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Assemblage diversity, cell density and within-slide variability: Implications for quality assurance/quality control and uncertainty assessment in diatom-based monitoring

Isabelle Lavoie[a,*], Stéphane Campeau[b]

[a] Institut National de la Recherche Scientifique, Centre Eau Terre Environnement, 490 rue de la couronne, Québec, QC, Canada G1K9A9
[b] Université du Québec à Trois-Rivières, 3351 boul. des Forges, Trois-Rivières, QC, Canada G9A 5H7

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ABSTRACT

This study was undertaken to evaluate the variability associated with the microscope analysis step in the application of the Eastern Canadian Diatom Index (IDEC: Indice Diatomées de l'Est du Canada), with the general objective of developing a suitable quality assurance/quality control (QA/QC) program for this biological index. For this purpose, we estimated within-slide variability (replicability) and inter-analysts variability (reproducibility), as a function of diatom assemblage diversity and slide cell density. Overall, our results show that variability associated with diatom assemblage characterization is low, which ensures that IDEC scores reflect environmental changes rather than variability at the microscope analysis step. The main recommendations ensuing from this study are (for the IDEC in particular but also for diatom-based monitoring in general):

(1) An error term of ±2 IDEC units corresponding to the within-slide variability (replicability) should accompany all reported IDEC scores.
(2) A deviation of ±3 points from the audit's IDEC scores should be considered as an acceptable difference. Considering the above-mentioned estimated error term of ±2 associated with all IDEC scores, an overall deviation of 7 would still be satisfactory.
(3) Samples showing low diversity (Hill's N2 ≤5) should automatically be submitted for QA/QC.
(4) A Bray–Curtis (analyst vs audit) similarity of ≥60% should also be included as a QA/QC criterion, and should increase to ≥70% for poorly diversified assemblages (Hill's N2 ≤5).
(5) A diatom valve density of ≤15 per field of view should be targeted in order to reduce variability at the enumeration step.

The results of this study illustrate how a relatively simple and straightforward approach to QA/QC can greatly strengthen the reliability of ecological inferences from an index based on a group of organisms with a high taxonomical diversity. It also highlights the importance of regular communication between analysts in order to maintain a high degree of concordance within taxonomical identification.

1. Introduction

The prime interest in using biomonitoring tools for water quality assessment is the integrated information that organisms provide regarding the health status of their environment. However, even if biomonitoring allows for overstepping point-in time measurements that can show a great deal of fluctuation over space and time, it is still subjected to a certain level of variability and errors at different scales (e.g., sampling, laboratory processing, taxonomic identification). Depending on their extent, these additive sources of errors and variability may potentially affect our ability to accurately measure biological differences between samples collected at sites with contrasting levels of perturbations (Barbour et al., 1999).

Diatoms (Bacillariophyceae) are photosynthetic unicellular organisms at the bottom of aquatic foodwebs and are widely used to assess water quality. Variability at any step of diatom-based monitoring has the potential of affecting the outcome of the biological assessment. However, it has been demonstrated that algal

* Corresponding author. Tel.: +1 418 651 3914.
E-mail address: ilavoie.bio@gmail.com (I. Lavoie).

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community attributes in general and diatom-based indices in particular are robust despite this variability (Kahlert et al., 2012; Prygiel et al., 2002), and environmental factors generally prevail over the variability associated with sampling, sample preparation and microscope analysis (Alverson et al., 2003; Lavoie et al., 2009). Using standardized protocols greatly diminishes sources of variability, and the establishment of quality assurance/quality control (QA/QC) programs is key for detecting errors and quantifying variability of sample processing at the diatom assemblage characterization step. Although central to any discipline for protocol validation, information on QA/QC approaches and criteria used in biomonitoring in general is difficult to find in the scientific literature. This is not to say that they do not exist, but rather that they may be scarce, not well-defined or published in grey literature or internal reports written in various languages. For diatom-based monitoring specifically, most of the available information on variability of diatom assemblage characterization and analyst performance validation relates to the Trophic Diatom Index (TDI; Kelly, 2001, 2013). Kahlert et al. (2016) review diatom identification and counting validation protocols in Europe from different published and non-published sources and also present useful information on this matter.

QA/QC protocols in chemistry or other disciplines are generally well established for laboratory procedures depending on the approach and the analytical instrument used. For example, detection limits, replicability, consistency, accuracy, and sensitivity are often evaluated for validation of analytical methods, and are used for laboratory accreditation. While some of these protocol validation criteria may also be applied to biomonitoring approaches, it is clear that the highly diverse and variable nature of biological data renders it more complicated to validate. For example, detection limit (using a blank sample as control) is irrelevant in the case of most assemblage-based indices because they do not measure an amount of a substance but rather, they express the structure of the species assemblage present in the sample. The fact that the data are usually multivariate (e.g., multi-species assemblage matrices) further complicates statistical measurements of uncertainties for biological data. Quantifying the error strictly inherent to the analyst's performance is also difficult; it relies on the verification of assemblage characterization by an audit and differences in the results may be partly influenced by factors such as assemblage diversity or cell density on the microscope slide. Partialling-out the error due to identification problems and within-slide variability therefore appears to be an essential assignment for adequate validation of the results.

The present study focuses on the assessment of uncertainties and variability of the water quality status obtained using the IDEC (Eastern Canadian Diatom Index; Lavoie et al., 2014) diatom-based monitoring tool, specifically at the diatom assemblage characterization stage of the process. Although this study is oriented toward the need to develop a sound QA/QC program specifically for the IDEC, we feel that the results, discussion and recommendations will undoubtedly be useful for other biomonitoring approaches. It is essential to mention at this point that the purpose of this study was not to assess the potential sources of variability at all steps involved in diatom-based monitoring. Rather, this paper intends to provide an error term for calculated IDEC scores as well as to define appropriate criteria for QA/QC of biomonitoring in general. We acknowledge that variability may be associated with operations conducted upstream in the biomonitoring processes, such as with sampling, but it is argued elsewhere that it is usually relatively low (e.g. Lavoie et al., 2005; Prygiel, 2001). Moreover, once the samples are collected and sent for water quality assessment using the IDEC, this source of variability is out of the hands of the analyst in charge.

The necessity to determine an uncertainty value and to develop an adapted QA/QC program for the IDEC emerged with its growing use in Eastern Canada, particularly in the province of Québec. Since the collection of the first samples in 2002, some 2000 diatom assemblages have been characterized and the IDEC has been used to evaluate the environmental integrity of over 700 Eastern Canadian streams. To date, biomonitoring of streams using the IDEC has been conducted for about 30 organizations in the provinces of Québec and Ontario for the purpose of one-time or continuous annual biological integrity evaluation. For example, the IDEC is currently being used for a before and after assessment of water quality in the context of a large-scale restoration program (by the provincial government in Québec) focusing on small agricultural streams. This type of survey requires a good knowledge of the variability associated with the use of the IDEC in order to adequately establish the proportion of the before and after difference in IDEC scores attributed to the error that is intrinsic to the approach.

We now have an appreciable amount of data that can be used for QA/QC, allowing a better examination of the appropriate criteria to apply as a measure of diatom analyst performance. Along with this purpose, we investigated "within-slide" variability as a function of diversity and slide preparation density (i.e. the concentration of diatom valves on the slide) with the aim of providing the IDEC with an estimated "uncontrollable" uncertainty factor associated with the microscope analysis step and the ensuing IDEC scores.

2. Material and methods

2.1. The Eastern Canadian Diatom Index (IDEC)

The IDEC is a diatom-based index developed as a tool for biological monitoring of stream water quality, and to supplement traditional stream monitoring protocols in Eastern Canada (Grenier et al., 2006, 2010; Lavoie et al., 2006, 2010, 2014). It integrates the effects of multiple stressors on lotic ecosystems, most particularly those related to eutrophication in agricultural and urban areas. IDEC scores reflect the distance, on a scale of 0–100, of a diatom assemblage from its specific reference assemblage, with 100 representing pristine conditions. The IDEC version 3.0 was developed based on 648 diatom assemblage samples, including 150 reference sites (Lavoie et al., 2014).

2.2. Previous history of IDEC-related QA/QC

As the use of a diatom-based approach for assessing stream biological integrity is relatively recent in Canada, only a few analysts have been involved with the IDEC since its first application in 2006. The provincial government analytical laboratory (Centre d'expertise en analyse environnemental du Québec (CEAEQ)) has the expertise for diatom slide preparation and taxonomical identification of the assemblages, and is currently the main user of the IDEC. At the time of writing this paper, only four additional laboratories (Université du Québec à Trois-Rivières (UQTR), Institut national de la recherche scientifique Eau Terre Environnemnt (INRS-ETE), and two private consultant companies) were involved with IDEC-based monitoring. These organizations were submitted to a QA/QC of 10% of analyzed samples starting in 2012. The evaluation of the analysts' performance against a reference (analysis conducted by an audit) was based on the difference in IDEC scores (delta IDEC) and on the Bray–Curtis similarity of the assemblage data. The Bray–Curtis similarity index (Bray and Curtis, 1957) ranges from 0% (complete dissimilarity) to 100% (identical assemblages) and assemblages showing $\geq$60% similarity are usually considered to be similar enough to be regarded as replicate samples (Gauch, 1982). It is fundamental to use these two measures of disparity and not only the delta IDEC values because two completely different diatom assemblages may yield the same IDEC