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Quantifying relative fish abundance with eDNA: a promising tool for fisheries management

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Running title: Quantifying fish populations with eDNA

Summary

1. Assessment and monitoring of exploited fish populations are challenged by costs, logistics and negative impacts on target populations. These factors therefore limit large-scale effective management strategies.

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2. Evidence is growing that the quantity of eDNA may be related to not only species presence/absence, but also to species abundance. In this study, the concentrations of environmental DNA (eDNA) from a highly prized sport fish species, Lake Trout *Salvelinus namaycush* (Walbaum 1792), was estimated in water samples from 12 natural lakes and compared to abundance and biomass data obtained from standardized gillnet catches as performed routinely for fisheries management purposes. To reduce environmental variability among lakes, all lakes were sampled in spring, between ice melt and water stratification.
3. The eDNA concentration did not vary significantly with water temperature, dissolved oxygen, pH and turbidity, but was significantly positively correlated with relative fish abundance estimated as catch per unit effort (CPUE), whereas the relationship with biomass per unit effort (BPUE) was less pronounced.
4. The value of eDNA to inform about local aquatic species distribution was further supported by the similarity between the spatial heterogeneity of eDNA distribution and spatial variation in CPUE measured by the gillnet method.
5. *Synthesis and applications*. Large-scale empirical evidence of the relationship between the eDNA concentration and species abundance allows for the assessment of the potential to integrate eDNA within fisheries management plans. As such, the eDNA quantitative method represents a promising population abundance assessment tool that could significantly reduce the costs associated with sampling and increase the power of detection, the spatial coverage and the frequency of sampling, without any negative impacts on fish populations.

Key-words: conservation genetics, gillnetting, monitoring, qPCR, salmonid, species-specific detection, water sampling,

Introduction

Inland fisheries management requires fish population assessments based on sound quantitative data on broad spatial and temporal scales. Fish population assessment and monitoring are in most cases implemented by governmental agencies and most of them are based on standard sampling methods using gillnets as sampling gear (Bonar, Hubert & Willis 2009; Pope, Lochmann & Young 2010; SFA 2011; Sandstrom, Rawson & Lester 2013). Estimating population abundance is one of the most common objectives of fisheries management. Methods for estimating absolute abundance (e.g. mark-recapture techniques) are often time-consuming and expensive and are consequently excluded from standard monitoring (Quist, Bonvechio & Allen 2009). Relative abundance estimation using gillnet data provides an index of absolute abundance, and is assumed to be directly proportional to density (Hubert & Fabrizio 2007). This is the most common method used to estimate fish abundance in lakes, giving abundance estimates precise enough for fisheries management (Bonar, Hubert & Willis 2009; SFA 2011; Sandstrom, Rawson & Lester 2013). However, these monitoring techniques may also be expensive in terms of financial and human resources, especially when performed in remote regions. There is also a general consensus that collection of data on fish populations should be conducted in a way that minimizes habitat disturbance and mortality (UFC 2014). There are alternative field collection techniques causing no mortality in the population being sampled, including hydroacoustic techniques, snorkelling surveys and fish ladder counts. However all of these also have limitations in a management context.

The analysis of environmental DNA (eDNA) could be a revolutionary tool to overcome logistical constraints when collecting fish population data (Lodge *et al.* 2012). The eDNA method can detect traces of DNA in cellular or extracellular form from sources such as feces,

secreted mucous membranes, gametes and skin cells (Lydolph *et al.* 2005; Haile *et al.* 2009; Taberlet *et al.* 2012). More specifically, eDNA released by fish in natural ecosystems is likely to originate predominantly from either mitochondria or cells and rapidly degrades/settles (Barnes *et al.* 2014) or settle (Turner *et al.* 2014; Turner, Uy & Everhart 2015) following release. The popularity of this method to assess presence/absence is growing fast and expectation of using species inventories collected via eDNA instead of traditional sampling methods is gaining broad interest (Jerde *et al.* 2011; Dejean *et al.* 2012; Thomsen *et al.* 2012a; Jerde *et al.* 2013; Mahon *et al.* 2013; Pilliod *et al.* 2013a).

Results from aquaria and small ponds suggest that eDNA could also be used to estimate population abundance (Thomsen *et al.* 2012b) or total biomass (Takahara *et al.* 2012; Pilliod *et al.* 2013a; Kelly *et al.* 2014; Klymus *et al.* 2015). Using eDNA concentration to estimate fish abundance could facilitate rapid sample collection, reduce the costs associated with data collection, and avoid negative consequences on the organisms of study (Lodge *et al.* 2012; Taberlet *et al.* 2012). This method has great potential to increase the power of detection as well as spatial coverage and frequency of sampling (Rees *et al.* 2014). Integrating eDNA analysis in fisheries management could help standardize fish population monitoring and enable early detection of declines in abundance. However, empirical studies supporting the clear occurrence of a relationship between eDNA concentration and species abundance in natural environments are still lacking.

Testing whether eDNA is a reliable indicator of species abundance or biomass represents a technical challenge, namely due to the uncertainty associated with estimated abundance and biomass. Also, eDNA concentration is expected to vary strongly with local biotic and abiotic factors, yet little is known about the effect of environmental factors on the production and

degradation of eDNA in natural ecosystems (Rees *et al.* 2014). In freshwater ecosystems, the concentration of eDNA is expected to vary as a function of the amount of eDNA released by individuals, the density of organisms, environmental conditions and the action of fungi and bacteria (Shapiro 2008; Takahara *et al.* 2012; Thomsen *et al.* 2012b; Barnes *et al.* 2014). Seasonal variation in environmental conditions is therefore likely to influence the concentration of eDNA due to the changing behaviour of the species, water stratification, temperature and ultraviolet radiation (Zhu 2006; Pilliod *et al.* 2013b). Overall, DNA fragments have been shown to persist for only a few weeks in freshwater ecosystem (Dejean *et al.* 2011; Thomsen *et al.* 2012b; Goldberg *et al.* 2013; Pilliod *et al.* 2013b; Piaggio *et al.* 2014; Strickler, Fremier & Goldberg 2015).

This study aimed to test the relationship between eDNA concentration and relative fish abundance as well as biomass using a large data set based on water samples from natural lakes and standardized population assessment. Specifically, we compared eDNA data to gillnet data of Lake Trout *Salvelinus namaycush*. Typical of cold and well oxygenated water in North America, this salmonid is highly prized by anglers, particularly in Québec, Canada, where there is a current priority to streamline and standardize large-scale population surveys to protect the species and ensure the sustainability of the fishery (MRNF 2011). In this context, we (i) developed specific primers and a qPCR probe for Lake Trout, (ii) documented the intra- and inter-lake variation in eDNA and (iii) tested whether eDNA concentration varies significantly with gillnet data and/or environmental conditions.

Materials and methods

STANDARDIZED GILLNET DATA

Twelve Lake Trout populations were selected for this study, covering the southern portion of the species distribution in Québec, Canada (Fig. 1). The size of the twelve sampled lakes varied between 44 and 6702 hectares (Table 1). These populations are part of the Québec Lake Trout monitoring program (MRNF 2011) and were sampled between 2007 and 2013. The government wildlife agency of Québec conducts standardized indexed gillnet surveys in about 50 Lake Trout populations visited every five years (MRNF 2011). The catches are used to estimate Lake Trout relative abundance and biomass as well as other population parameters (mortality, growth, etc.). Surveys are conducted in late summer by deploying standard multi-panel gillnets with meshes ranging from one to six inches, fishing overnight for approximately 24 hours with the number of stations sampled being proportional to the lake size (SFA 2011). These methods are based on North American standards (Lester, Bailey & Hubert 2009; Sandstrom, Rawson & Lester 2013). Relative indices of population abundance are expressed as catch per unit effort (CPUE, number of catch per overnight set) and biomass per unit effort (BPUE, average of total mass per overnight set) and are estimated using eight to 50 sampling sites depending on the lake size. Populations selected for this study cover a large gradient of fish abundance.

eDNA SURVEY

Water was sampled in all twelve lakes during the month of May 2013. In each lake, 1L of water was collected at 10 different sites, which were randomly distributed over the entire surface area. To ensure water column homogeneity, and to increase the similarity of environmental conditions between lakes, sampling was conducted in early spring, before thermal stratification

of the lake. At this period of the year, physico-chemical conditions are more homogenized and Lake Trout are much more likely to be evenly spatially dispersed in lakes compared to when lakes are stratified and they have to seek cold thermal refugia. Water samples were collected in the form of integrated samples taken from 0–5 m of depth. Integrated samples are assumed to be representative of the entire water column since they are collected in a non-stratified period of the year and may also reduce stochastic effects. Within each lake, water column temperature, dissolved oxygen, pH and turbidity (Secchi disc) were recorded at every meter above the deepest location in the lake (Table 1). To minimize eDNA degradation, samples were stored on ice until arrival at the lab (< 24 h) where samples were filtered through a 1.2µm glass microfiber filter (Whatman GF/C) using a peristaltic pump (Cole-Parmer: Masterflex L/S Modular Drive). Filters were then frozen at -20 °C until eDNA extraction. Sampling and filtration equipment was sterilized between each sample with 10 % chlorine bleach and rinsed with distilled water; this method was found to be efficient for control samples (Lacoursière-Roussel *unpublished data*). Different sampling teams using multiple filtration equipment surveyed the lakes, limiting the risk of cross-contaminations at the water sample collection step and filtration step between lakes.

MOLECULAR ANALYSES

DNA was extracted using the QIAshredder and DNeasy Blood and Tissue Kit (Qiagen, Inc (Venlo, Netherlands)) method from Goldberg *et al.* (2011) adapted for Whatman GF/C filters. Similarly to several eDNA studies (Takahara *et al.* 2012; Thomsen *et al.* 2012b; Pilliod *et al.* 2013a; Wilcox *et al.* 2013), we used quantitative PCR (qPCR, or real time PCR) to estimate eDNA concentrations due to the increased sensitivity compared to traditional PCR (Ellison *et al.* 2006; Wilcox *et al.* 2013). We used the TaqMan MGB™ technology with a 7500

Fast Real-Time PCR system (LifeTechnologies), which requires constructing primers and probes to amplify short DNA fragments and is very sensitive to mismatches. To reduce the effect of PCR inhibitors, all samples were diluted by a factor of five and amplified using Environmental Master Mix 2.0 (Life Technologies). TaqMan® Exogenous Internal Positive Control Reagents (Life Technologies) also supported no deviation of the amplification curves potentially caused by inhibitors among lakes (Appendix S1 in Supporting Information). Standard curves were constructed from whole genomic DNA extracted from fish tissues and diluted to absolute total values of 0.000256, 0.00128, 0.0064, 0.032, 0.16, 0.8 and 4 ng determined by PicoGreen® fluorescent staining (Quant-iT™ Invitrogen™ Molecular Probes®). Appendix S1 presents the details regarding extraction and amplification methods. The amplification was replicated six times for each sample in each lake. Six negative qPCR controls were run per plate.

The species-specific primers used to amplify the targeted 66bp of the mitochondrial COI gene were: LakeTrout_COI_F (5'- GGGCCTCCGTTGATTTAACTATC -3'), LakeTrout_COI_R (5'- TGGCCCCTAAAATTGAGGAA -3'), LakeTrout_COI_Probe (5'- CTCTCTTCATTTAGCTGGC -3'; position 369–434 of the 652bp COI fragment). To ensure species-specific amplification, primers and probes were designed to optimize the number of mismatches to other salmonid species that may coexist in the system, and to optimize the position of the mismatches (i.e. near 3' (Wilcox *et al*. 2013)). Sequences from salmonid species known to coexist with Lake Trout were aligned in Geneious 6.0.6 and primers and probes were designed using Primer Express 3.0 software (Life Technologies; see Appendix S2). The specificity of primers and probes were tested on tissue extracted DNA of four salmonids which may be found in the same lakes as Lake Trout in Québec (Rainbow Trout *Oncorhynchus mykiss*, Brook Charr *Salvelinus fontinalis*, Cisco *Coregonus artedi* and Lake Whitefish *Coregonus*

clupeaformis) and subsamples of the amplified products were sequenced to confirm that these truly represent Lake Trout DNA (Appendix S3). Moreover, the fact that lakes with some of the highest CPUE and BPUE values (Seneca and Maganasipi; Table 1) did not harbor other salmonid species support the contention that high eDNA values are not related to the amplification of closely related species.

STATISTICAL ANALYSIS

All statistical analyses were performed using R 3.0.3. Except for the Linear Mixed-Effects Model (see below), the quantification of the eDNA amount per site was obtained by averaging successful amplification replicates (Ellison *et al.* 2006).

To evaluate the variability of eDNA concentration within lakes, the Shapiro-Wilk test was used to verify the normality of distributions within lakes using the *shapiro.test()* function of the STATS library and Levene test for assessing the equality of variances among lakes using the function *leveneTest()* of the CAR library. The spatial autocorrelation of the amount of eDNA was evaluated to determine if closer samples showed similar eDNA concentration using Moran's *I* index from the *Moran.I()* function of the APE library; the Moran's *I* value reported is the I_{observed} less the value of I_{expected} under the null hypothesis. The precision of the within-sample unit replication and the spatial eDNA distribution was evaluated for each lake from the relative standard deviation error (RSE); over 20% is generally considered as high heterogeneity or inadequate sample replication (McCune & Grace 2002). To determine if the spatial variability of eDNA concentration within lakes was due to the spatial species distribution, eDNA variance was compared to the size of lakes and to variances generated from gillnet data among lakes using Spearman correlations using the *cor()* function of the STATS library.

To obtain linear unbiased predictors and to compare the effect of CPUE, BPUE and environmental conditions on eDNA concentration, a hierarchical Linear Mixed-Effects Model (LME) was used. In this model, the uncertainty of CPUE and BPUE measurements were considered by integrating 1000 permutations of each measure, generated from a normal distribution with a mean and standard deviation specific to each lake. This is an extension of a type II regression model with random effects (Sokal & Rohlf 1995; Legendre & Legendre 1998). After each iteration, the model's parameters were estimated with their corresponding standard error. Final parameters were calculated by averaging the corresponding parameters, and variances were calculated by considering the variance for each iteration and among iterations. *Sampling site* and *qPCR amplification* were used to integrate random technical and biological variability respectively. *qPCR amplification* was nested in *site* (6 amplifications per site) and *site* nested within *lakes* (10 sites per lake). CPUE and BPUE models were run separately using the function *lme()* of the NLME library. AIC scores was used to identify which model between CPUE and BPUE best predicts the observed eDNA data (Johnson & Omland 2004). However, BPUE data was missing in Seneca Lake and so this lake was removed from AIC comparisons. Since pH values could be recorded in only ten lakes, effect of pH was tested separately from the other environmental variables. A square root transformation was applied to normalize residuals based on Box-Cox power transformation with the *boxcox()* function of the MASS library.

Results

LAKE TROUT eDNA DETECTION IN NATURAL LAKES

Lake Trout eDNA was found in all sampled lakes. Among all sites in all lakes, the eDNA concentration varied from 2.6 to 4278.7 pg L^{-1} (average amplification within two different sites, both found in Sacacomie). No amplification of negative controls was observed in any of the assays. The R^2 values for the qPCR standard curve ranged from 0.993 to 0.999, and the efficiency ranged from 0.86 to 0.99.

eDNA VARIANCE WITHIN AND AMONG LAKES

Over the ten sites, standard deviation (SD) around eDNA average concentrations ranged from 19.9 to 1344.0 pg L^{-1}, depending on the lakes (Table 2). While variance of the eDNA concentration among amplifications was low (SD = 27.3 in average within sites), high eDNA spatial heterogeneity was observed within lakes (SD = 358.8 in average among sites; Table 2 and Fig. 2). The variance of eDNA concentration was not correlated to lake size ($R^2 = 0.005$) and the eDNA concentration was not more similar among closer samples than distanced ones. Only one lake showed significant spatial autocorrelation among sites (Mégantic, $P = 0.02$, $I = 0.11$ and $P > 0.1$ for the other lakes). The precision of eDNA concentration measurements did not depend on the averaged eDNA concentration; RSE ranged over 20% for 11 lakes (Table 2) and was not correlated to the average eDNA concentration within lake ($R^2 = 0.14$).

Extreme measurement of eDNA concentration may hypothetically be caused by sampling cells, other organic tissues or suspended sediment and potentially lead to abundance overestimation (Klymus *et al.* 2015; Turner, Uy & Everhart 2015). In our data, beyond 1.5 × IQR (interquartile range) of the overall distribution, there was a clear gap between concentrations

of an average 243 pg L^{-1} and extreme values of 848.1 – 1091.3 pg L^{-1}. All values >848.1 pg·L^{-1} were therefore flagged as outliers. Excluding outliers ($N = 7$), the maximum average estimated eDNA concentration was 480.6 pg L^{-1} (found in Seneca) and the highest standard deviation was 255.9 and found in Massawipi. eDNA concentration was not normally distributed within lakes when including all samples (i.e. $P < 0.01$ for all lakes except for Maskinongé and Massawipi), but the eDNA concentration was normally distributed for the majority of lakes when excluding outliers, except for Montauban, Vermont, 31-Miles and Matapédia Lakes ($P < 0.01$)). To reduce the influence of individual sites with extreme values, outliers were therefore excluded from further analyses and figures. Without outliers, the variance of eDNA concentration was significantly different among lakes ($P = 0.003$; Fig. 2), but was not correlated to lake size ($R^2 = 0.02$, $P > 0.05$). RSE was lower than 50% for all lakes and lower than 20% for four lakes (Brompton, Maganasipi, Maskinongé, Seneca; Table 2) and was not correlated to the eDNA concentration ($R^2 = 0.16$, $P > 0.05$).

The spatial heterogeneity observed from eDNA data was similar to the one observed from gillnet data. Namely, variance of fish abundance in terms of CPUE measured by gillnet catches was significantly correlated to the variance of eDNA concentration ($R^2 = 0.61$) although variances in BPUE and eDNA were not strongly correlated ($R^2 = 0.11$; Fig. 2).

eDNA CONCENTRATION CORRELATES WITH FISH ABUNDANCE

A significant association between eDNA concentration and fish abundance estimated from gillnet catches was found. While the measured abiotic factors varied among lakes (Table 1), none had a significant effect on eDNA concentration in the fitted models (Table 3). Although variance among gillnet catches was included within the model, eDNA was significantly

correlated to CPUE (R^2 = 0.45, P = 0.02, AIC = 2273.8). The relationship with BPUE was less pronounced and not significant (R^2 = 0.39, P = 0.42, AIC = 2276.5). Brompton was the only lake found outside of the confidence interval (i.e. all amplifications were found outside of the 95% confidence interval of the model; Fig. 3).

Discussion

Integrating eDNA with standard population assessment and monitoring could be of great value to improve the temporal and spatial data sets used for inland fisheries management. Due to the recent development of the technique, few studies (any organism included) have investigated the relationship between the concentration of eDNA in water samples collected in the field and the number or biomass of target individuals, particularly so in an applied fisheries and wildlife management context. For amphibians, Thomsen *et al.* (2012b) showed a correlation between eDNA concentration in natural ponds, and the density of individuals, and Pilliod *et al.* (2013a) found a correlation with density, biomass, and the occupied proportion of transects in streams. Takahara *et al.* (2012) found a positive correlation between fish biomass and eDNA concentration in two experimental ponds and Mahon *et al.* (2013) reported that positive detection increased with relative abundance of six fish species in the Chicago area waterway system. Our data adds to these studies and supports the view that eDNA may be used to trace the presence of Lake Trout and that eDNA offers potential to become a very useful management tool for estimating a relative abundance index for exploited fish populations assessment and monitoring. To the best of our knowledge, this is the first empirical demonstration of quantitative similarity between eDNA and gillnet data sets in order to estimate relative fish abundance among exploited lakes.

While it is clear that more in-depth studies need to be conducted pertaining to the understanding of processes responsible for eDNA diffusion in waterbodies, the results of this study support the view that eDNA can be used to estimate abundance of aquatic species and is likely to become an important management tool in ecology and management. Here we found that variance among eDNA samples within lakes was correlated to variance observed among CPUE values measured within lakes. It is likely that the variance in eDNA concentration is partly caused by the same stochastic factors that affect CPUE measurements. CPUE estimates are known to vary widely because fish distribution is patchy in space and time due to their distribution and activity patterns (Hubert & Fabrizio 2007). CPUE is defined mathematically as $CPUE = qN$ where q is the catchability coefficient or the probability of catching an individual in one unit of effort and N the absolute abundance of fish in the population. An interaction of biotic and abiotic factors influences the spatial distribution and movement of Lake Trout and its catchability in gillnets (Janoscik 2001) and we hypothesize that such factors may also influence the distribution of eDNA in lake volume. Like CPUE, we argue that eDNA may be considered an index of abundance, also influenced by a "catchability" coefficient, itself a mixture of detection and diffusion factors.

The relationship between CPUE and BPUE is not so straightforward. Indeed, for the same number of fish, two populations may exhibit differences in BPUE due to intrinsic life history characteristics. For instance, Lake Trout exhibit different ecotypes in lakes mainly due to differences in environmental conditions and/or prey field and feeding strategies (Zimmerman *et al.* 2009; McDermid, Shuter & Lester 2010). In this study, almost all populations were known to be strictly piscivorous, but two were known to be strictly planctivorous (Seneca & Sacacomie), and one (Maganasippi) exhibited both ecotypes (MFFP, *unpublished data*). Average size at age

is very different between these ecotypes, the planctivorous being much smaller (Bernatchez *et al*. *unpublished data*). Interestingly, the concentration of eDNA correlated more closely to CPUE than BPUE. The non-significant relationship between eDNA concentrations and biomass could potentially be explained by the fact that bigger fish do not necessarily produce more eDNA. An alternative and perhaps more likely explanation could be that these observations reflects the fact that a greater number of fish will distribute the eDNA more homogenously within lakes, thereby increasing the probability of eDNA detection when surveying large lacustrine systems.

The concentration of eDNA depends on the amount of DNA released from the organisms and the rate of DNA degradation (Dejean *et al.* 2011), both of which are expected to vary seasonally according to the ecology of the species and its environment (Goldberg *et al.* 2011; Barnes *et al.* 2014). Indeed, significant seasonal change in fish eDNA concentration has previously been reported (Turner *et al.* 2014). Higher temperatures increase DNA degradation by denaturing DNA molecules and increasing enzyme kinetics and microbial metabolism, but also have a significant effect on growth, metabolism, physiology, and immune function in fish and may therefore increase eDNA release, as suggested by Takahara *et al.* (2012). In this study, environmental conditions did not explain the eDNA variability observed within and among lakes. The latter is likely due to limited variation in the measured environmental conditions between lakes as a result of the sampling strategy whereby all samples were collected in a small period of time between ice cover melt and thermal stratification. Water temperature varied among lakes but can be considered as cold overall. Lake Trout habitat is defined by temperatures <15 °C and dissolved oxygen concentration > 4 mg L^{-1} (Plumb & Blanchfield 2009). In early spring, Lake Trout are typically found in the entire water column but as the thermal stratification begins, Lake Trout becomes confined to the hypolimnion where the species' environmental

criteria are met (Evans 2007). At the time of eDNA sampling in the 12 lakes, temperature and dissolved oxygen habitat criteria were met in the entire water column so Lake Trout was presumed to be randomly distributed in the lake volume (Martin & Olver 1980), thus potentially liberating eDNA more randomly in the water column. If sampling had taken place in summer at the time of thermal stratification, we hypothesize that eDNA would have been concentrated and isolated in the hypolimnion and would possibly rapidly settle (Turner *et al.* 2014). Lake Trout spawn during the fall (Scott & Crossman 1973), such that if sampling had been conducted at that time, the aggregation of fish and the release of sexual products to the environment would likely have led to biased and less representative eDNA concentrations compared to measurements taken in the spring. While high variance among sampling sites might be attributed to the nature and degradation stage of eDNA, the fact that eDNA was not spatially concentrated in a single area of the lakes is consistent with the expected spatial distribution of Lake Trout in spring.

From the perspective of better predicting relative fish abundance from eDNA concentration in natural systems, we propose that the timing of sampling should be standardized for a given species by considering seasonal variation in species behavior (i.e. aggregation, spawning migrations, period of larval development, philopatry and vertical movements) and temporal and spatial distributions of eDNA. Collecting gillnet data is costly and time consuming, and managers therefore have to deal with a certain level of risk to base decisions on CPUE and BPUE that cannot often be measured every year due to cost and logistical constraints. Similarly, in the present work, population abundance may have changed since the last gillnet data collection, but it was not possible to obtain CPUE estimates for the same year that eDNA was sampled due to the above constraints. It is therefore possible that eDNA may have provided a better estimation of the population abundance than gillnet data for 2013. The eDNA method

does not provide population parameters (e.g. fish condition, sex ratio and growth), but is likely to become an effective complementary method to gillnetting for increasing the spatial and/or temporal scale of a data set, or for allowing the sampling of remote and difficult to access regions. Importantly, the potential for false positives and negatives should still be taken into consideration (Roussel *et al.* 2015), but recent method improvements now offer a range of different manipulations to meet these technical challenges. The effect of different biodiversity compositions on primer efficiency should also be clarified (e.g. LOD/LOQ assessment). The limited understanding of how environmental conditions alter eDNA concentration also limits the use of DNA models such as the ones presented here to systems with similar environmental conditions.

CONCLUSION AND FUTURE PERSPECTIVES

In the last few years, eDNA has been shown to be successful in monitoring the presence/absence of rare, endangered, indicator and invasive species, assessing biodiversity and determining species historical patterns of distribution, population dynamics, ecosystem health and trophic interactions (Ficetola *et al.* 2008; Jerde *et al.* 2011; Mahon *et al.* 2013; Díaz-Ferguson & Moyer 2014; Mächler *et al.* 2014; Piaggio *et al.* 2014; Rees *et al.* 2014). Our results indicate that eDNA may additionally be used to quantify fish relative abundance in lakes. From a fisheries management perspective, such eDNA analyses represent a new step towards improving spatial and temporal coverage of population assessment and monitoring while being less invasive, less time consuming and less expensive. While resources generally support the survey of only a few lakes per year using the gillnet method, here eDNA sampling covering southern Québec was achieved in seven sampling days and required two technicians working on average

less than 2 h to cover an entire lake. With the use of buffer to preserve samples at room temperature (Renshaw *et al.* 2014) and new, rapid, accurate and portable biotechnology (e.g. the use of Light Transmission Spectroscopy (Egan *et al.* 2013)), eDNA offers huge potential in helping to overcome the logistical issues related to sampling in remote regions. We thus envision a bright future for eDNA quantification in a fisheries management context.

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Data Accessibility

eDNA and environmental data set are available from the Dryad Digital Repository doi:10.5061/dryad.6g53s (Lacoursière-Roussel *et al.* 2015).

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Supporting Information

Additional supporting information may be found in the online version of this article:

Appendix S1. eDNA extraction and amplification method.

Appendix S2. Primer and probe sequences showing mismatches to closely related salmonid species that may occur in the sampling area.

Appendix S3. Method used to ensure specificity of primers and probes.

Table 1. Gillnet data information (i.e. sampling year, number of sites, CPUE and BPUE and their variance), environmental conditions for each sampled lake (i.e. surface area, maximum depth, temperature, dissolved oxygen, pH and Secchi depth) and the presence of closely related salmonid species (i.e. Rainbow Trout *Oncorhynchus mykiss*, Brook Charr *Salvelinus fontinalis*, Cisco *Coregonus artedi* and Lake Whitefish *Coregonus clupeaformis*). Units are given in parenthesis, horizontal traits represent the missing data and X indicates that a species is present in the lake.

	Gillnet data information						Environmental conditions						Related species			
Lake	Year of data collection	No of sites	CPUE	CPUE Variance	BPUE	BPUE Variance	Surface area (hectares)	Max depth (m)	Temperature (°C)	Dissolved oxygen (mg L^{-1})	pH	Secchi (m)	*O. mykiss*	*S. fontinalis*	*C. artedi*	*C. clupeaformis*
Brompton	2010	14	2.1	3.2	1.6	1.2	1191	42.0	9.0	7.6	6.4	3.4	x			
Maganasipi	2007	14	13.3	52.2	14.0	80.9	919	—	5.9	11.2	6.3	7.0				
Massawipi	2012	18	8.6	22.1	8.2	14.5	1792	86.0	7.5	7.5	7.1	3.5	x	x	x	x
Maskinongé	2010	13	1.5	3.6	3.9	23.1	1018	28.0	7.4	11.1	5.9	2.0				
Matapédia	2009	41	5.1	6.3	6.3	27.9	3807	42.5	6.8	14.7	—	4.5		x		x
Mégantic	2011	27	1.6	3.2	2.1	5.5	2692	75.0	7.0	7.4	6.7	2.5	x	x		x
31-Miles	2013	50	3.0	4.6	3.6	12.2	4973	88.0	6.6	12.5	7.9	6.0			x	x
Montauban	2012	10	1.6	5.2	3.6	40.8	456	30.0	6.2	10.0	5.6	3.3		x		x
Sacacomie	2013	10	1.6	1.2	1.0	0.8	974	77.4	5.0	10.4	5.7	11.1		x		
Seneca	2010	8	27.5	34.3	—	—	44	—	6.0	10.2	6.3	6.3				
Témiscouata	2013	37	4.5	7.9	7.3	28.9	6702	75.0	6.1	14.4	—	4.0		x		x
Vermont	2012	10	3.2	2.4	4.2	10.1	849	35.0	7.4	11.5	6.2	6.0				x

Table 2. Lake abbreviations and the average, median, variance, standard deviation and relative standard error (RSE in percentage) of eDNA concentration among the 10 sampling sites for each lake with and without the outliers (i.e. 7 eDNA concentration values >848.1 pg·L^{-1})

Lake	Mean eDNA (pg L^{-1})	Median eDNA (pg L^{-1})	Variance eDNA	Standard deviation	RSE (%)
Complete data set					
Brompton	747	433	627274	792	34
Maganasipi	693	395	437240	661	30
Massawipi	308	196	65503	256	26
Maskinongé	34	33	397	20	19
Matapédia	82	37	13535	116	45
Mégantic	259	135	118253	344	42
31-Miles	111	48	15331	124	35
Montauban	30	18	1197	35	37
Sacacomie	455	20	1806416	1344	97
Seneca	623	437	236923	487	25
Témiscouata	73	55	4350	66	29
Vermont	40	21	3685	61	48
Without outliers					
Brompton	416	369	41191	203	17
Maganasipi	430	343	43865	209	17
Massawipi	308	196	65503	256	26
Maskinongé	34	33	397	20	19
Matapédia	82	37	13535	116	45
Mégantic	155	132	12300	111	23
31-Miles	111	48	15331	124	35
Montauban	30	18	1197	35	37
Sacacomie	30	19	1018	32	34
Seneca	481	416	37171	193	13
Témiscouata	73	55	4350	66	29
Vermont	40	21	3685	61	48

Table 3. Comparison among predictor variables (SE = standard error, z = z-statistic, *P* = *P* - values of the z-statistic) for both hierarchical Linear Mixed-Effects models separately (i.e. CPUE and BPUE). Models included all predictor variables. pH values were missing within two lakes and therefore pH results reported here are based on separate analyses excluding those lakes

Predictor variables	CPUE model			BPUE model		
	SE	*z*	***P***	*SE*	*z*	***P***
Gillnet data	0.24	2.31	0.02	0.56	0.80	0.42
Temperature	2.07	1.22	0.22	2.54	1.05	0.29
Dissolved oxygen	0.68	-1.12	0.26	0.87	-1.05	0.30
pH	2.75	0.72	0.47	3.13	0.69	0.49
Secchi depth	0.77	0.50	0.61	0.97	0.58	0.56
Site depth	0.07	-0.98	0.33	0.07	-1.42	0.16

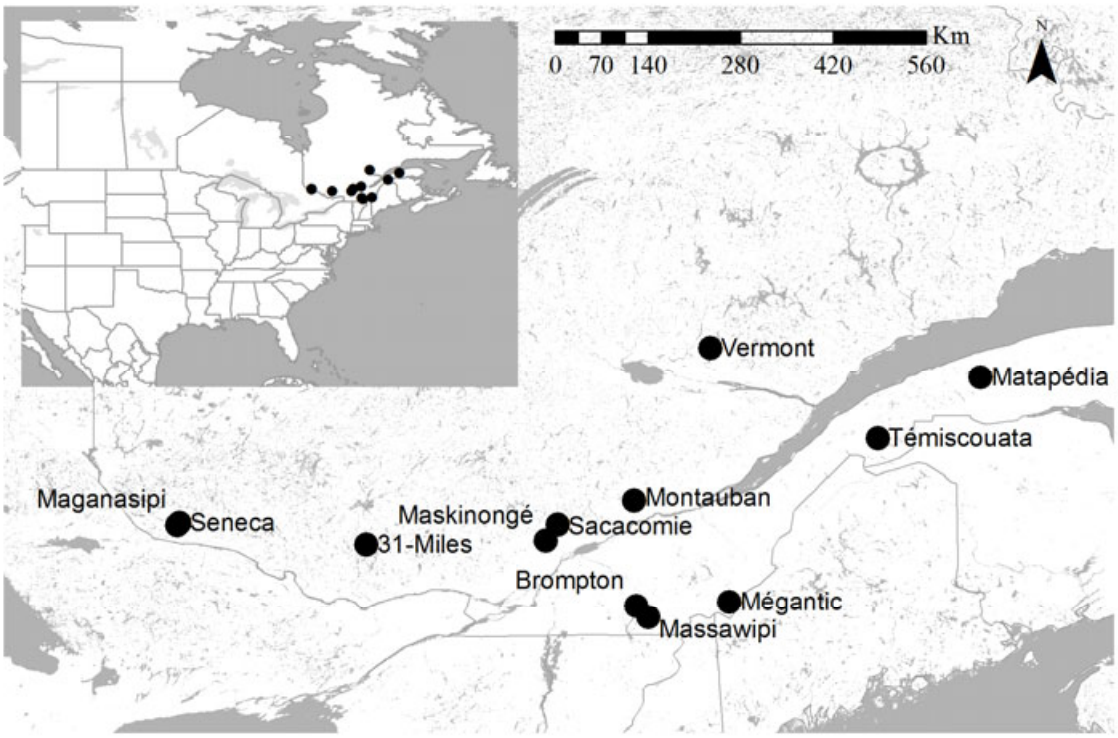

Fig. 1. Geographical locations of the sampled lakes in Québec (eastern Canada). eDNA was collected in ten 1L water samples within each of these lakes. Lake characteristics are reported in Table 1.

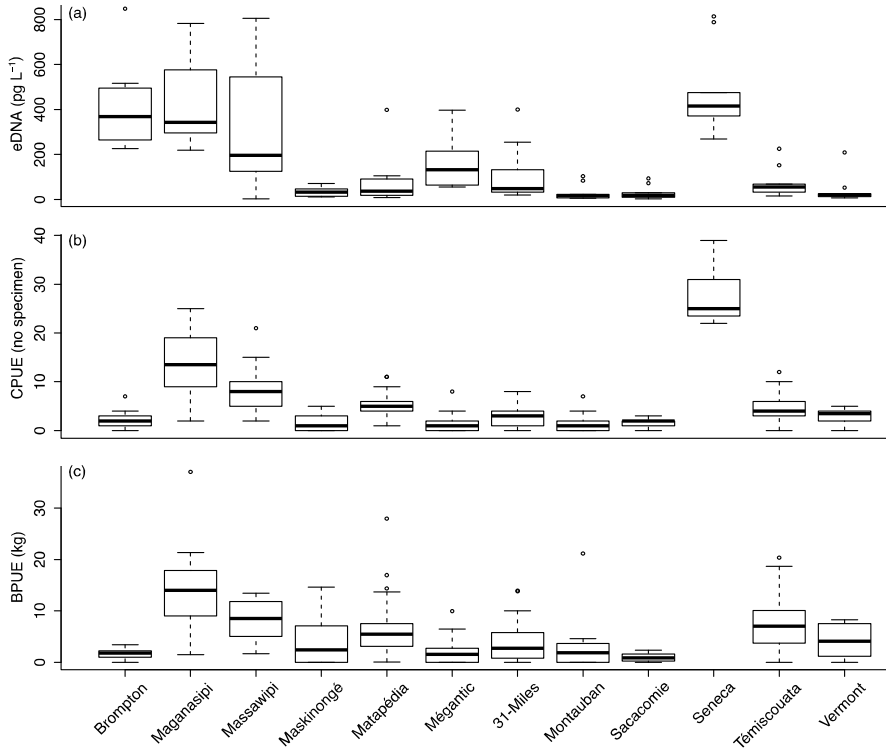

Fig. 2. Boxplot comparing variance among sites between (a) environmental DNA concentration (eDNA; pg L^{-1}) and gillnet data, including (b) number of catch per unit effort (CPUE; number of specimen per site) and (c) biomass per unit effort (BPUE; kg per site). eDNA outlier sites were removed and biomass data was not available for Seneca. The lines inside the boxes represent the median values, the top and bottom of the boxes represent the 75% and 25% quartiles and outliers are shown using empty circles (i.e. any data beyond 1.5 × IQR).

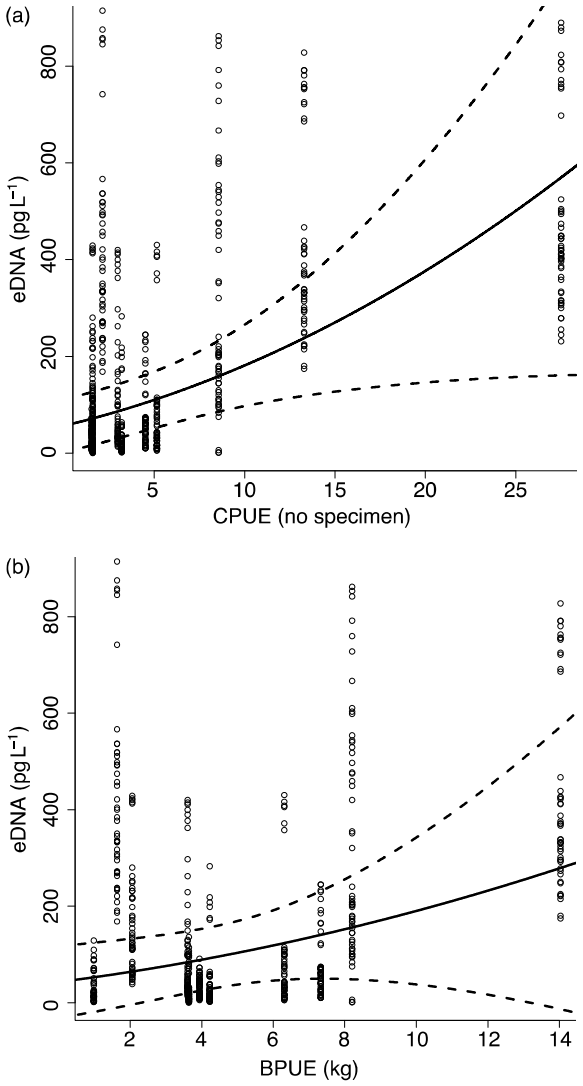

Fig. 3. Relationship between the concentration of environmental DNA (eDNA, in pg L^{-1}) and gillnet data including (a) the number of catch per unit effort (CPUE, number of specimen) and (b) biomass per unit effort (BPUE, in kg). Each dot represents a single DNA amplification and curves were fitted based on the coefficients of the hierarchical linear mixed model including the variability of CPUE and BPUE measurements (i.e. extension of bootstrapped type II regression model with random effects; see method section). Outlier sites were removed. Dashed lines depict the 95% confidence interval.