Tool Selection for use in Water Quality Based Citizen Science Projects

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Melissa L. Topping

Major Professor: Alan S. Kolok, Ph.D.

Committee Members: Erin Brooks, Ph.D.; Christine Parent, Ph.D.

Department Administrator: Timothy Link, Ph.D.

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Authorization to Submit Thesis

This thesis of Melissa L. Topping submitted for the degree of Master of Science with a Major in Water Resources and titled "Tool Selection for use in Water Quality Based Citizen Science Projects," has been reviewed in final form. Permission, as indicated by the signatures and dates below, is now granted to submit final copies to the College of Graduate Studies for approval.

Major Professor: ______________________________ Date: __________

Alan S. Kolok, Ph.D.

Committee Members: ______________________________ Date: __________

Erin Brooks, Ph.D.

______________________________ Date: __________

Christine Parent, Ph.D.

Dept. Administrator: ______________________________ Date: __________

Timothy Link, Ph.D.

Abstract

When citizen scientists conduct water quality monitoring, the results can include better educated communities, increased local political participation, and an increased breadth in the geospatial and temporal range of the sampling campaigns. Unfortunately, the value of data collected by citizen scientists is often criticized as being inaccurate and unreliable. To address these criticisms, it is necessary to quantify volunteer accuracy using different tools and data collection strategies. The first chapter of this thesis reviews current citizen science literature and introduces the following two chapters, which focus on tool selection for citizen science monitoring. In the second chapter of this thesis evaluates the capacity of citizen scientists to gather accurate nitrate data. Specifically, this research focused on the quantification of Hach © nitrate test strips, which volunteers analyzed visually or using a cell phone app. The objective of this work was to compare the accuracy of the two quantification methods. The objective of the final chapter is to review the literature and identify the potential for citizen science research methods to contribute to environmental monitoring efforts through the collection of biologic tissues.

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Dedication

This thesis is dedicated to my family and friends who supported me along this journey. Thank you for everything, I wouldn't be here without you all.

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Chapter 1: Literature Review of Citizen Science in Water Quality

The participation of volunteers in scientific discovery, or citizen science, began with the earliest observations of the natural world (Mckinley et al., 2015). Astronomy and ornithology have rich histories of volunteer involvement (Dickinson et al., 2010; Greenwood et al., 2007), with newer developments in technology shifting participation to other scientific disciplines (Mckinley et al., 2015). Citizen science has grown to encompass a variety of project types, from online "gamification" (Wiggins & Wilbanks, 2019), to research endeavors designed to influence local policy (Ottinger, 2010; Stepenuck & Genskow, 2019), to the combination of athletic adventure and data collection in adventure science (Horodyskyj et al. 2016), to more traditional projects focusing on biodiversity observations (Dickinson et al., 2010; Pettibone et al., 2017). Citizen science has quickly become a valuable tool for researchers to amass large datasets, but also proves beneficial for non-research purposes, such as educational and societal growth (Trumbull et al., 2000; Conrad & Hilchey, 2011; Huddart et al., 2016).

Societal Benefits

Citizen science is influencing the democratization of the scientific method and introducing young generations to scientific inquiry (Conrad & Hilchey, 2011; Mason & Garbarino, 2016). Democratization of science, or the concept of making scientific expertise more available to the public while simultaneously tasking researchers to work and integrate local knowledge into their research, is relatively new (Conrad & Hilchey, 2011). Researchers have identified that participants in these types of events often walk away encouraged and motivated to make a difference in their community (Storey et al., 2016). Community involvement in monitoring projects has also been suggested to build social capital through volunteer engagement, interaction between community stakeholders, and leadership opportunities (Whitelaw et al., 2003). It has also been suggested that communities involved with citizen science projects are often more sustainable and likely to be engaged in local issues (Whitelaw et al., 2003; Conrad & Hilchey, 2011).

Citizen science has many societal benefits, from community engagement to breaking down the scientific "ivory tower" (Pettibone et al., 2017), but it is important to acknowledge that it does not connect well with traditionally underrepresented groups in science (Pandya 2012). Many minority groups do not participate in citizen science because of scientific knowledge barriers (Evans et al., 2005; Ottinger, 2010), a lack of access to natural settings (Evans et al., 2005), and greater disconnects between research priorities and the values of minority groups (Pandya, 2012). To truly include historically marginalized groups in citizen science it is necessary for researchers and communities to collaborate to design projects that are place-based and culturally relevant to the participants (Pandya, 2012). Diversity in science has the potential to change participatory research for the better (Pandya, 2012) and make scientific democratization truly accessible to all.

Scientific Benefits

Other than societal benefits, citizen science has been a useful means to advance scientific knowledge (Bonney et al., 2009). As it has gained traction, projects that engage volunteers cover a wide variety of disciplines within the natural sciences (Dickinson et al., 2010; Stepenuck & Green, 2015). Projects range from skilled ornithological observations (Devictor, Whittaker, & Beltrame, 2010; Bonney et al., 2014), to genetic sampling for rare newt species (Biggs et al., 2015), to participation in at home genetic testing using kits such as 23andMe (Kuznetsov, Kittur, & Paulos, 2015), to water testing to identify drivers of eutrophication (Zhang et al., 2017). These projects are often accomplished on a range of spatial and temporal scales that cannot be achieved with the limitations of more traditional research (Theobald et al., 2015). It has been demonstrated that the more expansive a project, both spatially and temporally, the higher the likelihood that the data will be incorporated into a peer-reviewed article (Theobald et al., 2015). Citizen science has become an invaluable tool for ecological research and has changed the scale on which research can be conducted (Dickinson et al., 2010; Wei, Lee, & Wen, 2016).

While volunteers contribute to the scientific enterprise, these data are not always readily accepted by the scientific community (Bonney et al., 2014). Despite the hesitation of the scientific community, many researchers using citizen science argue that acceptance can be increased through further open-source technological advancements, development opportunities, and tool development (Bonney et al., 2014). Proper training and the incorporation of cell phone technologies are other options that researchers suggest for improving the reliability of volunteer data (Mason & Garbarino, 2016). Finally, a further understanding researcher's perceptions and hesitations about citizen science as a data collection strategy is also necessary before it is completely integrated into the scientific community (Burgess et al., 2017). These concerns need to be addressed, as participatory research is considered an underutilized resource for both scientific opportunity and data collection (Theobald et al., 2015), as well as an unrealized tool for societal benefit (Bonney et al., 2014).

Citizen Science and Water Quality Monitoring

Relative to environmental monitoring, citizen scientists have contributed to numerous water quality monitoring projects (Ely and Hamingson 1998; Stepenuck & Genskow, 2018). These projects are diverse (Stepenuck & Genskow, 2018), and include those that focus on: environmental DNA (or eDNA) to track invasive aquatic species (Miralles et al., 2016; Larson et al., 2017), long term trends in lake water clarity (Lottig et al., 2014), and trends in nutrient concentrations in urban waterways (Lévesque et al., 2017; Thornhill et al., 2017). Generally, the use of community members broadens the geospatial and temporal scales of the work (Devictor et al., 2010; Cooper et al., 2014; Stepenuck & Genskow, 2018).

Nutrients are a common water quality parameter that citizen science campaigns measure, as they are easily perceieved by volunteers (Lévesque et al., 2017; Peckenham & Peckenham, 2014; Muenich et al., 2016; Zhang et al., 2017; Ali et al., 2019) and they have research implications as drivers of eutrophication (Zhang et al., 2017). As with citizen science in general, the quality of water-related data produced by volunteer monitoring efforts is still questioned by researchers and regulatory agencies (Nicholson et al., 2002; Loperfido et al., 2010; Ali et al., 2019). There are a variety of verification methods that researchers use to address data concerns and they have concluded that validation techniques need to be both robust and replicable for the data to be accepted within the community (Jollymore et al., 2017). For example, Ali et al. (2019) conducted a suite of volunteer testing events to determine if experience level had any impact on the proportion of accurate observations by citizen scientists measuring nitrate, phosphate, and atrazine. Other research teams validate findings by instructing volunteers to collect two samples, one that they test in the field and the second for laboratory verification purposes (e.g. Muenich et al., 2016; Scott & Frost, 2017).

Cell Phone Technology for Data Enhancement

The advent of cellphone technology has revolutionized citizen science and has expanded the type of data volunteers can submit (Mason & Garbarino, 2016; Leeuw & Boss, 2018) and it has also been suggested that cell phones might be a way to further enhance the quality of data that volunteers contribute (Burke et al., 2006; Dickinson et al., 2010; Kolok, Schoenfuss, Propper, & Vail, 2011; Mason & Garbarino, 2016). With the advent of almost ubiquitous smart phone technology, volunteers are capable of providing rapid GPS located data that has the potential to aid in many different types of research (Burke et al., 2006; Dickinson et al., 2010; Kolok et al., 2011; Mason & Garbarino, 2016). There are a handful of smartphone applications, or apps, that are designed for water-focused citizen science projects. Creek Watch allows participants to monitor the quality of their local waters through the submission of pictures and parameters such as water level, flow rate, and presence of trash (Kim et al., 2011). HydroCrowd encouraged participants to record stream gauge measurements and submit them using their smart phones (Lowry et al. 2018).

Other than to submit data in real time, there are apps that serve as a tool for quantitative water quality measurements. For example, HydroColor uses built-in smartphone technology to sense the reflectance of water bodies for components such as sediment, chlorophyll, and organic matter (Leeuw & Boss, 2018). Similarly, the Deltares Nitrate app uses the phone's camera to assist with the quantification of nitrate concentrations and then allows users to share the results on an interactive online map. These tools have the potential to contribute consistent and reliable data for researchers (Leeuw & Boss 2018), but it is necessary to gauge how citizen scientists will interact with the technology. As with other volunteer appropriate tools, validation efforts are necessary to increase the reliability of the data (e.g. Peckenham & Peckenham, 2014; Meunich et al., 2016; Ali et al., 2019), and the same must be done with cell phone apps to ensure that volunteers can produce accurate data.

Crowdsourcing Biologic Material to Supplement Environmental Monitoring

While citizen scientists have participated successfully in water quality monitoring from a chemical perspective, their participation relative to pollution monitoring using biological indicators is less developed. There are notable barriers of entry for volunteer participation in ecotoxicological studies. From the scientific knowledge gaps about pollution on the part of participants (Ottinger, 2010) to the inherent diversity and chemical complexities of pollutants that exist in the environment (Gundert-Remy et al., 2015), ecotoxicology is not the most accessible field for citizen scientists.

While the chasm between citizen science water quality monitoring and ecotoxicology may seem vast, there are examples of citizen groups contributing to research that might appear too complicated for non-professionals. For example, volunteer participation in genetics research was not very common until recent advancement of genetic technologies and low-cost sequencing (Kuznetsov, Kittur, & Paulos, 2015). Now volunteers are proving themselves useful for genetics research (e.g. Biggs et al., 2015; Wilson et al., 2015; Miralles et al., 2016; Larson et al., 2017; Mori et al., 2017), a field in which there were many barriers to entry in the recent past.

The combination of citizen science and ecotoxicological pollutant monitoring may face many barriers to success, but through easily accomplished field work and intentional protocol design there are opportunities for important research. This idea was demonstrated successfully by a research team that used citizen scientists to collect dragonflies which served as biosentinel species for mercury in the environment (Eagles-Smith et al., 2016; Pritz & Nelson, 2017). Crowdsourcing tissue collection for metal contamination research has the potential for many exciting and diverse projects when carefully designed by researchers, as well as exposing volunteers to new types of citizen science projects.

The goal of this thesis is to evaluate tools, both chemical and biological, for use in water quality citizen science monitoring projects. In chapter two, citizen science tools to quantify nitrate will be assessed. The objective of this work was to compare the accuracy of the two quantification methods. More specifically, volunteer accuracy quantifying the Hach © nitrate test strips visually will be compared to the accuracy of data produced by volunteers using the Deltares Nitrate app. The success of this project will work to strengthen the trust that researchers can put in crowdsourced nitrate measurements, and simultaneously attempt to make citizen science a more legitimate form of data collection.

The final chapter of this work discusses the potential of citizen science participation in biological water quality monitoring through the collection of biologic tissues organisms. Crowdsourcing tissue collection for metal contamination research has the potential for many exciting and diverse projects when carefully designed by researchers, as well as exposing volunteers to new types of citizen science projects.

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Chapter 2. Tool Selection for Use in Water Quality Based Citizen Science Projects

Abstract

In this study we evaluated the capacity of citizen scientists to gather data on the aqueous concentration of nitrate using two quantification methods. First, volunteers quantified nitrate samples visually using test strips with a colorimetric scale that corresponds to concentration. Then, volunteers used cell phones equipped with the Deltares Nitrate app to quantify the concentration of their samples. The results of this testing indicated that the Deltares Nitrate app does not increase the accuracy of data collected and that visual testers are more likely to produce higher proportions of accurate results than their app testing counterparts. A second testing suite tasked volunteers to sample a continuum of nitrate samples using visual and app quantification methods. Both the app and visual testers experienced increased variation as concentration increased but also demonstrated that they can accurately quantify nitrate samples at or below EPA drinking water regulatory standards. The results of this study suggest that cell phone apps do not increase the accuracy of nitrate data, but both app and visual methods are suitable tools for collecting reliable data within EPA relevant ranges.

Introduction

Citizen science, the public's participation in scientific inquiry, is transforming the scientific narrative by increasing scientific literacy (Bonney et al., 2009; Shirk et al., 2012; Kuznetsov et al. , 2015; He et al., 2019), involving the public in research (Shirk et al., 2012), and providing researchers with novel approaches to large-scale questions (Dickinson et al. 2010; Shirk et al. 2012; Mason and Garbarino 2016; He et al. 2019). Citizen science has engaged countless volunteers in a wide range of subjects, from at home genetic testing (Kuznetsov, Kittur, & Paulos, 2015), to sampling cougar muscle tissues (Beausoleil et al., 2016), to tracking monarch butterfly migrations (Howard et al., 2010). Long running programs such as eBird (Dickinson et al., 2010) and CoCoRaHs (Reges et al., 2016) are examples of highly successful citizen campaigns, that have changed the narrative by involving thousands of participants over broad geographies. The meaningful data amassed by programs like these have been included in many peer-reviewed journals, and research indicates that the rate of publication using citizen collected data increases for projects with broader geospatial and temporal scales (Theobald et al., 2015). While research and publication opportunities exist using volunteer collected data, researchers argue that it remains an underutilized resource whose full potential has yet to be realized (Theobald et al., 2015).

Citizen scientists have participated in a broad range of water related projects (Stepenuck & Genskow, 2018), and some of these projects have been conducted over large geographies and long temporal periods. For example, volunteers have measured a variety of parameters, such as nutrient concentrations in the St. Lawrence River (Lévesque et al., 2017) , the presence or absence of the herbicide atrazine in the Mississippi River watershed (Ali et al., 2016), and the determination of total coliform levels in the surface waters of Hamilton, Ontario (Au et al., 2000). More explicitly, Thornhill et al. (2018) amassed a data set of over 1190 data points from 3 continents collected by citizen scientists to explore the effects of urbanization on the biological and chemical conditions of surface waters. Another study (Lottig et al., 2014) compiled volunteer observations from 1938 to 2012 to determine if temporal trends in lake-water clarity existed across the broad geographic range of eight midwestern states.

While the diversity of water quality-based citizen science programs continue to increase, a common critique of crowdsourcing is the lack of accuracy within the data collected (Nicholson et al., 2002; Loperfido et al., 2010; Ali et al., 2019). To address these concerns Ali et al. (2019) tested whether volunteer experience level impacted the accuracy of nitrate, phosphate, and atrazine measurements collected by groups of inexperienced, experienced, and expert level participants. They found that volunteer experience level influences the accuracy of results, but that unlike inexperienced users, both experienced and expert level users produce consistent and reliable measurements, indicating expert status is not a requirement to receive accurate data (Ali et al., 2019). Similarly, Peckenham and Peckenham (2014) conducted method testing for protocols and tools used by high school students to address concerns about precision and variability. They identified tool kits available to students and conducted a variety of tests on parameters such as pH, hardness, iron and nutrients. They found that method accuracy and precision varied among the metrics, with the nitrate assays showing good precision, and the chloride assays experiencing greater ambiguity near critical end-point values. They also concluded that with proper management and training of volunteers, precise measurements are achievable (Peckenham & Peckenham, 2014). Likewise, Muenich et al. (2016) found that volunteers were consistently able to produce nitrate concentrations that agreed with the results from laboratory tests, confirming the utility of their measurements.

Nutrient monitoring is an ideal task for citizen scientists. For one, anthropogenic nutrient loading is accelerating eutrophication processes, which is considered a “wicked” problem facing watersheds (Thornton et al., 2013). To address “wicked” problems, researchers argue that management approaches need to be flexible and adaptive, which is ideal for the inclusion of citizen science initiatives (Thornton et al., 2013). To supplement this, it has been demonstrated that volunteers have the capacity to accurately perceive nutrient parameters (e.g. Muenich et al., 2016; Lévesque et al., 2017; Ali et al., 2019). More specifically, the quantification of nitrate in surface waters is a parameter commonly analyzed in citizen science monitoring campaigns (e.g: Loperfido et al., 2010; Peckenham & Peckenham, 2014; Breuer et al., 2015; Muenich et al., 2016; Storey et al., 2016; Lévesque et al., 2017; Scott & Frost, 2017). Nitrate quantifying tools in citizen science projects rely on a colorimetric reaction, most commonly a modified Griess reaction (Nelson et al., 1954).

It has been proposed that the use of cell phone-based monitoring might be a way to further enhance the quality of data collected by the public (Burke et al., 2006), and this is particularly true when monitoring for nitrate. Cell phones have become a part of many citizen science projects, allowing volunteers the freedom to submit samples from the field (Burke et al., 2006; Dickinson et al., 2010; Kolok et al., 2011; Mason & Garbarino, 2016). For example, HydroColor, a smartphone app, has been used to measure water reflectance (Leeuw & Boss, 2018) while the Creek Watch app encourages photo submission of water levels, flow rates, and presence of trash (Kim et al., 2011). With respect to nitrate quantification, Deltares has developed a cell phone app to aid with quantification. Specifically, the app uses the phone's camera to assist with the quantification of nitrate concentrations allowing users to share their results on an interactive online map (see: https://www.deltares.nl/en/software/nitrate-app/). This app was identified and selected for this study because of its potential for integration into a citizen science campaign and novel status as an app that quantifies nutrients.

In this study citizen scientists were provided with Hach © nitrate test strips to evaluate nitrate concentrations in water. These strips can be evaluated visually or with the assistance of the Deltares nitrate app. The objective of this work was to compare the accuracy of data produced by volunteers using the two quantification methods.

Methods

Recruitment of Citizen Scientists

To compare the accuracy of visual and app methods for nitrate quantification, citizen science participants were recruited from various populations through the coordination of twelve separate testing events in Idaho and Washington. These events were hosted on university campuses, at local scientific meetings, and with high school groups from February 2019 to February 2020. The participants had varied skill levels and backgrounds, to adequately represent a population who might participate in a citizen science campaign (Table 1). The number or participants at these events ranged from 3 to 23 volunteers, with a total of 142 citizen scientists tested.

Table 2.1: Organizations that participated in testing events with their corresponding test dates and sample sizes

Testing Event	Date	Sample Size	Participant Type
ORED Staff	2/26/19	23	University of Idaho Staff
Idaho Commons	3/22/19	23	UI College students, Staff
ORED Open House	4/4/19	11	General college population
Spokane River Forum	4/16/19	16	Water Professionals, educators, general public
Columbia High School	5/28/19	15	High School Students
Palouse Basin Aquifer Committee Meeting	10/10/19	13	General public, water professionals, students
Ourgem Symposium	11/6/19	9	General Public, Water professionals
Idaho Water Institute Symposium	11/12/19	3	Water Resources graduate students, faculty
Idaho Commons	12/6/19	9	UI College Students
Idaho Water Quality Workshop	2/11/20	10	Water Professionals, Students, General Public, Faculty
Continuum test: visual samplers	6/24/19	5	Idaho Water Institute staff and interns
Continuum test: app samplers	1/24/20	5	Water Resource graduate students

Nitrate Test Strips

Volunteers measured nitrate concentration using Hach © test strips, which indicate discrete concentrations in parts per million (ppm) through colorimetric indication (Figure 1). The discrete colors correspond to concentrations of 0, 1, 2, 5, 10, 20, and 50 ppm. Test strip exposure pads are fully submerged in the water sample for 1 second and then removed. The color of the pad after a 30 second incubation time corresponds to the concentration of nitrate in the water. Test strips are both temperature and time sensitive, and any color recorded at a time other than 30 seconds will lead to either under or overestimation. The Hach © test strips also measure nitrite, but they will be disregarded for the remainder of this study. These test strips have been validated by other studies (see: Ali et al., 2019) and used in a variety of other citizen science monitoring programs (Loperfido et al., 2010; Muenich et al., 2016). The Hach © website notes that these test strips are accurate +/- one half of a color block (https://www.hach.com/teststrips).

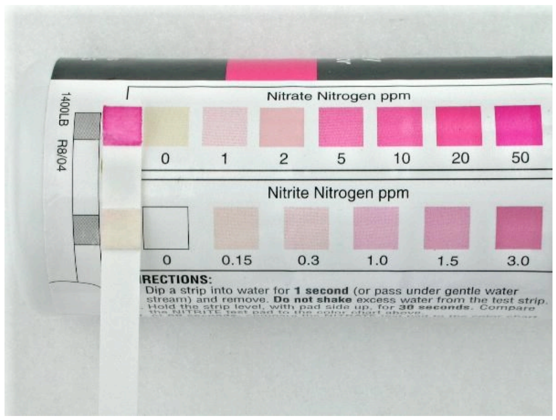

Figure 2.1: Nitrate test strip discrete colorimetric ranges and corresponding concentrations

Sample Preparation

Each testing event required volunteers to quantify nitrate concentrations in prepared spiked water samples. All the nitrate samples were prepared using KNO_3, then preserved and refrigerated (Ali et al. 2019). New solutions were made for each of the twelve testing events. The concentrations of all the solutions used in the testing events were confirmed analytically either by the University's analytical laboratory or by an in-house discrete analyzer (Seal AQ400: Method: EPA-114-C, Appendix A).

Study Design

Two experiments were conducted to address the objectives of this study. The first experiment ("App v. Eye") aimed to assess the accuracy of data produced by app and visual testers on two different nitrate concentrations. The second experiment ("Quantifying a Continuum of

Nitrate Concentrations") was conducted with the intent to further understand how each quantitative method performed on a continuum of nitrate samples.

App v. Eye

To accomplish the goal of the first experiment, 132 of the volunteers recruited were provided a water sample and instructed to quantify the nitrate concentration either visually or using the app. Volunteers were given a water sample and instructions to follow with no further verbal directions, as to not bias their test. More specifically, each tester was assigned to one of two data types. Visual methods traditionally make use of a categorical scale (Figure 2.1) and the app operates on a continuous concentration scale. To compare the tools fully it was necessary to account for the data type produced. Data type was assigned to the volunteer through different types of instructions which directed participants to either categorically or continuously assign their sample concentration. The categorical instructions required the participants to bin their sample into one of the discrete categories indicated on the Hach © bottle, unlike the continuous instructions which allowed participants to interpolate the concentration of their sample. The volunteers were instructed to use one of two analytical methods (referred to as "app testers" and "visual testers") to complete the nitrate test. The app testers used an iPad equipped with the Deltares Nitrate App to quantify the nitrate concentration of their sample. The visual testers quantified their sample visually using the colorimetric scale provided by the test strip instructions (Figure 2.1).

Once volunteers were placed into an analytical method category, they were provided one of two water samples. Two different concentrations of nitrate were chosen for this testing; 2 and 15 ppm. 2ppm was chosen because it directly corresponds to a categorical bin that both the continuous and categorical participants should be able to record. 15ppm was chosen because of its placement between two categories: 10 and 20. This concentration was chosen because it allowed the continuous samplers the freedom to interpolate both visually and using the app. There were 66 app and 66 visual samplers. There were 17 categorical and 16 continuous visual testers and 18 categorical and 16 continuous app testers at 2ppm. There were 17 categorical and 16 continuous app testers and 17 categorical and 15 continuous testers at 15ppm.

When comparing the results gathered visually to those generated using the app it was important to understand if the volunteers were accurately analyzing their sample or producing under- or over-estimations of the true concentration. For 2ppm the acceptable range of accurate responses included any value that fell within the two flanking categories (i.e. 1.1 to 4.9 ppm). Anything other than this range was considered an under or overestimation. For 15ppm, any category that flanked 15 (10 or 20ppm) were considered accurate. Values below 10ppm were under-estimations and any value above 20 was considered an over-estimation.

Quantifying a Continuum of Nitrate Concentrations

The second experiment was conducted with the intent to further understand how each tool performed over a wide range of nitrate concentrations. Ten individuals were tasked with quantifying a continuum of 25 randomized nitrate samples (Table A.2). Half of these samplers were instructed to visually quantify their test strip and categorize their samples into the discrete bins on the Hach © bottle. The other half of the samplers were instructed to continually quantify their 25 samples using the Deltares nitrate app. The 25 samples began at 1ppm and increased to 50ppm nitrate on every odd value (e.g. 1, 3, 5…47, 49, 50 ppm). Participants recorded their nitrate concentrations on a provided test sheet.

Statistical Analysis

In the first experiment, Fisher's exact tests were used to determine if data type had any impact on the accuracy of results. These tests were conducted at 2 ppm, 15 ppm, and as a composite for both visual and app testers. This test was chosen because of the small sample sizes, which would have been inappropriately small for a Chi^2 analysis. To determine if there was any difference in accuracy between the app and visual testers as a composite, the proportions of accurate to inaccurate responses were also compared using a Fisher's exact test.

The second experiment was conducted with the intent of understanding how each analytic tool performed when quantifying a continuum of nitrate samples. It was necessary to transform the continuous samples used by the visual testers into the same categories that the volunteers used. To do this the continuous values were binned into their corresponding category as per Muenich et al. (2016) who similarly compared continuous lab samples to categorical field samples (Table 2.2). This data was plotted and analyzed statistically using Spearman's correlation to determine the relationship between the binned continuum samples and the volunteer's recorded categories.

Table 2.2: Test strip categories used by visual testers and the ranges of continuous nitrate samples placed in those bins.

Hach © Test Strip Scale (ppm)	Assigned Continuous bin (ppm)
0.0	<0.5
1.0	0.5-1.5
2.0	1.6-3.5
5.0	3.6-7.5
10.0	7.6-15.0
20.0	15.1-35.0
50.0	>35.0

For the app testers, the responses did not need binning as both data types were continuous. The volunteer data was plotted against the true nitrate sample concentrations and fitted with a linear regression model.

All statistical analyses for this project were preformed either using JMP (v. 14.0) or Microsoft Excel (V. 16.33) software with $\alpha = 0.05$.

Results

App v. Eye

Visual Testers and Data Type.

Before comparing the analytic capacity of the app to visual quantification methods, it was necessary to look at each method individually. There were 34 volunteers who quantified their samples using visual methods, with 18 of those individuals that evaluated the strips using categories and 16 participants who interpolated their findings according to a continuous scale. None of the participants underestimated at 2ppm and both groups tended to overestimate when errors occurred. Categorical testers were accurate 89% of the time and continuous testers were accurate 75% of the time (Figure 2.2). The proportions of accurate to inaccurate results for both categorical and continuous methods were compared using a Fisher's exact test and the results were not significant (p=0.3872).

For visual testers at 15ppm there were a total of 32 volunteers who participated. 17 of those used categorical data types and the remaining 15 produced continuous results. In contrast to the samplers testing at 2ppm, continuous volunteers did underestimate at 15ppm. Continuous testers produced accurate results 80% of the time while categorical testers produced 100% accurate results (Figure 2.2). The proportions of accurate to inaccurate results for both categorical and continuous methods were compared using a Fisher's exact test and the results were not significant at 15ppm (p=0.0917). Finally, both concentrations were combined and analyzed as a composite with a Fisher's exact test. These findings were also not significant (p=0.719; Figure 2.3).

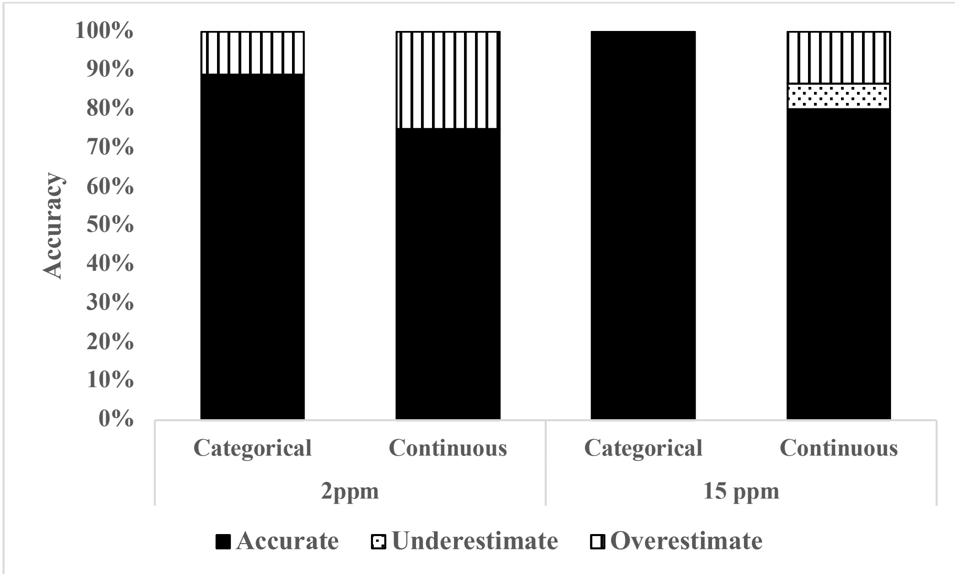

Figure 2.2: Graphical representation of percent accuracy for visual testers at both 2 and 15ppm NO_3-N for both categorical and continuous data types.

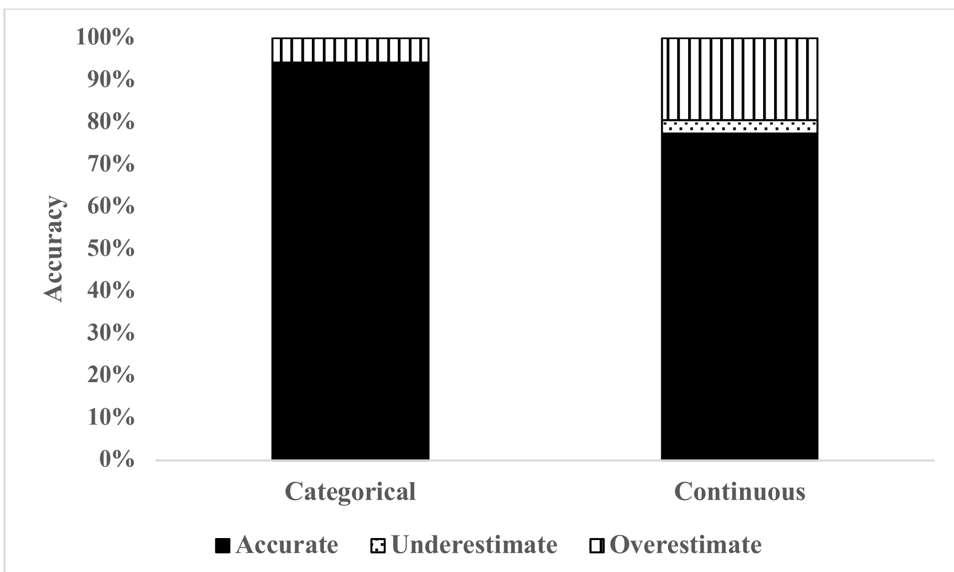

Figure 2.3: Composite percent accuracy for visual testers for both categorical and continuous data types.

App Testers and Data Type.

There were 33 volunteers who quantified their samples using the Deltares app at 2ppm, with 17 of those individuals categorizing their results and 16 participants who recorded values on a continuous scale. There were underestimations, accurate, and overestimation results produced by both continuous and categorical testers (Figure 2.4). Categorical testers tended to overestimate more often than continuous testers, who were more likely to underestimate. The proportions of accurate to inaccurate results for both categorical and continuous methods were compared using a Fisher's exact test and the results were not significant at 2ppm (p= 0.2818).

There were 33 volunteers who quantified their samples using the Deltares app at 15ppm, with 17 of those individuals categorizing their results and 16 participants who continuously quantified their findings. The only underestimation at this concentration was the result of continuous testers (6%). Continuous and categorical testers were more likely to overestimate (50% and 76% respectively) using the app at this concentration than produce accurate results. (Figure 2.4). The proportions of accurate to inaccurate results for both categorical and continuous methods were compared using a Fisher's exact test and the results were not significant at 15ppm (p=0.2818), or when both concentrations were combined and analyzed as a composite (p=0.1387, Figure 2.5).

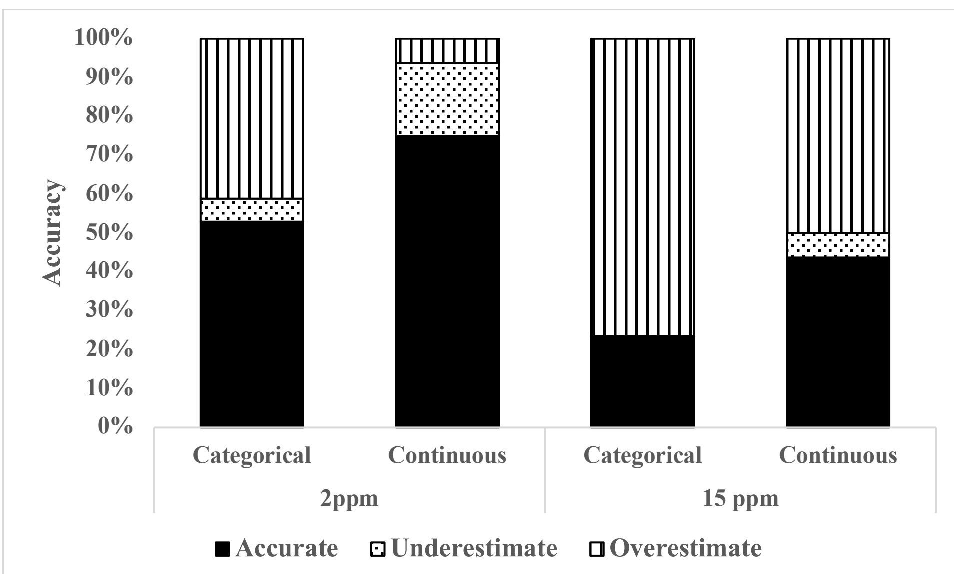

Figure 2.4: Graphical representation of percent accuracy for app testers at both 2 and 15ppm NO_3-N for both categorical and continuous data types.

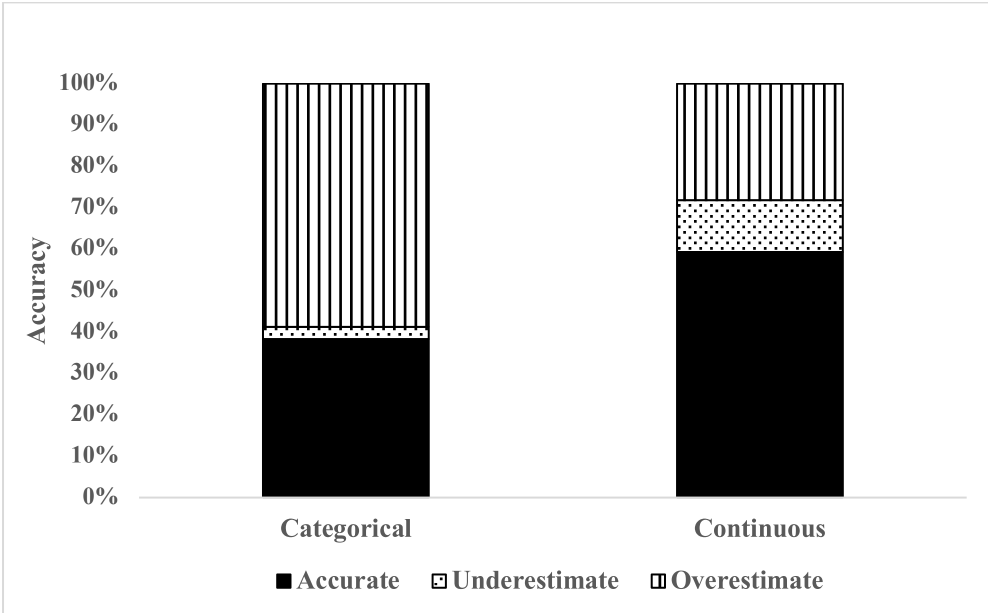

Figure 2.5: Composite percent accuracy for app testers for both categorical and continuous data types.

Analytic Tool and Accuracy.

To compare the two analytic tools, all the accurate responses were pooled from the 66 visual and 66 app testers, regardless of data type or concentration. The proportion of accurate to inaccurate responses were determined for each tool and then were analyzed using a Fisher's exact test. The results indicate that testers using visual methods are statistically more likely to be accurate than their app testing counterparts ($p<0.00001$; Figure 2.6). The data were further broken down into the proportion of accurate to inaccurate responses at the two concentrations then analyzed using a Fisher's exact test (Figure 2.7). The findings indicate that at 2ppm, visual and app methods are not statistically different ($p=0.1036$). At 15ppm, the visual testers are statistically more likely to be accurate than their app testing counterparts ($p<0.00001$*).

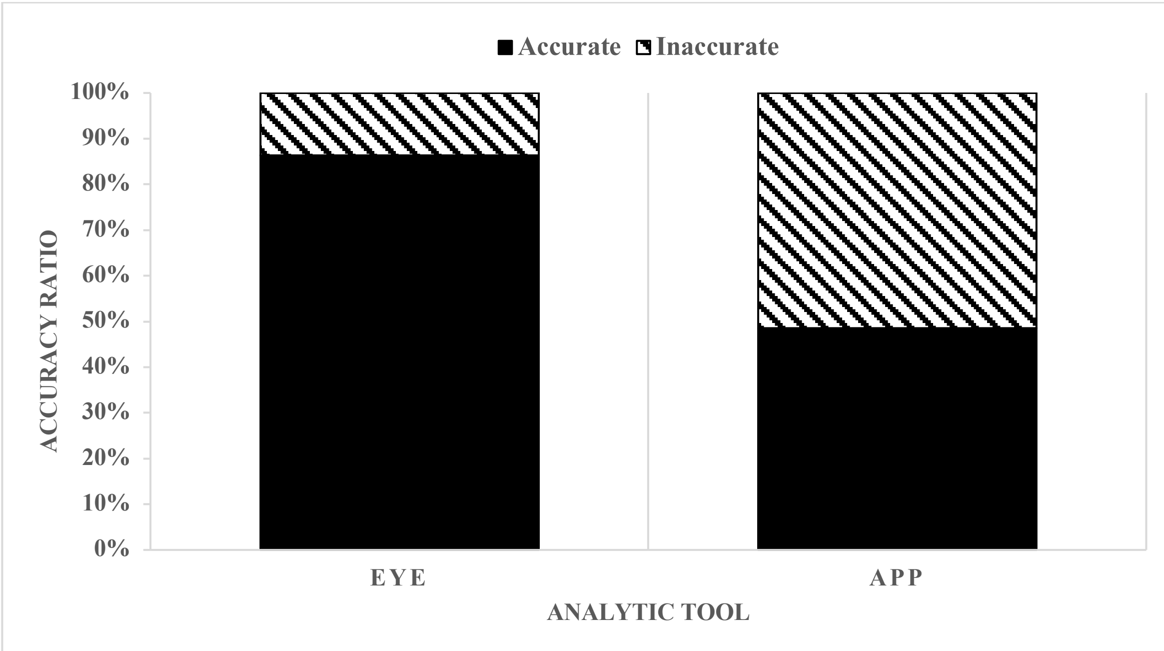

Figure 2.6: Graphical representation of composite accurate and inaccurate responses for both analytic tools.

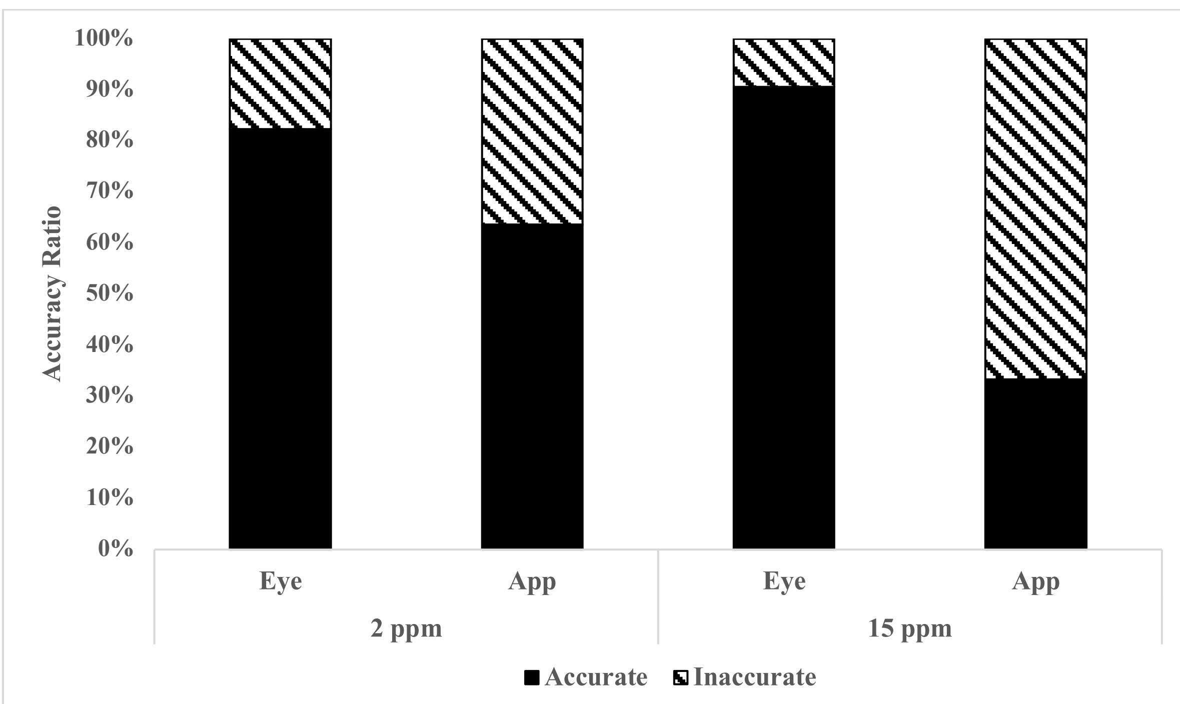

Figure 2.7: Graphical representation of app and eye accuracy at 2 and 15ppm NO_3-N.

Quantifying a Continuum of Nitrate Concentrations

The objective of this work was to assess how volunteers quantify a continuum of nitrate samples using either the app or visual quantification methods using the default data type for each tool. More specifically, the citizen scientists that quantified their results visually categorized the data according to the instructions on the Hach © bottle, while the app testers recorded the continuous data produced by the cell phone software.

Categorical Visual Testers.

To account for the non-linear categories produced by the visual testers, the continuous nitrate samples were binned into one of the six existing categories (Muenich et al. 2016; Figure 2.8). The Spearman's rank correlation coefficient was calculated to assess the relationship between binned concentration and volunteer category response. The actual bins and recorded categories were strongly correlated (ρ=0.8735, p<0.0001*).

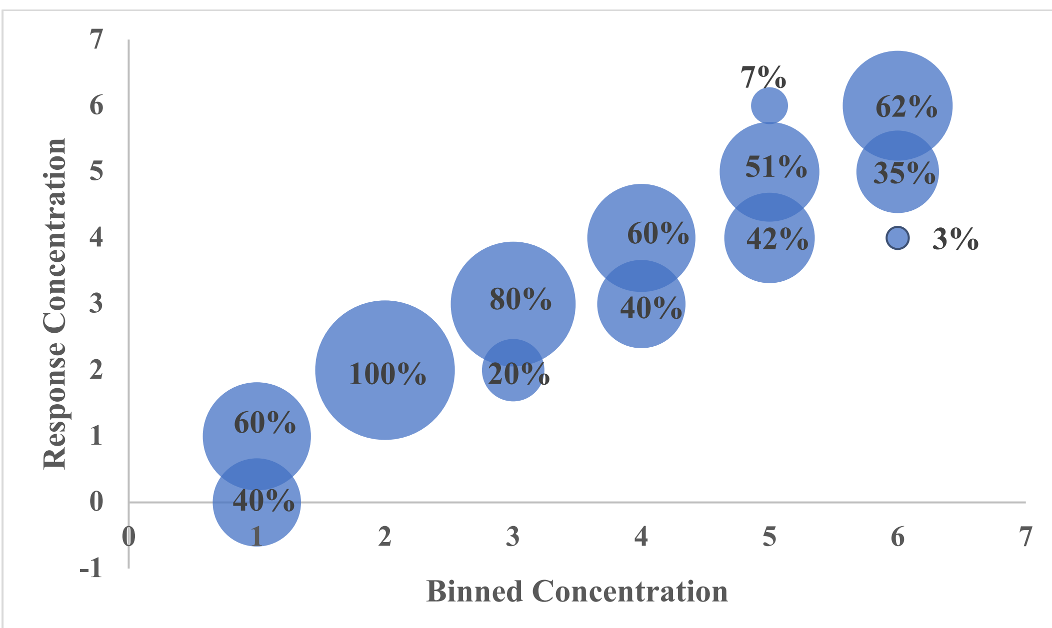

Figure 2.8: Binned nitrate concentrations and corresponding response categories produced by visual testers were analyzed using a Spearman's correlation.

Next the residuals were calculated by taking the volunteer's response category from the true concentration bin. These data were plotted against the actual concentrations (Figure 2.9). For the lowest four categories, not including zero (1-4) residuals were only off by one category. The higher concentration bins displayed higher residual ranges, indicating that as nitrate concentration increased, so did the range of categories that were recorded by the participants.

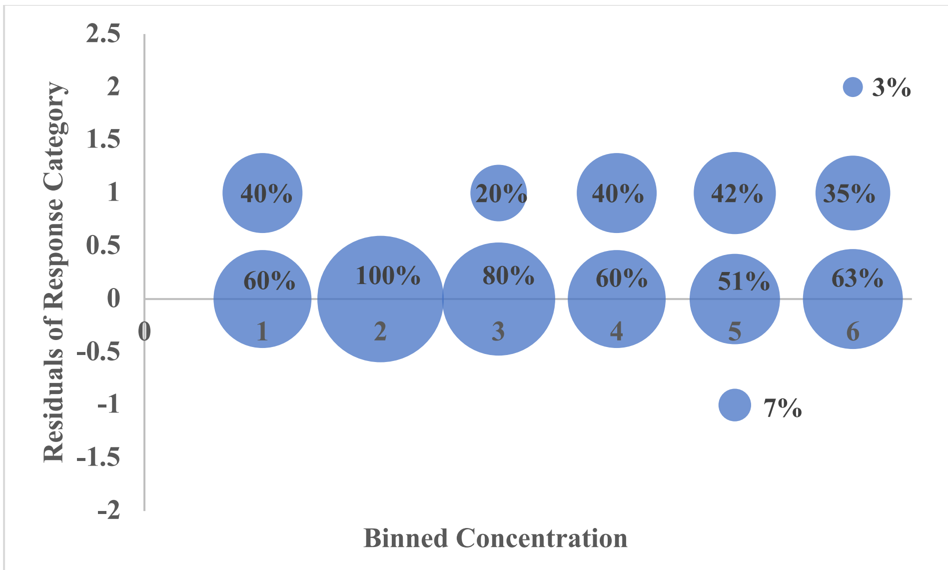

Figure 2.9: The residuals from visual testers were calculated and plotted against the true binned category. The bubble size corresponds to proportion of samples with the same residual.

When these data were broken into two groups the relationship (Spearman's rank correlation coefficient) between the actual bins and recorded categories were strongly correlated for both groups, with a higher correlation realized for categories 0-4 (ρ=0.8523, p<0.0001*) relative to that for the higher categories, 5 and 6 (ρ=0.6431, p<0.0001*; Figure 2.10).

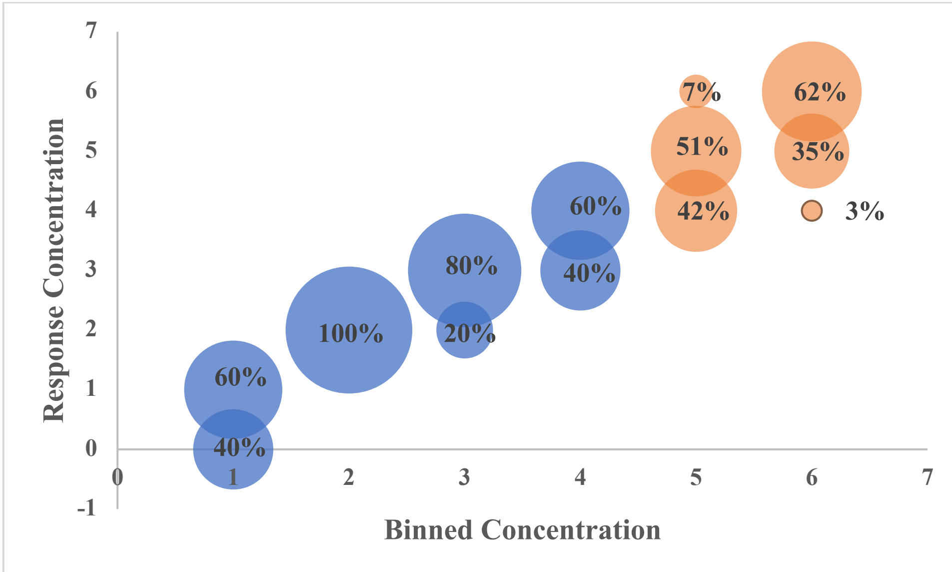

Figure 2.10: The actual binned concentrations compared to the response categories. The blue color corresponds to the samples in the lower range with smaller residuals and the orange data corresponds to the samples in the higher range with residuals of 3.

Continuous App Testers.

The continuous data produced by the app testers did not require the same binning process as the categorical data above and was simply plotted against the true nitrate sample concentration (Figure 2.11). A linear regression was modeled for the entire data set. The linear regression model for the data explains 77% percent of the variation in this modeled relationship (y=1.0369x +4.9569, R^2= 0.766, p=0.0011 *). A Nash-Sutcliffe efficiency (NSE) coefficient, an alternative goodness-of-fit index, was calculated to assess the accuracy of the observed versus expected relationship, which can supplement limitations of a linear regression's R^2 (E_f= 0.519) (Agrimet, 2019).

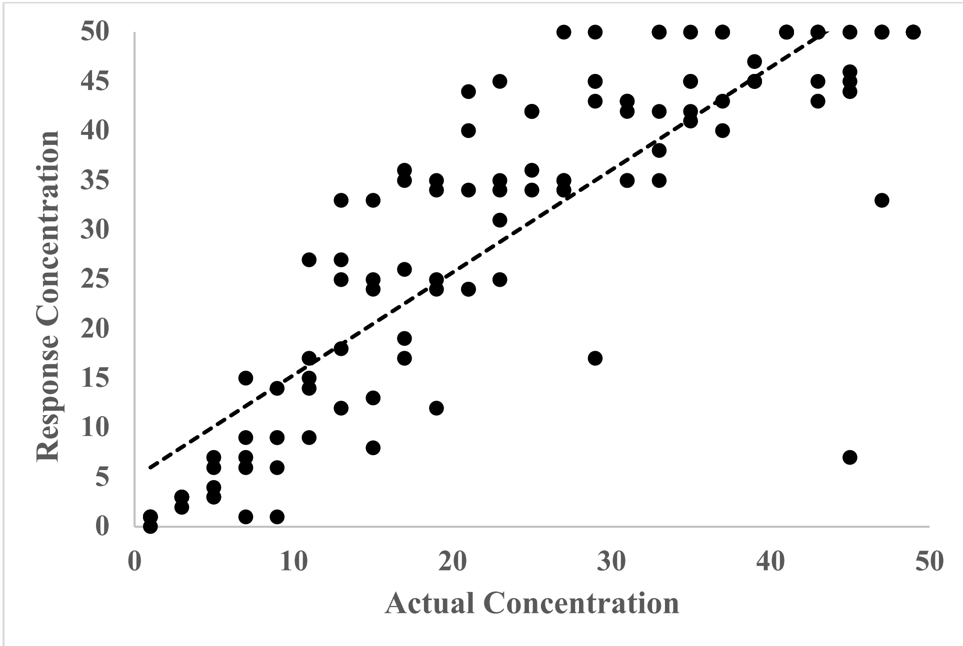

Figure 2.11: The linear regression comparing actual nitrate concentration to the response concentration produced by the app.

Next all the response concentrations from each tester were average at each testing concentration and plotted against the true concentration. Standard error values above and below this average were calculated and plotted (Figure 2.12). The upper and lower bounds (standard error) increases as the actual sample concentration increases.

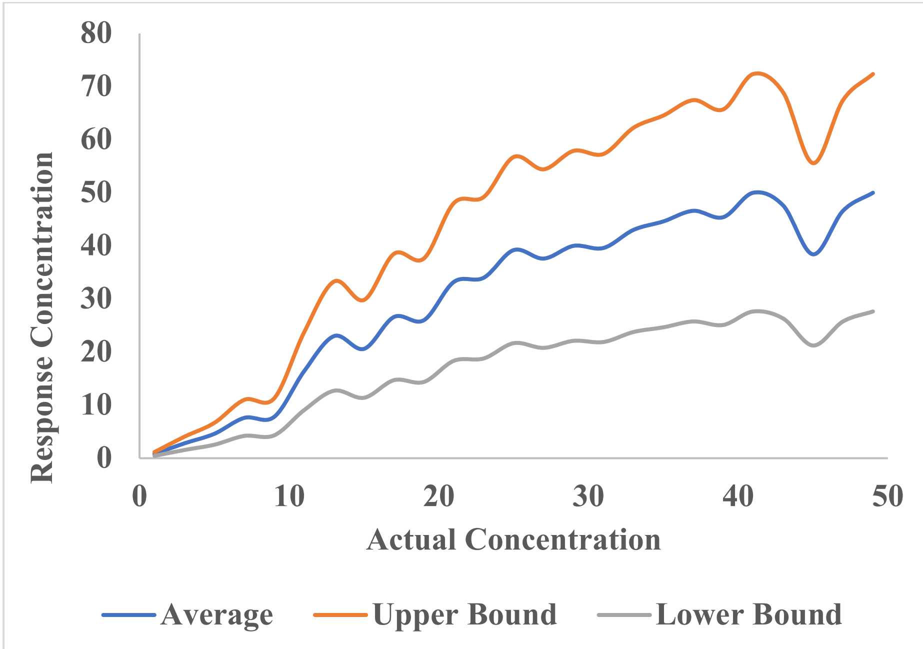

Figure 2.12: Standard error confidence bands of the relationship between actual and response concentration

Next the residuals were calculated by taking the volunteer's continuous response from the true nitrate concentration. These data were averaged at each concentration and plotted with their standard deviations against the actual concentrations (Figure 2.13). The lower ranges (0-15ppm) had the smallest residuals, which is similar to the findings from the categorical testing above. As concentration increases so does average residual and the variation associated. The average residuals tended to be negative, indicating that the app tends to overestimate the true nitrate concentration. A chi-square test of independence was performed to examine the relationship between residual sign (positive, negative, or zero) and the observed/expected values. The relationship between these variables was significant X^2 (1, N = 150) = 52.56, p <0 .00001.

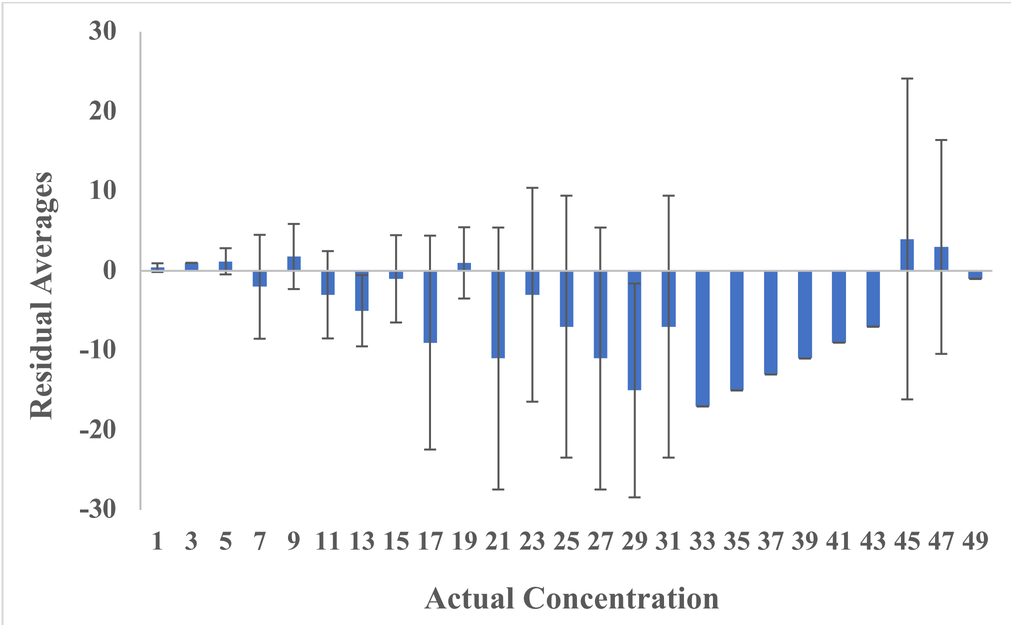

Figure 2.13: The average residuals plotted against the true concentration for the continuous app testers. The error bars correspond to the standard deviation of the residuals from all five app testers.

The continuous app data showed a similar trend to the categorical visual testers in that the data at the lower end of the nitrate continuum had smaller residuals. In order to treat the categorical and continuous testing events similarly, the continuous data was broken into two ranges. The lower range (0-15ppm) corresponds with the four lower categories from the categorical testing. The remainder of the data (15-50 ppm) falls into the two higher categories. These two data ranges were then plotted against the true concentrations and linear regression models were determined for each (Figure 2.14). The lower range model explained 66% of the variation of the data produced ($y=1.7321x - 3.125$, $R^2=0.6599$; $p<0.0001$*). The higher range's correlation is not as strong as the lower range, as the model produced only explains 45% of the data ($y=0.71x +16.78$, $R^2=0.453$; $p<0.0001$*). The slopes of these two relationships were significantly different from each other. The 95% confident intervals for the two slopes were [1.7321 ± 0.3663] and [0.7064 ± 0.1376] respectively.

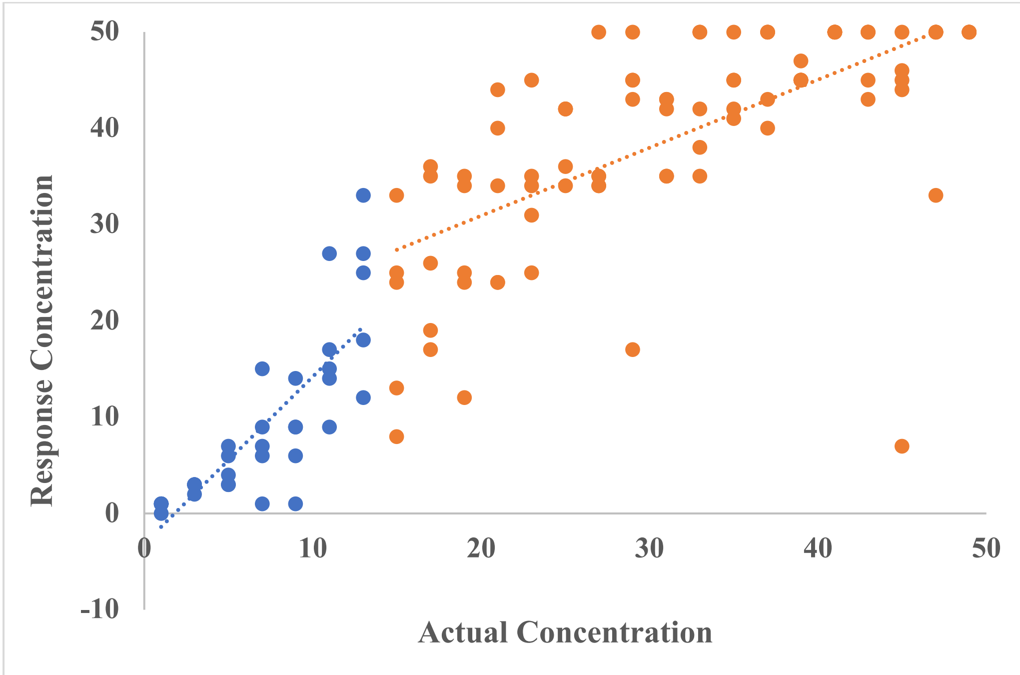

Figure 2.14: The linear regressions for continuous app testers comparing the actual concentration of nitrate to the recorded values. The blue range corresponds to the same categories (0-4) and the orange data corresponds to the samples in the highest categories (5-6).

Discussion

App v. Eye

Data Type.

To enhance the accuracy of crowdsourced data and ultimately the trust of researchers and regulatory agencies it is necessary that volunteers produce robust and repeatable data (Jollymore et al., 2017). One way to address this is to ensure that participants are using tools that provide the appropriate data type. For visual testers there was no difference in accuracy when using the tools to provide categorical as opposed to continuous data types. While the analysis of volunteer categorical data has been cited as one of the difficulties of crowdsourced data collection (Muenich et al., 2016), and while not significantly different than the continuous testers, these results suggest that categorical data types are still recommended over volunteer interpolation.

The Deltares nitrate app uses the phone's camera to produce a continuous value when quantifying the color of the test strip. There are a variety of other cell phone based colorimetric apps in the literature that aim to conduct high accuracy tests (Hong & Chang, 2014; Yetisen et al., 2014) that are cost effective (Chen et al., 2017), and easy for users to conduct (Hong & Chang, 2014; Chen et al., 2017). Many of these apps make use of cell phone cameras and then use specialized software to produce a concentration from the resulting test strip color. Examples include the detection of the mycotoxin zearalenone (Chen et al., 2017), the quantification of glucose, protein and pH in artificial urine (Yetisen et al., 2014), and the quantification of chlorine in water samples (Sumriddetchkajorn et al., 2013). All the cellphone apps discussed above provide data to the user on a continuous scale as the default setting of the software, and the expectation is that this data would be more accurate than a categorized output. The statistical difference between continuous and categorical data types for app testers was not significant. The app is programed to provide continuous values of nitrate, and as such it was expected to produce results of higher accuracy when testing continually as compared to categorically, which is not it's default data type.

The findings from these tests suggest the importance of data type and analytic tool alignment. The visual interpretation of test strips is designed to produce categorical results, while apps are designed to produce continuous results. Since there were no significant differences in accuracy within each tool based on how the data were collected, it is appropriate for citizen scientists to use the default data type of each tool.

When comparing the accuracy of the two tools in this study, the visual testers produced more accurate results than their app testing counterparts, indicating that specialized tools, in this case the Deltares Nitrate app, do not increase the accuracy of volunteer data. It is worth noting that the Deltares app was the only app studied in this research, and these findings might not hold true for other apps. Volunteers were also experiencing the Deltares app for the first time when they were testing. As noted on the Deltares "Tricks and Tips" webpage, practice using the app is best for producing high quality results (see: https://publicwiki.deltares.nl/pages/viewpage.action?pageId=127634730). This is not unique to cellphone apps in that other studies have demonstrated a level of experience increases the quality of data produced by volunteers (Kosmala et al., 2016). In addition to experience using a tool, it would also be valuable to integrate step-by-step instructions into cellphone apps to assist first time users. Researchers have identified this as a concern and have begun to develop apps with self-guided instructional materials included in the analysis process (Chow et al., 2018).

Citizen scientists have successfully used cell phone apps for colorimetric quantification of solutions (Kehoe & Penn, 2013; Kuntzleman & Jacobson, 2016). Cellphone cameras have been integrated into high school chemistry classrooms to teach about Beer's Law and the quantification of sample concentration using absorption spectrophotometry (Kehoe & Penn, 2013; Kuntzleman & Jacobson, 2016). For example, high school students in Minnesota successfully used cell phones to colorimetrically analyze solutions of food dye, sports drinks, and Iron (III) chloride using controlled laboratory light-settings to produce results of suitably quantitative data (Kehoe & Penn, 2013). Citizen scientists possess the capacity to use cell phones for analysis, but for these apps to be useful outside a controlled setting they must be equipped to accommodate for external variabilities.

Cell phone apps that generate continuous results from colorimetric assays can be biased due to lighting variation, angles, and device type (Shen et al., 2012; Yetisen et al., 2014; Karlsen & Dong, 2015). The findings from this work suggest that the Deltares app might also be sensitive to external factors. It is possible that lighting variations might have accounted for some of the overestimation produced by app users. If cell phone colorimetric readers are going to be robust, accurate, and repeatable they need to account for potential measurement variability (Shen et al., 2012; Yetisen et al., 2014). Ambient lighting is often one of the variables that influence app function most dramatically (Shen et al., 2012; Yetisen et al., 2014) and would certainly be a factor that citizen scientists face in the field.

Lighting issues are likely one of the reasons that the app testers tended to overestimate. It is also worth noting that these testers were first time users of the app, which may have resulted in them taking longer to complete the testing, resulting in further overestimations. App testers also had to coordinate instruction reading, an iPad tablet, and testing materials, while visual testers had one less variable to coordinate. The addition of the iPad may have increased the instructional time testers required, resulting in overestimations. To account for these variations, it would be worthwhile to conduct further testing with users who have had the chance to practice with the app software and become more comfortable with the workflow. Likewise, visual testers also tend to overestimate nitrate concentrations using the Hach © test strips when errors are made. These results are consistent with the findings of Ali et al. (2019), who demonstrated that inexperienced volunteers are more likely to overestimate their nitrate concentrations than their more experienced counterparts.

Overall the results from the "Data type" objective support the finding that first time volunteers quantifying their samples visually are more likely to produce accurate results than first time testers using the app. Apps have the potential to be useful for colorimetric analysis, but must be designed with the user in mind through the addition of instructional workflows (Chow et al., 2018) and self-correcting algorithms to account for external variables that can influence quantification accuracy (Shen et al., 2012; Yetisen et al., 2014; Karlsen & Dong, 2015). In the case of the Deltares app software that can correct for imperfect lighting or device angle when quantifying a test strip would be useful for citizen science contexts. It would be worthwhile to conduct these tests again with users who have used visual and app quantification methods before to assess how accuracy reflects a level of experience with a tool.

Quantifying a Continuum of Nitrate Samples

The second experiment assessed the variability of the eye as a visual categorical tool relative to the app as a continuous tool for the quantification of a continuum of nitrate samples. The default data type for each tool (eye: categorical, app: continuous) was chosen for this testing. For both groups, there was a statistically significant relationship between the true nitrate concentration and the results estimated by the volunteers. Quality assurance testing, such as the tests conducted here, have been used for a variety of other test strip apps such as glucose meters used by Diabetic patients (Hönes et al., 2008), the diagnostic accuracy of NicAlert, a test strip that identifies tobacco metabolites in saliva (Cooke et al., 2008), and test strip accuracy and precision for "Merckoquant" nitrate test strips for use in plant and soil monitoring applications (Jemison & Fox, 1988). While Cooke et al. (2008) calculated the sensitivity, specificity, and predictive values of their test strips, other research teams calculated the coefficients of variation to determine precision of their test strips (Jemison & Fox, 1988; Hönes et al., 2008). As with other analytic tools and methods, it is important to understand the range, precision, and calibration of a tool before it is assumed to produce reliable data, particularly in a water monitoring context (Skougstad, 1974).

The results produced by the app and visual testers all increased in variability as concentration increased. Concentrations between 1 and 15ppm (categories 0-4) experienced the lowest variability of the whole spectrum. This is expected for the visual testers, as the chromatic color change between concentration values becomes harder to perceive in ranges above 10ppm. When both data set were assessed for correlation, the visual testers produced a statistically strong association with the true concentration category (ρ=0.8523, p<0.0001*). The app's correlation was not as strong (R^2=66%, p=0.001*), but still significant.

Interestingly, visual testers tended to underestimate during this testing regime, which is counter to the results found in the first round of testing conducted in this study. Ali et al. (2019) found that nitrate testers will produce both underestimations and overestimations but are more likely to produce results higher than the true value. Visual testers may be underestimating because the color differences between categories 20 and 50 are difficult to discern and there were more samples in this range than any of the other ranges. In the opposite direction, the Deltares app results from this test tend to overestimate concentration. These findings also make sense with the findings from the first objective of this research, which also indicate that this app is prone to overestimation. These findings are likely the result of inconsistent lighting or shadows in the testing room.

The app and visual testers produced results of decreasing precision at medium to high concentrations which was determined by the residual analyses. These findings indicate that the accuracy and precision of data above 15 ppm, or the fourth category, is more variable than that at lower concentrations. From a regulatory perspective the sensitivity of the Hach © nitrate strip can still be useful because the range at which the strip is most accurate coincides with the EPA drinking water regulatory limits of 10ppm (EPA, 2020). Citizen scientists and the tools they're provided can accurately and repeatably quantify samples at or below the EPA regulatory limit for drinking water, making the data they provide still useful. While confidence in the true value of data above this range might be lacking, this finding is still useful for "hot spot" monitoring. The idea of "hot spot" monitoring argues that citizen scientists can be used for first tiered monitoring efforts to identify regions of concern for future monitoring (Kolok et al., 2011).

Conclusions and Implications for Citizen Science and Water Quality Monitoring

Taken in tandem, the results from this study and other similar research literature continue to support the reliability of data produced by citizen scientists. As demonstrated by others in the citizen science field, volunteers can visually quantify nitrate concentrations accurately within the limits of the tools they are provided (Muenich et al., 2016; Lévesque et al., 2017; Ali et al., 2019). The findings from this work support the use of categorical data types by volunteers analyzing for nitrate visually and continuous data types if testers are using an app-based quantification method. The findings from this work also indicate that both methods are prone to overestimation when errors occur.

To expand this research, further testing with the Deltares app could be conducted using other cell phone-based platforms. All the testing in this study was conducted using Apple iPads, and it would be worthwhile to assess how other cell phone readers would perform. Similarly, and as mentioned earlier, all the testers were conducted by first time app users. It would also be meaningful to assess how accuracy of results changes when volunteers are more comfortable with the Deltares app. Furthermore, there are timing inconsistencies between the instructions on the Hach © test strip bottle and on the Deltares instructions. Extended testing to assess optimal incubation time would certainly be worthwhile for consistency between platforms and accuracy of resulting data.

The use of cell phones in citizen science projects certainly has benefits for researchers such as GPS data, rapid data transmission and the platform for sensor data to be combined with human observation (Burke et al., 2006), and citizen scientists have successfully used them for colorimetric quantification in controlled laboratory settings (Kehoe & Penn, 2013; Kuntzleman & Jacobson, 2016). If apps are to be useful for citizen science monitoring it is crucial that they are designed with the general public in mind. The integration of algorithms to account for external variations such as light are necessary for the production of accurate data (Shen et al., 2012; Yetisen et al., 2014; Karlsen & Dong, 2015), especially for citizen scientists in the field who might experience a variety of conditions. Apps also need to be friendly to first time users or those who are less comfortable with cell phone technology. The integration of step-by-step instructions as a user works through the app would be a useful way to ease first time users into an app (Chow et al., 2018). Deltares has detailed instructions on their website but including directions that prompt the user through the testing experience would be useful for first time users. Supplemental educational materials, or similar engagement methods within the app platform would also be beneficial to users.

The combination of cell phone technology and citizen science monitoring offer a potential range of benefits for researchers and volunteers. For this combination to produce high quality data it is necessary that app selection and data quality produced by volunteers is analyzed thoroughly. The tests and findings from this research did not support the use of the Deltares nitrate app for increased accuracy in citizen science testing. While this app is not best for citizen science purposes, when properly designed to account for testing variations apps have the potential to be useful in citizen science projects. It is also worth reiterating that all the testers in this study were first time users, and supplementary testing with experienced users of both tools could further elucidate the findings from this testing. Finally, it was determined that both visual and app testers experience higher accuracy and lower variability at the low end of the nitrate scale. While accuracy decreases and variability increase as concentrations get higher, both the app and visual methods have potential to serve as reliable first-tier monitoring tools, as they can easily perceive concentrations of nitrate at regulatory significance.

Taken in tandem, these findings can further enhance the understanding that researchers have of nitrate data produced by citizen scientists. The validation testing of other tools for citizen science purposes has the potential to equip volunteers with the best possible tools for high quality data.

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Chapter 3: Crowdsourcing Biologic Materials to Supplement Environmental Monitoring

The objective of this review is to identify the potential for citizen science research methods to contribute to environmental monitoring efforts through the collection of biologic tissues. To fully elucidate the potential of citizen science in this realm, it is necessary to review the history of citizen science in the biological sciences, discuss citizen science in genetics research and tissue collection, and tie these themes together to identify the potential for citizen science to be integrated into environmental pollutant monitoring through the collection of biological tissues. Ecotoxicological monitoring for pollutants is inherently complex, and barriers to entry for volunteer participation will be identified. This review will also highlight the limited examples in the literature and discuss how their program design can be adopted by other researchers.

Citizen Science in Biological Sciences

Citizen science projects cover a wide variety of disciplines within the natural sciences (Dickinson et al., 2010; Stepenuck & Green, 2015), but few areas of research have benefited to the same extent as biology and biodiversity (Dickinson et al., 2010; Mason & Garbarino, 2016). The earliest citizen science projects focused on skilled ecologically-oriented observation (Greenwood, 2007; Mason & Garbarino, 2016) and arguably some of the most successful and widespread citizen science projects involve ornithological observation. For example, the Christmas Bird Count is a notable long-term project that amasses bird observation data annually. It is hosted by the National Audubon Society as a popular alternative to the Christmas hunt and has been occurring every year since 1900 (Silvertown, 2009), with recent years engaging with over 76,000 volunteers (LeBaron, 2018). Bonney et al. (2014) identifies at least 90 peer reviewed articles and book chapters that use citizen collected eBird data, displaying the true reach that crowdsourced data has accomplished.

Data contributions from volunteers have dramatically changed the course of fields such as biodiversity (Dickinson et al., 2010) and have the potential to cause a similar shift relative to environmental monitoring. While environmental monitoring for pollutants has inherent challenges to overcome before volunteers can participate, there is potential for innovation and growth, as demonstrated by the entry of volunteers into the field of genetics. Citizen science participation in genetics research is a perfect example of how properly designed programs and innovative technology can break down scientific barriers of entry.

Citizen Science and Genetics

There are several volunteer programs that focus on the gathering of biologic samples. In their review of biodiversity-based citizen science projects, Theobald et al. (2015) estimated that approximately 2% of identified biodiversity projects study genetic diversity, with the majority focusing on the monitoring of taxonomic diversity. Genetics was once a field that was inaccessible to volunteers, but with the advancement of genetic technologies and low-cost sequencing, citizen scientists can now more meaningfully contribute to genetic-based monitoring projects (Kuznetsov et al., 2015). The technological advancements ushered in by the Human Genome Project rapidly introduced the scientific community to new low-cost tools that have since been adapted to capture the data-amassing strengths of citizen science (Kuznetsov et al., 2015; Larson et al., 2020).

One such avenue for the incorporation of citizen science into genetic-based monitoring projects is through the collection of environmental DNA, or eDNA. Citizen science monitoring programs have adopted the use of eDNA in different types of projects because of it has the benefit of simple water or soil sample collection and cost effectiveness (Larson et al., 2020). eDNA testing is often used for wildlife species surveillance and has shown the sensitivity to identify the presence of rare species (Biggs et al., 2015; Sutherland, Roy, & Amano, 2015; Miralles, Dopico, Devlo-Delva, & Garcia-Vazquez, 2016; Larson et al. 2017; Larson et al., 2020). eDNA collection entered the citizen science world with the initiation of the great crested newt project in 2015. Biggs et al. (2015) tasked volunteers to collect eDNA samples from across the United Kingdom to detect the presence of a rare newt species. The research team concluded that eDNA collection was more effective than traditional techniques, such as nighttime flashlight surveys and egg counts, and that volunteers can survey successfully using this genetic method (Biggs et al., 2015). Other examples of successful eDNA citizen monitoring projects include detecting the presence of invasive crayfish in large north American lakes (Larson et al., 2017) and volunteers detecting invasive pygmy mussel (*Xenostrobus securis*) populations in Iberia (Miralles et al., 2016). The combination of easy sampling protocols, cost-effective monitoring, and a tool with the technological sensitivity to identify the presence of rare species makes eDNA monitoring an ideal method to be employed by citizen scientists (Larson et al., 2020).

Citizen scientists have also proven capable of collecting tissue samples for genetic work. For example, Mori et al. (2017) used citizen scientists to track the elusive and highly protected crested porcupine (*Hystrix cristata*) in Italy through the collection of discarded quills. Similarly, volunteers in Malaysia collected butterfly legs to help researchers monitor biodiversity. The legs were used to genetically identify different species in the area (Wilson et al., 2015). Guindon et al. (2015) identified angler volunteers to collect skin cells from the Atlantic tarpon (*Megalops alanticus*), a valued recreational species, to determine if DNA fingerprinting was a suitable method of tagging and tracking individual fish. More dramatically, researchers with the Washington Department of Fish and Wildlife used citizen scientists to collect cougar (*Puma concolor*) muscle tissue for population density genetic research and to determine if volunteer biopsy collection is a low cost alternative to more expensive capture-radiocollar techniques (Beausoleil et al., 2016). These examples demonstrate the innovative ways that researchers have included volunteers in their genetics research, which until the technological advancements of the 2000s, would have been inaccessible to citizen scientists.

Non-Genetic Tissue Collection

Tissue collection for non-genetic research has also been accomplished by citizen scientists. For example, fish scales were collected by volunteer anglers in England to further understand the spatial variability of both age and growth rates of invasive barbel (*Barbus barbus*) (Trigo, Roberts, & Britton, 2017). Researchers have also successfully tracked a variety of pathogens using biologic material, principally plant and insect tissues, collected by volunteers. For example, a research group based out of Northern Arizona University conducted a tick collection program to determine distribution of ticks and the prevalence of tick bites and associated diseases. This project was quite successful, as participants contributed over 16,000 tick tissue samples which the authors contribute to the public's desire to understand pathogens that threaten public health (Nieto et al., 2018). Citizen scientists have also successfully contributed to plant pathology research through the collection of infected leaf tissues. In the "Sudden Oak Death Blitz" project, volunteers collected symptomatic oak leaves to aid in research tracking the spread of this disease (Meentemeyer et al., 2015). Tissue collection has also ventured into environmental monitoring through the collection of samples that have been impacted by pollution. For example, the collection of sycamore leaves with tar spots, or a fungal infection that results from poor air quality, has helped researchers further identify areas of increased air pollution across England (Gosling, Ashmore, Sparks, & Bell, 2016). These examples elucidate the potential of volunteer tissue collection for non-genetic research.

Citizen Scientists and Ecotoxicology

The involvement of citizen scientists in the monitoring of pollution is not new to the literature, as volunteers have routinely been recruited to monitor for trash and litter, often in marine environments (Nelms et al., 2017). More recently, volunteers have been contributing valuable data to monitoring efforts that focus on small scale pollution events such as spills or air polluting incidents (Ottinger, 2010; Bera & Hrybyk, 2013; Hyder et al., 2017). The contributions by volunteers have begun to fill data gaps regarding small scale polluting occurrences, which are commonly underreported compared to larger accidents (Hyder et al., 2017). Citizen science observational and monitoring data of pollution events has proven valuable to researchers, but there is room for volunteer participation to grow in pollution monitoring, particularly when biologic sampling is involved.

There are several reasons why citizen science does not have a huge presence in the field of ecotoxicology. From the scientific knowledge gaps about polluting chemicals on the part of participants (Ottinger, 2010) to the inherent diversity and chemical complexities of pollutants that exist in the environment (Gundert-Remy et al., 2015), ecotoxicology is not the most accessible field for citizen scientists. Ecotoxicology refers to the study of fate, transport, and ultimate effect that a toxic compound or pollutant has in an ecosystem (Moriarity, 1985). Pollutants are diverse in chemical properties, which influences their fate and transport within an ecosystem (Kaviraj et al., 2014). This chemical diversity also influences how they can vary in toxicity, from innocuous in some organisms to deadly in others (Kaviraj et al., 2014). Scientific literacy is a barrier for participation in citizen science campaigns (Evans et al., 2005; Pandya, 2012), so it makes sense that a field as science-heavy and complicated as toxicology has yet to break into the citizen science world.

There are other important barriers of entry that limit volunteer participation in ecotoxicological monitoring of pollutants, such as the timing of a pollution event. As demonstrated by researchers, polluting events can display varying patterns of exposure and timing depending on a variety of external factors such as spring rains and herbicide application timing, as in the case of atrazine (Ali & Kolok, 2015). While sampling during a pollution event has been demonstrated by volunteer "bucket brigades" who collect air samples during periods of poor air quality in fence line industrial communities (Ottinger, 2010), it is more challenging to deploy citizen scientists to measure pulses of degraded water quality.

Other than timing concerns, it is necessary for the chemicals of interest to sequester in the tissues of the organism if citizen science collection for biomonitoring is to be useful. Many contaminants are persistent and sequester in the tissues of an organism, such as metals or persistent organic pollutants (Murdock, 2005). Other chemicals, such as certain pesticides, steroids, or volatile organic compounds are less suited for volunteer biomonitoring because they don't linger in the target organism. These chemicals would be nearly impossible to measure if collected by volunteers because they are often quickly metabolized and excreted (Murdock, 2005) on a time frame that would be too short for citizen scientists to capture.

The collection of biological materials to serve as indicators for environmental pollution by volunteers is promising but comes with a fair amount of challenges that need to be identified and avoided prior to collection efforts. Tissue identification is one such challenge. Identifying a target tissue or organism may exclude members of the public who are not qualified or experienced enough to collect samples. For example, the collection of cougar tissue for DNA analysis (Beausoleil et al., 2016) is a very different and much riskier collection regime than sampling for crayfish (Larson & Olden, 2016; Larson et al., 2017). Easily accomplished field work and sampling methodologies are necessity for the success of a tissue monitoring program using volunteers (Pritz & Nelson, 2017).

Addressing Barriers to Entry

Metal contamination of aquatic ecosystems is a reasonable starting point for this idea because barriers of entry are less severe. Persistent contaminants, such as metals (DeForest, Brix, & Adams, 2007; Richards and Borgeois 2014) are suitable for volunteer biomonitoring because they lack some of the sampling complications posed by organic pollutants. Metals often bioconcentration in the tissues of organisms and can serve as a time integrated sampling system of that environment (Rainbow, 1995). Metals enter the environment through natural and anthropogenic sources and result in contamination that affects many different parts of the ecosystem, from the organisms themselves to the soil and water they live in (Duruibe et al., 2007; Ali and Kahn 2018). Aquatic ecosystems are of interest because their sediments can serve as metal sinks through a variety of geochemical and physical processes, (Suedel et al.,1994; Ouédraogo et al., 2015). Focusing on aquatic systems for metal contamination is also ideal because of the many citizen science programs already working with these ecosystems (Stepenuck & Genskow, 2018).

Invertebrates are an ideal sampling group to introduce to environmental monitoring and citizen science for a few choice reasons. First, invertebrates already exist in the citizen science literature (e.g Howard, Aschen, & Davis, 2010; Eagles-Smith et al., 2016; Vitone et al. 2016; Larson et al., 2017; Pritz & Nelson, 2017; Vendetti et al. 2018), indicating their interest among citizen science communities. Macroinvertebrates are abundant in water systems, easy to collect, and are commonly used by regulatory agencies to indicate water quality of a system, so historical data may also be accessible (Barbour et al., 1999). Aquatic invertebrates are ideal bioindicators and could serve as integrated samples of short-term environmental exposure (Barbour et al., 1999) because of their close interaction with contaminated sediments, their restricted mobility, and tendency to bioaccumulate metals (Barbour et al., 1999; Quinn et al., 2003; Stankovic et al. 2013; Parmar et al., 2016). Finally, invertebrate research and collection does not come with the same ethical treatment considerations that accompany other vertebrate organisms, allowing for the involvement of volunteer scientists.

While the use of invertebrates in biomonitoring of metal has potential, there are several methodological concerns that researchers need to address prior to volunteer participation. Research permits, safety measures for participants, and sample collection consistency are some examples of variables that need to be identified and resolved prior to project initiation. Mercury sampling, for example, is prone to contamination through dust or particles, rain, or human skin or hair contact (USGS, 2017; Nelson et al., 2018). Moreover, shipping and storage protocols of samples to be analyzed for mercury require strict cooler/freezer packing (USGS, 2017; Nelson et al., 2018). The consistency of method details such as these are crucial for the data collected by citizen scientists to be scientifically sound.

The dragonfly mercury project is an example of a citizen science project that collected invertebrate tissue for pollutant monitoring. A team from the University of Maine (UM) and the National Parks Service (NPS) (Eagles-Smith et al., 2016; Pritz & Nelson, 2017) asked volunteers visiting national parks to collect dragonfly nymphs which can serve as biosentinels, or proxies, for mercury in the environment. In this project leaders or NPS staff support volunteers through the collection process and ensure samples are consistently collected, stored, and shipped for analysis. They developed a very detailed and thorough sampling guide that addresses many of the concerns discussed previously (see: Nelson et al., 2018). This project simultaneously offered participants an opportunity to learn about biodiversity discovery in the national parks. The research team ultimately wanted to further the understanding of how biotic and abiotic mercury interact in aquatic and terrestrial environments. Volunteers engaged in nymph collection and learned from park staff about biodiversity. This innovative project demonstrates the potential to successfully involve citizen scientists in easily achievable fieldwork, while simultaneously gathering important data about pollutants in the environment (Pritz & Nelson, 2017).

Conclusions

The collection of biological materials has potential to diversify the type of data and environmental research that volunteers can contribute to. Citizen scientist collected data already exists in the peer reviewed literature (Theobald et al., 2015) and there is potential for further environmental monitoring research and publications (Stepenuck & Green, 2015). It has contributed to analytically sensitive genetics research (e.g. Biggs et al., 2015; Wilson et al., 2015; Beausoleil et al., 2016; Miralles et al., 2016; Larson et al., 2017; Mori et al., 2017; Larson et al., 2020), and has the potential to supplement the environmental monitoring of metal contamination, as the UM and NPS groups have accomplished (Eagles-Smith et al., 2016; Pritz & Nelson, 2017).

In addition to collecting important bioindicator data for metals research, volunteers can be exposed to new types of citizen science projects, which could ultimately be beneficial for volunteer acquisition and retention. While opportunities for environmental monitoring through tissue collection are exciting and diverse, program design, target organism and pollutant, and other logistical planning must be carefully determined to make the most of the volunteer's efforts. As demonstrated by the UM and NPS teams in the implementation of their mercury and dragonfly project, this goal is achievable for both researchers and volunteers when programs are thoughtfully designed.

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Appendix A

Table A.1: Sample verification data for testing events. ASL= analytical sciences laboratory

Testing Event	Event Date	Expected concentration (mg/L)	Actual Concentration (mg/L)	Verified By
ORED Staff	2.26.19	2	1.9	ASL
		15	15	ASL
Idaho Commons	3.22.19	2	1.8	ASL
		15	16	ASL
ORED Open House	4.4.19	2	1.6	ASL
		15	15	ASL
Spokane River Forum	4.16.19	2	2	ASL
		15	15	ASL
Columbia HS	5.28.19	2	1.9	ASL
		15	16	ASL
Palouse Basin Aquifer Mtg	10.10.19	2	1.99	AQ400
		15	15.7	AQ400
Ourgem Symposium	11.6.19	2	2.0	AQ400
		15	15.82	AQ400
Idaho Water Institute Symposium	11.12.19	2	2.0	AQ400
		15	15.82	AQ400
Idaho Commons	12.6.19	2	2.18	AQ400
		15	15.2	AQ400
Idaho Water Quality Workshop	2.11.20	2	2.13	AQ400
		15	15.51	AQ400

Table A.2: Sample verification data for continuous testing events. ASL= analytical sciences laboratory.

Sample ID	Expected Concentration NO3 (mg/L)	Event 1: ASL Verification (6.22.19) (mg/L)	Event 2: AQ400 Verification (1.14.20) (mg/L)
A	1	0.97	1.00
B	3	3.0	3.01
C	5	4.9	5.00
D	7	7.0	7.02
E	9	8.9	8.40
F	11	11	11.49
G	13	13	13.42
H	15	15	15.61
I	17	17	17.78
J	19	18	19.61
K	21	20	22.23
L	23	22	24.67
M	25	24	26.13
N	27	26	28.46
O	29	28	29.60
P	31	30	30.99
Q	33	32	35.60
R	35	34	35.96
S	37	36	36.90
T	39	37	39.27
U	41	39	39.33
V	43	41	43.23
W	45	43	49.60
X	47	45	47.94
Y	49	47	49.80

Table A.3: Precision testing for Deltares App

Run	1 ppm	2 ppm	5 ppm	10 ppm	20 ppm	50 ppm
1	1	2	4	10	17	>50
2	1	2	4	8	30	>50
3	1	2	4	14	32	>50
4	1	2	4	11	32	>50
5	1	2	5	9	31	>50
6	1	2	4	10	29	>50
7	1	2	4	10	31	>50
AVERAGE	1	2	4.14	10.29	28.86	>50
STDEV	0	0	0.38	1.89	5.34	-

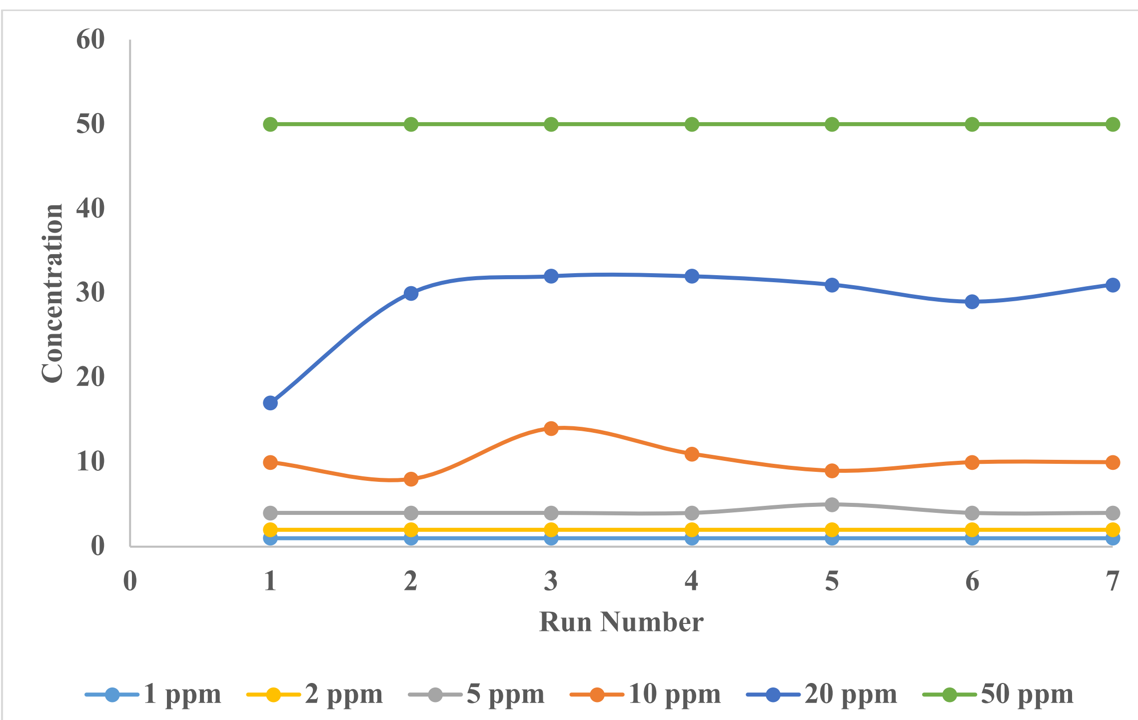

Figure A.1: Precision testing for Deltares app

Table A.4: Concentration change over time as measured by the Deltares App

Time (Sec)	1 ppm	2 ppm	5 ppm	10 ppm	20 ppm	50 ppm
10	0	0	1	4	8	34
20	1	2	3	8	15	46
30	2	3	4	8	32	>50
40	2	4	6	9	34	>50
50	3	3	6	9	37	>50
60	3	4	5	13	37	>50

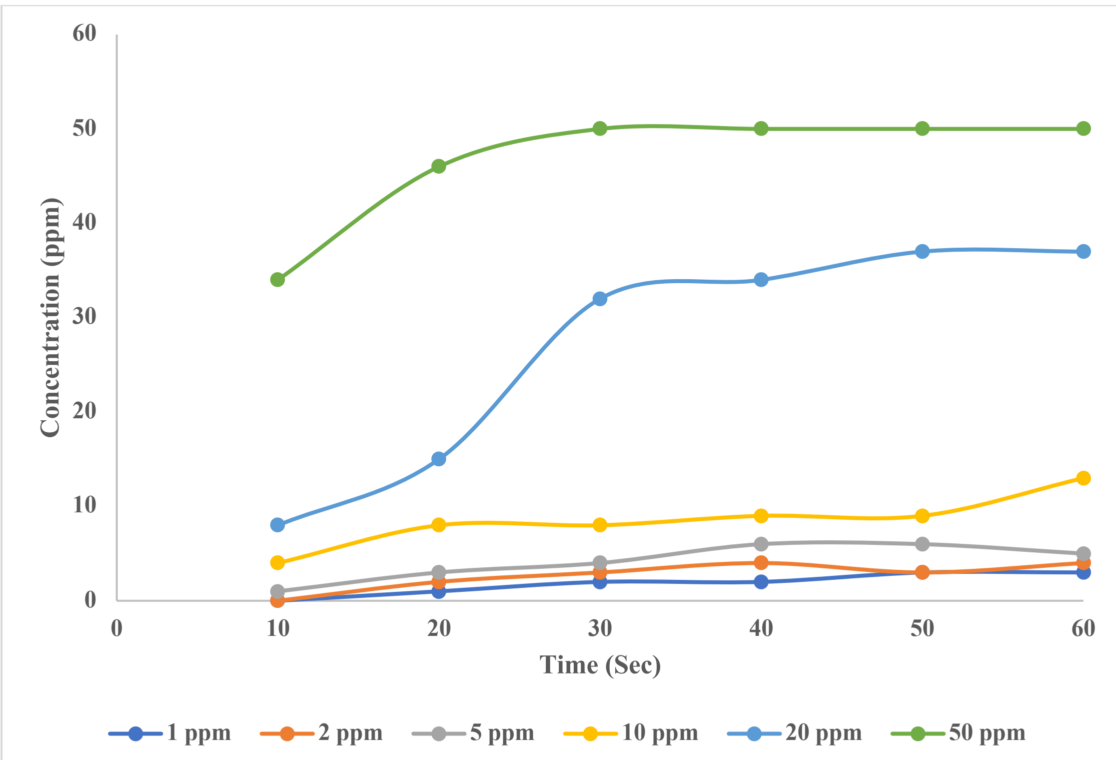

Figure A.2: Concentration change over time as measured by the Deltares app