Title: Democratizing water monitoring: Implementation of a community-based qPCR monitoring program for recreational water hazards.

Short title: Community-based qPCR monitoring for recreational water hazards

Sydney P Rudko[1], Ronald R Reimink[2,3], Bradley Peter[4], Jay White[5], Patrick C Hanington[1].

1. School of Public Health, University of Alberta, Edmonton, Alberta, Canada
2. Office of Campus Ministries, Hope College, Holland, Michigan, USA
3. Freshwater Solutions, LLC
4. Alberta Lake Management Society, Edmonton, Alberta, Canada
5. Aquality Environmental Consulting Ltd., Edmonton, Alberta, Canada

Corresponding author: Patrick Hanington, pch1@ualberta.ca

Abstract

Recreational water monitoring can be challenging due to the highly variable nature of pathogens and indicator concentrations, the myriad of potential biological hazards to measure for, and numerous access points, both official and unofficial, that are used for recreation. The aim of this study was to develop, deploy, and assess the effectiveness of a quantitative polymerase chain reaction (qPCR) community-based monitoring (CBM) program for the assessment of bacterial and parasitic hazards in recreational water. This study developed methodologies for performing qPCR 'in the field', then engaged with water management and monitoring groups, and tested the method in a real-world implementation study to evaluate the accuracy of CBM using qPCR both quantitatively and qualitatively. This study found high reproducibility between qPCR results performed by non-expert field users and expert laboratory results, suggesting that qPCR as a methodology could be amenable to a CBM program.

1.0 INTRODUCTION

Community based monitoring is now routinely used for conservation and environmental monitoring(1). Citizen science describes both a methodology of conducting large-scale research by recruiting volunteers, and refers to the process by which citizens are involved in scientific investigation as researchers. Citizen science can include community based monitoring (CBM) as a process of collaboration between government, industry, academia, and local community groups to monitor, track, and respond to issues (2–4).

The earliest incarnations of citizen science and CBM relied on volunteers as data collectors, but the discipline of CBM has grown and evolved. Recent arguments in favor of CBM suggest the field move away from a paradigm of "using citizens to do science" to an equal power relationship which views citizens as scientists, embracing some of the ideals of participatory action research (5).

CBM is poised to improve environmental decision-making. Its use has been on the rise due to budgetary constraints in both government and academia, but also because CBM can be a powerful methodology for generating large spatial or temporal datasets for monitoring/surveillance purposes. CBM improves scientific literacy, builds social capital, improves participation in local issues and benefits the environment (6,7). Traditional CBM programs have typically relied on volunteers to conduct biodiversity surveys, to conduct simple tests (i.e. Secchi disk tests for assessing water clarity), or to collect specimens and send them to central facilities for analysis. However, modern

66 monitoring methods conducted in academia, industry, and government have evolved
67 considerably to include large-scale spatial assessment methods, for example:
68 algal/cyanobacteria bloom-tracking satellites, next generation sequencing analysis, and
69 eDNA monitoring. CBM programs also must evolve and advance as new technologies
70 become available. In water monitoring especially, quantitative polymerase chain
71 reaction (qPCR) has emerged as the method of choice for conducting routine
72 compliance monitoring of water bodies (8).
73
74 Quantitative PCR methods for the detection of surrogates and hazards in water
75 have existed for decades and can be used to detect minute quantities of an organisms'
76 DNA in a complex matrix such as water, soil, or blood. qPCR is highly sensitive (in theory,
77 capable of detecting a single copy of organismal DNA) and is very specific for particular
78 regions of DNA. In the last decade, agencies responsible for monitoring the environment
79 and health have begun to capitalize on the potential of qPCR. Some of the greatest
80 strides have been made in health, especially after the USEPA EMPACT study, which
81 found that levels of enterococcus as measured by qPCR correlate with risk of human
82 gastrointestinal illness (9). Since then, strides have been made in correlating the amount
83 of human-associated *Bacteroides* with human health targets (10,11). Screening for
84 toxigenic cyanobacteria species is also moving towards molecular detection method. For
85 example, in Poland, initial screening for toxin genes in recreational waters is conducted
86 using qPCR, followed by immunochemical analysis to quantify the toxins (12). In related
87 fields like environmental monitoring some locales have moved to molecular methods

for monitoring for the veliger stage of invasive zebra (*Dreissena polymorpha*) and quagga (*Dreissena rostriformis bugensis*) mussels.

As the effectiveness of qPCR diagnostic tests continues to be realized, it is apparent qPCR is an excellent choice for CBM, or more broadly, a decentralized monitoring system. qPCR is a platform, and with the infrastructure in place, monitoring for additional targets becomes a matter of designing/validating a new test and running it on the established infrastructure. For this reason qPCR and related molecular techniques have been touted as grand solutions for point of care diagnostics in infectious disease monitoring, this future has not yet been realized (13,14). The idea of portable diagnostic technologies that can be used to detect multiple targets, which feed information into a surveillance system, is attractive for a number of reasons, but the development to implementation gap is often wider than one would expect.

It is often presumed that highly skilled personnel are required to execute molecular biology methods such as qPCR. Additionally, technologies to conduct testing portably have only just begun to emerge onto the market and have not been fully vetted. This study is, to our knowledge, the first of its kind to test the rigor of qPCR for detection/quantification of biological hazards and their surrogates in water through a CBM-implementation study. Here, we test the feasibility, reproducibility and reliability of implementing portable qPCR water monitoring amongst a variety of groups (government, NGO, and private enterprise). This was assessed both quantitatively, by

conducting our own measurements on CBM partner samples, and qualitatively, through surveying our user groups to capture their perceptions of the technology and its fit within their individual contexts and organizations.

2.0 MATERIALS AND METHODS

2.1 Implementation study design

We first connected with relevant stakeholders of recreational water in Alberta, and worked with them to determine their monitoring goals. Using a participatory research (PAR) approach, we then developed qPCR tests and testing methodologies that would fill these needs(15). Under this PAR approach, CBM partners selected study sites they felt would be appropriate, and we advised and assisted in this selection where it seemed appropriate. Since the goal of this study was to measure the effectiveness of a CBM monitoring program in a real world context, participants in the study were instructed to collect a duplicate sample or cut the filter membrane in half after filtration and send this to the university lab. Samples in our lab would be processed in an identical fashion to the field user to compare novice versus expert methodologies (Fig 1). Additionally, CBM partners sent their extracted DNA to our lab, which enabled us to also perform qPCR on their DNA extracts and to perform inhibition reactions.

2.2 Sample collection

131 Specific water collection methods are detailed below for each target of interest;
132 regardless of the volume collected, all samples were then filtered through a 0.4 µm
133 polycarbonate filter (Pall FMFNL1050) using an electric vacuum pump (Vaccubrand®).
134 CBM partners had the option of either collecting and filtering a duplicate water sample
135 for analysis, or cutting their filter membranes in half to be analyzed at the university lab.
136
137 **2.2.1 Avian schistosome monitoring:** Sample collection was conducted as
138 described in Rudko et al (2018). Briefly, 25L water samples, collected one litre at a time
139 across a shoreline up to ~1m deep were passed through a 20µm plankton tow. Debris
140 from inside were washed down using well water (this is not a contamination risk when
141 monitoring for avian schistosomes as these parasites are shed from snail hosts, and only
142 when those snail hosts also co-occur with locations where the bird definitive host's
143 feces are also present(16)) followed by a 95% ethanol wash and collection in sterile 50-
144 mL conical tubes.
145
146 **2.2.2 Toxin-producing cyanobacteria monitoring:** Sample collection was
147 conducted from watercraft operated by CBM partners on various lakes. Samples were
148 collected through a one-way foot valve attached to weighted 3/4" Nalgene tubing.
149 Samples were only collected from the euphotic zone as determined by a Secchi disk
150 measurement at each lake's deepest point. Ten sampling locations were selected for
151 each lake, with water being composted from each sampling location into a central
152 container. Water from this container was then poured into 50-mL conical tubes.

153 Equipment was decontaminated between lakes using quaternary ammonium compound
154 to prevent contamination between lakes.
155
156 **2.2.3 HF183 monitoring:** All samples were collected by scooping two 50ml
157 samples in sterile, conical, collection tubes from the surface water 15m from shore
158 every 150m along the entire perimeter of each participating lake.
159
160
161 **2.3 DNA extraction**
162 **2.3.1 In field method**
163 DNA extraction was conducted using the MI Sample Prep Kit (Biomeme)
164 according to the manufacturers' instructions. The MI sample prep kit is designed to
165 function in the field. Lysis is accomplished by placing a filter in the lysis buffer and
166 shaking for one minute. Next, the solution is passed through a syringe unit fitted with a
167 DNA binding column. The column undergoes two washes to remove proteins and salts,
168 and then is dried using an acetone buffer before elution. In 2018, the avian
169 schistosomes monitoring group was interested in transitioning to a DNA extraction
170 method that would allow for batch processing of samples. We therefore opted to
171 transition their program to the DNAeasy DNA extraction kit (Rudko et al. 2018). To set
172 up this remote laboratory in a cost-effective manner, equipment (centrifuge, heating
173 block, and vortex) were sourced from Dot Scientific (Supplementary Table 2), and

pipettes were from VWR. Sample blanks were conducted by partners every batch of 24 samples processed.

2.4 qPCR methods

2.4.1 Maintaining workflows: All master mix components were mixed in a clean room located at the University of Alberta and aliquoted into 0.2 ml thin wall PCR tubes (Axygen). All plasmid dilutions and preparation of positive controls occurred in a deadbox. Standards and reaction tubes were prepared independently to prevent cross contamination.

2.4.2 In lab qPCR method

Samples were quantitated relative to a plasmid standard curve which contained 50,000, 5000, 500, 50, 5 and 0.5 copies. Each of the gene targets below was synthesized (IDTDNA) into a puc19 plasmid vector (Genscript). Thermocycling was performed on the ABI 7500 Fast or the QuantStudio 3 using a standard, 40 cycle, two-step reaction. The thermocycling parameters were a 30 second hold at 95 degrees, followed by a 30 second denaturation cycle at 95 degrees, and a 60 degrees annealing cycle. Each qPCR reaction had a final volume of 20, and we added 5µL of DNA to each reaction.

2.4.3. Avian schistosomes: The *18S* avian schistosomes-targeting qPCR assay was performed as described in Narayaran et al (2015) and Rudko et al (2018). The LOD_{95} of this technique is 3.4 gene copies/ rxn (17) (Table 1S). qPCR master mix (IDT DNA) containing 1x Master mix, and 200nm forward reverse primer and fluorescein-labeled probe was used.

199 **2.4.4 Toxigenic (mcyE gene) cyanobacteria monitoring:** The mcyE gene
200 targeting qPCR assay was performed as described in Qiu et al. (2013) and Sipari et al.
201 (2010) (Table 1S). The LOD_{95} of this technique is 6.25 copies/5µL. qPCR master mix (IDT
202 DNA), containing 1x Master mix, and 200nm forward reverse primer and 125nm
203 fluorescein-labeled probe was used.
204
205 **2.4.5 HF183 bacteroides monitoring**: This 16S gene-targeting assay was
206 performed as described in Haugland et al. (2010). The LOD_{95} of this technique is 7.2
207 gene copies/rxn. qPCR master mix (IDT DNA), containing 1x Master mix, and 100nm
208 forward reverse primer and 80nm fluorescein-labeled probe was used (Table 1S).
209
210 **2.4.6 In-field qPCR method**
211 Mastermix components and concentrations were unchanged between the lab
212 method and the field method, nor were the thermocycling parameters. CBM partners
213 received 4 control tubes, which consisted of a negative control, and 3 standards
214 (5000,500, and 50 copies). They were instructed not to open these tubes to prevent
215 contamination. CBM partners also received 12 tubes to add their own samples DNA to
216 (Fig 1).
217
218 **2.4.7 Inhibition controls**

Inhibition controls were performed as described in Rudko et al. (2017) (20). Plasmid control DNA was spiked in excess into qPCR reactions containing 5µL of water sample DNA, and inhibition was defined as a 3-ct (i.e. 1 log) shift in amplification.

2.5 Creation of the field kits

Field kits given to CBM partners contained: the M1 DNA extraction kit (Biomeme), 1.5 ml snap-cap tubes, sample collection vials (Corning), a 20 micron plankton tow (Acquatic Research Instruments), 0.45 uM polycarbonate filter funnels (Pall, FMFNL 1050), a 20 µL pipette, a box of pipette tips, PCR tubes, a laptop (some Acer, some Chromebook) an Open qPCR thermocycler, all the necessary cables, and reaction strips (Fig 1, Supplementary Materials).

2.6 Training of CBM partners

CBM partners were provided with a training video, and a written protocol. Additionally, they were provided with two in-person training session. Typically we would demonstrate the method in our laboratory, and the second training session would on-site at their location, where the CBM partner would run their first samples.

2.7 Capturing CBM partners perceptions of the method

CBM partners (6 in total) were administered a survey with open-ended questions regarding the implementation of the method (Supplemental Table 3). All 6 CBM partners submitted a completed survey. Surveys were blinded from the researchers to

encourage honesty from participants; a research associate received the surveys via email and edited them to remove any personal identifiers before sending them to the analyst. Data were analyzed using deductive thematic analysis(21). Open coding was used, and codes were developed and modified as the analysis took place. Analyzing the codes enabled the identification of initial themes; these preliminary themes were refined to demonstrate interesting patterns in the data that were important to the successes or failures of the implementation. Themes were realized semantically (i.e. the explicit or surface meaning of the data), and latently, to identify and examine underlying ideas and assumptions that inform the semantic content of the data (22).

2.8 Ethics Statement

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This research was approved by the University of Alberta Human Research Ethics Board: Approval # Pro00048511.

2.9 Bland-Altman plots

Bland-Altman plots were created in GraphPad Prism 8 on the log transformed copy number per 5µL data. Log transformation was performed prior to conducting the analysis because this method assumes that the SD of method differences is uniform

262 across measurements, but it has been documented that variability in measurement
263 becomes greater when a larger value or amount of analyte is being measured (23,24).
264
265 **2.10 Statistics**
266 Statistical analyses were conducted in SPSS (version 25). Graphs were made in
267 GraphPad Prism 8. Limit of Detections were calculated using the POD/LOD calculator
268 (25). Maximum log difference was calculated as the upper 95% confidence interval of
269 average of the log difference between all sets of paired samples. Interclass correlation
270 analysis was performed in SPSS on the log-transformed data using a two-way random
271 effects model with average measures, and a type c model with a consistency definition.
272 A two-way random effects model was selected because it models both an effect of
273 operator and the sample, and assumes that both are drawn randomly from larger
274 populations.
275
276 # 3.0 RESULTS
277
278 ## 3.1 THERMOCYCLER COMPARISON
279 ### 3.1.1 Detection limits of the Open qPCR thermocyclers
280 The limit of detection 95 (LOD_{95}) of the Open qPCR thermocyclers is 63.4 gene
281 copies (GC)/5µL (lower limit 43.7 GC/5µL, upper limit 89.2 GC/5µL, n= 40, based on all
282 qPCR tests). This is approximately 1-log higher than the same assays (Avian
283 schistosomes $LOD_{95:}$ 3.4 GC/5µL;Toxic cyanobacteria LOD_{95}: 6.25 GC/5µL ; HF183 LOD_{95}:

284 7.2 GC/5µL) performed using our laboratory ABI 7500/QuantStudio 3 thermocycler. All
285 of these assays have been validated in previous papers, the names, sequences, and the
286 references for the primers and probes are found in Supplementary Table 1. Standard
287 curves performed optimally using the Open qPCR thermocyclers (Table 1).
288
289 **3.2 Comparison between machines**
290 Interclass correlation coefficients (ICC) were calculated to compare CBM partner
291 DNA extracts run on the Chaibio Open qPCR machine, and our laboratory ABI
292 7500/QuantStudio 3. In 2017, the ICC of the avian schistosomes assay was 0.88 (95% CI:
293 0.85 lower, 0.90 upper), and in 2018, it was 0.76 (95% CI: 0.56 lower, 0.866 upper), in
294 2018 this group used 2 Open qPCR machines, and this ICC is a pooled result of both of
295 these machines. In 2018, the ICC of the toxic cyanobacteria assay was 0.57 (95% CI: 0.1
296 lower, 0.86 upper) (Table 2). Maximum log differences were also calculated and ranged
297 from 1-1.5 depending on the test and year (Table 2).
298
299 **3.3 CBM PARTNER COMPARISON**
300 **3.3.1 Semi-quantitative analysis using Bland-Altman plots**
301 Reproducibility was assessed using the semi-quantitative Bland-Altman plot.
302 Bland-Altman plots graph the average of two measurements on the X-axis and the
303 difference between these measurements on the Y-axis. The Bland-Altman plot for avian
304 schistosomes monitoring for 2017 and 2018 show a linear pattern at lower copy
305 numbers, but at higher copy numbers show uniform variability (Fig 2). Bland-Altman

306 analysis of the toxic cyanobacteria test shows uniform variability within the limits of
307 agreement (1.96 times the standard deviation). A paired t-test using the log-
308 transformed data was used to compare the within-subject standard deviations of the
309 partner data compared to the lab-generated data. They were significantly different
310 based on an F-test and Welch's t-test ($p < 0.0001$, $F=6288$, mean difference ± SEM:
311 20326 ± 9843.
312
313 **3.3.2 Interclass Correlation Analysis**
314 ICC analysis was performed to compare user and lab samples. In 2017, the
315 Biomeme MI extraction kit was used for swimmer's itch monitoring. The ICC between
316 user and lab extraction samples was 0.539 (95% CI: 0.320 lower, 0.680 upper). The ICC
317 2018 for avian schistosomes monitoring was 0.593 (95% CI: 0.344 lower, 0.747 upper).
318 The ICC mcyE was 0.640 (95% CI: -0.250 lower, 0.896 upper) (Table 2). Maximum log
319 differences ranged from 1.3-1.4 (Table 2).
320
321 **3.3.3 Inhibition controls**
322 PCR Inhibition was tested on partners DNA extractions and on DNA extractions
323 performed in house. Between 5-8% of samples were slightly inhibited in both partner
324 and in house extractions. Cyanobacteria samples were most likely to be inhibited.
325 Inhibited samples were excluded from the analyses in this paper.
326
327

3.4 QUALITATIVE ANALYSIS

3.4.1 User Perceptions

User perceptions of the program were captured through a written survey that was administered to participants. The questions are available in Supplementary Table 3. Thirty-three percent (33%) of respondents stated that they had some prior knowledge of molecular biology, PCR (polymerase chain reaction), eDNA, or DNA based detection in general prior to the use of the qPCR field method. Fifty percent (50%) reported having low prior knowledge and one participant had no prior knowledge. The same 33% of respondents who reported some knowledge with molecular biology and methods also reported having performed some form of PCR in the past. The rest of respondents reported not having performed PCR (50%) and one participant did not remember. However, prior knowledge did not impact the training all users were provided.

3.4.2 Thematic analysis

User surveys underwent deductive thematic analysis whereby surveys were coded, and then codes were organized into themes (22). The codes identified and relevant excerpts from the surveys are presented in Supplementary Table 4. The first theme identified is “rapidly responding to hazards”. This theme captured the CBM partners’ perceptions on the speed of the qPCR method and their perceived ability to respond to issues quickly. The second theme identified was the question of who controls the CBM monitoring system. This theme emerged from CBM partners expressing a desire for independence and control over the interpretation of results. The

350 third theme identified was that the triangulation of training was valuable in that most
351 CBM partners suggested that the written and video protocols (complemented with a
352 few in person training sessions) were important to them and enhanced their learning. A
353 subtheme that emerged from this theme was “learning and communication”.
354
355 **4.0 DISCUSSION**
356
357 In this study, the accuracy of a community based qPCR-monitoring system was
358 assessed. We assessed the accuracy of the portable qPCR machines relative to a “core”
359 machine, and the ability for CBM partners to execute the method. Our analyses have
360 demonstrated that a CBM qPCR monitoring program can yield accurate results for
361 different targets (i.e.: eukaryotic versus prokaryotic); however, if the method itself is too
362 time consuming or challenging to be completed by relatively novice CBM partners, a
363 larger scale implementation of the CBM monitoring program could be less reliable.
364
365 Our intention was to implement a CBM qPCR system in a real-world context. As Fig 1
366 details, we began the development of this project by consulting with local stakeholder
367 groups and assessing their interest in the project and what types of biological hazards
368 and surrogates they might be interested in monitoring for. Our goal was to have
369 partners run a sufficient number of tests, not to prescribe a particular test for CBM
370 partners to run. Therefore, we adapted to the needs of our CBM partners and adapted a
371 variety of existing qPCR tests to the field equipment and testing protocol. Additionally,

372 some of the groups we worked with had their own scientific questions they wanted to
373 answer, and we facilitated this.
374
375 Our laboratory distributed all materials required to complete testing to users,
376 additionally we prepared all qPCR master mix components (enzyme mix, primers and
377 probes), and aliquoted these into individual reaction tubes for users. The purpose of this
378 was two-fold, to prevent contamination of CBM partners' qPCR reactions, and for
379 simplicity for partners. Our laboratory facilities are equipped with a PCR clean room, as
380 well as separate pre and post amplification rooms. By preparing reaction tubes and
381 controls, we could prevent CBM partners handling high copy number controls (a likely
382 source of contamination). Additionally, CBM partners were instructed not to open tubes
383 that had undergone qPCR. The Biomeme DNA extraction does not utilize pipettes, but
384 all users were supplied with filter-tipped 20µL pipettes to add their purified DNA into
385 their reaction tubes. Pre-preparing reaction tubes made running qPCR as simple as
386 adding the DNA and pressing "Start" on the Open qPCR machine.
387
388 Analysis of the qPCR data was also performed by our laboratory. CBM partners would
389 download their spreadsheets from the Open qPCR and send them either via email or
390 google drive to our labs, where we would analyze control data, and calculate copy
391 numbers and, where possible, organismal numbers for partners. Again, this was done in
392 attempt to preserve the simplicity of the method, and because analysis of qPCR data is
393 complex and requires an expert eye.

394

395 The CBM partners participating in this study ran 985 total samples over the two years of
396 this program. Deductive thematic analysis was performed to analyze CBM partner
397 surveys, which is a method of analysis by which codes and theme development were
398 directed by our existing research questions. Three primary themes emerged from this
399 analysis.

400

401 The first theme identified was “Rapidly responding to hazards”. Our CBM partners liked
402 that the “time requirement from the qPCR testing method was less than the traditional
403 operational time frame..." However, when asked about the time it took for the method
404 to be completed and if this time was appropriate, all of our CBM partners equated
405 rapidness of the method to a rapid policy response to hazards. This was likely not the
406 reality, as any hazard was dealt with formally through our existing regulatory system
407 which is currently still adapting to the implementation of qPCR methodologies for water
408 monitoring at their core facilities, and for which clear policy frameworks and courses of
409 action do not yet exist for qPCR test results. The only exception to this reality was the
410 avian schistosomes monitoring group, who liked “that [they] could use the next day to
411 change [their] field procedures and experimental designs".

412

413 The second theme was “Independence and verification of a CBM monitoring system”.
414 Two codes that emerged during analysis were CBM partners expressing a desire for
415 more independence and more control over the interpretation of results. Our study was

416 designed to remove data interpretation from participant's hands, and instead place it in
417 our own hands (with the vision that in a CBM monitoring system that data analysis
418 would be accomplished by a central data processor or the enforcement agency). We
419 thought this would be beneficial because the interpretation of qPCR data is not trivial
420 (especially for quantitative tests that can be correlated to organismal or health outcome
421 levels), and to prevent panic if CBM partners saw positive samples that, while
422 meaningful, might not constitute a real concern. Nonetheless, CBM partners said "the
423 only way these results would be more valuable would be to have a quantitative number
424 which would correlate to specific standard or relative unit conversation chart." In
425 reality, this was what we were doing for CBM partners, but this group expressed a
426 desire to conduct this independent of our assistance. Additionally, we had a group
427 suggest that they wished the data was published online, "If the data was available or if
428 there was a way to input the data online into a database. Then we could use the results
429 more easily," they said. Our CBM partners also expressed a desire to validate their
430 results and have access to quality control data. One user suggested "…a visual that
431 compared our results to yours so we have some idea of if we were capturing the results
432 accurately." Another specifically suggested that, "…third party verification can be one
433 method to enhance validity of the results," suggesting a desire for some oversight to
434 ensure data quality, but also a desire for CBM partners to know that they are
435 contributing meaningful and accurate results.
436

One of the biggest challenges for CBM programs is data validation, storage, and visualization. Many communities lack the ability to share CBM data online. However, tools are emerging to address this challenge, including the Lake Observer mobile app through the Global Lake Ecological Observatory Network, the DataStream through the Gordon Foundation, or the ABMI's NatureLynx. Allowing community partners to upload and visualize their results may help to create a sense that partners are part of something bigger than just their lake. It might allow them to contextualize their results relative to other water bodies, log additional environmental observations, or upload photographs of recreational waters. These apps can also be helpful to track long-term results, or to have the data incorporated into reporting by other agencies.

The third theme we identified was that the triangulation of training was valuable. CBM partners appreciated the three forms of training. Most CBM partners found "the training videos were really useful." CBM partners found the written protocol useful as a reference, but suggested that after "around 2-3 runs of the machine this resource was no longer needed." Most CBM partners stressed the importance of the in-person training and one user stated that "the in-person training went a long way in creating and (sic) increased comfort and confidence in the machine." Studies conducted assessing training in citizen science or CBM projects have found that multiple training sessions can improve data accuracy (26).

A subtheme that emerged during analysis was that CBM partners appreciated

459 the learning process. One user stated that they were "…always up for learning new
460 methodologies to answering scientific questions." CBM programs are often touted for
461 the positive learning experiences they create, and it is nice to see that ours also had
462 positive learning outcomes for participants (6). A number of CBM partners also
463 suggested that they appreciated the ability to communicate results quickly to their
464 volunteers or to residents on the lakes they worked on. This type of a CBM project could
465 greatly improve science literacy and communication.
466
467 The LOD_{95} is the lowest concentration of DNA that can be reliably detected in 95% of
468 samples; it is a measure of sensitivity. The Open qPCR thermocycler has a higher limit of
469 detection when using a Taqman fluorescein probe than our ABI core thermocyclers
470 (63.4 DNA copies/5µL versus >10 DNA copies/5µL across all methods). The field
471 thermocyclers are less sensitive than the core laboratory machine. Understanding this
472 change in detection limit is important to determining if the CBM qPCR system would be
473 effective for a particular test. For instance, if the concentration of the target that might
474 constitute a risk is below the LOD_{95} for the Open qPCR thermocyclers, “risky” samples
475 will appear negative as the thermocycler is not capable of detecting them. For example,
476 when we deployed the human-associated bacteroides HF183 CBM testing for
477 recreational shoreline source tracking in Michigan, USA, our CBM partners reported only
478 a single positive sample. However, when these DNA extracts were analyzed, 22.7%
479 (54/237), were found to be positive for between 15-35 copies DNA/5ul. Seven (0.07%)
480 of these samples approached the LOD_{95} of the Open qPCR thermocycler, and CBM

partners detected one of these samples. A recent study found that a HF183 gene copy number of 3220 HF183/100ml exceeds the USEPA benchmark risk of GI illness (10). This level is equivalent to a gene copy number of 161 HF183 GC/5ul—well above the detection limit of the Open qPCR thermocycler. Thus, outbreak scenarios would be clearly discernable. However, this also illustrates an example of how the monitoring project must be clearly rooted in a management outcome. If the intention of the monitoring program is to detect potential outbreak scenarios and initiate action, the increased detection limit is acceptable, yet if the management context is detection of leaking septic areas or source tracking fecal markers on a beach, this detection limit may be inappropriate to answer such questions. This example highlights the importance of working closely with CBM partners to understand their specific monitoring questions, and critically appraising and assessing if CBM qPCR is capable and appropriate to answer these questions.

ICC analysis for the avian trematode assays showed a very high level of agreement between the Open qPCR thermocycler and the core thermocyclers (Table 2). We can expect highly reproducible results between the core machines and the field units. The toxic cyanobacteria test showed much lower levels of agreement between the field thermocycler and the lab thermocycler. We discovered through analyzing the control standards that the heated lid on the field thermocycler was loose, and therefore was failing to engage properly with the tops of the reaction tubes (i.e. machine failure). However, from a quality control perspective, the fact that we were able to detect a

503 probable machine failure with a sample size comparison of merely 11 is extremely
504 promising for future larger scale CBM qPCR systems. It suggests that it would be
505 possible with a relative low number of samples being confirmed by a core facility or
506 quality control partner to detect user or machine error once a baseline level of
507 agreement for a single test had been established.
508
509
510 The comparison between CBM partners performing DNA extraction and myself
511 performing the DNA extraction was first assessed semi-quantitatively using the Bland-
512 Altman plot (Table 2). The results of this analysis for the almost all targets show a linear
513 and negative linear pattern at lower gene copy numbers. This can be due to bias
514 between methods, but can also be caused by a difference in the within-subject standard
515 deviation (24). This seems plausible as users with potentially very different skill levels
516 are performing the two methods. A paired t-test using the log-transformed data was
517 used to compare the within-subject standard deviations. They were significantly
518 different, which suggests that the linear pattern observed is due to an increased
519 variability in CBM partner data.
520
521 Partner extracted samples are typically lower in copy number than expert
522 extracted samples (Fig 2). This is likely due to differences in DNA extraction efficiency
523 between the CBM partners and myself. However, it seems more experienced users
524 become better at DNA extraction over time, as both the avian schistosomes monitoring

525 group and the toxic cyanobacteria monitoring groups seem to improve over time. (Fig
526 2).
527
528 Its unsurprised that the ICCs and maximum log differences would be higher
529 when comparing partner and expert extracted DNA samples due to the highly variable
530 nature of DNA extraction, and because the duplicate samples run in the central lab
531 could never be expected to contain exactly the same amount of organism. The ICCs of
532 the DNA extraction comparison ranged from 0.54 to 0.67, with maximum log differences
533 ranging from 1.3 to 1.4 (Table 2). It is important to note that for the avian schistosomes
534 monitoring program, a change was made in 2018 to establish a full functional remote
535 laboratory, and move these partners onto using the Qiagen DNAEasy DNA extraction kit.
536 This change was made at the request of the CBM partners, who would typically collect
537 and analyze hundreds of samples each field season. Details about the equipment in this
538 satellite laboratory can be found in Supplementary Table 2.
539
540 Ebentier et al. (2013) conducted a reproducibility analysis of five core laboratories on a
541 panel of microbial source tracking qPCR markers. They calculated reproducibility as the
542 maximum expected log difference (within 95% confidence) between the different
543 laboratories. Their analysis demonstrated reproducibility coefficients for different qPCR
544 assays were highly variable, between 0.09-0.66 log. The methods that were likely to
545 produce higher copy numbers, like Enterococcus qPCR testing via USEPA Method 1611,
546 showed higher reproducibility coeffients than methods that were likely to produce

547 lower copy numbers, like human associated bacteroides marker HF183. They also
548 analyzed the contribution to variability of a variety of factors (the sample itself,
549 equipment, procedures) to the measurement. Their paper concluded that when
550 protocols and reagents were not standardized, agreement between methods decreased.
551 They highlighted the need for standardization of protocols and consumables before
552 implementation of studies involving multi-laboratory experiments (27,28).
553
554 The maximum log difference of the CBM qPCR monitoring program higher than the
555 values reported in the Ebentier paper. Reproducibility between the same extract
556 performed by myself and the CBM partners ranged from 0.44 to 1.5 log, and
557 reproducibility coefficients of between partner and expert extracted split samples
558 ranged from 1.3 to 1.4 log (Table 2). It should be noted that the majority of the qPCR
559 methods deployed routinely detected copy numbers in excess of 1 log, thus we might
560 expect higher variability between replicates at these larger copy numbers (Fig 2). CBM
561 qPCR monitoring programs will likely generate data that does have higher variability. It's
562 important to weigh the pros of a CBM qPCR approach, notably that a CBM qPCR
563 approach may result in increased numbers of samples from across a larger geographic
564 area, and builds relationships and partnerships across sectors.
565
566
567 Rapid monitoring approaches, including CBM qPCR, should be deployed within
568 the context of a policy framework and management response plan that can support

569 acting upon the results generated. The response plan for samples that might constitute
570 a hazard should be clear to CBM partners. If response plans lack transparency, a CBM
571 partner who encounters a sample that contains a high level of an indicator organism,
572 but upon subsequent tests shows low or no risk, might be dismayed by a lack of
573 response by government. A CBM qPCR monitoring system in recreational water would
574 need to prioritize communication and understanding between regulators and CBM
575 partners, and would likely function best when addressing specific objectives(29).
576
577 Whether the rapid CBM qPCR monitoring system enables a more rapid response
578 to hazards is yet to be seen; however, CBM qPCR monitoring certainly has the
579 advantage of being able to generate data over a large geographic area and for
580 numerous hazards. It could be adapted to measure organisms not typically considered
581 in monitoring programs; as we have demonstrated in our study, the approach works
582 equally well for eukaryotic hazards like parasitic organisms as it does for the more
583 traditional prokaryotic targets like enteric bacteria. The flexibility inherent in CBM qPCR
584 makes this an attractive and adaptive platform for governments and communities to
585 answer management related questions for their watersheds.
586
587 Our vision for the CBM qPCR monitoring system was that data analysis would not
588 occur in the hands of CBM partners (Fig 1). Analysis of qPCR data, while not extremely
589 complex, does require a more comprehensive understanding of qPCR data; additionally,
590 data interpretation is typically the most erroneous component over CBM programs

591 (30,31). Despite our CBM partners desire for independence in data interpretation, we
592 feel that a central 'expert' should still be responsible for data interpretation in order to
593 ensure quality in reporting. This could be a single laboratory or a network of QC
594 partners. However, our successes establishing a field laboratory for avian schistosomes
595 and enteric bacteria monitoring in Michigan suggests that a CBM qPCR network could
596 operate effectively within a framework that paired CBM volunteers with quality control
597 partners that could also be operating remotely from the central agency.
598
599 Participants in our study expressed a desire to know how well they were
600 performing the method. This highlights an important component of a large-scale CBM
601 monitoring program: a compliance testing system which would test and train potential
602 participants to ensure the method is being conducted appropriately. We believe a this
603 must include third-party verification of a certain percentage of all samples tested. While
604 verification is important to ensure CBM partners are generating reliable results, it is
605 essential that communication be prioritized. This includes responding quickly to results
606 reported by CBM partners when a potential hazard is detected. It also includes being
607 honest with partners about their performance, and willingness by both the CBM and
608 regulatory partners to collect and assess additional samples when clarification or
609 confirmation is required.
610
611 **5.0 Conclusion**

612 To our knowledge, this is the first study to comprehensively test the accuracy of
613 a CBM qPCR water monitoring approach in a real-world context. Our results show that
614 when implemented in a controlled manner, such that a central body controls materials
615 and protocols, results can be highly reproducible. Our study also suggests that CBM
616 partners, whose buy-in would be required for ensuring program longevity, value the
617 method, the data, and what they could do with that data.
618
619 CBM qPCR could process a large number of samples from a wide geographical
620 area that could aid beach management for health and invasive species. CBM qPCR could
621 act as a valuable component of an environmental monitoring surveillance system, but
622 could also be a viable option for monitoring and management of rural drinking water
623 systems. qPCR is a platform, and therefore a myriad of diagnostic tests could be
624 deployed as needed in remote locations. While CBM qPCR programs may be more
625 variable than traditional monitoring programs, they could serve as a comprehensive
626 screening system for traditional monitoring programs. In many contexts, CBM qPCR
627 programs could be as accurate as traditional testing and have the potential to replace
628 traditional testing.
629

630 **6.0 ACKNOWLEDGEMENTS**

631 The authors would like to acknowledge the community partners who worked to
632 collect samples and perform qPCR reactions.
633

8.0 REFERENCES

1. Conrad CC, Hilchey KG. A review of citizen science and community-based environmental monitoring: issues and opportunities. Env Monit Assess [Internet]. 2011 [cited 2019 Mar 18];176:273–91. Available from: www.eman-rese.

2. Conrad CC, Hilchey KG. A review of citizen science and community-based environmental monitoring: issues and opportunities. Environ Monit Assess [Internet]. 2011 May 17 [cited 2017 Jan 4];176(1–4):273–91. Available from: http://link.springer.com/10.1007/s10661-010-1582-5

3. Obama Administration. Accelerating Citizen Science and Crowdsourcing to Address Societal and Scientific Challenges. The White House. 2015.

4. Serrano F, Holocher-Ertl T, Kieslinger B, Sanz F, Silva C. White Paper On Citizen Science For Europe. Annual Review of CyberTherapy and Telemedicine. 2013.

5. Lakshminarayanan S. Using Citizens to Do Science Versus Citizens as Scientists. Ecol Soc [Internet]. 2007 Dec 28 [cited 2019 Mar 18];12(2):resp2. Available from: http://www.ecologyandsociety.org/vol12/iss2/resp2/

6. Trumbull DJ, Bonney R, Bascom D, Cabral A. Thinking scientifically during participation in a citizen-science project. Sci Educ [Internet]. 2000 Mar 1 [cited 2017 Oct 4];84(2):265–75. Available from: http://doi.wiley.com/10.1002/%28SICI%291098-237X%28200003%2984%3A2%3C265%3A%3AAID-SCE7%3E3.0.CO%3B2-5

656 7. Whitelaw G, Vaughan H, Craig B, Atkinson D. Establishing the Canadian
657 community monitoring network. Environ Monit Assess [Internet]. 2003 [cited
658 2019 Mar 18];88(1–3):409–18. Available from:
659 https://link.springer.com/content/pdf/10.1023/A:1025545813057.pdf
660 8. Wade TJ, Calderon RL, Sams E, Beach M, Brenner KP, Williams AH, et al. Rapidly
661 measured indicators of recreational water quality are predictive of swimming-
662 associated gastrointestinal illness. Environ Health Perspect [Internet]. 2006 Jan
663 [cited 2019 May 3];114(1):24–8. Available from:
664 http://www.ncbi.nlm.nih.gov/pubmed/16393653
665 9. Wymer LJ, Brenner KP, Martinson JW, Stutts WR, Schaub SA, Dufour AP. The
666 EMPACT Beaches Project : Monitoring in Recreational Waters. Environ Prot. 2005;
667 10. Boehm AB, Soller JA, Shanks OC. Human-Associated Fecal Quantitative
668 Polymerase Chain Reaction Measurements and Simulated Risk of Gastrointestinal
669 Illness in Recreational Waters Contaminated with Raw Sewage. Environ Sci
670 Technol Lett. 2015;
671 11. Cao Y, Sivaganesan M, Kelty CA, Wang D, Boehm AB, Griffith JF, et al. A human
672 fecal contamination score for ranking recreational sites using the HF183/BacR287
673 quantitative real-time PCR method. 2018 [cited 2019 May 6]; Available from:
674 https://doi.org/10.1016/j.watres.2017.10.071
675 12. Ibelings BW, Backer LC, Kardinaal WEA, Chorus I. Current approaches to
676 cyanotoxin risk assessment and risk management around the globe. Harmful
677 Algae [Internet]. 2014 Dec [cited 2016 Oct 4];40:63–74. Available from:

http://www.ncbi.nlm.nih.gov/pubmed/26435706

13. Abou Tayoun AN, Burchard PR, Malik I, Scherer A, Tsongalis GJ. Democratizing Molecular Diagnostics for the Developing World. Am J Clin Pathol [Internet]. 2014 Jan 1 [cited 2017 Sep 26];141(1):17–24. Available from: https://academic.oup.com/ajcp/article-lookup/doi/10.1309/AJCPA1L4KPXBJNPG

14. Wood CS, Thomas MR, Budd J, Mashamba-Thompson TP, Herbst K, Pillay D, et al. Taking connected mobile-health diagnostics of infectious diseases to the field. Nature. 2019.

15. Baum F, MacDougall C, Smith D. Participatory Action Research. J Epidemiol Community Health [Internet]. 2006 Oct [cited 2019 Jun 7];60(10):854–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16973531

16. Blankespoor HD, Reimink RL. The Control of Swimmer's Itch in Michigan: Past, Present, and Future. MICHIGAN Acad XXIV. 1991;7–23.

17. Rudko SP, Reimink RL, Froelich K, Gordy MA, Blankespoor CL, Hanington PC. Use of qPCR-Based Cercariometry to Assess Swimmer's Itch in Recreational Lakes. EcoHealth. 2018;

18. Qiu Y, Yuan T, Zurawell R, Huang Y. Rapid Detection and Quantitation of Microcystin-Producing Microcystis Using Real-Time PCR. J Mol Biomark Diagn [Internet]. 2013 [cited 2017 May 30];S5. Available from: http://dx.doi.org/10.4172/2155-9929.S5-006

19. Sipari H, Rantala-Ylinen A, Jokela J, Oksanen I, Sivonen K. Development of a chip assay and quantitative PCR for detecting microcystin synthetase E gene

expression. Appl Environ Microbiol [Internet]. 2010 Jun [cited 2016 Jul 28];76(12):3797–805. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20400558

20. Rudko SP, Ruecker NJ, Ashbolt NJ, Neumann NF, Hanington PC. Investigating Enterobius vermicularis as a novel surrogate of helminth ova presence in tertiary wastewater treatment plants. Appl Environ Microbiol [Internet]. 2017 Mar 24 [cited 2017 Apr 10];AEM.00547-17. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28341675

21. Fereday J, Muir-Cochrane E. Demonstrating Rigor Using Thematic Analysis: A Hybrid Approach of Inductive and Deductive Coding and Theme Development. Int J Qual Methods [Internet]. 2006 Mar 29 [cited 2019 Dec 16];5(1):80–92. Available from: http://journals.sagepub.com/doi/10.1177/160940690600500107

22. Braun V, Clarke V. Using thematic analysis in psychology. Qual Res Psychol [Internet]. 2006 Jan [cited 2019 May 6];3(2):77–101. Available from: http://www.tandfonline.com/doi/abs/10.1191/1478088706qp063oa

23. Martin Bland J, Altman DG. Stastical Methods for Assessing Agreement Between Two Methods of Clinical Measurement. Lancet. 1986;

24. Bartlett JW, Frost C. Reliability, repeatability and reproducibility: analysis of measurement errors in continuous variables. Ultrasound Obstet Gynecol [Internet]. 2008 Apr 1 [cited 2019 Mar 20];31(4):466–75. Available from: http://doi.wiley.com/10.1002/uog.5256

25. Wilrich C, Wilrich P-T. Estimation of the POD Function and the LOD of a

Qualitative Microbiological Measurement Method. [cited 2015 Jun 22]; Available from: http://www.ingentaconnect.com/content/aoac/jaoac/2009/00000092/00000006/art00020

26. Ratnieks FLW, Schrell F, Sheppard RC, Brown E, Bristow OE, Garbuzov M. Data reliability in citizen science: learning curve and the effects of training method, volunteer background and experience on identification accuracy of insects visiting ivy flowers. Methods Ecol Evol [Internet]. 2016 [cited 2019 May 3];7(10):1226–35. Available from: http://cygnus-biologystudentjournal.wikispaces.

27. Ebentier DL, Hanley KT, Cao Y, Badgley BD, Boehm AB, Ervin JS, et al. Evaluation of the repeatability and reproducibility of a suite of qPCR-based microbial source tracking methods. Water Res [Internet]. 2013 Nov [cited 2017 Jul 20];47(18):6839–48. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0043135413005484

28. Shanks OC, Sivaganesan M, Peed L, Kelty CA, Blackwood AD, Greene MR, et al. Interlaboratory comparison of real-time pcr protocols for quantification of general fecal indicator bacteria. Environ Sci Technol. 2012;

29. Barzyk TM, Huang H, Williams R, Kaufman A, Essoka J. Advice and Frequently Asked Questions (FAQs) for Citizen-Science Environmental Health Assessments. Int J Environ Res Public Health [Internet]. 2018 [cited 2019 Jun 7];15(5). Available from: http://www.ncbi.nlm.nih.gov/pubmed/29751612

30. Hunter J, Alabri A, van Ingen C. Assessing the quality and trustworthiness of

citizen science data. Concurr Comput Pract Exp [Internet]. 2013 Feb 1 [cited 2017 Dec 15];25(4):454–66. Available from: http://doi.wiley.com/10.1002/cpe.2923

31. Cohn JP. Citizen Science : Can Volunteers Do Real Research ? Bioscience. 2008;58(3):192–7.

7.0 FIGURE LEGENDS

Fig 1. Implementation process of the CBM qPCR program. Cells with blue backgrounds are processes done in collaboration between the central laboratory and the CBM partners, yellow backgrounds indicate processes completed by the central laboratory, and red backgrounds indicate processes completed by the CBM partners.

Fig 2. Bland-Altman graphs of the difference between the CBM partners data and the central labs data. Limits of agreement (1.96 times the standard deviation) are bounded by the dotted lines. Top: Agreement of the 2017 Avian schistosome monitoring program. Middle: Agreement of the 2018 avian schistosomes monitoring program. Bottom: Agreement of the microcystin gene monitoring program.

9.0 TABLES

766

767 **Table 1. Standard curves of each assay performed on the Open qPCR and the core lab**

768 **machine.** Data shown represent the average of 5 runs (each consisting of two internal

769 replicates of each standard). Ideal standard curves have an efficiency of between 0.98

770 and 0.99, a slope of -3.32, and an efficiency of 100%, which can also be represented as

771 an amplification factor of 2, which suggests product has doubled every cycle.

	Copy number in standard	Cycle threshold (average, st dev)		r2	Slope	Amplification factor
Cyanobacteria mcyE assay						
Open qPCR	5000	30.1	0.27	0.980	-3.05	2.100
	500	33.2	1			
	50	36.3	1.6			
ABI qPCR	5000	28.7	1.5	0.990	-3.700	1.800
	500	32.2	2.4			
	50	36.1	0.91			
Human-associated bacteroides (HF183) assay						
Open qPCR	5000	25.6	0.25	0.99	-3.46	1.94
	500	29.2	0.24			
	50	32.9	0.28			
ABI qPCR	5000	24.8	0.13	0.98	-4.4	1.87
	500	28.1	0.31			

	50	33.7	0.26			
Pan-avian schistosome assay						
Open qPCR	5000	27.3	0.41	0.99	-3.03	2.1
	500	30.3	0.64			
	50	33.3	0.52			
ABI qPCR	5000	26.9	0.52	0.99	-3.03	2.1
	500	30.5	0.47			
	50	32.9	0.54			

772

773

774 **Table 2. Interclass correlation Coefficients and Maximum Log Difference.** comparing

775 the reproducibility of samples run on the Chaibio Open qPCR thermocycler and the ABI

776 7500 thermocycler/QuantStudio, and

	Comparison Of Partner-Extracted DNA Samples Performed On The Open qPCR Versus The Quantstudio 3/ABI 7500				
qPCR Test	**Interclass correlation coefficient**	**Lower 95% CI**	**Upper 95% CI**	**Maximum log difference**	**N**
Toxic cyanobacteria 2018	0.57	0.1	0.86	1.2	12
Toxic cyanobacteria	0.6	0.24	0.8	1.5	40

2019					
Avian schistosomes 2017	0.88	0.85	0.9	1	255
Avian schistosomes 2018	0.76	0.56	0.87	1	47
	Comparison Of Partner-Extracted And Expert-Extracted Split Samples				
	Interclass correlation coefficient	**Lower 95% CI**	**Upper 95% CI**	**Maximum log difference**	**N**
Toxic cyanobacteria 2018	0.65	-0.25	0.9	1.4	12
Toxic cyanobacteria 2019	0.67	0.366	0.83	1.3	39
Avian schistosomes 2017	0.54	0.32	0.68	1.4	255
Avian schistosomes 2018	0.59	0.34	0.75	1.3	70

10.0 SUPPLEMENTARY TABLES

781 **Table 1S: Primers and Probes used in this study**

Name	Sequence (5'-3')	Reference
Toxic cyanobacteria (mcyE gene targeting)		
127 Fwd	AAGCAAACTGCTCCCGGTATC	Qiu et al., 2015
186 Probe	/FAM/CAATGGTTAT/ZEN/CGAATTGACCCCGGAGAAAT /IABkFQ	Qiu et al., 2015
247 Rev	CAATGGGAGCATAACGAGTCAA	Qiu et al., 2015
Avian trematode (18S gene targeting)		
JVSF 18S Fwd	AGCCTTTCAGCCGTATCTGT	Narayanan et al., 2015
JVSP 18S Probe	/FAM/AGGCC/ZEN/TGCCTTGAGCACT/IABkFQ/	Narayanan et al., 2015
JVSR 18S Rev	TCGGGAGCGGACGGCATCTTTA	Narayanan et al., 2015
Human associated bacteroides HF183 (16S targeting)		
HF183 Fwd	ATCATGAGTTCACATGTCCG	Haugland et al., 2010.
BFDProbe	FAM/CTGAG/ZEN/AGGAAGGTCCCCCACATTGGA/IABkFQ/	Haugland et al., 2010.
BFDRev	CGTAGGAGTTTGGACCGTGT	Haugland et al., 2010.

Table 2S. Field method materials used in this study

Name	Manufacturer/Catalogue Number
Biomeme MI DNA extraction kit	Biomeme

DNAeasy DNA extraction kit	Qiagen (69506)
0.4 uM filters	Pall (FMFNL1050)
Vaccum pump	Vaccubrand ME 1C
PCR tubes and caps	Axygen (PCR-02-FCP-C and PCR-0108-LP-C)
Microcentrifuge tubes	Eppendorf (Z666548-250EA)
Open qPCR thermocycler (single channel)	ChaiBio.
20 µL Micropipette	VWR (470231-608)
Maximum Recovery Filter tips	Axygen (TF-20-L-R-S)
Pelican Storm Case	Pelican IM2450
20uM Plankton Tow	Acquatic Research Instruments
Primetime Gene Expression Master Mix	IDTDNA (1055772)
Computers	Google Chromebook and Acer Switch One.
DNAeasy DNA extraction equipment- found in the field laboratory	
Spectrafuge 24D	Dot scientific (C2400)
VorTemp 56 Shaking Incubator	Dot scientific (S2056)
Mortexer™	Dot scientific (BV-1005)
Mortexer™ 12 sample head	Dot scientific (BV1000-H15)

782

783

784

Table 3S. Questions administered to users in survey
Section 1. Prior Experience 1. How would you rank your knowledge with molecular biology,PCR (polymerase chain reaction),eDNA or DNA based detection in general prior to the use of the qPCR field method

o High prior knowledge

o Some prior knowledge

o Low prior knowledge

o No prior knowledge

2. Had you performed PCR (polymerase chain reaction) before attempting the qPCR field method? (highlight your response)

o Yes

o No

o I do not remember

Section 2. Training

3. Was the training on the qPCR field-testing sufficient?

3a. Did you utilize the written protocol/video?

3b. Was the in-person training valuable?

Section 3. DNA extraction

4a. Was the DNA extraction protocol simple?

4b.What could be improved?

Section 4. Operating the thermocycler

5. Did you find the operation of the Chai Bio Open qPCR (the thermocycler and computer) simple?

What could be improved?

6. What method of results reporting would you have liked to see?

Section 5.

7. Was the time spent on the method too long or just right?

8. Did the portable field qPCR unit fit well within your/your organizations normal monitoring tasks?

9. Did the portable field system meet your expectations?

10. Did you find the results obtained by qPCR valuable? What could make these results more valuable?

11. Do you think that field qPCR is appropriate to answer the questions your organization sought to answer in agreeing to participate in this trial?

12. Do you see any value for continued use of the field qPCR method within your organization?

13. Why were you interested in participating in this trial to begin with? Have your views on the use

of field qPCR changed since then?

PROJECT DEVELOPMENT

Consultation with stakeholders in your region. Meet potential CBM partners who might be interested in participating

Discuss what the goals of a CBM qPCR program would be, and how this program could fit within the partners organization.

ITERATIVE PROCESS OF DEVELOPMENT AND CONSULTATION

Development and validation of testing methods (field collection, DNA extraction, qPCR diagnostic tests).

Development of training materials for users, i.e. written protocols, videos, and a plan for in person training.

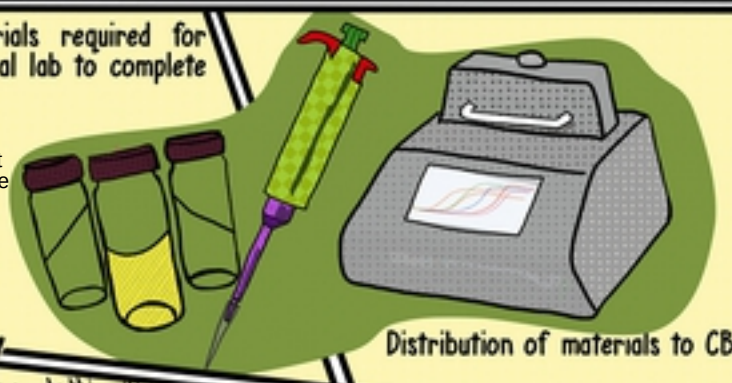
In-person, hands-on training session with CBM partners. Multiple in-person training sessions might be required.

CBM qPCR

qPCR master mix preparation, as well preparation of control standards. Allocation of these materials into reaction tubes.

Batch testing of qPCR master mix and standards in house to ensure reactions and controls function appropriately

Ordering of all materials required for CBM partner and central lab to complete their project

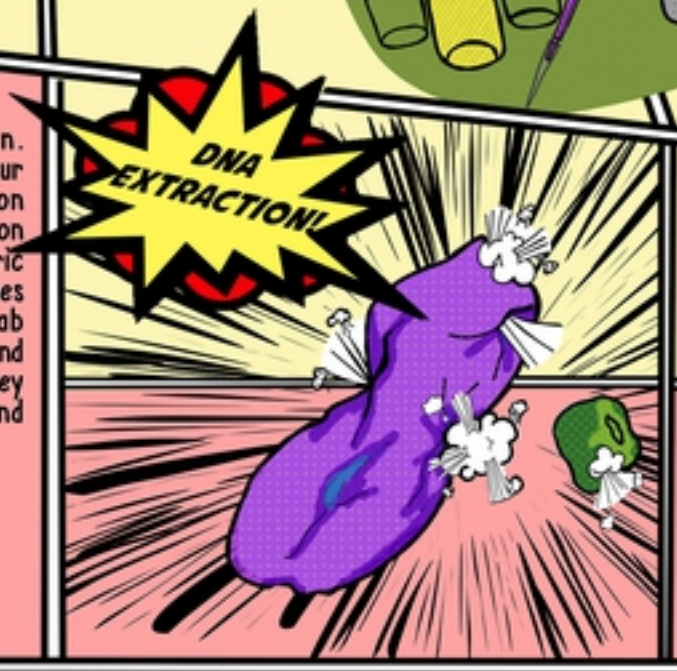


Distribution of materials to CBM partners

Sample collection. The central lab might help in selecting sample sites, or these sites could be dictated by the CBM partner to align with their current monitoring programs or goals.

AT SOME SITES TWO OR MORE SAMPLES SHOULD BE COLLECTED. THESE SAMPLES CAN BE FILTERED AND SENT TO THE CENTRAL LAB AND VERIFIED

Sample filtration. Filtration should occur soon after collection and can be achieved on site using an electric vacuum pump. Samples for the central lab should be filtered and stored frozen until they can be verified and extracted



qPCR reaction to confirm results of some water samples

qPCR reaction. This should include pre-made standard controls as well as samples

Users upload their data into spreadsheets (we recommend developing a spreadsheet with users ahead of time to ensure consistency and ease-of-use) and users share spreadsheet with central lab.

Central lab analyses samples, as well as controls. Analysis should be done promptly.

RESULTS SHOULD BE INTERPRETED AND REPORTED BACK TO USERS QUICKLY. RESULTS SHOULD BE PRESENTED TO USERS IN A MANNER THAT IS EASY TO UNDERSTAND AND IN A MANNER THAT FULFILLS THE USERS NEEDS. IF FOLLOW UP SAMPLING IS REQUIRED IT SHOULD BE REQUESTED AT THIS TIME.

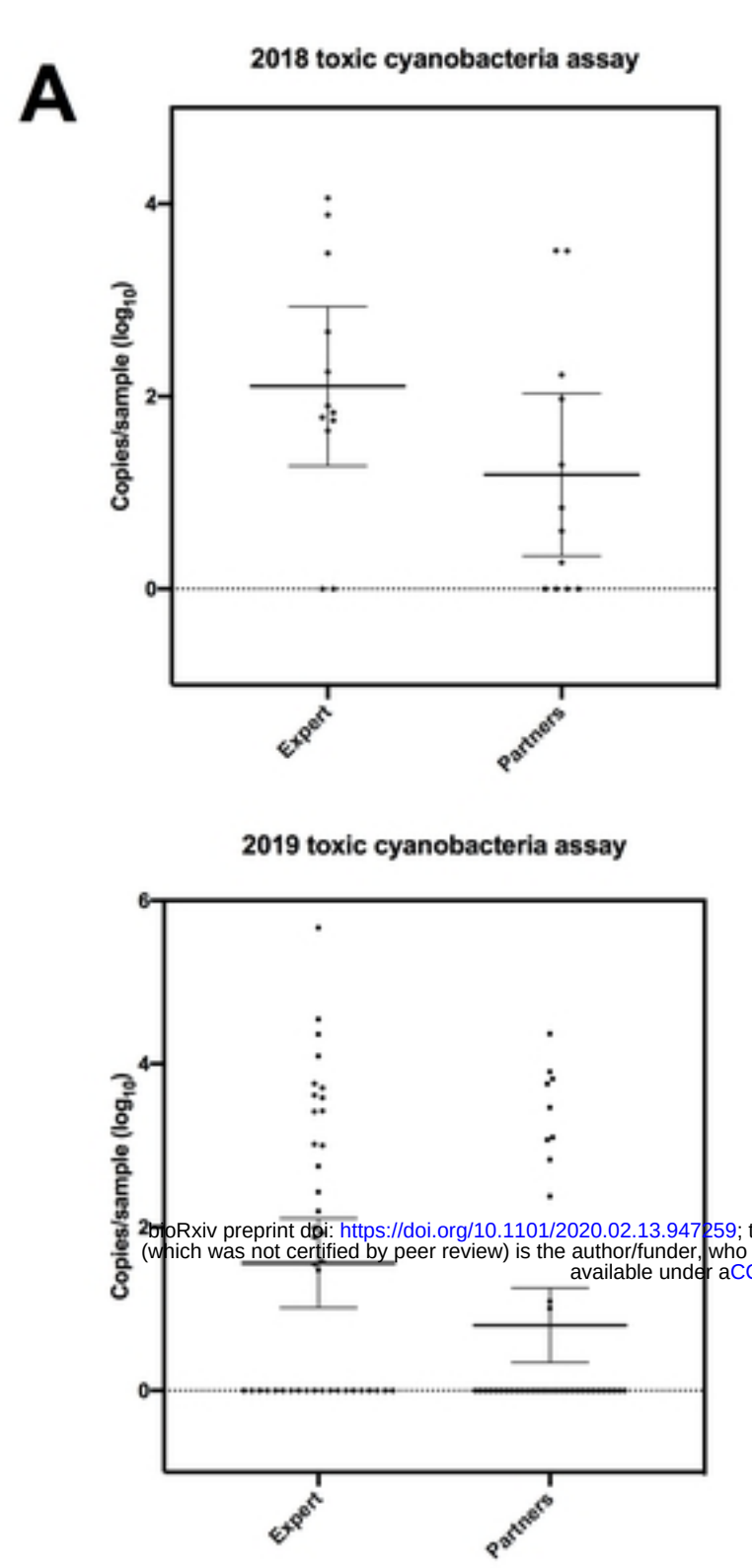
PROJECT WRAP UP

Discussion between CBM partner and Central Lab to discuss implications of results, recommendations going forward, or future projects or lines of inquiry that could be tested.

Dissemination of results. This could include creating reports, publications, co-presenting at stewardship meetings, or presenting results to regulatory agencies.

REFLECTION ON THE SUCCESSES AND FAILURES OF THE PROJECT, COLLABORATION ON HOW TO IMPROVE CBM PROGRAM DELIVERY

Figure 1



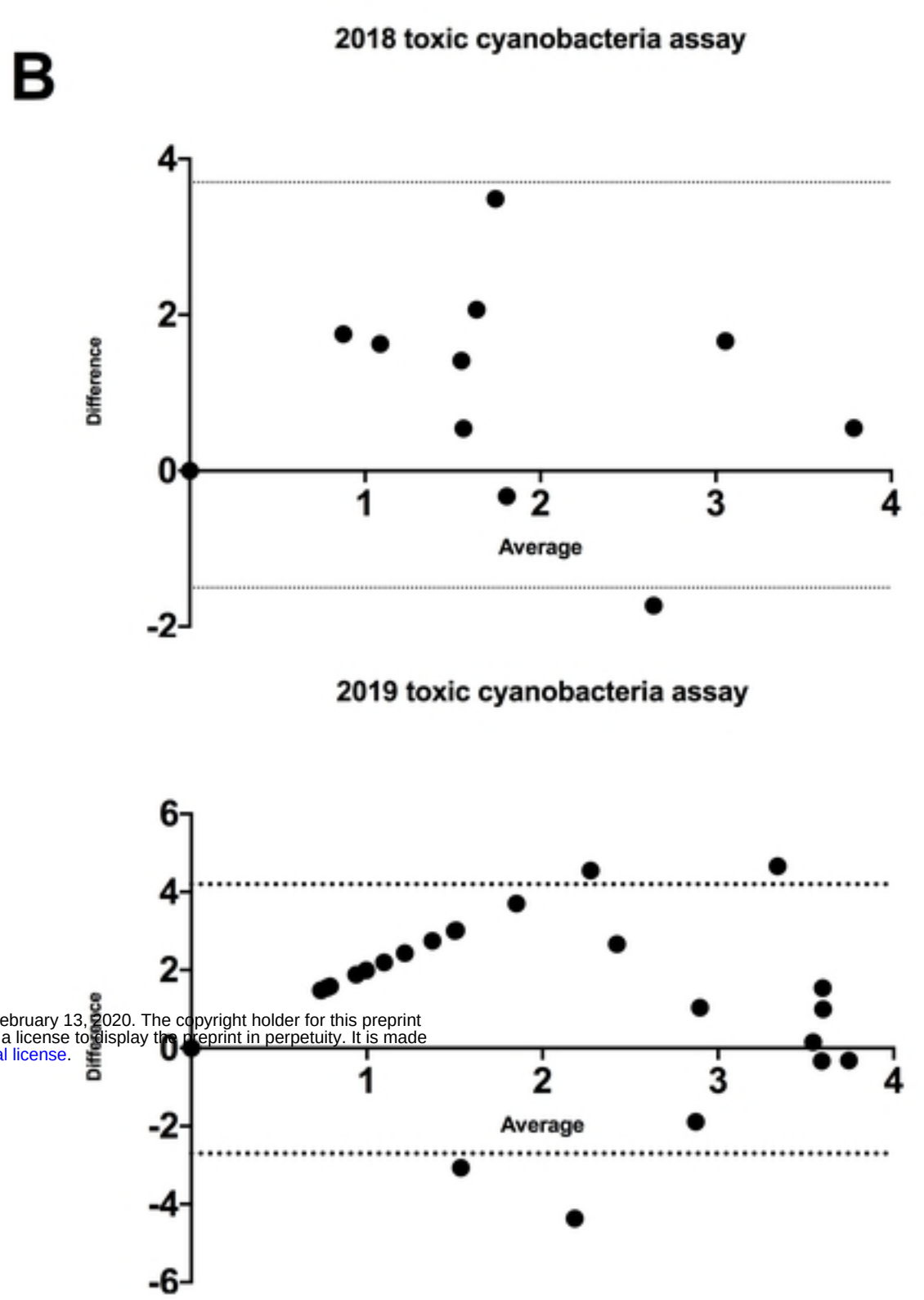


Figure 2