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SHORT COMMUNICATION OPEN ACCESS

Analysis of sea lamprey environmental DNA during lampricide treatment in a tributary of Lake Ontario

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Abstract – Sea lamprey (*Petromyzon marinus*) is a species of invasive parasitic fish in the Great Lakes region of North America. Accurate estimates of larval lamprey populations in lake tributaries are necessary for making control decisions regarding treatment of lake tributaries with 3-trifluoromethyl-4-nitrophenol (TFM), a piscicide toxic to lamprey larvae. Analysis of environmental DNA (eDNA) isolated from water samples is a recent innovation under consideration for sea lamprey biomonitoring. eDNA analysis was performed before and at three time points after TFM treatment in a tributary of Lake Ontario (Ninemile Creek) to assess presence of invasive sea lamprey. Lamprey DNA was detected in four out of four sample locations before TFM treatment and three of four locations 72 hours following treatment. No lamprey DNA was detected in any of the locations 4 months or 12 months after TFM treatment of the creek. These results are consistent with known effectiveness of TFM treatment and provide evidence for the potential of eDNA analysis as a tool for monitoring decline of larval sea lamprey abundance due to TFM treatments.

Keywords: Environmental DNA (eDNA) / sea lamprey / invasive species / TFM / lampricide / population monitoring

Résumé – Analyse de l'ADN environnemental de la lamproie marine lors d'un traitement au lampricide dans un affluent du lac Ontario. La lamproie marine (*Petromyzon marinus*) est une espèce de poisson parasite envahissante dans la région des Grands Lacs en Amérique du Nord. Des estimations précises des populations de larves de lamproie dans les affluents des lacs sont nécessaires pour prendre des décisions de contrôle concernant le traitement des affluents des lacs avec du 3-trifluorométhyl-4-nitrophénol (TFM), un toxique pour les larves de lamproie. L'analyse de l'ADN environnemental (eDNA) isolé à partir d'échantillons d'eau est une innovation récente envisagée pour la biosurveillance de la lamproie marine. L'analyse de l'eDNA a été effectuée avant et à trois moments après le traitement au TFM dans un affluent du lac Ontario (Ninemile Creek) pour évaluer la présence de lamproies marines envahissantes. L'ADN de la lamproie a été détecté dans quatre endroits sur quatre avant le traitement par TFM et dans trois endroits sur quatre 72 heures après le traitement. L'ADN de la lamproie n'a été détecté dans aucun des sites 4 mois ou 12 mois après le traitement TFM du ruisseau. Ces résultats sont conformes à l'efficacité connue du traitement TFM et prouvent le potentiel de l'analyse de l'ADN environnemental comme outil de suivi du déclin de l'abondance des larves de lamproie marine dû aux traitements TFM.

Mots-clés : ADN environnemental (eDNA) / lamproie marine / espèces envahissantes / TFM / lampricide / surveillance des populations

Petromyzon marinus, sea lampreys of the family Petromyzontidae are an invasive semelparous species within the Great Lakes basin of North America. Sea lampreys were first observed in Lake Ontario in the mid-1800s and fully invaded the Great Lakes basin through the early to mid 20th century (Christie and Goddard, 2003; Larson *et al.*, 2003).

The Great Lakes Fishery Commission (GLFC) was established in the 1950's to monitor sea lamprey populations and develop control methods because mortality caused by parasitic lampreys had devastating effects on populations of lake trout, *Salvelinus namaycush*, and salmonids among other

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host fish (Fetterolf, 1980; Christie and Goddard, 2003; Larson *et al.*, 2003). Since then, sea lamprey larvae have been identified in 66 tributaries of Lake Ontario (Adair and Sullivan, 2013; Hansen *et al.*, 2016).

Because hatched larvae (ammocoetes) burrow into sandy/silty locations for two to ten-plus years, before metamorphosing into the parasitic phase of their life cycle (Dawson *et al.*, 2015; Hansen *et al.*, 2016), current detection methods are expensive, labor intensive, and can be disruptive to vulnerable populations of several species including native lamprey (Gingera *et al.*, 2016). Methods include direct lamprey counting via electrofishing, trapping and application of granular bayluscide to tributaries (Slade *et al.*, 2003; Steeves *et al.*, 2003). Methods for population control can also be expensive and disruptive. For example, the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) is a mitochondrial uncoupler (Birceanu *et al.*, 2014) and is a primary control method for sea lampreys (Brege *et al.*, 2003; Christie and Goddard, 2003). Acute TFM toxicity is known to occur in several non-target species found in TFM-treated waters including the threatened lake sturgeon, *Acipenser fulvescens* (Boogaard *et al.*, 2003). In efforts to minimize off-target toxicity, the distribution, and concentration of TFM treatments has significantly decreased in tributaries (Brege *et al.*, 2003). The need to limit TFM treatments in waterways not inhabited by sea lamprey populations, and accurately determine lamprey abundance highlights the importance of developing non-invasive, inexpensive and accurate methods for detection of lamprey populations.

Analysis of environmental DNA (eDNA) is a molecular technique that can be effective in detecting relatively low DNA copy number from environmental samples such as soil, air and water from a wide range of species (Dougherty *et al.*, 2016; Gingera *et al.*, 2016). The ease, comparative low cost and non-invasive nature of conducting eDNA analysis can provide advantages over traditional population survey methods. In recent studies, eDNA techniques were used to detect the invasive rusty crayfish, *Orconectes rusticus,* in lakes within Gogebic County, Michigan and Vilas County, Wisconsin, USA. These efforts proved to be more successful than trapping detection methods (Dougherty *et al.*, 2016). eDNA was also shown to be more effective and sensitive in determining the threat of invasion of Asian carp species such as the silver carp, *Hypophthalmichthys molitrix,* and the bighead carp, *Hypophthalmichthys nobilis*, from the Mississippi River basin into the Great Lakes basin (Jerde *et al.*, 2011). Specific amplification of sea lamprey DNA has been performed from water samples collected from multiple sites across the Great Lakes basin (Gingera *et al.*, 2016). Since then, quantitative PCR (qPCR) analyses have been developed for sea lamprey that offer greater sensitivity (Bracken *et al.*, 2019; Schloesser *et al.*, 2018). With methodological improvements, these techniques are under consideration for implementation as a standard practice for determining lamprey abundance and distribution (Sullivan and Mullett, 2018). Nevertheless, eDNA analysis using end-point PCR is an inexpensive surveying technique that has the advantage of being relatively simple and accessible in many laboratories world-wide. The objective of this study is to evaluate the usefulness of a simple endpoint PCR assay to detect changes in lamprey environmental DNA before and after TFM treatment.

Ninemile Creek, a tributary to Lake Ontario which flows through parts of Cayuga and Oswego Counties, New York, USA, was treated with TFM in late May and early June of 2017. TFM treatment was conducted by employees of the Sea Lamprey Control Program of the GLFC administered by Fisheries and Oceans Canada and the United States Fish and Wildlife Service. Water samples were collected at four locations along Ninemile Creek (Fig. 1) on four separate dates: May 29, 2017 (early morning, pre-TFM treatment), June 2, 2017 (post-TFM treatment), September 22, 2017, and on June 9, 2018 one year following TFM treatment. Three water samples and a negative control sample consisting of commercially bottled drinking water were taken each time a sample was collected. Negative controls were collected and processed using the same protocol as the experimental samples to rule out on-site contamination.

Water sampling and DNA isolation were performed using the methods of Dougherty *et al.* (2016). Each water sample was collected in 250 mL sterile, individually wrapped Nalgene Analytical Test Filter Funnels (Thermo Fisher Scientific, Waltham, MA, USA). Each of the samples were filtered in the location where they were collected using 47 mm diameter cellulose nitrate (CN) membranes with 0.45 µm pores, a 1 L Nalgene Polypropylene Vacuum Flask (Thermo Fisher) and a CP7839 Hand Vacuum Pump (Actron, Warren, MI, USA). Each filtered CN membrane was removed using clean forceps and placed in 2 mL Eppendorf DNA LoBind microcentrifuge tubes (Thermo Fisher) containing 700 mL Longmire's buffer and placed in a dark, cold icebox (Longmire *et al.*, 1997). Following collection, microcentrifuge tubes were stored in the laboratory at 4 °C until DNA isolation.

DNA isolation from CN membranes was performed via chloroform/isoamyl alcohol extraction followed by isopropyl alcohol precipitation. DNA pellets were resuspended in 100 µl TE with low EDTA (10mM Tris, 0.1mM EDTA, pH 8.0).

Mitochondrial cytochrome oxidase C subunit I gene (COI) PCR primers were used in all analyses. Primer sequences and PCR conditions used were according to Gingera *et al.* (2016).

Forward: 5'-GGCAACTGACTTGTACCCCTAATAC-TTGGT-3'

Reverse: 5'-GGCTAAGTGTAAGGAAAAGATTGTTA-GGTCGAC-3'

Positive control DNA was obtained by performing PCR amplification of *P. marinus* genomic DNA using the above primers yielding a 225 bp product. Amplified DNA was gel isolated and quantified using a NanoDrop 2000 spectrophotometer (Thermo Fisher). Molecular mass of the expected 225bp DNA band was estimated (650 Da/bp) and serially diluted to 10^1 copies/µl. 5µl diluted stock (50 copies/reaction) were used as positive control template and was included in each experimental PCR.

The PCR performed for each eDNA extract consisted of 13.75 µl ultrapure H_2O, 2.5 µl BioReady rTaq 10X Reaction Buffer (Bulldog Bio, Inc. Portsmouth, NH, USA), 0.25 µl dNTPs (20mM), 2.5 µl primers (2.0 µM each), 1 µl (5U) BioReady rTaq (Bulldog), 5 µl template DNA in a total reaction volume of 25 µl. Cycling conditions were 95° C for 5 min followed by 55 cycles of 95 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s; 72 °C for 10 min with a terminal 4 °C hold. PCR products (expected band = 225 bp) were viewed on a 1.5%

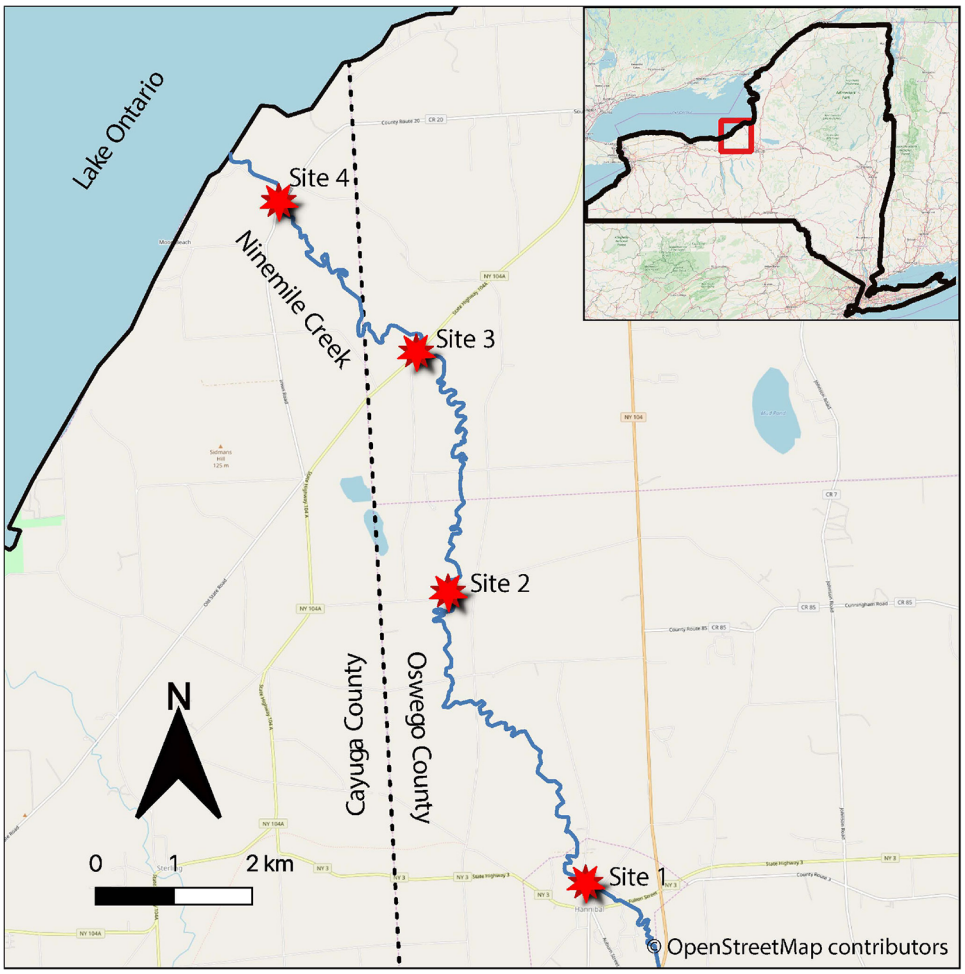

Fig. 1. Sampling sites along Ninemile Creek.

agarose gel stained with ethidium bromide. Each eDNA sample was subjected to a single PCR reaction.

All negative control samples collected during all four collection time points at each site were negative for sea lamprey DNA ($n = 16$). Seven and six total positive samples were found from the pre-TFM treatment time point and the immediate post-TFM treatment time point, respectively. All samples collected 16 weeks and one year post-TFM treatment from each site were negative for lamprey DNA (Tab. 1). Specificity of lamprey DNA amplification was analyzed via DNA sequencing of two positive PCR products (29-May-2017, site 4, sample 1; 2-June-2017, site 1, sample 1). 225bp PCR products were Sanger sequenced with both the forward and reverse PCR primers listed above. All sequence results aligned 100% with GenBank accession no. KX145312 (*Petromyzon marinus* COI).

Prior to the May 2017 TFM treatment, Ninemile Creek was treated in May 2014. An assessment of larval populations in the tributary conducted in August, 2016 estimated that residual larval lamprey – survivors from the 2014 treatment – and new larval populations were around 75,000 total larvae (Mullett and Sullivan, 2017). Ninemile Creek was subsequently treated with 182.4 kg liquid TFM, 2.0 kg Solid TFM and 0.2 kg granular bayluscide over a distance of 25.4 km in May, 2017. Our ability to detect sea lamprey presence pre-TFM treatment is consistent with this report on the state of sea lamprey infestation in Ninemile Creek at this timepoint. Our sampling sites spanned an approximate distance of 11.67 km from the opening of the tributary into Lake Ontario to Sample Site 1, the most upstream collection site. All sample sites fell within the range of the tributary that was treated. Exposure to TFM usually occurs over 12 hours for maximum lethality (Clifford *et al.*, 2012) and TFM concentrations for 2017 treatments were designed to eliminate 93% of the larvae in the population (Sullivan and Mullett, 2018).

Our detection of lamprey DNA four days post treatment in three of the four sample sites might be interpreted as detection of residual live larval lamprey. More probably, this result might reflect detection of DNA from decomposing larval carcasses. This finding is in line with the results of Gingera et al., (2016) where sea lamprey eDNA signals were detectable for several weeks following spawning and subsequent death of adults in the absence of TFM treatment. Future work is warranted to correlate TFM treatment efficacy, flow rate, weather, and other environmental parameters with temporal resolution of eDNA detection. Our finding of not detecting lamprey DNA 16 weeks

Table 1. Detection of sea lamprey DNA at corresponding sampling locations and dates.

Site locations	Replicate samples	Sampling date			
		29-May-2017 (pre-TFM treatment)	2-June-2017 (post-TFM treatment)	22-Sept-2017 (post-spawn)	9-June-2018
1 Lat:43.3241 Long:-76.5794	1	Yes	Yes	No	No
	2	No	Yes	No	No
	3	No	No	No	No
2 Lat:43.3575 Long:-76.6011	1	Yes	No	No	No
	2	Yes	Yes	No	No
	3	Yes	Yes	No	No
3 Lat:43.3850 Long:-76.6061	1	No	Yes	No	No
	2	No	No	No	No
	3	Yes	Yes	No	No
4 Lat:43.4022 Long:-76.6277	1	Yes	No	No	No
	2	Yes	No	No	No
	3	No	No	No	No

after TFM treatment is evidence of decrease in sea lamprey abundance to below a detectable level. This result alone, however does not yield precision data on the proportion of larval death. Caution should be taken as limitations in sensitivity of this assay led Gingera *et al.* (2016) to find false negatives in some lamprey infested waters. Nevertheless, this result agrees with the GLFC 2017 report in which larval populations assessed by electrofishing before and after treatment showed significant TFM-related mortality (Sullivan and Mullett, 2018). The August 2017 GLFC assessment found no evidence of residual populations and no recruitment was evident.

Sea lampreys within the Great Lakes usually enter the spawning phase of their life cycles after 12–18 months of parasitizing lake fish species (Hansen *et al.*, 2016). Starting in March, spawning lamprey travel into tributaries, often following a pheromone secreted by larval lampreys. Between March and July, lampreys spawn, and shortly thereafter die (Potter *et al.*, 2015). Our inability to detect lamprey DNA shortly following a spawning season (September, 2017) and a year following treatment (June, 2018) is further indication of population decline following TFM treatment. Since measurable larval populations were eliminated by August 2017 and no recruitment was evident (Sullivan and Mullett, 2018), pheromone-induced attraction of spawning adults to the creek may have been negligible (Bjerselius *et al.*, 2000; Hansen *et al.*, 2016). This may have contributed to our finding of no detectable lamprey DNA one year following TFM treatment. Overall, the results of this study suggest that there was a decline in larval populations post-TFM treatment that was detectable using eDNA surveying techniques.

Here, we took a simple endpoint PCR approach to eDNA analysis for presence/absence of a signal, with species specificity confirmed via DNA sequencing. Other techniques show promise for analytical improvements: Next-generation DNA sequencing/metabarcoding of environmental samples might provide insight into changes in population dynamics of multiple taxa in a single assay or in response to changing weather patterns or other environmental conditions (Valentini *et al.*, 2015; Cannon *et al.*, 2017). Likewise, qPCR assays could offer greater sensitivity (Bracken *et al.*, 2019; Schloesser *et al.*, 2018). Nevertheless, a major value of endpoint PCR is its simplicity, low cost, and availability in a wide range of academic and governmental laboratories. These factors make this technique a robust option for preliminary research and management-based molecular ecological monitoring, particularly in areas of low infrastructure. This study provides evidence for the utility of simple environmental DNA analysis in assessing population decline in aquatic environments.

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