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1 # Studying ecosystems with DNA
2 # metabarcoding: lessons from aquatic
3 # biomonitoring

4 **Alex Bush[1*], Zacchaeus Compson[1], Wendy Monk[1,2], Teresita M. Porter[3,4], Royce Steeves[5],**
5 **Erik Emilson[3], Nellie Gagne[5], Mehrdad Hajibabaei[4], Mélanie Roy[5], Donald J. Baird[1]**
6 1. Environment and Climate Change Canada @ Canadian Rivers Institute, Department of
7 Biology, University of New Brunswick, Fredericton, NB Canada
8 2. Faculty of Forestry and Environmental Management, University of New Brunswick,
9 Fredericton, Canada.
10 3. Great Lakes Forestry Centre, Natural Resources Canada, 1219 Queen Street East, Sault
11 Ste. Marie, ON Canada
12 4. Centre for Biodiversity Genomics and Department of Integrative Biology, University of
13 Guelph, 50 Stone Road East, Guelph, ON Canada
14 5. Department for Fisheries and Oceans, Gulf Fisheries Centre, 343 Université Avenue
15 Moncton, NB Canada
16 * Corresponding author: alexalbush@gmail.com
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19 Running title: Studying ecosystems with DNA metabarcoding
20
21 Word count: 6,950
22
23 Keywords: biodiversity observation, high-throughput sequencing, taxonomic resolution,
24 community ecology, environmental genomics
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27 # Abstract

28 An ongoing challenge for ecological studies has been the collection of data with high precision
29 and accuracy at a sufficient scale to detect effects relevant to management of critical global
30 change processes. A major hurdle for many workflows has been the time-consuming and
31 challenging process of sorting and identification of organisms, but the rapid development of
32 DNA metabarcoding as a biodiversity observation tool provides a potential solution. As high-
33 throughput sequencing becomes more rapid and cost-effective, a ‘big data’ revolution is
34 anticipated, based on higher and more accurate taxonomic resolution, more efficient detection,
35 and greater sample processing capacity. These advances have the potential to amplify the
36 power of ecological studies to detect change and diagnose its cause, through a methodology
37 termed 'Biomonitoring 2.0'.

38 Despite its promise, the unfamiliar terminology and pace of development in high-
39 throughput sequencing technologies has contributed to a growing concern that an unproven
40 technology is supplanting tried and tested approaches, lowering trust among potential users,
41 and reducing uptake by ecologists and environmental management practitioners. While it is
42 reasonable to exercise caution, we argue that any criticism of new methods must also
43 acknowledge the shortcomings and lower capacity of current observation methods. Broader
44 understanding of the statistical properties of metabarcoding data will help ecologists to design,
45 test and review evidence for new hypotheses.

46 We highlight the uncertainties and challenges underlying DNA metabarcoding and
47 traditional methods for compositional analysis, focusing on issues of taxonomic resolution,
48 sample similarity, taxon misidentification, sample contamination, and taxon abundance. Using
49 the example of freshwater benthic ecosystems, one of the most widely-applied non-microbial
50 applications of DNA metabarcoding to date, we explore the ability of this new technology to

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51 improve the quality and utility of ecological data, recognising that the issues raised have
52 widespread applicability across all ecosystem types.

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53 **Introduction**

54 Biodiversity loss and the risks it poses to ecosystem functions and services remain a major
55 societal concern (Cardinale et al. 2012), but due to a lack of consistently-observed data, there is
56 no consensus regarding the speed or severity of this decline (Vellend et al. 2013; Newbold et al.
57 2015). There are very few ecosystems in which we can quantify the magnitude of degradation,
58 nor can we discriminate among multiple stressors, both key goals for environmental monitoring
59 programs (Bonada et al. 2006). The power to detect change in ecological communities has
60 been hampered by sampling costs predominantly associated with human labour and travel. As a
61 result, ecosystem monitoring programs must manage a trade-off between the scope of a study,
62 including the phylogenetic breadth of taxon coverage and the resolution to which taxa are
63 described, and its spatial and temporal coverage (e.g. tropical forests Gardner et al. 2008;
64 marine sediments Musco et al. 2009). A history of such trade-offs has led to entrenched
65 practices relying on observation of a narrow range of taxa, which aim to provide a surrogate for
66 the full biodiversity complement, yet whose taxonomic, spatial or temporal relationships are
67 largely undefined (Lindenmayer & Likens 2011). The troubling reality is that management
68 decisions are informed by very limited and potentially biased information, generated by
69 approaches that no longer reflect our understanding of how ecosystems and species interact
70 (Woodward, Gray & Baird 2013).

71

72 Fortunately, technological advances offer the opportunity to generate high-quality biodiversity
73 data in a consistent manner, radically expanding the scope of ecosystem monitoring (e.g.
74 Turner 2014; Bush et al. 2017). One of the most promising of these is the technique of DNA
75 metabarcoding, which supports the massively-parallelised taxonomic identification of organism
76 assemblages within a biological sample. The application of this method in ecosystem
77 monitoring, termed “Biomonitoring 2.0” (Baird & Hajibabaei 2012) uses this approach to support

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78 the generation of higher level ecological knowledge that supports advances in our
79 understanding of metacommunity and food-web theory (Bohan et al. 2017). When fully realised,
80 DNA metabarcoding will provide a universal platform to identify any, and potentially all,
81 phylogenetic groups occurring within an ecosystem, including many taxa currently not
82 identifiable by expert taxonomists (e.g. streams: Sweeney et al. 2011; rainforest: Brehm et al.
83 2016; marine zooplankton: Zhang et al. 2018). As DNA sequencing capacity continues to
84 increase, there is a growing interest from ecological researchers and environmental managers
85 for guidance in how to apply these new tools, and to provide clear evidence of their value
86 relative to existing microscopy-based methods. However, it is important to emphasise that
87 comparisons between traditional morphological identifications and DNA sequences are far from
88 straightforward. For example, while metabarcoding can observe the occurrence of DNA
89 sequences within a specified environmental matrix (e.g. soil sample), it does not currently
90 discriminate between intact, living organisms and their presence as parts, ingested, or
91 extraneous tissue. While some may see this as a challenge to be overcome, to retrofit a new
92 method to an old system of observation, we view this as an opportunity to expand our universe
93 of interest and gain new insight into ecosystem structure and function (Bohan et al. 2017). Using
94 data from our own and other studies, we explore the uncertainties surrounding both traditional
95 and DNA-based observation approaches. Our examples are drawn largely from recent research
96 on river ecosystems, a research area with a long history and strong linkages with regulatory
97 application for assessing the state of the environment (Friberg et al. 2011; Leese et al. 2018).
98
99 Aquatic researchers have long recognised the challenges of taxonomic identification and
100 resulting limitations it imposes on the scale and scope of observational, experimental and
101 monitoring studies (Jones 2008). Freshwater monitoring programs rely upon a subset of taxa,
102 primarily aquatic macroinvertebrates, fish, or algae, with little consistency across environmental
103 agencies or regions (Friberg et al. 2011), and sparse spatial and temporal coverage and limited

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104 taxonomic resolution (e.g. Orlofske & Baird 2013) ultimately constrains outcomes to 'pass/fail'
105 (impacted/non-impacted; Clarke et al. 2006; Strachan & Reynoldson 2014), with causes of
106 degradation inferred rather than supported by direct evidence. After decades of research, our
107 ability to disentangle the influence of even the most basic drivers that impact the state of
108 freshwater ecosystems is still limited (Woodward, Gray & Baird 2013).

109 Our unit and universe of observation

110 The science of aquatic biomonitoring is based on the principle that site-level observations of
111 biological assemblage structure integrate responses to prevailing environmental conditions over
112 space and time, reducing the intensity of sampling required to detect stressor-related changes
113 in the environment, and providing an immediate signal of “ecosystem health” (Friberg et al.
114 2011). However, consistently observing more than a narrow range of taxa within an ecological
115 community has proved costly and impractical, with accuracy of identification often unrecorded or
116 difficult to quantify, and varying across taxa. The observation universe is further constrained by
117 sampling method (e.g. mesh-size of collection nets), rather than common phylogenetic or
118 ecological characteristics, with further downgrading or exclusion of groups that are difficult to
119 identify (e.g. Vlek, Šporka & Krno 2006). Even with the best taxonomic expertise available, it is
120 practically impossible to identify all specimens to species-level, since many early life-stages lack
121 necessary diagnostic features (Orlofske & Baird 2013). Species are subsequently aggregated at
122 higher taxonomic ranks, obscuring species-level responses, constraining our knowledge of
123 whether species’ environmental preferences are conserved or variable (Macher et al. 2016;
124 Beermann et al. 2018). In our view, the level of observation provided by direct morphological
125 identification of biological specimens in a sample is highly variable (typically referred to as
126 “lowest taxonomic level”), disconnected from ecological theory, and contains an unknown yet
127 potentially significant degree of bias (Jones 2008).

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128

129 Ecological field studies inevitably face budgetary constraints, and DNA metabarcoding offers the
130 potential to reduce many of the costs involved in routine morphological identification (Ji et al.
131 2013). While single-specimen DNA barcoding uses short genetic sequences to identify
132 individual taxa, often at the species-level, metabarcoding supports simultaneous identification of
133 entire assemblages of organism via high-throughput sequencing (Taberlet et al. 2012; Yu et al.
134 2012). Metabarcoding has now been applied in a wide range of aquatic ecosystems (e.g. rivers:
135 Hajibabaei et al. 2011; wetlands: Gibson et al. 2015; lakes: Bista et al. 2017) and used to
136 describe community composition in a wide variety of taxa (e.g. worms: Vivien et al. 2015;
137 insects: Emilson et al. 2017; diatoms: Vasselon et al. 2017).

138

139 When combined with appropriate bioinformatics tools, DNA-based identification can generate
140 lists of taxa that are typically far richer than those generated by morphological identification
141 (Sweeney et al. 2011; Gibson et al. 2015). This is further enhanced by expanding DNA barcode
142 reference libraries (e.g. Curry et al. 2018) and by machine-learning algorithms (Porter &
143 Hajibabaei 2018c). This has the potential to remove a significant impediment in field ecological
144 studies, which need no longer be constrained by available taxonomic expertise. This new
145 observation paradigm supports a definable universe of observation based on the types of DNA
146 barcodes sequenced (see also below).

147 Defining the universe of observation with metabarcoding

148 While metabarcoding offers the potential to observe a greater diversity of freshwater taxa, the
149 requirement to amplify extracted DNA to generate sufficient material for sequencing places
150 limitations on simultaneous, universal taxonomic observation. The selection of primers used to
151 amplify specific DNA sequence marker regions is crucial to any metabarcoding study, since they

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152 are necessarily tailored to the taxonomic groups under study (Hajibabaei et al. 2012; Gibson et
153 al. 2014). In order to expand taxonomic coverage, it is necessary to employ a range of primers
154 and marker sequences (see Fig.3 in Gibson et al. 2014). Considerable efforts have been made
155 to develop and refine primers for different taxonomic groups or species, and primers with broad
156 coverage for invertebrates have now been established (e.g. Hajibabaei et al. 2012; Elbrecht &
157 Leese 2017). However, amplification bias due to variable affinity among sequence variants for
158 amplification can distort the relationship between sample biomass and the number of sequence
159 reads (Elbrecht & Leese 2015; Zhang et al. 2018). Metabarcoding can therefore support a
160 taxonomically broad universe of observation, but outputs should be treated as occurrences and
161 do not currently support reliable estimation of organism biomass or abundance.
162
163 Before discussing the parallels and differences between morphology-based monitoring and
164 metabarcoding, two key issues must be highlighted: the distinction between bulk-community
165 sampling and environmental DNA (eDNA), and the choice of primers. eDNA samples focus on a
166 signal derived predominantly from traces of intracellular and extracellular DNA without
167 attempting to isolate organisms (e.g. from water or soil; Cristescu & Hebert 2018), whereas
168 bulk-community samples include eDNA, but target the collection of whole organisms. eDNA can
169 be effective in detecting biological signal from the environment, but the significant spatial and
170 temporal uncertainty of that signal clouds its application in observational studies. As a result, our
171 examples of metabarcoding below focus entirely on observations derived from bulk-community
172 samples that are otherwise identical to traditional monitoring surveys.

173 **Interpretation**

174 The statistical power and precision of any ecological assessment based on sample assemblage
175 composition depends upon how results are combined and scored, and how identification errors

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176 (i.e. false-presences and false-absences) can obscure the calibration of baseline composition,
177 limiting our ability to detect deviations from this baseline and infer that change has occurred
178 (e.g. Clarke et al. 2002; Clarke 2009). Although many sources of uncertainty affect our ability to
179 infer regional and landscape-level trends from site-level observations, these are difficult to
180 address with traditional approaches (Clarke 2009; Carstensen & Lindegarth 2016). To illustrate
181 this problem, we focus on how five sources of error involved in describing freshwater
182 biodiversity differ between morphological and metabarcoding workflows: a) taxonomic
183 resolution, b) replicate similarity, c) taxonomic misidentification, d) contamination, and e)
184 quantitative measures like abundance.

185 **Taxonomic resolution**

186 Biomonitoring 2.0 (Baird & Hajibabaei, 2012) employs metabarcoding to overcome the
187 taxonomic bottleneck of sample processing, removing a critical trade-off between sample
188 taxonomic resolution and the number of samples that can be studied (Jones 2008). Moreover,
189 sample metrics derived from higher taxonomic categories, such as family- or genus-level, make
190 a tacit assumption that species within those higher categories share similar environmental
191 responses, and possess similar ecological functions. However, when studies are able to
192 differentiate taxa at the species level, this assumption is false (e.g. nutrient and sediment
193 sensitivity; Macher et al. 2016; Beermann et al. 2018), and this can significantly influence study
194 outcomes (Hawkins et al. 2000; Schmidt-Kloiber & Nijboer 2004; Sweeney et al. 2011).
195

196 Observing taxonomic assemblages at genus- or family-level masks turnover in composition,
197 reducing our power to detect subtle changes among communities over space and time. As each
198 species is less common than its parent taxonomic group, there will be fewer observations with
199 which to establish reliable associations, and their inclusion could add noise to statistical models,
200 echoing the long-running debate about the value of rare taxa in biomonitoring (Nijboer &

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201 Schmidt-Kloiber 2004). This "noise" is not only due to the stochastic occurrence of uncommon
202 species, but also sampling error, which can be quantified before discarding data (Clarke 2009;
203 Ficetola, Taberlet & Coissac 2016; Guillera-Arroita 2016). We should therefore be particularly
204 cautious about concluding how taxonomic resolution affects the strength of statistical
205 relationships (Arscott, Jackson & Kratzer 2006; Martin, Adamowicz & Cottenie 2016). Instead,
206 our current challenge is understanding when these subtle changes, previously invisible to
207 traditional monitoring, are related to natural environmental factors or anthropogenic disturbance.
208
209 One criticism of DNA metabarcoding is that high taxonomic resolution is not valuable if those
210 taxa cannot be linked to a binomial taxonomic name, a limitation that emerges when barcode
211 reference libraries are incomplete (Curry et al. 2018). However, many methods of ecological
212 assessment evaluate community level characteristics such as alpha- and beta-diversity, that do
213 not retain taxon identity, particularly at the species-level (Pawlowski et al. 2018). For this
214 reason, interest in taxonomy-free approaches is increasing among those studying poorly-known
215 assemblages whose morphological identification is challenging (e.g. meiofauna or diatoms:
216 Vasselon et al. 2017). Moreover, new metrics could improve compatibility between
217 biogeographically separated programs (Turak et al. 2017). Nonetheless, to tie DNA-based
218 monitoring to historic surveys, and to assign ancillary information such as traits, it is still a
219 requirement to assign taxonomic names to identified sequences (e.g. Compson et al. 2018).
220 Based on the wealth of ecological information available that could complement DNA-based
221 ecological studies, and the considerable body of legacy data generated by historical studies,
222 including regulatory monitoring, increasing reference library coverage should be a priority for
223 management agencies transitioning to DNA-based surveys.

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224 **Replicate similarity**

225 Depending on the scale of observation, species are rarely distributed randomly or uniformly in
226 nature. For example, the distribution of macroinvertebrate taxa in streams is notoriously
227 dynamic, as species adjust to changes in both abiotic (e.g. flow velocity, substratum size) and
228 biotic (e.g. fish predation, mussel aggregation) factors (Downes, Lake & Schreiber 1993;
229 Vaughn & Spooner 2006). Heterogeneity may also result from stochastic processes such as
230 dispersal and colonization (Fonseca & Hart 2001), ephemeral resources (Lancaster & Downes
231 2014), or disturbance regimes at multiple scales (Effenberger et al. 2006). Indeed,
232 heterogeneity is so pervasive that a shift towards greater homogeneity within aquatic
233 communities could indicate human modification of the landscape (Petsch 2016). Given such
234 heterogeneity, the challenge for ecological studies or biomonitoring is to detect a sufficient
235 proportion of the community, whilst also minimising processing costs, so that further detections
236 are unlikely to alter the interpretation of subsequent analyses. Counting all individuals in a
237 sample can have value, but it is prohibitive for routine observational studies, and not cost-
238 effective for biomonitoring purposes (e.g. Vlek, Šporka & Krno 2006). Most studies therefore
239 employ subsampling (i.e. identifying a subset of individuals collected from the field) to reduce
240 the time, effort and cost of processing macroinvertebrate samples. However, reducing the effort
241 per sampling unit can significantly underestimate the richness per sample (Doberstein, Karr &
242 Conquest 2000; Buss et al. 2014) and although subsampling is standardized by volume, weight,
243 or number of individuals, it is often difficult to compare among survey methods and
244 biomonitoring schemes (Buss et al. 2014). Although sensitivity to subsampling depends on the
245 metric employed, subsampling can substantially increase the misclassification of site status
246 (Clarke et al. 2006; Petkovska & Urbanič 2010) and exaggerate the perceived rarity of many
247 taxa, whose exclusion from analyses may further bias interpretations of condition (Schmidt-
248 Kloiber & Nijboer 2004).

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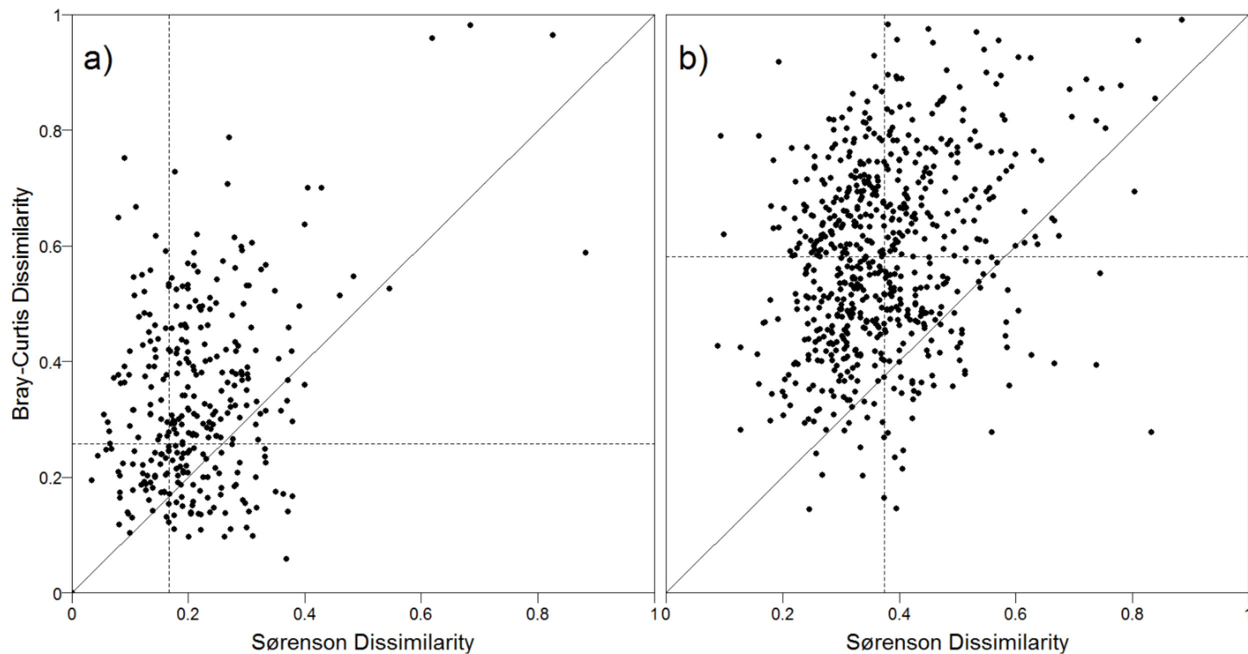

249

250 **Figure 1** - Dissimilarity between replicate samples based on presence/absence data
251 (Sørensen), and count data (Bray-Curtis) of morphologically identified macroinvertebrate
252 families from a) 417 CABIN (Canadian Aquatic Biomonitoring Network; ECCC 2018) surveys
253 (total n=1656, mean richness=16+/-4.8), and b) 787 surveys from the STAR-AQEM dataset
254 (total n=1673) from 14 European countries (mean richness=51 +/-18.4; (Furse et al. 2006;
255 Schmidt-Kloiber et al. 2014).

256

257 Regardless of the sub-sampling approach, a single sample only recovers a subset of the
258 community, particularly in heterogeneous environments (Fig. 1 & 2). As sampling effort
259 increases, either by area or time, more taxa are recovered until the rate of new discoveries
260 declines (Vlek, Šporka & Krno 2006). The rate of accumulation depends on taxon abundance
261 distributions, their dispersion, and ease of collection, including the effects of environment on
262 collection efficiency (Guillera-Arroita 2016). For example, a typical 3-minute kick-sample
263 recovered only 50% of the macroinvertebrates species, and 60% of the families, found in total
264 from six replicate samples (Furse et al. 1981). Other standardized protocols observe a similar
265 degree of turnover among replicates (Fig. 1).

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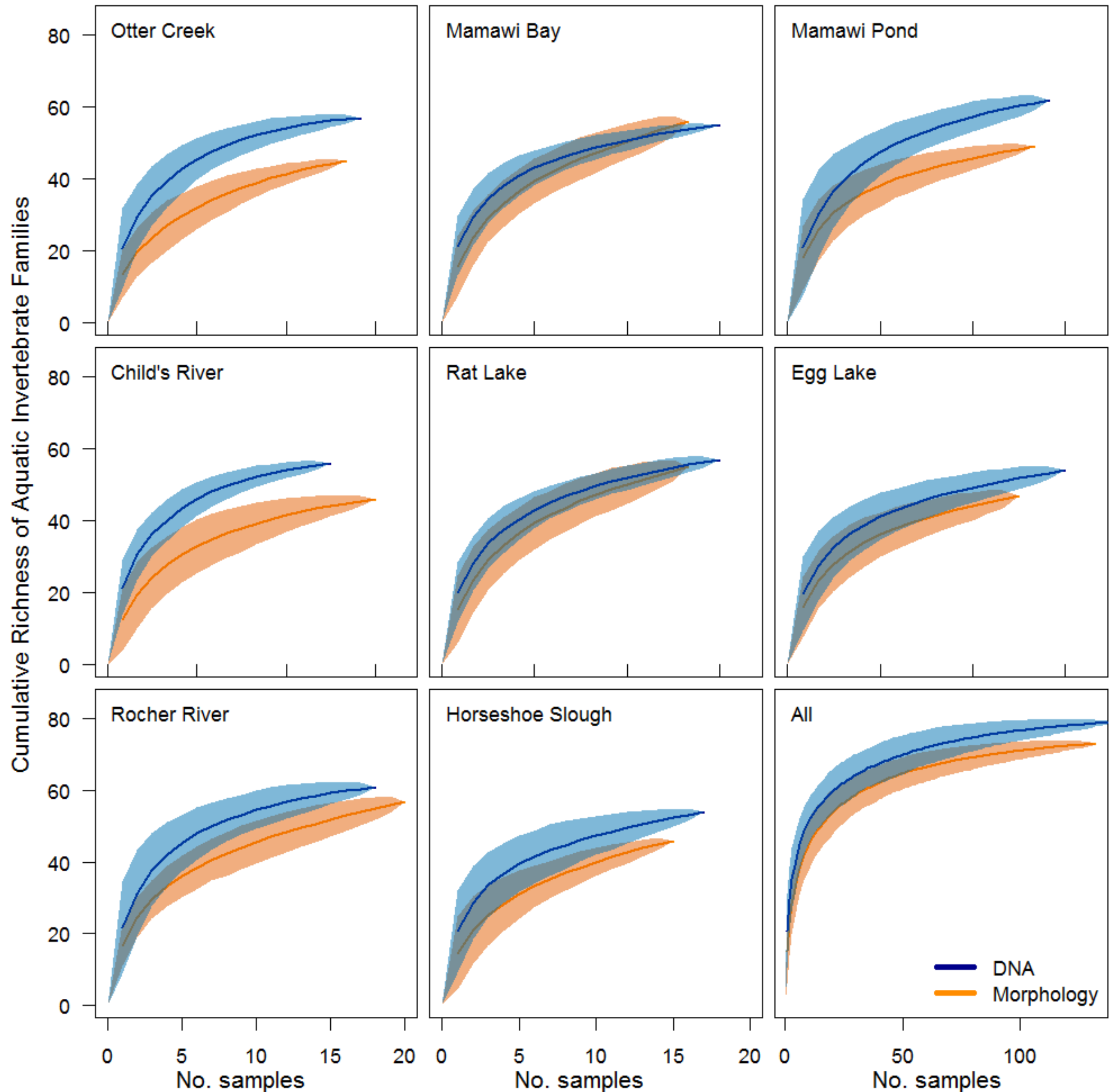

266

267 **Figure 2** - Accumulated richness (mean +/- 95% confidence interval) of aquatic invertebrate

268 families from 8 wetland sites in the Peace-Athabasca Delta, and for all samples combined (note

269 different scale). Samples were collected between 2011 and 2016 (updated from surveys

270 published in Gibson et al. 2015).

271

272 Metabarcoding can, in principle, substantially reduce this sampling error, since the entire

273 sample is processed (but see also limitations associated with primer selection discussed below).

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274 False absences can be further reduced by rarefying the number of taxa observed per read and
275 by analysing technical replicates (i.e. multiple DNA aliquots from sample extracts). Although
276 low-biomass, low abundance taxa may still be missed (Hajibabaei et al. 2012; Elbrecht, Peinert
277 & Leese 2017), metabarcoding detects a higher proportion of the target assemblage compared
278 to morphologically-identified samples (Fig. 2), thereby increasing the power of monitoring
279 programs to detect change.

280 **Misidentification**

281 Morphological identification of diverse taxonomic groups, such as invertebrates, is challenging,
282 as demonstrated by a lack of reliable species-level data generated by routine biomonitoring
283 programs. The probability of misidentifying an individual depends on the quality of the specimen
284 (e.g. is the specimen partial or complete? Is it mature or immature?), the availability and
285 completeness of identification keys, and the taxonomist's experience. Though most
286 biomonitoring programs now include a process for quality control and assessment to limit the
287 likelihood of misidentification, false positives and negatives are still common. For example, early
288 audits of the RIVPACS program showed that 8.3% of family occurrences were missed, and
289 approximately one false presence was added in every four samples (Clarke 2009). Similarly, an
290 audit of a range of European programs by Haase et al. (2006) found that after accounting for
291 misidentifications and sorting errors, samples were on average 40% dissimilar to their initial
292 composition. These errors compound the loss of taxa during sub-sampling, but remain difficult
293 to predict.
294

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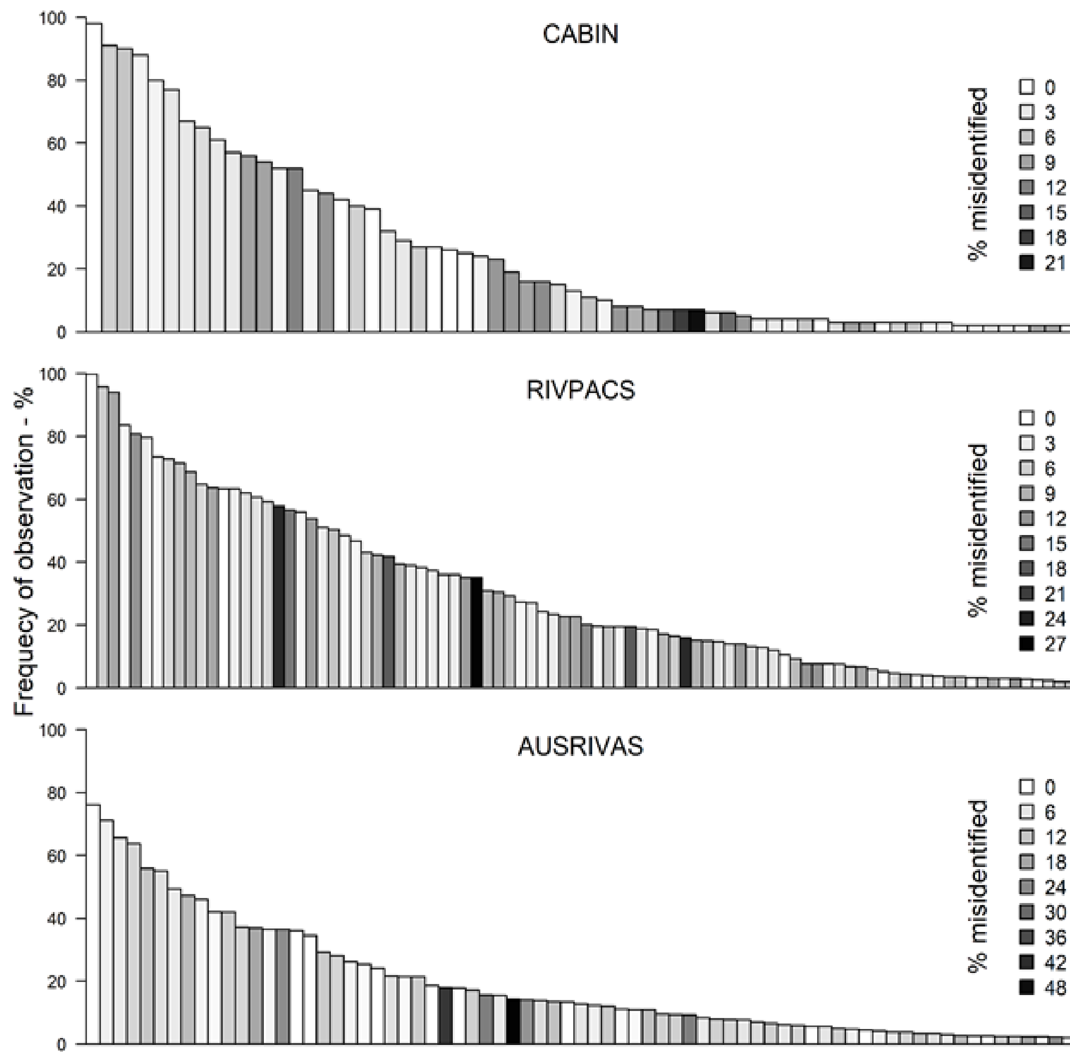

295

296 **Figure 3** - Families ordered by frequency of occurrence within three biomonitoring programs:

297 the CABIN (*n* = 540), the UK River Invertebrate Prediction and Classification System

298 (RIVPACS, n=2,504), and the Australian River Assessment System (AUSRIVAS *n*=1,516) from

299 Victoria. Shading reflects the likelihood taxa could be misidentified using the CO1 RDP classifier

300 v.3 (see Supplement 1 for further details).

301

302 A major advantage of metabarcoding over traditional morphological identification is the ability to

303 generate accurate identifications in a consistent manner (Orlofske & Baird 2013; Jackson et al.

304 2014). That said, the accuracy of metabarcoding still depends on the taxonomic coverage and

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305 quality of reference DNA sequences used for taxonomic inference as well as the bioinformatics
306 approaches employed (Porter & Hajibabaei 2018b). If organisms are misidentified at the time of
307 sequence deposition, reference library sequences become associated with an incorrect
308 taxonomic name. To minimise this challenge, the Barcode of Life Database (BOLD) stores
309 information on voucher specimens, supporting linkage of sequences to material in curated
310 reference specimen collections. Overall, database coverage for animals is expanding rapidly
311 (Porter & Hajibabaei 2018b) and is already relatively high for freshwater invertebrates. For
312 example, sequences exist for 95% of the genera observed in >1% of samples collected by the
313 Canadian national biomonitoring program (Curry et al. 2018; see also Leese et al. 2018). The
314 current BOLD reference library is better suited to identifying macroinvertebrate families routinely
315 observed in Canada, reflecting the greater effort on DNA barcode library development in that
316 country when compared to Australia and the UK (Figure 3). Consequently, at the time of writing,
317 a routine Bayesian classifier (Porter & Hajibabaei 2018a) is expected to misidentify 4.4%, 6.1%
318 and 7.7% of families within CABIN, RIVPACS and AUSRIVAS programs respectively. It cannot
319 be overstated that this is a significant improvement on the documented ability of current best-
320 available morphological identification, and is accompanied by an ability to drill down to species-
321 level, which will only improve as DNA libraries become more complete. To further improve DNA-
322 based identification by barcodes, agencies considering the transition to metabarcoding should
323 support targeted specimen collection, and accelerate the digitisation of existing museum-
324 collected material to improve geographic and taxonomic library coverage (Stokstad 2018).

325 **Contamination**

326 The detection sensitivity of metabarcoding has raised concerns that the number of false
327 positives will increase, particularly due to the adventitious introduction of DNA that did not
328 originate from the sampled site. Existing ecological sampling protocols often recommend
329 cleaning of equipment between surveys to reduce transfer of invasive species or pathogens,

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330 and a more rigorous version of this practice should be adopted as standard practice to reduce
331 the possibility of cross-sample contamination with DNA. Quality control and assurance practices
332 are particularly crucial in eDNA studies that amplify trace amounts of DNA; these studies often
333 include various controls, such as samples from localities that are believed to lack the target
334 taxa, extraction blanks, and equipment controls. A combination of replicate sampling and
335 appropriate controls can then quantify the rate of false-positives and false-negatives before
336 observations are confirmed (Ficetola et al. 2015). Thus, although it is difficult to eliminate the
337 possibility of cross-contamination altogether, it is possible to greatly reduce its occurrence and
338 precisely quantify the probability of errors to support study quality assurance and control.

339 **Quantitative measures of biodiversity**

340 As stated above, DNA metabarcoding results do not currently produce a reliable signal of
341 abundance or biomass (Elbrecht & Leese 2015). Nonetheless, it is equally misleading to
342 suggest that current biomonitoring practices are themselves able to effectively detect
343 differences in macroinvertebrate abundance without substantial effort. The difficulty of
344 processing samples, coupled with species' patchy distributions, means few studies can claim to
345 have truly quantified patterns of abundance for multispecies invertebrate assemblages (e.g.
346 Hawkins et al. 2000).
347
348 Obtaining a reliable estimate of taxon abundance or biomass can support studies of many key
349 ecological processes, but for the specific purposes of detecting compositional change,
350 abundance information is most useful when responses can indicate a shift in species
351 dominance without a change in composition. This is particularly true in depauperate systems, if
352 species are pooled at higher taxonomic levels, or rare taxa are discarded (Reynoldson et al.
353 1997). Nonetheless, differences in the composition of diverse assemblages are often sufficient
354 to discriminate among sites, even at relatively coarse taxonomic resolution (Thorne, Williams &

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355 Cao 1999; Hawkins et al. 2000), thus the challenge has always been the reliable identification of
356 those taxa. While count or relative abundance information may provide another axis for
357 discrimination, its inherent variability exaggerates the dissimilarity among replicate samples
358 (Fig. 1), rendering baseline conditions more variable, thus reducing statistical power to detect
359 change. These limitations are well illustrated by studies that have replaced quantitative count
360 data with qualitative categories or occurrence data (e.g. Wright et al. 1984; Armanini et al.
361 2013). These approaches have proved acceptable to practitioners precisely because count data
362 provide little or no incremental improvement to detecting differences among sites. Moreover,
363 approaches based on occurrence data illustrate a direct pathway to implement DNA
364 metabarcoding in routine biomonitoring programs.

365 **Performance**

366 The relative advantages of DNA metabarcoding over morphological methods are necessarily
367 contingent on the nature and scope of the question being investigated. Bonada et al. (2006)
368 reviewed the requirements of biomonitoring studies to detect the occurrence and intensity of
369 anthropogenic impacts, and Dafforn et al. (2016) explored their applicability to answer questions
370 over a range of spatial and temporal scales. As they are driven by regulatory needs, most
371 monitoring programs focus on relatively simple outcomes (e.g. local deviation from baseline;
372 categorical quality assessment), and thus can greatly benefit from increased precision and
373 statistical power. Recent freshwater ecosystem studies have demonstrated that metabarcoding
374 data can support detection of ecological change at a greater level of discrimination than
375 traditional approaches (Gibson et al. 2015; Elbrecht et al. 2017; Emilson et al. 2017). Although
376 regulators have thus far remained hesitant to transition to monitoring with metabarcoding, these
377 early studies have highlighted a lack of precision and consistency in the application of existing
378 morphological approaches, shortcomings of traditional morphological observation that too often
379 are either ignored or unrecognized by current practitioners.

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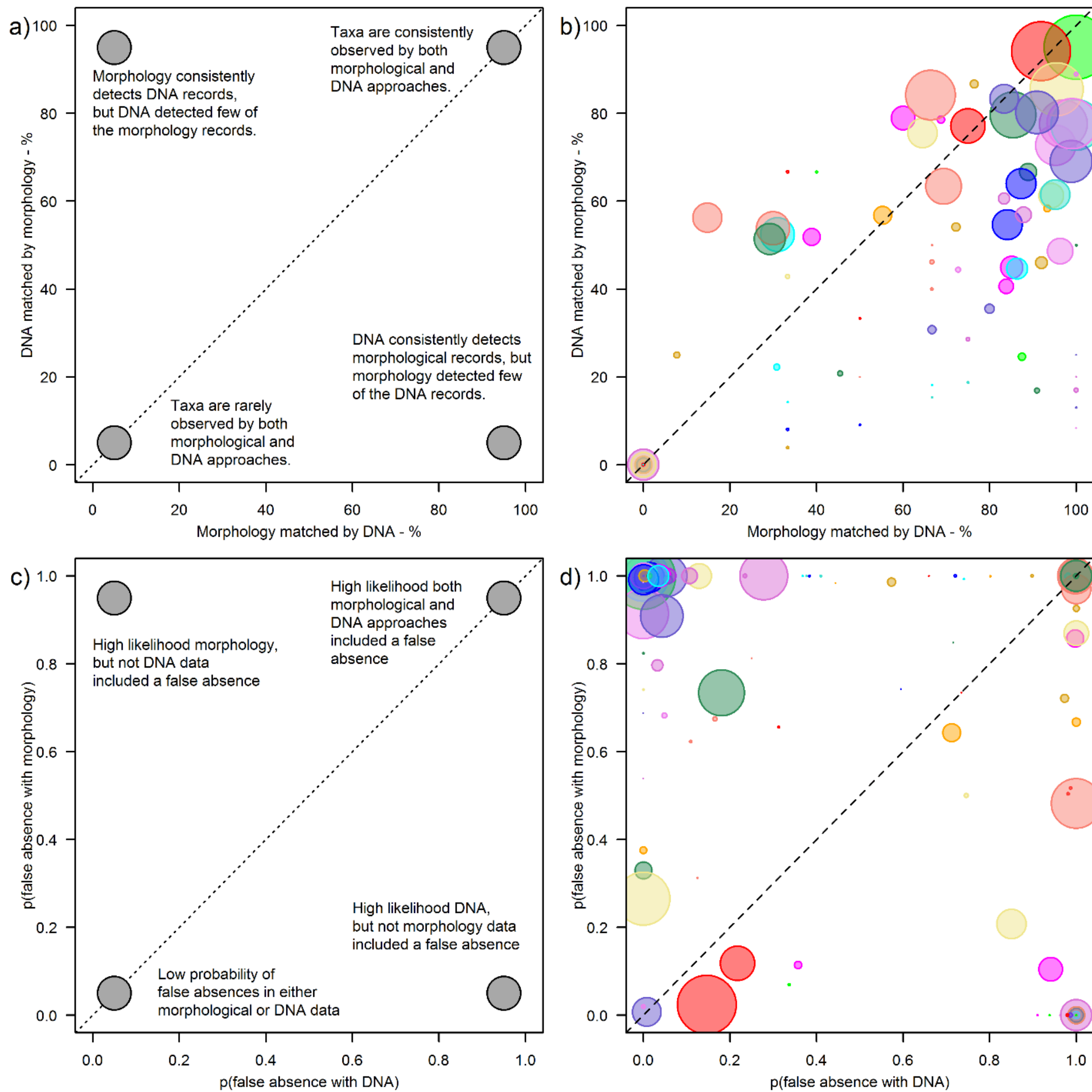

380
381 **Figure 4** Comparison of macroinvertebrate families (n=114) observed in pairs of standard 3-
382 minute river benthos kick samples (n=141 sites). Top row (a and b) shows the correspondence
383 between observations of each taxonomic family using either morphological identification or DNA
384 metabarcoding. Points are scaled relative to the number of morphological observations. Bottom
385 row (c and d) shows the probability that each method included at least one false absence for
386 each taxon (see Supplement 2 for code and raw data).
387

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388 Our purpose in developing DNA metabarcoding as an observational tool has been to explore its
389 ability to provide consistently-observed information to answer routine questions posed by
390 managers (e.g. is biological composition at a site significantly different from expectations, and if
391 so, is there evidence of impact?). Comparisons between metabarcoding and morphology-based
392 methods have involved sorting and identification of a sample using existing taxonomic keys,
393 followed by the reassembly of the sample for metabarcoding (Hajibabaei et al. 2012; but see
394 Gibson et al. 2015). These approaches have demonstrated that DNA metabarcoding recovered
395 ~90% of the taxa identified by morphology, and all false-absences were from taxa that
396 represented <1% of individuals. Most recently, we have also evaluated the similarity of taxa
397 recovered by metabarcoding using paired samples (Fig. 4; GRDI-Ecobiomics 2017). The
398 average similarity of morphological and metabarcoded samples at family-level was 73%, within
399 the range of variation expected for replicate samples (Fig. 1; Clarke et al. 2002). Of the families
400 observed by both methods, DNA observed 79% of the observations made by morphology,
401 whereas morphology only matched 61% of those made by DNA. Some families also appear to
402 be consistently under-represented or absent from this DNA dataset (Fig.4a-b, bottom-left), most
403 likely due to a combination of gaps in the reference library (aquatic mites and oligochaetes in
404 particular) and primer bias (Gibson et al. 2014; Elbrecht et al. 2017). Beyond mere overlap, a
405 better estimate of performance could be the likelihood each family was missed based on their
406 detectability in replicate samples (Fig.4b). Both methods are likely to have missed many families
407 at least once, but the mean and likelihood of multiple false absences was lower among
408 metabarcoding samples than for samples identified by morphology (Supplement 2).
409
410 While primer bias remains an issue, the composition recovered by DNA metabarcoding is
411 always likely to be a subset of all taxa in diverse systems. Nonetheless, metabarcoding provides
412 a step-change in taxonomic coverage, in terms of the taxonomic breadth of taxa observed,
413 improved taxonomic resolution, and fewer false negatives. Compared to traditional

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414 morphological methods, metabarcoding representing a major advance in how consistently we
415 observe the taxonomic structure of ecological communities.

416 Conclusions

417 Biomonitoring 2.0 (Baird & Hajibabaei 2012) envisaged the use of DNA metabarcoding to
418 generate consistently-observed biodiversity data to detect environmental change efficiently and
419 rapidly. This can be done with only minor modification of existing sample collection methods,
420 ensuring backwards compatibility with legacy data. Higher taxonomic resolution, more efficient
421 detection (Fig. 2), and the capacity to increase spatiotemporal coverage can all increase the
422 statistical power to detect change and diagnose its cause (Bonada et al. 2006).
423
424 Study design and interpretation should acknowledge the sources of uncertainty in both
425 morphological and metabarcoding approaches. Although abundance information is specified by
426 existing programs (Leese et al. 2018), it is not necessary to achieve biomonitoring goals and
427 many robust methods that use occurrence information already exist. Sources of uncertainty
428 associated with metabarcoding can be quantified and minimised more easily than morphological
429 approaches (e.g. Davis et al. 2018), and once standard operating procedures emerge, many
430 tasks can be automated, further reducing the risk of handling errors and the costs of sequencing
431 (Porter & Hajibabaei 2018c). A transition to large-scale observation by metabarcoding will take
432 time as sequencing still requires specialized technicians and facilities. However, as demand
433 grows, we anticipate organisations will outsource their DNA sample processing to specialist
434 labs, equivalent to the current use of private consultants for taxonomic and chemical analyses.
435 Currently, the cost of processing an invertebrate community sample (from DNA-extraction to
436 sequencing) is approximately half the cost of morphological identification by taxonomists, but as

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437 we have stressed, the divergent properties of each approach make it misleading to base
438 comparisons on costs alone.
439
440 We can only manage what we can measure, and at present the unknown magnitude and
441 consequences of global biodiversity loss emphasize the value of metabarcoding as a technique
442 to support improved ecological observation in all field studies of multispecies assemblages.
443 Moving forward, we expect the increasing number of metabarcoding studies to further refine the
444 uncertainties associated with observations, and the exchange of information should accelerate
445 as research activities in this area grow, spearheading large-scale implementation of
446 metabarcoding. Metabarcoding is also being used for increasingly novel applications, such as
447 the study of trophic interactions (Bohan et al. 2017), meta-community theory (Miller, Svanbäck &
448 Bohannan 2018), and ecosystem function relationships (Vamosi et al. 2017), and these
449 applications could generate substantial added value to existing or future biomonitoring programs
450 (Compson et al. 2018).

451 Acknowledgements

452 We thank Guy Woodward, Richard Marchant, Astrid Schmidt-Kloiber and Daniel Hering for
453 providing data from monitoring programs in the UK, Australia and EU. This work was supported
454 by the Ontario Genomics Institute and Genome Canada, NSERC, Environment and Climate
455 Change Canada program funds and the Canadian federal Genomics Research & Development
456 Initiative.
457

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