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ARTICLE

Improving herpetological surveys in eastern North America using the environmental DNA method[1]

Anaïs Lacoursière-Roussel, Yohann Dubois, Eric Normandeau, and Louis Bernatchez

Abstract: Among vertebrates, herpetofauna has the highest proportion of declining species. Detection of environmental DNA (eDNA) is a promising method towards significantly increasing large-scale herpetological conservation efforts. However, the integration of eDNA results within a management framework requires an evaluation of the efficiency of the method in large natural environments and the calibration of eDNA surveys with the quantitative monitoring tools currently used by conservation biologists. Towards this end, we first developed species-specific primers to detect the wood turtle (*Glyptemys insculpta*) a species at risk in Canada, by quantitative PCR (qPCR). The rate of eDNA detection obtained by qPCR was also compared to the relative abundance of this species in nine rivers obtained by standardized visual surveys in the Province of Québec (Canada). Second, we developed multi-species primers to detect North American amphibian and reptile species using eDNA metabarcoding analysis. An occurrence index based on the distribution range and habitat type was compared with the eDNA metabarcoding dataset from samples collected in seven lakes and five rivers. Our results empirically support the effectiveness of eDNA metabarcoding to characterize herpetological species distributions. Moreover, detection rates provided similar results to standardized visual surveys currently used to develop conservation strategies for the wood turtle. We conclude that eDNA detection rates may provide an effective semiquantitative survey tool, provided that assay calibration and standardization is performed.

Key words: amphibian, reptile, monitoring, eDNA metabarcoding, qPCR.

Résumé : Parmi les vertébrés, l'herpétofaune présente la plus grande proportion d'espèces en déclin. La détection de l'ADN environnemental (ADNe) constitue une méthode prometteuse pour augmenter considérablement les efforts de conservation à grande échelle pour l'herpétofaune. Cependant, l'intégration des résultats d'ADNe au sein d'un cadre de gestion nécessite d'abord une évaluation de l'efficacité de la méthode à grande échelle dans une environnement naturel, ainsi qu'une calibration des résultats d'ADNe avec ceux des méthodes d'inventaire actuellement utilisées en biologie de la conservation. À ces fins, nous avons d'abord mis au point des amorces spécifiques pour détecter la tortue des bois, *Glyptemys insculpta*, une espèce en péril au Canada, à l'aide de la PCR quantitative (PCRq). Le taux de détection de l'ADNe en PCRq a été comparé à l'abondance relative de cette espèce obtenue par des inventaires visuels dans neuf rivières du Québec (Canada). Deuxièmement, les auteurs ont mis au point des amorces multispécifiques pour détecter les espèces d'amphibiens et de reptiles présentes en Amérique du Nord à l'aide d'analyses métagénomiques de l'ADNe. Un index de probabilité d'occurrence des espèces, basé sur l'aire de répartition et le type d'habitat, a été comparé avec les résultats de la métagénomique pour des échantillons récoltés dans sept lacs et cinq rivières de la province de Québec. Les résultats confirment l'efficacité de la métagénomique de l'ADNe pour l'étude de la répartition des espèces d'amphibien et de reptile. De plus, les taux de détection de l'ADNe se sont avérés semblables à ceux obtenus par les inventaires visuels traditionnellement utilisés pour établir les stratégies de conservation de la tortue des bois. Les auteurs concluent que les taux de détection de l'ADNe pourraient fournir un outil efficace d'inventaire et de suivi semi-quantitatifs à condition que la calibration et la standardisation soient préalablement effectuées.

Mots-clés : amphibien, reptile, suivi de la biodiversité, métagénomique de l'ADNe, PCRq.

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A. Lacoursière-Roussel, E. Normandeau, and L. Bernatchez. Institut de Biologie Intégrative et des Systèmes, Pavillon Charles Eugène Marchand, Université Laval, Québec, QC G1V 0A6, Canada.
Y. Dubois. Ministère des Forêts, de la Faune et des Parcs, Direction de l'expertise sur la faune terrestre, l'herpétofaune et l'avifaune, 880 chemin Sainte-Foy, Québec, QC G1S 4X4, Canada.
Corresponding author: Anaïs Lacoursière-Roussel (email: anais.lacoursiere.1@ulaval.ca).

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Introduction

Herpetofauna, including the amphibians and reptiles, faces the highest proportion of declining species among the vertebrates (Alroy 2015; Böhm et al. 2013; Gibbons et al. 2000; Houlahan et al. 2000; IUCN 2016; Stuart et al. 2004). However, the lack of data on species distribution and population demographic trends currently limits the development of effective conservation strategies (Baillie et al. 2010; Howard and Bickford 2014; Stuart et al. 2004). Effective non-invasive monitoring tools are needed to properly assess the status of species, to locate populations for conservation efforts, and to describe population trends in relation to threats intensities and possible recovery actions (Campbell et al. 2002). Due to their ecology and life history traits, many amphibians and reptiles are very difficult to detect using classical monitoring methods (Bailey et al. 2004*a*; Couturier et al. 2013; Daigle and Jutras 2005; Lind et al. 2005; Price et al. 2012). To increase detection probability with practical efforts, surveys and monitoring programs are generally limited to a time window maximizing species detection (Erb et al. 2015; Massachusetts Cooperative Fish and Wildlife Research Unit and the Northeast Wood Turtle Working Group 2013; Petitot et al. 2014). Additionally, the time window optimizing species detection differs from one species to another, therefore, limiting the ability of developing simultaneous monitoring programs for the different species (Bailey et al. 2004*b*; de Solla et al. 2005; Pellet and Schmidt 2005). Improving distribution data for every species or for the entire range of a species within a jurisdiction territory using non-invasive methods is thus particularly challenging for large-scale herpetological management purposes.

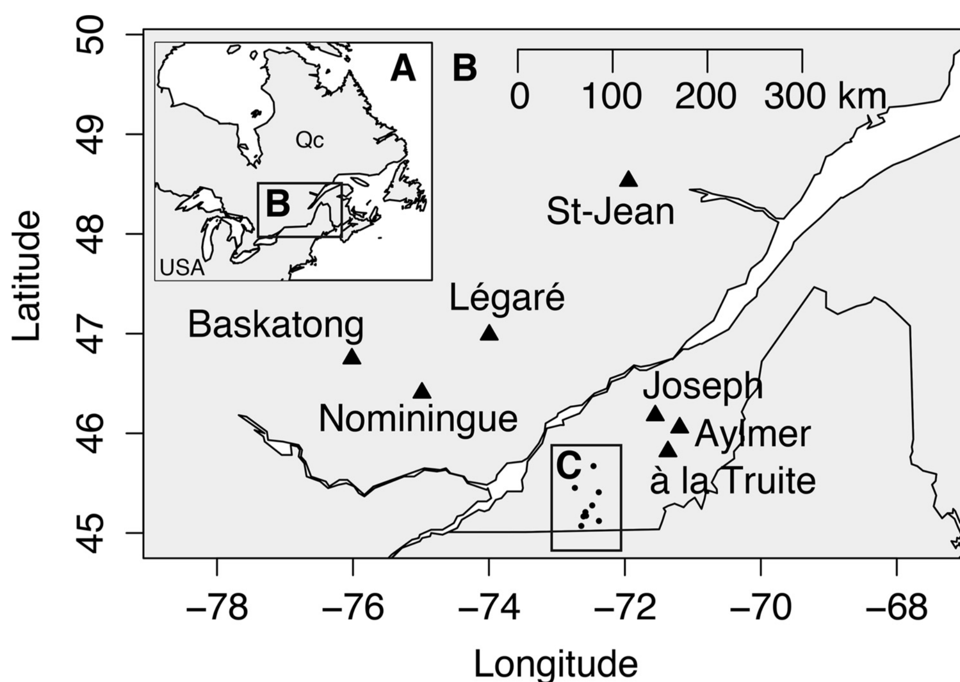
Analysis of environmental DNA (eDNA) is likely to become a revolutionary tool to increase both spatial and temporal scales of monitoring datasets for species of concern (Dejean et al. 2012; Jerde et al. 2011, 2013; Lodge et al. 2012; Mahon et al. 2013; Pilliod et al. 2013; Thomsen et al. 2012*a*). The eDNA method detects traces of DNA in cellular or extracellular form from sources such as feces, secreted mucous membranes, gametes, and skin cells (Haile et al. 2009; Lydolph et al. 2005; Taberlet et al. 2012). In addition to increasing the probability of detection of aquatic species compared to some traditional survey methods (Dejean et al. 2012; Jerde et al. 2011; Pilliod et al. 2013; Smart et al. 2015), using eDNA might also increase the observation time windows for surveys, enabling multispecies surveys and reducing the need for extensive taxonomic expertise and financial resources.

The successful detection of American bullfrogs (*Lithobates catesbeianus*) eDNA in natural ponds in 2008 (Ficetola et al. 2008) led amphibians to become model organisms for eDNA research (Thomsen and Willerslev 2015; Valentini et al. 2016). Recently, Davy et al. (2015) showed that eDNA could also be used to detect reptiles, namely freshwater turtles, in outdoor ponds. However, for both reptiles and amphibians, few studies have evaluated the efficiency of the eDNA method in large natural aquatic environments such as lakes and rivers, as well as the relative sensitivity of the method among taxa (see review in Thomsen and Willerslev 2015). In contrast to traditional monitoring surveys, false positive results may occur due to cross-contamination, sequencing errors, and large-scale transport of genetic traces (e.g., contamination from birds) (Lahoz-Monfort et al. 2016). The effect of environmental factors on eDNA concentration, diffusion, and advection in natural bodies of water (e.g., the effect of running water on the downstream transportation of eDNA) are also largely unknown. This new population data collection approach is thus challenged by uncertainty related to the environmental effects, samples collection, and laboratory procedures. Large-scale studies to test this efficiency and calibrate these methods to detect and quantify population are needed (Roussel et al. 2015).

Environmental DNA surveys may be designed to detect the presence of a single species (species-specific markers) or multiple species (i.e., eDNA metabarcode markers). By allowing the simultaneous sequencing of several million DNA fragments in water samples, eDNA metabarcoding analyses have a huge potential to assess biodiversity of aquatic species (Evans et al. 2016; Valentini et al. 2016). To date, the number of sequences obtained in eDNA metabarcoding does not allow a rigourous evaluation of eDNA concentration in water samples (but see Evans et al. 2016 and Thomas et al. 2016). Yet, a number of eDNA studies have shown positive correlations between the eDNA concentrations obtained from quantitative (qPCR) analyses and population abundance indices (Lacoursière-Roussel et al. 2016*a*, 2016*b*; Pilliod et al. 2013; Takahara et al. 2012; Thomsen et al. 2012*b*; Wilcox et al. 2013). However, the ability to quantify population abundance using eDNA is still debated (Iversen et al. 2015; Roussel et al. 2015; Tréguier et al. 2014) and the relative sensitivity of both methods is largely unknown.

In this study, we aim to evaluate the efficiency of eDNA to detect and quantify amphibian and reptile populations in natural habitats using both single- and multiple-species approaches. Species-specific primers with a Taqman probe were developed to detect the wood turtle (*Glyptemys insculpta*), a threatened species in Canada (Government of Canada 2015), by means of quantitative PCR (qPCR). The species-specific detection rate was then compared to its relative abundance obtained by the standardized visual surveys typically used by managers. Additionally, a pool of multi-species primers has been developed to detect North American amphibian and reptile species by eDNA metabarcoding analyses. Using these primers to collect eDNA samples could help to improve the current poor level of knowledge about distribution ranges and popu-

Fig. 1. Geographical locations of the sampled lakes in the Province of Québec (map A; eastern Canada). Environmental DNA was collected in seven lakes (black triangles in map B) and nine section rivers (black dots in map C).



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lation trends data that currently limits the development of effective conservation strategies for those species.

Methods

For species-specific analysis, detection rates obtained by qPCR were compared to the relative abundance of wood turtle obtained by standardized visual surveys in nine river sections in the Province of Québec (Canada; Fig. 1). For multi-species analyses, an eDNA metabarcoding dataset from samples collected in seven lakes and five rivers in Québec was compared to a species occurrence probability index (Fig. 1).

Population and community assessment

The relative abundance of the wood turtle was obtained from conventional visual surveys used by managers (Daigle and Jutras 2005). For each sampled river section, visual surveys were conducted by a team of three people walking between 2 and 4 km of riverbanks between 08h30 and 17h30. Rivers were surveyed on sunny days with air temperature above 15 °C between 26 April and 21 May 2013, before the complete emergence of vegetation. The total number of turtles during the sampling day was used as an index of relative abundance.

For each of the 34 local amphibian and reptile species (supplementary data, Table S1[2]), an occupancy analysis was conducted to obtain a categorical index of occurrence probability (0 to 3) for a given species in a given sampling location. This index (hereafter referred to as occurrence index, i) relies on the probability for a species to be present in a given location based on its distribution range and its habitat preferences. The species distribution range is based on validated species observations included in the Québec Amphibians and Reptiles Atlas database (AARQ 2013). The habitat preferences for each species are based on scientific literature (Desroches and Rodrigue 2004; Dodd 2013*a*, 2013*b*; Ernst and Ernst 2003; Ernst and Lovich 2009; Petranka 2010). The occurrence index is 0 when the sampled location is outside of the known distribution range of the species. When the sampled location occurs within the species distribution range, the occurrence index is 1 for terrestrial species like most snakes and the eastern red-backed salamanders (*Plethodon cinereus*). The occurrence index is either 2 or 3 for species with at least one aquatic life stage, depending on whether the sampled habitat is included (i = 3) or not (i = 2) in their preferred habitat types based on scientific literature (Table 1).

eDNA survey

Water samples were collected 22–24 July 2013 in sections of nine different rivers and 9–30 May 2013 in seven lakes in Québec (Fig. 1). Water was collected in the same sections of rivers than the wood turtle population assessment. Since rivers are shallower and more mixed compared to lakes, the amounts of eDNA are expected to be lower in lotic environments due to a greater degradation

[2]Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/gen-2015-0218.

Table 1. Occurrence index (*i*) used to validate the eDNA metabarcoding dataset for each local amphibian and reptile species within each sampled location.

Occurrence index	Distribution range	Habitat preferences
0	Outside of the known distribution range of the species	—
1	Within the species distribution range	Terrestrial species (most snakes and the eastern red-backed salamanders (*Plethodon cinereus*)).
2	Within the species distribution range	Aquatic species (for at least one life stage) for which the sampled habitat is not included in its preferred habitat types.
3	Within the species distribution range	Aquatic species (for at least one life stage) for which the sampled habitat is included in its preferred habitat types.

Note: For each species, the occurrence probability index is provided based on the species distribution range and the habitat preference based on atlas data and scientific literature, respectively (see Methods for references).

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rate from UV radiation (Thomsen et al. 2012*b*). A 2 L water sample was, therefore, taken from rivers in contrast to the 1 L sample from lakes. Ten water samples, separated by 100–200 m, were collected from each river for species-specific analyses, whereas seven samples randomly chosen were used for eDNA metabarcoding analyses (i.e., an equal number of sites for lakes and rivers for the eDNA metabarcoding; Table S2[2]). The wood turtle is protected against illegal collection and trade in most of its distribution range in North America (CITES 2015). In accordance with the recommendation of the Committee on the Status of Endangered Wildlife in Canada (COSEWIC), the exact locations are not provided for the wood turtle sampling sites to avoid illegal collection risk. The wood turtle is not expected to be present in the Mawcook River, but closely related species such as the common snapping turtle (*Chelydra serpentina serpentine*) and the painted turtle (*Chrysemys picta*) are known to be present, and this river was thus used as a negative control. All waterways were slow–moderate flowing (≤3.24 m^3/s), averaging 0.69 m depth (SD = 0.41) and 12.89 m (SD = 5.67) of width. In lacustrine systems, water samples were collected in seven lakes (Fig. 1). A 1 L water sample was taken from a boat at seven locations randomly distributed within each lake. To ensure the homogeneity of the water column, and to increase the similarity of environmental conditions among lakes, sampling was conducted in early spring, before thermal stratification of the lake. To represent the entire water column and reduce stochastic effects, water samples were collected in the form of integrated samples taken from 0–5 m of depth (i.e., oblique bottle filing along the water column) in a non-stratified period of the year.

For both lakes and rivers, water samples were stored on ice until they were filtered in less than 24 h after collection to minimize eDNA degradation (see delay between sampling and filtration in Table S2[2]). Water samples were filtered through a 1.2 μm glass microfiber filter (Whatman GF/C, 47 mm) using a peristaltic pump (Cole-Parmer: Masterflex L/S Modular Drive). 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 et al. 2016*b*). After filtration, filters were frozen until eDNA extraction.

Molecular analyses

To reduce potential laboratory cross-contamination, procedures for eDNA extraction from filters, PCR preparation, PCR, and qPCR cycling were all performed in different rooms. Samples from a specific location were all treated together, and the bench space was bleached prior to processing the next lake. Bodies of waters were processed in a randomized order. 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. To isolate and purify eDNA, 450 μL of ATL Buffer and 50 μL of Proteinase K (Qiagen) was added to the tubes containing the filter. Tubes were then vortexed and incubated at 56 °C overnight. After incubation, the filter and lysis solution mixture was centrifuged at 13 000 rpm within a QIAshredder tube, and then aliquoted equally to three different tubes. A total of 400 μL of AL Buffer was added to each tube, vortexed and incubated at 70 °C for 10 min. After the incubation, 400 μL of ethanol was added and mixed by vortexing. This mixture was then transferred to a DNeasy Mini spin column (Qiagen, Inc.) and centrifuged at 13 000 rpm. The spin column filter was washed using 500 μL of AW1 Buffer and was centrifuged at 13 000 rpm. Subsequently, the spin column filter was washed again using 500 μL of AW2 Buffer and centrifuged at 13 000 rpm. Purified DNA was then eluted in 20 μL of nuclease-free water, incubated at room temperature for 5 min and centrifuged at 13 000 rpm. The extracted DNA was then frozen at –20 °C until amplification.

Wood turtle semiquantitative analyses

Detection rates of wood turtle obtained by qPCR was calculated by the sum of the total number of positive amplifications for each of the nine river sections (six amplifications per site and 10 sites per river section). We used the TaqMan MGB™ technology with a 7500 Fast Real-Time PCR system (LifeTechnologies), which requires

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constructing primers and probes to amplify short DNA fragments. Quantitative PCR (qPCR) was used to detect the presence of eDNA due to the increased sensitivity to sequence mismatches in the target DNA compared to traditional PCR (Ellison et al. 2006; Wilcox et al. 2013) and its potential to reduce false negative results compare to results visualized from electrophoresis gel (A. Lacoursière-Roussel and L. Bernatchez unpublished data). We developed species-specific primers and probe to amplify the targeted 71 bp of the mitochondrial COI gene (Table 2). To ensure species-specific amplification, primers and probes were designed to optimize the number of mismatches to other reptile species that may coexist in the system, and to optimize the position of the mismatches (i.e., near 3′ (Wilcox et al. 2013)). Reads from reptile species known to coexist with wood turtle were aligned in Geneious 6.0.6 and primers and probe were designed using Primer Express 3.0 software (Life Technologies). The specificity of primers and probe were tested in vitro on DNA extracted from tissues of two turtles that may be found in the same river as wood turtle (i.e., common snapping turtle (*Chelydra serpentine*) and painted turtle (*Chrysemys picta*)). The final reaction volume was 20 μL, including 1.8 μL of each primer (10 μmol/L), 0.5 μL probe (10 μmol/L), 10 μL Environmental Master Mix 2.0 (Life Technologies), 3.9 μL di$H_2$0, and 2 μL DNA. The qPCR amplification was performed under the following conditions: 2 min at 50 °C, 10 min at 95 °C, followed by 70 cycles of 15 s at 95 °C and 60 s at 60 °C. Each qPCR plate included three wells that contained all reagents but no DNA template, to serve as a negative control, and three wells that contained all reagents and wood turtle extracted DNA template, to serve as a positive control. The data collected from the post-PCR read were used to make presence/absence calls. Wood turtle eDNA was called present when the target amplified above the target's threshold (i.e., level of fluorescence) calculated from the negative control reactions obtained from the default analysis settings in the 7500 software (Life Technologies). The degree of precision of descriptors should always be chosen with respect to the optimal degree of precision related to the particular ecological phenomenon being studied, and semiquantitative descriptor should be used when there is a low precision of the measurements (Legendre and Legendre 1998). Here, by avoiding the measurement of the eDNA concentration (here we thus refer to the eDNA quantification as a semiquantitative analysis), we aimed to avoid bias due to extreme values obtained by the nature of the secretion captured, including the physiological source, the state (i.e., intra- or extracellular), and fate (e.g., suspension time) of aqueous macrobial eDNA.

eDNA metabarcoding

Four pairs of primers were developed to amplify amphibians, whereas two primer sets were developed for reptiles (Table 3; see Table S1[2] for the species list targeted

Table 2. Primers and probe sequences used for qPCR analyses showing mismatches with closely related reptile species that may occur in the sampling area covered in this study.

Common name	Latin name	Forward primer mismatches (5′→3′)	Reverse primer mismatches (5′→3′)	Probe mismatches (5′→3′)
Wood turtle	*Glyptemys insculpta*	TGCCTCTGTAGACCTAACCATCTTT	AGTTGATAGCCCCTAAGATTGAAGATA	CTCTACACCTGGCCGGT
Painted turtle	*Chrysemys picta*	……………………………T…………	……………....T……..GA……………..C…	……..C…….T..A…..A….G
Common snapping turtle	*Chelydra serpentina*	…………C………..T….G…..T….T….C	………………………...A……………..A…	...C….T…..TT..A………..G
Blanding's turtle	*Emydoidea blandingii*	……………………………T…………	……...A……………...GA…………………	...C…C………..A…………
Northern watersnake	*Nerodia sipedon*	CC……..A….G………..GG...A……….C	…………....T….T….C...G……G…..C….GG	...C……….T….A…..A….A
Common garter snake	*Thamnophis sirtalis*	CC……..A….G………..GG...A……….C	……………T….T….C...G……G…..C….GG	...C……….T….C…..A….A

Table 3. Group-species mitochondrial primers for amphibians and reptiles for eDNA metabarcoding analyses.

Group-species	Gene	Forward primer (5′→3′)	Reverse primer (5′→3′)	Total length (bp)
Amphi_A	COI	GCiGGiGCYTCWGTAGA	iGGWGTTTGRTATTGiGAT	132
Amphi_B	Cytb	YCCATGAGGMCARATATCWTTT	ACKGARAAWCCiCCYCAAA	111
Amphi_C	COI	CMCTTYTiGGYGATGATCAAA	RGCTATATCAGGKGCTCCAA	143
Amphi_RANA	COI	TCWACYACACARTAYCAAACACC	CTCCTGCiGGGTCRAAAA	151
Reptile_TURTLE	COI	GCMGGiACMGGiTGAAC	GATATiGCiGGRGMTTTTAT	167
Reptile_SNAKE	COI	GCYGGYACiGGiTGAAC	TRAAGTTRATTGCYCCiAGGA	130

Note: Letters refer to the nucleic International Union of Pure and Applied Chemistry (IUPAC) codes. Species targeted within each group-species primer set are presented in Table S1[2].

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for each primer). The sequences available for the 34 local amphibian and reptile species were compared for five different genes: mitochondrial cytochrome *c* oxidase subunit I (COI), cytochrome *b* (cytb), 12S, 16S, and 18S ribosomal subunits. Including all available sequences from GenBank, BOLD (Ratnasingham and Hebert 2007) and data provided from the Biodiversity Institute of Ontario, University of Guelph (E. Anne Chambers, personal communication), 31 species had sequence information on the COI and cytb. Sequences for the 12S, 16S, and 18S regions were not available for many species to be considered for primer design; sequences for the 12S gene were available for 26 species, sequences for the 16S gene (generally partial and related to 12S) were available for 25 species, and the 18S gene was only available for 10 species (Table S1[2]). Sequences were aligned in Geneious 6.0.6 (Kearse et al. 2012) and genetically similar species were grouped using the neighbor-joining method (Saitou and Nei 1987) with a Tamura–Nei model (Tamura and Nei 1993). The COI sequences were absent only for three species: pickerel frog (*Lithobates palustris*) and ring-necked snake (*Diadophis punctatus*), which are unlikely to be present within sampling sites, and spotted salamander (*Ambystoma maculatum*), which is likely to be present. Therefore, we developed primers amplifying the cytb gene instead for the latter and the closely related species (i.e., primers Amphi_B in Table S1[2]). Primers (Table 3) were designed using the PRIMER3Plus software (Untergasser et al. 2012). Primers were tested in vitro using DNA extracted from tissue samples of 29 species including the species with the unknown COI sequences discussed above (see species list Table S1[2]). To increase DNA extraction yield, 180 μL ATL buffer was first added to each tissue separately and shaken with a tungsten carbide bead (Qiagen, Germany) at 27 Hz during 1 min using a TissueLyser (Qiagen, Germany). DNA was then extracted from tissue of each species using the DNeasy Blood and Tissue Kit (Qiagen, Germany) following the manufacturer's spin-column protocol. The amplification mixture contained 0.2 μL GoTaq Flexi DNA polymerase (ProMega, USA) with 2 μL GoTaq Flexi Buffer 5X, 0.8 μL dNTP, 0.65 μL $MgCL_2$, 0.5 μL of each primer (10 μmol/L), 3.84 μL diH_20, and 2.5 μL of DNA. For each species, the PCR mixture was denatured at 95 °C for 2 min, followed by 35 cycles (95 °C for 45 s, 54 °C for 45 s, and 72 °C for 45 s), and a final elongation at 72 °C for 5 min.

Library preparation

DNA amplifications were performed in a two-step dual-indexed PCR approach specifically designed for Illumina instruments by the Plate-forme d'Analyses Génomiques (IBIS, Université Laval). The first PCR was performed with group-specific primers for amplifying the eDNA of the target taxonomic group. The primers were tailed on the 5′ end with part of the Illumina TruSeq adaptors. The following oligonucleotide sequences were first used for amplification: ACACTCTTTCCCTACAC-GACGCTCTTCCGATCT (forward primers (Table 3)) and GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT (reverse primers (Table 3)). A second PCR was performed to attach remaining adaptor sequence (regions that anneal to the flowcell and library-specific barcodes). For the second PCR, the generic forward primer was AAT-GATACGGCGACCACCGAGATCTACAC[index1]ACACTCTTTCCCTACACGAC and the generic reverse primer was CAAGCAGAAGACGGCATACGAGAT[index2]GTGACTGGAGTTCAGACGTGT. Please note that primers used in this work contain Illumina-specific sequences protected by intellectual property (Oligonucleotide sequences © 2007–2013 Illumina, Inc. All rights reserved. Derivative works created by Illumina customers are authorized for use with Illumina instruments and products only. All other uses are strictly prohibited).

The first amplification mixture contained 25 μL Qiagen MasterMix, 0.5 μL of each primer (10 μmol/L), 22 μL diH_20, and 2 μL of DNA. For all samples, the PCR mixture was denatured at 95 °C for 15 min, followed by 35 cycles (94 °C for 30 s, 54 °C for 90 s, and 72 °C for 60 s), and a final elongation at 72 °C for 10 min. For each primer set, seven aliquots were amplified for each lake and river (i.e., one aliquot per sampling site). Products of the seven aliquots (10 μL) from the first PCR were pooled for each primer and each lake and river separately. Pooled products were then purified using Axygen PCR clean up kit following the manufacture's recommended protocol and the resulting DNA eluted in 25 μL water. Final DNA amplifications were performed in a volume of 24.5 μL, including 5 μL Q5 buffer, 0.25 μL AmpliTaq Q5 DNA

polymerase, 0.5 μL dNTP, 2.5 μL Illumina adaptor (10 μmol/L), 12.25 μL di$H_2$0, and 4 μL PCR1 mixture. The second PCR mixture was denatured at 98 °C for 30 s, followed by 10 cycles (98 °C for 15 s, 58 °C for 15 s, and 72 °C for 25 s), and a final extension at 72 °C for 2 min. Separate barcodes were used for each location and primer separately. The final PCR products were purified using Axygen PCR clean up kit and DNA eluted in 20 μL water. Libraries were quantified by PicoGreen and, for each location, samples were pooled in equal molar concentrations to maximize equal sequence depth per sample location. When both the Bioanalyzer (Agilent) and Quant-iT PicoGreen (Life Technologies) did not detect any DNA, 15.0 μL PCR2 mixtures were mixed nonetheless (see Table S3[2] for the concentration and volume for each primer and locations separately). To avoid sample contamination, manipulations were performed in a decontaminated UV hood with new equipment. Negative controls were included for all PCRs and showed no amplification. Negative controls were not pooled and sequenced, but since some species were detected in samples for which no PCR product was detected (see Tables S3–S5[2]), we emphasize that future eDNA metabarcoding studies should sequence negative control as well, especially in the context of predicting species distribution and conservation purposes.

Sequencing was carried out using an Illumina MiSeq (Illumina, San Diego, USA) at the Plateforme d'Analyses Génomiques of the Institut de Biologie Intégrative et des Systèmes (IBIS – Université Laval, Québec City, Canada) using a paired-end MiSeq Reagent Kit V3 (Illumina, San Diego, USA) and following the manufacturer's instructions. For sequencing, the amplicon pool was diluted to 4 nmol/L with molecular grade water, denatured, and then sequenced at 10 pmol/L following manufacturer's instructions. Raw sequencing reads were de-multiplexed using the MiSeq Control software v 2.3 into independent libraries for a total of 140, resulting from each primer set for each lake and river sample. De-multiplexed raw sequencing reads were provided in gzip compressed Fastq format. Although we performed paired-end sequencing, only the forward sequences were kept due to lower quality observed for some reverse reads, especially the reverse reads for the wood turtle primers. Run quality was assessed using FastQC version v0.11.3 and the amplified regions of interest showed a mean Fastq quality scores around 38 (see Table S6[2] for FastQC quality reports). Since the quality over the portion of interest was very good, no sequences were removed based on quality score. Sequences with ambiguous nucleotides were discarded. Only reads within 5 bp of the expected amplicon length were kept. Forward and reverse primers were then trimmed from the sequences based on their expected lengths. Similarity of the studied sequences to these in a database of the species of interest was assessed using BLAST+ v2.2.29 available on the website of the National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/). Reads with BLAST results showing less than 94% similarity with the reference databases were removed. Finally, taxon–locality combinations with less than five reads were assumed to have a read count of zero (Schnell et al. 2015), whereas a species with a count of five and more in a specific location was considered as present. Chimeric sequences were not removed, but no ambiguous identification of species had been found. Raw sequences reads were deposited in NCBI's sequence read archive (SRA, http://www.ncbi.nlm.nih.gov/sra) under accession number SRP071113.

Statistical analyses

All statistical analyses were performed using R 3.0.3. To ensure the independence between water samples for the detection and quantification of the wood turtle using qPCR, the coefficient of determination (R^2) was calculated between the number of positive amplifications per site and the spatial distribution of this site (i.e., upstream to downstream). Finally, a linear regression was applied to evaluate the relationship between the number of positive amplifications and the relative abundance of the wood turtle obtained from conventional visual surveys.

The proportion between the number of species detected by next-generation monitoring over the number of species expected was calculated separately for each occurrence index (i) (hereafter referred to as proportion of species detection). This proportion of species detection was then used to assess the sensitivity of the eDNA method to detect species and compare it among locations. To compare the sensitivity of the eDNA method among species, the proportion between the number of locations with positive detection obtained by next-generation monitoring over the numbers of location expected for this species has been also calculated separately for each occurrence index (hereafter referred to as proportion of location detection). Note that missing information did not allow to estimate the occurrence index for wood turtle in two lakes (Joseph and Légaré) and the northern dusky salamander (*Desmognathus fuscus*) in one lake (à la Truite) and, due to the lack of extraction product, no amplification has been done for the primers Amphi_C and Amphi_B for the rivers Jackson and Quilliam, respectively (see Table 5). Such predictions of expected presence are commonly used by wildlife managers to recommend specific surveys and protection measures based on a list of species that should be present at a given location. These proportions of presence over expected presence is also used to assess the abundance in the context of species status evaluation.

Logistic regression was used to evaluate the relationship between the occurrence index and the eDNA metabarcoding dataset using the glm() function of the stats library (R Core Team 2016). Boxplots were first used to illustrate the distribution data of the number of reads

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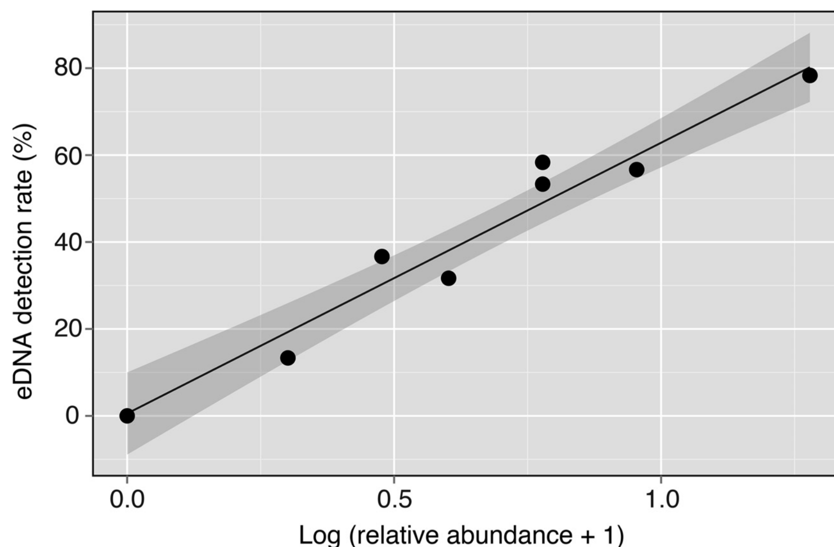
Table 4. Rivers sampled for the wood turtle semi-quantitative analyses, the relative abundance obtained by standardized visual surveys, the eDNA detection rates (i.e., total number of positive amplifications), the number of sites with positive amplifications, standard deviation (SD) of eDNA among sites, and relationship (R^2) between the number of positive amplifications and the spatial ranking from downstream to upstream.

River	Relative abundance	Total no. of positive amplifications	No. of sites with positive amplifications	SD	Spatial distribution effect (R^2)
Mawcook	0	0	0	0.00	—
Quilliams	1	8	3	1.87	0.03
Yamaska	2	22	7	2.49	−0.02
Jackson	3	19	8	1.79	0.21
Sutton	5	32	10	1.81	0.01
North Branch	5	35	10	1.90	0.02
Noire	8	34	8	2.55	−0.04
Missisquoi	18	47	9	2.21	−0.19
Renne	18	47	9	2.11	0.08

Note: For the latter, negative R^2 values depict a lower number of eDNA detected downstream than upstream, whereas positive R^2 depict a greater number of eDNA detected downstream than upstream.

Fig. 2. Relationship between percent eDNA detection rate and the $\log_{10}$ of the relative abundance obtained by standardized visual surveys of wood turtle. The associated 95% confidence interval is depicted in dark gray.



obtained for each occurrence index using the geom_boxplot() function of the ggplot library (Wickham 2009). To compare the sensitivity of the eDNA method among habitats, a t.test() function of the stats library was applied to compare the proportion of species detection between lakes and rivers. Similarly, to compare the sensitivity of the eDNA method between species group, a t-test was applied to compare the proportion of location detection between amphibians and reptiles.

Results

Wood turtle semiquantitative analyses

Except for the negative site control (Mawcook River), the qPCR results detected the wood turtle within all rivers (Table 4; see Table S2[2] for the number of positive qPCR amplification per site). Wood turtle was not detected in any of the qPCR negative controls. The number of positive amplifications and the spatial upstream to downstream distribution of the samples sites within a given river were not correlated (Table 4). However, the total number of positive amplifications per river was highly correlated with the relative abundance of the wood turtle obtained from visual surveys (Fig. 2; R^2 = 0.77, P = 0.002, F = 23.0, df = 7; Log_{10} (x + 1): R^2 = 0.96, P < 0.0001, F = 163.8). Similarly, the number of sites per river with positive amplifications was highly correlated with the relative abundance obtained from visual surveys (Log_{10} (x + 1): R^2 = 0.66, P < 0.01, F = 13.4, df = 7).

Community analyses

A total of 17 out of 34 probable species were detected using eDNA metabarcoding analyses: eight frog and toad species (Anura), four species of salamanders (Urodela), three snake species (Squamata), and two turtle species (Testudines; Table 5; Fig. 3). Among the 17 species that were not detected, 12 were not expected to be detected

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Table 5. Environmental DNA metabarcoding results including the number of amphibian and reptile species (i.e., species richness) and the number of reads for each species within each sampled location (i.e., lakes and rivers).

		Proportion of species				Amphibians												Reptiles				
	Species richness	*i*=3	*i*=2	*i*=1	*i*=0	American toad	American bullfrog	Eastern newt	Northern green frog	Mink frog	Northern leopard frog	Spring peeper	Northern two-lined salamander	Spring salamander	Gray treefrog	Wood frog	Spotted salamander	Red-bellied snake	Milk snake	Northern watersnake	Common snapping Turtle	Wood turtle
Lakes																						
Aylmer	4	3/8	1/12	0/5	0/9	–	–	–	2313	1118	1964	1116	–	–	–	–	–	–	–	–	–	–
Baskatong	4	3/9	1/10	0/5	0/10	1649	867	–	831	1878	–	–	–	–	–	–	–	–	–	–	–	–
Joseph	5	3/7	1/10	1/5	0/11	2165	1690	–	26	–	54	–	–	–	–	–	–	63	–	–	–	–
Légaré	1	1/7	0/10	0/5	0/11	–	–	–	–	–	15793	–	–	–	–	–	–	–	–	–	–	–
Nominingue	2	1/9	1/10	0/5	0/10	–	876	–	–	–	–	4032	–	–	–	–	–	–	–	–	–	–
St-Jean	1	1/7	0/6	0/3	0/18	–	–	–	1193	–	–	–	–	–	–	–	–	–	–	–	–	–
à la Truite	3	1/8	2/10	0/5	0/10	–	–	–	2577	–	–	–	–	–	–	460	421	–	–	–	–	–
Rivers																						
North Branch	10	7/12	2/7	1/6	0/9	6980	1116	–	37667	–	605	–	109	22	1570	26	–	–	186	–	–	12
Renne	5	4/10	1/9	0/6	0/9	–	9466	58	20888	–	–	–	–	43	–	–	–	–	–	–	5	–
Yamaska	9	7/13	2/6	0/6	0/9	104	484	55	33945	–	22	1541	58	–	974	–	–	–	–	–	28	–
Jackson	5	4/11	1/4	0/6	0/9	X	1102	8	30316	–	–	X	30	–	X	259	–	–	–	–	–	–
Quilliams	5	3/8	1/4	0/5	1/7	164	9039	X	51204	–	–	–	12	X	–	–	X	–	–	90	–	–
i=3						3/4	8/12	3/11	10/12	2/12	5/12	—	3/3	1/3	—	—	—	1/9	—	0/2	2/14	—
i=2						2/7	—	—	—	—	—	3/11	1/9	1/2	2/10	3/12	1/11	—	—	—	—	—

Note: For each occurrence index (*i*), the proportions of species detections have been calculated for each location, whereas the proportions of location detections have been calculated for each species. En dash (–) depicts that no sequence has been found for the species, whereas an X shows that no amplification has been done for the species. Em dash (—) shows that the species was not expected in any sampled locations for those particular species. Please refer to Fig. 3 for Latin names.

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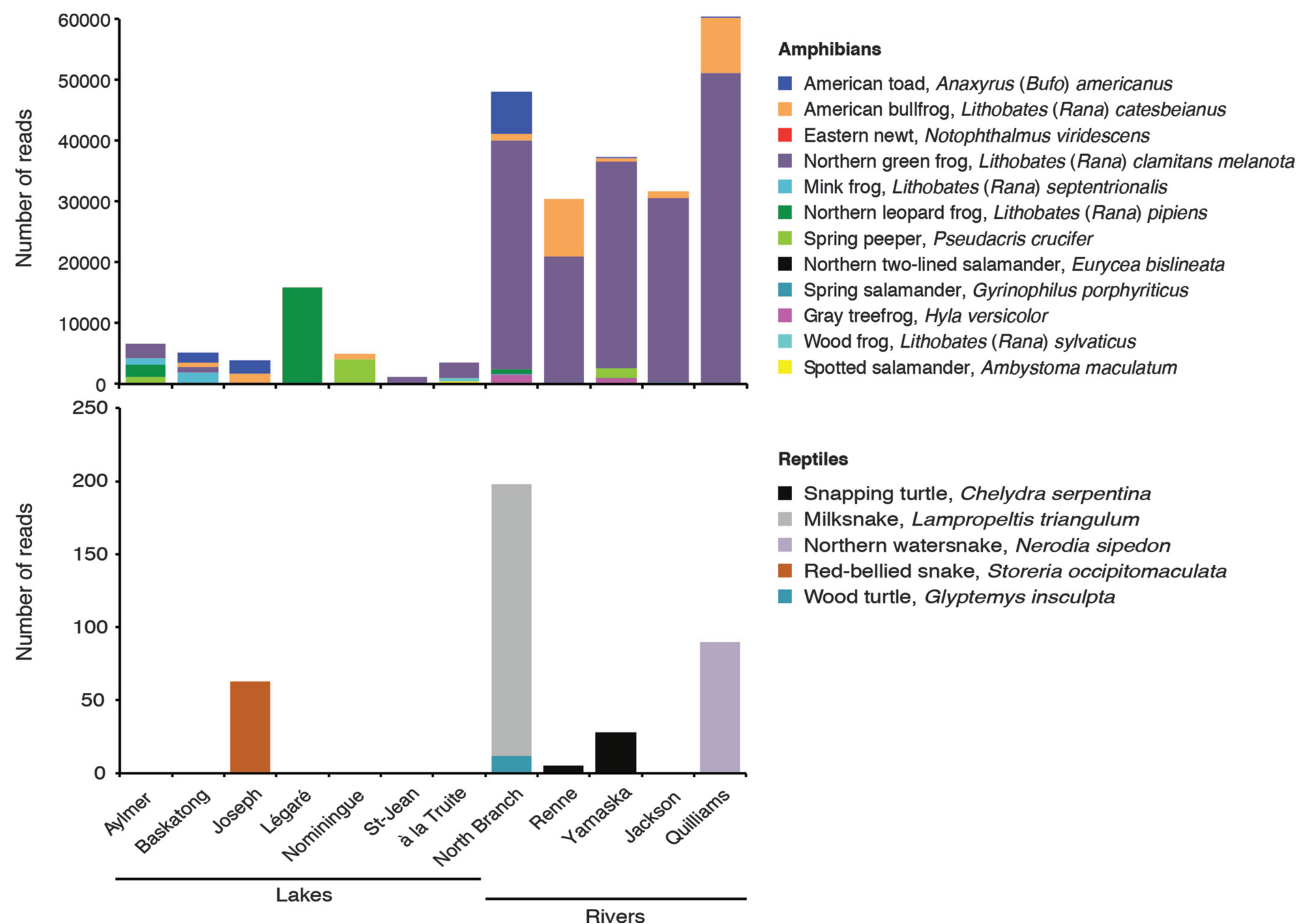
Fig. 3. Number of reads for each amphibian (upper graph) and reptile species (lower graph) within each body of water.



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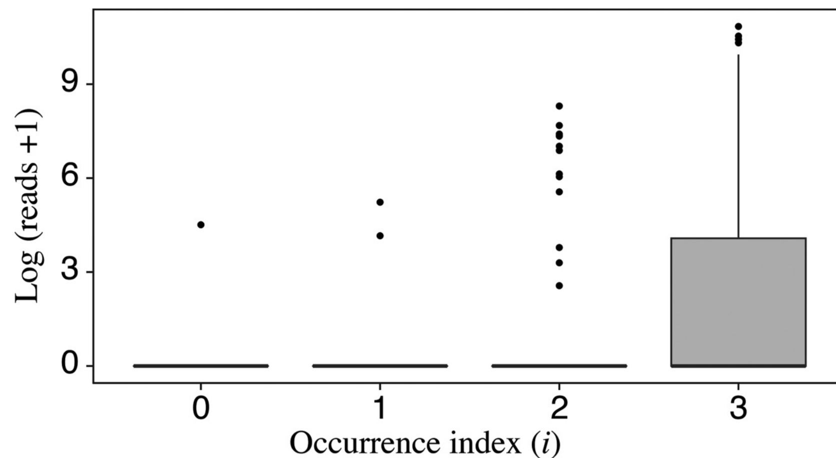
(i = 0 or i = 1). In total, after bioinformatic filtering (Table S4[2]), 249 274 reads were obtained, where 99.8% were amphibian species (amphibians: 248 890 reads; reptiles: 384 reads; see Table S5[2] for the number of reads for each primer and location separately). No amplification was detected in PCR negative controls. Despite the fact that a greater number of lakes were surveyed than rivers — albeit with an equal number of sites—, a greater number of species were detected and a greater number of sequences were found in rivers (5–10 species; 208 188 sequences representing 83.5% of the total reads) than in lakes (1–5 species; 41 086 sequences, 16.5% total reads; Table 5). Amphibians were found in all sampled lakes and rivers. Northern green frog (*Lithobates clamitans melanota*) was the most represented species in terms of the number of locations (i.e., 5 of 7 sampled lakes and all rivers) and the number of sequences (180 960 total reads); 96.2% sequences were found in rivers (174 020 reads in rivers vs. 6940 reads in lakes). The three snake species were found in one lake (i.e., redbelly snake (*Storeria occipitomaculata*)) and two rivers (i.e., northern watersnake (*Nerodia sipedon*) and milksnake (*Lampropeltis triangulum*)). The two turtle species were found in three rivers (snapping turtle (*Chelydra serpentine*) and wood turtle). The wood turtle was not detected in four rivers where it was expected to be found based on the qPCR results and visual surveys presented above.

As expected, species with a higher occurrence index were more often detected than species with a lower occurrence index (logistical regression: $P < 0.0001$, t ratio = 6.30, Residual deviance = 245.03, df = 386; Fig. 4). Ten species with a high occurrence index (i = 3) were detected (see the species list in Table 5 row proportion of location i = 3), representing 70.4% of the positive detections and a total of 234 667 reads. Only three species were not detected despite having a high probability of being detected (i.e., i = 3; northern watersnake (*Nerodia sipedon*), northern dusky salamander (*D. fuscus*), and painted turtle (*Chrysemys picta*) in 2, 3, and 12 locations, respectively). Four Anuran species (13 792 reads) and three salamander species (476 reads) were detected in aquatic habitats which are not included in their preferred habitat types (i.e., see the species list in Table 5; proportion of location i = 2). This represented 24.1% of the positive detections. Two terrestrial species were detected (i.e., i = 1: 63 reads of the red-bellied snake (*Storeria occipitomaculata*) in the Joseph Lake and 186 reads of the milksnake (*Lampropeltis triangulum*) in the North Branch River). This represented 3.7% of the positive detections. A single species out of its

Fig. 4. Data distribution of the number of reads for each species within each body of water for each occurrence index (see Table 1). The upper whisker extends from the hinge (i.e., the 75th percentile) to the highest value that is within 1.5 × IQR of the hinge, where IQR is the inter-quartile range.



expected spatial distribution range was found (i.e., $i = 0$: 90 reads for the northern watersnake in the Quilliams River), which represented 1.9% of the positive detections. GenBank BLAST supported that a unique mismatch in the identified sequence was probably due to intraspecific variability (i.e., 98.91% similarity), and the northern watersnake was the species with the closest match to the sequence.

The eDNA metabarcoding approach was more sensitive for rivers than lakes and for amphibians than reptiles. Species with a high occurrence index ($i = 3$) were more often detected in rivers than lakes (proportion of species detection averaged 45.2% and 23.7% for rivers and lakes, respectively; t-test $P = 0.01$, $t = -3.14$, df = 9.9; Table 5). The proportion of location detection was greater for amphibians than reptiles (the average was 49.3% and 6.3% for amphibians and reptiles respectively; t-test: $P = 0.005$; see Table 5 for the proportions for each species detected). Similarly, amphibians with moderate occurrence index ($i = 2$) were also more detected in rivers than lakes (proportion of location detection averaged 24.6% and 8.3% in rivers and lakes, respectively; t-test: $P = 0.008$, $t = -3.60$, df = 7.7).

Discussion

Increasing spatial distribution knowledge

Improvement of data on distribution range, presence, abundance, and population trends is a major goal of several recovery plans for amphibian and reptile species (Campbell et al. 2002; Lovett et al. 2007; Pavey 2004). Our results empirically support the effectiveness of the eDNA method to trace the presence of both amphibian and reptile species. The only inconsistency between the occurrence index and the eDNA metabarcoding dataset was the detection of the northern watersnake outside of its known distribution range in the Quilliams River. Interestingly, an observation of watersnake by a landowner was reported in 2015 in the same river section where water samples were collected in 2013 (Michel Delorme, personal communication). It was not considered as a valid observation because there was no photographic evidence and the location was approximately 50 km east from the known distribution range. This positive detection out of the known distribution range may help to identify sites for surveying to refine our sparse knowledge on this species. This species is likely to be designated threatened or vulnerable by provincial government in the coming years.

Effect of natural habitats on detection rates

Little is known about the origin (i.e., extracellular DNA fragments, mitochondria, cells, excretion, or eggs) and the dispersion of eDNA in natural ecosystems. Environmental conditions are likely to alter eDNA detection rates due to vertical and horizontal transport (advection and diffusion) and its effect on the eDNA release and degradation rates (Dejean et al. 2011; Pilliod et al. 2014). Without significant water flow, horizontal eDNA transport is expected to be limited due to the fact that eDNA collected from water samples has been suggested to be from cells and mitochondria that will rapidly sink to the bottom of the water body (Barnes et al. 2014; Turner et al. 2014; Turner et al. 2015). Low eDNA transport distances could thus potentially explain the lowest number of species and number of sequences detected in lakes compared to rivers, where eDNA is expected to be transported over large distances by currents (Deiner and Altermatt 2014; Jane et al. 2015). A lack of eDNA diffusion in stagnant water would mean that the sampling design needs to consider taking the samples at the exact site where the genetic material is released. Nevertheless, amphibian and reptile traces have been detected in lakes despite the fact that the sampling was not occurring on the edge of the lakes (i.e., where eDNA traces would have been expected to be higher). This result may underline a greater diffusion of eDNA than is expected, at least for

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abundant secretory animals such as amphibians (i.e., from moulting, mucus, larvae stage). However, due to the variability of environmental conditions, the ability to track eDNA of terrestrial species could potentially vary during seasons and might be higher during spring when the lacustrine environment is well-mixed. Therefore, the eDNA catchability may depend on water column stratification and horizontal sampling location in lakes, whereas the eDNA in rivers may represent a much larger scale of sampling given the flow of water allowing integration over space (Cannon et al. 2015; Deiner et al. 2015). To overcome a potentially greater eDNA degradation in rivers than lakes (Thomsen et al. 2012*b*), we doubled the volume of water that was filtered in rivers compared to lakes, but the environmental conditions may also have increased degradation rates in lakes compared to rivers (e.g., humic acid and tannin). A better understanding of eDNA diffusion and advection in large natural habitats is essential to optimize detection rates.

Variability of detection rates among species groups

Among species with a high occurrence index, detection rate was higher for amphibians compared to reptiles. This may be due to differences in life history traits (e.g., aquatic, high secretion rates such as mucus and moulting, eggs and larvae in the aquatic habitat vs. terrestrial eggs for turtles) and population density (Lacoursière-Roussel et al. 2016*a*; Mahon et al. 2013; Pilliod et al. 2013; Takahara et al. 2012; Thomsen et al. 2012*b*). For example, the three species with the greatest detection rates were northern two-lined salamander, *Eurycea bislineata*, found in 100% of the sampled locations where they were expected to be present (3/3 rivers), northern green frog (*Lithobates* (*Rana*) *clamitans melanota*) found in 83.3% of the sampled locations (10/12 locations), and the American toad (*Anaxyrus* (*Bufo*) *americanus*) found in 75.0% of the sampled locations (3/4 rivers) (Table 5). These species are known to be common, aquatic for all their life cycle with a high occupancy rate and may reach high abundance (Desroches and Rodrigue 2004; AARQ 2013). The northern green frog is the species with the highest occurrence index and likely the highest abundance in our sample sites, and had the greatest total number of sequences. The larval development (tadpoles) of the northern green frog can last up to two years (Desroches and Rodrigue 2004; Dodd 2013*b*). A high number of tadpoles could potentially explain the high levels of DNA detected. Moreover, since eDNA sampling in rivers occurred in July during spawning of the northern green frog, which occurs from June to August (Desroches and Rodrigue 2004), there was potentially a high number of eggs (i.e., high levels of genetic material with the mass of gelatin and the 1400–5300 eggs per spawning) and newly hatched tadpoles in the bodies of water at the sampling time. On the contrary, water sampling in lakes, where the proportion of species detection is lower than in rivers, was realized in early spring when the metabolism of amphibians is still slowed down by cold water and the reproduction activities of amphibian species commonly present in lakes has not begun.

Specific versus multi-species approach

Similar to conventional inventory methods, the eDNA method may generate false negatives due to limited sampling effort, but also due to limitations in the power of detection. The eDNA metabarcoding approach detected the presence of wood turtle in only one of the five rivers whereas it was detected within all five rivers using quantitative PCR (qPCR), which was, therefore, more sensitive than the eDNA metabarcoding monitoring. As it amplifies shorter DNA fragments (i.e., 71 bp DNA sequence for qPCR analyses vs. 167 bp sequence for eDNA metabarcoding analyses), qPCR may detect more highly degraded organic matter (Barnes et al. 2014; Dejean et al. 2011; Hajibabaei et al. 2006), thus improving detection ability. Although qPCR was more accurate and faster than next-generation monitoring for species-specific studies, eDNA metabarcoding still provided a powerful tool to screen the presence of herpetological species, including in habitats that have not been surveyed due to limited resources.

To date, the number of sequences obtained by eDNA metabarcoding cannot rigorously be related to species abundance, or even the number of DNA molecules in the water environment. This may be due to several factors, including (*i*) the disproportionate amplification from unequal primers binding among species, (*ii*) the nonlinearity of the amplifications during the PCR, (*iii*) the variability induced by the multiple manipulations (e.g., multiple beads purification), or (*iv*) the variability in pooling accuracy to collect similar numbers of reads among water bodies. The result of primer competition during the amplification is difficult to predict since it depends on the community composition and the relative abundance of each species. Moreover, the number of DNA copies does not increase linearly during the PCR cycles, whereby it first increases exponentially and then reaches a saturation plateau. Thus, despite the fact that the number of PCR cycles was equal among samples and primers, the number of amplified strands may vary significantly among species and samples as a function of the kinetics process during the PCR reactions. PCR initiation could be altered by the length, the sequence, and the ionic composition of the primer sets, as well as the occurrences of primer dimers (Higuchi et al. 1993; Liu and Saint 2002). The effect of the primer length should be studied, but here —and likely most of the similar studies— we preconized longer lengths of the primer–probe sets for qPCR analyses to ensure the specificity of the amplification (N_{total} = 69 bp) whereas shorter primer sets were used to optimize the amplification of overall species group for metabarcoding analyses (N_{total} = 37 bp). To the best of our knowledge, in nature, eDNA metabar-

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coding monitoring can only provide relative abundance indices based on the species detection rate from spatial replication with similar community composition and relative abundance in habitats with similar environmental conditions. However, Evans et al. (2016) showed a tendency to predict the abundance of species in mesocosm experiments based on eDNA metabarcoding results, and Thomas et al. (2016) recently suggested that quantitative DNA metabarcoding may provide estimate of proportional biomass when relative correction factors are applied. Therefore, more studies are needed to determine the limit of eDNA metabarcoding to provide abundance indices and intra- and interspecific variability of the eDNA release rate.

The use of eDNA as a quantitative tool

A salient result of our study was that eDNA semiquantitative results obtained by qPCR were highly correlated with the relative abundance of wood turtle obtained by visual surveys. Here we used a semiquantitative method (i.e., number of positive amplifications) instead of evaluating the eDNA concentration to predict population abundance from eDNA concentration (Lacoursière-Roussel et al. 2016*a*, 2016*b*; Pilliod et al. 2013; Takahara et al. 2012; Thomsen et al. 2012*b*; Wilcox et al. 2013). Similar to traditional methods, the eDNA method requires a sampling design adapted to the ecology of the species sought, but the eDNA concentration may also largely vary as a function of extreme measurements of eDNA concentration hypothetically caused by sampling cells, other organic tissues, or suspension sediment. Organic tissues or suspended sediment may potentially lead to abundance overestimation. Within a specific site, the eDNA concentration is thus likely to vary according to the rate of DNA released by individuals (interspecies variability, metabolic activity, and size of animals) and the environmental conditions (temperature, radiation, and water flow) (Barnes et al. 2014; Lacoursière-Roussel et al. 2016*b*). In contrast to the eDNA concentration, the number of positive amplifications provides evidence of a recent presence of the targeted species near the sampling area, which is likely to bring a more similar result to visual surveys, as suggested by our results. The maximum value is likely to be reached by a close-targeted individual or by multiple distanced individuals. In contrast, predicting population abundance based on the estimation of the eDNA concentration within a body of water is based on the assumption that higher individual densities release more eDNA within the system. A semiquantitative eDNA detection is based on the assumption that a greater number of specimens will distribute the eDNA more homogeneously within water bodies, thereby increasing the probability of eDNA detection when surveying large aquatic systems. However, this semiquantitative eDNA method is not likely to be applicable to well-mixed environments (e.g., ponds) or for aggregated species.

Increasing local genetic knowledge to improve eDNA species monitoring

Amphibian and reptile species are known to be genetically very divergent among species and even among geographical regions within species (Thomsen et al. 2012*b*; Vences et al. 2005, 2012). Despite the fact that the amplification of COI is often desirable to differentiate the species using the standard DNA barcoding procedures (Che et al. 2012), COI sequences are still often absent for amphibians and reptiles (Murphy et al. 2013). Here, COI sequences for many amphibians and reptiles were still absent from public databases, and the available sequences were not from individuals collected in Québec. Increasing our local knowledge of the genetic divergence of amphibians and reptiles of Québec at the COI locus would potentially improve the alignment of sequences (i.e., >94% similarity with the reference databases) and consequently improve the reliability of the eDNA metabarcoding analyses for herpetological monitoring. A set of mitochondrial 12S primers developed by Valentini et al. (2016) is expected to amplify all amphibians. Here, the 12S sequences were currently missing for 4 of the 19 amphibian species. Further studies are, therefore, needed to validate the efficiency of these primers for North American amphibian species. Sequencing a large number of individuals for each species from different regions is a crucial component to develop large-scale standardized eDNA monitoring programs and to ensure the successful implementation of the eDNA method within conservation strategies.

The potential of eDNA to improve herpetological conservation strategies

For wood turtle, the standardized visual surveys were limited to a single month during the spring between 09:00 and 16:00 on a sunny day (Bernier and Mazerolle 2009), but eDNA samples provided similar population abundance data in July, potentially increasing the time window for data collection. One challenge to increase knowledge about the population range of rare species is to decrease time needed to survey a given site and to increase the number of sites sampled (Dodd and Dorazio 2004). Environmental DNA offers the potential to facilitate species at risk monitoring because water sampling is faster than several traditional survey techniques and may increase the probability of detection (Dejean et al. 2012; Jerde et al. 2011; Pilliod et al. 2013; Smart et al. 2015). The eDNA also requires less work from expert taxonomists, for instance to identify species at various developmental stages (e.g., eggs and larvae).

Conventional methods used to document presence of species and to monitor population trends within a given distribution range are based on presence/absence and (or) count data to estimate abundance and occupancy (Mazerolle et al. 2007). Our results provide an empirical demonstration of the effectiveness of the eDNA method to efficiently characterize the presence of amphibians

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and reptiles in natural environments and show that eDNA may provide quantitative data comparable to measurements obtained by classical visual surveys. The extension or contraction of distribution ranges is one indicator of a species trend (Erb et al. 2015; Mackenzie et al. 2002; Zylstra et al. 2010) and is a common indicator in recovery plan objectives (e.g., Environment Canada 2014). Environmental DNA can be used to define species range data from different habitats and merge sampling efforts when resources are limited. Despite the fact that future work is needed to improve the detection rate of amphibians and reptiles, eDNA already represents a powerful tool for managers as a first step towards rapidly assessing species occurrence and, to some extent, their relative abundance.

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