# INTERREG V A FRANCE-SUISSE 2014-2020

(2014 - 2020)
Axe prioritaire-Priorité d'investissement-Objectif spécifique 2-1-2
AXE 2 : Protéger et valoriser le patrimoine naturel et culturel
6.c En conservant, protégeant, favorisant et développant le patrimoine naturel et culturel
OS 4 = Préserver et restaurer les écosystèmes fragilisés de l’espace transfrontalier

# SYNAQUA

(Ref : 2369 / 2016-36)
SYNérgie transfrontalière pour la bio-surveillance et la préservation des écosystèmes AQUAtiques

## LIVRABLE 39637

WP2.2.2 : Articles scientifiques

Responsables : UNIGE

## PRODUCTIONS SCIENTIFIQUES DU PROJET SYNAQUA

Les données produites au cours du projet SYNAQUA ont été valorisées scientifiquement par des publications dans des journaux scientifiques internationaux (peer-reviewed). Initialement 1 article de ce type avait été planifié, mais l'exceptionnelle originalité et qualité des données a permis de produire à ce jour plusieurs publications scientifiques.

Au total sept articles ont été produits, dont cinq sont déjà publiés, un est soumis et un autre est en préparation.

1. LEFRANÇOIS E, APOTHÉLOZ L, BLANCHER P, BOTREAU S, CHARDON C, CREPIN L, CORDIER T, CORDONIERA, DOMAIZON I, FERRARI B.J.D, GUÉGUEN J, HUSTACHE J.C, JACAS L, JACQUET S, LACROIX S, MAZENQ A.L, PAWLOWSKA A, PERNEY P, PAWLOWSKI J, RIMET F, RUBIN J.F, TREVISAN D, VIVIEN R, BOUCHEZ A (2018) Development and implementation of eco-genomic tools for aquatic ecosystem biomonitoring: the SYNAQUA French-Swiss program. *Environmental Science and Pollution Research* 25(34):33858-33866
2. VIVIEN R, HOLZMANN M, WERNER I, PAWLOWSKI J, LAFONT M, FERRARI BJD (2017) Cytochrome c oxidase barcodes for aquatic oligochaete identification: development of a Swiss reference database. *PeerJ* 5:e4122
3. VIVIEN R, WERNER I, FERRARI BJD (2018) Simultaneous preservation of the DNA quality, the community composition and the density of aquatic oligochaetes for the development of genetically based biological indices. *PeerJ* 6:e6050
4. VIVIEN R, APOTHÉLOZ-PERRET-GENTIL L, PAWLOWSKI J, WERNER I, FERRARI BJD (2019) Testing different (e)DNA metabarcoding approaches to assess aquatic oligochaete diversity and the biological quality of sediments. *Ecological Indicators* 106:105453
5. RIVERA S.F., VASSELON V., BOUCHEZ A., RIMET F. (2020) Diatom metabarcoding applied to large scale monitoring networks: optimization of bioinformatics strategies using Mothur software. *Ecological Indicators* 109:105775

6. VIVIEN R, APOTHÉLOZ-PERRET-GENTIL L, PAWLOWSKI J, WERNER I, LAFONT M., FERRARI BJD. High-throughput DNA barcoding of oligochaetes for abundance-based indices to assess the biological quality of sediments in streams and lakes. (article soumis à *Scientific Reports*, 2019)
7. APOTHELOZ-PERRET-GENTIL L et al. Inferring diatom index through metabarcoding: comparison of marker and methodologies. (article en préparation, octobre 2019)

Les pages de gardes des articles publiés sont données ci-après, et les articles complets sont fournis en annexe. Tous respectent l'obligation de communication exigée par Interreg France-Suisse.

# ARTICLES PUBLIES (2017-2020)

Environmental Science and Pollution Research
https://doi.org/10.1007/s11356-018-2172-2

**ECOTOX, NEW QUESTIONS FOR TERRESTRIAL AND AQUATIC ECOTOXICOLOGY**

## Development and implementation of eco-genomic tools for aquatic ecosystem biomonitoring: the SYNAQUA French-Swiss program

**Estelle Lefrançois[1,2] · Laure Apothéloz-Perret-Gentil[3] · Philippe Blancher[4] · Samuel Botreau[5] · Cécile Chardon[2] · Laura Crepin[2] · Tristan Cordier[3] · Arielle Cordonier[6] · Isabelle Domaizon[2] · Benoit J. D. Ferrari[7] · Julie Guéguen[2] · Jean-Christophe Hustache[2] · Louis Jacas[2] · Stephan Jacquet[2] · Sonia Lacroix[2] · Anne-Laurence Mazenq[5] · Alina Pawlowska[8] · Pascal Perney[2] · Jan Pawlowski[3] · Frédéric Rimet[2] · Jean-François Rubin[9] · Dominique Trevisan[2] · Régis Vivien[7] · Agnès Bouchez[2]**

Received: 29 March 2018 / Accepted: 26 April 2018

**Abstract**

The effectiveness of environmental protection measures is based on the early identification and diagnosis of anthropogenic pressures. Similarly, restoration actions require precise monitoring of changes in the ecological quality of ecosystems, in order to highlight their effectiveness. Monitoring the ecological quality relies on bioindicators, which are organisms revealing the pressures exerted on the environment through the composition of their communities. Their implementation, based on the morphological identification of species, is expensive because it requires time and experts in taxonomy. Recent genomic tools should provide access to reliable and high-throughput environmental monitoring by directly inferring the composition of bioindicators' communities from their DNA (metabarcoding). The French-Swiss program SYNAQUA (INTERREG France-Switzerland 2017–2019) proposes to use and validate the tools of environmental genomic for biomonitoring and aims ultimately at their implementation in the regulatory bio-surveillance. SYNAQUA will test the metabarcoding approach focusing on two bioindicators, diatoms, and aquatic oligochaetes, which are used in freshwater biomonitoring in France and Switzerland. To go towards the renewal of current biomonitoring practices, SYNAQUA will (1) bring together different actors: scientists, environmental managers, consulting firms, and biotechnological companies, (2) apply this approach on a large scale to demonstrate its relevance, (3) propose robust and reliable tools, and (4) raise public awareness and train the various actors likely to use these new tools. Biomonitoring approaches based on such environmental genomic tools should address the European need for reliable, higher-throughput monitoring to improve the protection of aquatic environments under multiple pressures, guide their restoration, and follow their evolution.

**Keywords** Metabarcoding · Bioindication · Water quality assessment · Diatoms · Aquatic oligochaetes

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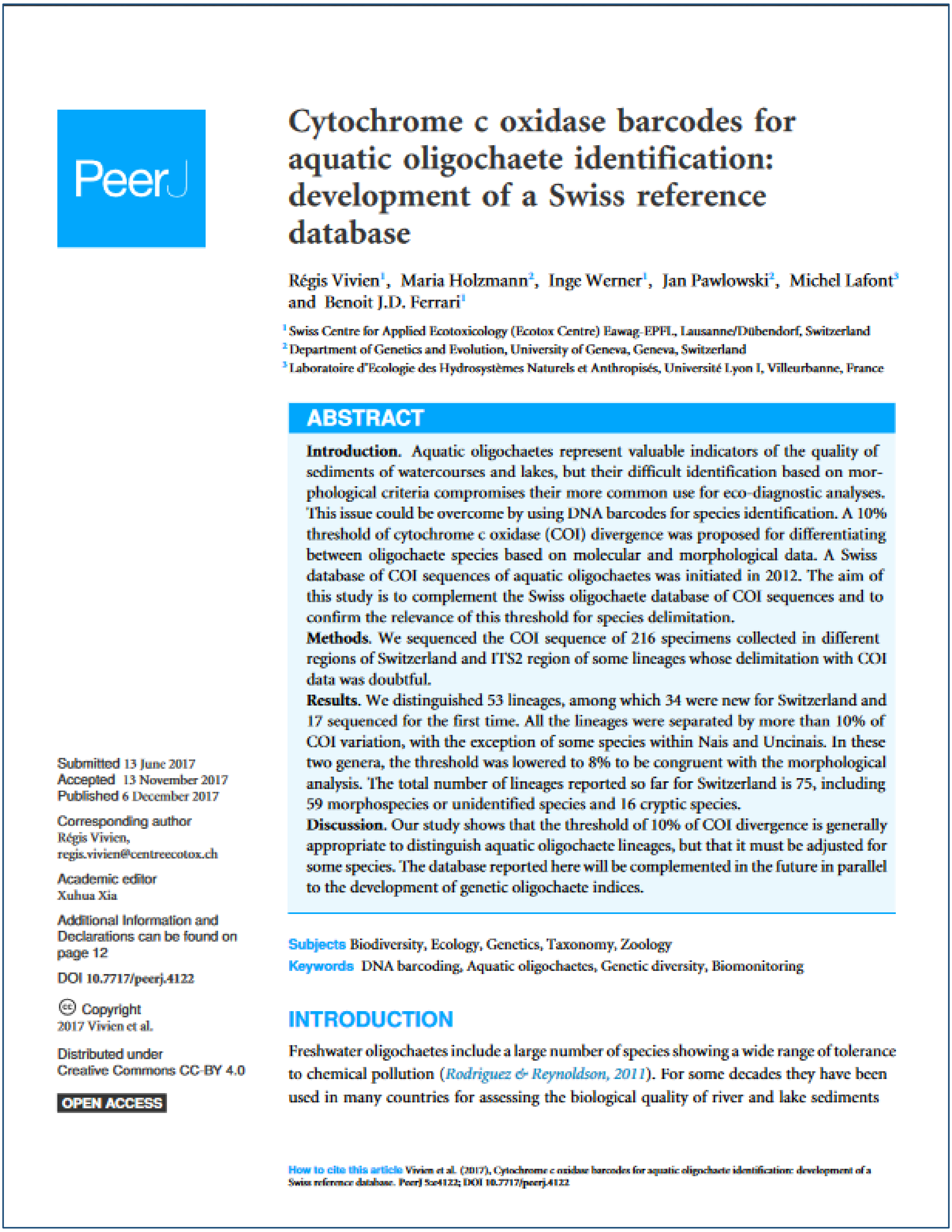

PeerJ

# Cytochrome c oxidase barcodes for aquatic oligochaete identification: development of a Swiss reference database

Régis Vivien[1], Maria Holzmann[2], Inge Werner[1], Jan Pawlowski[2], Michel Lafont[3] and Benoit J.D. Ferrari[1]

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## ABSTRACT

**Introduction.** Aquatic oligochaetes represent valuable indicators of the quality of sediments of watercourses and lakes, but their difficult identification based on morphological criteria compromises their more common use for eco-diagnostic analyses. This issue could be overcome by using DNA barcodes for species identification. A 10% threshold of cytochrome c oxidase (COI) divergence was proposed for differentiating between oligochaete species based on molecular and morphological data. A Swiss database of COI sequences of aquatic oligochaetes was initiated in 2012. The aim of this study is to complement the Swiss oligochaete database of COI sequences and to confirm the relevance of this threshold for species delimitation.
**Methods.** We sequenced the COI sequence of 216 specimens collected in different regions of Switzerland and ITS2 region of some lineages whose delimitation with COI data was doubtful.
**Results.** We distinguished 53 lineages, among which 34 were new for Switzerland and 17 sequenced for the first time. All the lineages were separated by more than 10% of COI variation, with the exception of some species within Nais and Uncinais. In these two genera, the threshold was lowered to 8% to be congruent with the morphological analysis. The total number of lineages reported so far for Switzerland is 75, including 59 morphospecies or unidentified species and 16 cryptic species.
**Discussion.** Our study shows that the threshold of 10% of COI divergence is generally appropriate to distinguish aquatic oligochaete lineages, but that it must be adjusted for some species. The database reported here will be complemented in the future in parallel to the development of genetic oligochaete indices.

**Subjects** Biodiversity, Ecology, Genetics, Taxonomy, Zoology
**Keywords** DNA barcoding, Aquatic oligochaetes, Genetic diversity, Biomonitoring

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Additional Information and Declarations can be found on page 12

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## INTRODUCTION

Freshwater oligochaetes include a large number of species showing a wide range of tolerance to chemical pollution (*Rodriguez & Reynoldson, 2011*). For some decades they have been used in many countries for assessing the biological quality of river and lake sediments

How to cite this article Vivien et al. (2017), Cytochrome c oxidase barcodes for aquatic oligochaete identification: development of a Swiss reference database. PeerJ 5:e4122; DOI 10.7717/peerj.4122

# Simultaneous preservation of the DNA quality, the community composition and the density of freshwater oligochaetes for the development of genetically based biological indices

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## ABSTRACT

**Introduction.** Oligochaetes are recognized as valuable bioindicators of sediment quality in streams and lakes. The development of an oligochaete index based on the identification of specimens using DNA barcodes requires a method for simultaneously preserving the DNA quality and information on the specimen density and oligochaete community composition. Absolute ethanol optimally preserves DNA but fixation of freshwater oligochaetes with this medium can cause disintegration and fragmentation of specimens. Here, we investigated the possibility to preserve oligochaete specimens in low-pH formalin and in neutral buffered formalin for up to four weeks before genetic analyses and tested if the addition of absolute ethanol to formalin-fixed oligochaetes resulted in a loss of specimens and/or species.

**Methods.** We performed guanidine extraction and polymerase chain reaction (PCR) amplification/sequencing of a fragment of the cytochrome c oxidase I (COI) gene on tissue fragments preserved in low-pH formalin for up to 3 weeks and in neutral buffered formalin for up to 4 weeks. In addition, we compared the density and taxonomic composition of formalin-fixed oligochaetes of several sieved sediment samples before and after the addition of absolute ethanol.

**Results.** The COI fragment of all oligochaete specimens preserved in neutral buffered formalin for up to 28 days was successfully amplified by PCR and obtained sequences were complete and of high quality. The amplification success rate for low-pH formalin fixed specimens declined after 7 days of storage. The addition of absolute ethanol to formalin-fixed oligochaete communities did not alter density or diversity estimates.

**Discussion.** Our results indicate that sediment samples can be stored in neutral buffered formalin for up to 4 weeks and the sieved material can then be transferred to absolute ethanol, without affecting DNA quality, density and community composition of oligochaetes. Based on these results, a protocol for preserving freshwater oligochaetes, describing all the steps from collection of sediments to preservation of the biological material in absolute ethanol, is proposed. This method of fixation/preservation is of relevance for establishing DNA barcode reference databases, inventories of genetic diversity and developing genetically based biological indices.

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# Testing different (e)DNA metabarcoding approaches to assess aquatic oligochaete diversity and the biological quality of sediments

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ARTICLE INFO

*Keywords:*
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Species diversity
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DNA metabarcoding
Cytochrome *c* oxidase
Genetic index

ABSTRACT

Aquatic oligochaetes are important bioindicators of sediment quality in watercourses and lakes, but their morphological identification to the species level is challenging and sometimes impossible. The use of DNA barcoding and metabarcoding could greatly facilitate the identifications of specimens and improve ecological diagnoses based on oligochaete communities. The aim of this study was to test how well metabarcoding approaches based on high throughout sequencing (HTS) could perform in assessing oligochaete species diversity and the biological quality of sediments. We analysed oligochaete communities at several sites in Swiss rivers, comparing morphological data to metabarcoding of mixed specimen, sieved sediment and total sediment samples. We amplified the cytochrome *c* oxidase (COI) marker using universal primers and/or specific primers to metazoans. Our results showed that metabarcoding analysis of mixed specimens allowed a more representative assessment of the biodiversity than the metabarcoding of sieved or total sediments. Although community structures obtained with the morphological and metabarcoding analyses were different in term of presence/absence of species and species abundances, the ecological diagnoses based on these two approaches largely agreed.

## 1. Introduction

Aquatic oligochaetes (annelids) represent important bioindicators of the quality of sediments in rivers and lakes (e.g. Howmiller and Scott, 1977; Milbrink, 1983; Lafont et al., 2010; 2012). Different biotic indices based on the analysis of oligochaete assemblages exist, such as the Oligochaete Index of Sediment Bioindication (IOBS) (AFNOR, 2016; Vivien et al., 2014), used in this study, that allows an assessment of the quality of fine/sandy sediments in watercourses. However, the difficulties associated with the identification of aquatic oligochaetes based on morphological features has hindered the use of this taxocenosis for ecological diagnostics. The morphological identification of oligochaetes requires excellent skills in taxonomy, and even those are not sufficient to identify all specimens present in a sample to the species level (Vivien et al., 2017). This affects the quality of the ecological diagnoses based on oligochaete indices.

DNA barcoding allows the rapid identification of a species by matching the sequence of a selected gene to a reference library and thus can overcome the challenges associated with morphological identification. The application of DNA barcodes to identify oligochaete species would facilitate their use in biomonitoring studies and improve ecological diagnostics of sediments. The barcode for the mitochondrial COI gene has been widely used for aquatic and terrestrial oligochaetes (e.g. Rougerie et al., 2009; Kvist et al., 2010; Martinsson et al., 2013). A 10% threshold of COI divergence has been suggested for segregating between aquatic oligochaete species (Zhou et al., 2010; Erséus and Gustafsson, 2009; Vivien et al., 2017; Prantoni et al., 2018). This threshold is generally appropriate to distinguish aquatic oligochaete lineages but must be adjusted for some species. For example, intraspecific divergence larger than 10% has been observed for some cryptic species of *Limnodrilus hoffmeisteri* (Liu et al. 2017) and interspecific divergence smaller than 10% has been reported for some species of Nais/Uncinais (Vivien et al., 2017), of Branchiodrilus (Martin et al. 2018) and of Enchytraeidae (Martinsson and Erséus 2018). A database of COI sequences of aquatic oligochaetes collected in Switzerland is currently being established (Vivien et al., 2017).

Metabarcoding based on high-throughput sequencing (HTS) allows the molecular analysis of a large number of samples and has been

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Original Articles

# Diatom metabarcoding applied to large scale monitoring networks: Optimization of bioinformatics strategies using Mothur software

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ARTICLE INFO

*Keywords:*
Diatoms
DNA metabarcoding
Biomonitoring
Bioinformatics

ABSTRACT

Benthic diatoms are routinely used as ecological indicators in rivers. A standardized methodology is based on biofilm sampling, species identification, and counting under microscope. DNA-metabarcoding is an alternative methodology that can identify species and assess their proportion based on high-throughput DNA sequencing. Sequence data is analyzed with bioinformatics tools, and several strategies can be chosen. The strategy choice can affect communities composition and structure, and therefore the resulting ecological assessment. We wanted to optimize the bioinformatics strategy to obtain the closest results to microscopy. This was done in the framework of the Mothur pipeline. Here, 447 samples from French rivers were analyzed in the monitoring context of the European Water Framework Directive. Samples were analyzed both with DNA metabarcoding and microscopy. A usual bioinformatics strategy in Mothur includes clustering DNA-sequences into Operational Taxonomic Units (OTUs). Different algorithms exist for this. From a subsample of 142 samples, we showed that some strategies (Furthest neighbor) gave closer results to microscopy than others (Opticlust) in terms of community structure and diatom index values. However, we showed that OTU clustering was not necessary for ecological monitoring: Direct taxonomic assignment of individual sequence units (ISU) gave similar results to those obtained in microscopy. Interestingly, direct assignment enabled the detection of more species 2 to 3 times faster in terms of computation time compared to the OTU strategy. However, it remained important to remove low quality and chimeric sequences; if not, biomonitoring results differed greatly from microscopy. We showed that it was preferable to have a loose taxonomical identification threshold instead of a stringent one. This allowed detecting more species, which could participate in the index calculation and increased its performance. Indeed, in diatoms, phylogenetically neighbor species often have similar ecologies, and this explains why it is preferable, in a biomonitoring framework, to identify more species with less stringency instead of identifying few species with stringency. Finally, the best strategy (direct assignment of filtered ISU with a loose taxonomical threshold of 60%) was applied to the 447 samples covering a large diversity of ecological qualities. These data were then used to produce quality index values, using a quantification correction factor taking into account species biovolumes. Compared to microscopy, the DNA-based method assigned the same quality class for 66% of the samples, and 72% of the samples had an index value (ranging from 0 to 20) with less than one point difference from microscopy.

## 1. Introduction

Diatoms are ubiquitous unicellular microalgae routinely used as biological indicators of water quality in Europe as part of the Water Framework Directive (European Commission, 2000). Current standard methods for water quality assessment using diatoms are based on the characterization of environmental assemblages that are subsequently used to calculate biotic indices (e.g. Rimet, 2012). Indices calculation requires identification of diatom taxa to the species level based on the morphology of their frustule. This microscopic identification is challenging because it requires a strong taxonomic expertise and is time-consuming thus limiting the use of diatoms for routine monitoring.

Diatom DNA metabarcoding was developed in recent years as an alternative method for diatom identification (e.g. Kermarrec et al., 2013; Zimmermann et al., 2015). By combining a DNA barcode reference database and bioinformatics processing steps, it is possible to

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*Abbreviations:* HTS, high-throughput sequencing; ISU, individual sequence units; OTU, Operational taxonomical unit

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## ARTICLES A VENIR (2019-2020)

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### High-throughput DNA barcoding of oligochaetes for abundance-based indices to assess the biological quality of sediments in streams and lakes

Régis Vivien[1*], Laure Apothéloz-Perret-Gentil[2, 3], Jan Pawlowski[2, 3, 5], Inge Werner[1], Michel Lafont[4], Benoit J.D. Ferrari[1]

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#### Abstract

Aquatic oligochaete communities are valuable indicators of the biological quality of sediments in streams and lakes, but identification of specimens to the species level based on morphological features requires solid expertise in taxonomy and is possible only for a fraction of specimens present in a sample. The identification of aquatic oligochaetes using DNA barcodes would facilitate their use in biomonitoring and allow a wider use of this taxonomic group for ecological diagnoses. Previous approaches based on DNA metabarcoding of samples composed of total sediments or pools of specimens have been proposed for assessing the biological quality of ecosystems, but such methods do not provide precise information on species abundance, which limits the value of resulting ecological diagnoses. Here, we tested how a DNA barcoding approach based on high-throughput sequencing of sorted and genetically tagged specimens performed to assess oligochaete species diversity and abundance and the biological quality of sediments in streams and lakes. We applied both molecular and morphological approaches at 13 sites in Swiss streams and at 7 sites in Lake Geneva. We genetically identified 33 or 66 specimens per site. For both approaches, we used the same index calculations. We found that the ecological diagnoses derived from the genetic approach matched well with those of the morphological approach and that the genetic identification of only 33 specimens per site provided enough ecological information for correctly estimating the biological quality of sediments in streams and lakes.

(article soumis à Scientific Reports, 2019)

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### Inferring diatom index through metabarcoding: comparison of marker and methodologies

Laure Apothéloz-Perret-Gentil et al.
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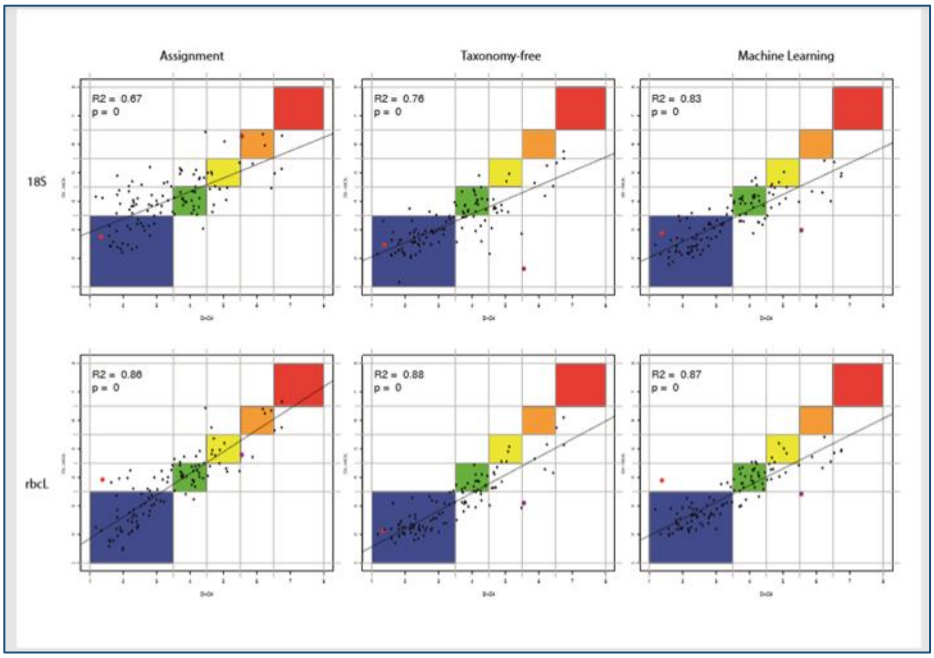
#### Abstract

Recently the use of metabarcoding has been more accepted and used to assess water quality in several countries. However, the choice of the barcoding marker as well as the methodology used to analyse the data is often led by research group and not well standardize. The aim of this study is to compare the two most used genetic markers in diatom metabarcoding as well as well distinct methodologies for data analysis and their impact on the Swiss Diatom Index. We analyse 112 epilithic samples from Swiss and French rivers for which ecological status were established based on morphological identification of diatom species. Both rbcL and the V4 region of the 18S were sequenced through Illumina sequencing technology for all samples and eDNA data were analysed to infer a molecular index based on taxonomic assignment, taxonomy-free approach and machine learning algorithms. We compared the different obtained molecular index with the traditional morphological index and highlighted the pros and cons of each method.



(article en préparation, octobre 2019)

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