less species biodiversity and abundance over time (Lamothe & Drake, 2019).

Therefore, it is important to determine the approximate number of AIS (if any at all) in the watershed that is planned for fish reintroduction (Lamothe & Drake, 2019). A relatively large AIS population remaining in the ecosystem may result in the repatriation efforts being futile if not removed/eradicated (COSEWIC, 2010). This may require continuous reintroductions to maintain the fish population at risk which may not be suitable for all fish species. Overall, repatriation efforts should aim to maximize positive biological interactions for imperilled fish species while minimizing the negative interactions when selecting areas for introduction (i.e., introductions should only occur in areas where occupancy probabilities of competitor or predatory species are low) (Lamothe & Drake, 2019).

Stocking approaches

a) Hatchery

Fish husbandry (breeding) in laboratories is challenging especially for imperilled fish species and has only been done for a few species throughout Canada such as the Atlantic Whitefish and Copper Redhorse (COSEWIC, 2010). Significant differences in behaviour can occur in bred populations compared to the naturally occurring populations. Fish initially developed in a hatchery can have altered behavioural traits compared to their wild populations if the habitat/living conditions in the hatcheries do not match the proposed stocked area (I.e., behavioural deficiencies in predation, foraging…etc.) (Lamothe & Drake, 2019). Studies have also proved that replicating habitat and ecosystem complexity and diversity in a hatchery/breeding is vital to induce natural behaviours. The lack of genetic diversity is another main problem with hatcheries where the primary incentive is to maximize the population, especially if the genetic traits are only compromised from a few of the naturally occurring individuals (Lamothe & Drake, 2019). Despite captive fish breeding improving over the past few decades, the main problem that may always be present is the reared fish adapting and becoming “domesticated” to the hatchery conditions (COSEWIC, 2010). An example of a successful hatchery program and facility in Nova Scotia is at Dalhousie’s Aquatron facility, in which the research team has helped rear Atlantic whitefish brood stock with support from Dalhousie members, DFO, and Coastal Action (Auld, 2022).

b) Translocation

May not be viable if the proportion of the extant population that is planned to be moved is already low in numbers and may not be self-sustaining in the long term, which could lead to expiration in both the original and the newly populated ecosystem (Lamothe & Drake, 2019). However, translocations could be a logical option for re-establishing fish populations if other areas have stable self-sustaining fish communities (COSEWIC, 2010). Translocations are most successful based on "right" source populations, where choosing a population should be focused in evaluating the genetic diversity and adaptive potential of the existing group and not just on geographic distance (distance between original population site and new area) (Lamothe & Drake, 2019). Previous studies have shown there is a positive correlation between local adaptation to the environment and geographically distinct populations, where it is recommended to first seek out original populations based on similar habitat characteristics to the new watershed that is planned to be restored (Lamothe & Drake, 2019). If existing populations are found in habitats that share few characteristics to the proposed restoration site, then the next priority is to determine the fish population(s) that have the most adaptive potential and genetic diversity to maximize the potential for self-sustaining fish populations (COSEWIC, 2010).

3.5 Environmental DNA

Environmental DNA (eDNA) detection is a recently developed monitoring tool used to confirm the presence of aquatic organisms without having to physically observe and/or catch the species of interest (Saccò et al., 2022). As a result, this makes this monitoring tool less environmentally disruptive compared to conventional monitoring techniques. In order to conduct the eDNA monitoring test, researchers first start by taking water samples of the ecosystem, in which any strands of DNA are purified and extracted to minimise any functional limitations for the final step (Saccò et al., 2022). The last part of the procedure involves detecting strands of eDNA through quantitative Polymerase Chain Reaction (qPCR), in which numerous copies of a specific sequence from the original eDNA strand are synthesized and detected through fluorescent signal amplification (Saccò et al., 2022). The qPCR test will be positive, if there is significant amplification detected, thus informing researchers and managers about the presence of the species in concern (Biomeme, 2019). EDNA has been primarily used in detecting species of interest such as aquatic organisms that are considered SAR, where these species may not have been physically sighted for a long period of time (Saccò et al., 2022). This monitoring tool has also been used for confirming early stages of invasions of AIS, which has given researchers and managers more time to prevent further introductions into the rest of the ecosystem (Loeza-Quintana et al., 2021). Specifically, eDNA was used in the Kejimkujik watershed in which the water samples were able to detect early invasion stages of both chain pickerel and smallmouth bass as well as SAR (Loeza-Quintana et al., 2021).

3.6 CABIN

The Canadian Aquatic Biomonitoring Network (CABIN) is a nationally standardized monitoring program (in contrast to the individual study sites described above) founded and maintained by Environmental and Climate Change Canada (ECCC) (Environmental and Climate Change Canada, 2022). This Network is primarily used to assess the water quality and ecosystem health of the watershed in focus by measuring the abundancy and diversity of benthic macroinvertebrates at that site (Living Lakes Canada, 2022). The program is continuously updated by ECCC to support the collection, assessment, reporting and distribution of monitoring data (Environmental and Climate Change Canada, 2022). In order to maintain national consistency, a training program with standardized protocols was created to certify new potential users of the CABIN program (Living Lakes Canada, 2022). As mentioned above, the common type of organisms that are used to determine both ecosystem health and water quality (biological indicators) in the CABIN monitoring program are macroinvertebrates such as molluscs, worms, insects, and crustaceans (Environmental and Climate Change Canada, 2022). Macroinvertebrates are commonly used as bioindicators due to these factors: sensitive to different types of environmental changes, found in all freshwater ecosystems, essential part of the ecosystem food chain, and assessments for these types of organisms have been well developed (Living Lakes Canada, 2022). Most of the freshwater ecosystem sampling is conducted by method called “kick netting”, which involves the assessor loosening the substrate that may have attached specimens by shuffling their feet or scrubbing the sediment with hands in front of an invertebrate net (Environmental and Climate Change Canada, 2022). It is critical that the invertebrate net is touching the surface of the substrate to reduce the potential number of invertebrates not entering the trap, there must also be no recent upstream activities prior to monitoring in order to prevent unwanted external disturbances when conducting the sample survey (Living Lakes Canada, 2022). Lastly, it is best for the user to walk in a “zig-zag” pattern across the water column to maximize area coverage and to capture certain organisms that may not be present in entire column (Environmental and Climate Change Canada, 2022). All collected data by the federal government (and some partners), is freely available through the Open Data portal, where the data is regularly updated (monthly) as more monitoring projects are conducted by various research groups and are stored in a CSV file format. Repeat visits to the same sites under different dates may also be conducted as well (Environmental and Climate Change Canada, 2022).

Chapter 4: Discussion

4.1 Evaluation of AIS treatments

4.1.1 Control/suppression methods

Physical barriers

In general, barriers are designed to prevent aquatic invasive species movement into other connected ecosystems through outlets and/or inlets (connected via streams, rivers…etc.). This type of control method would not be practical for aquatic areas that are completely isolated (ex: landlocked freshwater ecosystems). Most of the barriers may be effective at stopping major invasive species movement, however all barriers used still pose as a potential obstacle for native fish in the area that are incapable of passing it, especially those that are migratory, mobile for food, and life cycle dependent (salmon leaving and coming back when they have reached the end of their life cycle to spawn). Essentially, researchers must ask: do we accept the risk that only some native species will be able to bypass the barrier while other native aquatic organisms alongside chain pickerel and smallmouth bass are not able to pass thus impacting the rest of the ecosystem dynamics and biodiversity shift? There may have to be a decision of native species having priority over other species that cannot pass the barrier to get to its habitat.

Electrofishing

Electrofishing, if used properly, is a quick and effective method for removing invasive fish species that are within range of the electrical current and in previous studies is most effective at capturing medium to large-scale fish (Rytwinski et al., 2019). Despite the success of this removal method, there is a lot of potential risks associated with electrofishing which includes damage to non-targeted fish species that are stunned by the electrical current and injury to the team operating the vessel if not following proper procedures. Due to the risk of injury from improper handling of this device, the operators must be trained and certified to maximize safety for themselves and the crew, which in turn limits the amount of people that can conduct this activity. In addition, there is also a possibility that the electrical current produced may not stun all the chain pickerel in the targeted area if they are deeper than what the current can reach (> 6 feet). Lastly, electrofishing is relatively expensive to operate per day as well compared to the costs of other treatments.

Quadrafoil light traps

The quadrafoil light trap is quite specialized in terms of what it captures since only organisms under 5mm width can fit in the trap, attracted to the light source, and specifically chain pickerel and smallmouth bass larval stages are captured only in late spring and early summer based on its spawning time. This allows for efficient placement of these traps as pickerel only spawn twice a year (fixed spawning season) and to leave the traps near underwater vegetation (eggs are attached to this). Another advantage of this removal device is that it allows for time and resources to be concentrated elsewhere by the researchers since the device is capable of being left untouched for days and capture without any external aid/supervision (Gyekis et al., 2006). A disadvantage with using this trap is that it may be expensive relative to the groups budget for the restoration efforts especially if multiple light traps are purchased and deployed. Additionally, due to the size of this trap, it is life cycle (size) specific in which larger AIS individuals (>larval stage) cannot be captured with these traps and requires knowledge of spawning cycles of these two species in order to maximize the potential of this device. To achieve an overall maximum yield of AIS capture of varying sizes, both larval light traps and electrofishing should be practised simultaneously to achieve the maximum potential.

Conventional angling

Depending on the time and efforts invested, scientific angling can be an effective means to reduce/control an AIS population but will be hard (near impossible) to remove all AIS population in the ecosystem if the management goal is full eradication. This will get exceedingly harder as the catch per unit of effort (CPUE) reduces as there is less fish to be caught, thereby reducing the efficiency of this method of removal. However, scientific angling is the most cost-effective method for removing AIS and is also species specific since the user can decide whether to keep the fish species or not (based on identification). In addition, specific lures/bait can be used to target specific fish species based on prey preferences, which makes this type of treatment relatively modular (Feener, 2018). It also requires less experience and training compared to other types of treatment deployments as well, which allows for a greater access for participation and contribution towards removing AIS. However, significantly more time and effort needs to be spent angling in order to achieve similar results of other removal methods. Specifically, in 2019 two chain pickerel fishing tournaments facilitated the efforts in the extraction of tagged fish. Furthermore, angling can be quite effective when researchers know the preferred habitats of chain pickerel and smallmouth bass and can make predictions of where they would likely be found (Feener, 2017).

Rotary screw traps

As this trap relies entirely on the current flow of the watershed in order to rotate the cone (no motor), researchers must first determine if there are any areas with significant waterflow/currents before implementing this trap. If the waterbody is not considered landlocked and is interconnected to other watersheds (outlets or inlets) it would be best to establish the RST there to maximize the amount of fish capture since these channels/openings are typically narrower and shallow compared to the rest of the ecosystem. It is important to determine if there are flowing currents in the watershed as screw traps essentially acts as filtrations in which the “mouth” of the trap is positioned against the direction of the waterflow in order to capture all fish moving with the current. This trap maybe useful to implement if blocking the watershed with fish mesh barriers is not an option due to some species requiring open passages to migrate from the watershed due to life cycle stages (anadromous nature), food acquisition...etc. Lastly, this trap produces the least amount of injury/harm to captured fish species as the fish simply swim into the trap and are held in a live box (E.G Solutions, 2022). However, this can also be considered a liability, since AIS caught are fully functional (unlike electrofishing where the species are temporarily immobilized) and could prey on native fish species that also got trapped in the box. Therefore, researchers should frequently monitor the trap to collect any captured AIS and release native species as well to reduce the risk of AIS predation on native species.

4.1.2 Eradication methods (rotenone)

Since rotenone is a piscicide, various organisms that rely on gill respiration for oxygen uptake such as fish populations (both native and invasive), zooplankton, certain life stages of amphibians (I.e., tadpoles), and macroinvertebrates (especially members of the EPT taxonomic group) can be heavily influenced by this naturally occurring compound that may even lead to mortality from respiratory blockage. Due to the potential lethality of this compound, freshwater ecosystems that are confirmed (sighting/recordings) to be inhabited by SAR aquatic organisms and/or species that are endemic to a specific waterbody, should not have any rotenone treatment. There is a risk of losing the species completely even if capture and relocation of native populations occurs before treatment is conducted. However, the Piper Lake study has also demonstrated the concept that it is often best to completely eradicate an entire invasive species population (smallmouth bass) as well as other native species in the ecosystem. This is to prevent the spread of AIS populations into larger, noninvaded areas such as the St Mary's River that contains more biodiversity and important fish species such as brook trout and an Atlantic salmon population that is currently at risk.

Conclusion

The research conducted in this project revealed that there are quite a few differences between treatments that aim to supress and control AIS populations and treatments that are intended to completely eradicate the unwanted species from the ecosystem. The common advantage of using control and suppression methods is that they are species specific, in which all species (AIS and/or native bycatch species) caught in a specific trap (I.e., angling, RSTs, electrofishing, light traps, and fish barriers) can be identified and sorted out by operators/managers. This is critical as this allows minimum casualties of native species, which is especially important for species that are labelled at risk or are endemic to a location. This type of management method also does not pose any significant impacts to the ecosystem since the traps are only purposed to containing fish species. However, as mentioned previously, some traps (RSTs, electrofishing, and fish barriers) do pose a relatively small, potential threat to native fish species, where RSTs contain completely active species in its live box and there is a possibility that native fish caught can be preyed upon by invasive species that also got trapped. In contrast, electrofishing immobilises all fish within range of its current, thus the possibility of close-range predation is eliminated. However, this is the most damaging of all the control and suppression methods highlighted in this paper in which some previous studies have found that fish stunned could be left with internal gill bleeding or spinal injuries as examples. Fish barriers prevent exit/entry of the treated ecosystem, which is essential for stopping AIS spread into more ecosystems if the waterbody is not landlocked. However, it poses a problem to native fish species that have an anadromous lifecycle such as the Atlantic Whitefish or Atlantic salmon. Despite the disadvantages from these individual types of AIS treatment methods, they collectively pose no significant impact to the ecosystem they are deployed in and require no restoration measures from their activity. The second type of AIS treatment that is most used by managers is the application of piscicides, for this case rotenone (most accepted and used piscicide in North America), in which the management goal is the complete eradication of all AIS populations in the invaded ecosystem. Unlike physical treatment methods that aim to reduce/suppress current AIS populations and provides managers the option to return native bycatch species into the ecosystem, the application of rotenone and other types of piscicides kills all fish populations, including native fish species. Not only does chemical treatment leads to nonspecific widespread mortality of all fish present in the ecosystem, but it also poses a threat to aquatic species other than fish that use gill respiration as a means of oxygen uptake. Such examples of aquatic species include zooplankton, some lifecycle stages of amphibian species, and some invertebrate species as well including members of the insecta class (Ephemeroptera, Plecoptera, and Trichoptera-EPT). The EPT taxa was studied in-depth in this research paper as they are the principal biological indicators used to assess the overall health of freshwater ecosystems due to their sensitivity to environmental changes. Several international studies were reviewed and compared based on the length of time it took for EPT taxonomic diversity and abundancies to reach pre-treatment levels which would indicate successful ecosystem restoration after rotenone application. However, based on the ANOVA statistical analysis, there were no significant differences between pre-treatment and post-treatment compiled values from the assigned elapsed post-treatment monitoring time categories. This would suggest that rotenone may not have a significant impact on these ecologically sensitive communities and managers could then choose to repatriate lost native fish communities sooner than previously thought. Specifically, there would be sufficient food and resources to sustain the newly introduced fish species, especially for the larval stages as a result of the minimal changes of invertebrate communities before and after rotenone treatment to the ecosystem. In general macroinvertebrates act as the main diet for juvenile fish (or young of year) that are too small to feed on other bigger organisms (Gnohossou et al., 2009). It is therefore essential that freshwater ecosystems have enough macroinvertebrate taxa in order to sustain fish populations in the long term, so that young members of the population have a better chance of survival and therefore carry-on the next generation of their species. In addition, there are many studies that measure invertebrate taxa and/or abundance (including EPT taxa) to determine the natural recovery rate of studied freshwater ecosystems after rotenone application, making this group of taxa a suitable bio-indicator for measuring natural ecosystem restoration.

Chemical AIS treatments have the advantage over physical removals, as it is more likely that all AIS fish are eradicated and there is a much shorter span of treatment time compared to physical AIS removals, which must be practised yearly to keep populations under control. However, it should be noted that the treated ecosystem could still be significantly impacted if there is a lack proper management and utilization of rotenone, especially if there is ineffective or limited restoration protocols to reduce further damage. Consequently, there are numerous of restoration practises that could be employed in order to bring the overall health of the ecosystem back to near pre-treatment levels (in comparison to physical removals that require no restoration measures after AIS treatment). Such restoration methods, as discussed before, includes monitoring for natural succession of ecosystem indicator species such as EPT taxa (for Canadian sites using CABIN database to make reference to pre-treatment conditions). In addition, possible repatriation of fish species if deemed extirpated from area due to lack of ecosystem connectivity or succession taking too long (either those removed prior to treatment and kept in sanctuaries, or laboratory reared fish-especially for near extirpated or at-risk fish species to sustain remaining populations).

Recommendations

Theoretical guide for future AIS management in Nova Scotia:

1. *CMIST*

Use the CMIST tool to determine the likelihood and impacts of invasion in the non-invaded area of the species in concern. If the overall CMIST score for the area is less than 2.3, then ecosystem monitoring for potential early invasions of the species can occur on a less frequent basis. However, if the overall CMIST score for the area is greater or equal to 2.3, then ecosystem monitoring to detect early stages of invasion of the species should occur more frequently. Ecosystem monitoring for potential detections of early stages of AIS invasion should be conducted with eDNA sampling (go to the next step to determine the options for management from the results of eDNA monitoring).

2. *Environmental DNA monitoring*

a) If eDNA monitoring does not detect any signs of invasive species present in the ecosystem, then periodic monitoring should still take place in case of future introductions.

b) If eDNA monitoring of the area detects strands of DNA belonging to an invasive species then proceed to the next stage of management (step 3).

3. *Aquatic invasive species management options*

a) Control and suppression methods of AIS fish populations factors:

Ecosystem is relatively isolated to other bodies of water (only a couple of outlets) and there are vulnerable species present in the area that may be impacted by more extreme methods of AIS treatment (rotenone application). Secured funding for long term operations must also be secured to continue the annual removals of the invasive species population. The below listed treatment methods are relatively species specific in terms of capture, in which follow-up ecosystem restoration measures are not required due to their minimum impacts to the environment.

i) fish barriers:

Prevents further introductions and/or spread of AIS into other ecosystems, however barriers should only be implemented if it is certain that native aquatic species in the area do not frequently migrate between the proposed area of barrier implementation. However, this factor maybe wavered if the planned barrier will be preventing further AIS access into ecosystems that are considered ecologically sensitive. In order to ensure optimal function of barriers, funding must be secured in order to maintain/repair the infrastructure.

ii) electrofishing:

Electrofishing should be the main source of AIS capture and removal if there is sufficient funding for operating this treatment. It should be noted that this method is less effective in areas that are deeper than six feet, have relatively dense vegetation, and/or the water is saline. Only individuals certified in operating electrofishing gear should be using this equipment due to safety concerns.

iii) scientific angling:

Scientific angling, after electrofishing, is the most efficient method for removing AIS fish from the area. In order to maximise the effectiveness of this type of treatment, research should be conducted to determine if there are any lure/bait preferences for the targeted invasive species that other native fish species may be less attracted towards (to reduce bycatch). Due to the relative simplicity of this type of removal method, there should also be volunteer encouragement to maximize the catch rate of the AIS. Potential volunteers should also be educated to distinguish between AIS and native species, to ensure there is minimal accidental release of invasive species and/or collection of native fish especially fish that are SAR.

iv) larval light traps

These traps play a crucial role where they target newly hatched/spawned larval fish populations, contrary to other control/suppression methods that exclusively target larger individuals.
In order to maximize the effectiveness of these traps, nesting habitats and/or spawning grounds of invasive fish species should be identified as well as the season/time that fish species spawn and deposit their eggs. In doing so, managers can then strategically position these traps during spawning season to target the invasive juveniles, which can help control these invasive populations by removing the newly spawned generation.

v) rotary screw traps

This trap is specialised in capturing fish against a strong enough current of water that can propel the drum of this device. However, rotary screw traps apart from larval light traps, is the least efficient method for fish removal and should only be utilized if the implementation of fish barriers is not optimal due to native migratory fish in the area. Rotary screw traps should be installed in areas in which the current of the water is strongest and constant, typically in outlets of ecosystems if the area is not isolated/landlocked.

b) Eradication methods of AIS fish populations factors:

Invaded ecosystem is connected to other bodies of water that have not been invaded by the AIS and there is a risk that the species may spread to other areas that are more ecologically sensitive and more biodiverse in which it is optimal to apply eradication methods to prevent expansion. Rotenone is the most used and approved form of AIS treatment for eradication goals in Canada; however, this method can cause unintended impacts to the treated ecosystem in which restoration projects should be introduced after successful application of this piscicide. Proceed to step 5 to review potential management tool options for ecosystem restoration in Nova Scotia.

4. *Ecosystem restoration procedures:*

a) EPT monitoring surveys through CABIN

Before the area is treated with rotenone, it is recommended that ecosystem indicator species such members of the EPT group is monitored first to determine a baseline/reference for taxonomic diversity and abundancy levels of these macroinvertebrates with the CABIN sampling method. This will provide managers with a guide for the overall progress and success of the recovery of the ecosystem if pre-rotenone treatment values are defined. The relative time for both EPT taxonomic diversity and abundancy levels to reach near pre-treatment values should take between 4-7 years with natural recovery and succession.

b) Fish re-establishment

As rotenone is a naturally occurring piscicide, the treated area will have most if not all fish species (both invasive and native) killed from oxygen starvation and other aquatic organisms that depend on gill respiration. Therefore, to achieve a near "restored" freshwater ecosystem, there must be an option in which the original fish species are able to repopulate. The first and easiest option is the natural re-establishment of fish communities if the ecosystem is well connected to other waterbodies and most native fish are not endemic to the treated area and are not a species at risk. However, if one or more of these requirements are not met, then waiting for natural repopulation of lost fish communities may not be the most viable option and efforts should then be focused on fish repatriation. In order to maximise the potential of this restoration strategy, the treated ecosystem should be monitored again to ensure there are no remaining AIS individuals/populations that survived. Translocation of fish species should only be done if the fish species are approximately the same to the original population in terms of behavioural characteristics and the remaining population after the others have been transferred can sustain its population. If fish hatcheries are instead used to rear the population that will be transferred to the area, then there should be considerable efforts made to make the hatcheries mimic their new habitat (depends on resources available) to promote natural behaviours necessary for survival.

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Appendix

EPT taxonomic diversity changes relative to time elapsed after rotenone treatment

Table 1a)

	Stage 1: </= 1 year monitoring post rotenone treatment	
site	pre-treatment taxa	post-treatment taxa
Utah station 1	26	19
Utah station 2	31	16
Utah station 3	36	19
Utah station 4	32	25
Ogna River (spring treatment)	16	17
Ogna River (fall treatment)	16	19
Big Hawk	10	9
Black	12	7
Blackfoot	15	12
Margaret	10	8

Table 1b)

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
pre-treatment taxa	10	204	20.40	97.38
post-treatment taxa	10	151	15.10	34.54

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	140.45	1	140.45	2.13	0.16	4.41
Within Groups	1187.30	18	65.96			
Total	1327.75	19				

Table 2a)

	Stage 2: 1</=3 years monitoring post rotenone treatment	
site	pre-treatment taxa	post-treatment taxa
Ogna River (summer)	16	13
Big Hawk	10	4
Black	12	10
Blackfoot	15	14
Margaret	10	8
Montana (stream 1)	27	22
Montana (stream 2)	28	22
Black	12	9
Blackfoot	15	15

Table 2b)

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
pre-treatment taxa	9	145	16.11	46.36
post-treatment taxa	9	117	13	37.25

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	43.56	1	43.56	1.04	0.32	4.49
Within Groups	668.89	16	41.80			
Total	712.44	17				

Table 3a)

	Stage 3: 4-7 years monitoring post rotenone treatment	
site	pre-treatment taxa	post-treatment taxa
Big Hawk	10	12
Black	12	11
Blackfoot	15	14
Margaret	10	10
Margaret	10	13
Utah station 1	26	21
Utah station 2	31	24
Utah station 3	36	32
Utah station 4	32	38
Big Hawk	10	14

Table 3b)

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
pre-treatment taxa	10	192	19.2	115.51
post-treatment taxa	10	189	18.9	93.21

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.45	1	0.45	0.0043	0.95	4.41
Within Groups	1878.50	18	104.36			
Total	1878.95	19				

Table 4a)

	Stage 4: 8 years monitoring post rotenone treatment

site	pre-treatment taxa	post-treatment taxa
Big Hawk	10	15
Black	12	16
Blackfoot	15	19
Margaret	10	13

Table 4b)

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
pre-treatment taxa	9	145	16.11	46.36
post-treatment taxa	9	117	13	37.25

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	43.56	1	43.56	1.042	0.32	4.49
Within Groups	668.89	16	41.80			
Total	712.44	17				

EPT abundancy changes relative to time elapsed after rotenone treatment

Table 5a)

	Stage 1: </= 1 year monitoring post rotenone treatment	
site	pre-treatment abundancies	post-treatment abundancies
New Zealand (2 months)	94	8
Ogna River (5 months)	731	2253
Ogna River (10 months)	731	4574

New Zealand (4-12 months)	94	101
Big Hawk	537	338
Black	204	56
Blackfoot	120	326
Haukvatnet	1464	609
Lianvetnet	3575	1681
Margaret	328	60
Theisendammen	7360	2253

Table 5b)

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
pre-treatment abundancies	11	15238	1385.27	4952309.82
post-treatment abundancies	11	12259	1114.45	2084555.87

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	403383.68	1	403383.68	0.11	0.74	4.35
Within Groups	70368656.91	20	3518432.84			
Total	70772040.59	21				

Table 6a)

	Stage 2: 1</=3 years monitoring post rotenone treatment	
site	pre-treatment abundancies	post-treatment abundancies
Ogna River	731	223
Big Hawk	537	23
Black	204	62
Blackfoot	120	384
Margaret	328	187
Montana stream 1	1040	930
Montana stream 2	490	930
Black	204	115
Blackfoot	120	99

Table 6b)

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
pre-treatment abundancies	9	3774	419.33	97685.25
post-treatment abundancies	9	2953	328.11	127555.11

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	37446.72	1	37446.72	0.33	0.57	4.49
Within Groups	1801922.89	16	112620.18			
Total	1839369.61	17				

Table 7a)

	Stage 3: 4-7 years monitoring post rotenone treatment	
site	pre-treatment abundancies	post-treatment abundancies
Big Hawk	537	430
Margaret	328	1381
Black	204	160
Blackfoot	120	434
Margaret	328	454
Big Hawk	537	652

Table 7b)

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
pre-treatment abundancies	6	2054	342.33	28953.87
post-treatment abundancies	6	3511	585.17	176543.37

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	176904.08	1	176904.083	1.72	0.22	4.96
Within Groups	1027486.17	10	102748.62			
Total	1204390.25	11				

Table 8a)

	Stage 4: 8 years monitoring post rotenone treatment	
site	pre-treatment abundancies	post-treatment abundancies
Big Hawk	537	909
Black	204	220

Blackfoot	120	930
Margaret	328	1526

Table 8b)

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
pre-treatment abundancies	4	1189	297.25	32846.25
post-treatment abundancies	4	3585	896.25	285066.92

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	717602	1	717602	4.51	0.078	5.99
Within Groups	953739.50	6	158956.58			
Total	1671341.50	7				