Redesigning, rethinking *Fine Focus*

An Honors Thesis (HONR 499)
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REDESIGNING, RETHINKING FINE FOCUS

The purpose of design for any product or publication is to effectively communicate a message to a targeted audience. It also needs to help accomplish said product or publication’s end goal. While many people associate design with clothing or advertising, the items that require good design are much broader than that.

Fine Focus is an interdisciplinary, immersive learning class. Its objective is to publish a microbiology journal for undergraduate research twice annually. Each issue will include at least six research papers which have been edited by professionals in the appropriate field using a double-blind method. It also will have at least one student perspective.

The goal of the journal is to communicate the findings of the research as well as to create an opportunity for undergraduate students within the niche specialization of microbiology.

Part of the responsibility of the class is to publicize the journal using a website and advertisements. The website’s goal is to create an interactive experience through which to engage viewers and make them knowledgeable about Fine Focus. The goal of advertising is to grab people’s attention and tell them how to get involved.

I created a style guide, using my previous knowledge and new research of design methods, with the purpose of effectively communicating the journal’s research, creating brand recognition, meeting these goals for the website and advertisements.

I also compiled a broken down explanation of my design changes to ensure that future semesters of students will be able to keep the style of the publication consistent and professional.

ACKNOWLEDGEMENTS

I would like to thank Juli Metzger for advising me through this project. Her guidance allowed me to build on my communication skills and apply practices I’ve learned in journalism through writing in a different medium.

I would also like to thank John McKillip and the Fine Focus class for allowing me to pursue my vision for the future style of the journal and for supporting my decisions, even when they broke tradition.
WHY CARE ABOUT DESIGN?
The best design is one that isn’t noticeable. It’s not about being pretty or flashy; it’s about creating an experience and communicating a goal.

“If good design is doing its job, it is managing your perception of an experience in many ways—both obvious and not so obvious,” said New York City Mayor Michael Bloomberg in a recent interview in the annual design issue of Fast Company magazine. “How you feel, and therefore if whether you’re going to engage and buy, is directly influenced by the design of a website, a package or a business card.”

Everything people interact with has significant design purpose. A reader reads a magazine spread in a particular order because the designer instructed you to with their element placement. A viewer quickly located and uses a navigation bar on a website because a designer knew where they would look and gave them the tools they would want. A consumer stops to look at an ad because a designer knew what how to get them to look and what the ad wanted to quickly say.

A microbiology journal needs to have the same control with their design. It needs to have strong advertising and marketing so people know it exists, as well as a logical journal design to get readers to stay interested in its content.

Fine Focus is a microbiology journal for undergraduate research. It is assembled by a group of interdisciplinary students. Prior to fall, they never had a design major. As a result the design was an afterthought. The previous design decisions were based on aesthetics rather than function.

For example, previous classes had been using two logos. They said this was because one logo looked better on t-shirts and stickers while another looked better on print pieces. This is flawed because the entire purpose of a logo is for it to be recognizable as Fine Focus. I created a logo that has variations, so it can be applied to any of these mediums. It also more simply explains what the journal is by using a microscope with the “fine focus” knob highlighted in a different color. See the full transformation in “Style, explained.”

Similarly to how I considered the purpose of the logo, the marketing team worked with me to define the purposes of the different elements of Fine Focus: the website, promotional material and journal. The main purpose of the website is to create an interactive experience for users. The main purpose of promotional material is to entice people in less than two seconds and get them to follow or contribute to the journal. The main purpose of the journal is to create an approachable and readable outlet for sharing undergraduate scientific research. These were the driving forces behind the designs I created for each.

In general, the journal’s target audience and readership is made up of undergraduate students and people in the science community. Both admire logic and organization. Younger readers have become accustomed to modern, minimalist designs, which complement logic and organization. Also, with such a dense material, a simpler design will help communicate the content. This inspired the use of whitespace, simple color scheme and minimalist logo.

WHY CREATE A GUIDE?
Previously I have worked as a designer for two student media publications, The Daily News and Ball Bearings, and freelanced for the College of Communication, Information and Media. Last summer I interned as a print designer for The Denver Post, a top 10 news organization. In each of these different positions I was working with each publication’s individual style guides. This gave me the background experience to understand what tools a designer needs in order to follow a publication’s style.

The point of a style guide is to give a publication its own look, or brand. It provides consistency and familiarity for readers’ experiences. When I get on denverpost.com I know where to go to find the local news stories I am interested in. My dad knows where to go for sports. We can find those sections in the paper, too. When I see the front page, I know that the story with the largest headline is the most important for me to read. I also know where to look to find the story continues in the paper. The consistency and familiarity makes finding news in the The Denver Post efficient and enjoyable process.

A style guide is what sets those mandates. The same person doesn’t design every front page, an entire staff does. While individuals each have their own creative idea of how to present content, the basics have to stay the same. If I had given a news story a column-style headline, readers
would have been confused. Is it a column or is it news? It didn’t matter which headline was prettier; I had to pick the one that matched the style guide.

A single person will not be designing Fine Focus. The class has an added challenge since the participants will likely change each semester. They are also not guaranteed to have a design major involved. While the class will change, the product won’t. It will still be a microbiology journal for undergraduate students. Its target audience will remain constant. The class also won’t be able to get an entirely new group of followers every semester. So it needs to maintain and grow on current readership. The style guide can be passed down to the new students every semester. It will also ensure that the reader experience is familiar enough to maintain its readership.

The multi-platform style guide and the designs I created this year are Fine Focus, and will continue to push forward its purpose and success.
COLOR

* Fine print is never to run smaller than 10-pt font

COLORS CAN ROTATE

GRAYSCALE
* Fine print is never to run smaller than 10-pt font

COLORS CAN ROTATE

VARIATIONS
MONOCHROMATIC

* Fine print is never to run smaller than 10-pt font

VARIATIONS

FINE FOCUS
a microbiology journal for undergraduate research

FINE FOCUS
a microbiology journal for undergraduate research
COLORS

BLACK
FOR PRINT:
C - 0
M - 0
Y - 0
K - 100

FOR WEB:
R - 0
G - 0
B - 0
R - 128
G - 130
B - 133
R - 168
G - 169
B - 173

FOCUS BLUE
FOR PRINT:
C - 100
M - 50
Y - 0
K - 0

FOR WEB:
R - 0
G - 114
B - 188
R - 101
G - 153
B - 209
R - 149
G - 181
B - 222
R - 198
G - 213
B - 237
CHAMPAIGN/LIMOUSINES

JOURNAL TITLES
SECTION HEADLINES
LABELS, AUTHOR’S NAMES
GRAPHICS HEADLINES, AUTHOR’S LOCATION
IN-TEXT SUBHEADS

AHELLYA

Regular body font for print

Graphics body copy, corresponding author's location
Numbered references

45/39
30/47
16/18 BOLD
16/18 BOLD, 40 PERCENT
16/18 BOLD, LEDDING TO 25 FOR FIRST LINE

10/13, Ledding to 21 for new paragraphs, no indent, left aligned
10/13, 40 percent, left aligned
8/10, Ledding on first line to 16 for space
Photos need to have a 0.5 stroke around them.

To separate elements use a dotted line, 2-point stroke.

To identify a "perspective" from a journal, use the quotes above the kicker on the title page.

"PERSPECTIVE"

"DIRECT QUOTE FROM THE TEXT ON THE SAME PAGE AS THE PULL QUOTE GOES HERE AND HERE"

For a perspective piece, use pull quotes either vertically or horizontally.

Next to the author name in a perspective, include a mug shot of that person. The photo should be greyscale, taken on a plain background and cropped from forehead to chin.

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Copyright information should be included on each title page. It is centered in 8-point font a pica from the bottom.

PAGE SIZES
7.5"x10"

MARGINS
Top: 1.0417 in
Bottom: 0.6667 in
Inside: 1.1667 in
Outside: 0.6667 in

TIPS AND REMINDERS:
• Don't hit return or indent to create spaces, instead change the leading
• Dividing lines separate each element by three picas, two on the side with the larger element.
• Try to switch between dividing the page horizontally and vertically.
• Grey text (40 percent) is used for additional information, like the location of the authors or explaining a graphic
• Each title page needs to have copyright information on it and should start on the right page in a spread.
• Italicize Fine Focus on every reference as well as scientific terms
BDELLOVIBRIO BACTERIOVORUS PROTECTS CAENORHABDITIS ELEGANS FROM BACTERIAL PATHOGENS

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ABSTRACT

Bdellovibrio bacteriovorus is a naturally predatory bacterium that multiplies inside Gram negative prey bacteria. There is much interest in using Bdellovibrio as a living antibiotic to control infections by Gram negative pathogens. In recent years Caenorhabditis elegans has proven to be an attractive animal model of bacterial pathogenesis for a range of pathogens. We have used the C. elegans animal pathogenesis model to examine the ability of B. bacteriovorus to protect nematodes from four bacterial pathogens. In all cases, nematodes treated with B. bacteriovorus and the pathogen survived at a significantly higher level than nematodes treated with the pathogen alone. Treatment with B. bacteriovorus alone was nontoxic to the worms. We monitored the persistence of E. coli K-12 and E. coli OP50 in both B. bacteriovorus treated nematodes and control nematodes. E. coli K-12 levels were significantly lower in B. bacteriovorus treated nematodes than in control nematodes one day after Bdellovibrio exposure and E. coli K-12 was eliminated from the worm gut two days faster in B. bacteriovorus treated nematodes. E. coli OP50 also demonstrated significantly lower levels in B. bacteriovorus treated nematodes and faster elimination from the worm gut. The successful use of B. bacteriovorus as a therapeutic agent in C. elegans indicates that it may be useful as a living antibiotic in other animal systems.

INTRODUCTION

Bdellovibrio bacteria are intriguing because they naturally reproduce inside other Gram negative bacteria. The Bdellovibrio life cycle involves attachment to and penetration of prey cells, elongation inside the prey periplasm using prey components for growth, fragmentation into multiple cells, and finally, lysis of the prey cell (1). Because Bdellovibrio lyses prey as it multiplies, and because it cannot infect eukaryotic cells, there is growing interest in using Bdellovibrio as a “living antibiotic” (2). Numerous researchers have demonstrated in vitro killing of pathogens by Bdellovibrio, (3, 4, 5, 6) supporting the idea of using Bdellovibrio to control infections. Additionally, Bdellovibrio has been shown to attack prey within bacterial biofilms and reduce biofilm biomass (7, 8, 9). Two studies have put the living antibiotic concept into practice, demonstrating protection against Aeromonas hydrophila infection in fish and protection against Proteus penneri infection in shrimp through the use of Bdellovibrio (10, 11). Fish and shrimp mortality was significantly lower when the animals swam in water containing both the pathogen and Bdellovibrio as compared to animals in water containing only the pathogen. However, it was not determined whether the mechanism of Bdellovibrio protection was
simply a reduction of the pathogen level in the water, the killing of the pathogen within the animal, or a combination of the two. Until recently, the use of Bdellovibrio as an in vivo treatment for infection has been an intriguing, but theoretical option. In 2011 Atterbury et al. demonstrated Bdellovibrio could be used therapeutically to control Salmonella infection in chickens without negative effects on the birds (12). This was the first study to demonstrate in vivo efficacy of Bdellovibrio as a treatment for bacterial infection. Here we continue the use of Bdellovibrio as an in vivo therapeutic agent, but in the C. elegans bacterial pathogenesis model.

In 1999 Tan et al. first reported the use of the nematode C. elegans as an animal model for bacterial pathogenesis (13). Since then numerous researchers have demonstrated that this system can be used for multiple bacterial pathogens including Pseudomonas aeruginosa, Salmonella enterica, Serratia marcescens, and Staphylococcus aureus (14, 15). Genes identified in C. elegans as important in pathogenesis have been confirmed in mouse models of pathogenesis, validating the use of C. elegans as a pathogenesis model (16). Using C. elegans as an animal model for pathogenesis is attractive for numerous reasons such as low cost, short generation time, complete genome sequence and ease of genetic manipulation (17). When C. elegans are maintained in the laboratory they are grown on Petri plates containing lawns of nonpathogenic E. coli OP50 as their food source and the worms typically live two weeks (18). When grown on a pathogen instead of OP50, worm survival is greatly reduced (16).

Our lab has taken advantage of the well-studied C. elegans bacterial pathogenesis model system to examine the use of Bdellovibrio to protect C. elegans from bacterial infection. In this study, we first established an infection in the nematode and then examined the curative effect of a brief exposure to Bdellovibrio. We show that worms treated with both Bdellovibrio and a pathogen live significantly longer than worms treated with the pathogen alone. We also demonstrate that bacterial levels are lower and cleared faster in Bdellovibrio treated worms than control worms. This work demonstrates that Bdellovibrio can be used as a therapeutic treatment for bacterial infections in a well-defined animal model.

MATERIALS AND METHODS

NEMATODE AND BACTERIAL STRAINS
Wild type C. elegans N2 worms were used in all nematode assays. Worms and nonpathogenic E. coli OP50 were supplied by the Caenorhabditis Genetics Center (Minneapolis, MN). Worms were grown on nematode growth medium (NGM) with E. coli OP50 as the food source (18). Pathogens tested were E. coli K-12, Enterobacter aerogenes ATCC 15048, Pantoea agglomerans LS005, and Salmonella enterica serovar Typhimurium LT2 (19). B. bacteriovorus HD100 was used for all biocontrol assays (20). E. coli HB101 was used as the nonpathogenic control in the biocontrol assays since our early work in this system used B. bacteriovorus 109K, which does not infect E. coli OP50, but does infect E. coli HB101. However, all the experiments described here used B. bacteriovorus HD100, which does infect both E. coli OP50 and E. coli HB101. B. bacteriovorus HD100 was cultured using E. coli K-12 as prey according to standard protocols (21). B. bacteriovorus prey lysates were checked microscopically for active, motile B. bacteriovorus cells and an absence of prey cells. Prey lysates contained approximately 6 x 108 B. bacteriovorus cells per ml. The persistence assays utilized
kanamycin-resistant E. coli K-12 derivative strain JW863-1 (22), supplied by the E. coli Genetic Stock Center (New Haven, CT) and ampicillin-resistant E. coli OP50-GFP strain DB15, kindly supplied by J. Ewbank (Centre d’Immunologie de Marseille-Luminy, Marseille, France).

**PATHOGENICITY ASSAY**

Bacteria were grown overnight in LB broth and 50 μl culture was spread on 60 mm diameter NGM plates. Plates were incubated for two days at 25°C to establish bacterial lawns. *C. elegans* were reared on NGM with lawns of *E. coli* OP50 as the food source. One-day old adult worms were placed on NGM plates containing lawns of bacteria. Worm survival was monitored daily for the next nine days. Worms were considered dead when they did not respond to gentle prodding with a platinum wire. Surviving adult worms were transferred daily to fresh bacterial lawn plates to separate them from newly hatched juvenile worms. Each trial measured the survival of 30 worms per treatment.

**BIOCONTROL ASSAY**

Bacteria were grown overnight in LB broth and 50 μl culture was spread on NGM plates. Plates were incubated for two days at 25°C to establish bacterial lawns. *C. elegans* were reared on NGM with lawns of *E. coli* OP50 as the food source. One-day old adult worms were placed on NGM plates containing lawns of a pathogen or nonpathogenic *E. coli* HB101. After exposing the worms to the pathogen or HB101 for 48 hours (32 hours for *E. coli* K-12), worms were washed three times in Ca/HEPES buffer (21) to remove external bacteria. *E. coli* K-12 treated worms were exposed to *E. coli* for 32 hours instead of 48 hours because a 48 hour exposure to *E. coli* K-12 was too toxic and killed the majority of the worms. Washed worms were suspended in 1 ml of an active *B. bacteriovorus* prey lysate or 1 ml of Ca/HEPES buffer, then pelleted and placed on NGM plates with *E. coli* HB101 lawns. Worms were transferred daily on to fresh *E. coli* HB101 plates as described above for the biocontrol assays. Numbers of internal bacteria persisting in the nematodes after *B. bacteriovorus* or buffer exposure were determined daily using the protocol of Garsin et al. (23) with the following modifications. Briefly, 5 worms were placed on a LB agar plate containing the appropriate antibiotic (50 μg/ml) and washed twice with 4 μl M9 medium to remove surface bacteria. Washed worms were suspended in 20 μl M9 medium and ground with a pestle. 30 μl of M9 medium was added to the worm solution to bring the total volume up to 50 μl; the solution was diluted in Ca/HEPES buffer and plated on LB agar containing the appropriate antibiotic (50 μg/ml) for bacterial enumeration.

**E. COLI PERSISTENCE IN C. ELEGANS**

Nematodes were exposed to an antibiotic-resistant strain of *E. coli* (32 hour exposure for kanamycin-resistant *E. coli* K-12 derivative JW863-1 or 48 hour exposure for ampicillin-resistant *E. coli* OP50-GFP strain DB15) followed by three washes in Ca/HEPES buffer. The washed worms were suspended for 15 minutes in either 1 ml of an active *B. bacteriovorus* prey lysate or 1 ml of Ca/HEPES buffer, then pelleted and placed on NGM plates with *E. coli* HB101 lawns. Worms were transferred daily on to fresh *E. coli* HB101 plates as described above for the biocontrol assays.

**STATISTICS**

Kaplan-Meir survival analysis followed by pairwise logrank tests (24, 25, 26) was used to analyze *C. elegans* survival over time. The Mann Whitney test was used to analyze *E. coli* persistence data. Data analyses were performed using GraphPad Prism® 4 (27). The significance level for all statistical analyses was set at α = 0.05.
RESULTS

PATHOGENICITY ASSAY
We tested the pathogenicity of four species of bacteria, comparing them to the standard, nonpathogenic E. coli OP50 routinely used to maintain C. elegans. All four species tested were pathogenic when compared to E. coli OP50, greatly reducing worm survival (Fig. 1). The pairwise comparisons examining worm survival between the four pathogens indicated that all four pathogens were similar in pathogenicity (p=0.9926). We also tested E. coli HBI01 and found it to be nonpathogenic. Worm survival on E. coli HBI01 was not significantly different from worm survival on E. coli OP50 (p=0.5482). Worms grown on all four pathogens survived significantly less than worms grown on E. coli HBI01 (p<0.001) and worms grown on all four pathogens survived significantly less than worms grown on E. coli HBI01 (p<0.001). We proceeded to use E. coli HBI01 as the C. elegans food source when monitoring worm survival in our biocontrol assays rather than E. coli OP50 since our early work in this system used B. bacteriovorus strain 1091, which did not prey on E. coli OP50.

BIOCONTROL ASSAY
To determine whether B. bacteriovorus could protect nematodes from bacterial pathogens, we established infections in the nematodes, briefly treated infected worms with B. bacteriovorus, placed worms on non-pathogenic E. coli HBI01, and monitored worm survival for seven days. For all four pathogens tested, worm survival was significantly improved when worms were treated with B. bacteriovorus as compared to the pathogen alone (Fig. 2). For each pathogen, the pairwise comparison between worms treated with the pathogen alone and worms treated with both the pathogen and Bdellovibrio was highly significant (Table 1). Worm survival was unaffected by B. bacteriovorus treatment when worms were grown on nonpathogenic E. coli HBI01 (Table 1), demonstrating that B. bacteriovorus is nontoxic to worms. Bdellovibrio and pathogen treated
Table 1

P values for pairwise comparisons in the biocontrol assay survival curves.

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Comparison</th>
<th>HB101 vs. HB101</th>
<th>HB101 vs. HB101 + Bd</th>
<th>HB101 + Bd vs. Pathogen</th>
<th>HB101 + Bd vs. Pathogen + Bd</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli K-12</td>
<td></td>
<td>0.4658</td>
<td>&lt;0.0001</td>
<td>0.00047</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>E. aerogenes</td>
<td></td>
<td>0.4504</td>
<td>&lt;0.0001</td>
<td>0.00001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>P. agglomerans</td>
<td></td>
<td>0.7376</td>
<td>&lt;0.0001</td>
<td>0.0207</td>
<td>0.0093</td>
</tr>
<tr>
<td>S. enterica</td>
<td></td>
<td>0.7316</td>
<td>&lt;0.0001</td>
<td>0.1901</td>
<td>0.3292</td>
</tr>
</tbody>
</table>

*Bd indicates

Figure 2

Figure 2. Survival curves for C. elegans exposed to (a) E. coli K-12 (b) E. aerogenes (c) P. agglomerans and (d) S. enterica. Worms were treated with nonpathogenic E. coli HB101 (○), HB101 and Bdellovibrio (●), pathogen (□), or pathogen and Bdellovibrio (■). Worms were exposed to Bdellovibrio or control buffer on day one. Data are from three independent trials for each pathogen.
worms had significantly longer survival than worms treated with the pathogen alone. However, for three of the four pathogens, Bdellovibrio treatment was unable to restore the same level of worm survival as with the nonpathogenic E. coli HB101 control, and there were still significant survival differences between control worms and pathogen plus Bdellovibrio treated worms. S. enterica infection was the only one completely rescued by Bdellovibrio with no significant difference in survival curves between control worms and S. enterica plus Bdellovibrio treated worms (Table 1).

**E. coli Persistence in C. elegans**

We also monitored the persistence of one of the four pathogens (a kanamycin-resistant derivative of E. coli K-12) as well as ampicillin-resistant E. coli OP50 in both Bdellovibrio treated and control worms. One day after exposure to Bdellovibrio or a control buffer, E. coli K-12 levels were significantly lower in worms treated with Bdellovibrio compared to control worms (Fig. 3A). Levels of pathogenic E. coli K-12 decreased to undetectable levels in worms three days after Bdellovibrio treatment, while it took five days for pathogenic E. coli to drop below detectable levels in control worms. E. coli OP50 showed a similar trend in that bacterial levels were lower in Bdellovibrio treated compared to control worms (Fig. 3B). E. coli OP50 was also cleared to undetectable levels faster in Bdellovibrio treated worms and E. coli OP50, unlike E. coli K-12, persisted in the control worms for the entire seven day experiment. The limit of pathogen detection was five CFU per five worms.
DISCUSSION

While many have used C. elegans as a model for bacterial pathogenesis, we have extended that model to investigate control of four bacterial pathogens by Bdellovibrio. The non-vertebrate C. elegans has many advantages as an animal model for Bdellovibrio infection control studies including short life span, ease of manipulation, low cost, consumption of bacteria as food, and absence of ethical concerns. Our work in C. elegans supports and extends earlier work using Bdellovibrio as a therapeutic agent to control bacterial infections in chickens (12). Interestingly, the one log reduction in S. enterica by Bdellovibrio in chickens is similar to the reduction in E. coli K-12 levels we demonstrated in C. elegans (Fig. 3A). In agreement with the chicken study, our work demonstrated improved animal health with a single, discrete dose of Bdellovibrio. Using Bdellovibrio to control infection is often compared to bacteriophage therapy with Bdellovibrio having the advantage of a wider prey range than phage (2). Indeed, similar to our results, one group has demonstrated the ability of phage to protect C. elegans from Salmonella infection (28) confirming the robustness of the C. elegans model.

Our pathogenicity assay results demonstrate a clear difference in nematode survival between the four pathogens tested and the two non-pathogenic E. coli strains (Fig. 1). This highly significant survival difference is also reflected in the biocontrol assay comparing the HB101 treated worms with the pathogen treated worms (Fig. 2). Although E. coli K-12 is typically considered to be nonpathogenic in animal models and we referring to E. coli K-12 as a pathogen may seem inaccurate, others have also demonstrated that E. coli K-12 is pathogenic in C. elegans (29). E. coli OP50 is the strain typically used as a nonpathogenic food source for C. elegans; however we have demonstrated that E. coli strain HB101 is also nonpathogenic. Similar nematode survival curves between OP50 and HB101 have also been demonstrated by researchers examining the effect of bacterial nutrition on C. elegans lifespan (30). Interestingly, when survival is examined beyond ten days, worms live longer on HB101 compared to survival on OP50 (30).

Although Bdellovibrio provided intermediate protection from most pathogens, the significant improvement in survival along with the complete protection of Salmonella treated worms clearly demonstrates the protective ability of Bdellovibrio in this system (Fig. 2 and Table 1). The variation in Bdellovibrio protection of C. elegans from pathogens may be due to the difference in bacterial colonization of the worms. S. enterica serovar Typhimurium kills worms through a persistent intestinal colonization while E. coli kills through a non-persistent intestinal colonization (16). The ability of S. enterica to multiply within and distend the worm intestinal lumen, establishing a persistent infection after the worms are no longer being fed S. enterica cells (31), may provide a more concentrated source of pathogen cells to support increased Bdellovibrio growth and predation, leading to complete recovery from infection. Interestingly, these data suggest that the more numerous the pathogen cells are in the host, the more effective Bdellovibrio treatment may be for resolving the infection.

We followed the persistence of two E. coli strains in this system using antibiotic-resistant derivatives of E. coli K-12 and E. coli OP50 to examine the effect of Bdellovibrio on E. coli clearance from the worm. Pathogenic E. coli K-12 levels were significantly lower in Bdellovibrio treated worms one day after treatment and E. coli...
K-12 was cleared from the worms two days quicker in Bdellovibrio treated worms (Fig. 3A). This marked reduction in pathogenic E. coli levels by Bdellovibrio was enough to significantly improve worm survival, but not enough to restore worm survival back to the level seen in non-pathogen treated control worms (Table 1). Our results are based on a single, 15 minute exposure of the worms to Bdellovibrio and increased survival may occur with longer or repeated exposures of the worms to Bdellovibrio. We chose a 15 minute exposure to allow time for Bdellovibrio to attach to prey cells and begin invasion of the prey cell (2). Even without Bdellovibrio treatment, E. coli K-12 was cleared from the worms, in agreement with earlier research demonstrating that pathogenic E. coli does not establish a persistent infection in worms (16). Levels of nonpathogenic E. coli OP50 were also significantly lower and cleared faster in Bdellovibrio treated worms (Fig. 3B). However, unlike E. coli K-12, nonpathogenic E. coli OP50 was able to persist in the control worms for seven days. The levels of E. coli OP50 we detected in control worms on day one agree closely with those found by others investigating viable E. coli OP50 counts in C. elegans lysates (30), validating our work in this system.

C. elegans appears to be an ideal model system for refining and exploring the use of Bdellovibrio as a therapeutic agent. Since C. elegans is a bacterivore, exposure of the worms to pathogenic bacteria is simple and easy. The lower growth temperatures favored by C. elegans (20-25°C) compared to birds and mammals coupled with Bdellovibrio's optimal growth temperature of 28°C makes C. elegans an attractive animal system to investigate the use of Bdellovibrio as a biocontrol agent. We administered Bdellovibrio as a liquid treatment for precise, controlled dosing, but worms could also be treated with Bdellovibrio through placement on plaque plates (21) containing both the pathogen and Bdellovibrio. Our work prepares the way for future experiments with C. elegans and Bdellovibrio to examine additional pathogens, dosage and frequency of Bdellovibrio treatment, persistence of Bdellovibrio in worms, effect (if any) of Bdellovibrio on worm morphology, as well as other variables.

While an intriguing hypothesis, the use of Bdellovibrio as a feasible therapeutic agent has only been demonstrated in vivo in chickens against Salmonella (12). Here we extend that work by demonstrating significantly increased nematode protection from four different pathogens through Bdellovibrio treatment. In addition to being a well-studied pathogenesis model, C. elegans are much more tractable than chickens and our results lay the groundwork for future Bdellovibrio biocontrol studies in C. elegans. The presence of Bdellovibrio as a member of a healthy gut community in children (32), along with its lack of toxicity in birds and nematodes, suggests that it holds potential for therapeutic use. Our demonstration of protection by Bdellovibrio against multiple bacterial pathogens in the well-studied C. elegans pathogenesis model strengthens the validity of Bdellovibrio as a promising, future therapeutic agent.
ACKNOWLEDGEMENTS

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REFERENCES


UNDERGRADUATE RESEARCH IN THE SCIENCES AS A SERIES OF TRANSFORMATIVE OPPORTUNITIES

JILL BANGE
UNDERGRADUATE STUDENT, SENIOR
MAJORING IN MICROBIOLOGY
AT BALL STATE UNIVERSITY
EXPECTATIONS OF UNDERGRADUATES

Undergraduate students tend to find, from the moment of their arrival on campus until graduation, that they are held to a long series of ever-increasing professional expectations. Some are curricular standards set by the university, while others are evolving objectives the students decide for themselves. Students anticipate they should engage during lecture and laboratory courses, maintain high academic standings throughout their college career, and, potentially, pursue part-time employment. But perhaps one of the most pronounced expectations of an undergraduate is for the student to become socially and professionally involved on campus. The idea of campus "involvement" can be both vague and intimidating, especially to a new student.

Universities have numerous and varied organizations; these can be academic, social, faith-based, or service-oriented in nature, to name only a few. When a student is faced with many opportunities but has limited time, it can be a challenge to decide which of these commitments are worth pursuing with limited time. It is in an undergraduate student's best interest to choose activities that complement his or her area of study while promoting personal and professional growth. However, a student may be more interested in finding activities which build lasting, meaningful relationships with peers. This fundamental choice does not have to be a mutually exclusive one. None of the elements mentioned above are missing from undergraduate research experiences, which is why commitment to extended study outside of the classroom is one of the most valuable uses for an undergraduate's time. This is especially true of students majoring in biology. Research allows students to apply broad concepts learned in the classroom to original research problems in the field or laboratory setting, all of which enhances content comprehension, professional development, and peer interaction.

CONTENT COMPREHENSION AND TECHNICAL SKILL

The most immediate benefit of an undergraduate research experience is the ability to translate what is learned in the laboratory to one's understanding of scientific concepts learned in the classroom. A recent study by Hunter et al. indicated a common gain for students after an undergraduate research experience was perceiving "increased relevance of coursework" (2007). In a science lecture, broad and sometimes overgeneralized ideas are taught first, and eventually the finer details are covered. Research, however, begins by trying to answer a very specific question or solve a particular problem. For example, my first research experience involved determining the effects of different concentrations of carvacrol (a bactericidal extract from oil of oregano) on Bacillus cereus, a toxigenic bacterium associated with foodborne illness and ocular infections. Using a nematode model, Caenorhabditis elegans, I was able to quantify the effects of Bacillus toxins because the nematodes would ingest the bacteria and become infected. Although I had no background in cell biology or genetics at that point in my college career, my research advisor was able to build from my knowledge of basic biology and teach me about the organism I was studying.

Often, I encountered information while working in the lab before I had taken a course which covered those ideas—part of my Bacillus project involved transforming the bacterium with a specific plasmid vector that my advisor and I had designed. When I took genetics a few semesters later, I studied how bacteria are naturally competent. Research for me became a balance of relating concepts from the classroom to my project, and relating
my research back to the classroom to realize the real-world implications of what I was learning. This learning style does not stress memorization as much as application, which is more valuable considering scientific "facts" may change with breakthroughs (AAAS, 2011). Translating knowledge between the lab and the classroom allowed me to appreciate the complexity and importance of what I was studying, while giving me a better, more complete understanding of some of the more challenging theories. As classroom content is applied to a real-world setting, students performing research also begin to increase their technical skill set in the lab. Some of the first aspects of my research experience were becoming oriented with the lab and learning proper execution of basic bench skills, such as using aseptic technique or performing polymerase chain reaction (PCR). Bench work and instrumentation revealed the reality of research: it can often be tedious. But the practical experience was worthwhile in learning what the process of designing, executing, and analyzing an experiment is like from start to finish. One of the most valuable skills learned in research is the ability to troubleshoot problems when they arise. In the early phases of my Bacillus study, one nematode was to be placed in an individual well with agar on a 96 well plate. Then, each individual nematode could be studied separately as the Bacillus toxins began to take effect. Isolating a microscopic animal, however, turned out to be extraordinarily difficult. It was hard to avoid picking up multiple nematodes at a time, so the methodology for the project had to be amended. While this may sound like it would have been a frustrating experience, it was actually exciting and eye-opening. The difference between a real undergraduate research experience and a "canned" lab experiment that a student encounters in a basic biology class is that no one knows the "right" way to execute a research project. This gives the student ownership of the entire experiment and the freedom to be creative when adjusting for problems encountered during the process, and the end results are that much more rewarding when the project is completed. The three-hour labs designed for a classroom setting may give students some practice in bench techniques, but these skills are only applied to a piece of an overall research experiment. In an immunology lab, I read through a three-part protocol that stated parts one and two had been done for the students. This is not a criticism of the immunology course; it simply illustrates that students have a limited perspective of the goals in a research experiment and the process involved to acquire the end results in a short lab period. Furthermore, students may find it difficult to imagine application of methods they are using to solve real problems, even if they understand the concept being illustrated in a classroom lab. This is the advantage of undergraduate research: students are exposed to the scientific method from beginning to end, including the planning of the project and
the presentation of results. After participating in undergraduate research, I no longer look at a graph and see only the data, but also have an appreciation for the months and years of work that producing only one figure required. I have a more realistic conception of what research is like, and I am more able to understand how other scientists arrived at their results and conclusions because I have a sense of what they may have done in their own processes. In fact, one study indicated students who perform research-based activities rather than lab-based activities gain confidence in interpreting data (Brownell, 2012). When I have questions about a concept in microbiology, I can imagine how a scientist may have approached discovering an answer because I have been exposed to a number of techniques and instruments used in my field. Research enables a student to think critically within his or her own field rather than simply accepting facts in a classroom without being able to put the “pieces” together in a broader understanding of the world.

Performing undergraduate research shifts a student’s outlook on aspects of his or her own specific area of study. But the research process may also give students a new appreciation for other natural sciences as well, primarily because students will discover that subsets of science are not separated by as distinct boundaries as course curricula may indicate. While I primarily use techniques I have learned in microbiology courses in the research lab, I also find myself referring to knowledge I acquired in chemistry or physics classes to execute my project. For example, purifying the plasmids necessary for transformation of Bacillus requires a number of reagents. I relied on general chemistry knowledge to make these solutions at appropriate concentrations. And while not a topic I studied directly, having knowledge of the laws that govern forces and energy because of my physics education also helps me to understand the living systems in which I was interested. Physics, chemistry, and biology all build upon each other, something that is not stressed in lecture. Therefore, it was difficult for me to see how necessary my understanding of all of these disciplines was until I had research experience.

Undergraduate research gives a student appreciation for all of the “core curricular” sciences, but for students studying microbiology, research also allows for a better understanding of the relationship between the various disciplines within biology. For example, my research in environmental microbiology involved taking measurements such as pH, dissolved oxygen, water table height, and temperature of the water in which the algae of interest was growing. These data were considered when studying nutrient effects on algae because they can influence algal metabolism, as well as the presence of other microbes, and interactions between these communities also impact algal biomass and metabolism. Evaluating the influence of the environment on microorganisms helped me appreciate that the toxigenic bacterium I was studying in the food microbiology lab also changed depending on the conditions in which the cells grew, even though my work was in the laboratory and not in the field. I appreciated more the ability to carefully control variables and I became a more conscientious scientist. While working with Bacillus, I learned the importance of handling samples with precise, sterile techniques, and this training prepared me to more efficiently process hundreds of water samples in the environmental microbiology lab. My involvement in two laboratory projects has exposed me to the details within a subdiscipline, but has also enabled me to think critically about the broader concepts and implications of the subjects I am studying, and the problems and diagnoses I will make in my future training and career.
“SOFT” SKILLS AND PROFESSIONAL DEVELOPMENT

Developing a deeper understanding of the biological sciences through research is a critical and valuable undergraduate experience, and a student undertaking a research project might expect this to be an outcome of the process. What students may find surprising is that they also grow interpersonal skills immensely while engaging in research. Communication of scientific concepts becomes more comfortable as a student has more practice both reading and writing scientific literature. Utilizing primary literature—peer-reviewed publication of original scientific findings—is helpful in learning background information for a project, but it also adjusts a student to thinking and speaking in scientific terminology. As scientific studies produce information much faster than editions of textbooks can be produced, relying on scientific articles for supplemental detail of a broader classroom concept can be a critical piece of an undergraduate science education (Hoskins, 2007). The first time I read a seven-page piece of primary literature about Bacillus, I spent several hours deciphering the dense writing. I found later that this was a valuable investment of my time; I became more confident in speaking about my research to professors and other students because I understood the “language.” With enough practice, I could read a scientific article as fast as I could read anything else, and this gave me a sense of belonging to the scientific community.

The more a student reads primary literature, the better he or she will be able to compose a poster presentation, oral presentation, or manuscript in the future, and the more insightful their questions will become. Likewise, delivering an oral or poster presentation requires much practice to convey the essential information to an interdisciplinary audience. Successfully transferring the salient aspects of your work to a mixed audience involves not only a thorough understanding of your project on all levels, but a realization for how to “teach” and engage your audience as well. This concept is becoming more important with each passing year as new specialty areas develop within each subdiscipline of the life sciences. Without consideration of the audience at hand when rehearsing a presentation, the implications of a student’s finding may be lost on those who are not familiar with the jargon of a subspecialty. It is critical that a student presents his or her findings in a way that allows the scientific community to learn from the results and build from them in future studies. With careful preparation, especially in the background content of a presentation, a student can successfully and confidently convey findings from a study without overestimating the audience’s background, and without running overtime, two of the most common errors among students and experienced researchers alike. As a student gains more experience presenting, these presentations become less rehearsed and more of a conversation between the student and the audience. This is an exciting transformation, because students can begin to share ideas with peers about each other’s projects, and they become more interested and engaged in each other’s work as the conversation progresses. I encountered this at the 2014 Indiana Academy of Science conference, where a professor was presenting a poster on her study of the nervous system of the same nematode model which I used for my Bacillus project. As the conversation progressed, I was both learning from this professor and offering valuable information for her; it was a discussion that felt more collegial than instructional, which is atypical compared to most of my interactions with professors. Communicating and sharing ideas in this way builds a sense of fellowship between students and professors, so the student starts to feel less...
like a science major and more like a scientist through this process of contributing and collaborating. Collaboration is, in fact, an important piece of the research process. Even if a student is working on an individual project, he or she will often rely on peers who have more research experience for advice and wisdom. This student-centered learning, with the advising professor assuming the role of a facilitator rather than an instructor, builds students’ prowess in the lab and willingness to give input as to the direction of the research projects discussed. Teamwork in the lab makes the research projects more successful, but it also allows a students to form valuable friendships with others of their own discipline. Another research experience which I undertook relied heavily upon collaboration. During the summer of 2013, I studied in the Bonanza Creek Experimental Forest in Fairbanks, Alaska for three weeks with a professor and graduate student. We were assessing the effects of warming and nutrient addition on algal biomass and metabolism. This experiment had many components, and at times, it was difficult to keep the “big picture” in mind when I was focused on my comparatively small set of data. I was able to rely on the graduate assistant for help when I was trying to make sense of the results. She helped me have a better appreciation for the role of algae as primary producers, and I was able to keep the end goal of the experiment in mind because of her explanations. I began to see her as a mentor, but also as a friend, because we worked very closely over the course of those three weeks. But these friendships form regardless of the length or location of the project. I interact with students working in the same research labs as I on a more regular basis than many other students. Not only do we collaborate on our research together, but we have many of the same classes together as well, so some of the best connections I have had with peers during my college career have resulted from research experiences. My relationships with my faculty advisors have also grown and become more valuable than I anticipated as I have become more involved with research. At the beginning, I was being told what to do and how to work at the bench. I was being taught in the traditional way I was used to in a classroom, although it was one-on-one interaction. As my skills grew and I relied on my professors less for technical instruction, I felt more confident in expressing my take on the data or my ideas for amending the methodology. My advisors respected what I had to offer; I felt trusted and accepted as a scientist, even while I was still their student. Beyond that, my advisors have been incredible resources to me in realms outside of the research laboratory. They have written recommendation letters for me and edited my research presentations and posters, but they’ve also given me advice throughout my undergraduate career, which has been what I value most about our interactions. I can share experiences I’m having in class or in the process of applying for medical school, and they encourage me and give me a sense of what to expect as I move forward in my college years. Having a faculty member support me as I work to accomplish my goals has increased my confidence and improved my work, and has been easily the best aspect of my undergraduate research experiences.

By mentoring undergraduate students, faculty engage in service to their profession by training future scientists. Of course, the student is helping further that research project, but there is a great deal of commitment to the training of that student and investment in that student’s future given by the most dedicated faculty before those results emerge. “Service to the profession” has been heavily emphasized in my own research training; for example, I have been especially encouraged to be on a journal
“My advisors respected what I had to offer; I felt trusted and accepted as a scientist, even while I was still their student.”

As an undergraduate, students are prepared for this service through research training. Students will often read and discuss primary literature with each other or an advisor and learn how to critique an author's work thoroughly while still communicating the errors respectfully. Following an advisor’s example, more advanced students can also facilitate the training of some of the novice students in bench technique and general concept comprehension. Commentary on each other’s poster presentations and talks also models a professional conference, in which a scientist would field questions from colleagues and engage in dialogue about the study. In addition to professional service, students also serve their community through volunteerism. Our lab community, for example, organizes a fundraiser for Next Generation Nepal, which is a non-profit dedicated to returning trafficked children to their families. We use the “penny war” method of collecting donations and involve the science professors and students in the process. While not directly connected to our lab work, this collaboration for a greater cause on the part of a few research students has allowed us to contribute to society in both an academic and social capacity.

**PERSONAL GROWTH AND IDENTITY**

As students begin to build relationships with peers and faculty who are also involved in research, these students are engaging in a socialization process into the scientific community. Students in an undergraduate research experience are integrating the role of “scientist” as part of their identity, and they are learning that a scientist is so much more than someone who executes an experiment. As I enter into my last year of undergraduate research, I find that I begin to take on the role of a peer mentor while still being guided by advisors and other students. Mentorship is so closely connected to research because science involves a great deal of collaboration to be successful. The characteristics that I have appreciated in my own advisors—patience, enthusiasm, and respect—I have attempted to implement in my own attitude when working with other students. For example, when consulting a lab partner on methodology for the Bacillus project, I noticed that she had difficulty recalling some of the math concepts from general chemistry. I was able to find a new way of explaining the calculations that she hadn’t heard before which made sense to her. At the same time, my lab partner organized the methodology into a list and was able to walk me through what needed to be done. She saw the bigger picture of the project and how we needed to progress through each phase, whereas I was focused on the details of a particular step. We both assumed different roles in the partnership and were able to teach each other different aspects of the same research project, which was valuable leadership practice. In the future, my career as a physician will require a great deal of patience and commitment to mentorship of medical students and resident physicians. These partnerships are most successful when the members rely on each other's strengths, even though one is the "mentor" and one is the "mentee." It is difficult to be engaged and invested in one's own learning if one does not have an active hand in the learning process.
As a physician, I would expect my mentees to offer input regarding the subject material and I, as a mentor, would be willing to let the students take ownership over solving the problem at hand with guidance from me. I know this method has worked for me while I have been a mentee, and I think it is important to deviate from the traditional lecture-based learning to some degree so the students feel like a valued member of the class or group. This is what research does, and I believe my peer mentorship experience from research will translate easily to the medical field.

Beyond mediating discussion and encouraging my peers in science, research has increased my interest in developing methods of communicating scientific findings to the general public. Through my involvement as an editor for Fine Focus, I collaborate regularly with a marketing team, while my role is primarily for handling manuscript submissions. The interdisciplinary project has revealed to me the importance of packaging content in a way that is appealing and understandable for a target audience. This is a new concept to me; I am familiar with marketing products, but the intricacies of marketing information have become a more immediate challenge to me as someone striving to publish in the sciences. The frustration that scientists can feel when their findings are lost on an under-informed audience is expressed by Volpes’ *The Shame of Science Education* (1984):

> Public understanding of science is appalling. The major contributor to society’s stunning ignorance of science has been our educational system. The inability of students to appreciate the scope, meaning, and limitations of science reflects our conventional lecture-oriented curriculum with its emphasis on passive learning.

I would argue that while the public may have a limited view of some current scientific studies, scientists also have a minimal understanding of how to convey that information to a broad audience. Scientists write and talk for other scientists in the system of publication that currently exists. These are valuable data and analyses, but it is not for everyone. I would argue that undergraduate participation in research begins to encourage students to think about science from other perspectives so that the student can communicate to individuals of various educational backgrounds. For example, an ecology professor of mine once played an NPR interview of a paleontologist; this is a perfect example of an instance in which language had to be carefully tailored to speak to a particular audience, and this particular interviewee did so effectively. Undergraduates may, in their future careers, encounter situations in which they need to convey findings to the media or other public entities. Collaboration in research is a small step in developing these communication skills, because students are only working with other science majors. Nonetheless, students are bound to encounter diversity even within the sciences, and this student-centered, active learning process is excellent practice for conveying scientific content to a number of audiences.

To be certain, communicating scientific concepts is both exciting and challenging for any student new to research. A student is bound to encounter roadblocks throughout the research process which will require critical thinking and problem solving, especially when the original methods fail to produce acceptable results. These frustrations are combated by the desire to satiate one’s own curiosity as to how living systems operate, which develops as one becomes more attached to the research project, and more empowered in knowing that research can allow these discoveries to be had. This desire for understanding motivates a student to be flexible as he or she copes...
with the challenges associated with running an experiment. I felt tested when attempting to run a successful polymerase chain reaction (PCR) for my Bacillus experiment. PCR, like other tools in the arsenal of a microbiologist, involves sensitive reactions and is time-sensitive as well. It was important for me to be precise when working with small volumes of DNA, primers, and reagents. Even though I knew I had handled the samples carefully, it still took several attempts to generate copies of the plasmid I needed, and I was embarrassed I might have had poor technique. My advisor reassured me I was doing well, and that successful PCR is determined by a variety of factors, some of which may have been outside my control. With this in mind, I was able to be more patient with myself as I made more attempts at PCR, and this shift in attitude has translated over to my classroom work as well. I am less likely to get frustrated if, for example, I am trying to solve a chemistry problem that I don’t understand. Instead, I look for creative approaches to the question and persist until I find an explanation for the concept that makes sense to me. This patience and flexibility is crucial to the mindset of a college student, because balancing schoolwork can be difficult. Training in perseverance through the research process helps a student better face this obstacle.

Once I was able to solve problems on my own in the lab, I began to feel more ownership over the project which had been assigned to me. I was more comfortable working without supervision and I felt responsible for performing quality work, even though there would be no “grade” assigned to my research. This intrinsic motivation is harder to feel in a classroom setting. Classroom learning is passive, and students may not know how to integrate information that seems surface-level (Lopatto, 2009). The knowledge a student gains in a lecture doesn’t feel as though it “belongs” to the student because it is so readily given. But new knowledge generated in research almost has an emotional attachment associated with it, because the student knows first-hand the work required to discover this information. In this way, research is its own reward, and it fosters a desire for understanding in other realms of a student’s life.

The personal satisfaction and comprehension of scientific content are only gained, however, if the student is producing original results at the end of the research process. A research project which does not add new knowledge to the scientific community does injustice to both the student and fellow scientists. A typical classroom science lab, when the results are known at the process is designed to “work”, is helpful in illustrating a concept but does little to prepare a student for the reality of research as a career, in which results are elusive and methodology often needs revision (Chmielewski, 2009). Furthermore, if a student is not striving to solve unanswered problems through research, the student does not have new information to publish, and the opportunity to grow scientific writing and presenting skills is lost. One way to ensure that a student is building on prior studies but is developing novel results is by reading primary literature. Consulting scientific journal articles, whether for a course or for research, begins to feel more like participating in a dialogue than tedious work. I became more interested in scientific discovery as my research progressed, making me more willing to ask questions of my teachers and advisors when I was confused. Throughout primary and even secondary education, there is this fear associated with “being wrong” which can prevent students from engaging in classroom conversation. This anxiety quickly becomes outweighed during undergraduate education by the desire to know more as a student becomes more involved with research.
This internal drive brought on by research has allowed me to overcome the fears associated with the risk of trying something unusual. Adapting the attitude that a new experience will enable me, even if it may seem intimidating at first, has been a direct lesson of my undergraduate research experiences. I have learned not to feel anxious when I don’t know what to anticipate from a class or a job, because I have experience encountering "the unexpected" in the lab. For example, in the Bacillus experiment, the plasmids were designed with the addition of the gfp gene, so that once the bacteria transformed the vector, fluorescence would be an indicator of expression of products on the vector.

We used a flow cytometer to measure fluorescence, and we expected stressed Bacillus to express a particular gene on the vector and therefore fluoresce. We also expected our control bacteria not to fluoresce because they did not have the vector with gfp. However, our control Bacillus did fluoresce. The experiment was repeated, because it was assumed that we mislabeled our samples or some other aspect of the methodology went wrong. But again, the control bacteria fluoresced. Making sense of the unexpected was challenging and exciting, and it was concluded that when stressed, Bacillus must produce a primary metabolite that fluoresces. An experience that could have been frustrating ended up being enlightening, and it has allowed me to readily embrace new challenges.

**BROAD IMPACTS**

Engaging in an undergraduate research experience is a large undertaking. Scientific discovery involves active learning and adapting to new findings, a process initially uncomfortable to students accustomed to lecture-style lessons and rigid syllabi. Yet these challenges enable a student to grow in ways that a standard course could not allow. Students learn the complexity of the scientific method, and are able to appreciate and understand published literature after going through the process themselves. Students collaborate with faculty and peers to better communicate their findings and learn from the experience of others. Students come to realize that they are more capable in understanding and performing science than they could have known. The contributions which undergraduate research students make to the body of scientific knowledge are rewarding and stimulate further interest and motivation in scientific work. In my own experience, research has allowed me to feel immersed in the process of doing science and has made me more invested and interested in my own education. My undergraduate career would have been incredibly different without research as a tool to enhance my core understanding of science and improve my confidence in professional settings. I highly encourage all students participate in an undergraduate research experience to realize their full potential as a scholar and scientist.
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SUBMISSIONS

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EXPERTS PAGE

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*Fine Focus* is currently run by undergraduate students at Ball State University in Muncie, Indiana, under the guidance of a faculty advisor. We come from diverse academic backgrounds, including microbiology, digital publishing and marketing.

SPRING 2014

Back from left: Kyla Adamson, Adam Carr, Alicia Gorski, Anna Richter, Professor John McKee.
Middle from left: Matt Marano, Samantha Schwartz, Avery Sampson, Karah Mason.
Front from left: Jack Hesser, Grey Harris, Jonathan Miksaneck.

See behind the scenes of what *Fine Focus* has been doing since it began in 2013.

SINCE 2013

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FAQs

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How does the article review process work?
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The full instructions to authors can be found under the Submissions tab. Correctly formatted completed manuscripts are to be submitted electronically through the Open Journal System (OJS) to ensure confidentiality and proper management of your paper through the peer review process. Details about these simple steps are available on our website.

Who runs this journal?
Fine Focus is managed by Dr. John McKillip, Ph.D., at Ball State University, and run by a group of interdisciplinary undergraduate students. All aspects of graphics, marketing, website maintenance, and peer review are handled by these undergraduates under the direction of Dr. McKillip (although the peer reviews are actually completed by external members of our Editorial Board – see website for complete listing of these experts).
If not using a logo, use 80 pt. Champagne & Limousines, regular, all caps.

To separate elements use a dotted line, 5-point stroke in any style colors.

To add visual weight to contact information use the social media buttons. This makes it easy to identify how to reach the journal, which is the main point of creating a flyer. The size of the buttons shouldn’t change.

CHAMPAIGN/LIMOUSHINES

JOURNAL SLOGAN 31/37

LABELS 18/21 BOLD

REGULAR BODY TEXT 12/14.4
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VARIATIONS
COLOR VARIATIONS

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WHERE WE ARE
As an international journal, Fine Focus accepts article submissions and works with more than 50 editorial board members from around the world. We use a double-blind review system to ensure fair and accurate edits.

Initial submissions are from:
- Fort Wayne, Indiana
- New Albany, Indiana
- Indianapolis, Indiana
- Salisbury, Maryland
- Minneapolis, Minnesota
- Manila, Philippines
- Greensburg, Pennsylvania
- Spartanburg, South Carolina

CONFERENCES
To see where we will be go to www.finefocus.org.

- Indiana Academy of Science, 100th March 21, 2015
- Indiana Branch, American Society for Microbiology (BIASM) March 27-28, 2015, Brown County State Park, Nashville, IN
- National Conference on Undergraduate Research (NCUR) April 16-18, 2015, Iowa State University, Center, IA
- American Society for Microbiology (ASM) 115th May 30-June 2, 2015, New Orleans, LA

DONATE
We are a nonprofit journal and we rely on donations. Help us out at www.finefocus.org.

GET INVOLVED
Undergraduate students can submit microbiology research by going to finefocussubmissions.org. Microbiology professors and other professionals can become involved with Fine Focus as editors or reviewers.

WHO WE ARE
The American Association for the Advancement of Science (AAAS) call to action emphasizes the need for a re-evaluation of undergraduate biology education. Integration of creative student research into existing curricula and community-based participatory research are major themes of this announcement.

Fine Focus is a product-based course at Ball State University. It is uniquely poised to meet this call and action and is well positioned to take advantage of many rapidly evolving objectives in undergraduate science education. Utilizing the skill sets of dedicated undergraduate students spanning several departments, Fine Focus is a peer-reviewed academic journal with a mission to publish findings of international undergraduate microbiology research in both print and electronic platforms. By partnering with the American Society for Microbiology (ASM) as well as other scientific coalitions, participating students gain a multitude of experiences and establish permanent professional contacts in varied sub-disciplines of microbiology. Such experiences yield a working knowledge of scientific writing, editing, peer review, graphic design, and advertising, as they relate to dissemination of microbial research data through an academic journal. In order to be successfully implemented, contemporary undergraduate research in the biosciences must incorporate professional dissemination in addition to bench skills.

Fine Focus fills this unique niche. Our proposed work is the first international undergraduate journal specifically in microbiology. Fine Focus allows interested students the opportunity to see their research efforts through to fruition via publication while learning about the scientific peer review process at the same time.

SCOPE
We are an international journal dedicated to showcasing undergraduate research in all fields of microbiology. Fine Focus is managed entirely by undergraduate students from production to print.

MISSION
We publish original research by undergraduate students in microbiology. This includes works in all microbiological specialties and microbiology education.

VERSION 6
FINE FOCUS
CONCENTRATED ON STUDENT OPPORTUNITY

UNDERGRADUATE STUDENTS CAN SUBMIT MICROBIOLOGY RESEARCH BY GOING TO FINEFOCUSSUBMISSIONS.ORG.
MICROBIOLOGY PROFESSORS AND OTHER PROFESSIONALS CAN BECOME INVOLVED AS EDITORS OR REVIEWERS.

VARIATION
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VARIATIONS
STYLE, EXPLAINED

FINE FOCUS

A MICROBIOLOGY JOURNAL FOR UNDERGRADUATE RESEARCH
TO FUTURE DESIGNERS

The rationale behind the style changes I made are included in this report. I have detailed my thinking behind each piece of the style guide. This can be used as background for future designers to know the reasoning behind my design decisions, as well as have an idea of why certain designs didn’t work. I hope this will make it easier for future semesters to maintain a consistent style for the journal, website and promotional materials.

The main purpose of this redesign and the creation of the guide was to both create consistency for the publication as well as consider the most effective ways to make the journal successful and functional. My process was to rethink Fine Focus.

As a result, I created a professional and modern look. Scientists value organization and logic, and undergraduates are drawn to clean, contemporary designs. With information easier to access than ever, people also expect a highly usable and intuitive product.

The main purpose of the website is to create an interactive experience for users. The main purpose of promotional material is to entice people in less than two seconds and get them to follow or contribute to the journal. The main purpose of the journal is to create an approachable and readable outlet for sharing scientific research.

If you have any questions, either about the design or my reasoning behind it, please feel free to contact me.

Emma Kate Fittes
Email: ekfittes06@gmail.com
Twitter: @EmmaKateFittes
Curved lettering is hard to read. Design shouldn't make a reader turn their head unnaturally.

A circle is a restrictive shape.

The tiny pictures are very detailed and they get lost because they are so small. Logos can run at any size, so if this was smaller, it would be less clear.

This is unnecessary information especially since a journal hasn't printed yet.

Tiny words are hard to read, often logo is turned on its side, which also ruins readability.

The blue words don't hold extra significance, just randomly chosen.

The words repeat, so they are not adding any more information.

The class explained that they had two different logos because each worked for some mediums. For example, the circle logo worked best for t-shirts and stickers, but the other worked best for print and posters.

A logo should have enough options and variations to be consistent, no matter where it is used. The function of a logo is to create a recognizable image for your business, so having multiple defeats the purpose.
AFTER:

The challenge with redesigning a logo was to keep the design close enough to the original that it wouldn't interrupt any branding that has already happened. Also, the class wanted to keep certain elements, including the “Champaign circle shape and black and blue color scheme, however defined the blue so it will remain consistent.

The simple microscope is easily recognizable at any size, not over detailed. I chose microscope since it is a common and important tool for microbiologists.

The microscope is a tool to direct the eye towards the journal title, since it points directly to it.

The knob is known as the “fine focus” that allows a scientist to zoom in on the microorganisms. This is representative of the journals, which focuses on microbiology research and students, as well as the name sake. So, I added emphasis by changing the color.

I kept the lettering and bar style to keep the logo recognizable and kept the same description, but made it readable by keeping it straight and instructing that it is not to run smaller than at 10-point font. That text is not to be included in logos that would require it to be smaller than that.

I also created variations, so designers will have an option for any situation that arises. A successful logo should have an option from being used on the cover, a t-shirt or in an advertisement. This includes different color options, color, black & white and monochromatic as well as different basic design options. The next page has thumbnails of them.
The circle works well for t-shirts and stickers. Usually best in places where it is independent. This is the main logo.

Losing the circle opens this up for flexibility on posters and notepads. The bar can extend to the ends of the paper. It also is easy to make larger without taking up too much space, unlike the circle.

The final variations are for places where the logo needs to be very small. Like on a pen.
Revised mechanism of D-alanine incorporation into cell wall polymers in gram-positive bacteria

Nathalie T. Reichmann, Carolina Pica Cassignola and Angela Crudding
Section of Microbiology and IMM, Centre for Modern Biotechnology and Infections, Royal College of Science, London, London SW7 2AZ, UK

INTRODUCTION

Employing a treatment framework in which clinicians administer different drugs in strategic succession could both treat bacterial infections and select against the development of resistance. Technical University of Denmark's Lejla Imamovic and Morten Sommer argue today (September 25) in Science Translational Medicine. This new framework, which the researchers call collateral sensitivity cycling, could also help curb unnecessary antibiotic use, which is known to contribute to the emergence of drug-resistant superbugs.

METHODS

Employing a treatment framework in which clinicians administer different drugs in strategic succession could both treat bacterial infections and select against the development of resistance. Technical University of Denmark's Lejla Imamovic and Morten Sommer argue today (September 25) in Science Translational Medicine. This new framework, which the researchers call collateral sensitivity cycling, could also help curb unnecessary antibiotic use, which is known to contribute to the emergence of drug-resistant superbugs.

RESULTS

Employing a treatment framework in which clinicians administer different drugs in strategic succession could both treat bacterial infections and select against the development of resistance. Technical University of Denmark's Lejla Imamovic and Morten Sommer argue today (September 25) in Science Translational Medicine. This new framework, which the researchers call collateral sensitivity cycling, could also help curb unnecessary antibiotic use, which is known to contribute to the emergence of drug-resistant superbugs.

The body copy font is a sans-serif, which is usually used for web or digital products. That combined with the text being justified makes this dizzying and intimidating to read. The eye has less of a natural pattern to follow, and as a result readers can accidentally skip lines or get lost. This is not helpful for such a dense topic, which is already difficult to read based on content.
This microscope was pulled from the circle logo, I believe. In the logo, it is too small to tell because it is such a detailed illustration. It is not used anywhere else and looks childish with the thick line strokes. Since it isn't recognizable it isn't adding any information for the reader.

This is one of the most important pieces of information and it appears stretched at the bottom of the page.

The cover uses an Internet image instead of an original. The logo is flipped to be vertical. I assume this is done to fill space. However the reader now either has to tip their head 90 degrees or rotate the book. It is poor design to inconvenience the user. Design should be intuitive and consider their experience. Also, the words are repetitive and highlighted at random, adding no value.
The cover design pulls from the design of the logo. Since this is the first issue, it is important for people to recognize it. Using the logo design in a different way makes it memorable but recognizable. The design pulls the reader's eye in a "z" formation, starting at the top left in the white space, following the line of the microscope to the title, and through the blue line into the rest of the journal. It is simple, so it looks modern and approachable. An undergraduate journal should be appealing to its target audience, and a large chunk of ours is young undergraduate students. They generally have become accustomed to the use of white space and enjoy contemporary designs. It also only has the necessary information, so it is uncluttered and clear what this book is and what you will find inside.

The cover will always be a page on the right side of the spread, so it feels like the beginning of a chapter in a book. Also, this consistency will make it easy for a reader to navigate the journal. The headlines are left aligned and ragged, to match the rest of the text. The gray text is used for extra information to show that it holds less importance.
**ABSTRACT**

Life in a healthy stream can be severely impacted by changes in pH and other water quality parameters. This study reveals differences in diatom diversity and water quality characteristics in a central Pennsylvania stream. A healthy site was compared to three nearby sites affected by abandoned mine drainage during a May sampling in 2015. Permanent slides were made and microscopically assessed for diatom identification. The healthy stream contained eleven diatom genera while the site most impacted by mine drainage showed only one diatom, Eunotia exigua. Data were analyzed for Shannon diversity index and species richness. Water samples showed differences in pH, aluminum, sulfate, and iron. This work demonstrates the use of diatoms as bioindicators of stream health, and particularly in harsh environments can have an effect on overall growth characteristics of the diatoms. These factors, most importantly temperature, can have an effect on solubility of salts and gases found within waters especially those impacted with AMD. Thus leading to larger overall changes in water chemistry. Fluctuations in water chemistry throughout the year due to temperature change can have an effect on diatom species present as well as seasonal variation in diatom populations. Each diatom species has specific growth parameters and morphology giving us the ability to identify them by their frustules, making them good bioindicators of water quality. This study was undertaken to assess diatom diversity in a healthy stream and three sites downstream from the AMD outflow. The first site is a healthy stream, 40 m upstream of the AMD discharge with a pH of 7.2. The second site is at Hughes Borohole, 5 m below the source of AMD discharge due to surface mining with a pH of 6.0. The borohole site is impacted and currently devoid of vegetation. The third site is at a bridge 600 m below the AMD discharge, roughly 50 m from where the healthy and low pH waters mix, with a pH of 5.9. Figure 1 illustrates the four sampling sites.

**INTRODUCTION**

Abandoned mine drainage (AMD) is a perennial source of pollution in currently and previously mined areas throughout the United States. AMD impacted water is saturated with metals such as iron and may be very low in pH making it an inhospitable environment for the majority of aquatic life. Hughes Borohole is a source of AMD pollution that flows into the little Connemoga River near Fratage, Pennsylvania. The Borohole was drained in the 1990s and now contains water from miles of flooded underground coal mines in the area. The Borohole was capped in 2007 and was thought to be unimportant until 2010 when paleo-potentiometric conditions were realized. Since then, water with a pH as low as 3.0 has been observed at the Borohole at a rate of 100,000 gallons per minute and blinding the surrounding six acres with a red/brown iron precipitate. This drainage occurs in an area where water flows in contact with exposed rocks that have high concentration of sulfide minerals. Pyrite, also known as fool’s gold, is a common mineral found with coal in the eastern United States. The oxidation of pyrite and other sulfide-rich minerals causes the release of sulfuric acid and metal ions. If a stream has a limited buffer capacity, the pH will continue to decrease, thus increasing the oxidation reactions and the precipitation of metal. When the temperature of the water increases in the summer months, gases such as oxygen become less soluble and metals become more soluble. Diatoms are a group of photosynthetic algae which can survive in a wide variety of aquatic environments. Each diatom species has a differently shaped silica cell wall, called a frustule, which is used for microfossil identification. Different diatom species are found in two different microenvironments, they are either suspended in water (planktonic) or growing on a substrate (benthic). Environmental factors such as pH, light availability, and temperature may cause variation in frustule morphology.

Keeping the text ragged instead of justified makes it easier to read over longer periods of time. Increased leading between paragraphs helps break up long pieces of text which otherwise could look intimidating. I chose a serif font because they are easier to read in print since a user’s eye expects it and can more quickly recognize those words. I moved it down to 10-point font since that is easy to see still and less dizzying.

Information for the corresponding author and the keywords are pulled out separately from the rest of the text because these are additional tools for the readers. Since the amount of space these take up varies greatly in each journal, the white space on top can grow. That white space helps direct a readers eye to the content as well.

Each section has two picas of white space above the separation line and one pica below. The white space above directs the eye towards that section. The section headline or in-text subhead is then a point of entry into the text.

Overall, I try to switch between splitting pages vertically, like the Abstract, and horizontally, like with Materials and Methods. This variety will help break up the monotony. I also put the graphics (shown in the full template) throughout the results and discussion sections. These sections are where those graphics are referenced, and they help spit up the text. I made the graphics text gray to make it clear it is a separate element.
The homepage is static. There is nothing to interact with. A user would have no reason to stay on the site. Also, it is clear nothing will ever change. If it appears no new information will be added, a user would have no reason to return to the site. A site can be resourceful, but it has to be user friendly as well.

**BEFORE**

The lights are random and unprofessional

The dominant piece is a logo, which doesn’t add any information. Users expect this to be at the top, and generally skip to the center of the site, since that is where their information is typically.

The trend of having a navigation bar on the side had faded. Most users look for the bar at the top. It no longer has to be in a box for people to know it is clickable.

This is basic information, but probably what a user would skip over, since they know this if they are accessing the site.

This is the most important information to someone accessing the site, but it looks like an afterthought

This is likely the second reason someone would come to the site, other than to get involved, but it is at the bottom.
A white background is more modern and professional. The contrast with text helps readability.

A new, condensed navigation is at the top. It is more readable and users expect it there.

The photos run as a slideshow, adding a moving element to the homepage.

We created a slogan to explain the purpose behind the journal quickly.

One goal is to get followers. Both keeping it to the top and adding dominance with the circles help.

I added subheads that clearly show the two ways users can get involved.

Embedding a Twitter feed keeps the site fresh and updated. Users can stay connected after leaving.

Having contact and basic journal information here is a consistent resource that's easy to access.
Meet us page. Before the site only included two semesters and didn't provide much information beyond the names of the previous members. The two slideshows make it easy to add new semesters as well as have a specific place for behind the scenes photos. Since contact information is at the bottom of every page, it would have been redundant to add it to this page, or make it have its own page.
Experts (editorial board) page. Creating a map of the experts creates an interactive experience for a user and makes the list visual. It is easier to see the variety in location of the experts, which is a large selling point of the journal.
FAQs page. The FAQs page didn’t change drastically. The black text on the white background improves readability. The gray on the teal background has hardly any contrast and therefore isn’t readable.
Submissions page. The original submissions page didn't have anything interactive except for a couple links. As a user, seeing a screenshot of the Open Journal System site isn't helpful. Neither is including a poster that repeats the information on the homepage.

There is a separate page for document guidelines which is accessible in a drop down menu under submissions. Having a separate page is inconvenient. Also, having to scroll through the entire list to find what you need is inconvenient. The gray titles are hard to read.
AFTER:

SUBMISSIONS - EXPERTS - DONATE - MEET US - FAQs

INSTRUCTIONS TO AUTHORS:

DOCUMENT ORGANIZATION

ALSO:

Submissions page. For the new submissions page I put the deadline at the top in a distinctive color since that is the most important information. Then a user only needs to scroll down to see the guidelines. Usability tests have proven that users more naturally scroll for information rather than having to click around to other pages.

I added a quick navigation list at the top titled "Help me with" which will jump the user down to that section of the page. That way they don't have to scroll to access it.

Donations page. I also created a donations page. This way people can get more information before sending in money. The bar chart is a way to visualize the amount of donations compared to the benefits for the donor.
The abstract is unnecessarily long and repetitive. Having only one block of text is dizzying. The sans serif reduces readability.

The title doesn’t tell a viewer why to care. We only have two seconds to get their attention, so it needs to be obvious.

The Logo faded in the center interrupts that reading experience. There is no point of re-entry to invite a reader visually back.

One of the most important take-aways would be to continue to interact. The contact information needs to be a dominant element and easy to understand.
In advertisements I made a point to keep the visuals consistent with what a reader would find on the website. The purpose of the poster is to quickly answer viewer's questions, so I split it up using titles that make it clear which question that section answers.

The new logo is the dominant element to ensure the first thing people see is the name.

Contact buttons are the secondary item so they draw the eye from the logo. It is the second most important information on the poster.

The slogan tells the viewer why they should care. It focuses the poster on opportunity.

A visual representation of the international aspect of the journal is the next item that draws the eye further down in the poster. This helps guide the viewer to the rest of the information.

The abstract has been cut down, given a more inviting title and the main points are highlighted.

Instead of just listing the prizes for certain donations I created a visual representation.

Getting involved is an important take away and should be kept simple so it’s easy to remember.
**BEFORE:**

**Submission Deadline:**
*September 30th, 2014*

**Mission**
Fine Focus is a web and print journal dedicated to showcasing the research of undergraduate students, internationally, in all fields of microbiology. Fine Focus is managed entirely by undergraduate students from production to print.

**Scope**
Fine Focus publishes original research by undergraduate students in microbiology. This includes works in all microbiological specialties and microbiology education. Research in other biology disciplines will not be accepted unless the main emphasis of the work centers on microorganism(s).

**Conferences**
Visit our display at ASMCUE and at the ASM General Meeting in Boston (at the “Future Conferences” tables in the North Lobby area).

The photo of the staff is irrelevant to the point of the add, which is to get people interested in submitting or editing.

These descriptions of the journal are too long and awkwardly text wrapped.

The contact information is hard to find and QR codes are no longer popular. People shouldn’t have to download an app to get more information.

The submission deadline is important to know, but an ad’s first goal is to get people interested. This is not the draw to Fine Focus, but it’s the first thing a reader would see.
I wanted a new strategy for the advertisements. Not only should they be easily recognizable as our brand, but they should be informative. This quickly answers a reader’s first three questions: What is Fine Focus, why should I care and how do I get involved?

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MICROBIOLOGY PROFESSORS AND OTHER PROFESSIONALS CAN BECOME INVOLVED AS EDITORS OR REVIEWERS.

This is the technical description of the journal. The language is short and clear and also brings a second purpose to the logo. It’s first purpose is to be recognizable.

There are clear prompts of how to get involved for either undergraduate students or professors and professionals. This is an easy way to identify who we are targeting.

Making the slogan the third most dominant element gives a viewer the reason they should care. It answers what being a part of a microbiology journal can do for them.

This contact information should be included on any promotional material. This is always the end goal.
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We created the flyer to leave behind at conferences and to mail to universities interested in the journal. They can either be hung publicly or kept by someone for their personal information. Therefore, it was important to make it simple and eye-catching enough that it would stand out on a bulletin board, but also have clear directions for how to get involved and have more information. I used the same strategies as with the print ad.
VARIATIONS:

BLACK & WHITE

ONE COLOR