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Investigating Scientific Self-Concept in the General Chemistry Laboratory While Performing Course-Based Undergraduate Research Throughout a Pandemic

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INVESTIGATING SCIENTIFIC SELF-CONCEPT IN THE GENERAL CHEMISTRY LABORATORY WHILE PERFORMING COURSE-BASED UNDERGRADUATE RESEARCH THROUGHOUT A PANDEMIC

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INVESTIGATING SCIENTIFIC SELF-CONCEPT IN THE GENERAL CHEMISTRY LABORATORY WHILE PERFORMING A COURSE-BASED UNDERGRADUATE RESEARCH EXPERIENCE THROUGHOUT A PANDEMIC

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ABSTRACT

Climate change, waste management crises, and global pandemics emphasize the need for a thriving science, technology, engineering, and mathematics (STEM) workforce, and one of the jobs of STEM educators is to provide meaningful educational experiences that engage STEM students in scientific practices, broaden their worldview, and prepare them for a future career in the scientific field. Undergraduate research experiences have been shown to benefit STEM students, but few students can participate, especially at early stages in their undergraduate studies. To broaden participation in undergraduate research, an honors general chemistry laboratory course was converted into a course-based undergraduate research experience (CURE) in environmental chemistry. Specifically, in collaboration with the Cahaba Riverkeeper, a method for microplastics detection in natural water was incorporated into the laboratory curriculum. Microplastics are a ubiquitous environmental presence, and methods for their quantification in the environment are not standardized, making this topic timely and relevant for an environmental chemistry CURE. The timing of this work allowed for relevant data on the career intentions of STEM students throughout the COVID-19 pandemic to be collected. In addition, this work outlines the CURE

curriculum, including the optimization of a procedure to estimate microplastic pollution in standing water samples with Nile Red as a fluorescent tag, and the outcomes of its implementation, including microplastics estimates at various locations along the Cahaba River from 2020-2022, a summary of student projects from over four years, and this CURE's impact on our student's science identity, STEM persistence, and self-efficacy. Method optimization demonstrated that Nile Red is an appropriate microplastics detection method for use in general chemistry laboratories, Trends from two iterations of the CURE are consistent in showing that students who participated in this research experience saw greater gains in self-efficacy and science identity than did students in the traditional general chemistry laboratory. This CURE has the potential to be scaled up to a CURE network, allowing researchers at various institutions to estimate microplastic pollution in watersheds across the country and compare results under a common method.

Keywords: Course-based Undergraduate Research Experiences, Science Identity, COVID-19, Microplastics

DEDICATION

For my grandparents,

Margie and Max Franks (mimi and poppy),

For always supporting me in my pursuits, whether musically or academically, and always reminding me to see the funny side of life. I miss you both.

Ann and Gus Forakis (giagia and pappoús),

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LIST OF ABBREVIATIONS

COVID-19 – The novel coronavirus first identified in 2019

CURE – course-based undergraduate research experience

DBER – discipline-based educational research

EB – environmental behaviors

EFA – exploratory factor analysis

EI – environmental identity

FTIR – Fourier transfer infrared spectroscopy

GCLS – general chemistry laboratory sequence

HONLS – honors laboratory sequence

NR-B – The novel microplastics dying method using Nile Red and a water bath.

NR-S – The standard method for dying microplastics with Nile Red

SCCT – Social Cognitive Career Theory

SI – science identity

SIP – science identity prominence

SSE – science self-efficacy

STEM – science, technology, engineering, and mathematics

Py-GC/MS – pyrolysis gas chromatography coupled to mass spectroscopy

URM – under-represented minority

CHAPTER 1

INTRODUCTION

Climate change, pandemics, decreasing biodiversity, global hunger, and depleting fossil fuels are issues of a planetary scale that call for the efforts of a thriving science, technology, engineering, and mathematics (STEM) workforce. The growing need for STEM professionals is well understood. In 2010, President Obama allocated 1 billion dollars of funding as a part of his "Educate to Innovate" campaign with the goal of "preparing 100,000 new and effective STEM teachers over the next decade" and "broadening participation in STEM disciplines[1]." Despite this initiative, attrition rates for STEM students in undergraduate institutions remain startlingly high, and educational studies continually report dwindling interest in STEM[2-5]. In many institutions, STEM education consists of didactic teaching styles which have been proven ineffective compared to active learning strategies[6], and laboratory activities are mostly provide step-by-step instructions, neglecting to engage students in higher levels of cognition or scientific inquiry[7-9]. These instructional approaches allow students to be passive participants and lead some students to view their STEM courses as simply a requirement to complete rather than an interesting foundation for a career[5]. Therefore, STEM education must be improved through the inclusion of innovative, evidence-based practices to better support the emerging STEM workforce.

Efforts towards improving STEM education reach back as far as the early 1920s (the Journal of Chemical Education was founded in 1924) and spiked in the 1950s and 1960s as the United States strove to increase the number of scientists and engineers in its workforce during the space race[10]. In the 1980s and 1990s, discipline-based educational research (DBER) emerged as a formal effort from STEM educators and professionals to investigate how people learn the concepts of STEM, how people develop expertise in STEM disciplines, and to broaden participation in STEM[10]. These research areas have yielded several innovative, evidence-based STEM teaching methods that have gained prominence in the past several decades and focus on active learning strategies and scientific inquiry.

At least three methods of instruction have emerged over the past twenty-five years: Process Oriented Guided Inquiry Learning (POGIL), peer-to-peer instruction, and course-based research. POGIL focuses on applying scientific inquiry to lead students to key concepts while also honing their process skills, such as collaborative learning and critical thinking[11] (see POGIL.org). Peer-to-peer instruction has the student learn through teaching in a process of answering questions, discussing their answers with their peers, and answering again[12]. Finally, course-based undergraduate research experiences (CUREs) have gained prominence in laboratory courses for allowing students to perform novel experiments in the classroom and contribute new knowledge to the instructor's field[13, 14]. Despite the prominence of these teaching methods in DBER spaces, adoption of these strategies in the undergraduate classroom is slow and the issue of STEM attrition remains.

I. Identifying Causes of STEM Attrition

A STEM student's freshman year is crucial for determining whether they will persist to degree completion, especially for under-represented minorities (URMs)[15, 16]. Some have reported as many as 50% of undergraduate STEM students change their major during the first year[4]. Students with a wide range of STEM-related career interests, from biological sciences (neurology, environmental science, genetics), physical sciences (chemistry and physics), to pre-professionals (medicine, optometry, dentistry) participate in a general chemistry laboratory sequence during their first year of undergraduate studies. The general chemistry laboratory is a prime candidate for both fostering interest in STEM and developing process skills[16], thus, the role of the general chemistry laboratory on STEM persistence and attrition must be examined.

II. The Potential of the General Chemistry Laboratory

The goals of the general chemistry laboratory are a topic of considerable discussion and its execution varies widely, even within the same institution[17-20]. Reforms in the general chemistry laboratory have focused on moving from the "cook-book" model, where students follow a detailed procedure to perform a pre-determined experiment, towards an inquiry-based model, where students ask scientific questions, design procedures, and use original data to answer their questions[21-23]. The inquiry-based model places its emphasis on student autonomy, allowing them to make decisions and respond to failure in the same manner as STEM professionals.

Inquiry learning is broadly defined, but guidelines have been devised by the National Research Council (NRC)[24] based on the work of Schwab[25] which was adapted by Herron[26]. At its most supervised, the method is considered structured inquiry, where students perform an inquiry activity whose research question, step-by-step procedure, and conclusions are predetermined by the instructor. This method has been compared most to the traditional cookbook laboratory model. Moving towards more student autonomy is the guided inquiry process. In this model, predetermined research questions are provided to students by the instructor, but determining procedures and drawing conclusions are student driven. In this model, the results are unknown to the students, but the instructor has a good idea of what to expect. Finally, open inquiry places all decision-making power in the students' hands. The instructor only provides the background knowledge students need to devise an original research question in a particular area, determine the appropriate process, and draw conclusions from data they collected themselves. This form of inquiry reaches the highest levels of cognitive processing and is the most like the authentic research environment[27].

To reach higher levels of inquiry learning in the classroom, laboratory courses in a variety of STEM disciplines have been adapted into CUREs[28-31], allowing students to apply authentic scientific inquiry in a typically upper-division (third-year or fourth-year) classroom environment. Some introductory level CUREs have been developed in biology[32, 33], but chemistry CUREs in the literature are rarely, if at all, designed for the first-year, general chemistry student. Thus, there are opportunities for chemists to design and develop CUREs at the freshman level with the goal of positively affecting graduation rates for STEM students[13, 33, 34].

III. Social Psychology in STEM Education Research

Many efforts have been allocated towards understanding the factors that contribute to a student's decision to persist in STEM. Social psychological constructs, like attitude, science identity, self-efficacy, and outcome expectations, have been used to study the student's persistence in STEM, but science identity has been demonstrated to be most correlated with STEM persistence outcomes[35]. Science identity is a subset of an individual's identity scheme. The entire scheme of an individual's identity includes a set of meanings that define a person. Those meanings are then defined by the behavior of an individual that holds a certain identity[36]. Some STEM education studies define science identity as the extent to which an individual conceptualizes themselves as a good science student[37]. Additionally, individuals with a science identity might see themselves as "scientists" or as belonging to a scientific community. Some theories posit that one's identity works alongside other factors, such as self-efficacy, when students make career choices[35]. In a study by White et al. investigating the relationships of racial identity, science identity, science-self efficacy, and science achievement, they demonstrated that science achievement was significantly explained by science identity with science self-efficacy acting as a mediating factor[38].

While the role of the science identity as it relates to the pursuit of a STEM career has been examined, less attention has been paid to investigating the relationship between educational practices and the development of science identity. Science identity measures, like the Persistence in the Sciences (PITS) survey[39], are typically used to determine the effectiveness of educational interventions, but the relationship between practice and identity has not been studied directly. Because of the demonstrated role that science identity plays in an individual's decision to pursue a STEM career, STEM educators must understand which educational practices contribute to or deter the development of science identity.

IV. Authentic Science within CUREs

A CURE is an authentic instructional research experience that engages students in the entire scientific method, but the full potential of a CURE involves incorporating socially relevant topics that encourage both engagement with experimental design and connections to places, events, people, or objects that students are likely to encounter. As a result of widespread plastic use and unregulated waste management, microplastic pollution has become one such emerging, relevant research topic[40-43].

Connecting chemistry to the environment through the exploration of microplastics provides opportunities for students to generate personal research questions while providing instruction about chemical structure, decomposition reactions, analytical techniques, and sampling. Microplastics were first described in the environment in 2010, and have since been discovered in the marine environment, mountaintops, and inside of organisms[43-45]. Their role in the environment is not well understood and research methods to detect and quantify microplastics are not standardized[46, 47]. The most common method is visual detection with digital microscopy, but this method is unreliable[47, 48]. Other, more robust spectroscopic methods, like FTIR spectroscopy and Raman Spectroscopy, are more accurate but require time, expensive equipment, and highly specialized professionals. Thus, a fast, cost-effective, method for microplastic detection and quantification is yet to be found and is, therefore, a prime candidate for inclusion in a general chemistry CURE.

In collaboration with the Cahaba Riverkeeper, an honors general chemistry laboratory sequence was adapted into a CURE with a general theme of microplastics in the Cahaba River. The theme allowed students to explore the detection and quantification of microplastics in a local waterway and informed them of the different types of commercial polymers that they frequently encounter. The purpose of this experience was to further incorporate the scientific process and inquiry learning into the general chemistry laboratory experience. Additionally, the nature of this research allowed for an authentic field experience to be included in the form of a water sampling trip each semester with the Cahaba Riverkeeper. The intention of this work was to investigate the extent to which this project impacted various factors related to students' self-concept as a scientist.

V. An Overview of the Publications in this Work

The original intention of this study was to develop a novel research-focused laboratory curriculum including a field experience in general chemistry and to assess its impact on our student's scientific self-concept and behaviors towards the environment. The curriculum for this laboratory experience was designed in the first half of the year 2019, and the first iteration of the project began in the fall of 2019. Part of the way through the spring semester, when students were completing their original research projects, instruction at our institution was shifted to a remote format due to the novel coronavirus discovered in 2019 (COVID-19). Data collection for the previously described project had already begun prior to the Fall 2019 semester. The constructs we were measuring are relevant to the disruption in a similar manner to the use of the CURE, so we shifted the focus from the impact of the CURE to the impact of the disruption. This shift allowed us to use the pre-intervention survey data already collected as a baseline to compare with student responses after shifting to remote instruction.

Chapter 3 of this work includes two publications, the first published in 2020 and compares self-efficacy and science identity data of general chemistry laboratory students from before and after the onset of the pandemic (around March of 2020 for the USA). The second, published in 2022, compares similar data for three different incoming freshman cohorts enrolled in a general chemistry laboratory who experienced different levels of pandemic-related disruptions to their university instruction, the fall 2019 cohort (pre-pandemic), the fall 2020 cohort (early-pandemic), and the fall 2021 cohort (mid-pandemic). We felt this shift was crucial, as the timing of our original study allowed a rare opportunity to publish data from before and after the COVID-19 outbreak on important STEM persistence factors.

The fourth chapter of this work includes the results of the original study. It contains the CURE curriculum and an assessment of the outcomes of participating in the CURE, including the impact on student measures of self-efficacy, science identity, environmental behaviors, and knowledge of the relationship of chemistry and the environment.

The fifth chapter includes how a microplastics screening method was adapted and improved upon for use in the general chemistry laboratory. Included is the novel adaptation of a procedure that uses Nile Red to stain microplastics and the results from using this procedure on various sites along a significant central Alabama watershed, the Cahaba River.

CHAPTER 2

DEVELOPMENT AND VALIDATION OF A QUESTIONNAIRE TO INVESTIGATE STUDENT SELF-CONCEPT IN THE GENERAL CHEMISTRY LABORATORY

I. Theoretical Constructs used to Assess the CURE

The intention of our microplastics CURE was to provide an authentic experience to first-year students that promotes their view of themselves as scientists, increases the likelihood that they will complete a STEM degree, and helps them understand their role in the plastic pollution problem. Similar outcomes are sometimes measured using attitudinal surveys, but these surveys are poor predictors of behavior[1, 2]. Better measures of these outcomes employ a combination of survey items built around Identity Theory and Social Cognitive Career Theory (SCCT). To assess the outcomes of the microplastics CURE, a questionnaire was developed that would measure some desired outcomes of participating in this experience, such as persisting in a STEM degree and engaging in positive environmental behaviors. This questionnaire contained some original items in addition to items taken from other works. This section will summarize the constructs we intended to measure and the items that correspond to each construct.

a. Identity Theory

Identity theory as defined by Stets and Burke, was established to understand individual behaviors as they relate to the multiple groups and social categories that define the individual[3]. Identity theory seeks to explain behaviors through the relationship of an individual's societal roles, as individuals typically participate in a multitude of relationships and therefore contain multiple identities that influence how individuals behave in social settings[4].

Identity is defined as the set of meanings that defines who a person is. For example, someone who identifies as "a mother" or "motherly" may see mothers as nurturing or caring. As a result, they may behave in ways that communicate to themselves and to others that they are nurturing and caring to adhere to the "mother" identity[3]. Because an individual holds multiple identities in various societal roles, certain identities are context specific. One who identifies as "motherly" may perform behaviors to reinforce the identity in their home environment and may not do the same in a work environment where their prominent role is as "manager," "employee," or "colleague."

Identity formation in an individual begins early in life and peaks at adolescence as one's ability to conceptualize abstract concepts presents the need for one to find meaning within themselves[5, 6]. Though the formation of identities happens early, other individual processes continue to develop. As a result, identities are not stagnant. Throughout adolescence, an individual will undergo cycles of identity exploration and identity commitment, and these processes continue throughout one's life[7]. Thus, identity formation is a d ynamic process influenced by affective arousal and societal interactions[8].

Identity is commonly considered when seeking to predict behaviors and presents a promising avenue in assessing the effectiveness of environmental education in chemistry classes. Specifically, we are interested in examining the connectedness of one's identity as "scientist" (science identity) and their identity in relation to the natural environment (environmental identity).

b. Science Identity

Science Identity is defined as an individual's professional self-identification as a scientist and the pursuit of behaviors that reinforce this self-identity[9]. Science identity is associated with a student's interest in science and persistence into a science career. Science identity has predicted involvement in a science career or field after college, and influences intentions regarding research careers[9, 10]. In a survey of high school students, Chapman demonstrated that authentic science in the classroom can influence the development of one's scientific identity[11].

One's undergraduate study occurs in their formative years, where various identities are being formed and altered. Students may feel pressure to define their identity in an academic setting while defining identities in a social setting through making friends and joining extracurricular groups. Many students who enter college with aspirations to pursue a degree in STEM, especially under-represented minorities (URMs), change their majors during this time. In STEM education research, science identity is often considered as a significant predictor of academic and career outcomes in undergraduate students[12]. Someone with a strong or prominent science identity is expected to persist in a STEM degree and into a career in their field and is an extremely important factor to consider when developing educational interventions for STEM students.

c. Science Identity Prominence

The theory of identity prominence was developed to understand the interrelation of the multiple identities within an individual and is a stronger predictor of behavior[13, 14]. Identity prominence describes the extent of the importance of certain identities compared to others held by an individual[15]. The higher the prominence of an identity, the more central it is to the individual's self-concept, and therefore influences their behavior more strongly[16]. We sought to understand the role of participation in a CURE on persistence in STEM, thus we also consider the prominence of science identity when seeking to predict academic and career choices.

d. Environmental Identity

Additionally, environmental identity has been considered when seeking to predict pro-environmental behaviors. Stets (2003) defines environmental identity as the meanings one attributes to themselves as they relate to the natural environment[75]. It is a measure of one's perception of their ability to live in harmony with the environment. Learning experiences affect development and dedication to an environmental identity[17], and environmental identity is a predictor of environmental behaviors[18, 19].

e. Environmental Behaviors

We sought to measure participants' likelihood to engage in environmentally friendly behaviors. Biga organized environmental behaviors into three categories: environmental activism, environmental non-activism, and private sphere environmentalism[20]. Environmental activism involves behaviors that intentionally support organized environmental groups, such as protests, events, boycotts, and consumption of environmental media. Environmental non-activism represents the willingness to participate in passive behaviors that benefit the environment. These include paying higher taxes, supporting legislation that helps the environment, and supporting environmental regulation. Private-sphere environmentalism refers to environmentally friendly personal choices. These behaviors include purchasing fuel efficient vehicles, recycling, and choosing to walk or bike over driving. The intervention in this work is heavily involved with the issue of plastic pollution, so items were adapted that focused on behaviors involved with the plastic pollution issue to develop an environmental behaviors scale.

f. Social Cognitive Career Theory (SCCT)

SCCT is a useful model for explaining the factors that affect academic and career outcomes in undergraduate students. SCCT seeks to explain the interrelated aspects of career and academic choices, such as how career and academic interests develop, how career and academic choices are made, and how career and academic goals are achieved[21]. This theory stems from Social Cognitive theory which seeks to explain various cognitive and self-regulatory processes[22]. SCCT builds off this theory and highlights social cognitive processes that are relevant to career development: self-efficacy, outcome expectations, and goal representations[21].

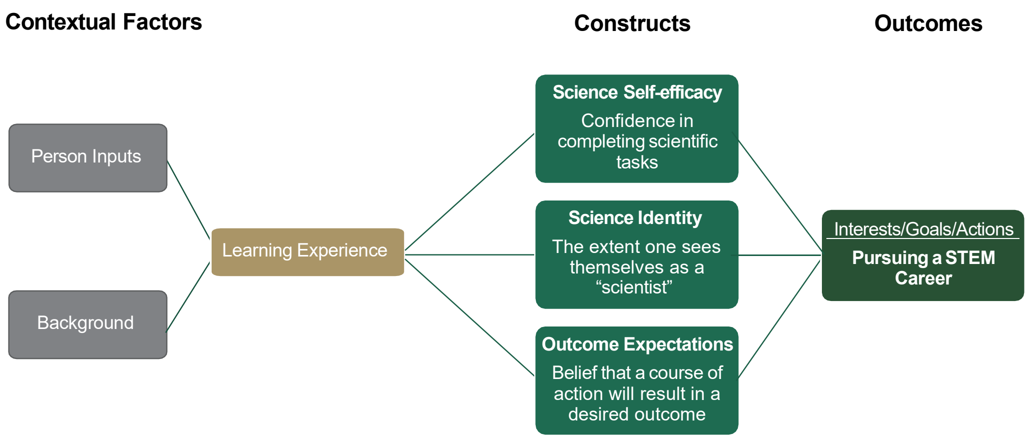


Figure 1. A condensed SCCT theoretical model with science identity. Lines show relationships between various social cognitive constructs, learning experiences, and one's interests, goals, and actions.

i. *Self-efficacy.* Self-efficacy represents a set of beliefs held by an individual that reflects their confidence in their ability to perform certain tasks[22]. Social cognitive theory states that self-efficacy is a crucial passive mechanism in one's decision making and is an operating factor on how one proceeds when faced with obstacles. SCCT has identified that some sources of self-efficacy stem from learning experiences and are specifically related to one's perceived accomplishments and emotional arousal[23].

ii. *Outcome expectations.* In SCCT, outcome expectations refer to one's perceived consequences of taking on a course of action[21]. Outcome expectations are concerned with an individual's answer to the question, "what will happen if I do this?" Outcome expectations work together with self-efficacy to determine decision making paths, with self-efficacy playing a stronger moderating role. Even if an individual perceives a positive outcome for taking a course of action, they may not take this on if they do not believe they are able to do it.

iii. *Goal Representations.* Goal setting is a significant aspect of career counselling methods. Social cognitive theory states that goals are a way an individual organizes their behavior and symbolizes the desired outcomes of their behavior[21, 22]. Bandura (1986) defines a goal as intention to act out a particular behavior or to achieve a particular outcome.

g. SCCT and STEM Education

STEM education researchers have used SCCT to predict career and academic behaviors. Specifically, SCCT has been used to predict choice of STEM major and interest in STEM careers[24]. One of the central tenants of SCCT is that self-efficacy is a mediating construct between the learning experience and an individual's interests, goals, and actions. While self-efficacy, outcome expectations, and goal representations are central tenants to SCCT, self-efficacy is the dominant factor in SCCT to predict career intentions in undergraduate students[25].

SCCT is also used to understand underrepresentation of women and minority groups in STEM fields. Betz and Hackett examined self-efficacy and career intentions in relation to one's identity as male or female. They showed that women were more likely to have high self-efficacy to in pursuing a traditionally female occupation versus a traditionally male occupation[26]. Undergraduate research experience programs tailored towards women and minorities have been developed using SCCT as a framework and have been shown to increase matriculation into entry level STEM occupations as well as participation in graduate research[27]. SCCT is reliable across gender, age groups, socioeconomic status, and age for predicting career behaviors[28].

h. Science Self-efficacy

Self-efficacy plays a broad role in the SCCT framework, but for use in a STEM education intervention, a more specific form of self-efficacy is considered, science self-efficacy. Science self-efficacy is defined as one's confidence in their ability to perform scientific tasks. Furthermore, science self-efficacy can be multidimensional with the two relevant domains to this project being chemistry self-efficacy and research self-efficacy.

Uzuntiryaki defines chemistry self-efficacy in three categories. This includes self-efficacy in cognitive ability (e.g. confidence in describing the structure of an atom, explaining chemical theories), psychomotor skills (e.g. confidence in setting up an apparatus in the lab, gathering data), and in everyday applications (e.g. confidence in identifying chemistry related careers, understanding news articles related to chemistry)[29].

Research self-efficacy is one's confidence in their ability to perform research related tasks (e.g. presenting a talk or poster, analyzing data, conducting an experiment)[23]. Research self-efficacy is included as a sub-category of science self-efficacy because performing research related tasks is a central tenant of completing a degree with a science major and is a major component of many STEM careers.

i. Chemistry and the Environment

This study also sought to identify underlying factors involved with students' perception of chemistry. One's perception of chemists and the chemical industry is a potential contributing factor to one's beliefs about chemistry and desire to pursue a science related career is. The chemical industry has been involved with some of the more well-known environmental disasters of our time. A student's awareness of the chemical industry's role in these disasters can influence the academic choices of students, especially students with a greater environmental identity.

Additionally, a potential direct effect of educating about the environment in chemistry education is that students will have a knowledge of the ways that chemistry and the environment are connected. A student who has knowledge of the relationship between chemistry and the environment might be able to notice chemical principles at work in their everyday life or recognize the ways chemistry relates to environmental issues. The framework used in this dissertation includes knowledge of the role of chemistry in the environment as a possible factor in the formation of scientific and environmental identities.

The remainder of this chapter will discuss three phases of development and validation: 1) A formative assessment (focus group) of General Chemistry 2 students, 2) selection of survey items and the development of original items, and 3) an initial administration of the survey for validation.

II. Formative Assessment to Frame the Research Questions

The intended participants for the CURE intervention were freshmen STEM majors at a large, research-focused university in the southeastern United States. It was important to understand students' perceptions of the term "chemistry" and "the environment" in a qualitative setting so that more meaningful quantitative data could be collected throughout the project.

a. Methods

Three focus groups were conducted in April of 2019 with the goal of understanding general chemistry laboratory student's perception of chemistry, the natural environment, and how the natural environment relates to chemistry. All students that participated in the focus groups were enrolled in a General Chemistry II Laboratory course at the time and represented a diverse group of STEM and some non-STEM majors. During a scheduled laboratory section, the study was explained to potential participants who were able to voluntarily opt into the focus group. No course-based incentive or otherwise was offered to participants. In total, 23 students opted into one of the three focus groups. Table 1 breaks down the demographics of the participants in the focus groups.

Table 1. Demographics of Focus Group Participants

Characteristic	**No. (%) (N = 23)**
Gender Identity	
Male	8 (34.8)
Female	15 (65.2)
Age	
18	7 (30.4)
19	13 (56.5)
20+	3 (13.04)
Academic Class	
freshman	16 (69.6)
sophomore	7 (30.4)
Major	
chemistry/biochemistry	1 (4.3)
healthcare mgmt.	1 (4.3)
biomedical science	5 (21.7)
neuroscience	5 (21.7)
public health	1 (4.3)
immunology	1 (4.3)
psychology	1 (4.3)
biology	4 (17.4)
philosophy	1 (4.3)
finance	1 (4.3)

Participants were asked a series of questions in a semi-structured format. Questions were established to guide the conversation and follow up questions were asked at the discretion of the interviewer. The questions were designed to answer the following three research questions established to guide the analysis of the focus group results:

R1: What do students think of when they hear “chemistry?”

- What terms come to mind when you hear the word chemistry?

- Describe what you think a chemist does? What jobs or career opportunities do you think of?
- What problems is chemistry used to solve?
- How have your chemistry courses and labs affected how you view chemistry?

R2: What do students think of when they hear "the natural environment?"

- What comes to mind when you think of the natural environment?
- What things have you read about on the internet concerning the natural environment?
- How have your chemistry courses impacted how you feel about the natural environment?

R3: What connections, if any, do students see between chemistry and the natural environment?

For R1, coding categories were determined to bin student responses. The categories represent the facets of the world, society, or nature that relate to their view of chemistry. The first category, "Academia" represents a student response that mentions chemistry in the academic setting, including topics from their chemistry courses or characteristics of their professors/instructors. "Industry" refers to student responses that refer to product manufacturing or the chemical industry, such as oil, cosmetics, factories, or engineering. The category "Research" refers to student responses that touched on data, science, or contributing to generalizable knowledge. In addition, many students mentioned the laboratory environment, whether they referred to reagents, glassware, laboratory attire, or any other element of the physical laboratory space, thus a category was defined as “Laboratory.” The “Socio-scientific issues” category represents responses that mention social issues related to science, like climate change, renewable energy, etc. The “Medicine” category refers to responses that mentioned the health industry, the medical profession, pharmaceuticals, etc. Lastly, students who mentioned adjacent life sciences such as biology and ecology were given the category “life sciences.”

Responses relevant to Research Question 2 (R2) were coded to determine how students interpreted the term "natural environment" and the role chemistry plays in that setting. If participants mentioned resource management, scarcity, or renewable/non-renewable resources, the category “sustainability” was given. The category “ocean” was given to participant responses that mentioned issues impacting the marine environment, such as ocean acidification and oil spills. Responses were tagged with “Industry” when students mentioned the impact of the chemical industry on the environment. The “air pollution” tag refers to mentions of CO_2 emissions or smog. Finally, the tag “plastic” was given when students mentioned the impact of plastic products on the environment. Several environmental issues mentioned could reasonably fit into more than one of the above categories. For example, an oil spill has a great impact on the marine environment, but is a result of the chemical industry’s involvement, thus two codes would be given: “ocean” and “industry.” The same applies to responses relevant to R1.

b. Results and Discussion

Results of coding responses relevant to Research Question 1 (R1) show that participants associated chemistry with a variety of factors. The categories tagged the most were Research and Socio-scientific Issues by a small margin. It is not surprising that Research was one of the most common tags (18%), as many students immediately mentioned "science" or "conducting experiments" when asked what they think about chemistry. Socio-scientific issues (18%) was mentioned when students were asked about the problems that chemists solve, such as renewable energy, pollution, and erosion. Participants also mentioned the laboratory (14%) and the medical profession (14%), followed by academia (11%) and industry (11%). Finally, the environment (9%) and life sciences (5%) were mentioned, but the least frequently. Analysis of R1 results shows that students' perception of chemistry is diverse and reflective of the many ways that chemistry is observed in society. This presents challenges to developing a survey because the students may see chemistry through a particular lens. However, this diversity presents an opportunity because students likely do not see chemistry as a single career choice.

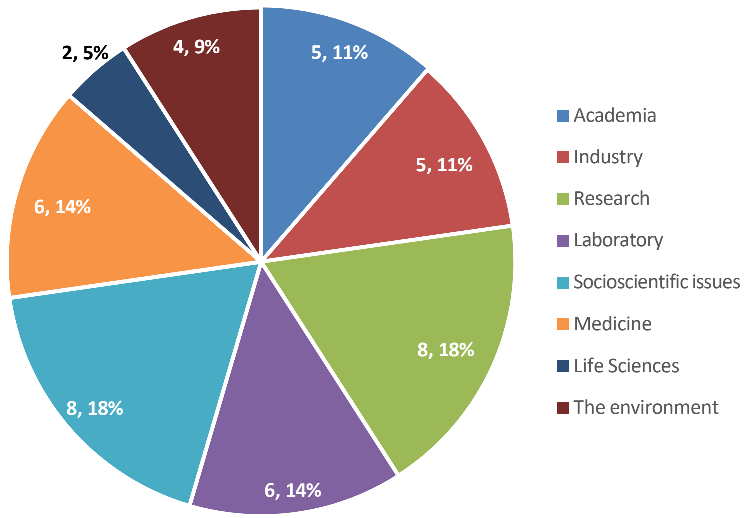


Figure 2. Focus group coding results for R1, “What do students think of when they hear ‘chemistry?’” The pie chart displays the count and percentage of each coding category.

For responses relevant to R1, a coding category was also defined to understand the affective nature of participants’ thoughts about chemistry. Statements regarding chemistry received one of three codes: positive, negative, or neutral. The positive tag refers to a statement that refers to chemistry as joyful, necessary, or useful, for example, if a student says that chemistry helped them understand acid rain. The negative tag was given when participants made a statement that implied negative feelings towards chemistry, such as referring to “deadly liquids.” Furthermore, categories were defined for participants who expounded on their reasoning behind affective statements toward chemistry. Codes for this category were determined qualitatively for each affective statement made. Some categories tagged were “complex,” “practical,” and “real-life applications.”

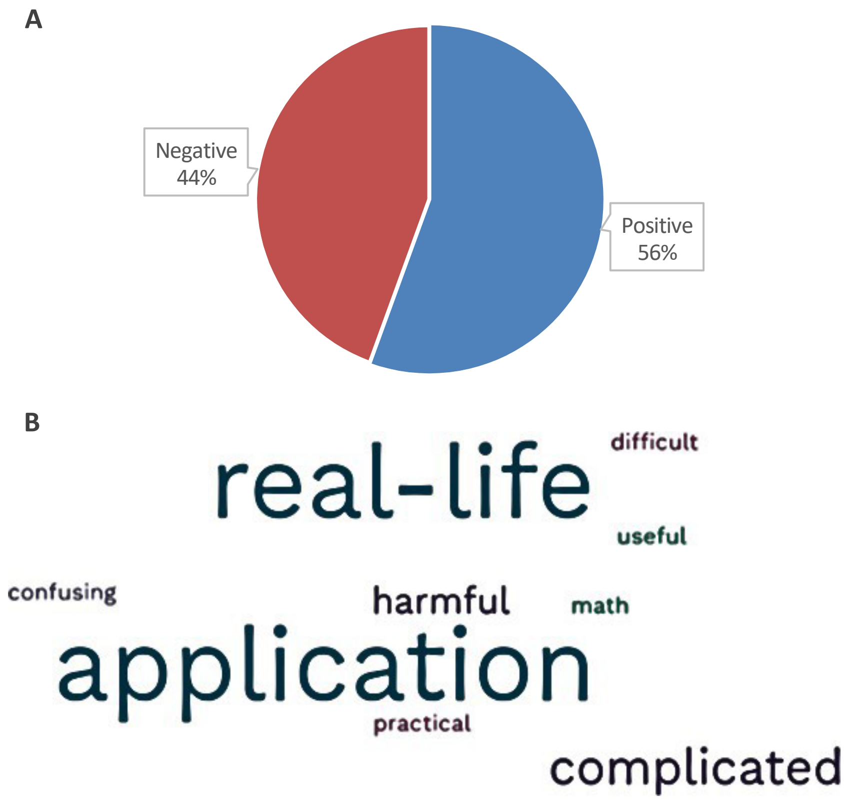


Figure 3. Coding results of participant affective statements regarding chemistry made during the focus group. A) Statements were coded as either "positive," "negative," or "neutral" if the participant mentioned chemistry. The pie chart contains the breakdown of all statements marked as positive or negative. Neutral statements were not included in this figure. B) A word cloud of coding results for both positive and negative student responses regarding chemistry. The size of the term indicates the frequency of that code in affective responses.

Regarding chemistry, a total of ten (10) statements were coded as positive. One participant mentioned that because of their chemistry courses, they found chemistry to be "…pretty fun." Additionally, one student mentioned that they planned to change their major to chemistry. Most positive affective statements made by students involved some sort of application of chemical concepts to their individual lives. Some of these statements involved the use of chemistry to solve societal problems, and others mentioned chemistry as useful and practical. Negative affective statements towards chemistry usually involved instances that the chemical industry has caused harm to humans or the natural environment. For example, one student brought up the Chernobyl nuclear plant catastrophe that resulted in decades of radioactive fallout and still impacts the area to this day. Some participants who made negative affective statements towards chemistry also acknowledged that while the chemical industry is the cause of environmental problems, chemists are also working to solve environmental problems. One participant used the phrase "with great power comes great responsibility" to describe the role of a chemist in our society.

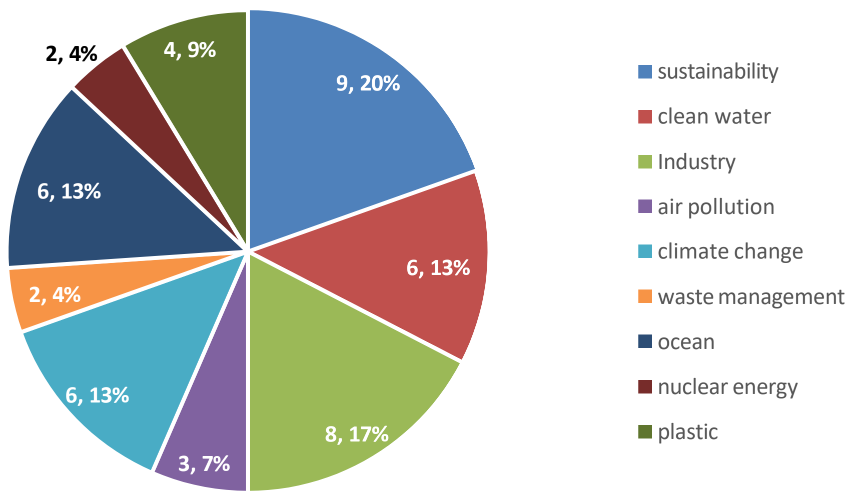


Figure 4. Focus group coding results for R2: "What do students think of when they hear 'the natural environment?'" The pie chart displays the count and percentage of each coding category.

Participants mentioned a variety of topics relating to the natural environment during the focus groups. The most common code given was "sustainability," (20%) which included statements about fossil fuel scarcity and food scarcity. Following this code, "industry" was tagged the most (17%). Participants mentioned the fossil fuel industry as well as the impact of manufactured products on the environment. Next, "ocean," "clean water," and “climate change” were mentioned a total of six times each (13%). The ocean tag was often attached to statements about the BP oil spill. This study took place in a university in Alabama, and several participants grew up in communities that were adversely affected by the BP oil spill. The remaining categories included “plastics” (9%), “air pollution” (7%), “ocean” (4%), and “waste management” (4%).

Based on qualitative analysis, it is apparent that students’ view of chemistry and the environment is complex and multi-faceted. Connections are made between the two concepts, with several students noting elements of chemistry that harm the environment and help the environment. Many students, however, were still referring to chemistry only in the academic sense. A chemistry laboratory intervention with a focus on the impact of anthropogenic materials on the natural environment should consider what mediates one’s understanding of chemistry’s role in environmental issues. The nature of this work is best served by comparing quantitative gains or losses over time, thus, a construct measuring the extent to which one is making these connections should be defined. Defining this concept and creating the items to measure it will be discussed thoroughly in the following section of this chapter (see *Knowledge of the Relationship of Chemistry and the Environment*).

III. Questionnaire Scales

A survey was developed and validated for use in this study. The survey is a combination of existing items from similar studies and original items written based on the focus group. The combined survey items were validated using Exploratory Factor Analysis (EFA) ensuring that each item's variance could be explained best by the intended construct. Table 2 provides an overview of all the items, their source, and response scale.

a. Science self-efficacy Scale

Six survey items were included to measure science self-efficacy. These items were selected and used without editing from two previous studies[25, 29]. One item from each of the three dimensions of chemistry self-efficacy as defined by Uzuntiryaki (2008). These items were developed to assess undergraduate chemistry students' confidence in the academic environment and are related to specific tasks relevant to an undergraduate chemistry course.

Additionally, a scale measuring research self-efficacy was developed by Byars-Winston (2017) to assess a research mentorship program for under-represented minorities in STEM. The items in this scale focus on confidence in research related tasks. Three items were chosen that noted tasks applicable to the chemistry laboratory, such as presenting a research talk and completing a science degree.

Table 2. Questionnaire Items by Measured Construct and Source

Item	Scale	Source
Science Self-Efficacy		
SSE1. Propose solutions to everyday problems using chemistry.	5-point Likert scale: "extremely confident" to "not confident at all	Uzuntiryaki (2008)
SSE2. Conduct an experiment to solve a scientific question.		Byars-Winston (2017)
SSE3. Collect data during the chemistry laboratory.		Uzuntiryaki
SSE4. Complete a degree with a science major.		Byars-Winston
SSE5. Present a research talk or poster.		Byars-Winston
SSE6. Interpret data in laboratory sessions		Uzuntiryaki
Environmental Behaviors		
EB1. I support policy that bans single-use plastic bags at retail stores.	5-point Likert scale: "strongly agree" to "strongly disagree"	Adapted from Biga (2006)
EB2. I often volunteer my time to environmental causes involved in waste mgmt. and recycling.		
EB3. I make special effort to avoid single-use plastics in my daily life.		
EB4. I support charging a deposit on recyclable materials.		
EB5. I often avoid using products from companies that generate excessive plastic waste.		
EB6. I go out of my way to put paper and plastic waste into proper recycling bins.		
Environmental Identity		
EI1. an advocate of the natural environment vs. disinterested in the natural environment	5-point scale from association with one descriptor versus the other	Biga and Stets (2003)
EI2. indifferent of the natural environment vs. very concerned about the natural environment		
EI3. very protective of the natural environment vs. not at all protective of the natural environment		
EI4. Not at all passionate about the natural environment vs. very passionate about the natural environment		

Table 2. Questionnaire Items by Measured Construct and Source (*cont.*)		
Item	**Scale**	**Source**
*EI5. Inferior to the natural environment vs. superior to the natural environment		
*EI6. In competition with the natural environment vs. in cooperation with the natural environment		
Science Identity		
On a scale from 0 (bad) to 10 (good), how do you view yourself as a science student?		
Science Identity Prominence		
SIP1. In general, being a scientist is an important part of my self-image.	5-point Likert scale: "strongly agree" to "strongly disagree"	Persistence in the Sciences (PITS, 2017)
SIP2. I have a strong sense of belonging to a community of scientists		
SIP3. Being a scientist is an important reflection of who I am		
SIP4. I have come to think of myself as a "scientist"		
Intention to pursue a science/STEM career		
On a scale from 0-10, how likely are you to pursue a science related career (for example: chemist, biologist, medical researcher, forensic scientist, policy maker, science educator, etc.)?	10-point scale	original Item
Knowledge of the Relationship between Chemistry and the Environment		
KR1. I can give more than 2 examples of ways chemists are working to help the natural environment	5-point Likert scale: "strongly agree" to "strongly disagree"	original scale
KR2. I recognize chemical principles at work in the environment in my daily life.		
KR3. I often see the relationship of chemistry to environmental issues		
*KR4. I am interested in learning chemistry the most when it relates to the natural environment.		
*KR5. I feel that my chemistry courses support relating content to environmental issues.		
*KR6. Environmental researchers apply chemical knowledge frequently in their work.		

*These items were added after analyzing the inter-item reliability and factor analysis results.

The six items were combined to create a science self-efficacy scale. The scale covers a wide range of tasks related to STEM degrees. Participants were asked to rate their confidence in their ability to complete each of the listed tasks ranging from "extremely confident" to "not at all confident." A high score on this scale indicates a participant who is extremely confident in their ability to perform a wide range of scientific tasks.

b. Environmental Identity Scale

Six items from Stets and Biga's Environmental Identity measure were used[30]. The scale is structured as a five-point scale between two extremes, and participants are prompted to mark the point on the scale that describes how they see themselves. The statements that represent dichotomous extremes are related to one's perception of themselves and the natural environment. The items were reverse coded such that marking all the same spot on the scale for each item resulted in an average score for environmental identity.

c. Environmental Behaviors Scale

Environmental behaviors were measured with a six-item scale adapted from Biga[20]. These items seek to determine the extent to which students engage in pro-environmental behavior. Items were adapted to specifically relate to environmental behaviors involving plastic because this was the main topic covered in the CURE. Participants were asked if they agree or disagree with 6 statements. A high score represents a high participation in pro-environmental behaviors.

d. Science Identity Scale

In a study to understand the role of science identity in the pursuit of a science occupation, Stets used a one item measure of science identity where students were asked to rate how they view themselves from good (10) to bad (0)[16]. Because this work took place in an academic setting, this item was used as a measure of science identity.

e. Science Identity Prominence Scale

Because we were also interested in the relationship of the science identity to an individual's other identities, four science identity prominence items were pulled from the Persistence in the Sciences questionnaire[31]. Participants are asked to rank their agreement with each statement from "strongly agree" to "strongly disagree." Items in this scale measure both the extent to which one identifies as a scientist and give an indication of the prominence of this identity in relation to the other identities held by an individual.

f. Knowledge of the Relationship Scale

Based on the results from the focus group from the previous section, six items were written to measure the extent to which general chemistry students recognized the connection between chemistry and the natural environment. This construct can be subdivided into two sub-domains. The first is the relationship of chemical principles to the natural environment.

Participants of the focus group had all recently enrolled in a general chemistry sequence. Several students noted that their professor explicitly pointed out environmental applications of the content during the lecture and noted additionally that these connections helped them recognize the real-life applications of chemistry. As a result, items were included in the "knowledge of the relationship" measure that asked participants to rate their aggreement with statements involved with recognizing chemical principles at work in the natural environment. These items include "I recognize chemical principles at work in the environment in my daily life," "I often see the relationship of chemistry to environmental issues," and "I am interested in learning chemistry the most when it relates to the natural environment." These items reflect the observations from the focus group that students benefitted from their professor's explicitly connecting chemical concepts to environmental issues in lecture. For a general chemistry CURE focused on environmental issues, these items can demonstrate that students are making those connections in the laboratory classroom as well.

The other sub-domain involved student perception of what is involved with a career in chemistry. Focus group results imply that general chemistry laboratory students percieve that chemistry is related to a wide variety of careers ranging from the industry to research, including the fact that chemistry is used to solve a variety of societal issues. Some participants in the focus group made statements that implied that they felt chemists have a positive impact on the environment, and other statements implied that chemists do not, for example, the mentions of the BP oil spill in the Gulf of Mexico and the Chernobyl nuclear plant. Items were written that sought to measure the extent to which students believed a career involving chemistry could also be involved with environmental work. These items included "I can give more than 2 examples of ways chemists are working to help the natural environment," "I feel that my chemistry courses support relating content to environmental issues," and "Environmental researchers apply chemical knowledge frequently in their work. The focus of these items is to examine students' perceptions of chemistry in the academic sense versus as a career. If a student were to have a strong environmental identity, they may want their future career to involve the environment, or at the very least, not directly contribute to the destruction of the natural environment.

Participants were asked to rate their agreement to the six statements ranging from "strongly agree" to "strongly disagree." A high score on this scale indicates the participant recognizes a strong connection between chemistry and environmental issues.

IV. Preliminary Administration of Survey and Validation

To ensure that participants responded to the questionnaire as intended, a preliminary administration was performed in the Fall of 2019. Exploratory factor analysis and a comparison of responses by racial and gender identity were performed following this administration.

a. Participants

Table 3. Demographics of Questionnaire Participants

Characteristic	**No. (%) (N = 800)**
Gender Identity	
Female	492 (61.3)
Male	301 (37.5)
Nonbinary/other	3 (0.4)
Prefer not to respond	4 (0.5)
Race	
White/Caucasian	453 (56.5)
Black/African American	117 (14.6)
American Indian	4 (0.5)
Asian	152 (19.0)
Pacific Islander	1 (0.1)
Middle Eastern	22 (2.7)
Hispanic	41 (5.1)
Other	10 (1.4)

In total, 800 questionnaires were completed in the initial application of this questionnaire in the Fall of 2019. All the participants were enrolled in a general chemistry laboratory course at the time. Several demographic questions were asked to gain insight into the sample of participants. Participants were asked to indicate their gender identity from one of four options: "female," "male," "nonbinary/other," and "prefer not to respond." Participants were also asked to indicate which racial identity most describes them. This item was taken without editing from the most recent racial identity item in the United States Census. The item choices were as follows:

- White (for example: German, Irish, English, Italian, Polish, French, etc.)
- Black or African American (for example, African American, Jamaican, Haitian, Nigerian, Ethiopian, Somali, etc.)
- American Indian or Alaska Native (for example: Navajo Nation, Blackfeet Tribe, Mayan, Aztec etc.)

- Asian (for example: Chinese, Filipino, Asian Indian, Vietnamese, Korean, Japanese, etc.)
- Native Hawaiian or Pacific Islander (for example: Native Hawaiian, Samoan, Chamorro, Tongan, etc.)
- Middle Eastern or North African (for example: Lebanese, Iranian, Egyptian, Syrian, Moroccan, Algerian, etc.)
- Hispanic, Latino, or Spanish origin (for example: Mexican, Mexican American, Puerto Rican, Cuban, Salvadorian, Dominican, Columbian, Etc.)
- Some other race, ethnicity, or origin

Lastly, they were asked to indicate the total number of terms (semesters) they had been enrolled at the institution. Table 5 shows a breakdown of the demographics of questionnaire participants.

b. Statistical Methods

i. Cronbach's Alpha. Alpha values were calculated for every scale to determine whether participants answered each item similarly. Scales with alpha scores above 0.7 are considered adequate[32]. Low alpha scores indicate that additional items may be necessary and were addressed on a case-by-case basis.

ii. Exploratory Factor Analysis (EFA). Survey validation was performed via EFA using SPSS and was used to assess whether items were loaded into the correct measured construct. EFA attempts to explain the variance in large datasets using abstract components extracted via Principal Component Analysis (PCA) with the purpose of reducing the number of variables considered to predict an outcome. The components should represent each of the intended measured constructs in the questionnaire, and each item from the construct's scale should be more highly correlated to that component than the others. In other words, the items of one scale should "load" together beneath the same abstract component. This indicates that the items are measuring the same construct as intended.

iii. Kaiser-Meyer-Olkin Measure of Sampling Adequacy (KMO) and Bartlett's Test of Sphericity. To determine whether the sample was appropriate for factor analysis, KMO's measure of sampling adequacy and Bartlett's test of sphericity was performed. KMO's test determines whether the sum of the partial correlations between variables is large or small relative to the sum of correlations. A high value indicates that factor analysis will yield distinct, reliable factors. Bartlett's test of sphericity determines whether a correlation matrix is an identity matrix, i.e. whether correlations between variables are all zero (0). A significant ($p \leq .05$) result means that some of variables in the dataset are correlated and that factor analysis can be performed.

iv. T-tests. Construct averages of White participants were compared against the construct averages of representative minority groups using independent samples t-tests. Also, averages from male participants were compared to the averages of female participants. If construct averages were not normally distributed, a non-parametric test (Mann-Whitney U-test) was used. Results were considered significant if $p \leq .050$.

c. Results

Table 4. Inter-item Reliability of Questionnaire Scales	
Scale	**Cronbach's Alpha**
Science Self-efficacy	.837
Environmental Behaviors	.816
Environmental Identity	.864
Science Identity Prominence	.913
Knowledge of the Relationship	.731

i. *Inter-item Reliability.* All scales were found to have adequate inter-item reliability (>.7) according to Cronbach's alpha. The values for each scale are shown in Table 6.

ii. Factor Analysis Results. KMO's test determined that the sampling adequacy for factor analysis was excellent (.907)[33]. Bartlett's test also reflected that there were correlations between the variables in the dataset (<.001).

PCA was used to extract components from the questionnaire results. The results are shown in Table 3. Eigenvalues represent the ratio of variance in the dataset explained by the extracted component. To determine which components should be considered further in the analysis, the accepted criterion of an eigenvalue greater than 1.0 was used. As a result, five components were identified that explained a cumulative 63.9% of the variance in the questionnaire results.

Table 5. Principle Component Analysis Results

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	6.748	29.337	29.337	6.748	29.337	29.337	3.407	14.815	14.815
2	3.462	15.052	44.389	3.462	15.052	44.389	3.190	13.869	28.684
3	1.888	8.209	52.598	1.888	8.209	52.598	3.186	13.852	42.536
4	1.346	5.854	58.451	1.346	5.854	58.451	2.946	12.808	55.344
5	1.261	5.481	63.933	1.261	5.481	63.933	1.975	8.588	63.933
6	.831	3.615	67.547						
7	.732	3.181	70.728						
8	.636	2.764	73.492						
9	.597	2.597	76.089						
10	.569	2.475	78.565						
11	.539	2.345	80.910						
12	.510	2.216	83.126						
13	.480	2.089	85.215						
14	.451	1.962	87.177						
15	.425	1.847	89.024						
16	.391	1.701	90.725						
17	.375	1.629	92.354						
18	.356	1.549	93.903						
19	.336	1.460	95.364						
20	.324	1.409	96.773						
21	.285	1.239	98.012						
22	.273	1.185	99.197						
23	.185	.803	100.000						

Correlations between questionnaire items and the generated components were assessed, and the rotated component matrix displaying the results is shown in Table 4. The results allowed for target constructs to be matched to the generated components. Component one was identified as science self-efficacy, as all the items from the self-efficacy scale loaded with this component. All the items from the environmental behaviors scale loaded with component two, so this component was identified as environmental behaviors. Component 3 was identified as science identity, with all four science identity items loading with this component. Component 4 was identified as environmental identity, with five out of six of the environmental identity items loading under this component. All

Table 6: Rotated Component Matrix

Item	Component 1	Component 2	Component 3	Component 4	Component 5	Component 6	KMO
SSE1	.668						0.915
SSE2	.790						0.893
SSE3	.794						0.871
SSE4	.623						0.924
SSE5	.621						0.911
SSE6	.803						0.888
EB1		.590					0.924
EB2		.691					0.924
EB3		.746					0.925
EB4		.581					0.927
EB5		.786					0.91
EB6		.589					0.95
EI1				.732			0.922
EI2				.835			0.886
EI3				.723			0.924
EI4				.813			0.885
SIP1			.860				0.889
SIP2			.843				0.919
SIP3			.892				0.856
SIP4			.797				0.924
KR1					.722		0.934
KR2					.778		0.906
KR3					.784		0.905

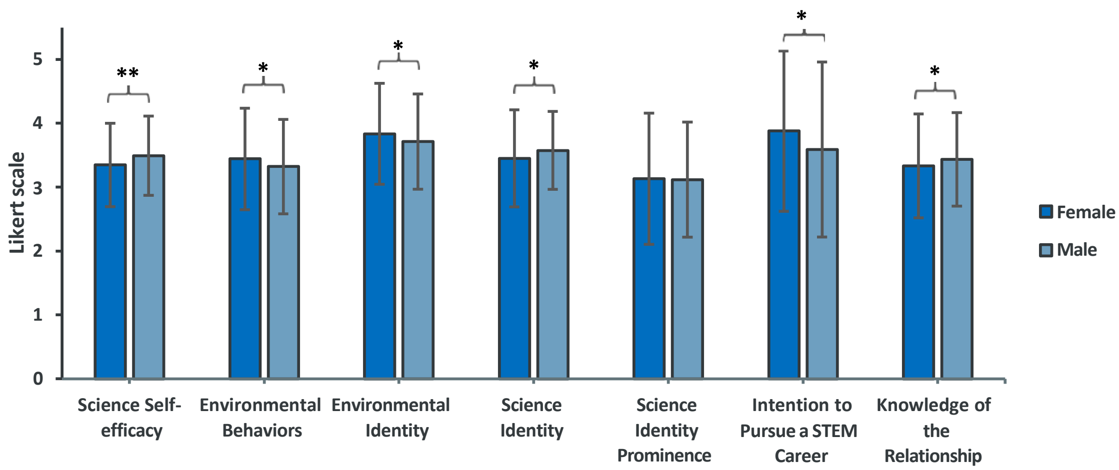
Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.
Rotation converged in 6 iterations.

original items intending to measure one's knowledge of the relationship of chemistry and the environment loaded with component 5. Overall, these results show that every item loaded with the other items from the same scale and support the use of these items cumulatively as indicators of their intended constructs.

iii. *STEM Persistence between Sex Groups*. It is well documented that women are underrepresented in many STEM fields[34]. While it was not a focus of this study, we asked participants to indicate their gender identity in the questionnaire because the scales chosen were often used in studies that seek to understand the gender gap in STEM, especially science-self efficacy, and science identity. Additionally, it is important to know the gender identity breakdown for constructs relating to the natural environment as this will provide insight into the efficacy of environmental interventions in the general chemistry laboratory based on one's gender identity. Therefore, this section is dedicated to discussing the differences observed between male and female identifying participants for all constructs over time.

Figure 5 displays the whole group averages broken down by male and female participants. Only male and female participants are shown because a representative sample of nonbinary identifying participants was not obtained in the initial questionnaire administration. Male participants had significantly higher average scores for the following constructs: science self-efficacy ($t(780) = -3.01$, $p = .001$, $d = -0.22$, 95% CI [-0.37, -0.08]), science identity ($t(734.6) =$

-2.50, p = .007, d = -0.18, 95% CI [-0.33, -0.04]) and knowledge of the relationship between chemistry and the environment (t(783) = -1.79, p = .037, d = -0.13, 95% CI [-0.28, -0.01]). Female participants had significantly higher averages for the following constructs: environmental behaviors (t(781) = 2.11, p = .018, d = 0.16, 95% CI [0.01, 0.30]), environmental identity (t(789) = 2.16, p = .016, d = 0.16, 95% CI [0.01, 0.30]), and intention to pursue a STEM career (t(590.5) = 2.94, p = .002, d = 0.22, 95% CI [0.08, 0.20]). No difference was



observed for science identity prominence (t(687) = 0.22, p = .408, d = 0.01, 95% CI [-0.13, 0.16]).

Figure 5. Whole group construct averages broken down by sex. Nonbinary results are not shown as a representative number of responses from nonbinary identifying individuals was not obtained. Error bars show the standard deviation from the mean. Asterisks indicate statistically significant differences, where ** means p ≤ .001 and * means p ≤ .05.

Trends observed after breaking down the data by gender identity demonstrate that construct averages vary slightly between male and female participants. It appears that male participants have higher confidence in their ability to perform well in STEM courses, as the item for science identity is to rate the extent to which you see yourself as a good science student. This tracks with the trend observed for science self-efficacy, as male participants might see themselves as better science students because of their confidence in doing scientific tasks, or vice versa. The phenomenon of male identifying individuals having greater scientific confidence than female identifying individuals is well documented, however the differences observed here are small in effect.

It also appears, based on these results, that female participants both have a stronger connection to the natural environment regarding their identity and participate in more pro-environmental behaviors regarding plastics than male participants. This may be connected to a cultural view of women as being "caring" and "nurturing" which could explain why female participants relate more strongly with items in these scales.

Female participants' intention to pursue a STEM career average was higher than those of male participants despite having lower affirmative averages for self-efficacy and science identity. Interpretation of this observation is complicated because the values differed between each cohort with some groups reporting lower science self-efficacy than others (see Chapter 3). Still, the strength of the intention to pursue a scientific career for female participants is correlated to a more prominent science identity than male participants, and supports other research that has shown that science self-efficacy is not the singular construct leading to an individual's decision to pursue a career in STEM[12]. Overall, these differences are small in effect, which supports aggregating the data collected in future analyses.

iv. *Racial Identity*. The metrics used to understand gender gaps in STEM disciplines are useful to address racial STEM gaps as well. Science identity and science self-efficacy are significant components of an expanded SCCT model to understand under-represented minority's career and academic decision-making regarding STEM. For this analysis, only racial groups with a representative sample (at least 30) of participants were compared. White participants constituted the largest racial group in this study (56.5%), followed by Asian participants (19.0%), and Black participants (14.6%).

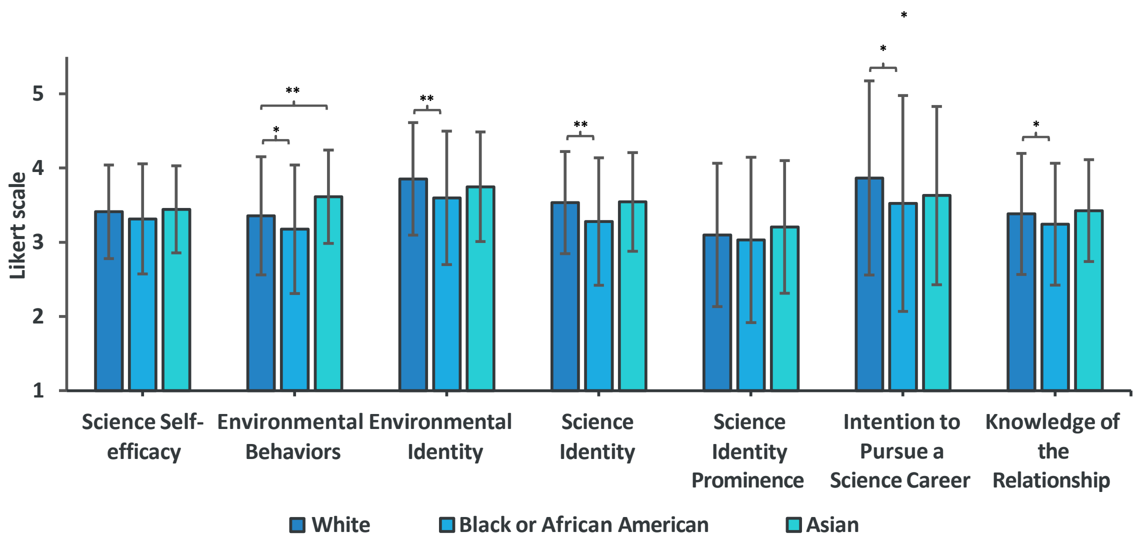


Figure 6. Construct averages for white participants versus other minority groups. Only racial groups with a representative number of responses were considered in this analysis. Error bars show the standard deviation from the mean. Asterisks indicate statistically significant differences where * means $p \leq .05$ and ** means $p \leq .001$.

There were some observed differences in the constructs based on reported race (Figure 6). White participants reported greater environmental behaviors than black participants ($t(561) = 2.14$, $p = .016$, $d = 0.22$, 95% CI [0.02, 0.43]) and lower environmental behaviors than Asian participants

($t(323.3) = -3.60$, $p = <.001$, $d = -0.34$, 95% CI [-0.52, 0.15]). White participants reported greater environmental identity than black participants $t(567) = 3.11$, $p < .001$, $d = 0.32$, 95% CI [0.12, 0.53] and was no different when compared with Asian participants ($t(602) = 1.50$, $p = .067$, $d = 0.14$, 95% CI [-0.04, 0.33]). The same trend was observed for science identity (vs. Black participants: $t(156.7) = 5.64$, $p = .001$, $d = 0.36$, 95% CI [0.15, 0.56]; vs. Asian participants $t(603) = -0.15$, $p = .40$, $d = 0.01$, 95% CI [-0.20, 0.17]). White participants also reported significantly greater measures of intention to pursue a STEM career than black participants ($t(568) = 2.46$, $p = .007$, $d = 0.26$, 95% CI [0.05, 0.46]) and Asian participants ($t(603) = 1.96$, $p = .026$, $d = 0.18$, 95% CI [0.00, 0.37]). Lastly, white participants reported significantly higher averages than black participants for the knowledge of the relationship scale ($t(562) = 1.65$, $p = <.049$, $d = 0.17$, 95% CI [-0.03, 0.38]).

The results of these analyses demonstrate that participants of different racial groups are answering the questionnaire slightly differently for certain constructs. The implications drawn will be minimal, as the under-represented minority's experience in the general chemistry laboratory was not a focus of this work. However, is important to discuss the differences observed.

Black participants scored lower on average than white participants in five out of seven scales. This is consistent with other reports concerning STEM persistence measures that control for racial identity, as a lack of representation in STEM, academic support, and other factors contribute to the incorrect notion that under-represented minorities do not belong in STEM. It should be noted that the observed differences are small in effect, each around or less than a third of a standard deviation.

An alternative explanation could be that black participants in this study were less susceptible to social-desirability bias, which explains the phenomenon of social science research participants' tendency to respond in a way that would be seen as favorable to others[35]. This bias can result in under-reporting of behaviors that are perceived as "bad" and over-reporting of behaviors that are perceived as "good." As a result, some participants in this study might have felt pressured to report more pro-environmental behaviors, as this may have been perceived as "good" behavior in the climate of a university environment. Further research must be conducted to determine the impact of social-desirability bias on STEM persistence questionnaires, especially for under-represented minorities.

Results demonstrate that some of the predictor variables used may operate differently for White, Black, and Asian groups. Thus, interpreting a raw response value for construct scales is not as valuable as interpreting change over time when comparing racial groups. The works herein that use data from this questionnaire do not separate by racial identity, but the studies have been designed such that change over time is analyzed instead of averages at a single time point. Control groups are used where possible to further isolate the effect of other variables, such as the COVID-19 pandemic and participating in a CURE, on environmental identity and STEM persistence measures.

V. Conclusion

Chapter 2 has outlined the background, theory, development, and assessment of the measure used in the following chapters of this thesis. The original purpose of this study was to design, implement, and assess an environmental chemistry CURE in the general chemistry laboratory. For this purpose, a questionnaire was designed to assess social cognitive predictors of career intentions and positive environmental behaviors for general chemistry students. Results of exploratory factor analysis demonstrated that the scales assessed their intended construct, and the questionnaire was determined to be adequate for this study. Subsequently, after all data collections were performed, the entire dataset was analyzed for differences between important demographic groups in STEM education. It was found that women and under-represented minorities responded differently to the questionnaire than male and white participants, but the differences observed are consistent with others in the literature. Future work will discuss the differences observed in the dataset regarding the COVID-19 pandemic and the impact of CUREs on scientific self-concept development in the general chemistry laboratory.

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CHAPTER 3

MONITORING STEM PERSISTENCE OF GENERAL CHEMISTRY LABORATORY STUDENTS THROUGHOUT THE COVID-19 PANDEMIC

PART 1

THE IMPACT OF COVID-19 ON THE ACADEMIC PLANS AND CAREER INTENTIONS OF FUTURE STEM PROFESSIONALS

by

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THE IMPACT OF COVID-19 ON THE ACADEMIC PLANS AND CAREER INTENTIONS OF FUTURE STEM PROFESSIONALS

Abstract

Future STEM professionals are a key part of dealing future disasters like that of COVID-19, but the pandemic may result in a gap in individuals joining the STEM work force. In the present work, we offer a picture of our student's identity as scientists and intentions to pursue a science career from before and after the transition to online instruction that occurred as part of the initial phase of the pandemic response. Additionally, we asked our students to describe the ways this transition has affected their academic plans to provide an in-depth look into their intentions. Data collection involved the administration of a questionnaire to first-year general chemistry laboratory students at the beginning and the end of the spring 2020 semester (January-May). The data indicate that there was no significant change to our student's identities and intention to pursue a career in science during spring 2020, and our students written responses indicate that they are making short term academic changes that could affect their graduation date, but do not suggest any serious changes to career plans. We conclude that the initial transition to online instruction due to the pandemic had a minimal effect on our student's career intentions, and future work can use this data to better understand the long-term effects of the pandemic on STEM students.

Keywords: First-Year Undergraduate / General; Laboratory Instruction; Distance Learning / Self-Instruction; Student / Career Counseling

The COVID-19 pandemic has caused the greatest disruption to our society in decades, and its effects are far-reaching. There has never been a greater need for the work of medical professionals and scientists in addressing public health issues. There is concern that the crisis will lead to a decline in the number of students continuing their education and entering the science, technology, engineering, and mathematics (STEM) workforce due to complications caused by the pandemic.

We had an opportunity to offer a snapshot of the impact of the pandemic on our student's intentions immediately after the disruption of instruction in the spring of 2020 because we were already studying factors contributing to a student's decision to pursue a career in science. During the semester our university, like many others, transitioned to online learning in response to the COVID-19 pandemic. We compare data that was collected before and after this transition on constructs related to career intentions: science identity, science identity prominence, and intention to pursue a science career.

The undergraduate years are a time of significant change within an individual's identities. Students enter college with aspirations to pursue a degree in STEM, but many change their majors several times throughout their undergraduate studies[1-2]. Science identity is defined as an individual's professional self-identification as a scientist, and the pursuit of behaviors that reinforce this self-identity[3]. In STEM education research, science identity is often used as a predictor of academic and career outcomes in undergraduate students such a student's interest in science and persistence into a science career.

It has predicted involvement in a science career or field after college, and influences intentions regarding research careers[3-9].

The theory of identity prominence was developed to understand the interrelation of multiple identities within an individual. Identity prominence is the extent to which an individual views an identity as more important than others or more central to their self-concept[10]. A person might see I as both a doctor and a parent, but one of these identities may play a stronger role in their decision making. A person's behavior is most strongly guided by the identity that they view as most important compared to their other identities. Identity prominence has been shown to be an excellent predictor of behaviors[10-12] and students with a more prominent science identity will have a greater likelihood of pursuing a STEM career[3]. The purpose of this study is to use these constructs to gain insight into the effect of COVID-19 related education transitions to online learning on our students' career and academic plans.

While this is a limited perspective into the effects of the pandemic, it is crucial for STEM education researchers to begin the process of assessing its impact on STEM students. Understanding the effects of the initial transition to online learning will provide a comparison for long-term studies. This is one of the first steps in developing the ways to support STEM students most effectively as we continue instruction.

I. Methods

a. Study Participants

Participants of this study were undergraduate students enrolled in a general chemistry laboratory course at a large public research university in the southern United States. At this institution, the general chemistry laboratory sequence (GCLS) fulfills the laboratory requirements for the core curriculum and a broad range of STEM majors. The general chemistry sequence also contains an honors option (HONLS) for students in the honors program at this institution. All participants were enrolled in the second course of the GCLS or the HONLS during the spring 2020 semester. The laboratory courses were one credit hour per semester and met for a 3-hour period once a week. Each section was taught by a team of one graduate student and one undergraduate student.

The approach of the GCLS is generally described as guided inquiry. Detailed procedures are typically only provided when necessary for safety or to reduce complexity. The content of the laboratory reinforces content from the lecture course and includes instruction on proper use of common quantitative equipment and glassware. At the beginning of the semester, the HONLS completes three of the same experiments as the GCLS, then, for the remainder of the semester, they complete a project-based assignment where students propose scientific questions and attempt to answer these questions by collecting data.

Pandemic related closures caused all instruction to be moved online after an extended spring break ending on March 27^{th}, 2020. From that point on, laboratory meetings were held using virtual meeting software (i.e. Zoom). Each week, students watched a video that depicted a graduate teaching assistant (TA) performing a lab activity from the GCLS curriculum. The videos included depictions of the equipment and glassware pertaining to this lab activity as well as data collection. The students were assigned a weekly lab report in which they would record qualitative and quantitative data from these videos and draw conclusions on the experiment. In Zoom sessions, a graduate TA would lead a discussion on the experiments and results.

b. Survey Administration

Students were asked to participate in this study by their instructor of record at the beginning of the semester through Canvas in the spring 2020 term (IRB#300003153). They were provided a hyperlink to the survey administered through Qualtrics™. The landing page of this survey was an informed consent statement outlining the study. This survey was available at the beginning of the semester (weeks 1-2) and at the end of the semester (weeks 12-13 of a 14-week term). Participants were offered course credit in the form of bonus points on their final point total if at least 90% of students completed the survey at the beginning of the semester, but bonus points were not offered for completion the survey at the end of the semester.

c. Survey Structure

Table 1: Survey Items Grouped by Measured Constructs

Construct	Q#	Type	Item
Science Identity	Q1	10-point scale	On a scale from "not at all good" to "very good" (1-10), how do you view yourself as a science student?
Science Identity Prominence	Q2	5-point Likert scale	In general, being a scientist is an important part of my self-image
	Q3	5-point Likert scale	I have a strong sense of belonging to the community of scientists
	Q4	5-point Likert scale	Being a scientist is an important reflection of who I am
	Q5	5-point Likert scale	I have come to think of myself as a 'scientist'
Career Intentions	Q6	10-point scale	On a scale from 1-10, how likely are you to pursue a science related career (for example: chemist, biologist, medical researcher, forensic scientist, policy maker, science educator, etc.)?
Effect of COVID-19	Q7	Free response	Have your plans for the Fall 2020 semester changed as a result of the COVID-19 pandemic?
	Q8	Free response	Describe the ways your plans have changed or may change for the Fall 2020 semester as a result of the COVID-19 pandemic.
	Q9	Free response	Have your long-term plans changed as a result of the COVID-19 pandemic?
	Q10	Free response	Describe the ways your long-term plans have changed or may change.

The pre-semester survey was a 6-item survey (Q1-Q6 from Table 1) associated with science identity, science identity prominence, and career intentions. The end-of-semester survey included the same 6 items plus four items (Q7-Q10) that were included to measure the types of changes our participants made in response to the transition to online learning. These items are grouped as shown in Table 1. The free response format was used to elicit genuine responses from our participants and to avoid prompting negative behaviors (i.e. taking fewer hours, dropping out, transferring, etc.).

Science identity was measured by a single indicator (Q1) as used by Stets et. al (2017). Students were asked to rate how they view themselves as a science student from 0-10 with zero (0) being "not at all good" and ten (10) being "excellent." A higher rating on this indicator represents a more positive view of oneself as a science student[5].

Science Identity prominence was measured using four items (Q2-5) from Stets et al. (2017). The responses were coded from 1-5 with one (1) being "Strongly Disagree" and five (5) being "Strongly Agree." Responses were averaged to obtain a science identity prominence score. A high score on this scale means that participant has a prominent science identity. Inter-item reliability for these items was analyzed using pre-survey data collected at the beginning of the spring 2020 semester from the participants of this study. The reliability of these items was found to be strong (a=0.904).

Participants were also asked about their intention to pursue a career in science using a single item (Q6). A response of zero (0) indicates that they are not likely at all to pursue a science career, and a response of ten (10) indicates that they are extremely likely.

In the post-survey only, participants were asked if their plans for the Fall 2020 semester have changed due to the COVID-19 pandemic (Q7: yes/maybe/no). If participants selected 'yes' or 'maybe,' they were asked to explain how their plans have changed in a follow up free-response question (Q8). We also asked all participants if their long-term plans have changed because of the COVID-19 pandemic (Q9: yes/maybe/no). If participants answered 'yes' or 'maybe,' they were then asked to explain how their long-term plans have changed or may change in another free-response question(Q10).

d. Free-Response Coding

All free responses were read independently by two reviewers to identify emerging themes. Three themes were identified: changes to academic plans, changes to extracurricular experiences, and concerns about online instruction. Once the themes were agreed upon, we developed questions to quantify the frequency and any directionality of the student's response to each theme.

1. Will this change affect student's four-year plan to graduation?
2. Did the student indicate that an extracurricular experience was affected by the pandemic?
3. Did the student indicate concern about taking courses in an online environment?

The responses were then independently coded as described below by two reviewers. Coding was accepted when both reviewers coded an item in the same way, and items that did not agree were reconciled by discussion.

Using the first coding question, we determined whether each student's response indicated that they were making a change to their academic plan. If a response indicated such a change, we further decided if this change would potentially decrease the amount of time to graduation (such as enrolling in more credit hours over the coming summer 2020 semester), increase the amount of time to graduation (such as delaying coursework for the summer 2020 or fall 2020 semester), or result in no change. If a student's response did not indicate any sort of academic change, they were not counted in this category.

Using the second coding question, we determined whether a response indicated a change in the student's extracurricular involvement (such as research experiences, shadowing opportunities, etc.). If a student discussed these experiences in their response, we further decided if an extracurricular experience was gained, lost, or unchanged. If a response did not discuss extracurricular involvement, they were not counted in this category.

Using the third coding question, we determined whether a response expressed concern about the transition to online instruction. If such a concern was addressed, they were counted as concerned about online instruction. If a response did not indicate such a concern, they were not counted in this category.

Based on this method of analysis, each response could potentially touch on multiple themes. For example, if a student indicated that they will no longer be enrolling summer courses because they did not want to take courses online, they were categorized as both increasing their time to graduation and expressing concern about online instruction. This method was chosen because our three themes are not mutually exclusive and could occur within the same free-response answer.

II. Results

a. *Science Identity, Science Identity Prominence, and Intention to Pursue a Science Career*

Pre- and post-survey data were collected for the spring semester for both the GCLS (pre: n=243, post n=83) and the HONLS (pre: n=63, post: n=40). Pre-survey data indicate that the GCLS and HONLS students are different enough to treat separately ($p<0.01$). The HONLS students demonstrated significantly higher science identity, science identity prominence, and intention to pursue science indicators. Since the two sequences were taught differently, treating two groups separately will offer two different perspectives on the ways science identity and career intentions were affected.

We observed a decrease in survey participation from pre-survey data collection to post-survey data collection. Levene's test for equality of variances was used to address unequal sample sizes. We compared the pre- and post-survey data for both groups individually and found that the variances were not significantly different; therefore, we used an independent samples t-test in which equal variances are assumed to compare pre/post data. (see supporting information).

In the GCLS and HONLS, scores for science identity, science identity prominence and intention to pursue a science career did not change significantly during the spring semester ($p<0.01$). This is consistent with the observation that identities are relatively stable once formed[8], however, the first year of undergraduate study is important in determining whether students will continue in STEM[2]. Thus, it is valuable to study how student's identities might be changing during the first year. It is also possible that normal gains are not seen since we do not have data from prior years, but there is no evidence that the transition to online learning due to the pandemic negatively impacted the constructs that were measured.

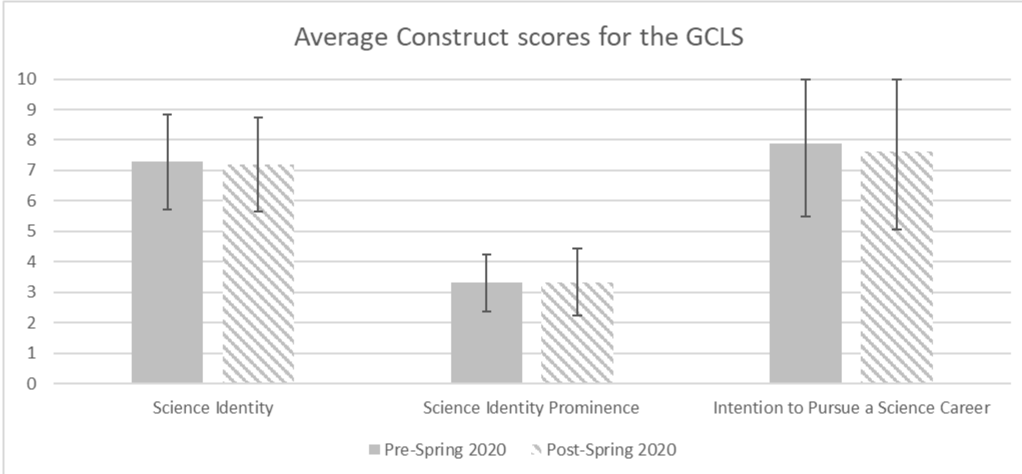

Figure 1. Science identity, science identity prominence, and intention to pursue a science career scores for the GCLS during the spring 2020 semester. None of the changes were found to be statistically significant, including intention to pursue a scientific career, even though it appears to decrease. Error bars represent the standard deviation of the sample from the mean.

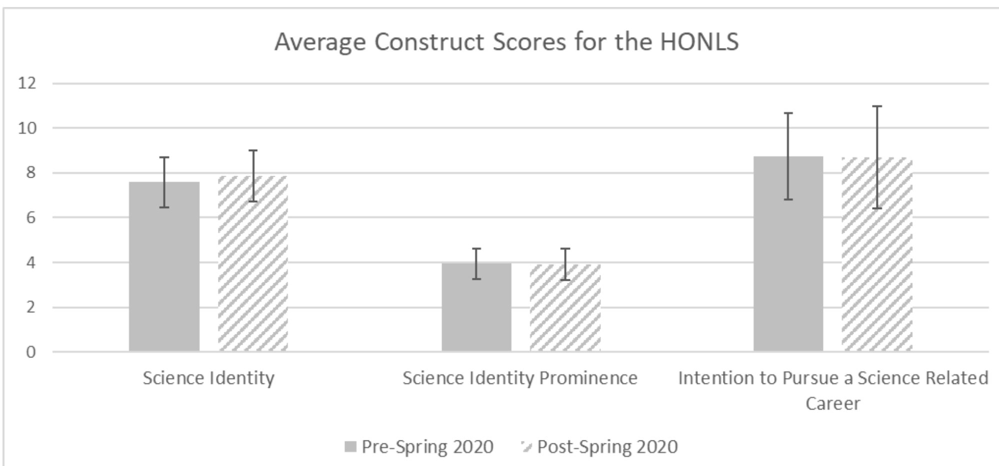

Figure 2: Science identity, science identity prominence, and intention to pursue a science career scores for the HONLS during the fall 2019 and spring 2020 semester. Data is included that was collected at the beginning (first 2 weeks) of the fall 2019 semester. No significant changes were observed for any construct score in the spring 2020 semester. Error bars represent standard deviation of the sample from the mean.

b. Free Response Data

Though there was no significant change in science identity, science identity prominence, or intention to pursue a science career, approximately half (49%) of the respondents indicated that their short-term plans were either going to change or may change because of the transition to online learning. Of that group, 66% reported that they planned to change their academic plan. A significant portion (39%) indicated that they were delaying or suspending coursework, and a small percentage (9%) intended to take additional summer courses. The remaining students (18%) indicated a change, but it was unclear whether this change would increase or decrease their time to graduation. The number of students delaying or suspending their coursework could result a lag in student's academics that could delay their continuation into professional programs and STEM careers.

A small percentage (12%) described a lost extra-curricular opportunity due to pandemic related disruptions. These experiences, such as summer research internships and shadowing opportunities, are an important contributor to retention in STEM[13]. While it is not surprising that students lost these opportunities, it is valuable to document the percentage of first year students that had intended to participate. Future work should investigate how these students subsequently found alternative experiences, future in-person experiences, or chose to leave STEM.

Additionally, a portion (31%) of respondents described concern about online instruction for the upcoming semesters. This observation is consistent with our expectations, as many students and instructors across the nation have undergone a similar transition and share this concern. Online concern could be prominent in our results because of the timing of our data collection and does not demonstrate a direct connection to constructs involved with career intentions.

III. Conclusions

In this study, we present data from before and after transitioning to online learning in response to COVID-19 that offers insight into its immediate effect on the career and academic intentions of STEM students. The measures of science identity, science identity prominence, and intention to pursue a science career were used as indicators of career intentions. The data suggest that these predictors were not significantly affected by the transition to online learning in response to COVID-19. Our results also suggest that the students in this study are making short-term academic choices to delay coursework which could result in a later graduation date. This could result in a delayed entry into the STEM workforce even though these students still intend to pursue a STEM career.

These results represent only a short-term perspective on the onset of COVID-19, but this data will be useful for future work as it demonstrates the lack of an immediate effect on STEM student's career intentions. Thus, those studying the long-term effects of the pandemic will have a comparison of before and after the initial disruption. This situation is ever evolving, and short-term data will allow those who continue this work to better understand the long-term impact of COVID-19.

IV. Limitations

The pandemic related disruption and the transition to online learning during spring 2020 resulted in a significant decrease in survey participation. The results are presented after controlling for the difference, but there is concern that the post-survey respondents represent the most motivated or attentive students.

As a result, the observation of no significant change may result in more positive immediate outlook than is present. However, there is a slight increase in pre-registration for the subsequent organic chemistry course (from 466 in fall 2019 to 479 in fall 2020), so it is not likely that the drop-off in survey participation is a result of a change in student's STEM intentions. Thus, it is important that the data, even if incomplete, is available to others interested in studying career intentions.

Supporting Information

The Supporting Information which contains our survey instrument and more details on the statistical analyses performed is available on the ACS Publications website at DOI: 10.1021/acs.jchemed.0c00646

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CHAPTER 2 PART 2

COVID-19 AND THE INCOMING CHEMISTRY STUDENT: THE EFFECT OF THE PANDEMIC ON SELF-EFFICACY AND IDENTITY

by

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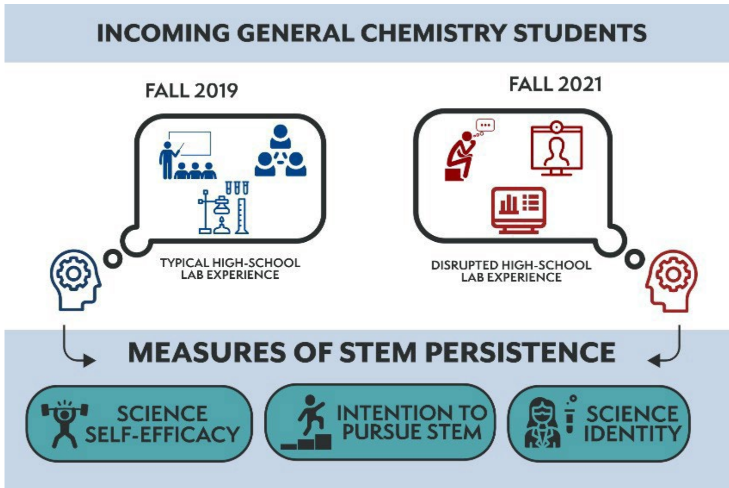
Format adapted and errata corrected for dissertation

COVID-19 AND THE INCOMING CHEMISTRY STUDENT: THE EFFECT OF THE PANDEMIC ON SELF-EFFICACY AND IDENTITY



Abstract

The COVID-19 pandemic has changed the way students and educators are able to interact in higher education settings, and timely investigations into its impact on science, technology, engineering, and mathematics (STEM) students are of critical importance. In a continued effort to provide student data to chemistry educators, we offer a comparison of three years of incoming undergraduate cohorts. Herein, we compare data from incoming general chemistry students for the fall of 2019 through the fall of 2021 to offer a picture of student intention to pursue a career in STEM over time during the pandemic. Results demonstrate that our most recent cohort (Fall 2021) has an increased intention to pursue a STEM degree coupled with a decrease in scientific self-efficacy. We discuss potential causes of these observations and offer strategies for fostering self-efficacy in the chemistry laboratory.

Keywords: General Chemistry, Laboratory Instruction, Career Counselling, Distance Learning

Educational institutions significantly altered their policies and procedures due to the coronavirus pandemic beginning in the winter of 2019 (COVID-19). For the United States, disruptions to face-to-face instruction began in the spring of 2020 and many institutions adopted a remote or hybrid model during the 2020-2021 academic calendar. This year-long gap in face-to-face instruction has the potential to affect educational practices for years to come, and timely investigations into its impact on student learning will help chemical educators address the challenges or impediments identified and implement best practices.

For students who seek a variety of science, technology, engineering, and mathematics (STEM) degrees, the general chemistry laboratory is one of the first experiences in an academic laboratory during undergraduate study. STEM majors also have a reputation of high attrition rates, with one statistical report documenting as many as 50% of STEM majors change their major within the first year of undergraduate study[1]. Due to the placement of general chemistry in STEM curricula as a first-year course for a variety of STEM majors, addressing STEM attrition becomes in part the responsibility of chemical educators, and understanding students' background as it relates to experience in the laboratory is necessary to create a learning environment in which students can meaningfully engage.

While a core focus of the general chemistry laboratory is introducing students to the laboratory environment, many first-year STEM students benefit from experiences that occurred during the final 1-2 years of their secondary (high-school) education that directly impact the way they will interact with the general chemistry laboratory. Due to the COVID-19 pandemic, the current population of STEM students may have had severely disjointed secondary laboratories, and for some students, they might have been completely absent or remote. For the chemical educator to best support the current era of STEM students, it is essential to identify the ways in which pandemic related disruptions have altered incoming students' background and their likelihood to persistence in STEM thereafter. Therefore, data regarding STEM persistence must be made available to chemical educators so they may identify and take appropriate measures to close gaps in their students' development as scientists.

Social Cognitive Career Theory (SCCT) seeks to explain the way academic interests develop and how career decisions are made[2]. In SCCT, self-efficacy and science identity are frequently used as predictors of student retention in STEM[3-9]. Self-efficacy, or one's belief in their ability to complete a course of actions to achieve a certain goal, is a mediating factor between the learning experience and one's interests, goals, and actions[2,10]. Self-efficacy has been used to predict success in high-school STEM advanced placement courses and the completion of an undergraduate STEM degree[11,12]. Additionally, science identity has been investigated alongside self-efficacy as a factor in STEM persistence. Stets et al demonstrated that the extent to which an individual sees themself as a good science student predicts their persistence to a degree[13]. Byars-Winston improved the SCCT model by including science identity as a mediating factor in decision-making alongside self-efficacy[14].

Over the past three years, we have collected data from three incoming fall cohorts: pre-pandemic (Fall 2019), early-pandemic (Fall 2020), and mid-pandemic (Fall 2021). We used data from Burbio®'s School Opening Tracker to determine the extent to which each cohort experienced disruptions to their education in their final year of high school[15]. Pre-pandemic students did not have any pandemic-related disruptions to instruction, early-pandemic students had two-to-four months of disruption beginning March 26th, 2020 when the state of Alabama announced all schools would be remote for the remainder of the school year, and mid-pandemic students had varying disruptions for over a full year. We previously reported that pre-pandemic and early-pandemic students showed no differences in science identity and intention to pursue a career in science[16]. In the present work, we offer a similar comparison of the pre-/early-pandemic cohorts to the mid-pandemic cohort. These observations offer insight into how the year-long disruption impacted students and suggest that chemistry instructors should consider how to address an audience that may need different instructional support.

The primary goal of this study was to determine the extent that secondary educational disruptions affected our most recent cohort's self-efficacy, science identity, and STEM career intentions compared to those from the beginning of and before the pandemic. This work is the only to date that has compared data from before and during the COVID-19 pandemic regarding self-efficacy and science identity of STEM students. The data presented herein is of critical importance for chemical educators to support current students and to understand the extent of the impact of COVID-19 on the future STEM workforce.

I. Methods

a. Study Participants

Participants in this study were enrolled in the general chemistry laboratory sequence (GCLS) of a large public research university in the southern United States. The GCLS at this university consists of two courses offered over two semesters that fulfill the laboratory requirement for a broad range of STEM majors. Each of these one-credit hour courses are taught by a team of a graduate student teaching assistant and an undergraduate student teaching assistant. The first course, general chemistry I laboratory (CH116), is primarily taken by STEM majors during the first semester of residency at the university. The approach of the GCLS is described as modified guided inquiry. Detailed procedures are typically only provided when necessary for safety or to reduce complexity. The content of the laboratory reinforces content from the lecture course and includes instruction on the proper use of common quantitative equipment and glassware.

Table 1: Breakdown of Incoming Cohorts 2019-2021

Cohort	**Semester**	**Number of Participants *(N)***	**Likely Disruptions**
Pre-pandemic	Fall 2019	790	No disruptions
Early-pandemic	Fall 2020	721	2-4 months of remote or hybrid instruction
Mid-pandemic	Fall 2021	649	Greater than 1 year of remote or hybrid instruction

For this study, only first-semester students in the CH116 course were surveyed. Data from three cohorts of participants were compared over three years, from fall 2019 to fall 2021. These cohorts are categorized by their proximity to pandemic-related instructional closures. Table 1 breaks down data collections by timing, number of participants, and description of likely educational disruptions. The pre-pandemic cohort is considered as a negative control, having their studies unaffected by the pandemic. The early-pandemic cohort is defined as having two to four months of instruction disrupted and the mid-pandemic cohort is defined as having over a year's worth of instruction disrupted.

b. Survey Administration

Students were asked to participate in this study by their instructor of record at the beginning of the semester each fall through Canvas (IRB#: 300003153). They were provided a hyperlink to the survey administered through Qualtrics. The landing page of this survey was an information statement outlining the study. This survey was available during the first two weeks of a fourteen-week term. Participants were offered course credit in the form of bonus points on their final point total if at least 90% of students completed the survey.

c. Survey Structure

Participants completed a 15-item questionnaire at the beginning of each fall semester. Table 2 gives all items separated by source and measured construct. The first item contained an information statement and consent question. Items two through nine contained measures of science self-efficacy, science identity, and intention to pursue a STEM career. The remaining 6 items gathered demographic and course-specific information.

Science self-efficacy was measured using three items from a chemistry self-efficacy scale17 (Q2, Q4, and Q7), as well as three items of research self-efficacy created by Byars-Winston[14] (Q3, Q5, and Q6). Participants were given the prompt: "The following items ask about your confidence in completing various scientific tasks. Please answer with the response that most describes you. How confident are you in your ability to..." Responses were organized on a 5-point Likert scale ranging from "not confident at all" (1) to "extremely confident" (5). The six responses were averaged to obtain a science self-efficacy score ranging from one (low self-efficacy) to five (high self-efficacy).

Science identity was measured by a single indicator (Q8)[13]. Students were asked to rate how they viewed themselves as a science student from 0 to 10 with zero (0) being "not at all good" and ten (10) being "excellent". A higher rating on this indicator represents a more positive view of oneself as a science student. Participants were also asked about their intention to pursue a career in science using a single item (Q9). A response of zero (0) indicated that they do not intend at all to pursue a science career, and a response of ten (10) indicated that they have strong intentions to pursue a STEM career.

Table 2: Survey Items Broken Down by Measured Construct

Construct	**Item #**	**Type**	**Item Text**
Science Self-Efficacy	2	5-point Likert scale *(extremely confident to not confident at all)*	Propose solutions to everyday problems using chemistry
	3		Conduct an experiment to solve a scientific question
	4		Collect data during the chemistry laboratory
	5		Complete a degree with a science major
	6		Present a research talk or poster
	7		Interpret data in laboratory sessions
Science Identity	8	10-point scale	On a scale of 0-10, how would you rate yourself as a science student?
Intention to Pursue STEM	9	10-point scale	On a scale of 0–10, please rate how likely you are to pursue a science or STEM-related career *(e.g. chemist, biologist, medical researcher, forensic scientist, engineer, policymaker, science educator, etc.)*

d. Statistical Analysis

SPSS was used to perform all analyses on survey data. Inter-item reliability for the six self-efficacy items was confirmed using Cronbach's alpha using the entire dataset (a=0.841, n=6124). Values above 0.70 are acceptable for items within the same construct grouping18. Data for science self-efficacy, science identity, and intention to pursue a science career were compared between the 2019-2021 cohorts via comparison of means t-tests. To deal with unequal sample sizes, Levene's test for equality of variance was performed before all comparison of means testing. For groups with unequal variance, Welch's t-test was used.

II. Results

a. Science Self-efficacy, Science Identity, and Intention to Pursue a STEM career

Average values for science self-efficacy, science identity, and intention to pursue a science career were calculated and compared from fall of 2019 to fall of 2021. Table 3 contains the descriptive statistics for each collection broken down by construct.

Construct	***Collection***	***Mean***	***Std. Deviation***	***Std. Error Mean***
Science Self-efficacy	2019	3.4042	0.64958	0.02311
	2020	3.4166	0.63338	0.02359
	2021	3.2910	0.61805	0.02426
Science Identity	2019	7.00	1.440	0.051
	2020	7.10	1.427	0.053
	2021	6.91	1.368	0.054
Intention to pursue a STEM career	2019	7.54	2.622	0.093
	2020	7.70	2.585	0.096

Table 3. Descriptive Statistics for Science Self-Efficacy, Science Identity, and Intention to Pursue a Science Career

Figure 1 shows the comparison of means between collections from 2019-2021 separated by construct. From 2019 to 2020, we observed no difference between the means for self-efficacy, $t(1509) = -0.37$, $p = .709$, $d = -0.02$, 95% CI [-0.12, 0.08], science identity, $t(1509) = -1.39$, $p = .164$, $d = -0.07$ 95% CI [-0.17, 0.03], and STEM career intentions, $t(1509) = -1.16$, $p = .245$, $d = -0.06$, 95% CI [-0.16, 0.04]. We then compared the means from the 2021 collection to the previous two collections. We observed an average self-efficacy score in 2021 that was lower than both 2019, $t(1437) = 3.36$, $p = 0.001$, $d = 0.18$, 95% CI [0.07, 0.28], and 2020, $t(1368) = 3.71$, $p < .001$, $d = 0.20$, 95% CI [0.09, 0.31].

The means for science identity differed significantly between 2020 and 2021, t(1368) = 2.35, p = .011, d = 0.14, 95% CI [0.03, 0.24]. For intention to pursue a STEM career, the mean for 2021 was higher than both 2019, t(1437) = -6.14, p < .001, d = -0.33 95% [-0.43, -0.22], and 2020, t(1368) = -4.8, p < .001, d = -0.27 95% CI [-0.37, -0.16].

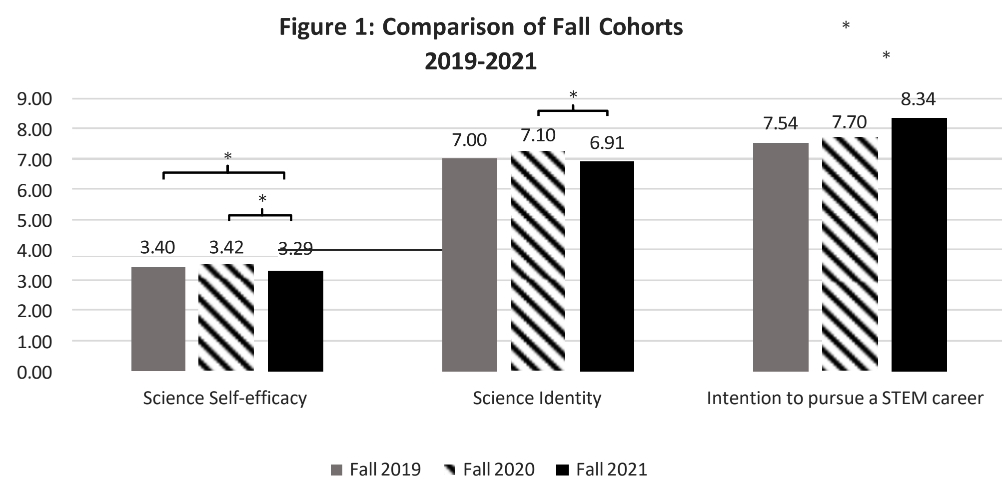


Figure 1. A comparison of three fall undergraduate student cohorts from 2019 to 2021. The figure shows the science self-efficacy, science identity, and intention to pursue STEM averages for each cohort. Independent samples comparison of means t-tests were used to differentiate between groups. *p<0.050.

III. Discussion

Fall 2019 and fall 2020 data are consistent with previously published results that construct scores for STEM students were mostly unaffected by the onset of pandemic-related closures[14]. Specifically, the 2019 freshman cohort reported similar averages for science identity and intention to pursue a STEM career at the beginning and the end of the spring 2020 semester, so it is not surprising that similar construct scores were observed between the cohorts from fall 2019 and fall 2020. Initially, we expected the pandemic's onset to affect opportunities for STEM students between March and August of 2020. However, there are many confounding variables that could have resulted in similar construct scores between the fall 2019 and fall 2020 cohort, who, at the time, had only experienced the effects of COVID-19 for several months.

The mid-pandemic cohort who experienced hybrid or remote instruction in their last year of high school reported lower science self-efficacy scores than the prior two cohorts (fall 2019 and fall 2020) and lower science identity scores than the prior year (fall 2020), though both differences had weak effect sizes. According to Burbio®, school disruptions varied significantly throughout the 2020-2021 school year for Alabama[15]. Most schools met remotely at the beginning of the school year, but by the end of the spring 2021 semester, most were back to face-to-face instruction with fluctuations throughout (see supporting information). It is not surprising that lower self-efficacy scores were observed among our most recent student population, but the weak effect size indicates this difference may not be generalizable.

Surprisingly, the mid-pandemic cohort (2021) reported higher STEM career intentions than the prior two cohorts with a small-to-moderate effect size. Increased interest in STEM-related careers, like medicine, has been observed during times of societal hardship and when the media takes an interest in the medical profession19. In 2021, the Association of American Medical Colleges reported an 18% increase in applications to medical school. This observation was called a “Fauci effect” due to the prominent media coverage of Dr. Anthony Fauci’s efforts to inform the public throughout COVID-19[19]. Many students who designate a pre-medical emphasis in their studies pursue a STEM degree, though we are unsure if our respondents considered “physician” a STEM career when completing our questionnaire. Despite this, the timing of this effect is consistent with our data collection, and its proximity to our observed change in STEM career intentions should be noted.

The apparent increase in our incoming student’s intention to pursue STEM might point to an influx of individuals who are interested in STEM entering the undergraduate sphere. While STEM career intentions tend to form initially at an early stage of life, the phenomenon of STEM attrition within the first one-to-two years of undergraduate study is well documented especially among groups historically marginalized by STEM, such as women and under-represented minorities[1,20-22]. In the pandemic’s aftermath, it will be more important than ever to create a welcoming and inclusive environment in the chemistry classroom. Several strategies to reduce equity gaps in chemistry classrooms were outlined in a recent article, and include implementing group work, validating student’s identity as scientists, and allowing students to make mistakes, among other factors[23].

Due to the universal nature of the pandemic, it is possible that other institutions might observe similar fluctuations in self-efficacy. As a result, it is important to discuss strategies that promote student confidence in chemistry classrooms. In general, evidence-based instruction practices, such as student-centered instruction and active learning, have been identified as beneficial for STEM students regarding their self-efficacy and retention in STEM programs[24-

[26]. Active learning especially might have been difficult to implement in remote and hybrid learning environments and will be critical with the return to face-to-face instruction in addition to other practices that promote self-efficacy, like undergraduate research experiences and student mentorship programs[27-28].

In the wake of the COVID-19 pandemic, chemical educators will benefit from the continued push towards including evidence-based instructional practices and creating a welcoming environment in postsecondary chemistry classrooms. While our data cannot support the direct influence of absent laboratory experiences, the laboratory is an environment where beneficial instructional practices are easily incorporated, like inquiry, active learning, and peer instruction. We advise caution with incorporating strategies made prominent throughout the pandemic excessively, such as recorded lectures and virtual attendance. These adaptations were necessary throughout the pandemic, but it is crucial to lean into practices that are best served by the face-to-face environment.

IV. Limitations

A limitation of this study is the potential for regional effects, as students who attend the university where this study was performed are primarily from five counties in the southeastern United States. This may make extrapolation of these data to other regions difficult, as state policies and procedures regarding the COVID-19 pandemic have varied widely in the United States. Additionally, science identity and intention to pursue a STEM career were one-item indicators, which limits the implications that can be drawn from the observed scores. The measure used for science identity has been validated previously[13], and similar one-item measures have been used for intention to pursue a STEM career[29]. The present work demonstrates the importance of continuing to collect long-term data on self-efficacy and science identity in the aftermath of COVID-19. Our future work will include collecting data for the upcoming cohort (fall 2022) to determine if the observed changes are consistent. Additionally, future work should investigate the relationship between educational practices in laboratory settings and self-efficacy among introductory STEM students.

V. Conclusions

In the present work, we offer a comparison of three incoming groups of general chemistry students over three years (2019-2021). This comparison allows us to observe changes in factors relevant to STEM interest and persistence over time since the onset of the pandemic. Specifically, we were interested in the differences between the most recent incoming student group (fall 2021) and previous student groups. Our results show that the Fall 2021 cohort had lower self-efficacy and higher STEM career intentions than the previous two cohorts, but the effect sizes indicate these results may not be generalizable outside our student population. Still, our observations provide a valuable comparison to other chemical educators who are concerned with the state of chemistry students and STEM persistence throughout the COVID-19. This work further emphasizes the need for continued investigation of these and similar constructs in undergraduate chemistry courses and suggests that chemistry educators may need to monitor students' sense of community and science self-efficacy.

The Supporting Information is available at https://pubs.acs.org/doi/10.1021/acs.jchemed.2c00312.

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CHAPTER 4

A CURE FOR MICROPLASTICS: INTRODUCING FIRST-YEAR STUDENTS TO ENVIRONMENTAL CHEMISTRY THROUGH UNDERGRADUATE RESEARCH

by

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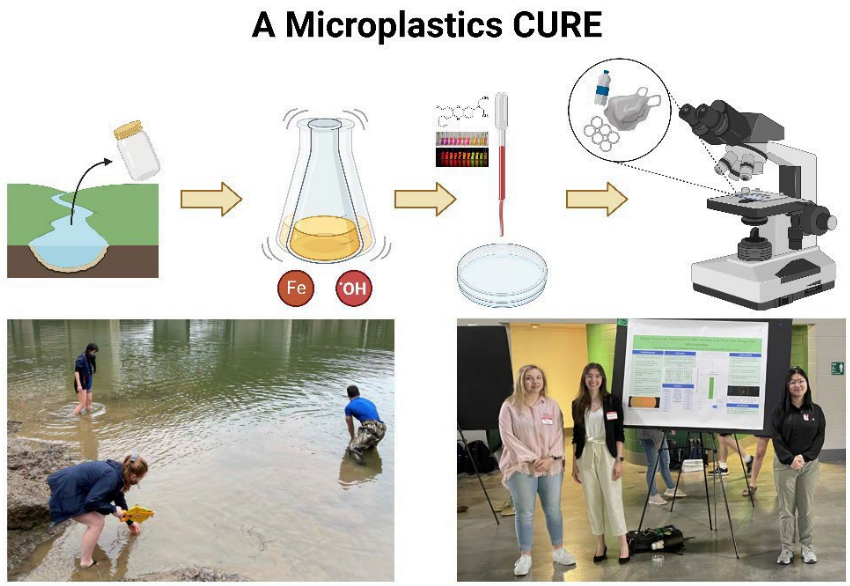
Abstract

Course-based undergraduate research experiences (CUREs) have been shown to result in increased STEM persistence, but such experiences are rarely available for first-year STEM students. This work outlines the development and integration of a CURE into a year-long undergraduate general chemistry laboratory sequence involving the detection of microplastics in a local watershed with Nile Red. This CURE was implemented over three academic years in collaboration with a local environmental preservation organization. Samples were collected as part of a field sampling trip and a pre-/post- questionnaire was administered each semester. The questionnaire investigated how this CURE impacted students' self-concept as scientists and behaviors regarding plastic use. Additionally, interviews were conducted with CURE and non-CURE laboratory students. Outcomes of participation in the CURE versus the traditional (non-CURE) sequence are discussed, including pre/post questionnaire results, inductive coding of interviews, and microplastic counts in Cahaba River water samples. Results demonstrate that this CURE is beneficial for students' scientific self-concept and results in self-reporting of environmentally conscious behaviors around plastic use. This experience is appropriate for first-year STEM students but can be scaled to an upper-division laboratory or scaled down to a one-to-three week-long laboratory activity.

Graphical Abstract



I. Introduction

Participating in undergraduate research benefits science, technology, engineering, and mathematics (STEM) students. These benefits include increased interest, engagement, and persistence in STEM[1-4]. Despite this, undergraduate student participation in research experiences remains low, especially in spaces where the traditional research mentor and student ratio is 1:1[3, 4]. There are many factors that contribute to the low participation rate including faculty selectivity, limited space, scheduling, costs, and safety regulations. To address these limitations, chemistry laboratory courses have been adapted into course-based undergraduate research experiences (CUREs)[5-8], which give students an opportunity to participate in undergraduate research in a classroom setting.

CUREs are rarely available to first-year students but have been shown to positively affect graduation rates for STEM majors[9-11]. CUREs are a more equitable way for STEM students to conduct original research throughout their undergraduate careers. These laboratory courses are typically upper-division (third-year or fourth-year) courses, though some early undergraduate CUREs have been developed for introductory biology[11, 12]. Students at a late stage in their undergraduate studies are far less likely to transition to entirely new areas of study, thus, there exists an untapped potential for CUREs to benefit STEM students much earlier in their careers. Therefore, general chemistry is a fertile area for designing novel CURE curricula and investigating its impact on students' self-concept as scientists.

While in a CURE, students review relevant literature, perform original experiments, analyze data, and present their findings for novel questions in the instructor's field of study. They are unique in that they provide crucial early research experiences to undergraduate STEM students, but chemistry CUREs often lack sample collection in the field. Field experiences have been shown to be beneficial for problem solving confidence and ability in biology[3, 13]. CUREs with field experience are somewhat limited to field-based disciplines, with several iterations present in biology and ecology[9, 11, 12]. There then exists an opportunity for the inclusion of a field-based CURE in general chemistry. Developing more field-based CUREs will not only introduce early STEM students to the entire scientific process but will provide more students with the opportunity to conduct authentic research in a field setting[14, 15].

a. Quantifying Microplastics in a Local Waterway as a CURE Investigation

The full potential of a CURE involves incorporating societally relevant topics that both encourage engagement with the CURE and develop a knowledgeable, well-rounded STEM workforce. Microplastic pollution has become an emerging, interdisciplinary research topic because of commercial plastic consumption and waste management[16-19].

Microplastics are a ubiquitous environmental presence that are not well understood in environmental and biomedical research, and methods to detect and quantify microplastics are not standardized[20, 21]. The most common method is visual detection with digital microscopy, but this method is unreliable[21, 22]. Other more robust spectroscopic methods, including FTIR spectroscopy and Raman Spectroscopy, are more accurate but require time, expensive equipment, and highly specialized professionals[23-25]. A fast, cost-effective method for microplastic detection and quantification has not been identified. This gap offers many opportunities within a teaching laboratory. Students can use real-world data to propose a variety of research questions ranging from improving a technique to observing microplastic content in authentic samples. Student results are expected to require error analysis and there are opportunities to discuss how research findings can be published even when scientists know that a better experiment might yield more confidence in the results.

In this CURE, students in a general chemistry laboratory sequence designed original research projects regarding microplastics detection. To answer their research questions, they collected and analyzed water samples for microplastic pollution from a local freshwater source, the Cahaba River, in collaboration with our community partner, the Cahaba Riverkeeper. This partnership was important because it allowed for authentic field experience and allowed students to meet an external stakeholder.

To ensure that our project met student learning goals and investigate how the experience might impact science identity and persistence in STEM, we collected quantitative and qualitative data. The data covers three years of implementation with over 300 CURE participants and others in a traditional laboratory curriculum. The quantitative data was collected with a pre/post survey and qualitative data was assembled from interviews of CURE participants (N=12) and non-CURE participants (N=12). These interviews were semi-structured to allow students to self-report meaningful experiences with the CURE. Specifically, participants from the CURE sequence and the traditional sequence were asked to discuss elements of their laboratory courses that made them most feel like a scientist and most confident in doing scientific work.

II. Course Design

a. Learning Objectives

The following laboratory activities, homework assignments, and assessments were designed and modified in a first-year general chemistry laboratory at a large public university in the Southeastern United states. The activities were field tested in the 'honors' sections of the laboratory sequence; however, these sections used the same common laboratory equipment present for other sections. Some equipment used in this CURE may not be available for the standard curriculum of other general chemistry laboratories, like FTIR spectroscopy and fluorescence microscopy, but collaborations with other laboratories or other common visual and spectroscopic techniques can be employed.

In line with the existing laboratory pedagogy at the institution, activities were designed to engage students in active-style inquiry learning. All activities are described in the following sections, and all laboratory handouts, assignments, and instructional materials are included in the Supporting Information (see Appendix B). To guide the design of this activity, the following learning objectives were chosen for this CURE:

1. Design an experiment using analytical and spectroscopic techniques to answer a scientific question.
    a. Evaluate the efficacy of an experiment using accuracy and precision measurements.
    b. Explain the theory and application of FTIR spectroscopy in microplastics research.
    c. Determine the identity of an unknown plastic material.
    d. Analyze natural water samples for microplastic pollution using fluorescence microscopy.
    e. Apply proper pipet and dilution techniques to a new context.
2. Explain the connection between individual choices and behaviors to ecological settings in a natural waterway.

3. Apply chemical theory to explain phenomena observed in the laboratory.

b. Outside Stakeholders

CUREs, like any project-based intervention, require positive student engagement and buy-in for effective implementation. One way to promote this in a CURE is the inclusion of outside stakeholders, expanding the impact of student work beyond the classroom. This CURE began as a collaboration between the UAB Chemistry Department and the Cahaba Riverkeeper, who oversees water quality analyses of Birmingham's most significant watershed, the Cahaba River. The Riverkeepers were monitoring microplastic values using common field procedures but were interested in developing a more accurate method and increasing the number of samples collected. The chemistry department offered to explore both goals by involving general chemistry students as part of the honors laboratory course. Therefore, the course acts as an analytical laboratory for analyzing environmental samples, modeling authentic analytical laboratories, who process samples for toxicology, ecology, and environmental science researchers. Student data from this CURE that reached an appropriate level of precision is then summarized and given to the Riverkeeper for dissemination to the broader community.

c. Field Experience

In each semester of this microplastics CURE, general chemistry students participated in a sampling trip alongside scientists from the UAB Chemistry

Department and the Cahaba Riverkeeper. Several Cahaba River sampling sites from the eleven in the Birmingham area were chosen based on student safety, availability of parking, proximity to the university, and ecological significance to the watershed. One week of class time was dedicated to facilitating the trip, which consisted of a tour of the Cahaba Riverkeeper office and lab, carpooling to the chosen sampling site, and assisting students as they collect one liter standing water samples. The sampling trip incorporated an important interdisciplinary connection to the general chemistry laboratory curriculum in the realm of authentic environmental science, which is often absent from analytical labs. This allowed individuals who have diverse scientific interests to participate in this CURE and demonstrated the potential for scientific work to be collaborative and interdisciplinary.

d. CURE Overview

Microplastics were first detected in the environment in 2010 and their impact on human health has been an area of wide concern[16-20]. Laboratory exercises for use in teaching laboratories involving microplastics have been described, but these procedures rarely require more than one laboratory period[26, 27]. CUREs involve immersion into the research topic of interest, and as such, require a significant amount of class time to accomplish. This microplastics CURE was integrated into a 2-semester honors general chemistry laboratory sequence but can easily to be adapted into a one semester or one-to-three week-long activity as needed. We adopted the 2-semester model with the knowledge that some students may join the lab for the second semester only, thus, we allowed students who only participated in semester 2 to be grandfathered into established projects.

A summary of the course calendar for semester 1 of the CURE is shown in Table 1. The first five to six weeks were allotted to perform several traditional general chemistry laboratory experiments, followed by a midterm practical. Then, a sampling trip was performed in the week following the practical. Weeks 9-15 of semester one included activities to prepare students for their original research projects to be completed in semester 2.

Table 1. An Overview of Semester 1 (CH126)

Week	In-class Activities	Out of Class Activities
1-6	Traditional gen chem lab activities	Graphing with Excel Experimental Design
7	Midterm Practical	N/A
8	Cahaba River Sampling Trip	N/A
9	Intro to Polymer Chemistry (POGIL)	Writing a Research Question
10	CURE day 1: Writing a Research Question	N/A
11-14	CURE day 2-4: Analyzing Microplastic Content	Proposal Draft Submissions
15	Final Presentations	N/A

Table 2 shows a summary of semester 2. The first section of the course was the same as semester 1, though only five weeks long. This section concluded with a midterm practical, followed by another sampling trip to the Cahaba River. The second part of the course was defined as “open research.” These weeks were allocated for students to complete their original research projects, and this period concluded with a final presentation at the university’s spring research exposition.

Table 2. An Overview of Semester 2 (CH128)

Week	In-class Activities	Out of Class Activities
1-5	Traditional gen chem lab activities	Materials List and Timeline Submission
6	Midterm Practical	N/A
7	Cahaba River Sampling Trip	N/A
8-14	CURE: Open Research	Poster Draft Submissions
15	Spring Research Expo	N/A

e. Microplastics Screening

The microplastics screening method used in this CURE is based on an upper division laboratory activity designed by Scircle et. al[26] utilizing fluorescence microscopy and Nile Red as a hydrophobic fluorescent tag. Nile Red is a common method for screening environmental samples for microplastics and has been used in various contexts to confirm the presence of polymers in environmental samples, drinking water, and biota[28-31]. This method capitalizes on the hydrophobicity of polymer particles in comparison to particles naturally present in natural water samples, as the dye adsorbs to polymer surfaces mainly via Van der Waals interactions[32]. Such interactions are negligible between the dye and inorganic sediment; however, the dye can adhere to biologically derived organic matter present in natural water sources and presents the possibility of false positive results. Thus, a digestion step is added to target natural organic matter and exclude chemically resistant anthropogenic materials, like plastics. In this experiment, Fenton's reagent was used.

f. Traditional General Chemistry Labs and Midterm Practical

To ensure that CURE participants acquired the same laboratory skills developed in the traditional sequence, a select number of one-off laboratory activities were included in addition to a midterm practical each semester. Traditional activities were pulled from the existing general chemistry curriculum of structured inquiry, one-off laboratory activities covering basic quantitative techniques (volumetric pipetting, titration, calibration curves) and instrumentation (UV-visible spectroscopy). For each activity, students completed a pre-lab assignment, a pre-lab quiz, and reported results of their experiments on an in-class assignment.

The midterm practical was an individual demonstration of competency with select quantitative techniques and associated calculations. Students were given the glassware necessary to answer a scientific question and given 20-30 minutes to gather the data needed. The practicals were taken directly from the traditional curriculum and laboratory activities were chosen that were aligned with the techniques addressed in the practical exams. For example, if the practical involved UV-visible spectroscopic measurements, at least two laboratory activities that used this technique were chosen to be completed before the midterm.

g. Cahaba River Sampling Trip

Each semester, students participated in a sampling trip that occurred the week after the midterm practical. This trip acted as the field experience element of the CURE, and often involved direct interaction with expert scientists from the Cahaba Riverkeeper. The goal of the sampling trip was to introduce chemistry students to environmental research methods and to expose them to the work of the Riverkeeper organization. Each trip began with a brief tour of the Riverkeeper office located in Vestavia Hills, Alabama, and followed with preparing sampling jars for transport to the sampling site. Students then carpooled to a common sampling site in semester 1, and after a demonstration of proper grab sampling technique, each gathered a one-liter Cahaba River water sample. Samples were then transported to the laboratory space for short term storage, or in 4 degree C freezers for long term storage. In semester 2, students determined which sampling sites were necessary to answer their research questions. For example, some sampling sites were located upstream from a wastewater treatment plant, and others were located downstream. Additionally, some students were interested in other environmental samples, such as sediment and larger plastic debris, so students were allowed to alter their sampling methods to suit their project.

h. Student Safety

Students were asked to carpool to the sampling sites if able. Students who were unable to safely drive or carpool were excused from the trip, and a grab sample was collected for them. Safety guidelines were modelled after the following policy at https://www.ccis.edu/about/policies/student-field-trip. Sampling locations were scouted prior to every excursion to ensure student and

TA safety.

Sampling trips were held only during daylight hours. Evening sections were invited to attend with other sections or samples were collected for them. Students were required to wear closed toed shoes during sampling, as grab sampling for microplastics requires at least 2-feet of depth, which made it necessary to wade into the river for some sampling locations. Students were not expected to travel to collection sites unsupervised. At least one graduate TA and Riverkeeper scientist was present at every sampling trip to ensure proper sampling and student safety at the site. All participants were always in the eyesight of the TAs during sampling and, prior to sampling, guidelines for water safety were described for the students. Lastly, a "buddy system" was required for students entering the water.

After sampling, all procedures other than imaging were completed in a teaching laboratory. Students were required to wear proper attire in the lab at all times (i.e. close-toed shoes, long pants, goggles). Fenton's reagent requires 30% hydrogen peroxide, and students were required to wear long sleeves or a lab coat when using it. Filtrates throughout the procedure were discarded in the sink.

i. CURE Weeks/Open Research

The student facing materials described in this section are included in Appendix C. In semester 1, CURE weeks were added so that students could become familiar with the background and standard methods for microplastic screening. Specifically, three original activities were added. First, students completed a guided-inquiry activity on the basics of polymer chemistry. Next, students completed an in-class activity designed to lead them through a literature review to determine a research question. Finally, students performed a standard screening procedure with Nile Red on environmental samples collected during the semester's sampling trip.

The purpose of the polymer chemistry guided inquiry assignment was to give students a basic understanding of commercial polymers, the source of a majority of microplastic pollution. One three-hour period was allotted for students to complete the activity. The assignment introduced the basics of polymer chemistry, including polymer classes, nomenclature, and polymer characteristics and is available in the supporting information. Students were given a homework assignment the previous week to complete the assignment individually with no outside help. They were told that the assignment was intended to teach them, not assess their learning, so they should guess even if they did not know the answers. In the next class period, group members came together and filled out a group worksheet comparing their answers, explaining their initial thought process, and reaching a consensus. Finally, the group presented their final answers to the TA who graded based on a key, and students were given the opportunity to correct their answers and provide an explanation.

The following week, students completed an activity (Writing a Research Question) designed to demonstrate the process of literature review, reference management, and proper ACS formatting of references. In this assignment, students downloaded free citation management software (Zotero, Mendeley, etc.)

and compiled a small set of references relevant to a desired project on the topic of microplastics quantification. Their submission for this activity was a research question, a short description of their proposed project, and at least five references in ACS format. In the remaining weeks leading up to the final, students performed a standard analysis procedure using Nile Red to stain for microplastics in positive control samples and natural samples collected during the sampling trip, and they analyzed images of their samples taken with fluorescence dissecting microscopy (Nikon AZ100) via ImageJ.

The CURE weeks in the second semester began with a review of the research questions developed in the prior course. Students that were new to the sequence were allowed to join groups with vetted research questions and the groups were allowed to modify their research questions with approval. Weeks 6-15 in semester 2 were designated as “open research,” meaning this was the time allotted for students to complete their original projects. A pre-lab assignment that described the group's laboratory objectives and procedures was due 48 hours prior to each meeting since each group project might have different objectives each week. A progress report was due withing 24 hours after each laboratory meeting. This report provided written observations and initial interpretation or visualization of the data. This allowed the TAs to model the role of research advisor and provide help with troubleshooting, analyzing data, and ensuring sufficient progress.

j. Out-of-class Activities

Students in the CURE were assigned several homework assignments throughout the sequence. The goal of these assignments was to introduce students to concepts relevant to their project (experimental design, excel, literature review) and to assist students in the completion of their final research proposal (semester 1) and final poster presentation at the university's undergraduate research exposition (semester 2), respectively. In the first half of semester 1, students used excel to generate a calibration curve using a provided dataset and used the results to comment on the acceptability of the curve for their Graphing with Excel assignment. For the Experimental Design assignment, students were introduced to basic terms related to experimental design and used the new terminology to assess example experiments. Students then submitted a draft introduction and methods sections for their research proposals for the remainder of semester 1. In semester 2, homework assignments served as deadlines for students to demonstrate progress in their original projects. First, students were given a deadline to submit a materials list and timeline for their project so the proper reagents could be ordered. Leading up to final poster presentations, students were given deadlines to submit poster drafts for evaluation and feedback by TAs.

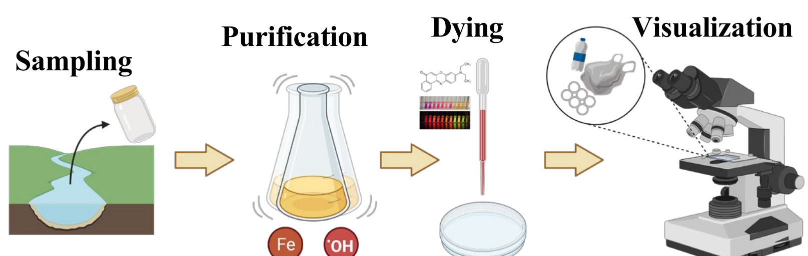
k. Final Presentations

A final presentation was required each semester. In the first semester, students presented a background section leading to their research aims as a 7-10 minute presentation. This presentation allowed for peer review and instructor feedback. Presentations were evaluated via a combination of TA feedback (70%), peer feedback (25%) and participation (5%). TAs and students used a rubric that evaluated the overall group presentation and the presentation skills of individuals. As a part of their participation grade, students were required to ask at least one question to one other student group during the time allotted for questions.

l. Spring Research Expo

In the second semester, students presented the results of their experiment as a poster to the university-wide undergraduate research day exposition. These posters were initially evaluated by the TA and departmental faculty in class using a rubric similar to the one used by the exposition, and students were allowed to modify their poster prior to attending the exposition.

III. Methods



a. Microplastics Screening

Figure 1. Workflow for microplastics screening. In this laboratory activity, 1-L standing water samples were collected from the Cahaba River and filtered for suspended solids. The solids were then purified by a digest with Fenton's reagent. The remaining solids were then dyed with Nile Red and visualized via digital fluorescence microscopy.

Grab samples were collected in 1-L wide mouth amber glass jars from one of eleven sites along the Cahaba River. Samples were taken in at least two feet of depth so that jars could be completely submerged and upstream from the person sampling so that fabric particles were not introduced. The jar was submerged with the opening face down and flipped while underwater to collect water below the surface. Samples were transported from the sampling site to a 4 °C cooler until filtration. Suspended solids were removed from the water via vacuum filtration on a polycarbonate track etched (PCTE) filter membrane (⌀ = 47 mm, pore size = 1.0 μm), and the filtrate was discarded.

Fenton's reagent was prepared by dissolving iron (III) sulfate in water in a 1:15 ratio (m/v), then adding around 3mL of concentrated sulfuric acid (before the total final volume is added), creating a 0.05 M stock solution named "Fenton's reagent stock". The entire PCTE filter membrane including solids was then placed in an Erlenmeyer flask and 20 mL of the Fenton's reagent stock was added. Organic material was dissolved by the addition of 30% hydrogen peroxide starting with slow addition of three 10-mL aliquots of hydrogen peroxide. The flask was left on the benchtop for 5 minutes, then heated slightly on a hot plate until bubbles began to form. Each aliquot was allowed to react completely until bubbles stopped forming before the addition of another. More 10-mL aliquots were added to the mixture if organic matter was observed in the flask after the first three. The reaction is exothermic, so the flask was allowed to cool before the next step. The PCTE filter was rinsed with DI water and removed from the flask.

The mixture was filtered again on a new PCTE filter to separate Fenton's reagent from the remaining solids, and the filtrate was discarded. Nile Red (99%) was obtained from ThermoFisher Scientific, Inc. A Nile Red stock solution was prepared by dissolving solid Nile Red in acetone in a 1:1 ratio (m/m) named "Nile Red stock". A working solution was then prepared by performing a 1:20 dilution of the stock Nile Red solution in acetone. The remaining solids were then transferred from filter membrane to a clean beaker with around 30 mL of water. Then, around 3mL of the working Nile Red solution was added to the solution and stirred. The mixture was then left for at least an hour before a final filtration was performed with another PCTE filter. The filter was then placed in a sealed petri dish for imaging.

Imaging was performed using a Nikon AZ-100 dissecting scope. A camera (Andor-Zyla 5.5 sCMOS) was used to capture images with the microscope. Images were analyzed using the open-source software, ImageJ. Particles were counted visually, sized, and identified as either a fiber, shard/fragment, or sphere.

b. STEM Persistence Measures

Social psychological constructs related to STEM persistence were measured using a 20-item survey as collected for our interpretation of the effects of the 2019/2020 pandemic[33, 34]. The survey contained items adapted from the Persistence in the Sciences measure (PITS), the Chemistry self-efficacy scale, a research self-efficacy measure developed by Byars-Winston, and several environmental behavior items adapted from Biga[35]. Other original items were added to gauge students' intention to persist in STEM and their ability to connect chemical phenomena to real world issues (named Knowledge of the Relationship). For example, students were asked to rate their agreement with the statement, "I can give two or more examples of ways chemists are working to help the natural environment." Most items were answered via a 5-point Likert scale ranging from "strongly agree" (5) to "strongly disagree" (1), or "extremely confident" (5) to "not confident at all" (1) for self-efficacy items. Items that measure the same constructs were averaged together to obtain an average construct score.

Only two items did not follow this Likert scale, which both were answered with a 10-point scale: a science identity item asking students to rate themselves as a science student and a STEM persistence item asking students to rate their likelihood to persist into a science career, with 0 being the lowest and 10 being the highest score. Table 2 shows each item broken down by construct and source.

Table 3. Questionnaire Items by Measured Construct and Source

Item	Scale	Source
Science Self-Efficacy		
SSE1. Propose solutions to everyday problems using chemistry.	5-point Likert scale: "extremely confident" to "not confident at all	Uzuntiryaki
SSE2. Conduct an experiment to solve a scientific question.		Byars-Winston
SSE3. Collect data during the chemistry laboratory.		Uzuntiryaki
SSE4. Complete a degree with a science major.		Byars-Winston
SSE5. Present a research talk or poster.		Byars-Winston
SSE6. Interpret data in laboratory sessions		Uzuntiryaki
Environmental Behaviors		
EB1. I support policy that bans single-use plastic bags at retail stores.	5-point Likert scale: "strongly agree" to "strongly disagree"	Adapted from Biga
EB2. I often volunteer my time to environmental causes involved in waste mgmt. and recycling.		
EB3. I make special effort to avoid single-use plastics in my daily life.		
EB4. I support charging a deposit on recyclable materials.		
EB5. I often avoid using products from companies that generate excessive plastic waste.		
EB6. I go out of my way to put paper and plastic waste into proper recycling bins.		
Science Identity		
SI1. In general, being a scientist is an important part of my self-image.	5-point Likert scale: "strongly agree" to "strongly disagree"	Persistence in the Sciences (PITS)
SI2. I have a strong sense of belonging to a community of scientists		
SI3. Being a scientist is an important reflection of who I am		
SI4. I have come to think of myself as a "scientist"		
Knowledge of the Relationship between Chemistry and the Environment		
KR1. I can give more than 2 examples of ways chemists are working to help the natural environment	5-point Likert scale: "strongly agree" to "strongly disagree"	original scale
KR2. I recognize chemical principles at		

work in the environment in my daily life.
KR3. I often see the relationship of chemistry to environmental issues
KR4. I am interested in learning chemistry the most when it relates to the natural environment.
KR5. I feel that my chemistry courses support relating content to environmental issues.
KR6. Environmental researchers apply chemical knowledge frequently in their work.

c. Participants

Students enrolled in the traditional (non-CURE) general chemistry laboratory and the CURE general chemistry laboratory were asked to complete the questionnaire in week 1 and week 13 of each full 14-week term. Participants were recruited by the instructor of record for the respective laboratory section who explained the purpose of the study and offered course credit as 3 bonus points to the course point total if students completed the survey. Participants were asked to generate a unique, 10-character identifier so questionnaires could be matched over time. Code matches were accepted if the Hamming's distance between the two codes was less than two (2). In the 2021-2022 academic year, 27 CURE participants and 23 non-CURE participants provided matched survey data. Only survey data from this year will be compared over time due to the influence of the COVID-19 pandemic on previous iterations of the CURE.

d. Quantitative Analysis

i. *Paired t-test*. To determine whether CURE participation correlated with increased STEM interest, identity, and self-efficacy, paired t-tests were used for matched student data. If the differences between pre- and post-test scores were not normally distributed, a non-parametric test (Wilcoxon signed rank test) was used. Differences in student's self-reported STEM interest, identity, and self-efficacy were determined to be statistically significant if $p \leq 0.05$.

ii. *Independent-sample t-test*. Construct averages for STEM identity, interest, and self-efficacy of participants in the CURE sequence were compared against the construct averages of participants in the traditional sequence within each data collection (pre- and post-) using independent samples t-tests. If construct averages were not normally distributed, a non-parametric test (Mann-Whitney U-test) was used. Averages between the CURE and traditional sequence were considered significantly different if $p \leq 0.05$.

e. Qualitative analysis

Inductive coding was used to identify emerging themes from student interviews. A specific focus was given to identifying CURE elements that students brought up when discussing what they felt was most beneficial about the lab, what made them feel the most confident doing science (science self-efficacy), and what made them feel the most like a scientist (science identity).

Codes were assigned independently by two coders and discrepancies were addressed collaboratively by the two original coders until a consensus was made. Mainly, themes were pulled from the following interview questions:

1. What did you find to be most beneficial about your general chemistry laboratory?
2. Describe a moment in your general chemistry laboratory where you felt most confident doing science.
3. Describe a moment in your general chemistry laboratory where you felt most LIKE a scientist.

IV. Results

a. Microplastics Identified via Nile Red

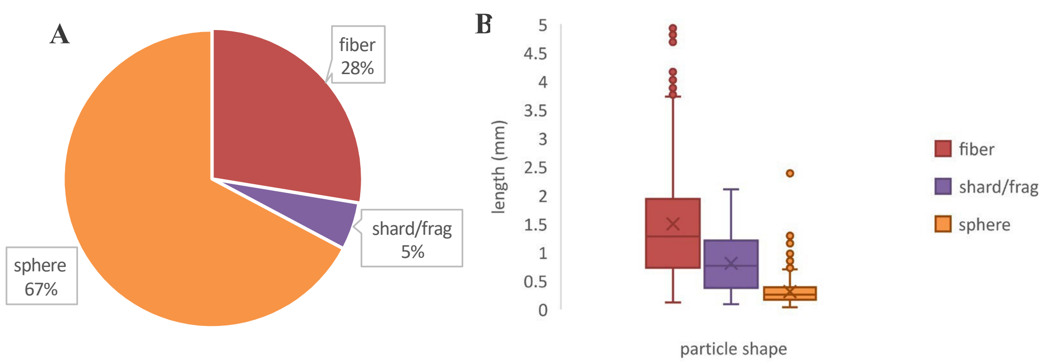


Figure 2. A summary of the size and shape of the microplastics identified in this CURE course since March of 2022. A) A breakdown of total particle counts by shape. B) Average particle length (mm) broken down by particle shape. Averages are shown with an x and outliers are shown as points.

Microplastics were observed in all environmental samples collected by students. Observations ranged from 1 particle per sample to as many as 112 particles per sample. Images showed three different particle shapes and students were able to quantitify them in the laboratory after images were provided by the

TAs. Most of the particles identified were fibers (381 particles) and spheres (929 particles). The remaining particles were shards/fragments (72 particles). Fibers were the largest particles on average (1.50 mm), followed by shards/fragments (0.81 mm) and spheres (0.31 mm). Twenty-eight particles were outside of the range to be considered microplastics (>5 mm) and were therefore excluded from the analysis.

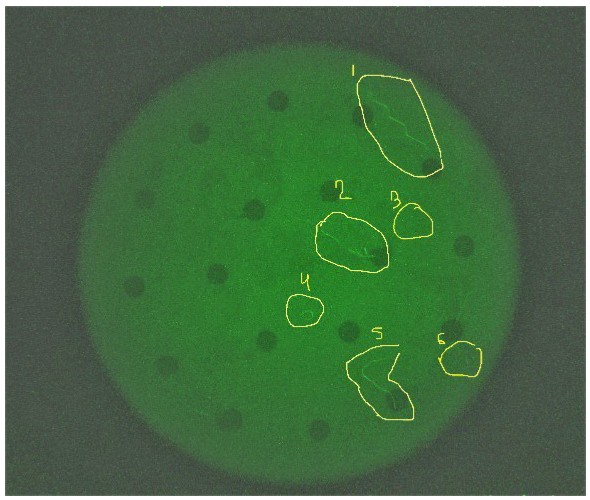


Figure 3. Imaging results of a Cahaba River water sample after processing, dying, and imaging. Fluorescing particles are circled and numbered. Images were taken at 1x magnification using a Nikon AZ-100 dissecting scope with an Andor-Zyla 5.5 sCMOS.

b. STEM Persistence Questionnaire

Figure 4 summarizes the differences in means for each STEM persistence construct broken down by the laboratory sequence (CURE or Traditional) for two subsequent iterations of the CURE. Only the differences between matched questionnaires are shown.

In the 2021-2022 academic year, CURE students reported gains in all measured constructs (SSE: $t(26) = 9.09$, $p < .000$, $d = 1.75$, 95% CI [1.14, 2.35]; EB $t(26) = 2.03$, $p = .050$, $d = 0.39$, 95% CI [0.00, 0.78]; SI: $t(26) = 2.77$, $p =$

.010, d = 0.533, 95% CI [0.12, 0.93], KR: t(26) = 2.16, p = .040, d = 0.42, 95% CI [0.02, 0.81]). The traditional sequence reported a significant gain for science self-efficacy only (t(22) = 4.38, p < .000, d = 0.91, 95% CI [0.42, 1.40]), with no change observed for all other constructs (EB: t(22) = 1.69, p = .105, d = 0.35, 95% CI [-0.07, 0.77]; SI: t(22) = 1.16, p = .260, d = 2.41, 95% CI [-0.18, 0.65]; KR: t(22) = 0.87, p = .393, d = 0.18, 95% CI [-0.23, 0.60]).

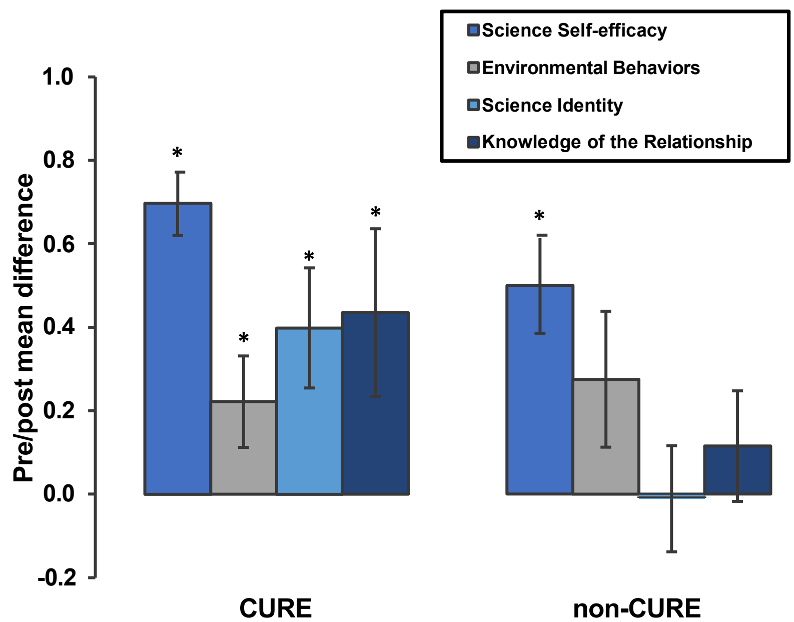


Figure 4. A summary of questionnaire results broken down by CURE and non-CURE participants in the 2021-2022 academic year. Bars show the pre/post difference in construct means. A negative value indicates a decrease in that construct's score for that group at that time interval. Error bars show the standard error from the mean. An asterisk indicates a statistically significant difference from in pre- versus post-semester administrations where * means p < .050.

c. Inductive Coding Results

Nineteen themes were identified via inductive coding of 12 CURE student interviews and 12 non-CURE student interviews. For some themes, one or more sub-themes were defined as necessary to connect different responses under the same conceptual umbrella. Sub-themes, counts of each theme identified, and sample quotes are shown in Table 3. The most prominent theme identified was named “hands-on experience.” Responses that were coded under this theme mentioned an aspect of the physical laboratory environment. For example, a student who mentioned handling reagents, laboratory PPE, or specific laboratory equipment would have their response coded under this category. Following hands-on experience, the most prominent theme was named “do-it-yourself.” This category includes responses where students reflected on their decision-making as it relates to the laboratory environment. Such responses mentioned writing one’s own procedure, experimental design, and having autonomy in the laboratory space.

The following theme was named “real-life application.” Responses that received this code demonstrated the students felt they could apply knowledge gained in the laboratory course to their career or future research lab. Responses were also coded under “problem solving” if students mentioned iteration, troubleshooting their experiments, or failure. Additionally, several student responses mentioned successful experiments, collecting data, and confirming their hypotheses. These responses were coded under “obtaining results.” The code “understanding” was given when students expressed that they felt they could understand the concepts of the laboratory. The code “visual confirmation” was given to responses that highlighted a visual element of the laboratory that confirmed their successful completion of the experiment. For example, a student

Table 3. Inductive Coding Results for Student Interviews

Themes	Sub-themes	Sample Quote	Total Count
presenting results	research expo, answering questions as an expert	*"I think it was the presentation that we had... I was really stressed about it.... And we've got it done. And I was so proud of myself."*	8
hands-on experience	handling reagents, use of equipment, efficiency, lab apparel	*"I feel like I actually kind of learned how to use some of the equipment. ...when they mentioned that equipment, I kind of had some background knowledge on how to use it. So that really helped me understand"*	22
visual confirmation	taking pictures, changing color, solid formation	*"I felt like the moment was whenever the solid formed in the little paper, and because... when you lift it up, and you could see [it], I think I was crying because I was just so happy. And that was like the moment where I really felt like, okay, I can do it. I am doing right, we can do it."*	10
connecting to lecture	N/A	*"... I guess it was just it came a lot easier to me because also I was able to see my scientific work in-person. Say I learned a titration in lecture, I was able to see that to fruition. And I felt like, oh, this is practical."*	9
obtaining results	confirming hypothesis, data collection, experiment working	*"...we went through the whole experiment by ourselves, and we got the results that were predicted... But it's just very satisfying and rewarding when you actually get the results that you know that you've written your prelab about."*	12
do-it-yourself	Procedure writing, experimental design, autonomy, start to finish, decision making	*"It was definitely great getting to build my own experiment and seeing [how things work] in the real world, and definitely experiencing failure... I'm going to be doing research in a lab next fall. So, I think it's definitely prepared me for when things don't go my way"*	20
real-life application	chemistry is everywhere, career prep, research lab prep, background knowledge, purpose	*"When we were giving our proposals... this is like, this is why I want to do the things that I want to do in the future. Because, like showing people like how far you've thought and how in depth you can go with this topic and what it actually means for them."*	18
problem solving	failure, troubleshooting, iteration	*"we had a problem like me and a lab partner had to solve the issue with an experiment, why wasn't it wasn't working well. And then I remember afterwards, when we were discussing what was going on and having to write that up in a paper. I think that was kind of cool."*	17
understanding	N/A	*"I just feel like I understood the concept really well. And I think all three of us understood the concept really well, which really helped our confidence."*	10
other	Field experience, online learning, cookbook, fear of failure, teamwork	*N/A*	N/A

response that mentioned a color change would receive this code. Responses that expressed that students could see the connection of the material covered in the laboratory to their lecture course received the code “connecting concepts to lecture.” Finally, the code “presenting results” was given to student responses that mentioned disseminating results, either in a public format or in the classroom. When student responses were unique or limited to fewer than 5 mentions, the responses were placed in a category titled "other". These themes include online learning, difficulty, field experience, teamwork, cookbook, and mentorship.

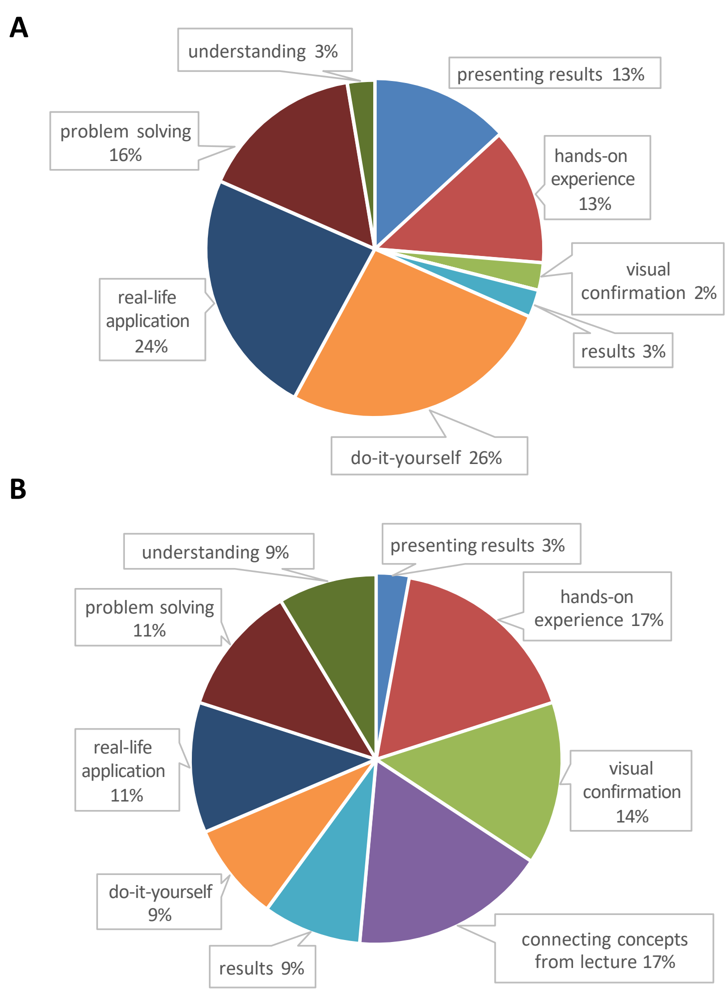


Figure 5. Interview coding results for A) CURE participants and B) non-CURE participants.

Figure 5 shows the frequency of emergent themes in responses from CURE students versus traditional students. For CURE students, the most prominent themes were do-it-yourself (26%) real-life application (24%), problem solving (16%), and presenting results/hands-on experience (13%). For traditional laboratory students, the most prominent themes were connecting concepts from lecture/hands-on experience (17%) and visual confirmation (14%).

V. Discussion

The implementation of a CURE that involves microplastics is appropriate for any chemistry department. We have demonstrated that first-year students were able to design experiments and collect valid measurements to answer their research questions. Student surveys and interviews indicated that the experience had a positive influence on students' scientific self-concept and their perception of the plastic pollution problem. The procedure was easily adapted into the existing general chemistry laboratory curriculum, and several techniques were reinforced throughout the microplastics analysis, such as dilution (preparing a working solution of Nile Red dye), titration (several groups performed EDTA titrations for hard water content in Cahaba River water), use of a pH probe, and establishing a calibration curve.

While this CURE adaptation included an abridged selection of cookbook labs from the traditional sequence, this experience has the potential to be scaled up or scaled down depending on the needs of the institution. For example, an elective laboratory research course could complete the entire CURE sequence in one semester by leaving out traditional cookbook labs, if desired. An upper division instrumental course could apply spectroscopic techniques such as Raman, FTIR, ICP-OES, and/or GC/MS (with a pyrolysis unit) to identify known and unknown microplastics as a summative project to demonstrate the capabilities of different chemical instruments for environmental chemistry research.

Figure 6. Students assisting each other in collecting grab samples at the “little Cahaba confluence” sampling site.

Lastly, this experience has the capacity for large groups of students in a non-honors general chemistry laboratory curriculum. A structured version of the experience could be completed in one-to-three laboratory periods, if desired. The recommended procedure would involve an introduction to microplastics and processing of known microplastic samples (week 1), processing of unknown environmental samples (week 2), and fluorescence image analysis (week 3). Furthermore, a similar project-based experience would be appropriate for chemistry classes and summer programs and the secondary level.

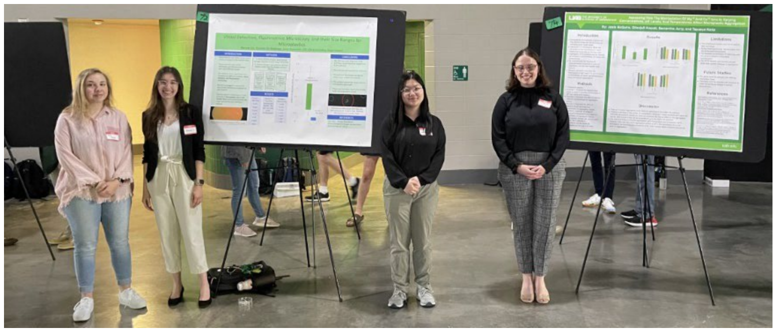


Figure 7. CURE Participants presenting their research at the institution's spring undergraduate research expo.

Questionnaire data from only one academic year is summarized in this article, because learning modalities shifted throughout the first two iterations of this CURE due to the COVID-19 pandemic, making data comparisons between CURE and non-CURE participants a challenge. The academic year presented is the first in which the learning modalities were consistent between the two student groups (fully face-to-face as opposed to hybrid and remote learning).

Additionally, the questionnaire was incorporated in this work to ensure that CURE students achieved similar or improved gains in STEM persistence constructs. The data is not meant to be extrapolated to all CUREs, as this was not within the scope of this work. Investigations into the CURE model have reported similar gains in relevant social psychological constructs, and we direct the reader to these studies for a more in-depth analysis of the relationship between CUREs and STEM persistence[3, 9, 36-39].

Questionnaire data from 2021-2022 demonstrate that this CURE experience was beneficial to general chemistry students. CURE students saw significant gains in all four target constructs while the traditional sequence only saw significant gains in science self-efficacy. In a laboratory experience that places a strong emphasis on plastic pollution, it is not surprising that students felt more strongly about the items within the environmental behaviors measure. The items were adapted slightly to place a stronger emphasis on behaviors around plastic use. Thus, this CURE experience appears to impact students' perception of their role in the plastic pollution issue and results in them reporting stronger beliefs around plastic use.

One theme that was absent from CURE student's interview responses was "connecting concepts to lecture," which was one of the most prominent themes in traditional student responses. The structured inquiry, traditional laboratory experience is designed to touch on all the major topics covered in the general chemistry lecture course. Topics present in both the lecture and lab include titrations, dilutions, calorimetry, and kinetics. The CURE experience touches on several topics covered in the lecture, but the emphasis is placed on applying these techniques to a research area of interest. Seeing that there is not a strong emphasis placed on covering environmental chemistry in the general chemistry lecture course, it is also not surprising that CURE students felt the experience did not connect to the lecture course. These results imply that the one-off cookbook style laboratory does accomplish the goal of helping students see the concepts from lecture play out in the laboratory.

Results from Nile Red microplastics screening can provide insight to the shapes and types of microplastics that might be present in the environmental matrix sampled, but do not confirm the chemical composition of recalcitrant particles. We found mostly spheres and fibers in the samples analyzed for this work. Clothes fibers are a particularly important microplastic. They are commonly found in environmental samples and are likely the result of the commercial use and degradation of polymeric textiles. The spheres observed could be primary microplastics but are more likely the result of very small particles (<30 μm) fluorescing. A more time-intensive screening could more accurately determine the shape and size of very small particles but was outside the scope of this work.

Particles isolated from 1-L grab samples are typically too small for ATR-FTIR spectroscopy; however, determining an exact chemical composition of these particles would require a spectroscopic instrument with capabilities for digital microscopy (μ-Raman, μ-FTIR). These instruments are not commonly available for use by students at the general chemistry level, so adaptations may consider focusing on larger microplastics (>500 μm) that can be isolated from sediments via density separation or from commercial products like cosmetics.

VI. Conclusions

This work summarizes the implementation, integration, and outcomes of a year-long, first-year general chemistry CURE on the topic of microplastics quantification with Nile Red. In this experience, students review literature, design an original research project, execute the project in class, and present their results at a university-wide research exposition. This CURE was easily integrated into 3-4 honors sections of a general chemistry laboratory for four iterations (2019-2023). Before and after participating in the CURE, participants

completed a questionnaire measuring their science self-efficacy, science identity, environmental behaviors, and their knowledge of the relationship of chemistry to the environment. Additionally, interviews were conducted at the end of each year-long CURE iteration. Results demonstrate that this microplastics CURE experience resulted in increased self-efficacy, more likelihood to engage in positive environmental behaviors regarding plastics, and increased knowledge of the relationship of chemistry to the environment. Additionally, the size and shape of microplastic particles identified in Cahaba River water samples by students were summarized. This CURE is appropriate for the general chemistry laboratory and has the potential to be scaled back to a one-to-three-week activity for large groups of students or incorporated into an upper division research or instrumental course.

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CHAPTER 5

MODIFICATION OF A FAST, ACCESSIBLE MICROPLASTICS SCREENING METHOD AND ITS IMPLEMENTATION ON CAHABA RIVER WATER SAMPLES

by

JOSH FORAKIS

In preparation for *Analytical and Bioanalytical Chemistry*

Format adapted for dissertation

Abstract

Microplastics have emerged as a persistent anthropogenic pollutant in outdoor and indoor environments, and little is known about their impact on humans and other living organisms. Spectroscopic methods are commonly employed to accurately quantify microplastics in the environment, but these methods require time, expensive instruments, and expert knowledge to interpret. A more common field test has been employed by ecologist and conservation groups using digital microscopy to visually detect microplastics. However, this method lacks accuracy and precision due to the requirement that the scientist properly identifies solids collected. Thus, there is a current need in the field of plastic pollution research. Nile Red is often discussed as a candidate to improve upon the imprecise visual detection method, but Nile Red itself has several challenges including background fluorescence. To improve upon established procedures utilizing Nile Red to quantify environmental microplastics, a novel water bath step (NR-B) was designed and incorporated in a variety of experiments. An optimal NR-B dying time was determined, a comparison of NR-B and the standard dying method (NR-S) on various polymers (EPS, PS, PP, and Nylon) was performed, and lastly, environmental samples from several locations along the Cahaba River were processed and analyzed according to the optimal NR-B method. Results demonstrate that the NR-B method does reduce background fluorescence for all tested polymers except for EPS. This optimized Nile Red procedure is an improvement on existing methods and is appropriate for screening environmental samples for microplastics. Recommendations for

microplastics researchers using Nile Red in teaching laboratories and quantitative studies are also discussed.

I. Introduction

Synthetic polymer particles ranging from 1 μm – 5 mm in diameter, known as microplastics (MPs), have become a prominent form of environmental pollution. Reports of their presence in fresh water, seawater, drinking water, and even human blood samples are becoming more frequent[1-4]. Despite the global nature of MP pollution, measurement methods are still in their infancy, and the accuracy and precision of these methods are just beginning to be discussed. Quantifying MP pollution in aquatic, terrestrial, and indoor environments, and determining its impact on humans is of critical importance. Yet, studies quantifying MPs in environmental samples are often not comparable due to the lack of standardized analytical methods. The three most common analytical techniques for quantifying MPs are micro-Raman spectroscopy (μRaman), micro-Fourier Transform Infrared spectroscopy (μFTIR), and pyrolysis-Gas Chromatography/Mass Spectroscopy (Py-GC/MS)[4-6]. These methods are used individually to confirm the presence of plastics in the environment, but analyzing microplastic pollution is complex due to the large number of polymeric materials used commercially. Additionally, these commercial products contain additives and degradation products from UV weathering[7-12]. These factors make any spectroscopic analysis complicated due to the many possible spectra resulting from the mixture.

The complexity of spectroscopic methods means that they are not often employed by environmental organizations. These organizations have primarily been identifying microplastics through a method known as visual detection[13, 14]. This method relies on an individual's expertise and lacks acceptable levels of precision[10, 15]. Spectroscopic methods are much more accurate, but analyzing samples can take hours and requires specialized experts to interpret the data output. Thus, a fast-screening method is necessary for environmental organizations who are interested in determining the severity of microplastics pollution in their natural source of interest and whether more robust analyses are necessary.

Some groups have employed a fluorescent dye to selectively tag microplastics in natural water samples as an improvement on the visual method[16, 17]. This method improves both the accuracy and efficiency of the visual detection method. In addition, the procedure is less demanding and requires less expensive equipment than spectroscopic methods. A frequent candidate as a fluorescent tag for microplastics is Nile Red, but limitations exist. Heejun and colleagues have reported the issue of background fluorescence in Nile Red analysis, where the fluorescence of microplastics can be confounded by fluorescence from the surrounding background[16, 18-20]. In published reports, the dye is applied by simply adding directly to the filter substrate or allowing the filter to be submerged in the dye for a period. The resulting images can be difficult to interpret due to the low fluorescence intensity of dyed microplastics and the amount of background noise. Thus, we explored conditions to increase particle-dye interaction in this process.

Fluorescent microscopic analysis for microplastics typically involves one or more filtration steps to isolate suspended solids from environmental matrices. These solids can be inorganic (rocks or soil), metallic (aluminum), or organic matter (leaves, paper). The dye cannot bind to the inorganic or metallic components but can bind to the organic matter. Thus, a digestion step that does not degrade polymers is often used to decompose the organic matter. After digestion, the dye is applied in the dying step. Some steps in this process have been optimized previously, such as a comparison of various oxidizers in the digestion step[21], but one less examined is the fluorescent dye application.

Nile Red is commonly used in these studies, as it is often used to stain tissues[22]. The dye fluoresces more intensely as it's solvent polarity decreases, fluorescing most in a hydrophobic environment[22]. It can also stain polymers, and behaves somewhat differently depending on the chemical properties of the polymer[23]. The dye most likely adsorbs to polymer surfaces via Van der Waals interactions and some dipole-dipole interactions, depending on the polymer[17].

In most studies, Nile Red is applied directly to the isolated solids on top of a hydrophilic polycarbonate track-etched (PCTE) filter paper. This method does not allow enough time for adequate polymer adsorption by Nile Red. The results can display significant background fluorescence and low particle fluorescence. Heejun et al. presented a "plating" method of dye application, where a sample is fully submerged in a dye solution overnight[20]. While this method does increase particle dye interaction time, the filter membrane is also left in the solution overnight and can still cause background fluorescence. This work seeks to improve upon the dye application further by applying a novel modification to this method through submerging the particles and dye in water overnight (NR-B), attempting to minimize background fluorescence and maximize particle-dye interaction time.

H1: The NR-B method will yield results with less background fluorescence and greater particle fluorescence for all polymers than the standard Nile Red dyeing method.

Four commercial polymers were chosen to assess the NR-B modification that represented a range of chemical composition: polystyrene (PS), expanded polystyrene (EPS), nylon, and polypropylene (PP). Three positive control microplastics samples were prepared for each polymer type and were subjected to the NR-S and NR-B method. Additionally, fourteen 1-L river water samples were fully processed in accordance with the NR-B method as proof of concept and so the method could be incorporated into a novel microplastics course-based undergraduate research experience in the general chemistry laboratory (see Chapter 4).

II. Methods

a. Positive Control Microplastics

Positive control microplastic standards were made from post-consumer goods, except for PS. PP was purchased from commercially available PolyPellet^{TM} beads. EPS was collected from packaging materials, and Nylon was purchased from commercially available yarn without color dyes (Value Solid Yarn by Craft Works from Michael's). PS standards were purchased from ThermoFisher Scientific (Cat#: 041922.03). Polymer identity was confirmed on a Bruker ATR-FTIR for all polymers.

PP and EPS were blended separately in 200 mL of pre-filtered, deionized water for 10-15 minutes until sufficient mechanical degradation had taken place. The mixture was first poured through a cheese cloth to separate large microplastics from small microplastics left in the filtrate. Then, the filtrate was passed through a 1 μm pore size PCTE filter membrane to isolate particles. Nylon microplastics were produced by cutting up the nylon yarn into 1 mm strips.

b. River Water Sampling

Cahaba River water was collected from five different sites in 1-L pre-rinsed wide-mouth amber glass jars (ThermoFisher^{TM}: 341-1250). Bottles were submerged face down and turned over to fill the jar with water below the surface. Samples were collected upstream from the researcher, so samples were not contaminated by polymer fibers originating from the individual sampling.

Before collection, jars were rinsed 3 times with surface water. After collection, the samples were stored in a 4°C cooler until analysis. Throughout all the following procedures, some precautions were taken where applicable to minimize the introduction of microplastics through processing. All glassware was triple rinsed prior to use. Solutions were pre-filtered where possible. The work was performed in a well-ventilated fume hood, and cotton lab coats were worn.

c. Wet Peroxide Oxidation

Natural samples were filtered through a hydrophilic polycarbonate track-etched (PCTE) filter paper (⌀ = 47 mm, pore size = 1.0 µm) using a standard vacuum filtration setup. The isolated solids were kept on the PCTE filter. A stock solution of Fenton's reagent was prepared by dissolving 7.5 g of iron (III) sulfate per 500 ml of water and adding around 3 ml of concentrated sulfuric acid to produce a 0.5 molar solution. The entire filter paper with isolated solids and 20 ml of the prepared Fenton's reagent was added to an Erlenmeyer flask. Then, three 10ml aliquots of 30% H_2O_2 were added. The reaction was carried out to completion before each subsequent aliquot of H_2O_2 was added. The process was repeated until no visible organic material remained or the addition of more H_2O_2 reacted no further. The solution was the filtered again.

d. Dying Procedure

Nile Red Standard method (NR-S): To create a stock Nile Red solution, solid Nile Red (ThermoFisher™: AC415711000) was dissolved in acetone to obtain a 1g/mL stock solution. A working solution was made by performing a 1-in-20 dilution of the stock in acetone. Then, 2-3 ml of the working solution was added directly to the filter and isolated solids using a glass pipet. Nile Red Bath Method (NR-B): Analytical water (30 mL of Milli-Q) was used to carefully transfer the remaining solids to a pre-rinsed beaker. 2-3ml of the working solution was then added to the beaker containing the isolated solids. The mixture was stirred for 1 minute every 10 minutes six times (every 5 minutes for the 30 minute trial). The beaker was covered with parafilm and left to sit for various time points to determine the optimal dying time, then the optimal time was used for all subsequent experiments. The solution was filtered again through another PCTE filter. The filter containing the remaining solids was stored in a covered petri dish until imaging.

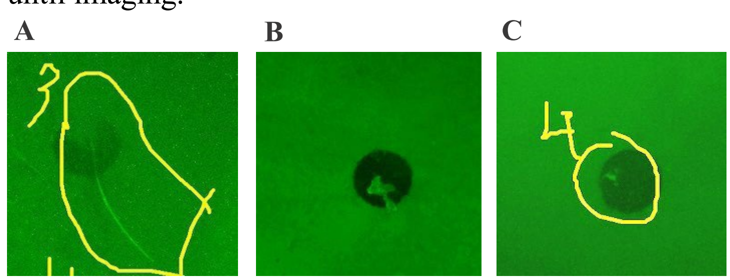

Figure 1. An example of each fluorescing particle shape classification where A) fiber, B) shard/fragment, and C) sphere.

e. Imaging and image analysis

Samples were imaged using a Nikon AZ-1000 dissecting scope with fluorescence capacity, and photos were taken using an Andor-Zyla 5.5 sCMOS. The filter profile used was the GFP filter profile (excitation: 457-487nm; emission: 502-538 nm) which most resembles Nile Red's emission spectrum in acetone, as Nile Red’s emission and excitation are solvent and polymer dependent[17, 23, 24].

ImageJ, an open-source program for analyzing image data, was used to count particles and quantify the fluorescence intensity of Nile Red-stained, positive control microplastics. For each image, a color intensity threshold was set so that all fluorescing particles in the image were selected, and the ‘analyze particles’ function was used to gather the area and fluorescence intensity of every particle. Background fluorescence was subtracted by measuring three areas where no fluorescence was observed and subtracting the average value from the raw fluorescence intensity value for every particle.

ImageJ was also used to determine the shape, size, and fluorescence intensity of environmental microplastics dyed with Nile Red[18]. Particles were classified in one of three shape categories: fiber, shard/fragment, or sphere. Fibers were particles that appeared stringlike in the image, or whose length was significantly longer than its width. Shards/fragments were particles that appeared in a variety of shapes and could not be classified as either a sphere or fiber. Spheres were particles that appeared uniformly round in the image. Figure 1 shows an example of each shape classification. Particle size was determined using either the straight line or freehand line tool, depending on the particle's shape. After known length is established, the line tool provides the diameter/length in centimeters.

f. Statistical Methods

Fluorescence intensities for all the particles in a sample were aggregated and background fluorescence was subtracted from the raw values so that particle number did not affect the fluorescence intensity values compared. Average particle fluorescence intensities were compared between samples using independent samples t-tests. For samples that were not normally distributed, a non-parametric test was used. Differences were considered significant if $p<.050$.

g. Experimental Design

First, PP was used in an experiment to determine the optimal dying time to be used for all other plastics. A typical general chemistry laboratory period of 3-hours was considered when determining dying times. PP particles were dyed in accordance with the NR-B method and left in the mixture for 30 minutes, 1 hour, and 1 week. Three trials were performed for each dying time. The time that resulted in the most fluorescence for the least amount of time was used in all subsequent experiments. To determine the dying efficacy of the novel dying method, all control microplastic species were processed with both the NR-S method and the NR-B method. Lastly, three Cahaba River samples were collected from five sampling sites. Three negative control trials and all the environmental samples were processed via the NR-B method.

III. Results

a. Optimal Dying Time

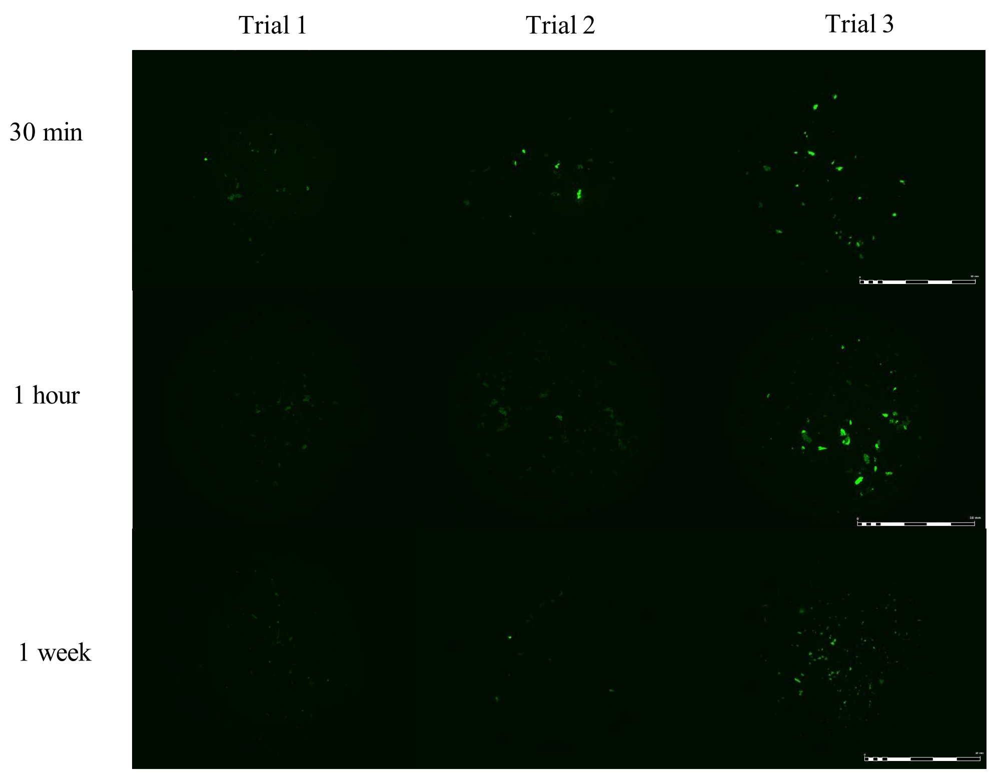

Figure 2. Images of PP dyed via the NR-B method for various amounts of time. Samples were imaged using a Nikon AZ-1000 dissecting scope with fluorescence capacity, and photos were taken using an Andor-Zyla 5.5 sCMOS. Images were taken at 1x magnification, and scale bars show 10 mm. False color images show relative intensity of fluorescing particles in green.

Figure 2 shows the imaging results and Figure 3 shows fluorescence intensity of Nile Red stained microplastics with dying times of 30 minutes, 1 hour, and 1 week respectively. On average, PP particles dyed for one week fluoresced more intensely than those dyed for 30 minutes ($t(627) = -24.12$, $p = <.001$, $d = -1.32$, 95% CI [-1.43, -1.20]) and those dyed for one hour ($t(821) = -$

4.28, p = <.001, d = -0.29, 95% CI [-0.42, -0.15]). In addition, Particles dyed for one hour demonstrated significantly more fluorescence than particles dyed for 30 minutes (t(430) = -16.75, p = <.001, d = -1.03, 95% CI [-1.16, -0.90]). For the remaining experiments using NR-B in this study, particles were dyed for 1 hour.

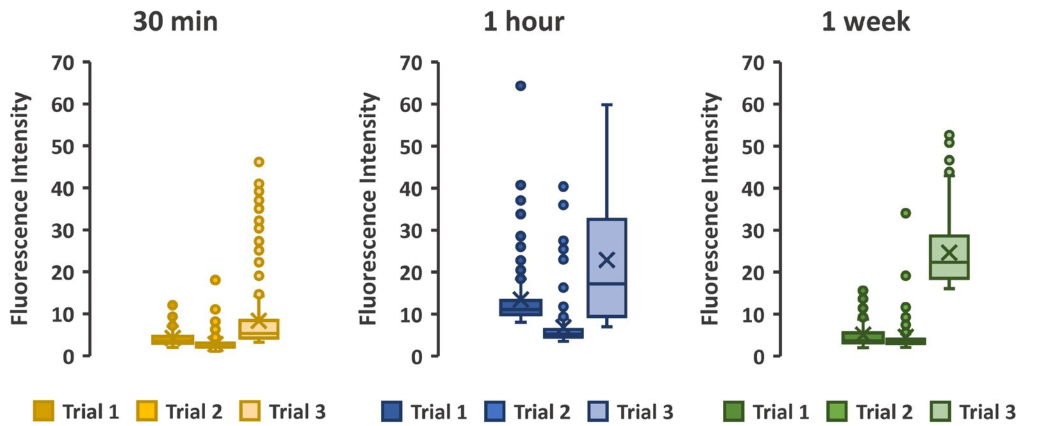

Figure 3. Average fluorescence intensity of PP particles dyed with the NR-B method for different amounts of time. Each trial contained a different number of particles, so fluorescence intensities were averages for each trial. Fluorescence was quantified in ImageJ. Several background measurements were taken of each photo and the average fluorescence of the background was subtracted off all the values of the fluorescing particles.

b. Polymer Comparison of NR-B and NR-S

Figure 4 shows the results from imaging each polymer after being dyed in accordance with the NR-S method and the adapted NR-B method. Between trials, all dyed polymers appear similar except for trial three of PS dyed via the NR-S method. Background florescence was observed for all polymers dyed with the NR-S method except for trial three of PS, and all three trials of EPS.

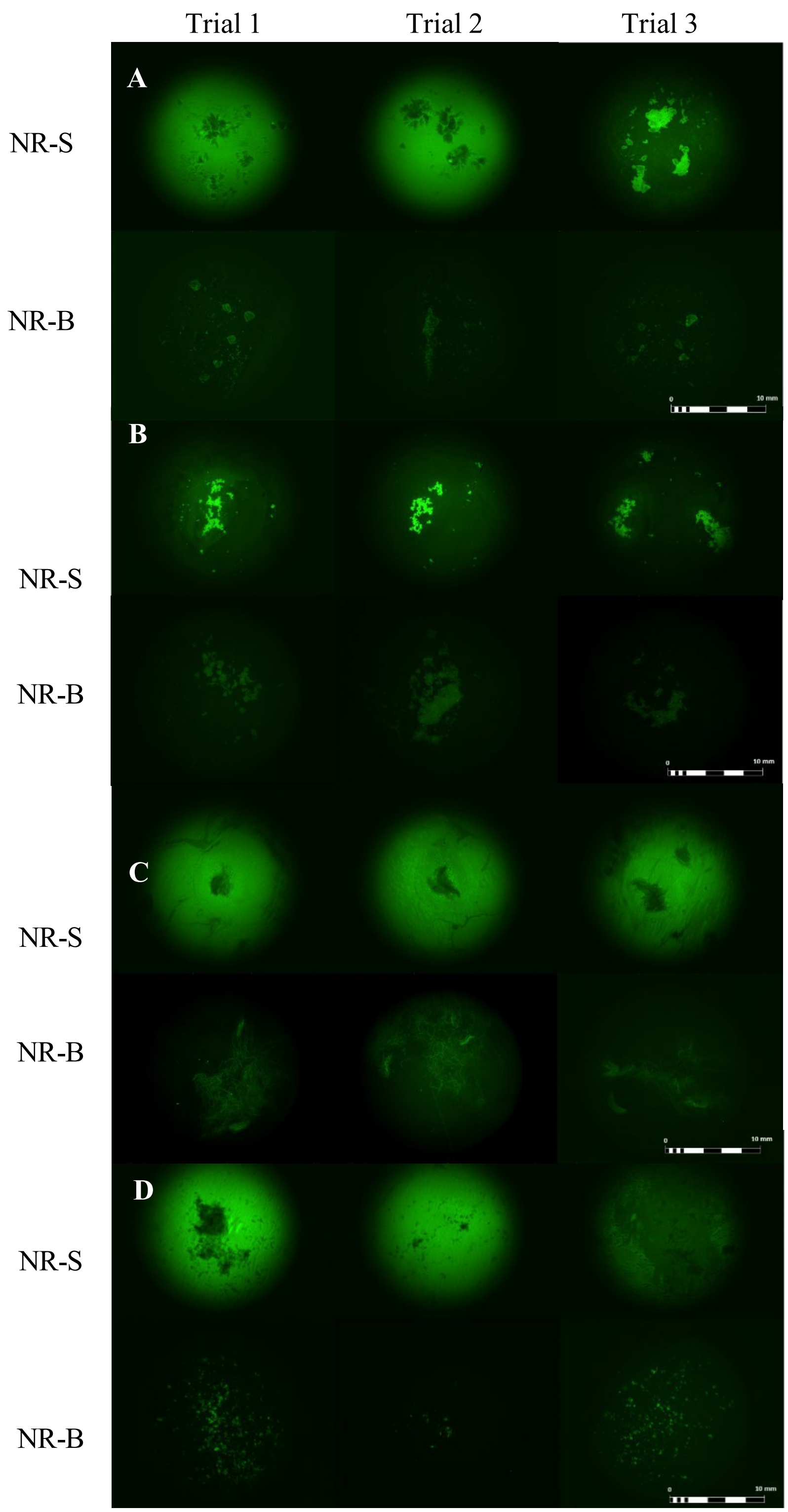

Figure 4. False color imaging results from the polymer comparison of the NR-B and NR-S method. For the NR-B method, all particles were dyed for 1 hour based on the results from Figure 2. Three trials of each polymer were completed with both methods, where A) PS, B) EPS, C) Nylon, and D) PP.

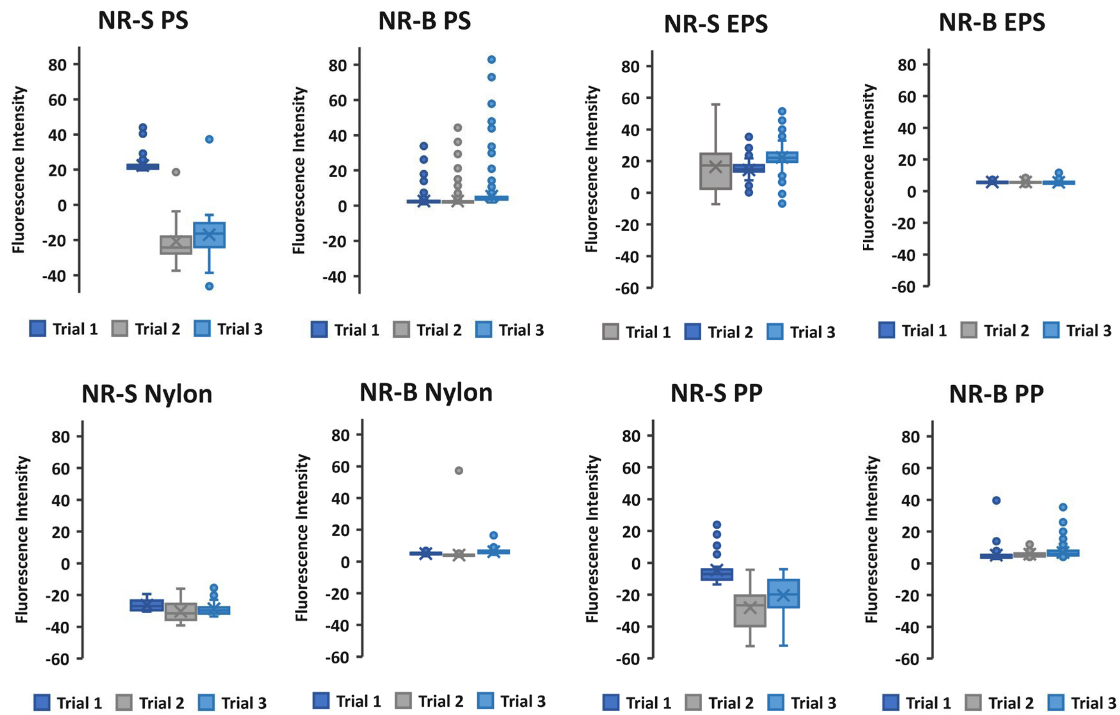

Figure 5. Fluorescence intensity of all polymers died via NR-B and NR-S. Intensity values were determined using ImageJ. Several background areas were selected, and the average background fluorescence was subtracted from the fluorescence values of all particles. Negative values indicate the background area fluoresced more intensely than the particles. The x shows the average, and outliers are shown as points.

Figure 5 shows the florescence intensity of the polymers stained by the NR-S method and the NR-B method. A negative value for fluorescence intensity indicated that the background fluoresced more intensely than the particles. Both PP ($t(106.4) = 16.7$, $p = <.001$, $d = 5.66$, 95% CI [5.40, 5.92]) and Nylon ($t(83.6) = 57.1$, $p = <.001$, $d = 17.6$, 95% CI [17.1, 18.1]) dyed with the NR-B method showed greater fluorescence than the same polymers dyed with the NR-S method. PS dyed via NR-S showed greater fluorescence than the NR-B method when particles from all three trials were combined for comparison ($t(215.8) = -7.17$, $p = <.001$, $d = -1.41$, 95% CI [-1.55, -1.26]), but two out of three NR-B trials fluoresced more intensely than their subsequent NR-S trial (Trial 1: t (21.1) = 9.09, p = <.001, d = -7.12, 95% CI [-7.46, -6.78]; Trial 2: t(106.4) = 16.7, p = <.001, d = 6.68, 95% CI [6.18, 7.17]; Trial 3: t(28.5) = 8.26, p = <.001, d = 3.05, 95% CI [2.65, 3.45];). Lastly, NR-S dyed EPS fluoresced more intensely than NR-B dyed EPS (t(181.5) = -13.6, p = <.001, d = -1.87, 95% CI [-2.07, -1.67]).

c. Cahaba River Samples

In total, 14 environmental samples and three negative control (milli-Q) samples were processed, dyed via the NR-B method, and imaged for microplastics. Figure 6 displays the average counts of fluorescing particles for all trials from each sampling site as well as average particle size (area). All sites had three trial samples analyzed except for the Grant's mill sampling site, of which only two samples were processed. Some fluorescing particles were noted in the negative control samples.

Particle counts for all sampling sites had no statistically significant difference when compared the negative control (HWY 280: t(2.1) = -1.8, p = .116, d = -1.37, 95% CI [-3.2, 0.5]; Moon River: t(2.1) = -2.70, p = .055, d = -2.2, 95% CI [-4.3, 0.0]; Old Looney Mill: t(2.1) = -2.5, p = .062, d = -2.1, 95% CI [-4.1, 0.11]; Hoover East: t(2.1) = -1.5, p = .138, d = -1.20, 95% CI [-2.9, 0.7]). A comparison with Grant's Mill could not be made, as only two samples were analyzed. ImageJ analysis of particle size demonstrated that the largest particles were found in the negative control on average (mean area = 1.83 mm). The particles found in Cahaba River water had <1 mm area on average. Some particles identified were too large to be considered microplastics (>5 mm in length) and were thus excluded from the analysis.

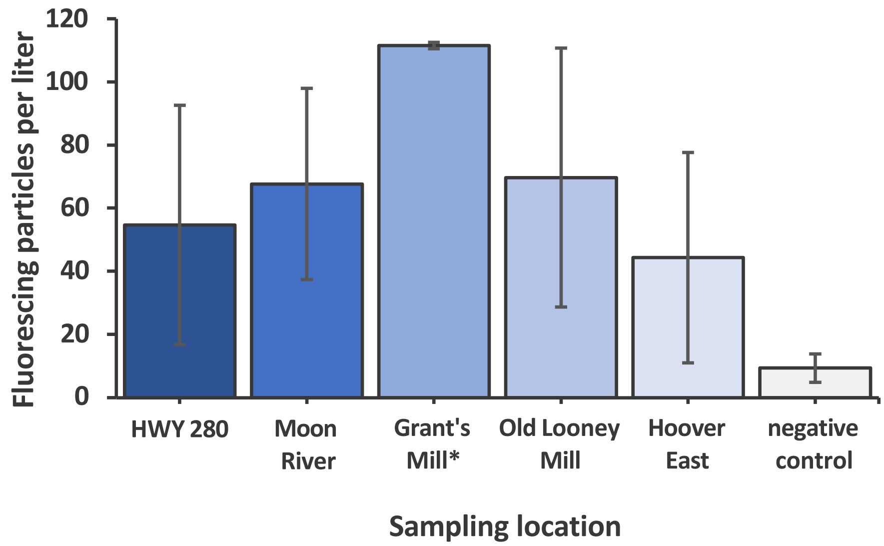

Figure 6. Counts of fluorescing particles from various sampling sites along the Cahaba River. All samples were processed and imaged according to the NR-B method. Microplastic counts were estimated via thresholding on ImageJ. Error bars represent standard deviation from the mean. Post-hoc comparisons were performed of all environmental samples against the negative control, and no statistically significant differences were observed. *Only two samples were collected for the Grant's Mill sampling site.

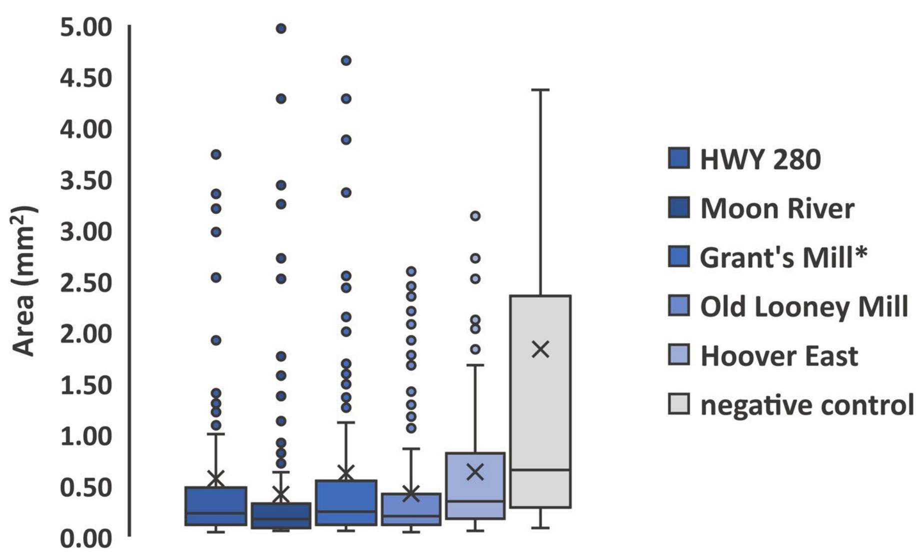

Figure 7. Size distribution of fluorescing particles identified in environmental samples and the negative control trials. The 'X' shows the average area of fluorescing particles isolated from samples taken at that site. Outliers are shown as free points along the vertical axes.

IV. Discussion

To determine optimal dying time for the NR-B method, times were chosen with a typical general chemistry laboratory period in mind (3 hours). A dying step lasting thirty minutes to an hour would mean the processing of environmental samples could be completed in its entirety by student groups in one laboratory period, and imaging could occur outside of the classroom or in a subsequent laboratory period. The longest period (1 week) was chosen because an entire week must pass in between laboratory sessions if the procedure was split.

The results of the time trial experiment demonstrated an optimal dying time of one-hour given the constraints of a three-hour laboratory period. Particles dyed for one week fluoresced most intensely on average when compared to the other groups, however, this can be attributed in part to the average particle fluorescence in trial three, which was much higher than the other trials for all groups (Figure 3). It is likely that the greater fluorescence intensity observed in this trial resulted in a greater overall average particle fluorescence for the three trials in the ‘1 week’ group. Though the mean fluorescence intensity was statistically greater for the ‘1 week' group than the ‘1 hour' group, this difference was weak in effect (d = 0.29) and had a magnitude just over one arbitrary fluorescence intensity unit. Thus, dying particles for one week may increase the fluorescence intensity of dyed particles on average, but the magnitude is not great enough to justify splitting the procedure into two-weeks when adapting into a general chemistry laboratory curriculum. This may be due to Nile Red's fluorescence decay over time, which is a reported phenomenon when considering dyed microplastics[25-27]. The data imply that some Nile Red degradation may occur when dying particles for one week.

Furthermore, particles dyed for thirty minutes fluoresced the least compared to the other groups. The difference in fluorescence intensity between the '30-minute' group and the 'one-hour' had a large enough effect size (around one standard deviation) to justify requiring the mixture to be left for one hour as opposed to 30 minutes. It is possible that there is a time dependency of the adsorption of Nile Red to polymer surfaces. Therefore, leaving the polymer/water/dye mixture for an additional 30 minutes appears to promote adsorption of Nile Red to the surface of PP. PP is a polymer composed of only branched hydrocarbons, and thus is conducive to hydrophobic interactions with Nile Red, however, additional polymers, especially those with hydrophilic groups (polyethylene terephthalate, polyesters, etc.) should be subjected to NR-B time trials as well to corroborate this explanation.

Variability between trials was also present in the polymer composition experiment (Figure 4&5). For PP dyed via NR-S, trial 3 particles fluoresced much more intensely than the two previous trials. This contributed to the overall difference observed between NR-S dyed PP and NR-B dyed PP, however, trial one and trial two of NR-B dyed PP showed significantly greater fluorescence than their subsequent NR-S trial. Nylon and PS particles dyed with NR-B consistently showed greater fluorescence than those dyed with NR-S, implying that the NR-B method promotes more particle dye interaction than the NR-S method for these two polymers and reduces background fluorescence.

EPS was the only polymer in which the NR-S method resulted in greater fluorescence than the NR-B method. EPS's primary chemical composition is the exact same as PS, both of whom have the same monomer, styrene. The main difference between the polymers is that EPS is created from PS starting material by expanding trapped gases inside of PS through heating, creating the foamy consistency observed in commercial products made from EPS. Processing PS into EPS results in a secondary polymer structure with much larger gaps and a greater surface area than the starting PS. Thus, EPS may be more susceptible to Nile Red absorption due to the impacts of physically altering PS in the formation of commercial products.

The NR-B method was successful in dying particles isolated from Cahaba River water, though confirmation of the fluorescing particles via IR or Raman microscopy was not performed in this study. Nile Red has been known to result in false positives by dying recalcitrant organic matter in environmental samples[26, 27]. The Fenton's reagent digest used in this work has been tested, resulting in adequate digestion of organic matter and minimal effect on polymer composition, but some forms of organic matter can remain, like chitin. Therefore, the Nile Red method has limitations in its ability to reliably quantify microplastics in the environment. For quantitative studies, the Nile Red method alone does not provide sufficient information to draw meaningful conclusions and thus requires a complementary spectroscopic method. However, when considered as a preliminary screening method with the purpose of determining the importance of monitoring microplastic pollution in a particular watershed or to introduce microplastic quantification methods in lower-division undergraduate chemistry courses, the Nile Red method is accessible, fast, inexpensive, and relatively easy to interpret compared to spectroscopic methods. This work demonstrated that the NR-B method improves the interpretation of this method's outputs, and applying the method on natural water samples resulted in plausible microplastic counts.

a. Limitations and future work

This study highlighted a pervasive issue in studies attempting to quantify microplastics, which is the difficulty of obtaining a pure sample to use as a negative control. Microplastic pollution is ubiquitous in the outdoor and indoor environment, meaning laboratories can also introduce routes of microplastic exposure. The negative control used in this study did show fluorescing particles when imaged, but the quantity was far fewer than each of the environmental samples, though this difference was not statistically significant due to the number of trials processed for each experimental group. As a result of completing this work, some recommendations for other microplastics researchers were identified that can help minimize the number of microplastics introduced to samples processed in the laboratory:

- Minimize the use of plastic in all steps of the analysis, including sampling, storage, filtration, and imaging.

- Perform experiments in a well-ventilated area to reduce airborne microplastic exposure.
- Wear a lab coat made of 100% cotton or other biologically derived material (not polymers).
- If possible, pre-filter all solutions used in the experiment with the same pore-size used throughout the study and pre-clean all glassware with NoChromix™ or some other strong detergent.

Another limitation in this work was the polymers chosen in the polymer comparison experiment. While they represent a range of polymer composition, they were also chosen based on availability. Future studies that incorporate this method should include more polymers with hydrophilic functional groups and especially those of increasing environmental concern (fibers, tire wear, poly-vinyl chloride).

Lastly, this study used a method of thresholding when quantifying fluorescing particles that incorporates some amount of researcher error, as the fluorescence threshold is adjusted for each image according to the observed particle fluorescence. There are works that highlight the importance of the thresholding method used in microplastics studies with Nile Red, and that some thresholding methods may result in over-estimating or under-estimating microplastics[26]. Future works may compare thresholding methods with plastics dyed via NR-B on accuracy of microplastic quantity.

V. Conclusion

Microplastics are a pervasive environmental and indoor presence, and quantifying these particles is a challenge. Assessing the impact of these particles on living organisms requires not only a reliable method for their quantification, but a fast, accessible screening method for use by environmental organizations and STEM educators. This work examined a microplastics screening method using Nile Red as a fluorescent microplastic tag, and its issue regarding background fluorescence. With the inclusion of a novel water bath step (NR-B), the Nile Red microplastics screening method was improved and implemented on a variety of polymers and Cahaba River water samples. The results of these analyses demonstrate that the entire procedure of processing environmental samples for microplastic screening with Nile Red (including the NR-B dying step, not including imaging) is efficient enough for one typical general chemistry laboratory period. The NR-B method was found to be more effective at staining three out of four of the polymers chosen for this work, and fourteen environmental samples were successfully screened for microplastic pollution using the NR-B method. This output from this method includes fluorescing particle count, size, fluorescence intensity and shape. Polymer composition cannot be determined unless a complementary spectroscopic method is employed. Future work should investigate the dying efficiency of more polymers, especially those with hydrophilic functional groups, and methods of reducing exposure pathways of microplastics in the laboratory environment to improve the ability to attain clean negative controls.

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CHAPTER 6

CONCLUSIONS AND FUTURE DIRECTIONS

I. Overcoming CURE Barriers: Towards an Inclusive Chemistry Classroom

My background before graduate school was as a high school biology teacher at YEP Prep Public Schools in Houston, Texas. I jumped directly from completing a Bachelor of Science in biochemistry with no focus on education into the world of teaching science to ninth graders. It was in this role that I would complete my first "research project" in the classroom. In my second year, I converted my course into a project-based curriculum, where every unit included a summative project demonstrating various biological phenomena, such as plant adaptations, cell structure, and ecosystem dynamics. Though biology was not my primary discipline, this experience provided the foundation for what would become my graduate thesis. As I gained experience using the educational tools provided to me in the district's intensive alternative teaching certification program, I fell in love with facilitating scientific inquiry with my students.

As a Ph.D. student in a chemistry program at a large research-focused institution, I had the rare privilege and opportunity to take on several roles not often available to graduate students. I was able to teach several sections of general chemistry lecture as the instructor of record. While this role is less relevant to the themes discussed in this dissertation, one important takeaway I observed came because of something I did on the first day of class in every section. I had my students generate a word cloud based on the question “How do you feel about chemistry right now?” While this always precedes an impassioned speech about how my belief is that all students CAN learn and be successful in chemistry, I always found that the largest word in the resulting word cloud was “nervous” or something very similar. This is very much in line with my observations as a secondary educator as well, as many students are quick to assign themselves as a “science person” or, more often, “not a science person.”

I found that understanding students’ perception of STEM is an interest of many other discipline-based educational researchers, especially chemistry education researchers. Chemistry has maintained the popularized image of a “weed-out” course, where eager freshman STEM majors go to flunk and switch their major to something perceived as less difficult. I spent a long time during my first year as a graduate student asking myself the question: “Why do so many students feel that chemistry is not for them?” This led to one of the major focuses of this work, the role of science identity.

I had a background in education, an undergraduate major with a heavy focus on chemistry, and experience redesigning courses. What I needed to complete my work was a course to redesign, hence my role as the “coordinator” or “lead teaching assistant” of the honors general chemistry laboratory sequence at UAB. I had spent much time reading about teaching laboratories and I discovered a collective effort to incorporate more authentic experiences into STEM curricula, specifically with a focus on inquiry and active learning. I saw several talks on CUREs and found that the CURE model was very similar to the project-based model I used at YES Prep. I decided to incorporate the ethos of all the training I received as a secondary educator in an investigation of a first-year general chemistry CURE.

Now, having completed four iterations of this CURE, I have gained insight into the challenges and benefits of taking the classical "cookbook" laboratory model and redesigning for student-centered, inquiry instruction. However, in conversations with other STEM educators, I have learned that many CURE barriers remain and that the full benefit of undergraduate research in the classroom has not yet been discovered. For the remainder of this section, I will discuss several takeaways and recommendations for other educators and researchers who wish to incorporate or research CUREs.

One of the most helpful parts of creating this CURE was the fact that the laboratory courses at UAB were already staffed with competent graduate and undergraduate TAs. This allowed me to spend more time designing and running the course, troubleshooting experiments, and coordinating with the Cahaba Riverkeeper. For an intensive CURE, I would highly recommend having two TAs (or one TA and one instructor) per each section of twenty-four students. Facilitating a CURE is a lot like having a large research group, and I found that managing many student projects to be difficult, yet not impossible. It certainly may not have been possible without additional graduate students as TAs, although I did spend one year as the only graduate TA in the course's four sections with several undergraduates working alongside me. In that situation, the course did take up a massive chunk (75%) of my time.

Depending on the nature of the research being performed, it may be necessary to structure the course in a certain way. For example, in the second semester of my microplastics CURE, I incorporated a deadline for a materials list to be compiled and submitted for every project group. This allowed me a reasonable amount of time for me to submit orders for reagents (as several groups requested materials not immediately on hand, though many were readily available in the stockroom and easy to obtain), and delays were common when ordering reagents and materials due to a variety of factors. In addition, other checkpoints should be incorporated as deadlines into the course calendar so all projects remain on track. In the course, homework assignments were all designed to have students create their final presentation one piece at a time. In between each step was an opportunity for students to receive TA/mentor feedback. Overall, the course assignments in a CURE should mirror the experience of being in a research group as much as possible, such as including peer-review, iteration, and designing one's own experiments.

It is possible that many of the successes involved with this CURE are tied to the setting of the research, a large, research-focused (R1) university. One limitation of the work was that, for honors labs, students self-select into the experience, and many participants were already interested in a career in science or research. While gains in scientific self-concept were observed and documented in chapter four, CUREs would reach an entirely new population of students if incorporated into community college and even high school courses (though the term CURE would not apply and could be changed to Course-based Secondary Education Research Experience (CSERE)). Students at this level may be more impacted by educational experiences that influence the development of science identity. Thus, it is crucial that funding lines be delegated to institutions that serve first-generation students, non-traditional students, and under-represented minorities in STEM because research experiences are beneficial for them.

Lastly, good classroom culture and student buy-in are crucial for those wanting to design a CURE. A huge factor in helping me achieve these for my CURE was collaborating with the Cahaba Riverkeeper and including the field sampling trips. For any research experience in a course, there must be some final product associated with the work done in the course, and it's best for this to be something outside the course, like a publication or conference presentation. The final product can be scaled according to the needs and resources available to the educator. I had my students present at a research exposition within the university. Some suggestions for scaling this down might be inviting an expert in the field to attend final presentations, compiling data from student projects to summarize in a school newsletter or local environmental publisher or hosting your own research exposition and inviting colleagues to attend.

Ultimately, the most enticing goal is to help your students collect publishable data and to include them as co-authors. This not only benefits the scientific community but gives students opportunities and exposure as scientists. To accomplish this, guidelines should be set in the course for high-quality data. A threshold for precision and reproducibility should be determined, and students should know that publishing is a possibility. If publishing is not feasible, it is possible to use student data as justification to alter the course or procedure. In my experience, students are motivated to produce quality research even if the results are only used to improve future iterations of the course.

II. The Big Picture: Collaborative Analysis of Microplastics

Before 2019, I was not aware of the presence of microplastics in the environment, or the fact that in three years they would be identified in human blood. At the time, I was searching for an external organization to collaborate with for my CURE project. My relationship with the Cahaba Riverkeeper began with a cold call. Through my Google research, I found that the Riverkeeper were one of the primary entities performing water quality testing on the Cahaba River, and I wanted to find a way for the students in my lab to get involved. After introducing myself to Myra Crawford, the executive director of the Riverkeeper, and explaining the project to her, she described a pilot study performed by her son, Shaun, also an employee of the riverkeeper, to investigate the presence of small, anthropogenic, chemically resistant particles in the river, known as microplastics. If was after this phone call that I began an extensive literature review on methods for detecting microplastics and determining their quantity in the environment.

I found that despite discovering environmental microplastics as early as 2010, the field was still in relative infancy and many challenges were present in measuring these particles. Namely, microplastics constitute a wide range of chemical makeup, meaning targeted analyses for microplastics must be able to screen from rubber particles to water bottles to PVC pipe. At the time, Nile Red was prominent in microplastic studies, with one recent study published in Nature using the dye. After deciding the method to be a good fit for my laboratory course, I based our "standard" microplastics detection procedure on dying with Nile Red. Chapter 4 summarized the experiments conducted to optimize the method so meaningful data could be obtained. After conducting the CURE over four years, I found that all my students were able to produce presentable data, understand the guiding principle of the method, and even work with me after completing the course to compile particle counts for publication.

I presented the outcomes of the first three years at the Biennial Conference on Chemical Education in a CURE symposium and found that several other educators were interested in trying the CURE at their institution. In the same symposium, I heard about the incredible work of other STEM educators to create CURE networks, where standard materials are made available to anyone who has interest. One such CURE network was BASIL, which had students determine the function of unknown proteins using computational methods. It was after participating in this symposium that I saw the potential for Aa CURE network to help close a critical gap in microplastics research: standardization and comparability.

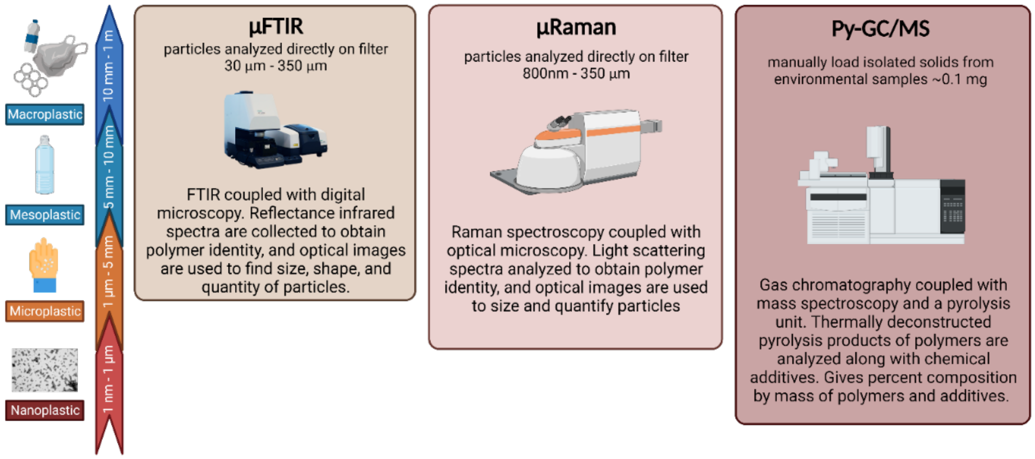

Figure 1: A comparison of three analytical methods for microplastics quantification in the environment.

While I found the method to be the most accessible for my student's projects, Nile Red is not especially quantitative. Even though it capitalizes on properties specific to plastic particles, the method has been found to overestimate certain microplastics and neglect others. Most commonly, spectroscopic methods, like Fourier Transform Infrared microscopy(µFTIR) and Raman microscopy (µRaman), and thermogravimetric methods, like pyrolysis gas chromatography coupled with mass spectroscopy (Py-GC/MS), are used to reliably quantify plastics, as both methods provide information on the chemical makeup of environmental polymers. Even though these methods are much more reliable, studies that use these methods still report inconsistent results. Below is an excerpt from a proposal I wrote in collaboration with the National Institute of Standard and Technology (NIST) for the National Research Council on this very problem:

"MP studies have used each method (µFTIR, µRaman, and Py-GC/MS) individually for identification[28]. Studies that offer a comparison of different analytical techniques for MP quantification often only compare two out of the three techniques and have shown that they often produce entirely different results[23, 29]. Thus, the field of MP measurement needs a direct comparison of all three prominent quantification methods to determine if one, two, or all three are necessary to accurately quantify MPs. One study compared µFTIR and µRaman with another thermal decomposition method, known as thermal extraction desorption GC/MS (TED-GC/MS) with a focus on instrumental restrictions, sample preparation, time input, and detection limits[30]. This pioneering comparison showed that the most appropriate methodology in MP studies is influenced by the research question, highlighting the need for a decision-tree analytical workflow for MP quantification. A direct comparison of µRaman, µFTIR, and pyro-GC/MS, the most common MP quantification methods, is therefore a crucial part of building a foundation for the field of MPs analysis to grow. No study has compared these three directly for accuracy and precision using the same preparation methods on the same samples in the same laboratory. The NIST Hawaii laboratory is an opportune location for the completion of this work, as it is one of the only locations with all three instruments under the same roof.

A recent interlaboratory comparison of MP quantification methods in laboratories across the world yielded drastically different results when given pre-counted samples[7], thus, the field of plastic pollution suffers greatly from a lack of comparability between studies due to the drastic variation of quantification methods, as well as varying interrater reliability among individual methods. The proposed study will provide a more comprehensive understanding of how the three major analytical methodologies focused on MPs can and should be combined to create a robust, accurate, and efficient procedure for the detection and quantitation of MPs in environmental matrices.

OBJECTIVES

1. *Compare the accuracy and precision of µRaman, µFTIR, and Py-GC/MS for quantifying MPs of various size classes with NIST plastic standards.*
2. *Develop a complementary workflow to quantify ensemble and size-fractionated MPs in environmental samples."*

A CURE network in the field of microplastics research requires an accurate method that is also accessible to educational institutions. Existing resources at primarily undergraduate institutions (PUIs) may allow for certain analyses to be performed, but not others. It may be necessary for certain institutions with fewer resources to focus on tasks that require more individuals to participate, such as performing sampling trips with a high school class, as sampling is relatively easy and safe, and sending those samples to a CURE laboratory course at a local university or community college. Additionally, universities may not have access to all or any of the techniques listed above. Thus, some laboratory classrooms may focus on sample preparation and processing so analysis can be completed by Master's or Ph.D students.

Ultimately, the goal of collaborative analysis of microplastics requires just that: collaboration. Not every institution must have every instrument to provide meaningful data to the broader community. What it does require, though, is a standardized workflow as described above. Thus, an analytical comparison of prominent microplastics methods is still greatly needed, and the resulting workflow would identify niche areas that could be filled by the laboratory classroom. In addition to the broader impacts of disseminating microplastics data to the community, involving undergraduate and high school students directly benefits them. A major conclusion of this work is that transitioning from "student" into "scientist" requires immersive, authentic experiences that have real-life impacts. Future efforts in the realm of microplastics research should investigate the role of the CURE classroom, as the complete potential of both have yet to be seen and could be accomplished through the summative efforts of environmental scientists, educators, and most importantly, student-scientists

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APPENDIX A

CHAPTER 2-4 IRB APPROVAL

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM
Office of the Institutional Review Board for Human Use

470 Administration Building
701 20th Street South
Birmingham, AL 35294-0104
205.934.3789 | Fax 205.934.1301 | irb@uab.edu

APPROVAL LETTER

TO: Forakis, Josh

FROM: University of Alabama at Birmingham Institutional Review Board
Federalwide Assurance # FWA00005960
IORG Registration # IRB00000196
(IRB 01) IORG Registration #
IRB00000726 (IRB 02)

DATE: 21-Jun-2019

RE: IRB-300003153
Changing Environmental and Chemical Attitudes Through Research Based Instruction

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The IRB reviewed and approved the Initial Application submitted on 17-Jun-2019 for the above referenced project. The review was conducted in accordance with UAB's Assurance of Compliance approved by the Department of Health and Human Services.

Type of Review: Exempt
Exempt Categories: 1
Determination: Exempt
Approval Date: 21-Jun-2019
Approval Period: No Continuing Review

Documents Included in Review:

- pptletter.190617.docx
- infosheet(survey)190617.docx
- exempt.190617.docx
- surveyquest.190617.docx
- focusgroup.190617.docx
- infosheet(FG).190617.docx

APPENDIX B

CHAPTER 4 SUPPLEMENTAL MATERIALS

I. CH126/128 (Microplastics CURE) Lab Manual*

1. Known Samples – Students analyze simulated environmental samples spiked with MP standards.
2. Cahaba River Water Samples – Students perform the same procedure with the water samples collected from the sampling trip.
3. Image Analysis – Student use ImageJ to identify number, size, and shape of microplastics from their imaged samples.

*Note: Only the procedure for weeks 1 & 3 are given. The exact same protocol was given to students for CURE weeks 1 & 2 in CH126

Detection of Microplastics Day 1 – Known Samples

This experiment was developed and written by A. Scircle and J. V. Cizdziel and reivsed by J. Forakis;
based on Detecting and Quantifying Microplastics in Bottled Water using
Fluorescence Microscopy: A New Experiment for Instrumental Analysis and
Environmental Chemistry Courses,
Journal of Chemical Education: 2019

PRE-LAB INSTRUCTIONS

In Your Notebook Outline a procedure in your own words that will allow you to collect the required data for each of the exercises below. Anticipate the type of data you will collect and prepare space in your notebook to record the data (data tables or space for observations).

Before arriving at the laboratory you should have the answers to the following questions in your notebook.

. Why are microplastics an environmental concern and what are some of its potential impacts?
. Write the balanced chemical reactions associated with Fenton's reagent. Why is it used in this lab?
. What are some chemical factors that might affect how strongly a dye adheres to certain microplastics?
. What measure(s) can determine the validity of your microplastics count for a known sample?
. What does infrared spectroscopy measure?

For Your Safety You must wear your goggles whenever you are in the laboratory.

The solutions encountered in this experiment must be handled with care. If small volumes of these solutions come in contact with your skin, you should wash the affected area with lots of water. If a large spill occurs, you should also notify your instructor. Avoid waste: do not obtain more reagent than you really need. All solutions and reaction mixtures can be disposed of in the sink.

The Hydrogen Peroxide used in this experiment is highly concentrated and a highly reactive oxidizer that will stain skin and damage clothing. Wear clothes to this lab that you are not afraid of damaging. <u>**LONG SLEEVES REQUIRED**</u>

Gloves must be worn when handling the hydrogen peroxide and when cleaning up any spill that occurs during this laboratory period.

Objectives fter this laboratory experience you should be able to:

Perform a digestion using Fenton's reagent
Use dye to estimate microplastic count in a natural water sample

Background (Text used from Civizidel et. al)

Microplastics

It is hard to believe that our grandparents or great grandparents grew up in a world without plastic. The advent of cheap and durable plastic introduced many conveniences and has partly led to our "throw-away" society. Since about the 1950's plastic production has skyrocketed, with world production surpassing 320 million tons in 2016. The improper disposal and mismanagement of plastic has resulted in plastic pollution in both terrestrial and aquatic environments. This global problem has resulted in growing concern about the fate and effects of plastic pollution.

Microplastics, small plastic particles < 5-mm in diameter, include microbeads once used in personal care products and fragments of weathered and degraded plastic. The small plastic particles are harming aquatic organisms which can mistake them for prey. Even smaller plastic particles (nanoplastics) can be incorporated into plankton at the base of the food chain. Ingestion of microplastics by aquatic organisms can cause digestive, hormonal, and reproductive problems. Ingestion of microplastics by wildlife also poses a potential route for human exposure. Seafood, including filter-feeders oysters and mussels, as well as shrimp, stem from coastal areas where microplastic particles congregate. The consumption of beverages sold in plastic containers (e.g. bottled water) pose an additional route for human exposure to microplastics. How great a threat microplastics are to wildlife and humans is the subject of intense scrutiny and multiple studies.

From an analytical chemistry perspective, detecting, identifying and quantifying microplastics is an especially challenging problem. The difficulties include (1) reliably distinguishing microplastic particles from natural materials (as weathered particles may develop biofilms or adhere other inorganic particles), (2) difficulty in categorization, as "plastic" can refer to thousands of unique formulations of organic polymers with different applications, colors, additives, morphologies and properties, (3) contamination issues, as synthetic polymers are ubiquitous in clothing and everyday lab equipment, and (4) a lack of standardized methodology for sampling and analysis (which means that studies are often difficult to compare). But analytical chemists are by nature problem solvers, and many analytical techniques varying widely in complexity and fundamental properties, have shown promise in characterizing microplastics.

This laboratory exercise will provide hands-on training with one promising approach, fluorescence microscopy, and simultaneously reinforce the principles of fluorescence that you learn in instrumental analysis. More specifically, you will isolate the microplastics from the water samples (bottled water or water from a nearby waterbody) and stain them with Nile Red dye, which is lipophilic and adsorbs on plastic surfaces (but not so much on inorganic materials like sand). The dye can be made to fluoresce providing a sensitive means to detect the particles using fluorescence microscopy. It is important to note that the Nile Red method does not identify the type of microplastic, that would require micro-spectroscopy, such as micro-FTIR or micro-Raman.

Fenton's Reaction

In this experiment, you will use fenton's reagent to degrade the organic material in your natural sample. Figure 1 depicts Fenton's reaction. Iron(II) is oxidized by hydrogen peroxide to iron(III), forming a hydroxyl radical and a hydroxide ion in the process. Iron(III) is then reduced back to iron(II) by another molecule of hydrogen peroxide, forming a hydroperoxyl radical and a proton. The net effect is a disproportionation of hydrogen peroxide to create two different oxygen-radical species, with water (H+ + OH−) as a byproduct.

The radicals produced by this reaction are highly reactive, non-selective oxidizers and are prime candidates for the oxidation and breakdown of organic material. This reaction is highly volitale and exothermic, and temperature control is imperative. Iron acts as a catalyst in this reaction because it is not used up by this reaction and remains in its original form once the reaction has completed. Iron does this by both accepting and donating electrons from H_2O_2 .

:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO\bullet + OH^- \quad \textbf{(1)}$$

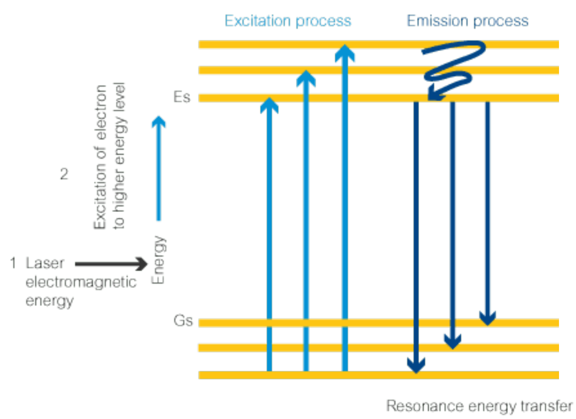
$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HOO\bullet + H^+ \quad \textbf{(2)}$$

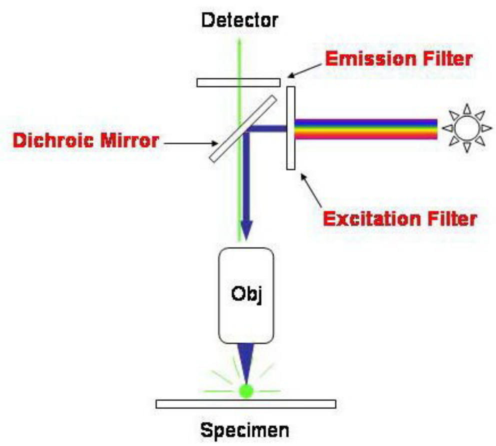
$$2\ H_2O_2 \rightarrow HO\bullet + HOO\bullet + H_2O \quad \textbf{(net reaction: 1+2)}$$

Figure 1. Fenton's reaction

Fluorescence Microscopy

The final step in your experiment is quantifying microplastics in each sample using fluorescence microscopy. Fluorescence microscopy is a powerful instrumental technique commonly used in biological studies to detect cells or specific (fluorescently tagged) molecules in cells or in larger tissues. The technique has also found use in genetics where proteins can be modified to exhibit fluorescence and their movement and function within a cell or tissue can be readily observed. Here, we use Nile Red dye to detect and quantify microplastics. Whereas there are many variations of fluorescence microscopes available, the basic principles and schematics of the technique remain the same (Figure 1). Briefly, fluorescence microscopy works by applying a light source through an excitation filter, which then hits a dichroic mirror which reflects the excitation light downwards onto a sample. The sample (in our case, microplastic particles stained with Nile Red dye) is excited to a higher electronic energy state, and subsequently relaxes back to ground state while emitting a photon. This emitted photon is lower in energy (longer wavelength) than the excitation light, and can pass through the dichroic mirror and reach the detector (in most cases a camera and/or the eyepiece of a microscope). Meanwhile any reflected photons will retain the shorter wavelength and be unable to pass through the dichroic mirror and thus can't reach the detector.





Infrared Spectroscopy

To learn the identity of the microplastic in your sample, you will analyze them using Fourier Transform Infrared Spectroscopy. This technique is based on the theory that molecules absorb specific wavelengths of light based on their structure. IR spectroscopy deals with the infrared region of light (14000-4000cm^{-1} wavelength). This is a relatively low frequency (and subsequently low energy); therefore, molecules will not re-emit the radiation. Instead the molecules will exhibit some form of vibration which takes the form of bends and stretches on the molecule's structure. IR is most useful to determine the functional groups present in an unknown sample, and can additionally be used to identify a known sample if the generated spectra matches a reference spectra. You will compare your generated spectra to reference spectra made available in the lab.

Exercise 1: Wet Peroxide Oxidation with Fenton's Reagent

Obtain a known microplastics sample from your TA. Record the unknown number in your lab notebook. Place a clear PCTE filter in a Buchner funnel so that the filter covers all the pores in the filter holder. Turn on the vacuum line and prime the filter with a small amount of DI water, then pour your known water sample through the filter. Make sure that no water enters the vacuum line. You will have to dump the excess water from the chamber several times.

Once you are finished filtering, keep the collected solids on your PCTE filter. Transfer your PCTE filter with the suspended solids from your known water sample to a clean 250ml Erlenmeyer flask. Add 20ml of iron(II) catalyst solution and 10ml of 30% hydrogen peroxide. Let sit on the benchtop for five minutes, then heat on a hot plate slightly (do not set higher than 3 on the knob, the reaction is exothermic and will produce more heat). If the solution starts to bubble violently, take the solution off the heat until the bubbles subside. If there are no bubbles forming and you still see visible organic matter, add 10ml of hydrogen peroxide. Repeat up to three times until no visible organic matter is present. If a cloudy, orange solution forms (resulting from precipitated iron compounds) ask your TA to add a couple drops of sulfuric acid to your flask.

Exercise 2: Dying of Microplastics Sample

Once the reaction has finished and there's no visible organic matter in your sample, pour your fenton's solution through a buchner funnel using a PCTE filter membrane. If available, look at your filter under the light microscope in the lab Iif available) to identify any possible microplastics. Remove 2-3 large pieces of plastic from the filter paper to be used for IR analysis. If there are no large plastic pieces, your TA will provide you with a plastic to use for IR analysis.

Transfer the solids on your PCTE filter into clean 50ml beaker using ~30ml of DI water, meaning, spray the surface of your filter directly so that the solids will be washed from the filter into the beaker. Prepare a working solution of Nile Red by diluting .5ml of Nile Red stock solution to 10ml with acetone. Add roughly 2-3ml of working Nile Red solution to each of your microplastics water baths. Stir well. Set aside for up to an hour.

Exercise 3: ATR-FTIR Analysis of Microplastics

Take your large microplastics removed from your natural water to be analyzed using IR spectroscopy (ATR-FTIR, Agilent). Follow the protocols from the video and the printed instructions at the instrument. Open the software names "OPUS" on the desktop and enter the user password. Be sure the stage is clear, then select Measure -> Measurement -> Take Background. Once the background measurement is complete, load your sample onto the stage, and adjust the lever so that it will clamp onto your sample with a moderate amount of pressure. Click Take Measurement. Adjust the range of your spectra so all the peaks are visible. Once you have generated a spectra, take a photo and compare it to the reference spectra provided to you. Identify specific peaks and wavenumbers that are similar between your spectra and the reference. Report the identity of the microplastic from your water sample on your in-class assignment.

Exercise 4: Preparation Of Known Samples for Imaging

Locate your water baths. Filter each water bath on a PCTE filter taking care to place all solids on the filter. Carefully transfer the the PCTE filter with solids on top to a clean labelled petri dish. Use the following convention when labelling your petri dishes: [section number] – [Group number] – [sample number]. Your group will be assigned a group number before you attend lab. You should determine on your own how to number your samples in your laboratory notebook. For example, you could use the format in the table below:

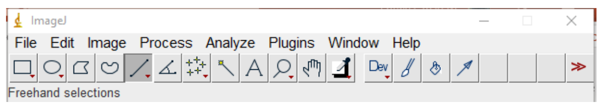
Sample Description	Sample number
Known sample trial 1	1
Known sample trial 2	2
Known sample trial 3	3
Cahba River Water sample trial 1	4
Cahba River Water sample trail 2	5
Cahba River Water sample trial 3	6

Therefore, if I'm a part of group 4 in section 8M, I would lable my first petri dish: **8M-4-1**. It's extremely important that you follow this naming convention, or your samples may not be imaged. Give your labelled samples to your TA for storage.

Detection of Microplastics Day 3 – Image Analysis

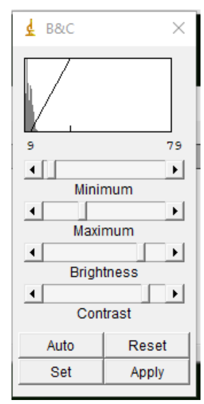
Exercise 1: Determining Inter-rater Reliability with a Pre-counted Image

- Download ImageJ
  - Go to: https://imagej.nih.gov/ij/download.html
    - Click the link that matches your system to begin download.
  - Extract the contents of the folder to your documents. Click "**ImageJ.exe**" to run imageJ
  - Detailed download instructions:
    - Mac: https://imagej.nih.gov/ij/docs/install/osx.html

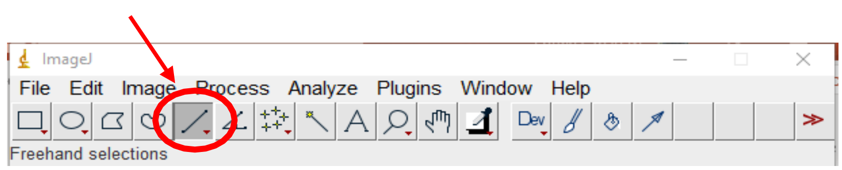


    - Windows: https://imagej.nih.gov/ij/docs/install/windows.html

- Uploading and adjusting images
  - In this first exercise, you will use image J to estimate the MPs in a sample that's already been counted. You will each analyze the image individually, then compare results on your in-class assignment.
    - Your TA will have the correct counts for the known samples

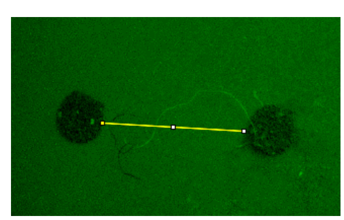


  - Find the folder with your group's images using This Link. You should have a folder of "Known MP samples" and "unknown MP samples." Download the images to your computer.
  - Open ImageJ. Select File -> Open, then select the image in your "Known MP sample" folder.
  - Select Image -> Adjust -> Brightness/contrast. Adjust the brightness and contrast until fluorescing particles are clearly visible from the background. The screenshot shows the

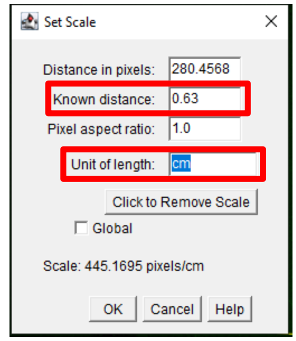


settings that worked.

- Set Scale
  - Select the "Straight Line" tool.
  - Draw a straight line between two of the dark circles on your image so that the edge of the line touches the edge of the circles.

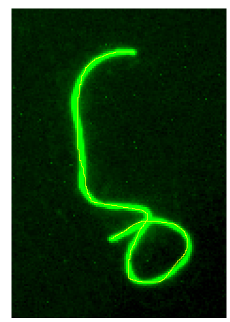


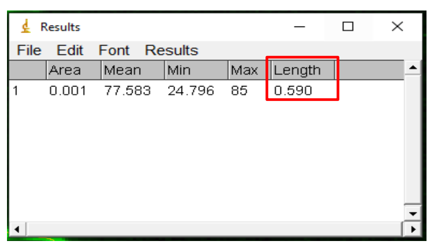
- Select Analyze -> Set Scale, and set known distance to “0.63”, and sent unit of length to “cm.” Click “OK.”



- You can now Identify and measure the length of fluorescing particles in your image.
  - Each member of your group should count the particles in the “known image” individually then compare results. Calculate the precision between all of your group members counts.

- Measure fluorescing particle
  - Identify a fluorescing particle and assign in a MP type, such as “fiber”, “fragment”, “shard”, or “sphere”. For example, I’d classify this particle as a fiber
  - To measure the length of a particle, select the best line tool for that particle. For example, for this particle, a “freehand line” was used which can be selected by holding down on the line tool button.
  - Trace the length of your fluorescing particle with the line tool of preference (the yellow line is the trace made for this particle).
  - Click Analyze -> Measure. The results of the analysis will pop up in another window. Copy the “length” data (cm) into your notebook.
  - Repeat for all the particles identified in your image. Keep a table that shows the number of particles found along with the type and length (cm) of each.
  - Ask your TA for the determined count for the image and calculate accuracy.





Exercise 2: Counting Fluorescing Particles in Unknown Images

- Open the folder labelled “Unknown MP samples” in your groups file. (If time allows, images of your Cahaba River Samples will be made available for counting). There will be three unknown images to analyze. Each member of your group can take one.
  - If your group has fewer than three members, you only need to analyze one per person.
- Repeat the procedure from the previous exercise (including setting the scale for each new image).
- Summarize the results of your analysis including average length of fluorescing particles and counts of each particle shape on your in-class assignment.

II. Instructor Notes for the Microplastics CURE

1. Materials list

Name of Reagent	Product#	Amount
Nile Red	Fisher: AC415711000 Acros: 415711000	100mg
PCTE Filter Membranes (47mm diameter, 10µm pore size)	Fisher: NC1831462	100
Hydrogen Peroxide (30%)	H325-100	100ml/ group
Iron(II) sulfate heptahydrate	Fisher: AA1449830 Alfa Aesar: 1449830	7.5g/L
Sulfuric acid (conc.)		3ml
Ceramic Buchner funnels	NC0480557	1 per # of groups in one section.
Polystyrene standard (M.W. 900,000)	Fisher: AA4194303 Alfa Aesar™: 4194303	100mg
Low density polyethylene (LDPE) standard (500 micron)	Fisher: AAA1023922 Alfa Aesar™: A1023922	100mg
Petri Dishes		5/group

2. Note regarding microplastic contamination:

a. Great care should be taken to avoid plastic contamination throughout the procedure. Some precautions taken in this experiment were the use of glass pipets instead of plastic disposable pipets, having students wear cotton lab coats to prevent contamination by polymer fibers in clothes, triple rinsing glassware (glassware can also be washed with noChromix to remove residual microplastics), filtering reagents, using analytical water (Milli-Q), maintaining adequate air filtration in the lab or working under a fume hood.

3. Preparation of microplastic standards (week 1)

a. MP standards can be ordered from any chemical provider such as Fisher Scientific. For this activity, microplastic standards were made by blending, milling, or cutting up commercial plastics.

b. Polypropylene (PP) – PP microplastics standards were made using 100% polypropylene Poly-pellets (used to fill weighted blankets). Beads were blended in around 250ml of DI water for 10-15 minutes. Microplastics were isolated from larger plastics by pouring the mixture through cheesecloth. The remaining filtrate was then filtered again with a vacuum filtration apparatus to obtain PP microplastics.

c. Nylon – Nylon yarn was purchased commercially and microplastics were made by cutting the yarn into 0.5 mm length pieces.

d. Polystyrene and Expanded Polystyrene (PS or EPS) – EPS microplastics were obtained by using commercial packaging. PS microplastics were provided by the Kharlampieva group or purchased through Fisher.

4. Preparation of simulated environmental samples (week 1)

a. Simulated environmental samples were made in 1-L wide-mouth amber glass jars. Any one-liter container will work, especially mason jars.

b. Each 1-L container was filled with the following:

i. 0.75 g soil – potting mix purchased commercially, milled with a mortar and pestle prior to addition to decrease digestion time with fenton's reagent.

ii. 0.75 g sand

iii. 0.50 g MP standard

- A code was used to distinguish between unknown identities. Unknown 1 was PP; Unknown 2 was Nylon; Unknown 3 was PS.

iv. Around one liter of DI water (or Milli-Q water if available).

c. Combine and mix thoroughly. Particles should remain suspended in the mixture, not dissolved.

5. Collection of environmental samples

a. 1-L grab samples were collected at each sampling trip using wide-mouth amber glass jars (mason jars are preferred).

b. Locate an area of around 2 feet of depth.

c. If standing in the water, position yourself so that the sample is taken upstream from where you are standing.

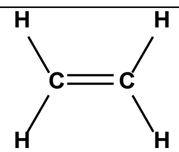
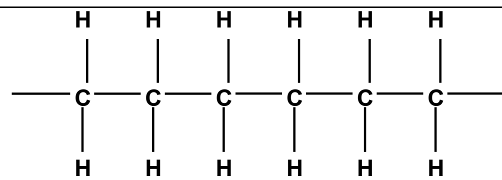
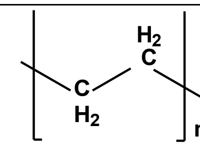
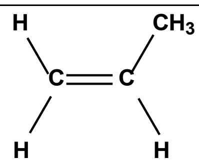
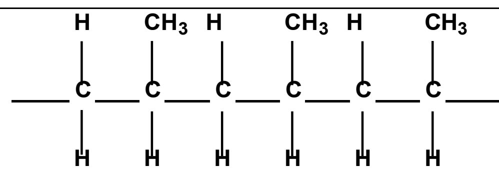
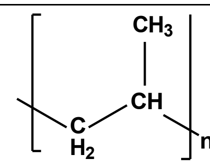
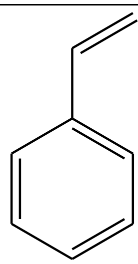
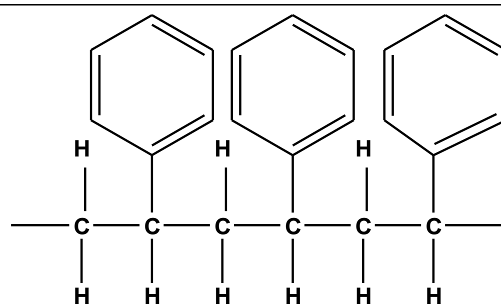
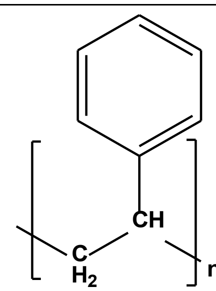
d. Prime the container by rinsing it three times with river water.

e. With the container facing down, submerge the container and turn it over underwater so that the sample is collected just below the surface.
f. Close the container and label with location, time, date, and group number.
6. MP analysis with Nikon AZ100 Dissecting Scope
a. Log in to the computer using the SCOPE account. password: scope (all lowercase)
b. Turn on microscope, monochrome camera, fluorescence source and brightfield source then open Nikon software. You'll be prompted to log in. They'll set you up an account if you are trained, until then, I'll log into my account.
c. A dialogue box will open. Choose the Andor-Zyla camera from the list of cameras.
d. The Nikon will boot up. Do not adjust the height of the microscope while it's calibrating.
e. Locate the filter cube rotator. Insert the EGFP filter cube into the #1 spot, removing the Long Pass GFP filter that is located there. (be sure to replace back before you leave)
f. Turn the rotator to the #4 spot (brightfield) and center your sample (after removing the petri dish lid) beneath the scope. Focus first on the very center of the filter.
g. Open the source light so that the camera is open (pull out metal rod and adjust level to 100% camera) and focus the microscope on your sample. Keep the objective at 1x for the time being.
h. Once the sample is in focus adjust shutter speed until the light is appropriate. Then, switch to the #1 spot (EGFP) and adjust shutter speed and binning until fluorescing particles are visible. Remember the settings for both profiles.
i. Optional: Set up multichannel capture if not already set. Capture only GFP and Brightfield channels
j. Select acquire -> multichannel -> capture multichannel image. You will be instructed to change the filter profile to the appropriate setting for each photo. Adjust lighting based on the settings that worked from step 8.
k. Capture first image, then rotate filter profile as directed. Capture brightfield image once light settings are adjusted.
l. Rotate around the remainder of the filter and capture up to 6 additional images for the rest of the sample. Save files as [sample name](a-f).
m. Close software when complete, return filter cubes to correct location, turn off camera, scope, and light sources. Cover the microscope.

III. Guided Inquiry Polymer Chemistry Assignment

Polymers are some of the most commercially available products made in the chemical industry. Many of the things you see around you right now are thanks to polymers. The table below shows three common polymers and their monomers. The **monomer** column shows a single monomer molecule. The **polymer (extended structure)** column shows a few repeating units in the polymer made from that monomer. The **polymer (condensed structure)** column shows how the chemical formula for that polymer is written.

Model 1 – Vinyl Monomers and Polymers

Monomer	Polymer (extended structure)	Polymer (condensed structure)
 ethylene		 polyethylene
 propylene		 polypropylene
 Styrene		 polystyrene

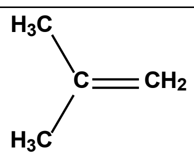
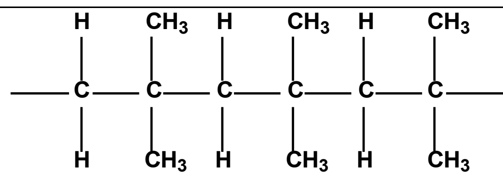
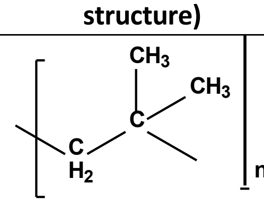
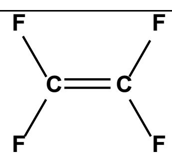
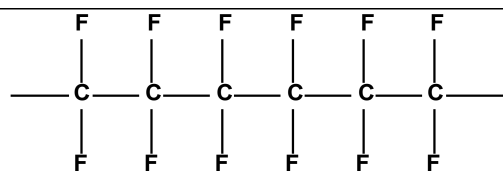
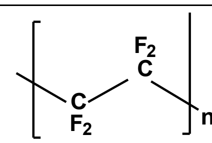
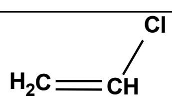
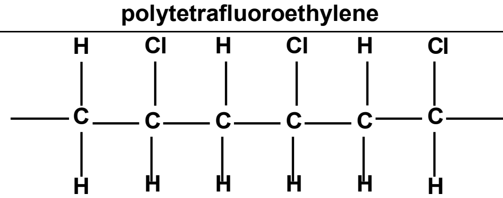
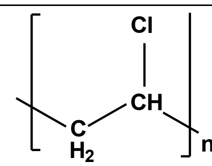
Critical Thinking Questions

1. Describe, using your own words, the difference between the monomer molecules and the polymer molecules presented in the model.
   The monomer molecules are individual molecules, and the polymers look similar, but are linked together in long chains. The monomers all contain double bonds, but the polymers do not.

2. Look at the condensed structure for each polymer. What do you think "n" represents? What is the numerical value of n based on the extended structure of each polymer?
"n" is the number of repeating units that make up the polymer. Basing it's value off the extended structure, n = 3

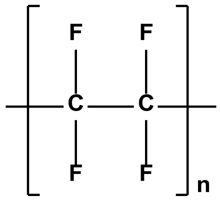
3. Based on what you have learned from the model above about monomers and polymers, create an analogy that relates a monomer and a polymer to a real-life example.
Any analogy works here where a monomer is a small building block, and a polymer is made up of repeating units of that building block. A good example is a lego block and a lego tower.

4. Fill in the following table with the appropriate polymer or monomer structure **and name**. (you may draw on paper and upload a picture to submit)

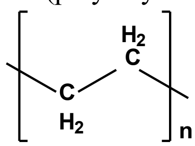
Monomer	**Polymer (extended structure)**	**Polymer (condensed structure)**
 isobutylene	 polyisobutylene	 polyisobutylene
 tetrafluoroethylene	 polytetrafluoroethylene	 polytetrafluoroethylene
 Vinyl chloride	 poly(vinyl chlroride)	 poly(vinyl chloride)

The model below contains the structure and description of a few common synthetic polymers. Use your critical thinking and knowledge of chemistry to help you answer the questions

Teflon (polytetrafluoroethylene)



PE (polyethylene)



Model 2 – Uses of Synthetic Polymers

Critical Thinking Questions

1. Observe the chemical structure of Teflon. What aspects of its chemical structure might contribute to its “non-stick” properties? (Hint: consider what you know about periodic trends concerning fluorine.)

Fluorine is the most electronegative element, meaning that it hangs onto its electrons the most tightly out of any other element on the periodic table. It’s electrons are not available for any sort of interactions with other compounds resulting it its ability to create non-stick surfaces.

2. When forming a synthetic polymer into a particular shape, multiple polymer molecules are stacked together. Draw a simple picture to demonstrate how HDPE and LDPE can be stacked with itself (e.g. if you have four HDPE molecules, draw how they would be stacked together. Do the same for LDPE.). Based on your pictures, which type of PE can be stacked more easily or closer together?

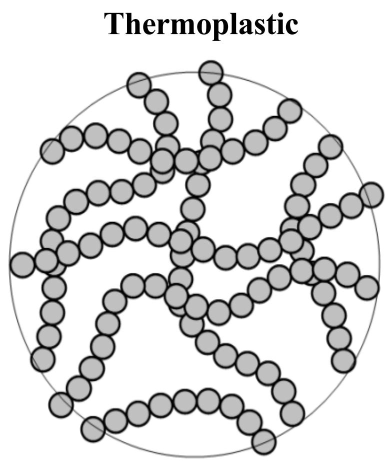
The image for HDPE should show that the linear molecule can be easily stacked with other linear molecules. For LDPE, the picture should show that the branches could make stacking more difficult.

3. As seen above, HDPE and LDPE are used for very different applications. So much so, that HDPE and LDPE constitute two entirely different categories of recyclable plastics (#2 plastics are HDPE and #4 plastics are LDPE). Use your answer to the previous question to explain why HDPE can be formed into more rigid and durable products and LDPE is typically used for flexible products like plastic bags.

HDPE can stack itself much more tightly than LDPE, resulting in a much denser structure. This dense structure results in its ability to be made into rigid containers. LDPE’s branches cause it to have a much lower density resulting in it being made into flimsy products like plastic bags.

Model 3 – Thermoplastics versus Thermosets

Polymers can be largely classified into two major categories depending on their structure and response when heated. These two categories are **thermoplastics** and **thermosets.** The model below shows a simplified structure of a thermoplastic and thermoset that emphasizes the way that multiple polymer chains interact with eachother.



Critical Thinking Questions

1. Observe the simplified structure of both types of polymers. Describe in your own words the difference between the simplified structures of a thermoset and a thermoplastic.

Thermoplastics contains separate linear molecules while thermosets are connected together with crosslinks.

2. The interactions of certain polymer molecules can be compared to a bowl of spaghetti, where polymer chains overlap and tangle with each other. Heating a polymer would then be like using a fork to separate polymer chains from each other, however, not all polymer chains can be separated from one another. Using this analogy, describe what you think would happen to a thermoplastic if it were heated, as well as a thermoset.

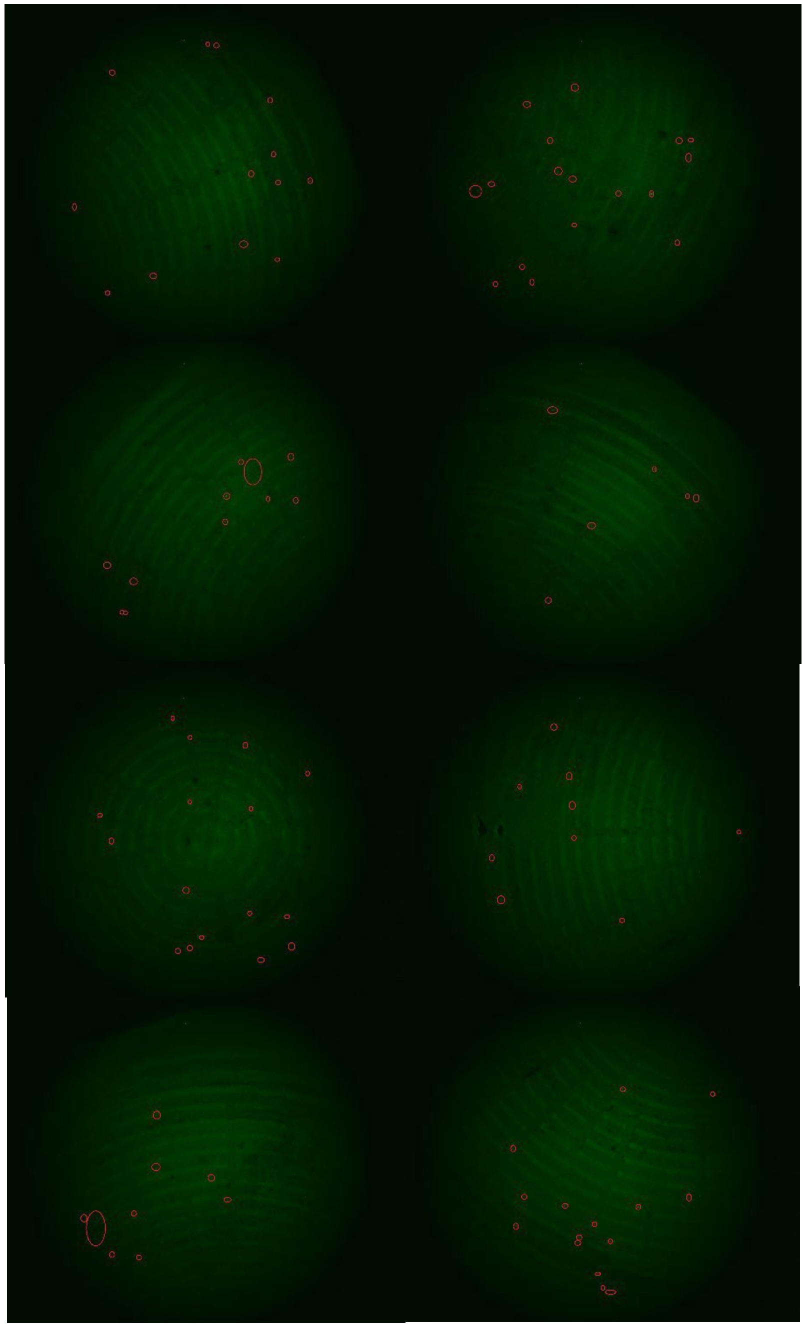
Thermoplastics could be separated with heat because the molecules are not attached to each other. Thermosets are crosslinked together, so the addition of heat would cause strain and eventually breakage of the chains.

3. Certain polymers in the plastic industry can be melted down and formed into a variety of shapes, like bottles, bags, and pipes. Other polymers, however, cannot be melted. When they are heated, they become brittle and break. Using the model above, predict which of these would responses (melting down or becoming brittle) would happen to a thermoplastic and a thermoset. Explain your reasoning.
   The thermoplastic could be melted down and remolded and the thermoset would become brittle and break.

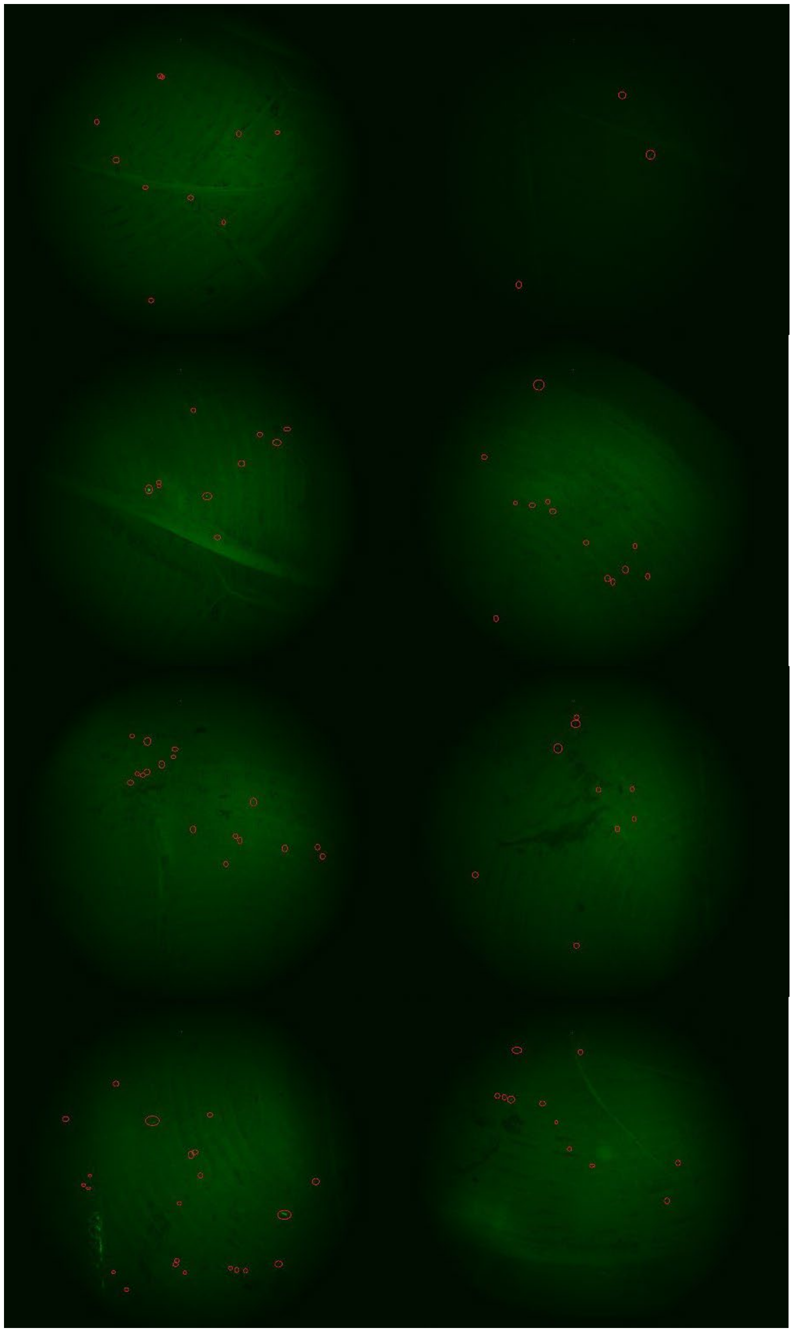
APPENDIX C

MICROPLASTICS IMAGES SUMMARIZED IN CHAPTER 5

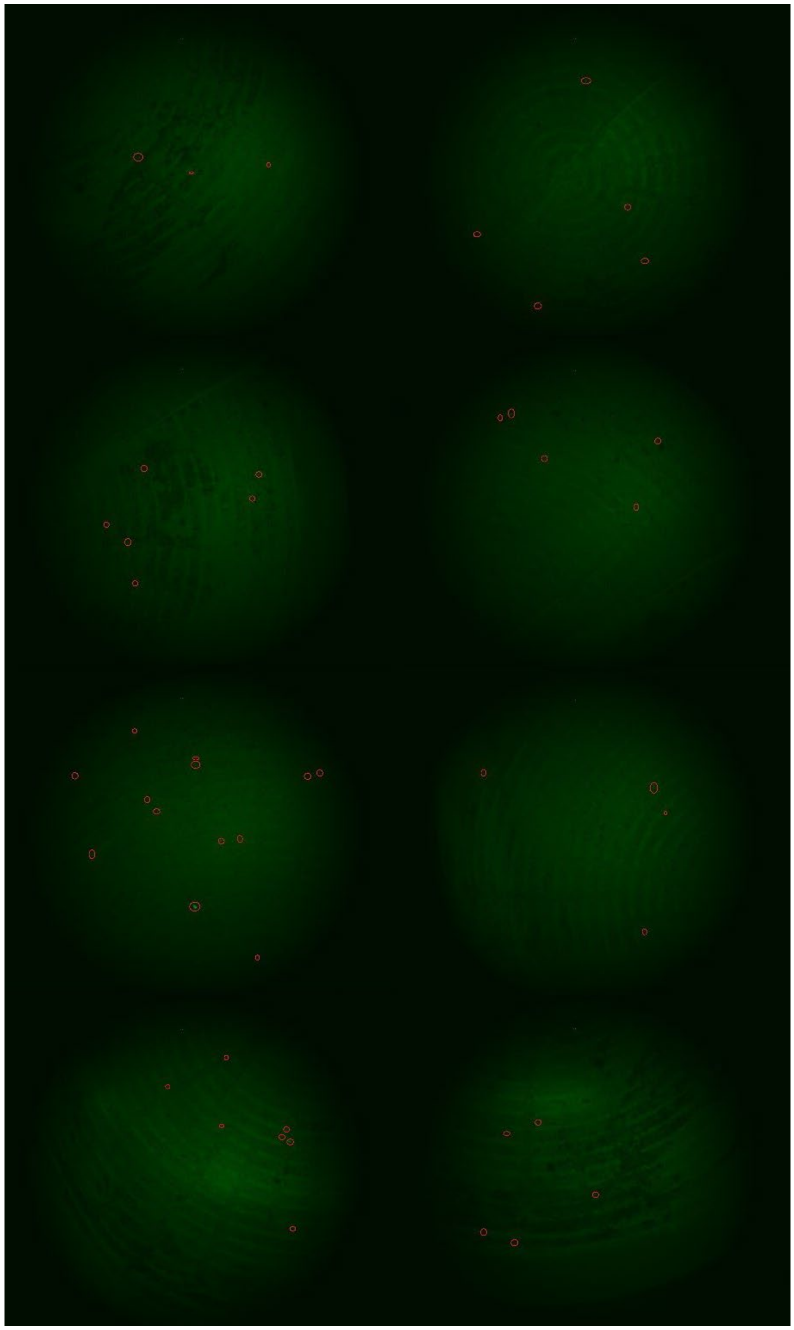
Highway 280 Trial 1



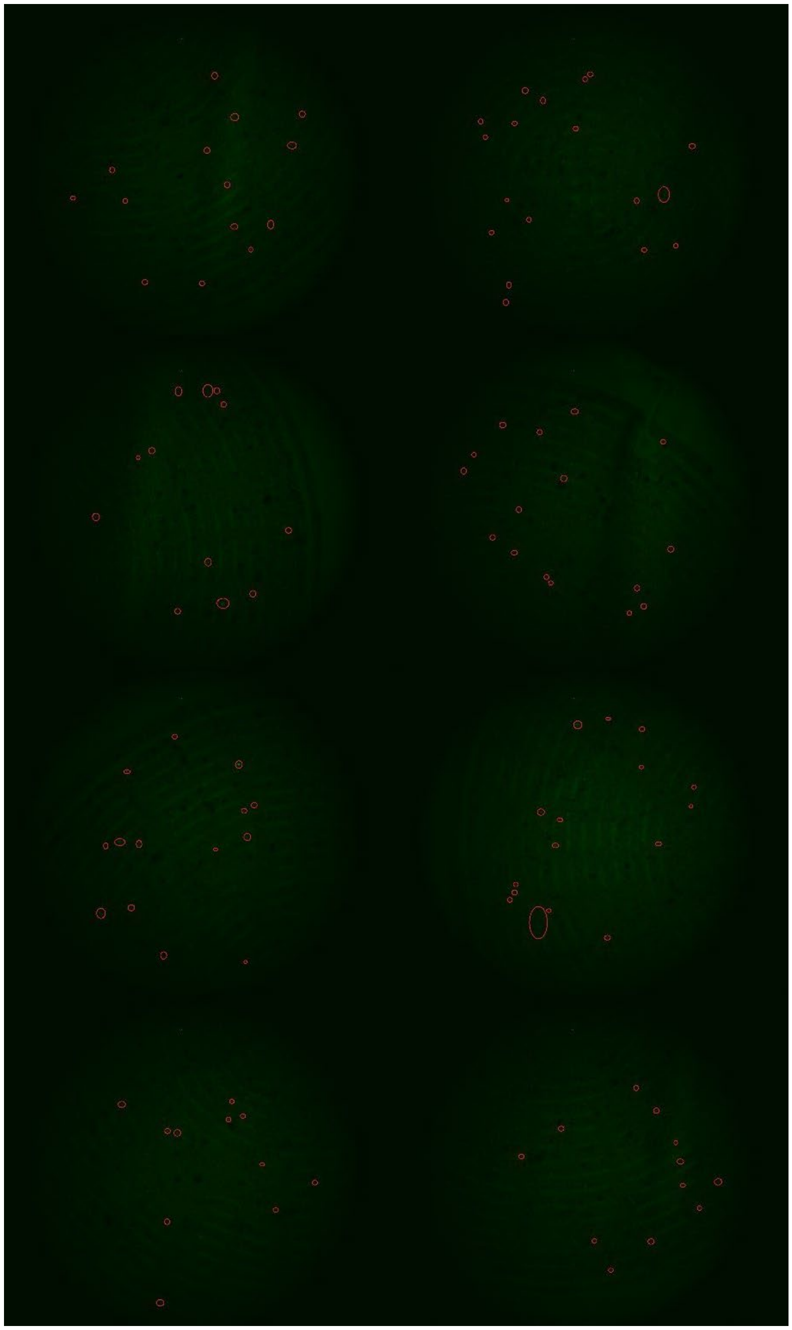
Highway 280 Trial 2



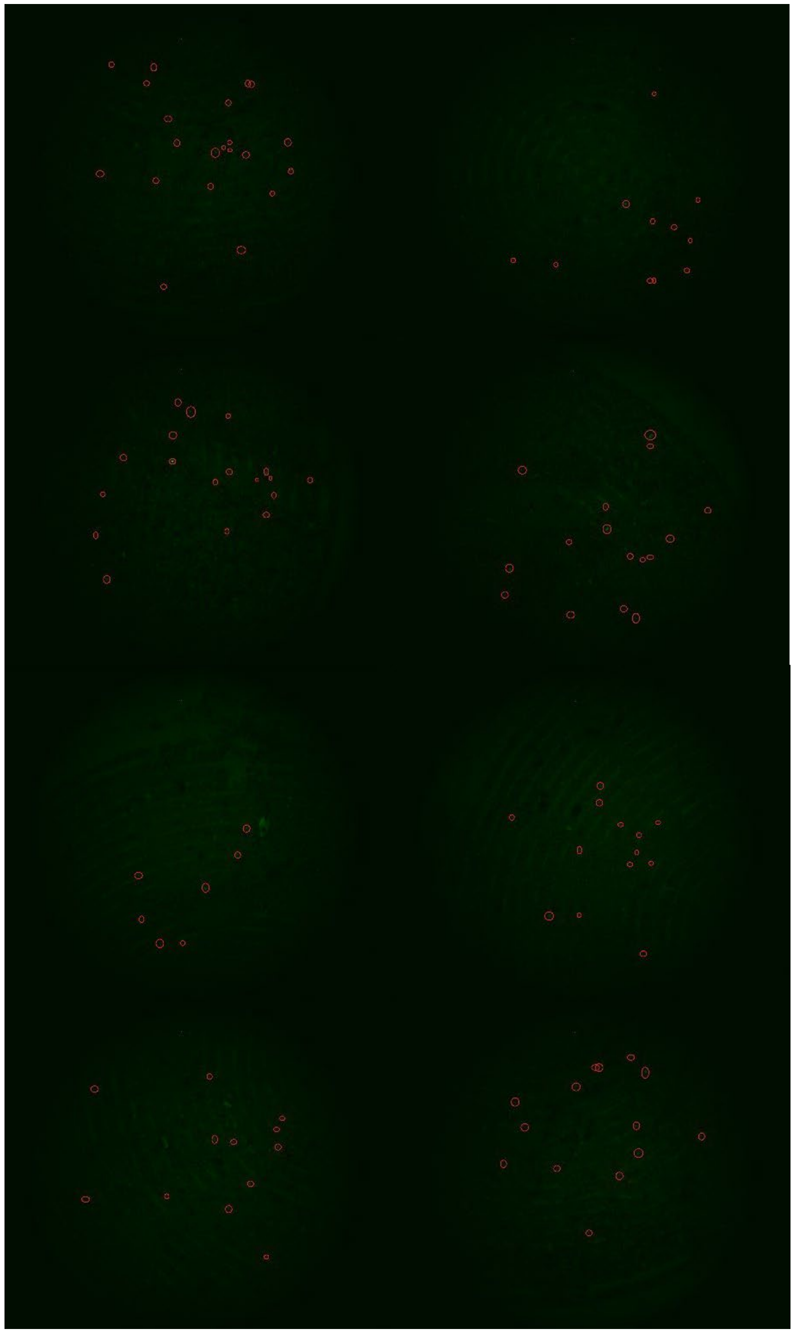
Highway 280 Trial 3



Hoover East Trial 1



Hoover East Trial 2



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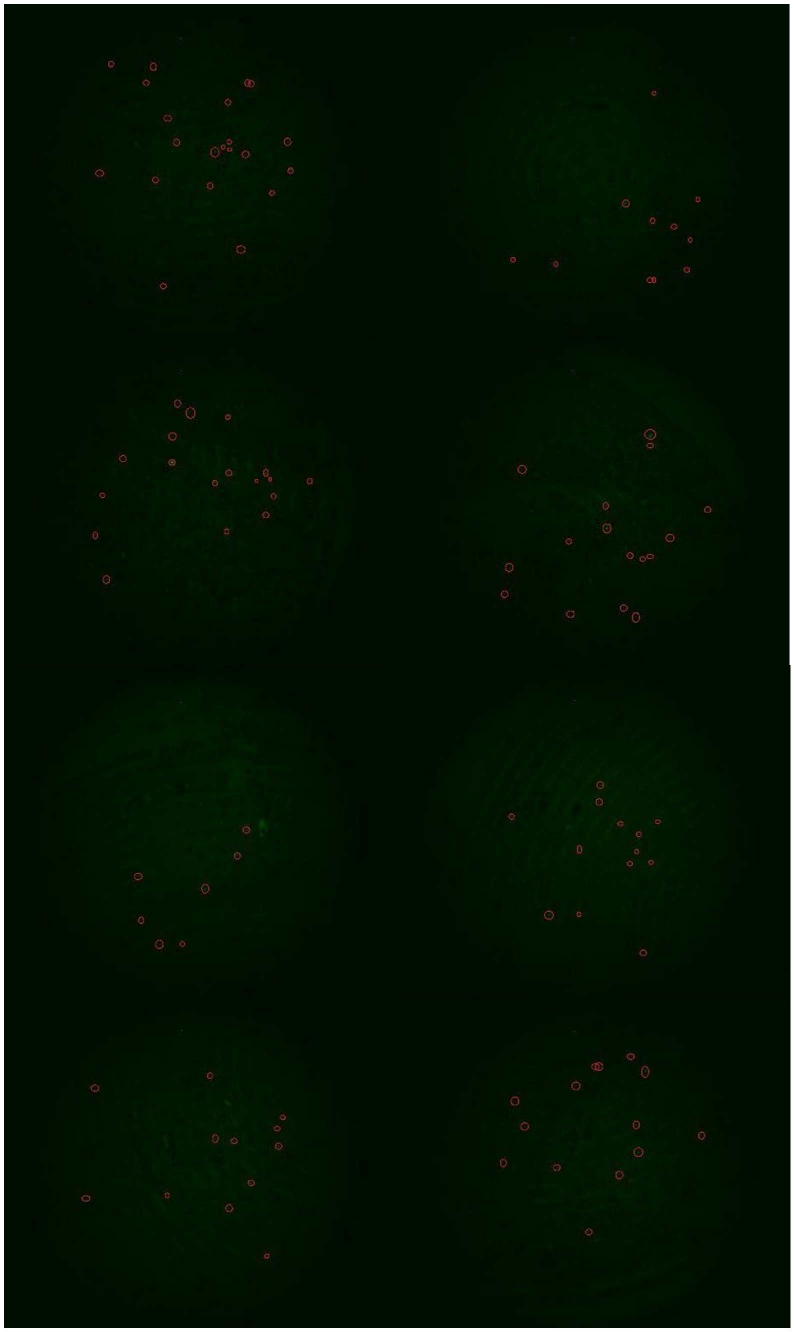
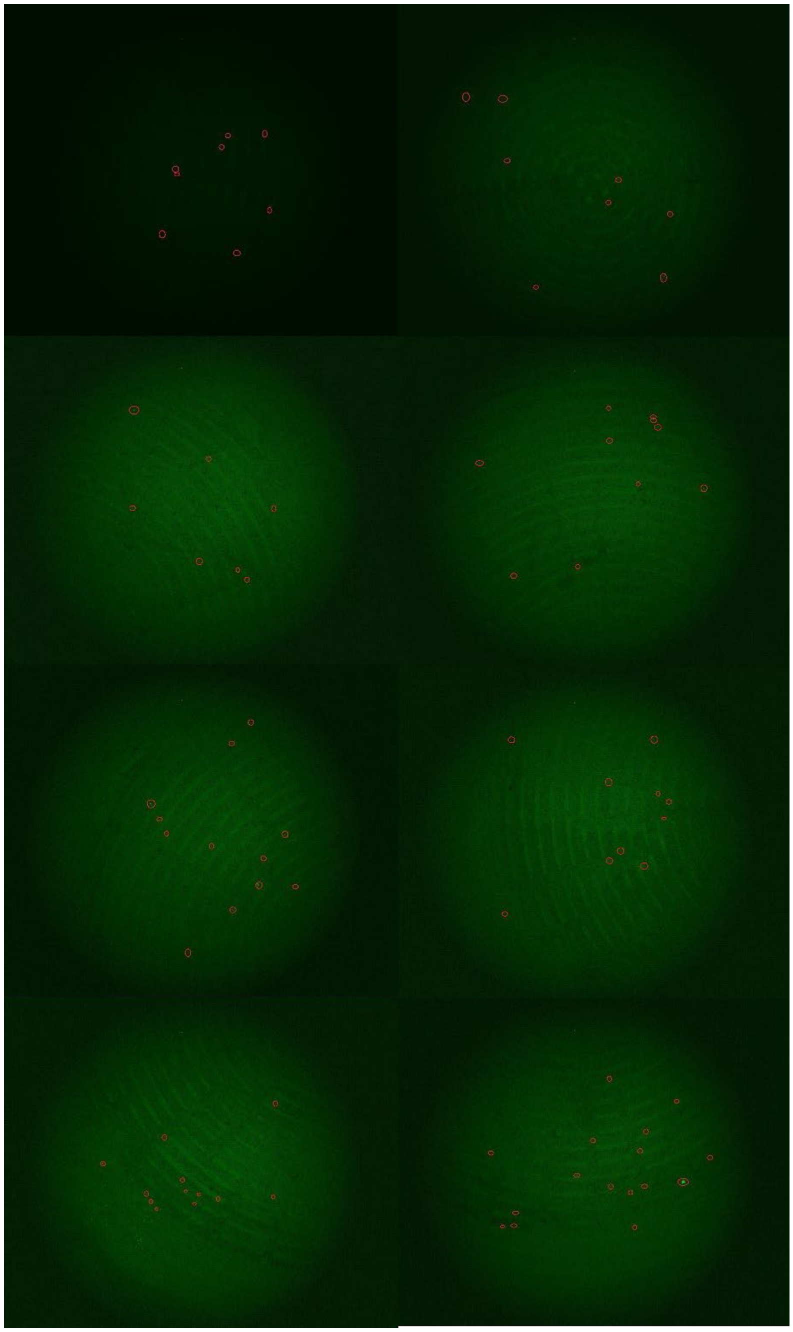
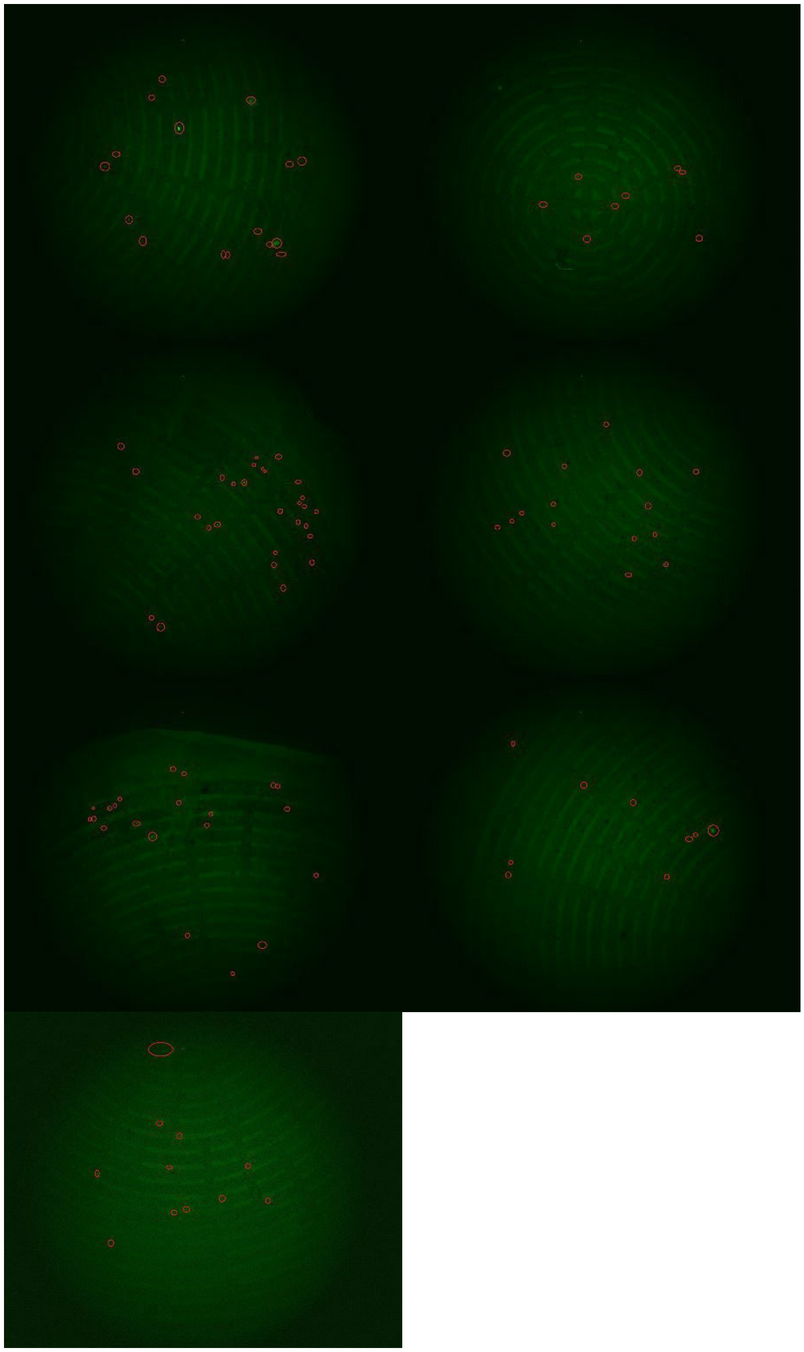


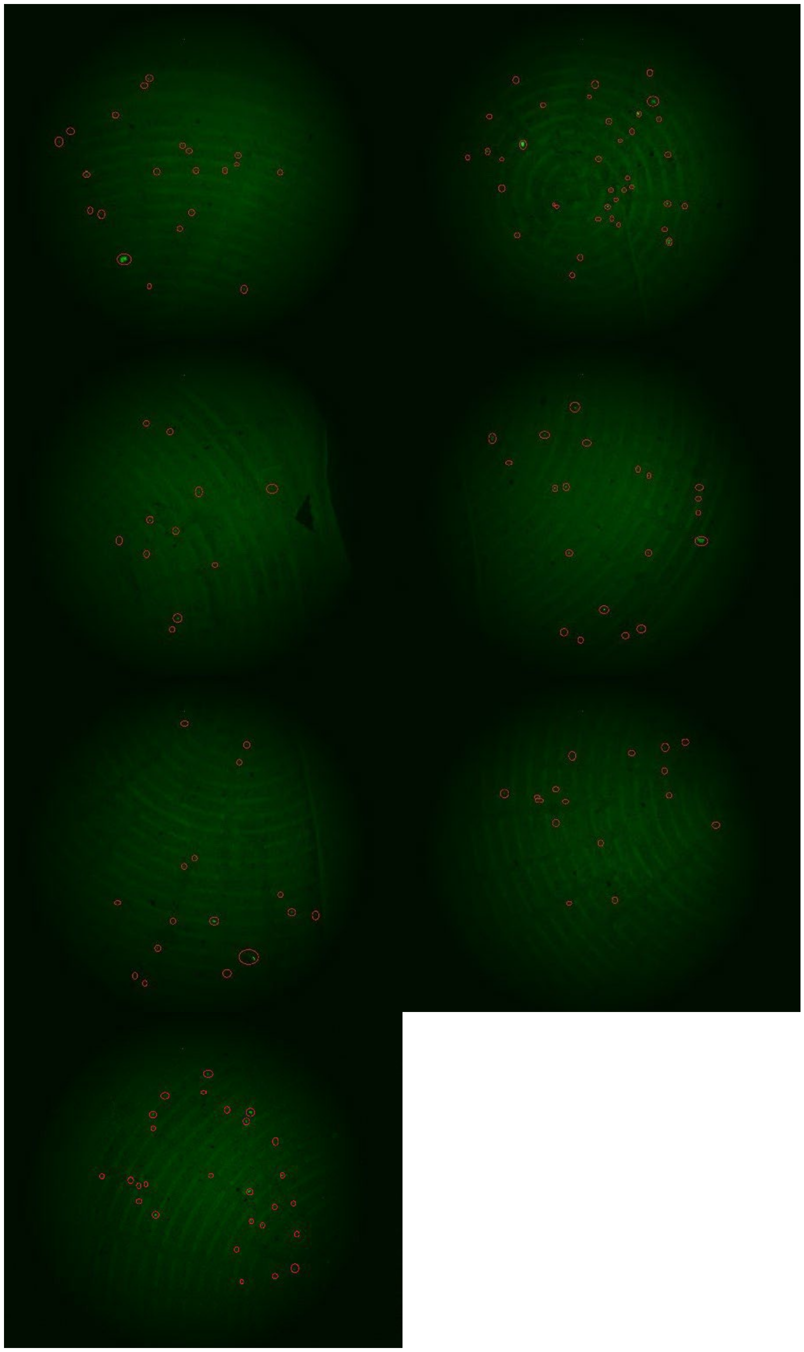
Moon River Trial 1



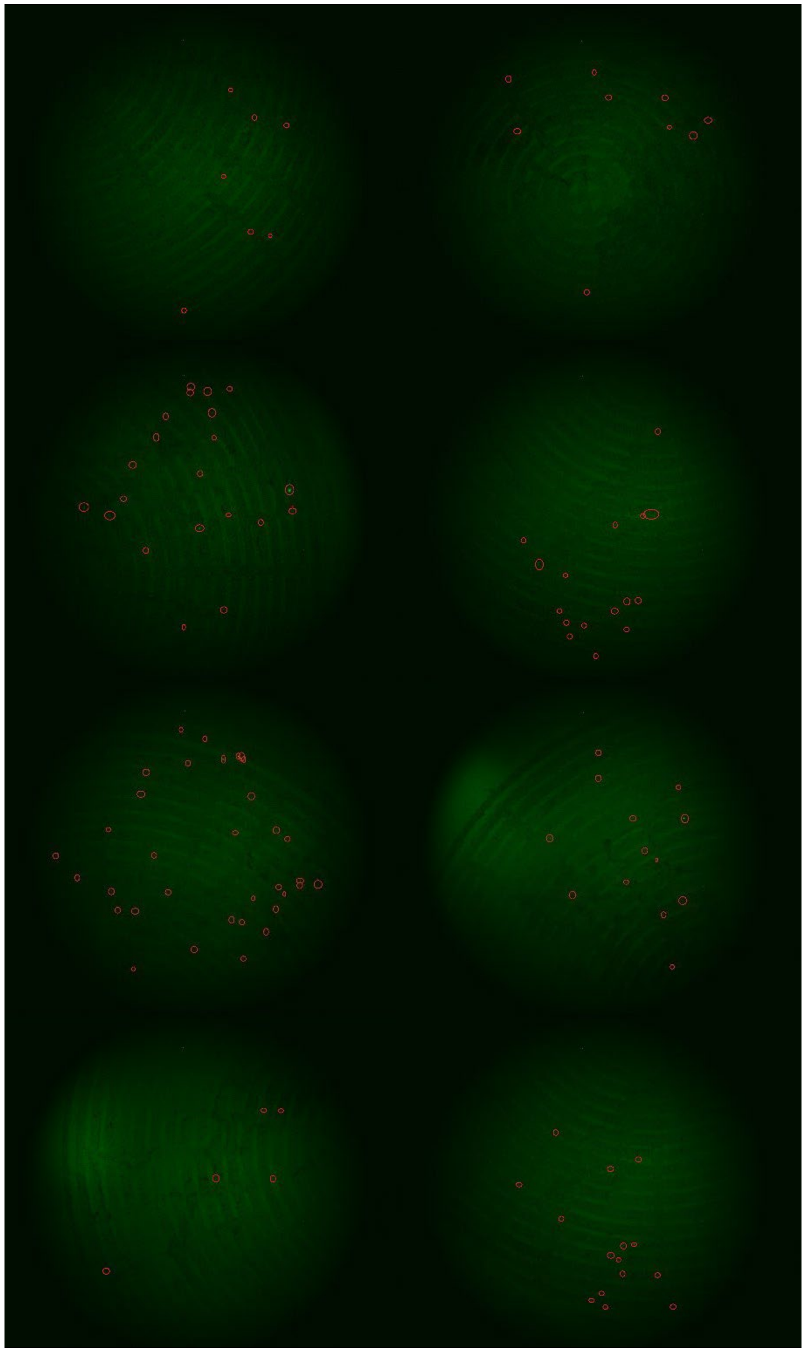
Moon River Trial 2



Moon River Trial 3



Old Looney Mill Trial 1



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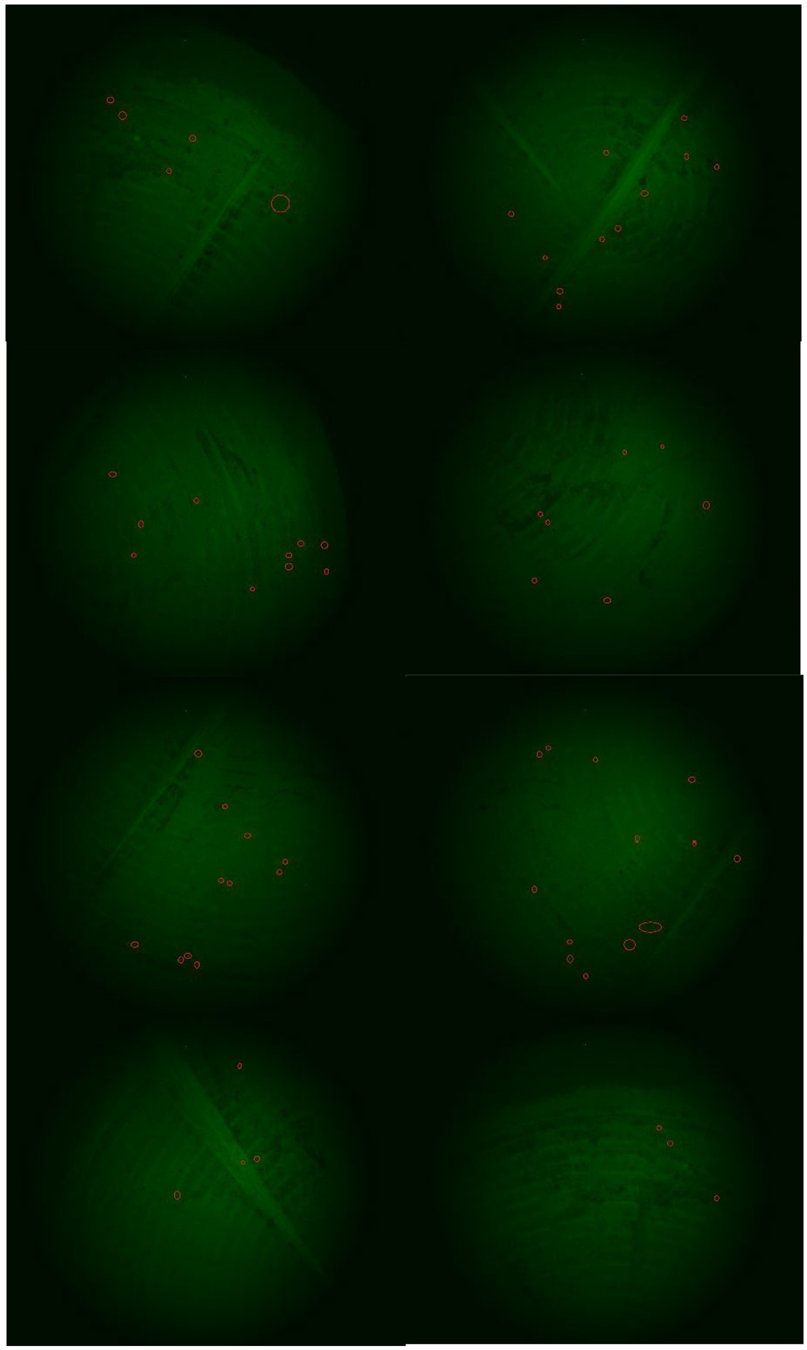
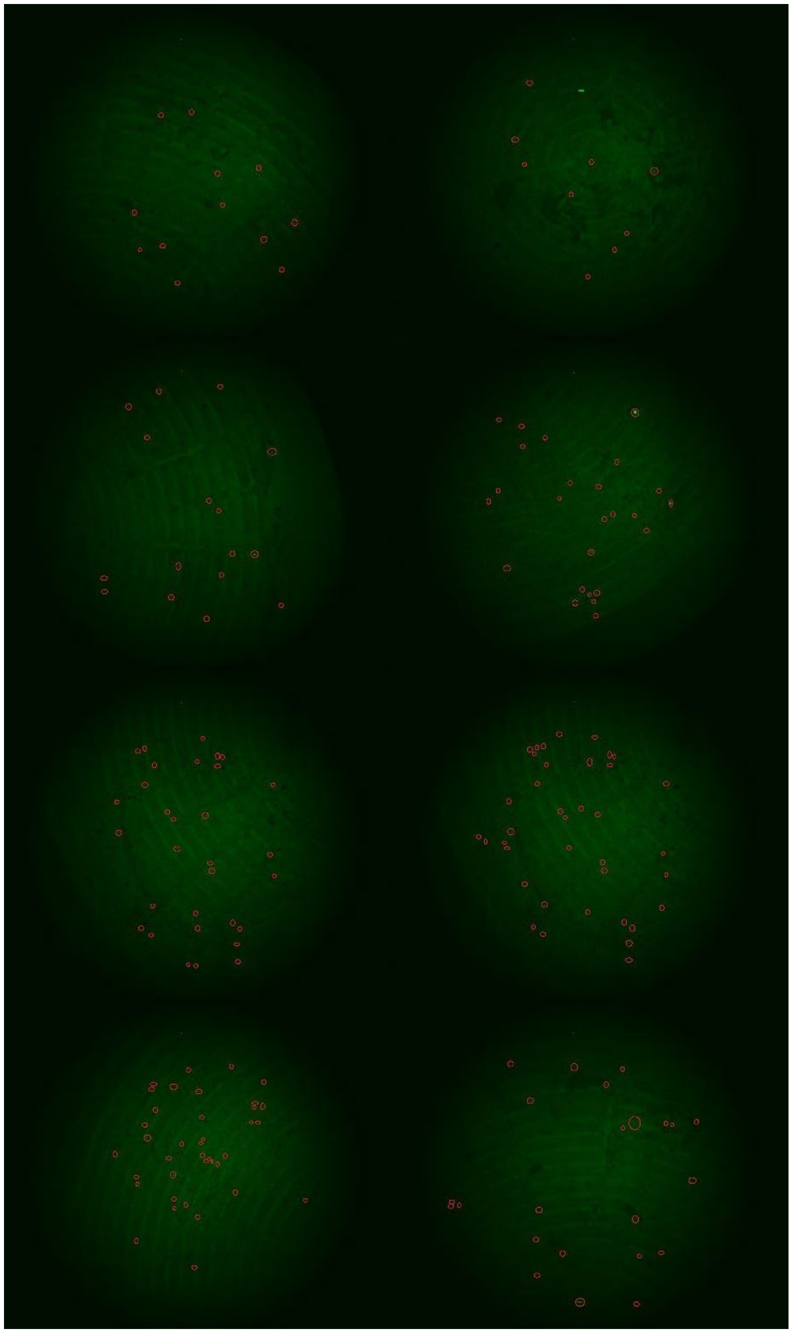
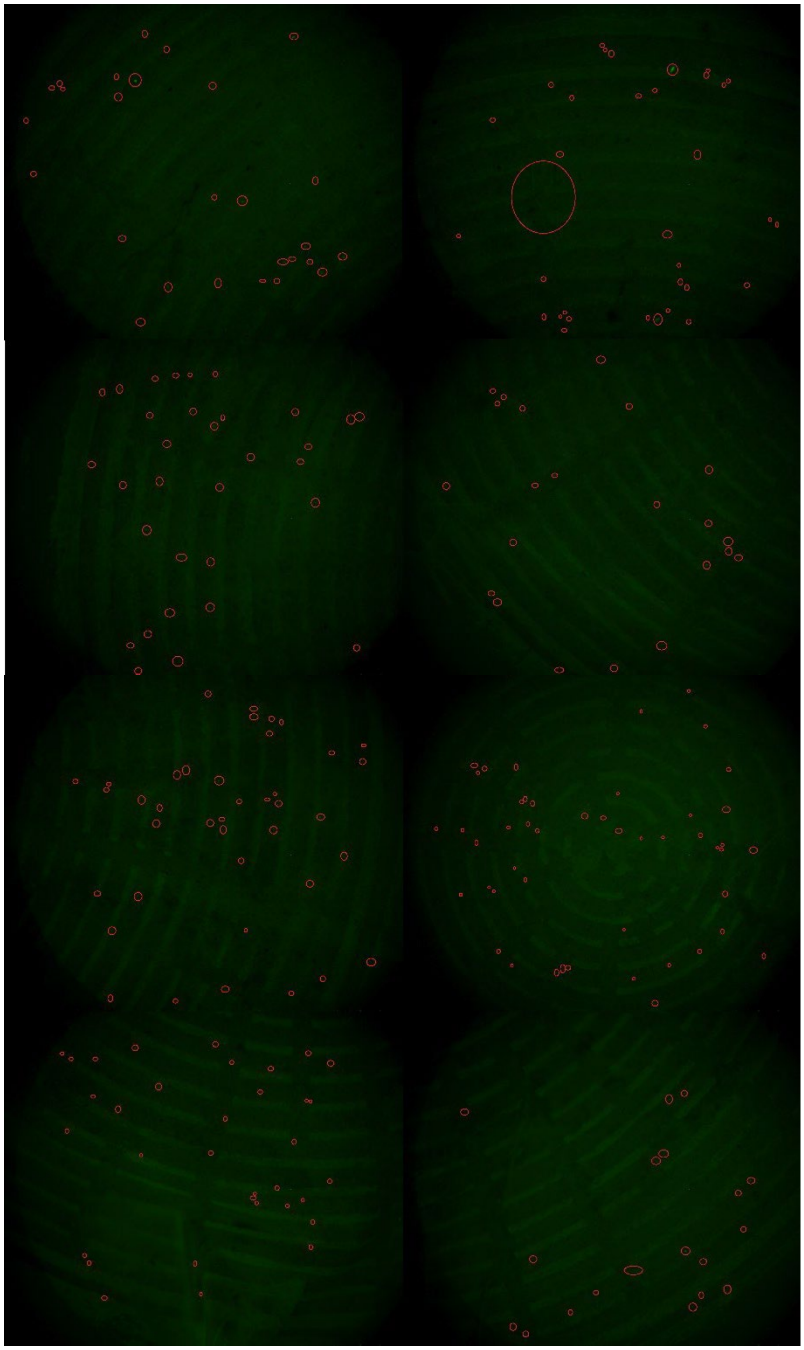


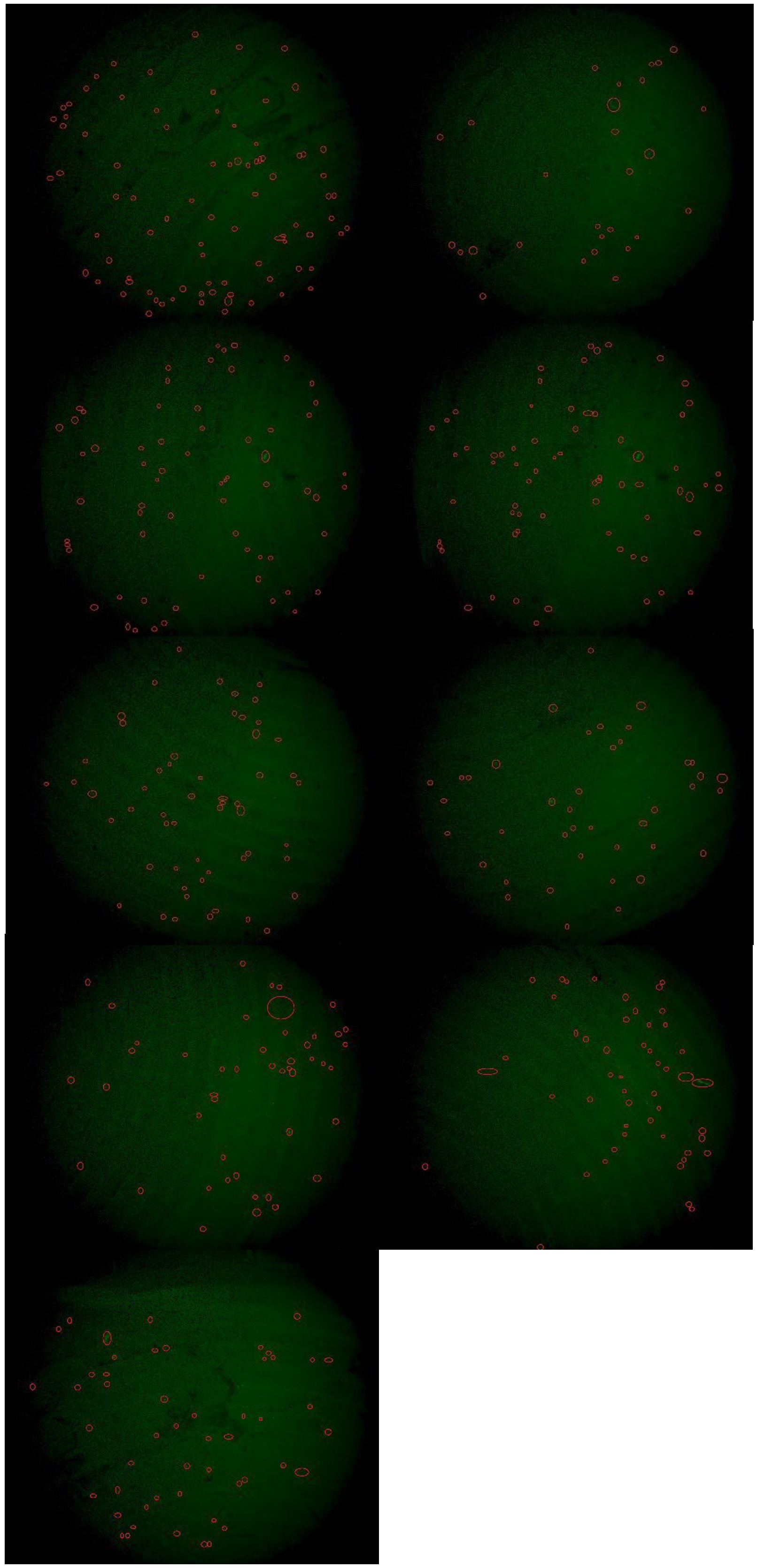
Old Looney Mill Trial 3



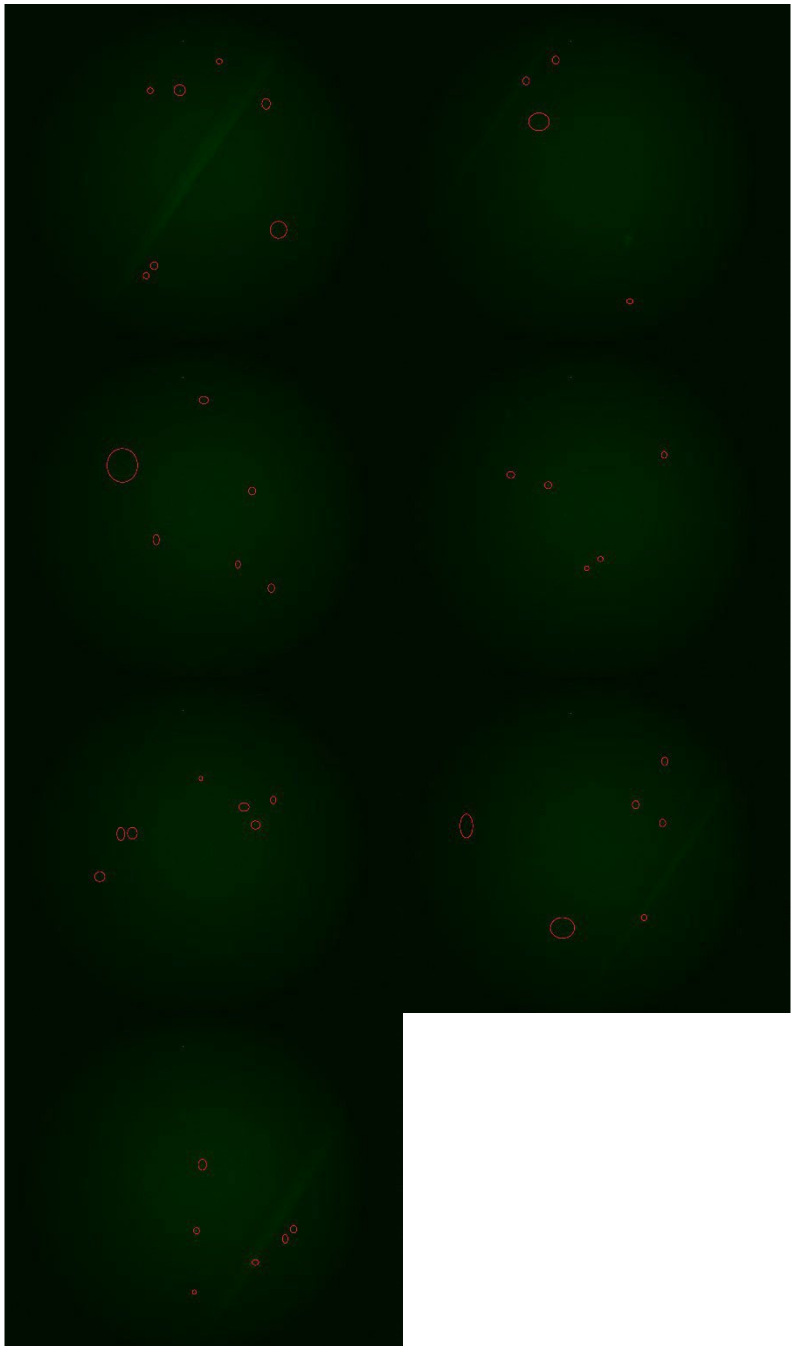
Grant's Mill Trial 1



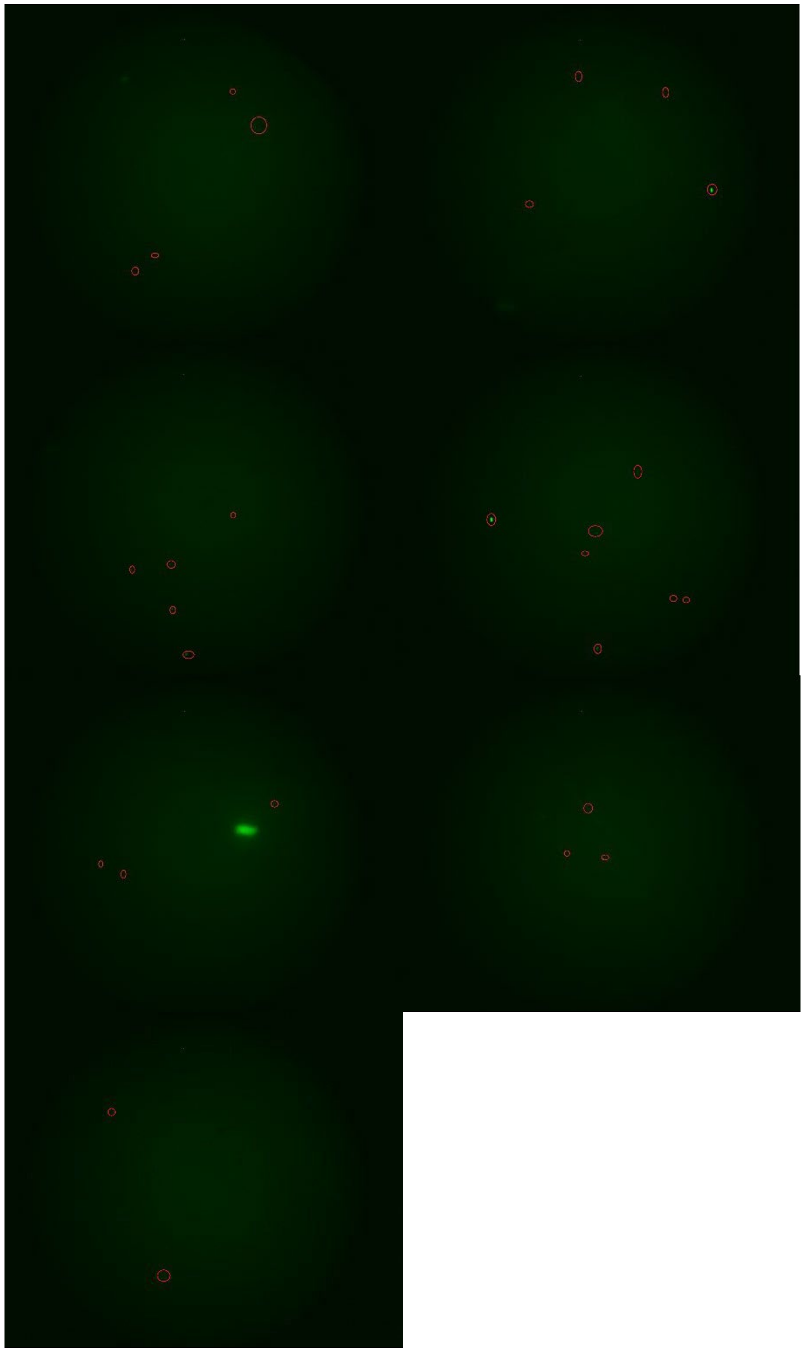
Grant’s Mill Trial 2



Negative Control Trial 1



Negative Control Trial 2



Negative Control Trial 3