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Applications of Multimedia Resources Developed as Part of the Virtual Cell Animation Collection in Undergraduate Introductory Biology

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Applications of Multimedia Resources Developed as Part of the Virtual Cell Animation
Collection in Undergraduate Introductory Biology

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Dedication

To Brittain, Battle and Weller, this is dedicated to you for your love and support throughout this journey. You have been the driving force behind this step in my life and the support system that has made it successful. I love you with all of my heart.

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The results of this journey would not have been possible without the support and encouragement from a great number of individuals. First and foremost, thank you to Dr. Alan R. White for being a mentor, colleague, and friend throughout these efforts. Your passion for STEM education introduced me to a field that has become near and dear to my heart. I cannot sufficiently communicate just how much your support and guidance throughout this has meant to me. I would also like to thank my dissertation committee of Bert Ely, Johannes Stratmann, Rekha Patel, and Michael Seaman for their support of my research. Thank you for taking a chance on a research topic that is not typical in the realm of biological sciences and for supporting my efforts throughout.

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Abstract

Calls for reform in undergraduate STEM education have arisen from an increased need for well-trained biology graduates in the future scientific workforce. To address this need, many institutions have focused on a pedagogical restructuring of instructional practices to promote deeper conceptual understanding of core biological concepts. This study investigates the implementation of multimedia resources as a possible reliable supplement to undergraduate introductory biology and aims to provide empirical evidence on the instructional best practices of their use. As a central part of this study, we focus on one specific multimedia package, the Virtual Cell Animation Collection, due to its developmental adherence to research-supported multimedia design guidelines. Using resources from this one central source, we focus on the implementation of dynamic animations in biology instruction as part of three individual aims. **Aim One** concentrates on the comparison of static and dynamic images incorporated into a lecture-centered traditional classroom setting. Results show that the use of animation as part of instruction on two major introductory concepts resulted in significantly higher learning gains than when lectures only incorporating static imagery, suggesting their ability to promote learning on the topics. **Aim Two** investigates the use of dynamic molecular animations as part of instruction outside of the classroom as either preparation for or reinforcement of classroom instruction. Results show that

animations assigned as either preparation for or reinforcement following classroom instruction on three common introductory concepts produced significantly higher learning gains than a non-treatment control group. Additionally, there was no significant difference in the direct comparison of the two outside of class interaction treatments.

Aim Three focuses on the use of online learning module as a stand-alone method of instruction on two core topics. Results from this aim demonstrate the ability of these stand-alone learning modules to outperform traditional instruction. The focus on introductory biology instruction from the aspect of inside the classroom, outside of the classroom, and independent of the classroom provides an encompassing view of the major settings for student concept introduction. Together these results provide empirical evidence for the use of multimedia resources in the introductory biology classroom, ultimately answering the call for reform and redesign in the undergraduate STEM classroom.

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List of Symbols

α	Crohnbach's Alpha
χ^2	Chi-squared Value
d	Cohen's d
F	F Value
G	Normalized Gain Score
K	Fleiss' Kappa
M	Mean
N	Sample Size
p	P-Value
SD	Standard Deviation
t	T value

Chapter 1

Introduction

1.1 An Introduction of Discipline-Based Education Research

Understanding the intricacies of how students learn has been an important aspect of instruction since the inception of the education system. Historically, instructors on all levels of education have routinely evaluated teaching practices and used results to develop new and better instructional strategies for the classroom (Stigler & Hiebert, 2009). However, with the push for performance in many current educational environments, an instructor's understanding of effective teaching has become less of a personal investigation in pedagogy and more of a practice in "teaching to a test" (Bond, 2008; Jennings & Bearak, 2014; Marchant, David, Rodgers, & German, 2015). With these superficial educational practices becoming somewhat commonplace for many in the teaching culture; a return to a deeper, more scientifically-based approach to understanding student learning is needed. Such investigations, and their research-based outcomes, are the foundation for the ever-evolving field of education research (Dolan 2012; Singer 2012; NCER 2016). Education researchers have an interest in student learning on many different levels, ranging from elementary to postsecondary instructional settings. Despite this broad spectrum, the goal of the field of education research remains the same; an overall improvement in quality of

education (NCER 2016). Examples of some individual aspects of this goal include the improvement of student achievement, bridging the gap between high and low performing students, and increasing access to and completion of college educational experiences (NCER 2016). The importance of education research has been evidenced with an increase in funding from a number of government supported associations and organizations. A subsection of these being longstanding, well-known entities such as the Institute of Education Sciences, the United States Department of Education, the National Science Foundation, and the Howard Hughes Medical Institute (Grant Funding Resources for Educational Initiatives, 2016). The benefits of education programs resulting these funding opportunities have been repeatedly noted and reported in multiple venues (Hudson & Ewert, 2015; National Academies of Sciences, 2016; Woods, 2015). However, while the efforts of the education research community as a whole have proven worthwhile over the years, instructors have noticed that the benefits that are noted in one field of study do not necessarily relate to all subjects equally (Cummings 2011; Singer 2012) .

The compartmentalization of educational research in the sciences began to flourish in the field of physics in the 1970s as a response to a push from the National Science Foundation to advance curriculum development and redesign in the field (Cummings, 2011). The initial call for change came as a result of the “space race,” however further developments in discipline-based education research began as educators in the realms of science, technology, engineering and mathematics noticed a distinct difference in the way that instruction takes place in their fields (Matthews,

1994). Researchers noticed, that even more so than in other fields, instruction as a part of STEM education requires the incorporation of many of smaller concepts to form complex associations that ultimately result in deeper understanding (Smart, 1996). While the formation of complex associations is not unique to the STEM discipline, it is seemingly more difficult due to the small, often unseen nature of many of the components that make up many scientific mechanisms (Singer, Nielsen, and Schweingruber 2013). Adding to the difficulty is the importance of avoiding misconceptions early in the process, as misunderstanding in the smaller conceptual subunits can result in inadequate mental models of the larger processes which could ultimately hinder the learning process (S.-C. Chen, Hsiao, & She, 2015; Hegarty, 1992). In addition, the intricacies of many visual depictions, such as graphs and charts, are unique to certain STEM disciplines and the way with which students interact with them can be specific to their field (Singer, Nielsen, and Schweingruber 2013). Conveyance of the proper interpretation of these figures is paramount to a true understanding in the sciences, and the investigation of how to promote these interpretations may be best conducted by those with a firm grasp on the discipline (Cummings 2011; Talanquer 2014; Singer 2012). To account for this and the other unique aspects of STEM instruction, education researchers in the STEM fields formed a community centered on the expansion of a more discipline-based form of education research (Singer 2012) .

This newly expanding aspect of the education research community commonly referred to as discipline-based education research (DBER) was formally outlined as a part of a report from the National Academies Press in 2012. In this report, the council

noted that successful endeavors into DBER focus on investigating teaching and learning using the full spectrum of research methods while retaining the priorities and practices of the instructional discipline (Singer 2012). However, the development of DBER practices was not intended as a stand-alone venture, it was instead meant to coincide with the findings and guidelines of other more general investigations into learning as a whole (Cummings, 2011; DeHaan, 2011). The National Research Council outlines the goals of discipline-based education research as follows:

- a.) understand how people learn the concepts, practices, and ways of thinking about science and engineering;
- b.) understand the nature and development of expertise in a discipline;
- c.) help identify and measure appropriate learning objectives and instructional approaches that advance students toward those objectives;
- d.) contribute to the knowledge base in a way that can guide the translation of DBER findings to classroom practice; and
- e.) identify approaches to make science and engineering education broad and inclusive.(Singer, 2012)

Coppola, et al. (2013) suggests that the second of these goals, understanding the nature and development of expertise in a discipline, truly outlines the nature of DBER and sets it apart from other aspects of education research. Understanding what comprises expertise in a field allows researchers to focus their efforts on the specific nuances involved in the learning of these conceptual focal points. As an example from the perspective of learning science specific graphs and charts, previous research has

highlighted the difficulties students have in making connections between science concepts and their graphical representation (Nixon, Godfrey, Mayhew, & Wiegert, 2016; Z. H. Wang et al., 2012). A researcher's expert understanding of the practical use of graphs in a scientific setting could provide insight into the formation of better research questions that will bridge the gap in student understanding (Coppola and Krajcik 2013; Singer, Nielsen, and Schweingruber 2013). In addition, expertise can allow instructors to better inform students on how science specific skills are used in the field, helping them to make practical connections between techniques and the underlying concepts (Coppola and Krajcik 2013; Singer 2013; Singer, Nielsen, and Schweingruber 2013). Making such connections between scientific concepts and scientific practice could lower common barriers in science instruction and ultimately lead to the formation of both stronger conceptual foundations and practice (Steve Olson & Riordan, 2012). With this level of focus on the specific needs of the STEM disciplines, the DBER community has continually advanced since its inception and the investigation of content specific topics remains the focus today (Singer 2012).

Early endeavors into discipline-based education research were met with mixed reviews. Specifically in the field of physics, early physics education researchers noted that their fellow physicist found their work "simple" and "not appropriate" to the field (Cummings, 2011). One physics education researcher recalled an encounter with a colleague where his research was referred to as a "gimmick of your own creation and variety- that is not going to add anything to this enterprise" (Cummings, 2011). Despite the trials of these early days, the efforts of the DBER community and the results of years

of investigation into STEM learning have led to multiple national reports on improvements to learning the science fields (Brewer & Smith, 2011; S. Olson & Loucks-Horsley, 2000; Steve Olson & Riordan, 2012). Researchers have continued to morph their investigations to focus on theoretical framework that has been shown to be successful in other realms of study (Clark & Mayer, 2011; Freeman et al., 2014; Haak, HilleRisLambers, Pitre, & Freeman, 2011). As a result of these efforts, the implementation of research-supported, “best practices” continues to rise in the STEM classroom, and new research into science specific classroom redesigns have begun to flourish (Talanquer, 2014). With this increased acceptance and the accompanying exposure in high level science journals, such as *Science* (Linn, Palmer, Baranger, Gerard, & Stone, 2015; Mervis, 2007; Ruiz-Primo, Briggs, Iverson, Talbot, & Shepard, 2011), the DBER community strives to continue the innovation and reform that have resulted from its recent formation.

1.2 Biology Education Research

One of the most recent branches in the DBER evolutionary tree focuses specifically on the subject of biology (Singer, Nielsen, and Schweingruber 2013). Similar to the more established DBER efforts in physics, chemistry, and engineering; education researchers in biology have only more recently focused on understanding and learning in the realm of life sciences (Singer 2013). Original research focused on learning in the field of biology stemmed from a concern for the university laboratory curriculum in the 1930s (Singer 2012). These initial endeavors investigated the perceived shortcomings of introductory biology instruction and proposed alternative designs for the associated

laboratory courses (DeHaan, 2011; Gerard, 1930; Nelson, 1931). Despite these early efforts, the field of biology education research (BER) suffered from a lack of organization that prevented its expansion until biology researchers began to follow the lead of more established research in physics and chemistry education (DeHaan, 2011). With a recent increase in both the number and quality of journals publishing articles focused on learning in the biological sciences, the biology education research community has only reached its maturity within the last twenty years (Singer 2012). Dirks (2011) reported on the results of a meta-analysis looking at 195 individual studies that met criteria used to define biology education research and grouped their findings into three categories based on their research focus. The majority of the studies included in this analysis were found to be published between 2001 and 2010, which highlights the birth of this newly emerging field. The categories described in this report (below) outline the efforts of education research in the field of biology and provide a description of how biology education research has emerged:

1. **Student Learning and Performance:** Studies that focus on student-centered instructional techniques and methods designed to enhance learning. Studies included those investigating alternative laboratory designs, supplemental instruction, and methods for concept introduction outside of the classroom. This category also includes studies centered on how students in biology learn as a whole (metacognition and cognitive psychology centered) and the outcomes of certain subgroups of learners.

2. **Student Attributes and Beliefs:** Studies that focus on student motivation and beliefs in learning biology and how these attributes affect learning outcomes in the biology classroom. Additionally, student motivations and their possible contribution to the progression to graduate studies and pursuit of science professions also fall into this line of research.
3. **Concept Inventories and Validated Instruments:** Studies that focus on the development of concept inventories and assessment instruments that accurately gather information on student understanding of concepts specific to the learning in the life sciences. The results of these efforts are often used to evaluate learning outcomes in the other two categories of research shown in this study. (Dirks, 2011)

More recent endeavors in biology education research have focused on the response to various calls for action in undergraduate STEM education (Brewer & Smith, 2011; Steve Olson & Riordan, 2012). One of these from The President's Council of Advisors on Science and Technology (Steve Olson & Riordan, 2012) noted that the economic forecast over the next decade points to a need for an increase in college graduates in the STEM fields by approximately one million students. This need is exacerbated by current statistics showing a remarkably low number (< 40%) of students that complete the STEM curriculum after declaring their major as a freshman (Steve Olson & Riordan, 2012). To meet this need in undergraduate STEM education, an emphasis has been placed on what can be done to increase both the enrollment and the retention of STEM majors while better preparing students with the skills that will be

required in the future workplace (Bradforth et al., 2015; Steve Olson & Riordan, 2012).

While reform on all levels of science education would undoubtedly be beneficial, the focus of many of these calls to action tend to fall in the realm of undergraduate education as noted by Bradforth:

“We call for immediate change at all levels of research to improve the quality of university STEM education. It is no longer acceptable to blame primary- and secondary-school teachers for the deficits in STEM learning at the university level.”(Bradforth et al., 2015)

These recent national calls to action have drawn attention to a number of issues unique to undergraduate biology education and have resulted in the shaping of new exploratory research questions that will come to define the future of the field. Dolan (2012) describes a resulting future focus of biology education research as centered upon:

- investigation into the effects of teaching practices on long time concept retention ((Steve Olson & Riordan, 2012);
- differences in motivation across the demographic cross-section of undergraduate biology students (Singer, Nielsen, and Schweingruber 2013);
- outcomes of change in teaching strategies at both the classroom and institutional level (Brewer & Smith, 2011)
- development of STEM specific research skills in undergraduate students (National Research Council, 2003); and

- development of accurate and effective measures of student learning in the sciences (Singer, Nielsen, and Schweingruber 2013).

With expansion and evolution, the biology education research community strives to provide insight on each of these aspects of undergraduate biology instruction. As the field continues to evolve, this focus could go a long way to improving how students learn in the complicated field of biology (Brownell et al. 2014).

1.3 Examples of Research-Based Best Practices in Undergraduate Biology Education

A number of efforts in the field of biology education research have resulted in the publication of what are known as “research-based best practices” (eg: Aronson and Silveira 2009; Caldwell 2007; Freeman et al. 2014). These practices are meant to provide instructors with guidelines and techniques that have been shown to be beneficial to the learning process of specific students (Niebaum, Cunningham-Sabo, & Bellows, 2015). Numerous examples of instructional best practices can be found for a variety topics and across multiple levels of education (eg: Daniels, Bizar, and Zemelman 2001; Epper and Bates 2001; Rao, Viswanadhan, and Raghunandana 2015). While their usage can vary greatly depending on both the institution and the instructor, one example of an area where “best practices” could be very beneficial is with graduate teaching assistants and new faculty. Many graduate students and new instructors in the sciences begin their teaching careers with a firm understanding of the discipline but with little background in education (Blouin & Moss, 2015; O’Neal, Wright, Cook, Perorazio, & Purkiss, 2007). Without ample experience or training in the art of instruction, these individuals can be left to learn by doing. While many instructors in the past have found success with this

method, it often takes long periods of time with large amounts of failure. As part of their training, many new professors and graduate assistants are now provided with instructional guidelines, derived from research-based best practices (Aronson & Silveira, 2009; Caldwell, 2007; Rao et al., 2015). These guidelines allow new instructors to focus their efforts on strategies that have been shown to work while, alleviating the frustration of failure from less structured techniques.

One example of a recent “best practice” garnering a large amount of attention is the use of active learning strategies in the science classroom (Freeman et al., 2007, 2014). Active learning strategies place emphasis on student interaction with material as part of a structured, instructor-led environment in the classroom (Handelsman, Ebert-May, Beichner, Bruns, & others, 2004). This typically requires a transition from the traditional instructor-led lecture to a more student-centered learning atmosphere (K. L. Anderson, 2016). The benefits of such a shift in focus were shown by Freeman (2014) in his meta-analysis comparing classrooms implementing active learning strategies to those that rely heavily on a traditional classroom lecture. Results from this study shows an increase of 0.47 standard deviations on test scores when students learned using active learning over traditional lecture. In addition, students in this study who learned biology concepts in classrooms using traditional lecture style techniques were shown to have a 1.5 times higher chance of course failure than those in an active learning-centered classroom (Freeman et al., 2014). Such positive results have led many instructors to publish their experiences with active learning strategies so that others may integrate them into their own course (Eichler & Peeples, 2016; Linton, Pangle,

Wyatt, Powell, & Sherwood, 2014). Adoption of active learning strategies in the STEM classroom has also benefited from the recent popularity of “flipped classrooms” (Bergmann & Sams, 2012; Berrett, 2012; DeLozier & Rhodes, 2016). This alternative structure introduces students to course concepts in a setting outside of the traditional classroom, thereby freeing up course time for in-class activities (DeLozier & Rhodes, 2016). The flipped approach has incorporated many different techniques to introduce concepts to students prior to class. Several examples of these techniques are online learning modules (Stelzer, Gladding, Mestre, & Brookes, 2009), case studies (Herreid & Schiller, 2013), videos (Persky, 2015), and reading assignments (Freeman et al., 2007). While some have been shown to be more effective than others, the level of student preparation outside of the classroom has been shown to be integral to the success of most flipped approaches (Gross, Pietri, Anderson, Moyano-Camihort, & Graham, 2015). The continued investigation of active learning and its incorporation into a flipped classroom approach will no doubt become a focus of future endeavors in biology education research, and the outcomes of such research will shape future best practices in biology education. It should, however, be noted that STEM instructors are still reluctant in adopting active learning and flipped classroom as part of the classroom setting (Andrews, Leonard, Colgrove, & Kalinowski, 2011; Egan et al., 2014; Walker, Cotner, Baepler, & Decker, 2008). Therefore, providing instructors resources to help mediate these changes are an important aspect of future work in biology education research.

One additional example of instructional best practices that has become the focus of many recent biology education research publications has been improvement and redesign of laboratory instruction. Previously introduced calls for action in undergraduate biology education (Brewer & Smith, 2011; Steve Olson & Riordan, 2012), have drawn attention to the need for a more authentic laboratory research experience in hopes of introducing students to what they will actually experience upon entering the workforce. As a result, restructuring of the undergraduate biology laboratory has focused on giving students settings that more closely mimic actual biology research (Brownell et al. 2012; Spell et al. 2014). Laboratory curriculum redesigns have ranged from predesigned large scale, open-ended experiments (Wang et al. 2015), and inquiry-based designs (Russell & Weaver, 2011) to faculty led learning communities (Harvey, Wall, Luckey, Langer, & Leinwand, 2014; Zinn, Foreman, Masso, Ouimette, & Zinn, 2015) and computer-based laboratory modules (Wang et al. 2015; Zhang 2011). With such a large variety to choose from, the decision to implement these techniques depends greatly on course size and structure. A study conducted by Spell et al. (2014) noted that faculty involved in authentic research experiences identified class size, cost, and time (amongst others) as barriers to implementation of these laboratory best practices in a cross-section of universities nationwide. It is possible that these hindrances can be mitigated using instructional approaches, and the best way to implement these new laboratory environments will continue to be the focus of investigations in biology education research in the future (Brownell et al. 2015; Spell et al. 2014).

One aspect of instruction that contributed to the development of recent many research-based best practices is the use of multimedia resources to supplement the learning process (Heyden, 2004; Stelzer et al., 2009; Williams, Aubin, Harkin, & Cottrell, 2001). Multimedia resources have been designed for a variety of different courses and throughout many different educational fields (Clark & Mayer, 2011; Mayer, Dow, & Mayer, 2003). While the benefits of these endeavors have been shown in a number of different studies, their method of implementation tends to depend greatly on the field of study (Baker, 2009; Milovanovic, Obradovic, & Milajic, 2013; Wald, 2008). The subject of undergraduate biology is no exception to this. Various multimedia formats have been developed to supplement learning throughout the undergraduate biology curriculum (Clark & Mayer, 2011; Heyden, 2004; Rhodes, Rozell, & Shroyer, 2014), and range from videos and animations depicting important scientific concepts (Azer, 2012; Reindl et al., 2015), to stand alone online learning modules focused on introduction of material outside of the classroom (Khalil, Nelson, & Kibble, 2010; Zhang, 2011). As part of the research presented in this study, we focus on the investigation of the use of such multimedia resources in the introductory biology classroom environment. To ascertain a true understanding of their efficacy, this study focuses on two aspects of multimedia resources: their development and their implementation.

1.4 Development of Multimedia Resources using Research-Supported Design Elements

Regardless of format, it is imperative that multimedia resources used in instruction follow research-supported guidelines as part of their development (Mayer, 2014; O'Day, 2010). Research in the field of cognitive and education psychology has led the formation

of a set of such guidelines that can be used in the development of multimedia resources (Clark & Mayer, 2011; Mayer, 2014; Mayer & Moreno, 2002). Adherence to these guidelines throughout the development process has been shown to result in more effective implementation as well as greater learning outcomes from these resources (Mayer & Moreno, 2002; O'Day, 2010; Plass, Homer, & Hayward, 2009). One of the most highly regarded sets of multimedia design guidelines was published by Mayer et al. (2005), and many effective resources follow a strict adherence to these "Principles of Multimedia Learning". As a result, the acceptance of these principles in the multimedia development community is widespread and has been noted repeatedly throughout the literature (Chang, Quintana, & Krajcik, 2010; O'day, 2006; O'Day, 2010; Plass et al., 2009). These seven principles are introduced below (as seen in (Mayer & Moreno, 2005)) and provide guidance to the creation and development of multimedia animations for educational use.

1. **The Multimedia Principle**- This principle focuses on the relationship between the narration embedded as part of animation and how it contributes to the learning process. Experimental results show an increase in learning when narrations and visuals are presented together as compared to individually. The multimedia principle is the basis of animation design theory and provides a backbone on which the other principles were designed. A focus on adhering to the presenting of narration and animation together is paramount when producing effective dynamic imagery.
2. **The Spatial Continuity Principle**- This principle focuses on the presentation of onscreen text and animation together. Experimental results show greater learning

when onscreen text is presented in close proximity to the animation which it represents as compared to when text is presented at a farther distance. Animation design based on this principle should focus on assuring that all onscreen text is presented in close spatial proximity to the information which it represents in order to promote proper learning outcomes.

3. **The Temporal Continuity Principle**- This principle focuses on the student's ability to relate narration and animation within a given time frame. Experimental results show greater learning when corresponding narration and animation are presented together at the same time rather than when separated by time. This shows a need for proper timing built into animation design.
4. **The Coherence Principle**- This principle focuses on the incorporation of extraneous information into animation. Experimental results show that greater learning was achieved when all extra music, words, video, etc. are excluded from animation design rather than included. This suggests that these additions introduce distraction rather than promote learning when incorporated into animation design.
5. **Modality Principle**- This principle focuses on the differences in the learning outcome when animation is accompanied by narration versus onscreen text. Experimental results show greater learning when voice narration coincides with animation as compared to onscreen text. In theory, presenting information in a text format overloads the learner from a visual processing perspective. This can be alleviated as a part of animation design when concepts are presented as an auditory narration instead.

6. **Redundancy Principle**- This principle focuses on the effectiveness of incorporation of animation, narration, and onscreen text simultaneously as a part of educational imagery design. Experimental results show greater learning when animation and narration are presented simultaneously in absence of extraneous onscreen text. This seemingly coincides with the modality principle. Proper animation design will therefore successfully incorporate narration and text separate of one another in order to promote greater learning outcomes.
7. **Personalization Principle**- This principle focuses on the way in which narration is presented as a part of animation design. Experimental results show that narration presented in a conversational format promotes greater learning than narration that is presented in a formal format. Speech format allows for the learner to personalize the content presented and therefore take ownership in the learning process.

In addition to these original seven principles of development, cognitive psychology has provided a number of other research-based guidelines that have been applied to multimedia design. Of these, some of the most impactful have focused on methods that reduce the cognitive load of those interacting with the resources being developed (Mayer & Pilegard, 2014). Cognitive load focuses on the appropriation of a learners' cognitive resources when they are attempting to learn new material or participate in a problem solving activity (Chandler & Sweller, 1991; Sweller, 1988). During these events, learners must focus their cognitive abilities on the processing of information and the use of their knowledge to make connections between novel concepts (Valcke, 2002).

Chandler (1991), notes that cognitive load can be broken into three separate types;

extraneous cognitive load, intrinsic cognitive load, and germane cognitive load.

Extraneous cognitive load is placed on learners by the presentation aspects of the multimedia resources itself. Intrinsic cognitive load is determined by the interactivity of the specific concepts being presented and is outside of the developmental aspects of animation. Germane cognitive load involves the processing and the mental compartmentalization of information presented within the resource. Assuming that a learner has a finite level of cognitive skill to delegate, if a learning tool occupies too many of these resources with extraneous information and needless processing, there can be a negative effect on the learning outcomes (Chandler & Sweller, 1991). As a result, there has been a focus on reducing the extraneous cognitive strain of multimedia tools, animations included, throughout the development process. This focus led Mayer and Pilegard (2014) to formulate three additional principles for multimedia design and cognitive strain reduction in learning. The first of these, “the principle of segmentation”, focuses on allowing the learner to view animations at a user-dictated pace in smaller conceptual pieces as opposed to one large, possibly overwhelming chunk (Mayer & Pilegard, 2014). Experimental results comparing the benefits of segmented to non-segmented animations show a high mean effect size ($d = 0.79$) when individuals are allowed to view the smaller segments as opposed to the larger chunks of information (Mayer & Pilegard, 2014). The second principle, “the pre-training principle” notes that learners have a greater outcome from educational multimedia when they have been previously introduced to general concepts to be presented prior to interacting with the resource. Experimental effect size when comparing pre-trained

students to control was again high ($d = 0.75$) suggesting a benefit to a pre-training model (Mayer & Pilegard, 2014). Lastly, the third principle, “the modality principle”, notes that students show greater learning outcomes when multimedia resources include spoken narration as opposed to written. Comparison of treatment groups again shows a high mean effect size ($d = 0.76$), suggesting a benefit of spoken narration (Mayer & Pilegard, 2014). With adherence to these additional principles throughout the development process, instructors can help reduce the cognitive load for learners viewing multimedia resources as part of instruction (Reindl et al., 2015). This refocusing of cognitive skills exclusively on the key conceptual connections that need to be made in a lesson can therefore allow students to bridge the gaps in learning that were previously prevented by extraneous cognitive strain (Mayer & Pilegard, 2014). As a result, developers of animations and other multimedia resources should pay close attention to both the principles of multimedia learning and cognitive load reduction when designing new materials. The animations investigated as part of this study, which comprise the Virtual Cell Animation Collection, have been developed with a strict adherence to each of these principles and aim to effectively promote learning in undergraduate biology students.

1.5 The Virtual Cell Animation Project

Previous investigation of multimedia resources for use in an educational setting has shown their drastic variability in both accessibility and quality (Azer, 2012; Raikos & Waidyasekara, 2014). While the development of course specific content is not

uncommon, the world of educational multimedia is currently dominated by textbook publishing companies that release their resources to students as part of a textbook package (O'Day, 2010). Despite the usefulness of these resources as an educational tool (Speckler, 2014) their accessibility is often restricted to those who purchase their materials. While this practice could be profitable for publishers, the benefits of the provided materials are ultimately limited to only those who purchase their educational packages.

As a possible alternative to the high cost of these publisher-produced educational resources, many instructors have created free-to-use videos and animations that are often posted to online sites such as YouTube. While some of these resources can be effective in an instructional setting, studies have shown their potential to be inadequate (Azer, 2012; Raikos & Waidyasekara, 2014). These outcomes could be a direct result of a developmental freedom that does not adhere to published guidelines of multimedia design (Clark & Mayer, 2011; Fleck, Beckman, Sterns, & Hussey, 2014). This places an emphasis on the development of resources that are both effective and free-to-use for both students and instructors.

The Virtual Cell Animation Collection addresses this emphasis in the development of their multimedia resources. As a part of the development process, the Virtual Cell team has applied research-based principles of multimedia design to produce a collection of high quality molecular animations and learning modules. Additionally, these resources are all free-to-use and openly accessible to both instructors and their

students alike. A strict adherence to content accuracy and guidelines for development is maintained by the Virtual Cell's group of content experts (McClellan et al., 2005; Reindl et al., 2015). Currently, this multimedia collection consists of 25 animations (Table 1.1) outlining concepts common to molecular and cellular biology, each of which are freely available for both streaming and downloading at the project's website (<http://vcell.ndsu.edu/animations>). The Virtual Cell Animation Collection exemplifies its popularity with approximately 23,000 registered users that have completed the optional registration process, as well as the Virtual Cell Animation Collection's YouTube channel (<http://www.youtube.com/user/ndsuvirtualcell>) which currently boasts approximately 44,000 subscribers and over 12,000,000 viewings. To appeal to a demographic that is well-versed in a mobile electronic environment, the team has also developed a free Apple iOS application (<http://itunes.apple.com/us/app/virtual-cell-animations/id427893931?mt=8>) that has been downloaded from the Apple app store approximately 200,000 times to date.

In addition to the continued development of molecular animations, the Virtual Cell Animation team has recently focused on the integration of their animations into stand-alone online learning modules. These modules are aimed at effectively presenting difficult biological concepts in a setting independent of a physical classroom or instructor. The successful development of these learning modules would provide effective, research-based resources that instructors can use outside of the classroom, thereby allowing time in-class to be devoted to alternative teaching strategies. In addition, the development of effective online learning modules could provide students

Table 1.1- Current List of Topics Covered by the Virtual Cell Animation Collection	
Topic	Duration
Introduction to a Cell	
Through the Virtual Cell	6:45
Cellular Processes	
Protein Trafficking (Golgi)	3:27
Protein Modification	3:49
Protein Recycling	3:15
Insulin Signaling	4:42
Constitutive Secretion	3:29
Regulated Secretion	3:24
Mitochondrial Protein Transport	3:22
Mitosis	6:10
Meiosis	5:27
Molecular Processes	
RNA Transcription	2:50
Regulated Transcription	3:36
mRNA Processing	2:30
mRNA Splicing	2:55
Protein Translation	3:32
Bacterial Gene Expression/Lac Operon	3:23
Cellular Energy Conversion	
Biological Gradients/ATP Synthase	3:47
Cellular Respiration/Electron Transport	3:49
Photosynthesis (Light Reactions)	5:04
Photosystem II	4:31
Glycolysis (Overview)	3:10
Glycolysis (Reactions)	5:09
Citric Acid Cycle (Overview)	3:17
Citric Acid Cycle (Reactions)	4:24
Energy Consumption	4:34

an alternative to in-class instruction for topics that they may have a solid previous general understanding. This could serve as a conceptual review for upper-level students that need to be reintroduced to a topic prior to more detailed classroom instruction. To date, Virtual Cell learning modules have been developed on the concepts of cellular respiration, meiosis, energy flow, and insulin signaling. Together with the core animations, these online learning modules aim to provide instructors and students alike with effective multimedia resources for use in undergraduate biology. The research specifically presented as part of this study investigates the use of these Virtual Cell resources in the introductory biology classroom.

1.6 Focus of the Current Investigation

As the college classroom continues to evolve, campuses have begun to increasingly incorporate technology into the learning process (Asthana, 2008; Bernstein, 2013). This incorporation not only provides professors a way to supplement instruction in the classroom but has also led to the creation of courses that are based entirely online (Bernstein, 2013). With this increased focus on the use of multimedia resources to aid in content delivery, research into aspects of their proper development and implementation becomes crucial. To combat discrepancies in learning with multimedia outlined in the literature (Tversky, Morrison, & Betrancourt, 2002), the Virtual Cell Animation Collection has set out to research a comprehensive series of molecular animations that provides instructors confidence that concepts presented are being presented both accurately and effectively (McClellan et al., 2005; Reindl et al., 2015). In

an effort to examine the efficacy of these resources and to possibly provide clarity to an otherwise murky literature base, this investigation evaluates the use of animations and online modules produced by the Virtual Cell Animation Collection as part of undergraduate introductory biology instruction. Initial small scale (n= 55) results testing the efficacy of the animations show that students viewing Virtual Cell animations have greater conceptual learning outcomes compared to a control group (McClean et al., 2005). The current study continues this investigation of the implementation of Virtual Cell Animations with an expansion to a large-scale introductory biology classroom that is common at many institutions nationwide (A. C. Smith et al., 2005). The aspect of classroom size provides a variety of challenges to instruction that have been repeatedly noted in STEM education (Eichler & Peeples, 2016; Moravec, Williams, Aguilar-Roca, & O'Dowd, 2010; A. C. Smith et al., 2005; Walker et al., 2008). As a result of these challenges, we feel that the conclusions of the research conducted in this environment will provide a more practical relevance than if it were conducted in a smaller controlled setting. In addition, the research presented here will provide evidence of learning gains for instruction augmented with multimedia resources and show their ability to be implemented despite the challenges of class size. Using the three study aims outlined below, we investigate the use of Virtual Cell resources in introductory biology from three different aspects of instruction. Background information on each of these aims is presented as part of the chapters in this dissertation.

1.7 Study Aims and Research Questions

- 1.) Aim One: As a part of the first aim of this study, we investigate the comparison of static and dynamic imagery as an instructional aide within the presentation of classroom lectures. Results of this aim will answer the question, “how does learning with dynamic animations as part of a classroom lecture influence students’ conceptual understanding in introductory biology as compared to learning with static graphics?”
- 2.) Aim Two: As a part of the second aim of this study, we investigate the use of dynamic animations outside of the classroom as either preparation for instruction or as reinforcement of concepts presented in the classroom. Results of this aim will answer the question, “how does learning differ when students view animations before instruction as preparation or after instruction as conceptual reinforcement when compared to a no-intervention group?”
- 3.) Aim Three: As part of the third aim of this study, we investigate the efficacy of stand-alone, online learning modules as a means of concept introduction outside of the classroom. Results of this aim will answer the question, “to what extent do stand-alone, online learning modules aide in instruction of introductory biology concepts compared to a traditional classroom lecture?”

1.8 Utility of the Conclusions of this Research

The recent influx of multimedia in the undergraduate classroom has placed a need for research-supported development and implementation strategies on their use. To address deficiencies in the literature concerning the use of multimedia in the classroom we investigate the use of resources produced by the Virtual Cell Animation Collection to supplement instruction in introductory biology. Conclusions of this investigation will provide empirical evidence on the efficacy of using Virtual Cell animations as part of undergraduate biology instruction. Evidence such as that reported here will also aid in the development of new and innovative instructional “best practices” for use in the classroom. These instructional best practices will provide instructors with research-supported resources that are easy to implement and can be trusted to convey important introductory concepts common to most institutions. While such resources would be practical for all instructors, they may be most beneficial to those who are new to the classroom such as new faculty and graduate teaching assistants who are searching for help in their pedagogical approach.

While the Virtual Cell Animation Collection is not the only source for biology focused multimedia, the results presented here represent its ability to outperform some other forms. For example, the online video database YouTube contains a plethora of representations of various biological concepts. A simple YouTube search of photosynthesis returns over 220,000 responses. On the surface this may seem beneficial to those in search of instructional resources, however many of these have been shown

to be inaccurate and in some cases they have actually promoted misconceptions concerning certain topics (Azer, 2012; Raikos & Waidyasekara, 2014). A set of research-supported multimedia resources that can be easily accessed and integrated in instruction can provide instructors with reliable resources to supplement their instruction. In addition, use of resources from one central collection also provides students a sense of consistency throughout the instructional period which may help in the learning process. Ultimately, the outcomes of the research presented here aim to foster student understanding of introductory biology concepts through interaction with well-developed multimedia resources and thereby answer recent calls to action for improvement of in STEM education (Brewer & Smith, 2011; Steve Olson & Riordan, 2012). This includes the promotion of deeper conceptual knowledge and the formation of a stronger conceptual foundation which could lead to a greater number of well-trained STEM graduates in the workforce of tomorrow.

1.9 Definition of Key Terms

- 1.) **Active Learning** – An educational strategy focused on promoting the interaction of students with the material being taught in a class. This typically involves activities that require students to actively engage in the learning process through investigation of problems associated with course concepts, or communication of concepts with classmates and instructors. Some examples of active learning strategies include small group discussion, classroom debate, case studies,

problem solving activities, and inquiry-based assignments. (Allen & Tanner, 2005; Freeman et al., 2014; Haak et al., 2011)

- 2.) **Biology Education Research** – A branch of discipline-based education research focusing on the investigation of instruction and learning in the field of biology. Originally developed as an aspect of education research due to the unique challenges that instruction in the life sciences typically present. (S. Singer, 2012; Susan R. Singer et al., 2013)
- 3.) **Cognition** – The act of acquiring knowledge through the processes of thought and experience. In the context of the research presented here, cognition refers to the mental process that a student uses to gather knowledge and process information leading to conceptual understanding of biological topics. (Chandler & Sweller, 1991; Sweller, 1994; Tanner, 2012)
- 4.) **Cognitive Load Theory** – The aspect of cognitive theory that focuses on the directing of an individual’s cognitive resources to a specific task. In the context of the research presented in this study, this refers to the use of one’s cognitive ability to aide in the acquisition of knowledge and content understanding. Cognitive load theory suggests that an individual’s cognitive resources are limited and effective instruction should focus on occupying these abilities with only essential aspects of the learning process. (Chandler & Sweller, 1991; Sweller, 1988, 1994; Valcke, 2002)
- 5.) **Discipline-Based Education Research** – A focused approach to traditional education research strategies where a specific discipline is the center of

investigation into student learning. Discipline-based education research in the STEM sciences began in the field of physics and has since branched out to cover many aspects of STEM education. (Coppola & Krajcik, 2013; S. Singer, 2012)

- 6.) **Dynamic Imagery** – A form of educational aide developed with a series of moving pictures often accompanied by either written or spoken narration. In relation to the research presented in this study, dynamic imagery typically refers to animation. However, in other settings dynamic imagery can also refer to video and computer generated models. (McClellan et al., 2005; Reindl et al., 2015; Yarden & Yarden, 2010)
- 7.) **Flipped Classroom** – A pedagogical model of instruction where concept introduction typically takes place outside of the classroom using video lectures, computer-based instruction, or some other form of instruction. As a result, classroom time is open for the implementation of active learning techniques where students use the knowledge gained outside of the class to interact with material as part of a more inquiry-based form of instruction. (DeLozier & Rhodes, 2016; Pierce & Fox, 2012)
- 8.) **Introductory Biology** – The series of basic biology courses in which most students enroll as part of their undergraduate studies. These courses serve as an introduction of basic biology principles and focus on a basic understanding of biological concepts. At the university in the research presented here, introductory is defined as the Biology 101 and Biology 102 courses typically

designated for students who are majoring in a STEM science. (Brewer & Smith, 2011; Momsen, Long, Wyse, & Ebert-May, 2010; A. C. Smith et al., 2005)

- 9.) **Learning Module** - A multimedia form of instruction typically focused on presenting information using a series of narrations, images, and assessments. Learning modules are often considered to be a stand-alone form of instruction, where students can interact with the material presented on their own time and without the aid of an instructor. (Huang, 2005; Khalil et al., 2010; Lancellotti, Thomas, & Kohli, 2016)
- 10.) **Multimedia** – Media consisting of more than one type of expressive representation typically including images, videos, animations, spoken or written narration, or other form of communicative content. In reference to the study presented here, multimedia is computer-based content combining imagery and narrations to present educational information to students. (Asthana, 2008; Mayer, 2009, 2014)
- 11.) **Static Imagery** – A form of educational imagery that presents information as a non-moving, typically 2-D representation of a concept. Static imagery can include both pictures as well as text and can contain arrows or other forms of visual shorthand to guide viewers through the figure. Static imagery is the most prominent form of graphic found in most introductory biology textbooks. (Lai & Newby, 2012; Paivio & Clark, 1991)
- 12.) **STEM** – Acronym for Science, Technology, Engineering and Mathematics. These fields are typically grouped together due to their interconnection in the

academic world and their similar attributes in relation to both practice and learning. (J. Brown, 2012; Knowles, 2014; Mervis, 2007)

13.) Virtual Cell Animation Collection - Originating at North Dakota State University, this team of cellular and molecular scientist focuses on the creation of dynamic animations and learning modules depicting key concepts of undergraduate introductory biology. The multimedia resources tested in this study are all a part of the Virtual Cell Animation project. (McClean et al., 2005; Reindl et al., 2015)

1.10 Structure of this Document

Each of the three aims investigated in this study is detailed in its own individual chapter comprised of a comprehensive literature review followed the results of experimentation. Results are reported in a modified manuscript format consisting of peer-reviewed journal articles that have been accepted for publication followed by additional experimental extensions and follow-ups. Each article is cited in full at the beginning of the chapter where it is included and the beginning of the cited article is noted by subheadings.

Chapter 2

Virtual Cell Animations as Part of Classroom Instruction in Introductory Biology

The recent push for reform in the realm of undergraduate STEM education has resulted in the redesign of many introductory level science courses (Brewer & Smith, 2011; Woodin, Carter, & Fletcher, 2010). Biology is no different in this aspect, as many life science instructors have focused on a change in the classroom. Reform efforts have included a variety of different instructional strategies ranging from an increase in active learning in the classroom (K. L. Anderson, 2016; Eichler & Peeples, 2016), to the increased use of multimedia to supplement instruction (Harrison & Hummell, 2010; Reindl et al., 2015). While each of these methods has shown their individual merits, the actual extent of instructional change depends greatly on the instructor and the dynamics of the course in which they are to be used (Allen & Tanner, 2005). One report suggests that instructors in the STEM sciences rely more on the traditional lecture format of delivering course content than other fields of education (Eagan et al., 2014). This reluctance to change delivery style can be attributed to numerous factors including the difficulty of the content itself and the previous failures that instructors have

Note: section 2.1 has been adapted from Goff, E., Reindl, K., Johnson, C., McClean, P., Offerdahl, E., Schroeder, N., White, A. (2017, in press). Variation in External Representations as part of the Classroom Lecture: An Investigation of Virtual Cell Animations in Introductory Photosynthesis Instruction. *Biochemistry and Microbiology Education*.

experienced when they have reformed their delivery style (Andrews et al., 2011; Kuiper, Carver, Posner, & Everson, 2015). Even with the prevalence of a traditional instruction style, the STEM classroom still presents a number of opportunities for reform. To address these opportunities, we focus the first aim of this study on the use of educational imagery, also known as external representations, in the traditional lecture-centered style of classroom instruction that is typical in many biology courses today. Specifically, we investigate the differences in the learning outcomes resulting from two forms of these external representations; static, non-moving depictions of concepts (slide images), and dynamic, moving representations of biological concepts (animations).

Research into the comparison of static and dynamic external representations as part of instruction has previously lead to no clear conclusions about what format leads to the greatest increase in learning outcomes and in which environment they are most beneficial (Tversky et al., 2002). In an attempt to account for the shortcomings of previous experimentation, we concentrate our efforts on the implementation of external representations from one single research-supported source, the Virtual Cell Animation Collection (McClellan et al., 2005; Reindl et al., 2015). Using resources produced as part of the Virtual Cell Animation Collection, we focus on answering the question, “how does learning with dynamic animations as part of a classroom lecture influence students’ conceptual understanding in introductory biology as compared to learning with static graphics?” Dynamic animations developed as part of this collection follow research-supported guidelines of animation design that have previously been shown to promote conceptual understanding in undergraduate students (Harrison &

Hummell, 2010; Mayer & Moreno, 2002). As a result, we hypothesize that students exposed to dynamic animations produced by the Virtual Cell Animation Collection as part of the classroom lecture will outperform those exposed to static imagery on an assessment of conceptual understanding. We concentrate on two concepts that have previously been shown to be a source of difficulty in introductory biology students; photosynthesis (Parker et al., 2012) and mitosis (Ozcan, Yildirim, & Ozgur, 2012). Each of which is investigated individually to assess the effects of different types of external representations on the learning outcomes of these topics. The findings of such are reported independently below.

2.1 Variation in External Representations as part of the Classroom Lecture: An Investigation of Virtual Cell Animations in Introductory Photosynthesis Instruction

Abstract

The use of external representations (ERs) to introduce concepts in undergraduate biology has become increasingly common. Two of the most prevalent are static images and dynamic animations. While previous studies comparing static images and dynamic animations have resulted in somewhat conflicting findings in regards to learning outcomes, the benefits of each have been shown individually. Using ERs developed by the Virtual Cell Animation project, we aim to further investigate student learning using different ERs as part of an introductory biology lecture. We focus our study on the topic of photosynthesis as reports have noted that students struggle with a number of basic photosynthesis concepts. Students ($n = 167$) in ten sections of introductory biology laboratory were introduced to photosynthesis concepts by

instructional lectures differing only in the format of the embedded ERs. Normalized gain scores were calculated, showing that students who learned with dynamic animations outperformed students who learned from static images on the posttest. The results of this study provide possible instructional guidelines for those delivering instruction on photosynthesis in the introductory biology classroom.

Introduction

External representations (ERs) (such as drawings, images, and animations) have been established as a crucial aspect of classroom instruction (Cook, 2012; Schonborn & Anderson, 2010). This is profoundly evident in undergraduate biology education. Scientific mechanisms are ripe with complex step-wise processes that require the association of many individual concepts to fully understand. One example of this is the production of ATP during cellular respiration. While some students may grasp surface level concepts of energy production, a deeper understanding of respiration requires the integration of many additional components such as the transport of electrons and the formation of concentration gradients. Learning the complexities of this process could be a truly monumental task if a student is provided with only a text. However, ERs provide students with an effective medium to help formulate more accurate mental models.

In the past, the most prominent mode of representation seen in the biology classroom has been the static pictures found in textbooks and lecture slides. Despite the stationary representation of concepts, these types of ERs function to guide learners through the stages of biological mechanisms using components linked together by

arrows and written explanations (Wright, Fisk, & Newman, 2014). The strength of these static images is that, due to their stationary nature, they provide learners the opportunity to self-regulate their processing of the material in the way they are most capable of understanding (Hegarty, 1992; Paas, Van Gerven, & Wouters, 2007). These benefits have been explored in a number of studies where static images were found to either outperform or be equivalent to other ERs at introducing various concepts (Lai & Newby, 2012; Rieber, Boyce, & Assad, 1990; Rieber, Hannafin, Rieber, & Hannafin, 1988; Schnotz, Böckheler, & Grzondziel, 1999; Wong, Castro-Alonso, Ayres, & Paas, 2015). Despite these positive results, the over use of arrows and other forms of visual shorthand to guide learners through static images has been suggested to inadvertently increase cognitive load, resulting in the creation of inaccurate mental models and student misconceptions (Höffler & Leutner, 2007; Wright et al., 2014). While static images have been shown to be effective in depicting some aspects of life science education, it is plausible that dynamic mechanisms may be more effectively taught using other forms of ERs.

With recent advancements in technology, the development and implementation of dynamic animations has begun to rise in popularity (O'Day, 2010; Stith, 2004). The dynamic nature of animation can be used to show multiple stages in intricate biological process in a step-wise, moving series of on-screen events (McClellan et al., 2005). In the field of biology, researchers interviewed students to gather opinions on different ERs and found that the benefits of animation are most evident in complex biomolecular processes (Rundgren & Tibell, 2010). While the variation in complexity of different

biomolecular processes is outside of the scope of the current study, these outcomes suggest the ability of animation to convey difficult concepts effectively. Rundgren and Tibell suggested this was a result of the dynamic nature seen in many biomolecular interactions. Research into the use of animations as an educational resource has also shown their ability to outperform other ERs in variety of different studies (Rhodes et al., 2014; Thatcher, 2006; Williamson & Abraham, 1995; Yarden & Yarden, 2010). In addition, it has been suggested that animations not only lead to improved immediate recall of concepts, but can also increase concept retention over time (O'day, 2007). These outcomes have been shown to be independent of both class size (Ardac & Akaygun, 2005), and animation complexity (Jenkinson & McGill, 2012). Despite many studies showing the benefits of dynamic animation, some researchers have suggested that they can actually hinder the learning process. For example, it has been suggested that some animations can present information in a "here then gone" manner that places a strain on the short term memory of viewers, a process which can result in reduced conceptual understanding (Ayres & Paas, 2007; Chandler & Sweller, 1991; Hegarty, 2004).

Extracting a message from an ER, whether static or dynamic in nature, is likely mediated by a number of different factors. Schonbron and Anderson (Schonborn & Anderson, 2010) group these factors into three categories affecting student interpretation of an ER: conceptual knowledge (student understanding of a concept), reasoning ability (student use of cognitive skills), and mode (makeup of the ER itself). Two aspects of this model, prior knowledge (representing conceptual knowledge) and

spatial ability (representing reasoning ability), have been suggested to contribute to student success with both static and dynamic ERs (L.-J. ChanLin, 1998; Lai & Newby, 2012). Previous studies have explored the importance of prior knowledge on learning outcomes when concepts were presented in both static image and dynamic animation formats. Results from these studies show greater conceptual understanding across both ERs by learners with higher levels of prior knowledge as compared to those with lower levels (L. ChanLin, 2001; Nerdel, 2003). Researchers suggested learners with greater prior knowledge were able to focus on the relationships between concepts presented instead of decoding the image itself. These sentiments have also been reiterated in relation to spatial ability. Spatial ability defines how well an individual is able to process their visual field and organize the information into their own mental representation (Carroll, 1993; Höffler, Precht, & Nerdel, 2010). Researchers have shown that students with higher spatial ability levels experience greater learning outcomes across both static and dynamic ERs when compared to student with lower spatial ability (Lai & Newby, 2012; Rieber et al., 1990, 1988; Schnotz et al., 1999; Wong et al., 2015). Much like prior knowledge, spatial ability was suggested to assist students with the formation of cognitive connections regardless of the ER used to present the information.

While both prior knowledge and spatial ability have been shown to influence student learning, the contributions of different ERs may vary for students exhibiting lower levels of each. For example, students with low spatial ability were found to have greater learning outcomes when information was introduced in animated form as compared to using static images (Hegarty & Kriz, 2008; Höffler et al., 2010; Mayer &

Moreno, 2002; Mayer & Sims, 1994). By comparison, higher spatial ability students that were presented with the same conditions showed no difference in conceptual understanding. Researchers proposed the “ability-as-compensator” effect of spatial ability, which suggests that animation acts as a “cognitive prosthetic” providing those with lower spatial ability an expert model of interactions rather than having the learner make the conclusions themselves (Höffler & Leutner, 2011). Likewise, students who exhibited lower levels of prior knowledge also showed significantly greater learning outcomes when presented with concepts in an animated form as compared to static images (L.-J. ChanLin, 1998; Moreno, Mayer, Spires, & Lester, 2001; Nerdel, 2003; Rhodes et al., 2014). This could again be feasibly attributed to the compensatory abilities of animation, aiding students in the creation of mental models where they previously have none. Together these studies suggest that the strength of animation may lie in its propensity to compensate for shortcomings in both the prior knowledge of learners and their ability to process and organize the presented content into their own mental models.

Despite the aforementioned benefit of both static and animated ERs, the literature provides no clear conclusions as for whom which format works best and with what topics that it does so. The purpose of this study is to compare these two forms of educational ERs in an introductory biology (Bio101) course at a large public university in the Southeast. Our study is centered around a classroom lecture on the topic of photosynthesis, as research has shown a prevalence of misconceptions on the topic (Parker et al., 2012; Södervik, Virtanen, & Mikkilä-Erdmann, 2015). The decision to use

the classroom lecture as the setting of our research stems from the role of lecture as the primary conceptual introduction to new topics in introductory biology at the institution where our study was conducted. Using normalized gain scores from assessments on photosynthesis, we aim to answer the question, “how does learning with dynamic animations influence students’ understanding of photosynthesis compared to learning with static images?” Previous studies suggesting the compensatory effects of animation lead us to believe that an introductory biology course with students who differ on many cognitive levels could benefit greatly from the use of dynamic ERs (Höffler & Leutner, 2011). As a result, we hypothesize that students’ who view photosynthesis animations as part of classroom lecture will exhibit greater normalized gain scores as compared to their static imagery counterparts.

Common Misconceptions on the Topic of Photosynthesis

Introductory biology students often enter into their undergraduate studies with varying levels of previous exposure to the topic of photosynthesis in their high school education. In general, instructors assume that students have been introduced to photosynthesis as a key component to energy flow in the environment where sunlight is used to produce stored chemical energy in the form of sugars (“Next Generation Science Standards,” 2016). The specifics of this process however are regularly the source of misunderstanding amongst introductory biology students. Common examples of photosynthesis misconceptions often focus on the role of sunlight in the molecular dynamics of the system, the reactions involving carbon dioxide and its contribution to

sugar production, and the connection between photosynthesis and cellular respiration in the flow of energy in the ecosystem (Parker et al., 2012; Wilson et al., 2006). Photosynthesis, like many biological concepts, requires the knowledge of many smaller components that must be grouped together to result in understanding of the full process. Various ERs of such mechanisms have previously been shown to aid in making these connections for a number of different topics (Ardac & Akaygun, 2005; Katsioloudis, Dickerson, Jovanovic, & Jones, 2015; O'Day, 2010). As a result, we focus this study on the use of ERs produced by the Virtual Cell (VCell) Animation project to teach the topic of photosynthesis in the introductory biology classroom.

The Virtual Cell Animation Collection

The VCell Animation Collection (NSF awards: 0086142, 0618766, and 0918955) is a free-to-use series of animations developed using research-based principles of multimedia instructional design to represent the introductory concepts of cellular and molecular biology. The VCell Animation team consist of an expert group of cellular and molecular biology researchers that work to assure accuracy of the information presented (McClellan et al., 2005; Reindl et al., 2015). Currently, the VCell Animation Collection consists of 25 animations which are available for either streaming or downloading in a variety of different formats from the project's website (<http://vcell.ndsu.edu/animations/>). In addition to the project website, the VCell Animation Collection also has a YouTube site (<http://www.youtube.com/user/ndsuvirtualcell>) with approximately 44,000 subscribers

and a free Apple iOS application (<http://itunes.apple.com/us/app/virtual-cell-animations/id427893931?mt=8>) with approximately 175,000 downloads. The VCell development team has recently focused on investigating the performance of the collection in a variety of classroom environments. It is the goal of these investigations to provide teachers with effective instructional resources that can be used to present concepts to students both inside and outside of the classroom. Details on the investigation into a comparison of the photosynthesis ERs in a classroom lecture environment are outlined below.

Methods

Participants and Treatment Groups

The participants were enrolled in an introductory biology lab course at a large university in the Southeast (Table 2.1). Introductory biology lab is taught congruently with the introductory biology lecture course and covers the same basic concepts. The lab sections were chosen as the setting for this study due to their flexibility in lecture and instructor. Study participants self-enrolled in one of 39 sections of introductory biological sciences laboratory (Biol101L) offered by the university. From the 39 sections we randomly selected ten sections to participate in this investigation, five sections ($n = 81$) were randomly assigned to receive instruction using Treatment One and five sections ($n = 86$) were assigned to receive instruction using Treatment Two. Treatment One introduced students to the basic concepts of the photosynthesis as part of a lecture presentation using a photosynthesis animation from the VCell Animation Collection. As

Table 2.1 - Demographic Breakdown of Sample Population											
Graphical Format	Gender		Ethnicity					Year in School			
	Male	Female	Caucasian	African American	Asian	Hispanic	No Response	Freshman	Sophomore	Junior	Senior
Static	19	67	74	5	5	1	1	44	29	8	5
Dynamic	26	55	73	4	2	1	1	63	11	5	2

part of the development process, the VCell team created a series of step-wise static images depicting the biological processes that can be viewed as part of their animations. These static images are posted on the VCell website and can be freely accessed. Treatment Two introduced photosynthesis in a lecture presentation using these static images (labeled “advanced look” on the VCell website) as a step-wise series of figures to denote a multi-step process. The same instructor presented the lecture to all the groups in the study, and their presentation slides were identical except for the ER used.

Assessment Instruments

Student conceptual understanding was assessed using instruments chosen by the research team in this study (Appendix A). The diagnostic pretest consisted of ten questions not used in the analysis of this study that were focused on basic understanding of a variety of biological concepts, ten questions used as a test of prior knowledge in this study focused on basic understanding of photosynthesis specifically (*Cronbach’s* $\alpha = 0.55$), and five questions covering student background information. As a part of this background information we included the following question with a five-point Likert scale used to gather information on students’ feelings toward learning with multimedia resources: “I learn best when information is presented in a visually stimulating (ie: animations/video) fashion.”

The posttest assessment was a ten-question instrument (*Cronbach’s* $\alpha = 0.54$) constructed using isomorphic questions focused on photosynthesis from the pretest. Length of the instrument was designed to remain relatively short so as to prevent

interfering with laboratory time. Questions were selected from two Biology textbooks (Brooker, Widmaier, Graham, & Stiling, 2017; Reece et al., 2014) and were slightly modified to fit the level of the course in this study. These textbooks were chosen as they are used as the primary text for students in the introductory biology course in this study and represent a large market share of biology text used nationwide. In addition, questions from these texts are commonly used as formative assessments for students in many university classrooms. Modification was conducted to make questions more appropriate for introductory learners and consisted of removing confusing phrasing and images that were more representative of upper-level biology course concepts. Weighted Bloom's Index was calculated and found to be 36.67, suggesting a lower to mid of cognitive skill level (Freeman, Haak, & Wenderoth, 2011a).

Student demographic information was obtained from the University registrar (gender, race, year in school, and previous enrollment in biology 101) and matched to student performance on the aforementioned assessment. Student identifier data was removed from the dataset.

We accounted for differences in instructional style by recording all lectures and assigning two randomly selected lectures (one from each treatment group) to three separate reviewers for assessment of conceptual introduction. Reviewers were asked to view recorded lectures and note, in a yes/no fashion, if five listed photosynthesis concepts were covered adequately in both treatments (Appendix B). In addition, reviewers were asked to note in a free response manner any differences in general

instructional styles that were noticed while viewing these recordings. Reviewer ratings suggest that concepts were presented similarly in both treatments, and the interrater reliability coefficient showed that reviewers rated the presentations consistently between coders (*Fleiss' K* = 0.93). No instructional style differences were noted by reviewers and general comments stated, "Both lectures (treatments) were very similar in style and material provided."

Experimental Procedures

At the beginning of the semester, all students enrolled in the selected laboratory sections were given the diagnostic pretest assessing baseline conceptual understanding on the topic of photosynthesis. At the midpoint of the fall semester, students were introduced to the topic of photosynthesis using the experimental treatments outlined above (Fig. 2.1). Treatments were administered at similar meeting times throughout the specified week of study in the semester. Previous studies have suggested that incomparable instructional procedures and inequivalent graphic quality can result in the misrepresentation of the outcomes of direct comparison between static and dynamic formats (Tversky et al., 2002). To account for incomparable instructional procedures, we used the same instructor across all class sections and an external instructor assessment for comparable lectures across all treatments. In addition, we controlled for inequivalent graphics by using static images that were developed in the production of the photosynthesis animations used in Treatment one.

Following instruction, students were assigned a ten-question posttest assessment designed to examine student knowledge on the concepts of photosynthesis.

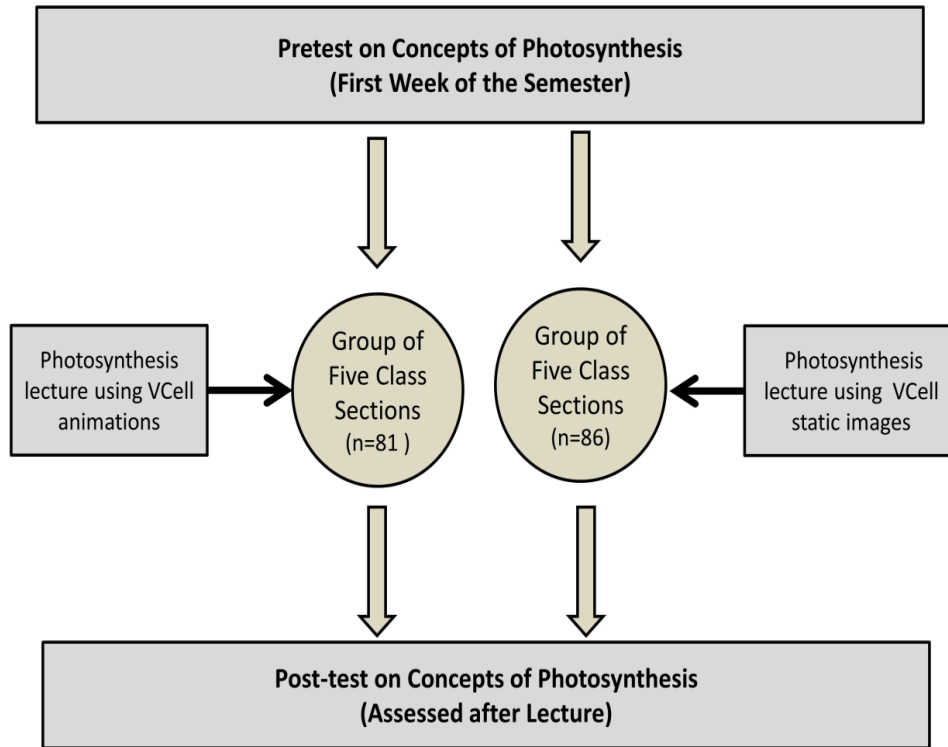


Figure 2.1 - Timeline of the experimental design presented.

Statistical Analysis

For the identified aspects of student performance, descriptive statistics were compiled and inferential analysis run comparing treatment groups using the R statistical programming package (The R project for Statistical Computing, 2015). Normalized gain score $[G = (\text{post score \%} - \text{prescore \%}) / (100 - \text{prescore \%})]$ was calculated from assessment results to provide an analysis of student learning (Hake, 1998). *P*-values were obtained using independent *t*-test across treatment groups and 95% confidence

intervals for improvement differences between treatments were calculated. Cohen's *d* was used to describe the magnitude of the difference between group means. Possible contributing extraneous variables were explored using principle component analysis which identified correlating factors contributing to variation in the sample population (Abdi & Williams, 2010). Follow-up exploratory factor analysis was conducted to identify possible contributing variables using a primary loading cutoff threshold of 0.40 (Child, 1990). This method was selected to identify a more parsimonious model from a larger set of possible explanatory variables. Due to previous research suggesting a possible effect on student achievement, the following preliminary factors were selected for analysis: previous enrollment in the course, high school GPA, student standardized test scores, multimedia learning preferences, year in school, ethnicity, major, and gender. After variable reduction, analysis of covariance (ANCOVA) was used to investigate the effect of the variables identified using the previous methods on posttest assessment scores. One-way ANOVA was conducted to test for effect of class section on posttest score.

Results

Analysis of pretest results show no significant difference ($t(164.75) = 0.19, p = 0.85, \text{Cohen's } d = 0.02$) in baseline scores between treatment groups (Animation: $M = 3.81, SD = 1.92$; Static: $M = 3.76, SD = 2.12$). However, subsequent analysis of posttest scores show a significant treatment effect ($t(153.18) = 4.59, p < 0.001, \text{Cohen's } d = 0.71$). To measure overall achievement, normalized gain scores were calculated using scores

from the photosynthesis pretest at the beginning of the semester and posttest scores collected after the classroom lectures. A comparison of variation in ERs shows that students who viewed dynamic animation as part of a classroom lecture on photosynthesis have higher normalized gain scores on a concept assessment as compared to those who viewed static images (Fig. 2.2). Analysis of the data shows that students who learned with dynamic animations ($M = 0.52$, $SD = 0.50$) performed significantly better ($t(109.92) = 2.73$, $p < 0.01$, *Cohen's d* = 0.52) on the posttest than students who learned with static images ($M = 0.12$, $SD = 1.32$).

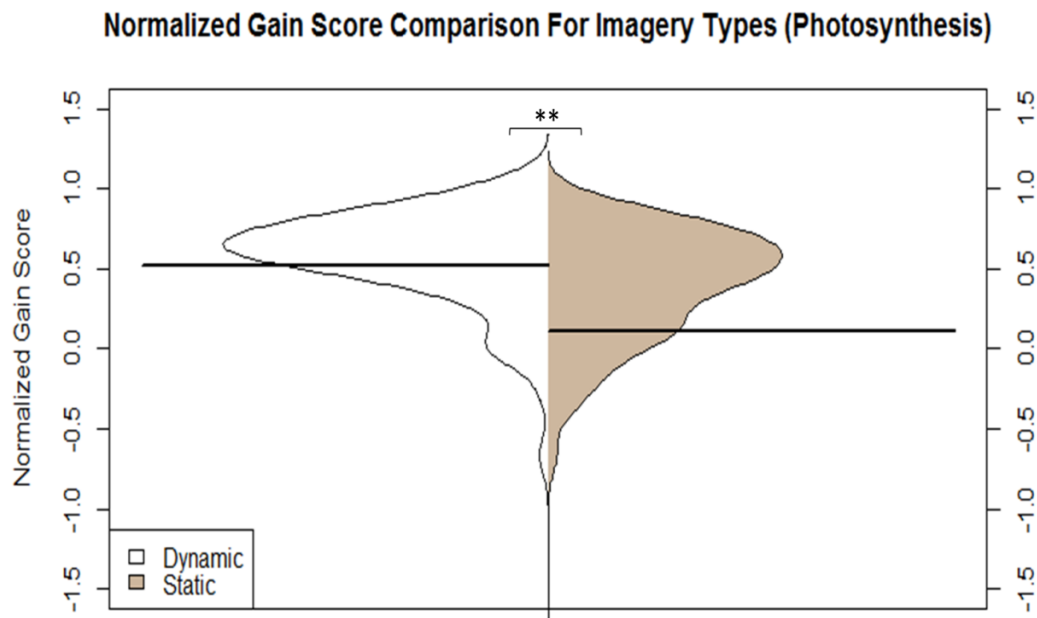


Figure 2.2 – Normalized gain score summary data for comparison of external representations as part of photosynthesis classroom lecture (** $p < 0.01$).

Analysis of selected extraneous variables

Previous studies have pointed out the potential for misinterpretation of normalized gain scores (Theobald & Freeman, 2014), therefore we conducted further

investigation using analysis of covariance to examine possible predictors of posttest assessment scores. In order to select possible extraneous variables, we used principle component analysis followed by factor analysis on demographic variables gathered for each participant. Demographic variables were treated individually based on factors suggesting prior knowledge (previous enrollment in the course and high school GPA), student standardized test scores (total SAT and ACT composite scores), feelings towards multimedia learning (learning preference as defined in methods), and general demographic information (year in school, ethnicity, major, and student gender). Factor analysis identified only SAT composite score as a major contributor to the variability of our sample with a primary loading factor of 0.71. None of the other factors reported primary loading factors above the 0.40 threshold. However, due to previous research results suggesting an effect of gender (O'Day, 2010; Wong et al., 2015) and student learning style (Carlson, 1991; C.-M. Chen & Sun, 2012) on learning outcomes with multimedia we decided to include gender and multimedia learning preference as separate factors in our analysis. Analysis of covariance was then conducted using the possible covariants of pretest score, SAT score, student gender, and multimedia learning preference as possible contributors to posttest score. The analysis showed no significant influence of these possible extraneous variables on posttest scores (Table 2.2).

However, the results indicated there was a significant difference between the static ER ($M = 6.16, SD = 2.11$) and the dynamic ER ($M = 7.50, SD = 1.49$) treatment groups on posttest scores ($F(1, 112) = 10.43, p = 0.002$). Subsequent analysis indicated an effect size of $d = 0.71$.

Table 2.2. - Analysis of Variance Table for Possible Extraneous Variables					
Variable	df	Sum Sq	Mean Sq	F Value	p-Value
Treatment Condition	1	33.45	33.45	10.43	0.002 **
Pretest Score	1	1.22	1.22	0.38	0.53
SAT Composite Score	1	5.28	5.28	1.65	0.20
Student Gender	1	3.69	3.69	1.15	0.29
Multimedia Learning Preference	4	4.90	1.23	0.38	0.82
Residuals	112	359.34	3.21		
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$					

Analysis of data for possible section effect

In order to account for a possible effect of student section we created subsections defined by each treatment condition and conducted one-way analysis of variance on the effects of student section on normalized gain score. Student section showed no significant contribution to normalized gain scores for either the dynamic animation treatment ($F(4, 81) = 0.35, p = 0.84$) or static treatment ($F(4, 76) = 0.69, p = 0.60$).

Discussion

In this study we focused our investigation on the role of educational ERs as part of an introductory biology classroom lecture on photosynthesis. After randomly assigning ten laboratory sections to one of two treatment conditions, we directly compared normalized gain scores for students that were introduced to photosynthesis concepts by one of two treatment conditions: lecture presentations with embedded dynamic photosynthesis animations or lecture presentations with embedded static photosynthesis images. We accounted for factors previously suggested to contribute to

conflicting results in the literature by controlling for instructional style and graphical quality (Tversky et al., 2002). This experimental design element adds strength to the results of our study and aims to clarify prior contradictions in the literature. Comparison of normalized gain scores show greater learning gains by students who viewed a photosynthesis lecture with embedded VCell animations ($t(109.92) = 2.73, p < 0.01, d = 0.52$). These findings suggest instruction incorporating dynamic animations could promote greater student conceptual knowledge on the topic of photosynthesis. With the previously reported prevalence of student misconceptions surrounding the topic (Parker et al., 2012; Södervik et al., 2015), our results could provide instructors confidence in the incorporating VCell animations into their photosynthesis lecture. In addition, this study presents new findings in the oft debated contributions of static and dynamic ERs in the realm of introductory biology education.

While these results suggest the benefits of the inclusion of animations as part of an in-class lecture on photosynthesis, we were concerned that normalized gain score comparison alone may lead to misinterpretation of the data. To account for this we analyzed data using a predictive model for student posttest scores followed by analysis of covariance. Results showed no significant effects of tested extraneous variables. However, they again suggested a treatment effect on student posttest scores ($p = 0.002$), providing further support of the benefits of dynamic VCell animations over static images on the topic of photosynthesis. Interestingly, these methods did not identify significance from one specific factor, prior knowledge, that has been shown to effect learning gains in previous studies (Hegarty & Kriz, 2008; Höffler & Leutner, 2011). In our

study we measured prior knowledge using student scores on the pretest assessment that was administered weeks prior to the experimental instructional treatment. This process allowed us to minimize the risk of a pretest sensation effect and provided confidence that the pretest acted solely as an assessment of prior knowledge (Campbell & Stanley, 2015). It should however be noted that the mean pretest assessment score was very similar for both the dynamic ($M = 3.81$, $SD = 1.92$) and the static ($M = 3.76$, $SD = 2.12$) treatments. As a result, it is plausible that the uniformity of pretest scores across both treatments mitigated any significant effects of prior knowledge in this current study.

One additional factor previously shown to effect outcomes of ER comparisons is that of spatial ability (Höffler & Leutner, 2011). Prior studies have assessed spatial ability levels using a variety of instruments ranging from a box puzzle (Daly, 2012) to a perception test for sense of direction (Hegarty, Richardson, Montello, Lovelace, & Subbiah, 2002). While these instruments have proven effective in previous studies with smaller sample sizes, due to the course design of introductory biology at this institution their implementation in this study was not practical. We do assume that students in our study have varying levels of spatial ability; however we do not have instrumental results to attest to this. Hence, we suggest that future studies investigate the role of spatial ability in learning from VCell animations.

Results of this study bolster the discussion on the benefits of dynamic animations in an otherwise murky literature base. While we did not directly assess

spatial ability in our study, these findings support the previously suggested “cognitive prosthetic” properties of dynamic animations which aids students in the production of accurate mental models throughout the learning process (Höffler & Leutner, 2011). The learning gains seen in this study suggests that conceptual knowledge on the topic of photosynthesis as measured by a posttest may benefit from lectures augmented with dynamic animations. These results could provide insight into lecture based instruction using multiple ER formats.

Limitations and Further Research

We note that these results could be considered somewhat preliminary. This is due to limitations of the study due to the context of how, when, and where the study was conducted. For example, the results of this study represent data from participants in one semester at one institution. In order to make the conclusions of this study more generalizable to the undergraduate population, additional studies at other institutions could be conducted. However, we would encourage researchers to improve the measurement instruments used in this study. Since this study was classroom-based, our instrument was multiple choice and relatively short in length. The brevity of the measure could have been one reason why the reliability of the instrument was found to be relatively low ($\alpha < .60$). Future research could implement item response theory in the construction of the measurement instrument. This would provide greater evidence of the analytical abilities of the instrument while subsequently adding to the empirical evidence on the use of external representation in the introductory biology classroom.

Improving the measure, collecting content and construct validity evidence for the measure, as well as including free response questions as part of the learning outcome tests could provide additional insights as to the impact of learning with dynamic animations compared to static imagery.

Further investigation into the comparison of static and dynamic formats should also include measures on content retention over a period of time. The original design of the study presented here included data from a follow-up assessment given to students one week following the instruction period. However we were unable to utilize a delayed test due to the structure of the course. Future studies on this topic will include a follow-up period of examination and that will analyze student retention on the concepts of photosynthesis.

Finally, we feel that it is important to compare ER formats in the classroom lecture using a variety of different topics within the VCell animation collection. Photosynthesis is a topic that typically requires the interconnection of many smaller factors in order to achieve the goal of conceptual understanding; however this is not the case with all introductory biology concepts. While VCell animations have been developed to help viewers make these smaller connections, not all topics in the collection are equally complex. Further investigation using a variety of different topics could therefore provide insight into which topics provide the most benefit when using different ER formats.

Conclusions

Undergraduate biology instruction often requires the understanding of complex processes which contain many smaller interacting elements. As a result, undergraduate biology is ideal for the investigation of the contributions of different ERs in students' learning. Previous research comparing learning outcomes have found somewhat conflicting results. Despite this, researchers have noted prior knowledge and spatial ability as key contributors to student performance when exposed to different forms of educational ERs. In this study we focused our comparison on static images versus dynamic animations as part of a classroom lecture on the topic of photosynthesis, and accounted for prior knowledge by using pretest scores in the calculation of normalized gain. Using graphics developed for the VCell Animation Collection and following experimental guidelines suggested by Tversky (2002), we centered this study in the introductory biology laboratory classroom. This setting allowed us to control for instruction style while preventing interference in the larger lecture courses. Results of our investigation show that students who were introduced to photosynthesis concepts using dynamic VCell animations as part of the classroom lecture scored significantly higher on a posttest assessment than those who received instruction using static images ($t(109.92) = 2.73, p < 0.01, d = 0.52$). Analysis of covariance using a number of possible extraneous variables shows no significant contribution to student posttest scores by the variables tested. Results did however show a significant effect of treatment condition on posttest score ($p = 0.002$). These results, along with the suggestions for future research provided above, aim to provide insight into possible instructional "best practices" for

those who are teaching with various ER formats as a part of introductory biology instruction on the topic of photosynthesis.

2.2 Continuation of Findings on Photosynthesis Instruction

In order to obtain a cross section of the student enrollment of one entire year at the institution where this study was conducted, we continued the experiments outlined above during the following spring semester. Following the procedure presented previously, we randomly selected four additional introductory biology laboratory sections and assigned them to treatment conditions (2 sections received the dynamic animation treatment and 2 sections received the static image treatment). This provided data on 14 total sections (7 sections per treatment), spanning one full school year, and representing a total of 214 total introductory biology students. Using the same instrument designed to assess understanding of concepts related to photosynthesis as previously reported (Appendix A); we calculated student achievement as normalized gain scores and analyzed the effect of treatment conditions. Results of treatment effect over the course of one full school year, and statistical analysis of possible extraneous contributors to assessment scores are presented below.

Results

Normalized gain scores were calculated across two semesters of study in order to assess student achievement on the topic of photosynthesis (Hake, 1998). Comparison was made between treatment groups where students either viewed dynamic animation ($n = 102$) or static images ($n = 112$) as part of a classroom lecture on the topic of

photosynthesis. Analysis shows that students who learned with dynamic animations ($M = 0.49$, $SD = 0.50$) had significantly higher learning gains ($t(153.4) = 2.90$, $p < 0.01$, $d = 0.40$) than students who learned with static images ($M = 0.13$, $SD = 1.17$) (Fig. 2.3/Table 2.3).

Analysis of Selected Extraneous Variables

Further investigation was conducted using analysis of covariance to examine for possible extraneous contributors to student posttest scores. Identical variables were tested as before and were selected based on reports suggesting their possible contribution to learning with multimedia resources (Bray, 2007; L. ChanLin, 2001; Ching, Basham, & Jang, 2005; Hannon, 2014; Höffler, 2010). Demographic variables were based on factors suggesting prior knowledge (previous enrollment in the course and pretest score), student standardized test scores (total SAT and ACT composite scores), feelings towards multimedia learning (learning preference as defined in methods), and general demographic information (year in school and student gender and ethnicity). Student year in school was classified as either underclassman (freshman/sophomore) or upperclassman (junior/senior). Additionally, student ethnicity was classified as either white or underrepresented minority (URM). Analysis of covariance shows no significant contribution of any of the extraneous variables tested (Table 2.4). However, the results did again show a significant influence of treatment group on assessment scores ($F(1, 92) = 12.84$, $p < 0.001$).

Normalized Gain Score Comparison For Imagery Types (Photosynthesis)

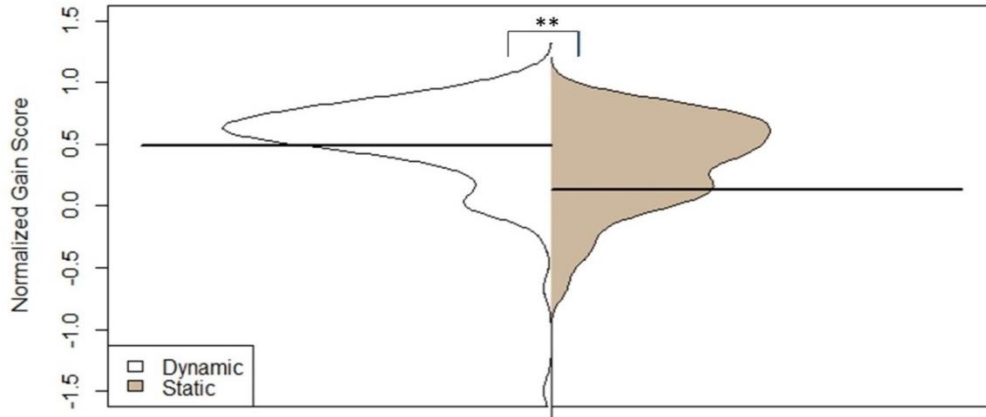


Figure 2.3 – Normalized score comparison based on imagery types on the topic of photosynthesis (** $p < 0.01$).

Table 2.3 - Descriptive Statistics for Photosynthesis Instruction		
Normalized Gain Score		
	Dynamic	Static
Min	-3.00	-8.00
1st Quart	0.41	0.13
Median	0.60	0.40
Mean	0.49	0.13
3rd Quart	0.74	0.63
Max	1.00	0.89
Std. Dev.	0.50	1.17
95 % CI	0.11 < μ < 0.59	

Table 2.4 - Analysis of Covariance Table for Possible Extraneous Variables (Photosynthesis)					
Variable	df	Sum Sq	Mean Sq	F Value	p-Value
Treatment Condition	1	41.67	41.67	12.84	< 0.001 ***
Pretest Score	1	0.04	0.04	0.01	0.92
Previously Enrollment	1	1.17	1.17	0.36	0.55
Total SAT Score	1	1.70	1.70	0.52	0.47
ACT Composite Score	1	2.34	2.34	0.72	0.40
Multimedia Learning Preference	4	2.33	0.58	0.18	0.95
Year in School	1	1.27	1.27	0.39	0.53
Student Gender	1	3.87	3.87	1.19	0.28
Student Ethnicity	1	5.98	5.98	1.84	0.18
Residuals	92	298.51	3.25		
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$					

Analysis of data for possible section effect

To account for a possible effect of student section, subsections were created defined by treatment condition and one-way analysis of variance was conducted on the effects of student section on normalized gain score. Student section showed no significant contribution to normalized gain scores for neither the dynamic animation

treatment group ($F(6, 95) = 1.22, p = 0.30$) nor the static image treatment group ($F(6, 105) = 0.33, p = 0.92$).

2.3 Extensions of Research (Mitosis Instruction)

As a continuation of the investigation into the use of external representations in the classroom lecture, we further attempted to answer the research question for aim one by conducting addition study on the topic of mitosis. Similar to photosynthesis (Parker et al., 2012; Södervik et al., 2015), the concept of mitosis has been noted as a common source of misconceptions in introductory biology students (Ozcan et al., 2012). Among the misconceptions noted by Ozcan (2012) are the confusion of the role of interphase in the cell cycle, an inability to denote the type of cells where mitosis occurs and an inability to track chromosome count and actions throughout the stages of mitosis. While it is common that introductory biology students have previously been exposed to a basic introduction on the topic of mitosis and the cell cycle, their understanding of the topic does not typically persist into their undergraduate studies (Dikmenli, 2010; Ozcan et al., 2012). To account for this, we target the second stage of our investigation on using various forms of external representations in a classroom lecture on the topic of mitosis. Like many biological concepts, the stages involved in the cell cycle and mitosis occur in a series of stepwise processes that have often been shown to benefit from dynamic moving images (McClellan et al., 2005; McElhaney, Chang, Chiu, & Linn, 2015; O'Day, 2010). As a result, we hypothesize that students who attend a lecture on mitosis that incorporates dynamic animations from the Virtual Cell

Animation Collection will show higher learning gains than students who attend a lecture on mitosis that only incorporates static non-moving images. As a part of this study, we focus on instruction solely at the level of the in-class environment. Results from these additional experiments on the use of dynamic instruction in the classroom will provide further evidence on possible best practices of in-class instruction using dynamic animations. Together with the results on previous work on the topic of photosynthesis, we aim to answer the question “how does learning with dynamic animations as part of a classroom lecture influence students’ conceptual understanding in introductory biology as compared to learning with static graphics?”

Methods

Methods and participants of this additional stage of investigation into the use of external representations in the classroom are similar to those previously described in this chapter. We do, however, outline the specifics of this extension below.

Participants and Treatment Groups

Over the course of two semesters, participants in this study self-enrolled in one of 51 sections of an introductory biology laboratory course. Sixteen total sections were randomly selected to participate in this investigation; ten from the fall semester and six from the spring semester. Eight sections ($n = 133$), five from the fall and three from the spring, were randomly assigned to receive instruction using treatment one and eight sections ($n = 122$), five from the fall and three from the spring, were assigned to receive instruction using treatment two. Treatment one introduced students to the basic

concepts of the mitosis as part of a lecture presentation using an animation from the Virtual Cell Animation Collection to augment the lecture. Treatment two introduced mitosis in a lecture presentation using static images (labeled “advanced look” on the Virtual Cell website) as a series of figures to denote a multi-step process as part of instruction. The same instructor presented the lecture to all the groups in the study, and their presentation slides were identical except for the type of external representation used.

Assessment Instruments

Conceptual understanding on the topic of mitosis was assessed using instruments developed by the research team in this study (Appendix C). The diagnostic pretest consisted of ten filler questions not used in the analysis of this study, ten questions used as a test of prior knowledge focused on basic understanding of mitosis concepts ($\alpha = 0.53$), and five questions addressing student background information. As a part of this background information we included the following question with a five-point Likert scale used to gather information on students’ feelings toward learning with multimedia resources: “ I learn best when information is presented in a visually stimulating (ie: animations/video) fashion”.

The posttest assessment instrument was comprised of ten questions ($\alpha = 0.53$) using the identical mitosis questions from the pretest. Questions were again selected from two commonly used Biology textbooks (Brooker et al., 2017; Reece et al., 2014) and were modified to fit the level of the course in this study. Weighted Bloom’s Index

was calculated and found to be 40.00, suggesting a lower to mid of cognitive skill level (Freeman et al., 2011a).

We again accounted for differences in instructional style by recording all lectures and assigning two randomly selected lectures (one from each treatment group) to three separate reviewers for assessment of conceptual introduction. Reviewers were asked to view both recorded lectures and note, in a yes/no fashion, if five listed mitosis concepts were covered similarly between both treatments (Appendix D). Reviewer ratings suggest that concepts were presented similarly in both treatments, and the interrater reliability coefficient showed that reviewers rated the presentations consistently between coders (Fleiss' $K = 0.99$).

Experimental Procedures

Experimental procedures were similar to those used as part of the photosynthesis manuscript presented previously in this chapter. At the beginning of the semester, students enrolled in this study were given the diagnostic pretest assessing baseline conceptual understanding on the topic of mitosis. At the appropriate point in the semester, students were introduced to the topic of mitosis using the experimental treatments outlined below (Fig. 2.4). To account for incomparable instructional procedures (Tversky et al., 2002), the same instructor was used across all class sections and an external instructor assessment for comparable lectures across all treatments. Inequivalent graphics (Tversky et al., 2002) were accounted for by using static images that were developed in the production of the mitosis animations used in treatment one.

Following instruction, students were assigned the ten-question posttest assessment instrument designed to examine student understanding on the topic of mitosis.

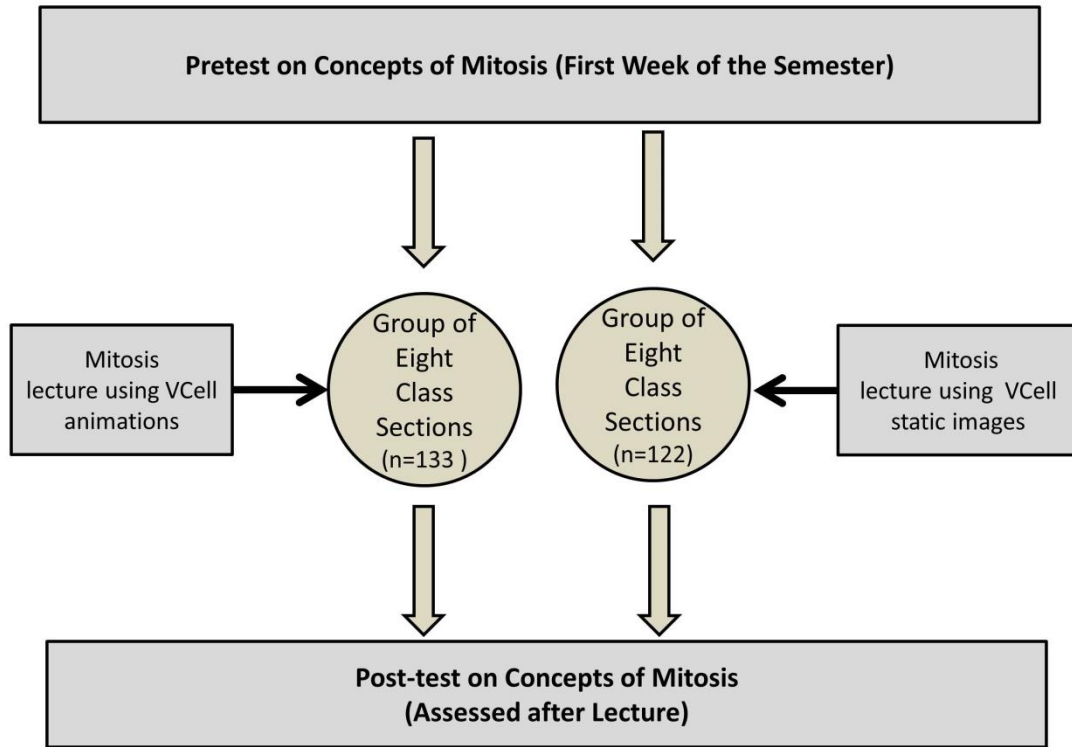


Figure 2.4- Experimental design outline for aim two of our study.

Statistical Analysis

For the identified aspects of student performance, descriptive statistics were compiled and inferential analysis conducted comparing treatment groups using the R statistical programming package. Normalized gain score was calculated from assessment results to provide an analysis of student learning (Hake, 1998). *P*-values were obtained using independent *t*-test across treatment groups and 95% confidence intervals for

improvement differences between treatments were calculated. Cohen's d was used to describe the magnitude of the difference between group means. Analysis of covariance was used to investigate the effect of possible explanatory variables on student assessment score.

Results

Normalized gain scores were calculated from pretest and posttest scores in order to assess student achievement on the topic of mitosis. Comparison of treatment conditions were made between students who either viewed dynamic animation or static images as part of an in-class lecture on the topic of mitosis. Analysis shows that students who learned with dynamic animations ($M = 0.60, SD = 0.49$) had significantly higher learning gains ($t(229.23) = 4.71, p < 0.001, d = 0.59$) than students who learned with static images ($M = 0.27, SD = 0.62$)(Fig. 2.5/Table2.5).

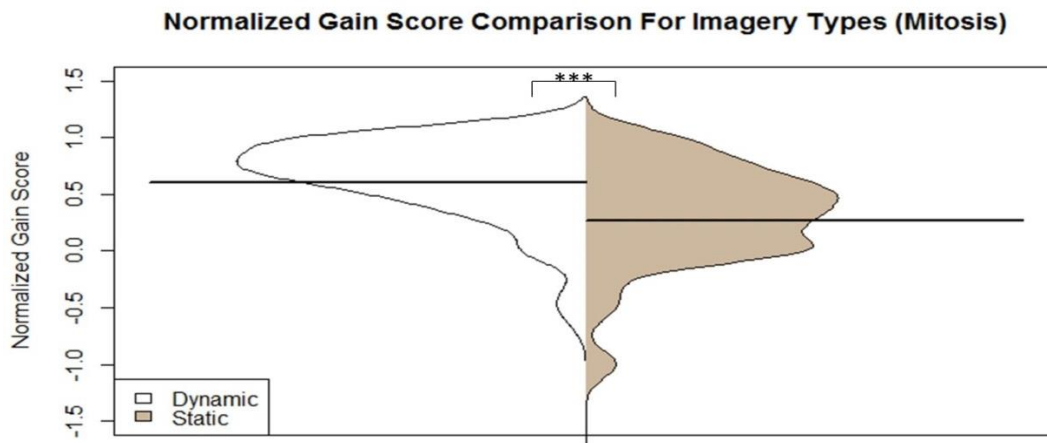


Figure 2.5 - Normalized score comparison based on imagery types on the topic of mitosis (** $p < 0.001$).

Table 2.5 - Descriptive Statistics for Mitosis Instruction		
Normalized Gain Score		
	Dynamic	Static
Min	-3.00	-4.00
1st Quart	0.50	0.00
Median	0.72	0.33
Mean	0.60	0.27
3rd Quart	0.88	0.60
Max	1.00	1.00
Std. Dev.	0.49	0.62
95 % CI	0.19 < μ < 0.47	

Analysis of Selected Extraneous Variables

Identical extraneous variables were selected for analysis on the topic of mitosis and were based on the same criteria outlined previously in this chapter. Student year in school was again classified as either underclassman (freshman/sophomore) or upperclassman (junior/senior). Additionally, student ethnicity was classified as either white or underrepresented minority (URM). Analysis of covariance shows only a contribution of SAT composite score to posttest assessment ($F(1, 103) = 8.39, p < 0.01$)

from the extraneous variables tested (Table 2.6). In addition, the results did again show a significant influence of treatment group on assessment scores ($F(1, 103) = 13.28, p < 0.001$).

Table 2.6 - Analysis of Covariance Table for Possible Extraneous Variables (Mitosis)					
Variable	df	Sum Sq	Mean Sq	F Value	p-Value
Treatment Condition	1	23.56	23.56	13.28	< 0.001 ***
Pretest Score	1	2.74	2.74	1.55	0.22
Previously Enrollment	1	2.02	2.02	1.14	0.29
Total SAT Score	1	14.88	14.88	8.39	0.004 **
ACT Composite Score	1	1.34	1.34	0.76	0.39
Multimedia Learning Preference	4	16.28	4.07	2.29	0.06
Year in School	1	1.82	1.82	1.03	0.31
Student Gender	1	1.82	1.82	1.02	0.31
Student Ethnicity	1	0.01	0.01	0.00	0.95
Residuals	103	182.68	1.77		

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Analysis of data for possible section effect

To account for a possible effect of student section, subsections were again created defined by treatment condition and one-way analysis of variance was

conducted for the effects of student section on normalized gain score. Student section showed no significant contribution to normalized gain score for the dynamic animation treatment group ($F(7, 125) = 1.87, p = 0.08$). The static image treatment group however did show an effect based on section of enrollment ($F(7, 114) = 3.64, p = 0.001$).

2.4 Discussion of Learning with Virtual Cell Animations as Part of In-class Instruction

The current literature on the use of external representations as part of instruction in the STEM sciences has not provided a clear conclusion as to which form of imagery is most beneficial (Ardac & Akaygun, 2005; Tversky et al., 2002). In an attempt to add to the somewhat murky literature and provide empirical evidence of the possible advantages of implementation of dynamic animations as part of the classroom lecture, we focused on two introductory biology concepts that have been previously shown to be a common source of misconception; photosynthesis (Södervik et al., 2015) and mitosis (Ozcan et al., 2012). In addition, previously outlined confounding aspects of experimental design (Tversky et al., 2002) were addressed by focusing on one specific collection of molecular animations and their corresponding static images (Reindl et al., 2015). Results spanning one full school year (two semesters), show that students who were presented content with classroom lectures that were augmented with dynamic animations showed higher normalized gain scores for both the topic of photosynthesis ($t(153.4) = 2.90, p < 0.01, d = 0.40$), and mitosis ($t(229.23) = 4.71, p < 0.001, d = 0.59$) as compared to those who were presented content implemented with only static graphics. Misconceptions relating to both photosynthesis and mitosis show that students may

have difficulty interpreting the step-wise processes that often entail the tested concepts (Ozcan et al., 2012; Parker et al., 2012; Södervik et al., 2015). Dynamic animations have been previously shown to help students form more accurate mental models of when they are used to present such difficult sequential processes (O'Day, 2010; Williamson & Abraham, 1995). Using images created as part of the Virtual Cell Animation Collection, we show that dynamic animations can aide in the conceptual understanding of the topics of photosynthesis and mitosis in introductory biology students. The formation of accurate base representations of these introductory concepts could provide learners with a stronger foundation on which to build their knowledge while matriculating through the undergraduate program of study. With such stronger foundations, students could be better equipped to further their understanding of more difficult upper-level concepts (Brewer & Smith, 2011). It is therefore logical that deeper understanding based on firm conceptual foundations could increase student retention rates in STEM majors, as is represented by GPA (DeBerard, Spielmans, & Julka, 2004), and likewise produce a more knowledgeable workforce upon graduation. Both of which answer the calls for reform in undergraduate science education outlined in recent reports (Brewer & Smith, 2011; Steve Olson & Riordan, 2012).

In addition to a simple treatment effect on learning outcomes, we also use both experimental and statistical means to show that the change in achievement was independent of other possible extraneous variables. Experimentally, we acknowledge the possible confounding that can arise with use of a pseudo-experimental design.

However, we attempted to control as many of these factors as possible by randomizing

sections to treatment and controlling for instructional influence throughout the experiment. Despite these attempts, we do recognize that the effects of this pseudo-experimental design are still evident, as is shown by a section effect in one branch of the mitosis experiment. Statistically, we attempted to control for extraneous variable using analysis of covariance focused on extraneous contributors that have been previously tied to multimedia learning (L. ChanLin, 2001; Hegarty & Kriz, 2008; Ruiz-Primo et al., 2011; Wong et al., 2015). Analysis shows a significant effect of only total SAT scores on student posttest score that is seen only in the mitosis aspect of this series of experiments. While SAT has previously been suggested to be a predictor of undergraduate academic success (Hannon, 2014), we feel that this correlation with achievement in our experiments may speak more to a student's cognitive ability. Student who have greater achievement on standardized tests have been shown to exhibit a higher level cognitive processing (Frey & Detterman, 2004). Likewise, cognitive ability has previously been shown influence interactions with multimedia resources (Ayres & Paas, 2007). This influence may be more prevalent on a more familiar concept, such as mitosis, than it would be on a relatively unfamiliar one, such as photosynthesis. However, we do note that not all students enrolled in introductory biology enter their undergraduate institution with SAT scores. Additionally, the other common college entrance examination, ACT composite score, did not show a significant effect in our study. As a result, we acknowledge that SAT may play a role in the ability for students to learn certain introductory biology concepts; however we feel that these effects are likely small and are probably associated with student cognitive processing level.

Limitations

Future investigations into the comparison of static and dynamic formats could add an aspect of content retention over a period of time. Previous studies have suggested that interaction with course materials using dynamic animations could increase concept retention over time (O'day, 2007). While a retention aspect was outside of the realm of this study, it would be interesting to see the effects of different graphical formats on concept retention in the future.

While the sample size and scale of these experiments provides a more realistic view of the undergraduate population at the university of this study, a smaller, completely randomized study may provide additional insight into the results presented here. Such a design could allow for collection of qualitative data on the use of different graphical formats and provide understanding on why one type may be more beneficial than the next. Such qualitative data could aid in the development process of future multimedia resources and lead to additional insight in to best practices of implementation.

2.5 Conclusions on Learning with Virtual Cell Animations as Part of In-class Instruction

In response to inconclusive literature on the effects of dynamic animation as part of introductory biology instruction we aimed to answer the question, “how does learning with dynamic animations as part of a classroom lecture influence students’ conceptual understanding in introductory biology as compared to learning with static graphics?” Using animations developed according to research-supported guidelines of

design (McClellan et al., 2005; Reindl et al., 2015), and an experimental design aimed at eliminating confounding elements of instruction (Tversky et al., 2002) we investigated animations on two topics that have previously been associated with misconceptions in introductory biology students; photosynthesis and mitosis. Results of our study span one full academic year of instruction and provide a representative sample of the introductory biology students at our institution. Results suggest that in regards to instruction on both photosynthesis ($p < 0.01$) and mitosis ($p < 0.001$), students who were introduced to concepts with a lecture incorporating dynamic animations showed significantly higher achievement than those who were presented with instruction using only static graphics. Statistical control of possible extraneous variables shows only total SAT score as a possible contributor to student posttest scores. Results of this study provide empirical evidence that the use of dynamic animations to convey difficult introductory biology concepts can provide students with greater understanding of specific topics which may provide a stronger knowledge base to build upon as part of their undergraduate studies.

Chapter 3

Virtual Cell Animations as Part of Instruction Outside of the Classroom

Calls for reform in STEM education have focused on the interaction of students with course content both inside and outside of the classroom (Brewer & Smith, 2011; Steve Olson & Riordan, 2012). Traditionally, these interactions have focused on the use of various homework assignments that typically take place after instruction in a classroom setting (Gieger, Nardo, Schmeichel, & Zinner, 2014; Malik, Martinez, Romero, Schubel, & Janowicz, 2014; Planchard, Daniel, Maroo, Mishra, & McLean, 2015). Such reinforcement strategies have been shown to positively influence students' achievement (Anliker, Aydt, Kellams, & Rothlisberger, 1997; Demirci, 2010), however their motivation to complete these assignments can vary greatly (Planchard et al., 2015). In addition to reinforcement activities, the recent push for the adoption of “flipped” classroom environments has also placed an emphasis on the interaction of students with content prior to classroom learning as a means of preparation (K. L. Anderson, 2016; DeLozier & Rhodes, 2016; Eichler & Peebles, 2016; Persky, 2015). These preparatory activities have also been shown to be integral to a classroom environment centered on active learning (Gross et al., 2015), and instructors have designed a number

Note: section 3.1 has been adapted from Goff, E., Reindl, K., Johnson, C., McClean, P., Offerdahl, E., Schroeder, N., White, A. (2017, in press). Learning about Concentration Gradients and ATP Synthase with Animations Outside of the Classroom. *Journal of Microbiology and Biology Education*.

of assignments that aim to provide proper preparation for such activities (Eichler & Peebles, 2016; Lineweaver, 2010; Persky, 2015). However, reports have shown that the adoption of these instructional formats are less prevalent amongst STEM educators than that of other fields (Eagan et al., 2014), and implementation has not been successful in all classrooms (Andrews et al., 2011). With variation in both the efficacy and fashion by which students are interacting with course content outside of the classroom, investigation into this variability becomes imperative. In this study, we investigate the student/content interactions that are implemented either as preparation for classroom instruction or reinforcement following instruction in the physical classroom.

With the implementation of flipped classroom environments still in its infancy amongst STEM educators (Andrews et al., 2011; Eagan et al., 2014), we focus this study on introductory biology courses where a traditional lecture style is still the norm. The institution where this study was conducted is no exception to this, as the majority of the biology instructors still use a traditional lecture-centered content deliver style. Using this uniform traditional delivery style as the basis on in-class content interaction allows us to focus our study on the interactions outside of the classroom. We concentrate on these interactions to answer the research question, “how does learning differ when students view animations before instruction as preparation or after instruction as conceptual reinforcement when compared to a no intervention group?”

To facilitate student/content interaction, we focus on the use of multimedia resources developed as part of the Virtual Cell Animation Collection. Multimedia has

become an increasingly prevalent aspect of education (Asthana, 2008; Heyden, 2004), and the development of reliable resources for use in the classroom has subsequently grown in importance with this rise (O'day, 2006; O'Day, 2010). In addition, multimedia resources have been repeatedly used as both student preparation and concept reinforcement in many undergraduate courses (Malik et al., 2014; Persky, 2015; Phillips, 2015; Rhodes et al., 2014). As a result, we focus on the use of dynamic animations to supplement outside of the classroom assignments. This concentration will provide empirical evidence for the use of animations as part of introductory biology instruction, and give insight into which format of outside of the classroom student/content interaction is best. Due to the number of recent reports suggesting the benefits of multimedia resources as preparation in a flipped classroom (Persky, 2015; Pierce & Fox, 2012), we hypothesize that students who view animations as preparation will show higher learning gains than both those that view animations as reinforcement and a non-treatment control. In order to examine this, we investigate the use of animations in three topics: concentration gradients as they relate to ATP synthase activity, mRNA processing, and translation. These topics comprise components of the introductory concept of cellular respiration and the central dogma of molecular biology. Both of these topics represent common sources of misconception amongst introductory biology students (Capa, Yildirim, & Ozden, 2001; Shapiro, 2009; M. K. Smith, Wood, & Knight, 2008; Songer & Mintzes, 1994), and knowledge into the learning of these concepts will provide building blocks on which to correct these misconceptions. Results of the investigation into these aspects of learning are outlined below.

3.1 Learning about Chemiosmosis and ATP Synthesis with Animations Outside of the Classroom

Abstract

Many undergraduate biology courses have begun to implement instructional strategies aimed at increasing student interaction with course material outside of the classroom. Two examples of such practices are introducing students to concepts as preparation prior to instruction, and as conceptual reinforcement after the instructional period. Using a three group design, we investigate the impact of an animation developed as part of the Virtual Cell Animation Collection on the topic of concentration gradients and their role in the actions of ATP synthase as a means of pre-class preparation or post-class reinforcement compared to a no-intervention control group. Results from seven sections of introductory biology ($n = 732$) randomized to treatments over two semesters show that students who viewed animation as preparation ($d = 0.44$, $p < 0.001$) or as reinforcement ($d = 0.53$, $p < 0.001$) both outperformed students in the control group on a follow-up assessment. Direct comparison of the preparation and reinforcement treatments shows no significant difference in student outcomes between the two treatment groups ($p = 0.87$). Results suggest that while student interaction with animations on the topic of concentration gradients outside of the classroom may lead to greater learning outcomes than the control group, in the traditional lecture-based course the timing of such interactions may not be as important.

Introduction

Recent calls for reform in STEM education cite the need for increased student interaction with course content both inside and outside of the classroom (Brewer & Smith, 2011; Steve Olson & Riordan, 2012). Traditionally, lecture-centered instruction has accounted for content delivery in many large-enrollment undergraduate classrooms. These student/content interactions inside the classroom have more recently become focused on strategies such as active learning and inclusion of authentic research in undergraduate laboratory environments (Allen & Tanner, 2005; Freeman et al., 2007; Spell et al., 2014). Research around such strategies has noted their benefits on a number of different occasions (Aronson & Silveira, 2009; Sara E. Brownell et al., 2012; Freeman et al., 2014), however their levels of adoption can fluctuate across educational settings (Davis, 2003; White et al., 2014).

To date, there has been little research to determine the most effective way to engage students with the instructional material outside of the formal classroom setting. Instructional strategies designed to promote interaction outside of the classroom can vary widely depending on instructors' pedagogical practices, course subject, and course level. Despite this, these interactions outside of the classroom have been widely shown to promote greater learning outcomes (10, 48). Examples of such successful engagement strategies include textbook reading assignments (French et al., 2015), worksheets (Lee, 2014), viewing of animations and videos (Long, Logan, & Waugh, 2016), online modules (Hill, Sharma, & Johnston, 2015), and instructor mediated blogs

(Ferdig & Trammell, 2004). These methods can be characterized broadly into two main categories: methods for pre-class preparation, and methods for post-class concept reinforcement.

The purpose of this study is to compare these two distinctly different categories of student interaction outside of the classroom, as well as compare both of these strategies to a no-intervention control. Recent innovations in the development of online instructional resources have provided students a platform where they can interact with course material on their own time and in their own environment. Hence, we investigate the use of such multimedia resources in support of learning in introductory biology.

Review of the Literature

Student preparation has long been a key aspect of undergraduate instruction. Traditionally, preparation strategies have required students to read material in the textbook prior to attending class (Aagaard, Conner, & Skidmore, 2014; French et al., 2015). While these reading assignments have been shown to be promote student preparation, motivation to complete such activities can fluctuate (Aagaard et al., 2014; Hodges et al., 2015; Persky, 2015). Research conducted by Gross, et al. (Gross et al., 2015) supports the role of preparation by noting that students who interacted with content prior to class performed 12% higher on follow-up exams than students who did not. To capitalize on outcomes such as these, various methods to promote student motivation and completion of these preparatory activities have been developed. Examples include the use of reading quizzes (Hodges et al., 2015), online learning

modules (Hill et al., 2015), and monitored discussion groups (Lineweaver, 2010). While classroom instructional styles following the preparation assignments can vary, the learning outcomes appear to be positive. This places a possible emphasis on pre-class preparation in the learning process in many introductory biology students.

Not unlike preparation, post-class concept reinforcement has also been used to increase conceptual understanding (Wieman & Arbaugh, 2014). Reinforcement assignments can vary in their specific format, and can be associated with a grade or simply left to the discretion of the student (Gieger et al., 2014; Hauk, Powers, & Segalla, 2015; Malik et al., 2014; Tas, Sungur-Vural, & Öztekin, 2014). While reinforcement assignments have been shown to increase exam scores in numerous studies (Bowman, Gulacar, & King, 2014; Malik et al., 2014; Planchard et al., 2015; Santoro & Bilisoly, 2015), motivation to complete such assignments has again been shown to vary (Planchard et al., 2015). One method that instructors have implemented as a means of tracking the progress of such reinforcement assignments and hopefully increase student participation is the use of web-based multimedia learning resources (Bowman et al., 2014; Hauk et al., 2015; Lazarova, 2015; Malik et al., 2014). Recent studies have shown that in many situations these online, computer-based assignments lead to higher achievement on concept assessments than a more traditional paper-based format (Feng, Roschelle, Heffernan, Fairman, & Murphy, 2014; Hauk et al., 2015; Shaw, 2015). These online resources are typically available to students at their convenience and may provide them a sense of technological familiarity that could motivate their completion. The rising popularity of multimedia resources in the undergraduate classroom makes

investigation into their development and implementation an important emerging aspect of education research. Therefore, we focus on the use of one of these multimedia resources, animation, in our investigation into learning outside of the classroom.

The field of biology is particularly well adapted to the use of multimedia resources as many biological processes are suggested to be more effectively depicted using animations than their static counterparts (Boucheix & Schneider, 2009; Höffler & Leutner, 2011; Yarden & Yarden, 2010). As a means of conceptual introduction, dynamic animations have been shown to provide students accurate depictions of biological concepts in a way that allows them to make connections that could ultimately lead to greater understanding (Katsioloudis et al., 2015; Lowe, 2003; Yarden & Yarden, 2010). With proper concept introduction prior to class being such an important aspect to some learning environments (Gross et al., 2015), animation could contribute to the preparation process. Likewise, with reports of the efficacy of online multimedia as a means of reinforcement assignments (Bowman et al., 2014; Hauk et al., 2015; Lazarova, 2015), the integration of animation as reinforcement given after class could promote learning in introductory biology students.

The research presented here investigates the learning outcomes of students introduced to the topic of concentration gradients and their role in ATP synthase activity. These topics constitute key components of the mechanisms involved in cellular respiration, and are typically presented as part of introductory biology instruction. Misconceptions concerning cellular respiration and its many components have been

shown to be prevalent in many introductory biology students (Driver & Bell, 1986; Songer & Mintzes, 1994). Furthermore, these misconceptions have been shown to be persist even after repeated instruction and advancement through the biology curriculum (Alparslan, Tekkaya, & Geban, 2003; Mann & Treagust, 1998; Seymour & Longden, 1991). With the ever evolving field of cellular and molecular biology, such misconceptions could prove detrimental to the learning process of students attempting to form foundational mental models in introductory biology (McDermott, 1991; Wright et al., 2014). Results of this study aim to provide empirical evidence of how different methods of student engagement with the material outside of the classroom can affect learning gains.

Research Question

While the benefits of both preparation and reinforcement have been individually researched, a deeper understanding as to which instructional strategy is more effective in a traditional classroom is needed. Here we conduct an investigation into the comparison of these two strategies as a means of increasing student engagement with material in undergraduate introductory biology. As part of a three group design, we also look at the contribution of both preparation and reinforcement as compared to a control group that received neither treatment.

The research question guiding our study was, “How does learning about concentration gradients and ATP synthase differ when students view animations before or after instruction compared to a no-intervention group?” Previous research has

supported the introduction of course material prior to classroom instruction (Gross et al., 2015; Lineweaver, 2010). However, it has also been noted that these benefits may be a result of instructional practices in the classroom and not the preparation assignments themselves (Jensen, Kummer, & Godoy, 2015). With the reported fluctuation in effectiveness of in-class instructional strategies (Andrews et al., 2011), this could suggest that the role of preparatory activities could vary drastically between courses. By contrast, reinforcement assignments following classroom instruction have consistently lead to higher achievement when students complete them as compared to when they do not (Anliker et al., 1997; Bowman et al., 2014; Planchard et al., 2015). Constructivist theory (Driver & Bell, 1986) might suggest that in regards to a traditional lecture-centered classroom, reinforcement assignments could facilitate the “concept application phase” of learning where students apply previously learned material to new content related problems. In regards to this study and the use of animation as a means of reinforcement, this could apply to the accurate formation of mental representations of scientific mechanisms. In addition, animations could also act as a metacognitive organization strategy that could lead students to greater understanding (Dunlosky, Rawson, Marsh, Nathan, & Willingham, 2013). Based on these theories, we hypothesize that students who view animations as reinforcement of instruction on topics related to concentration gradients and ATP synthase will outperform those who view animations as preparation for class instruction on an assessment focused on the presented concepts. The findings of this research will provide insight into instructional “best practices” in regards to the use of animation as introduction and reinforcement of

introductory cellular respiration concepts. Understanding of the best timing for implementation of animated instructional resources could provide instructors with guidance on strategies that encourage the highest learning gains in introductory biology students.

Materials and Methods

Participants and Treatment Groups

Participants ($n = 732$) were enrolled in the introductory biology course at a large public university in the southeast United States during either the fall or spring semester and all research was conducted in accordance to IRB protocol # 0004606. In this quasi-experimental study, sections were randomly assigned to one of three treatments. The “preparation” group ($n = 133$) consisted of two class sections (one fall and one spring) that viewed an animation developed as part of the Virtual Cell Animation Collection on concepts related to concentration gradients and ATP synthase prior to attending a lecture-centered class session on the topic. The “reinforcement” group ($n = 316$) consisted of three class sections (two fall and one spring) that viewed the same animation as a means of reinforcement after they attended a classroom lecture on the topic. The “control” group ($n = 283$) consisted of two class sections (one fall and one spring) that only attended a classroom lecture on concentration gradients and ATP synthase. This group did not view the animation on the topic neither prior to nor following instruction. All course instructors ($n = 5$) were determined to have similar instructional styles and content delivery strategies. Multiple observations of each

instructor revealed that all instructors dedicated ~75% of class time to lecture augmented with ~25% of class time devoted to other interactive techniques (ie clicker questions, think-pair-share, etc.). Two of the instructors taught more than one section in this study; however, to control for possible instructor bias, their treatment group varied between sections. Variation in treatment group size was due to uncontrollable variability in student enrollment between course sections. Such variation in course section size is common at this university and instructors typically do not vary teaching strategies between sections as a result of their enrollment numbers.

Assessment and Measures

The assessment used to obtain information on student conceptual understanding was a ten-question instrument ($\alpha = 0.66$) constructed using questions selected from two commonly used Biology textbooks that were slightly modified to fit the level of the course in this study (Appendix E). Length of the instrument was designed to remain short so as to prevent interfering with the course syllabus while maximizing participation among students. Modifications to make questions more appropriate for the introductory level consisted of removing confusing phrasing and images that were more representative of upper-level biology course concepts.

Assessment questions were categorized by the authors according to Bloom's taxonomy as requiring either lower-order cognitive skills (LOCS), comprised of questions from knowledge, comprehension or logic Bloom's levels, or higher-order cognitive skills (HOCS), comprised of questions from analysis, synthesis or evaluation Bloom's levels

(Crowe, Dirks, & Wenderoth, 2008). Six of the questions were determined to require lower-order cognitive skills, while four of the questions were determined to require higher-order cognitive skills suggesting an overall low to middle-order of cognitive skill level.

In order to obtain background information concerning student preference for multimedia learning, we included the following question with a five-point Likert scale (1 = Strongly Agree; 5 = Strongly Disagree) used to gather information on students' feelings toward learning with multimedia resources: "I learn best when information is presented in a visually stimulating (ie: animations/video) fashion".

Student demographic information was obtained from the University registrar and matched to student performance on the aforementioned assessment. Student identifier data was removed from the dataset.

Instructional Animation

The instructional animation used in this study was entitled "ATP Synthase (Gradients)" which is a part of the Virtual Cell Animation Collection (NSF awards: 0086142, 0618766, and 0918955). This set of multimedia resources was developed using the research-based principles of multimedia design (Mayer, 2009; Mayer & Moreno, 2002), and are free to use for both instructors and students. The Virtual Cell Animation Collection currently consists of 24 animations available for either streaming or downloading in multiple formats from the project's website (<http://vcell.ndsu.edu/animations/>).

Experimental Procedures

Considering their introductory status, students were all assumed to have had a similar basic introduction to cellular respiration and its components as part of their high school instruction. A sampling of secondary science standards notes that this includes a basic knowledge of concentration gradients, with little application as associated with cellular respiration. At the appropriate point on the instructional calendar, students were introduced to the topic of biological gradients and their role in the functions of the ATP synthase molecule using the experimental treatments outlined below (Fig. 3.1). All sections were conducted similarly in a traditional, lecture-centered style. Due to the quasi-experimental design of this study and the fact that students' participated outside of class, we minimized potential confounding variables when possible. For example, student participation in the viewing of animations was monitored and those who did not fully complete all assignments were excluded from the research results. All animations were uploaded to the Blackboard LMS page for the course and student participation with the content was tracked using the statistical features of the Blackboard software package. Course structure did not allow for pretesting of students in this study, however, following instruction they completed a ten-question assessment instrument (Appendix E) designed to examine student knowledge on the given topic.

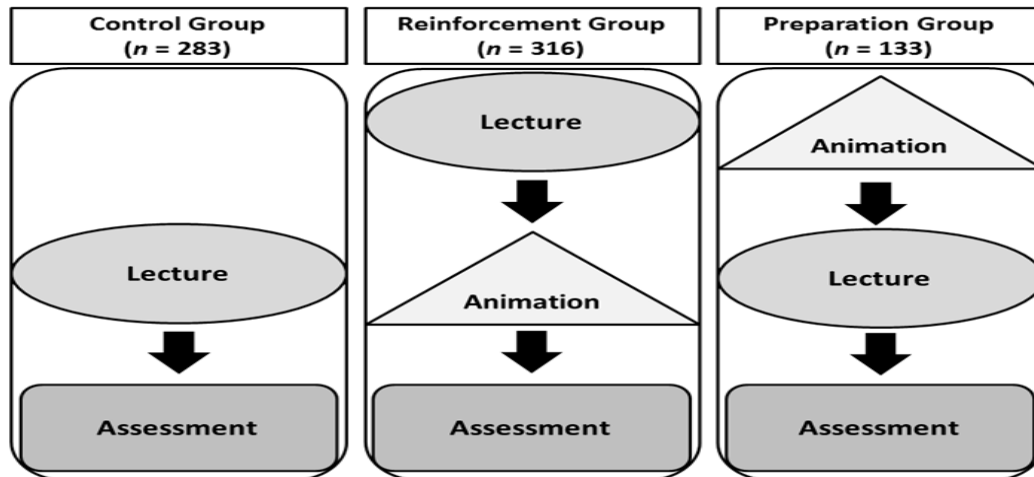


Figure 3.1- Experimental treatment groups as defined by the timing of their interaction with VCell animations.

Statistical Analysis

For each condition, descriptive statistics were compiled and inferential analysis run comparing treatment groups using the R statistical programming package. Student achievement was measured by their score on the assessment instrument following treatment. Analysis of Covariance (ANCOVA) was initially used to investigate the effect of possible explanatory variables on assessment score. Variables selected were based on previous suggestions of their contribution to learning with multimedia resources. Following ANCOVA, Tukey’s analysis (Hoaglin, Mosteller, & Tukey, 1983) was used to compare assessment scores across treatment groups and calculated *p*-values, and 95% confidence intervals for differences in means between groups.

Results

Previous studies have noted the possible confounding effects of various demographic factors on learning with multimedia resources (Bray, 2007; Ching et al.,

2005; Islam, Rahim, Liang, & Momtaz, 2011; O'Day, 2010; Wong et al., 2015). Therefore we used statistical methods to examine possible contributors to assessment scores. Demographic variables were based on factors suggesting prior knowledge (previous enrollment in the course), student standardized test scores (total SAT and ACT composite scores), feelings towards multimedia learning (learning preference as defined in methods), and general demographic information (year in school, student gender, and student ethnicity). In an attempt to account for the inability to conduct a pretest, we included both student standardized test scores and previous course enrollment as a proxy for previous knowledge. Student year in school was classified as either underclassman (freshman/sophomore) or upperclassman (junior/senior). Likewise, student ethnicity was classified as either white or underrepresented minority. ANCOVA shows no significant contribution to assessment scores by any of the extraneous variables tested (Table 3.1). However, the results show a significant influence of treatment condition on assessment scores ($F(2, 360) = 14.92, p < 0.001$).

Table 3.1 - Analysis of Variance Table for Possible Extraneous Variables (ATP)

Variable	df	Sum Sq	Mean Sq	F Value	p-Value
Treatment Condition	2	161.95	81.48	14.92	< 0.001 ***
Multimedia Learning Preference	4	5.56	1.39	0.26	0.91
Gender	1	0.06	0.06	0.01	0.91
Ethnicity	1	4.08	4.08	0.75	0.39
Year in School	1	6.97	6.97	1.28	0.26
SAT Composite Score	1	0.30	0.30	0.05	0.82
ACT Composite Score	1	1.78	1.78	0.33	0.57
Previous Enrollment	1	0.94	0.94	0.17	0.68
Residuals	360	1966.06	5.46		

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Three-Group Comparison of Conditions

In a three group comparison, students who viewed animations on concentration gradients and ATP synthase activity as either pre-class preparation ($M = 6.43, SD = 2.46$) or post-class reinforcement ($M = 6.55, SD = 2.12$) both had higher mean scores on the concept assessment compared to students in the control group ($M = 5.37, SD = 2.35$)(Fig. 3.2, Table 3.2). Post-hoc comparison of means using Tukey's analysis shows that when compared to the control group both the preparation group ($d = 0.44, p < 0.001$) and the reinforcement group ($d = 0.53, p < 0.001$) scored significantly higher on the assessment instrument (Fig. 3.3). Comparison of means between the preparation group and the reinforcement group shows no significant difference between these two treatment groups ($p = 0.87$) (Fig. 3.3).

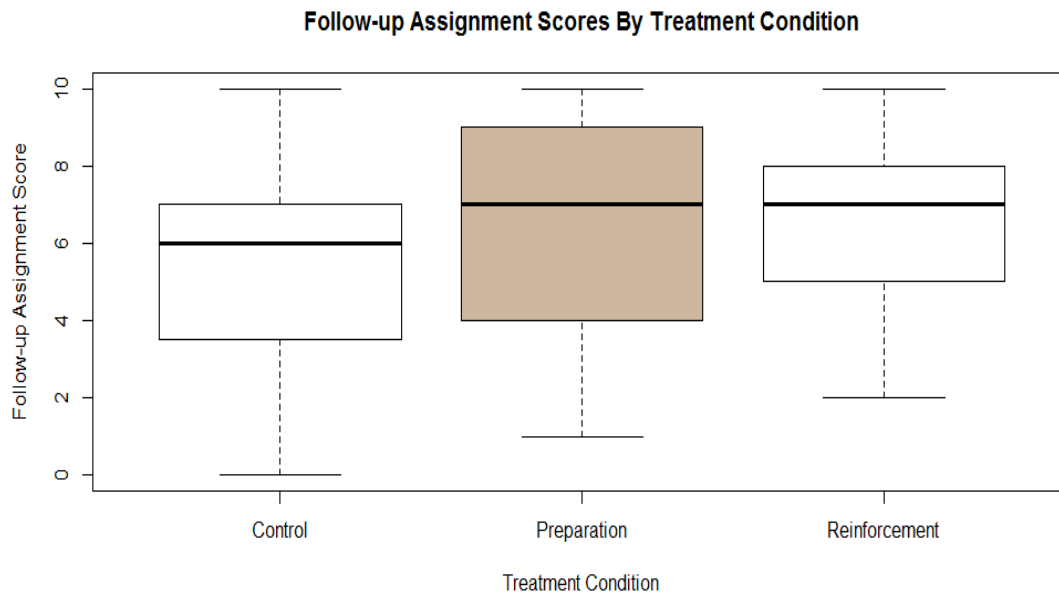


Figure 3.2 – Descriptive statistics for mean score on the follow-up assignment by treatment condition. (ATP)

Table 3.2- Descriptive Statistics for Comparison of Means (ATP)			
	Control	Preparation	Reinforcement
Min	0.00	1.00	2.00
1st Qu.	3.50	4.00	5.00
Median	6.00	7.00	7.00
Mean	5.37	6.43	6.55
3rd Qu.	7.00	9.00	8.00
Max	10.00	10.00	10.00
SD	2.35	2.46	2.12

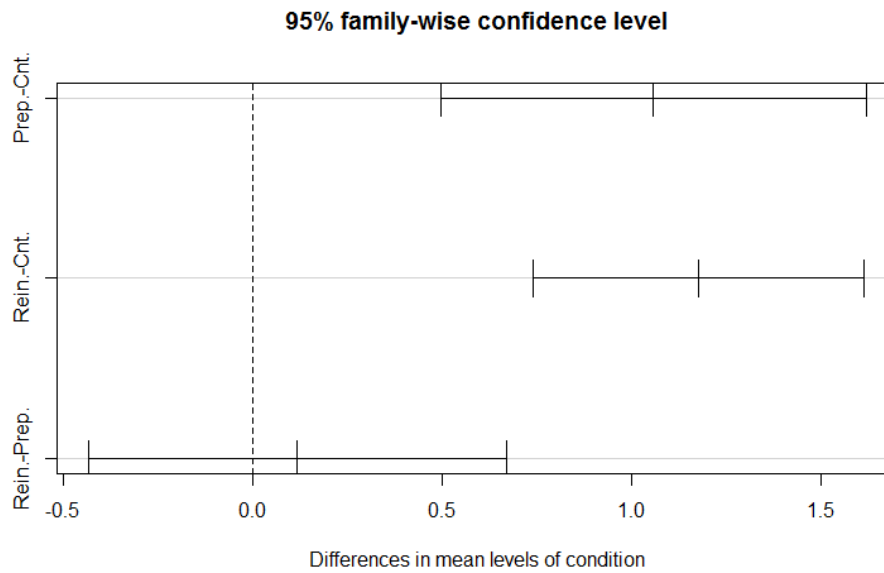


Figure 3.3 – 95% confidence intervals for comparison of means between treatment groups (ATP)(Rein. = Reinforcement Group, Prep. = Preparation Group, Cnt. = Control Group).

Discussion

Strategies to increase student interaction with material outside of the classroom typically requires participation in activities that either prepares students for classroom instruction or that reinforces concepts that have been presented in the classroom (Anliker et al., 1997; McLaughlin et al., 2014; O'Flaherty & Phillips, 2015; Planchard et al., 2015). As a possible resource for these methods we investigate the use of an animation on the topic of concentration gradients and their role in ATP synthase produced by the Virtual Cell animation project. None of the possible extraneous variables examined in this study were shown to contribute to assessment scores on the topic of concentration gradients and their role in the actions of ATP synthase. This is of particular interest considering most introductory biology courses are populated by a largely diverse group of students. Multimedia resources that can be effective despite this variability could be beneficial to introductory biology instructors seeking alternative methods of instruction. We do however note that the sample in this study is representative of one institution and may not be representative to all universities. Future extensions of the study presented here would benefit from the investigation of a more homogenous sample of student backgrounds.

The experimental focus on the use of animations provides evidence that perhaps multimedia can be a reliable means of content interaction outside of the traditional, lecture-centered classroom, regardless of timing. Reports show that many STEM educators either still rely on this traditional method of content delivery or have

experienced negative results when using active learning in the classroom (Andrews et al., 2011; Egan et al., 2014). This is also the case at the university where this study was conducted, as the introductory biology instructors typically still use these traditional instructional methods. In this study, we wanted to focus on the specific timing of student interactions outside of the class and not the instruction itself. As part of this focus, the use of a lecture-centered classroom environment allowed us to control for as many possible confounding factors as possible in regards to the instructional style, while still maintaining a robust, representative sample population. Our results suggests that in such a setting, student/content interaction is beneficial but there is no significant difference in learning outcomes between when students interacted with content as either preparation or reinforcement. However, it would be of interest to see if these results could be replicated in an environment where in-class instruction differs in style, such as a more active learning centered class design. Jensen et. al. (2015) suggests that in such an environment preparation may not be as significant as the classroom instruction itself. Comparison of the results between these two instructional methods could further the understanding of when the implementation of animations outside of the classroom are most effective.

We hesitate to make broad scoping generalizations of these findings due to the relatively short length and lack of full validation of our assessment instrument.

However, our results showed that regardless of timing, students that were exposed to animations outside of the classroom performed higher on an assessment on the topic of concentration gradients than the control group. These results support the call for

increased student interaction with biology concepts outside of the classroom and point to dynamic animation as an effective means of this interaction. Further expansion on this research could provide a deeper understanding of both student preparation and reinforcement in the learning process.

Limitations and Future Studies

We acknowledge that the quasi-experimental design of this study introduces a number of possible confounding variables. Our attempts to account for this using random selection of classroom section, and the random assignment of classroom sections to treatments helped to minimize the impact of many of these potential confounds. However, future investigations could benefit from a completely randomized experimental design. This design would allow for smaller sample sizes that could be assessed more comprehensively to gain insight into the learning process. Together with the current study, the results of such a randomized study could aid in making more powerful conclusions concerning the use of animations outside of the classroom. In addition, we feel that it is important to compare student performance using a variety of different topics within the Virtual Cell Animation Collection. Concentration gradients and their role in the actions of ATP synthase is considered relatively novel to students in introductory biology. It would be of interest to see how our results would compare to students that are introduced to a more familiar topic (mitosis for example). Further investigation using a variety of different topics and multiple replications could therefore

provide insight into which topics provide the most benefit when used as either preparation or reinforcement of concepts.

Conclusions

Recent calls to action in the field of undergraduate STEM education have placed a focus on the interaction of students with course materials outside of the classroom setting. Two instructional practices that have been implemented in a number of introductory biology classes to meet these needs are pre-class assignments focused on student preparation prior to class and post-class assignments that place an emphasis on concept reinforcement. In this study we focus on the benefits of these two strategies by using animations on the topic of concentration gradients and their role in the actions of ATP synthase developed by the Virtual Cell Animation Collection. Ultimately, the results of our study show that Virtual Cell animations on the topic of concentration gradients led to equally high achievement when used as either preparation prior to instruction or reinforcement following instruction as compared to a non-treatment control group. These findings, together with the results of the presented future extensions, aim to provide introductory biology instructor empirical evidence on the “best practice” for implementation of Virtual Cell animations in instruction. These practices could provide insight into the use of animations as part of introductory biology instruction and how the timing of their implementation could affect the level of student understanding and achievement.

3.2 Extensions of Research (mRNA Processing Instruction)

In continuation of the findings on student/content interaction outside of the classroom setting, we extend our investigation to the topic of mRNA processing. This topic comprises an important aspect of the central dogma of molecular biology that has been shown to be a source of confusion for some introductory biology students. (Fisher & Lipson, 1982; Leonard, Kalinowski, & Andrews, 2014; Shapiro, 2009). Results from this additional stage of investigation on the use of dynamic animations outside of the classroom will provide further evidence of possible best practices of instruction using Virtual Cell animations. Together with the results of our previous findings, we again aim to answer the question, “how does learning introductory biology concepts differ when students view animations before or after instruction compared to a no-intervention group?”

Methods

Methods and participants of this additional stage of investigation into the use of Virtual Cell animations outside of the classroom are similar to those previously described in this chapter; however, the specifics of this extension are outlined below.

Participants and Treatment Groups

Participants ($n = 545$) were enrolled in the introductory biology course during the fall semester. Sections were randomly assigned to one of three treatments. The “preparation” group ($n = 109$) consisted of one class section that viewed a Virtual Cell

animation on mRNA processing prior to attending a classroom lecture on the topic. The “reinforcement” group ($n = 219$) consisted of one class section that viewed a Virtual Cell animation on mRNA processing as a means of reinforcement after they attended a classroom lecture on the topic. The “control” group ($n = 217$) consisted of two class sections that only attended a classroom lecture on mRNA processing. This group did not view animations on the topic either prior to or following instruction. Variation in treatment group size was again due to uncontrollable variability in student enrollment between course sections.

Assessment and Measures

The assessment used to obtain information on student conceptual understanding was a ten-question instrument ($\alpha = 0.59$) constructed to fit the level of the course in this study (Appendix F). Weighted Bloom’s Index was calculated and found to be 33.33, suggesting a low to middle-order of cognitive skill level (Freeman et al., 2011a).

Student preference for multimedia learning was gathered using the following question with a five-point Likert scale: “I learn best when information is presented in a visually stimulating (ie: animations/video) fashion”.

Student demographic information was obtained from the University registrar and matched to student performance on the aforementioned assessment. Student identifier data was removed from the dataset.

Experimental Procedures

At the beginning of the semester, students were given a ten-question pretest assessment to gather information on their prior understanding of mRNA processing concepts. At the appropriate point on the instructional calendar, students were introduced to the topic of mRNA processing using the experimental treatments outlined previously (Fig. 3.4). Instruction was conducted at similar meeting times throughout the semester and all sections were conducted in a similar style. Following instruction, students completed an identical ten-question assessment instrument (Appendix F) designed to examine student knowledge on the concepts of mRNA processing. With the inclusion of pretesting into the experimental procedures for this extension of the research, learning outcomes were calculated in the form of normalized gain scores. Normalized gain scores are considered a more accurate representation of student learning than posttest scores alone (Hake, 1998). Therefore, results were analyzed using gain score values for this extension instead of posttest scores.

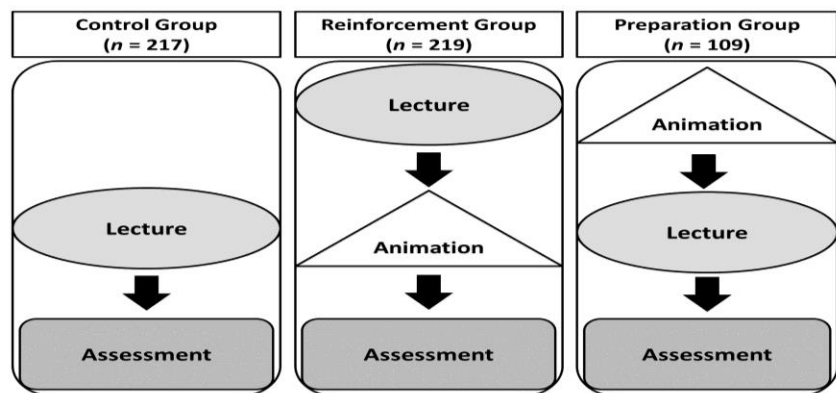


Figure 3.4 - Experimental treatment group as defined by the timing of their interaction with Virtual Cell animations (mRNA Processing).

Statistical Analysis

For each condition, descriptive statistics were compiled and inferential analysis run comparing treatment groups. Analysis of covariance was initially used to investigate the effect of possible explanatory variables on student assessment score. Subsequent Tukey's analysis (Hoaglin et al., 1983) was used to compare normalized gain scores across treatment groups and calculated p -values, and 95% confidence intervals for differences in means between groups.

Results

In order to investigate the possible confounding effects of number different demographic factors on learning with multimedia resources we again used statistical methods to examine possible contributors to assessment scores. Demographic variables were based on factors suggesting prior knowledge (previous enrollment in the course), student standardized test scores (total SAT and ACT composite scores), feelings towards multimedia learning (learning preference as defined in methods), and general demographic information (year in school, student gender, and student ethnicity). Student year in school was classified as either underclassman (freshman/sophomore) or upperclassman (junior/senior). Likewise, student ethnicity was classified as either white or underrepresented minority. ANCOVA shows no significant contribution to assessment scores by any of the extraneous variables tested (Table 3.3). However, the results show a significant influence of treatment condition on assessment scores ($F(2, 229) = 7.40, p < 0.001$).

Table 3.3 - Analysis of Variance Table for Possible Extraneous Variables (mRNA Processing)					
Variable	df	Sum Sq	Mean Sq	F Value	p-Value
Treatment Condition	2	1.74	0.87	7.40	< 0.001 ***
Multimedia Learning Preference	4	0.50	0.12	1.07	0.37
Gender	1	0.16	0.16	1.33	0.25
Ethnicity	1	0.11	0.11	0.95	0.33
Year in School	1	0.07	0.07	0.57	0.45
SAT Composite Score	1	0.01	0.01	0.01	0.82
ACT Composite Score	1	0.21	0.21	1.81	0.18
Previous Enrollment	1	0.03	0.03	0.23	0.64
Residuals	229	26.86	0.12		
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$					

Three-Group Comparison of Conditions

In a three group comparison, students who viewed animations on mRNA processing as either pre-class preparation ($M = 0.60$, $SD = 0.32$) or post-class reinforcement ($M = 0.65$, $SD = 0.29$) both had higher mean scores on the concept assessment compared to students in the control group ($M = 0.45$, $SD = 0.47$)(Fig. 3.5, Table 3.4). Post-hoc comparison of means using Tukey's analysis shows that when compared to the control group both the preparation group ($d = 0.37$, $p = 0.001$) and the reinforcement group ($d = 0.51$, $p < 0.001$) scored significantly higher on the assessment instrument (Fig. 3.6). Comparison of means between the preparation group and the reinforcement group shows no significant difference between these two treatment groups ($p = 0.61$) (Fig. 3.6).

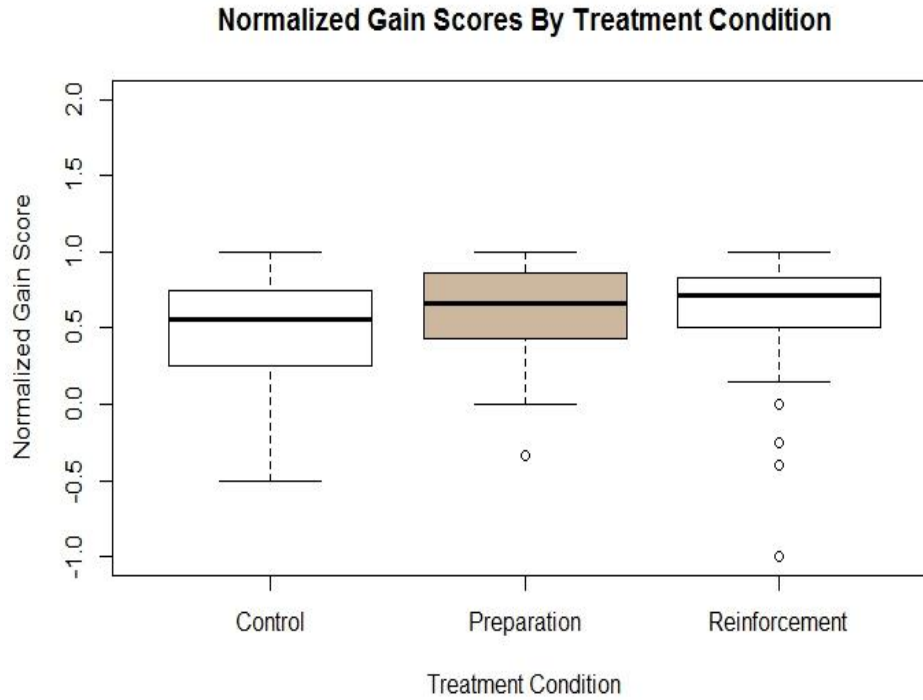


Figure 3.5 – Descriptive statistics for normalized gain score by treatment condition (mRNA Processing).

Table 3.4- Descriptive Statistics for Comparison of Means (mRNA Processing)			
	Control	Preparation	Reinforcement
Min	-3.00	-0.33	-1.00
1st Qu.	0.25	0.43	0.50
Median	0.56	0.67	0.71
Mean	0.45	0.60	0.65
3rd Qu.	0.75	0.86	0.83
Max	1.00	1.00	1.00
SD	0.47	0.32	0.29

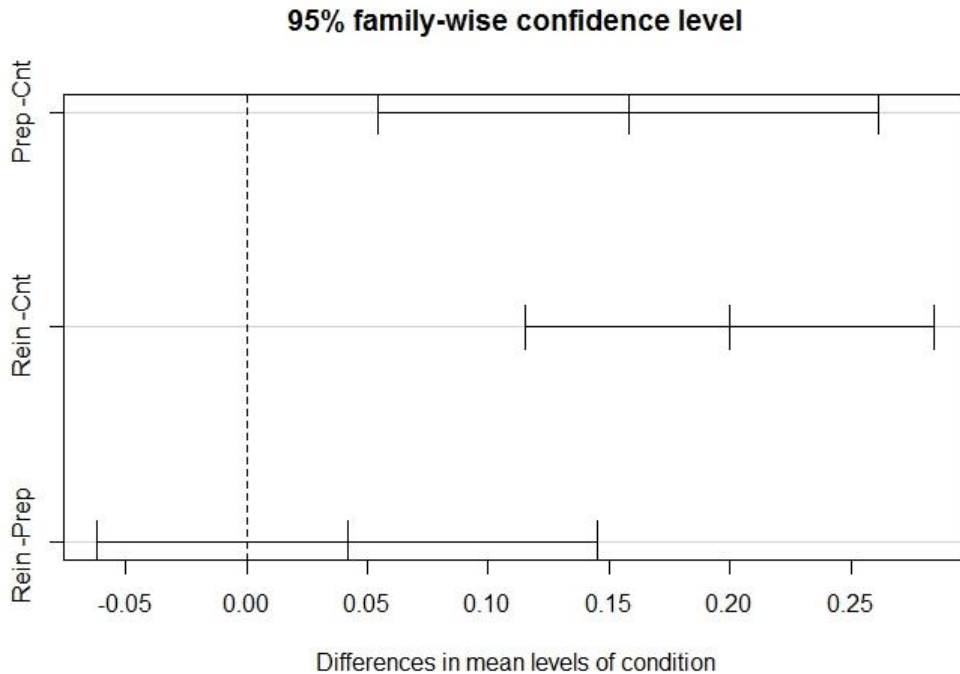


Figure 3.6 – 95% confidence intervals for comparison of means between treatment groups (mRNA Processing) (Rein. = Reinforcement Group, Prep. = Preparation Group, Cnt. = Control Group).

3.3 Extensions of Research (Translation Instruction)

In continuation of the findings on instruction outside of a classroom setting on the previous two topics outlined above, we complete our investigation with the topic of mRNA translation. This, again, comprises an important aspect of the central dogma of molecular biology that has been shown to be a major area of misconception for some introductory biology students. (Fisher & Lipson, 1982; Leonard et al., 2014; Shapiro, 2009). In addition, the concept of molecular inheritance, which is influenced by the actions of translation, has been reported by numerous studies as a point of misunderstanding for many undergraduate students (Khodor, Halme, & Walker, 2004;

Marbach-Ad, 2001; Wood-Robinson, Lewis, & Leach, 2000). As a principle source of many introductory biology misconceptions, investigation into the topic of translation allows us to further our findings on the use of Virtual Cell animations in introductory biology instruction while providing evidence of best practices for student/content interaction outside of the physical classroom. Together with the results of the two previous topics, we again aim to answer the question, “how does learning introductory biology concepts differ when students view animations before or after instruction compared to a no-intervention group?”

Methods

Methods and participants of this additional stage of investigation into the use of Virtual Cell animations outside of the classroom are similar to those previously described in this chapter; however, specifics of this extension are outlined below.

Participants and Treatment Groups

Participants ($n = 526$) self-enrolled in an introductory biology course during the fall semester. Sections were again randomly assigned to one of three treatments. The “preparation” group ($n = 199$) consisted of one class section that viewed a Virtual Cell animation on translation prior to attending a classroom lecture on the topic. The “reinforcement” group ($n = 223$) consisted of two class sections that viewed a Virtual Cell animation on translation as a means of reinforcement after they attended a classroom lecture on the topic. The “control” group ($n = 104$) consisted of one class section that only attended a classroom lecture on translation. This group did not view

animations on the topic neither prior to nor following instruction. Variation in treatment group size was again due to uncontrollable variability in student enrollment between course sections.

Assessment and Measures

The assessment used to obtain information on student conceptual understanding was a ten-question instrument ($\alpha = 0.55$) constructed to fit the level of the course in this study. Questions were selected from two common concept inventories on translation and molecular biology (Elrod, 2007; “Q4B Concept Inventories | Questions For Biology,” 2016) Weighted Bloom’s Index was calculated and found to be 50.00, suggesting a middle-order of cognitive skill level (Freeman et al., 2011a).

Student preference for multimedia learning was again gathered using the following question with a five-point Likert scale: “I learn best when information is presented in a visually stimulating (ie: animations/video) fashion”. Student demographic information was again obtained from the University registrar and matched to student performance on the aforementioned assessment. Student identifier data was removed from the dataset.

Experimental Procedures

At the appropriate point on the instructional calendar, students were introduced to the topic of translation using the experimental treatments outlined previously (Fig. 3.7). Instruction was conducted at similar meeting times throughout the

semester and all sections were conducted in a similar style. Following instruction, students completed a ten-question assessment instrument (Appendix G) designed to examine student knowledge on the concepts of translation. With the inclusion of pretesting into the experimental procedures for this extension of the research, learning outcomes were again calculated in the form of normalized gain scores. Normalized gain scores are considered a more accurate representation of student learning than posttest scores alone (Hake, 1998). Therefore, results were analyzed using gain score values for this extension instead of posttest scores.

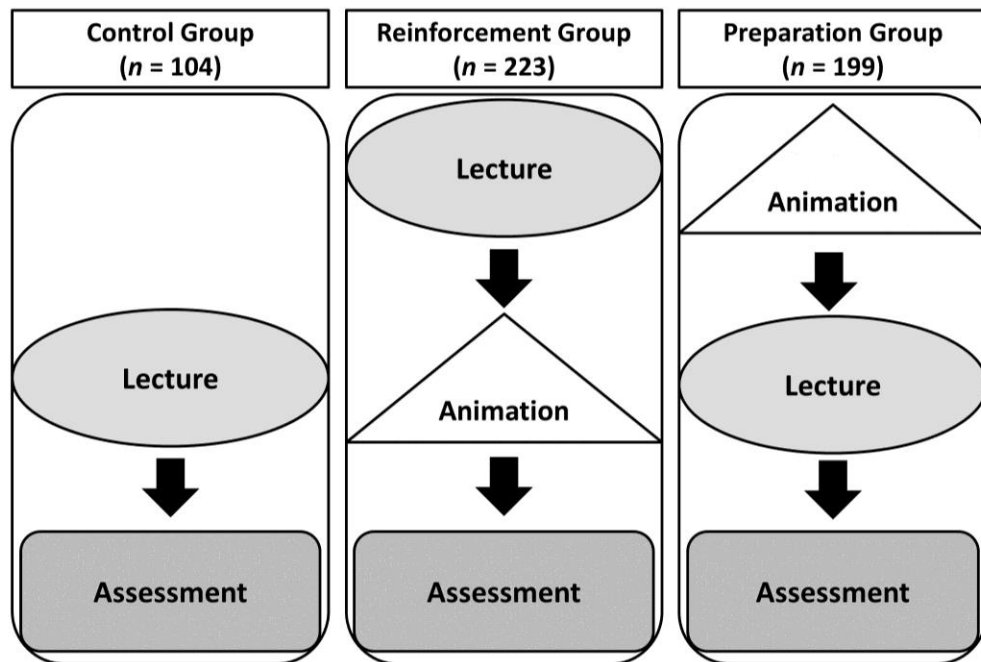


Figure 3.7- Experimental treatment groups as defined by the timing of their interaction with Virtual Cell animations (Translation).

Statistical Analysis

For each condition, descriptive statistics were compiled and inferential analysis was used to compare treatment groups. Analysis of covariance was initially used to

investigate the effect of possible explanatory variables on student assessment score. Subsequent Tukey's analysis (Hoaglin et al., 1983) was used to compare assessment scores across treatment groups and calculated p -values, and 95% confidence intervals for differences in means between groups.

Results

We again used statistical methods to examine possible contributors to assessment scores using previously noted variables identified as possible confounders in multimedia learning. Demographic variables were based on factors suggesting prior knowledge (previous enrollment in the course), student standardized test scores (total SAT and ACT composite scores), feelings towards multimedia learning (learning preference as defined in methods), and general demographic information (year in school, student gender, and student ethnicity). Student year in school was classified as either underclassman (freshman/sophomore) or upperclassman (junior/senior). Likewise, student ethnicity was classified as either white or underrepresented minority. ANCOVA again shows no significant contribution to assessment scores by any of the extraneous variables tested (Table 3.5). However, the results show a significant influence of treatment condition on assessment scores ($F(2, 231) = 4.40, p < 0.05$).

Table 3.5 - Analysis of Variance Table for Possible Extraneous Variables (Translation)					
Variable	df	Sum Sq	Mean Sq	F Value	p-Value
Treatment Condition	2	0.74	0.37	4.40	0.02 *
Multimedia Learning Preference	4	0.04	0.01	0.15	0.93
Gender	1	0.02	0.02	0.17	0.68
Ethnicity	1	0.10	0.10	1.22	0.27
Year in School	1	0.02	0.02	0.23	0.63
SAT Composite Score	1	0.18	0.18	2.18	0.14
ACT Composite Score	1	0.02	0.02	0.21	0.65
Previous Enrollment	1	0.06	0.06	0.74	0.39
Residuals	231	19.56	0.09		
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$					

Three-Group Comparison of Conditions

In a three group comparison, students who viewed animations on translation as either pre-class preparation ($M = 0.22$, $SD = 0.30$) or post-class reinforcement ($M = 0.25$, $SD = 0.25$) both had higher normalized scores on the concept assessment compared to students in the control group ($M = 0.14$, $SD = 0.28$)(Fig. 3.8, Table 3.6). Post-hoc comparison of means using Tukey's analysis shows that when compared to the control group both the preparation ($d = 0.28$, $p < 0.05$) and the reinforcement group ($d = 0.41$, $p = 0.001$) had significantly higher learning gains on the assessment instrument (Fig. 3.9). However, comparison of means between the preparation group and the reinforcement group shows no significant difference between these two treatment groups ($p = 0.48$) (Fig. 3.9).

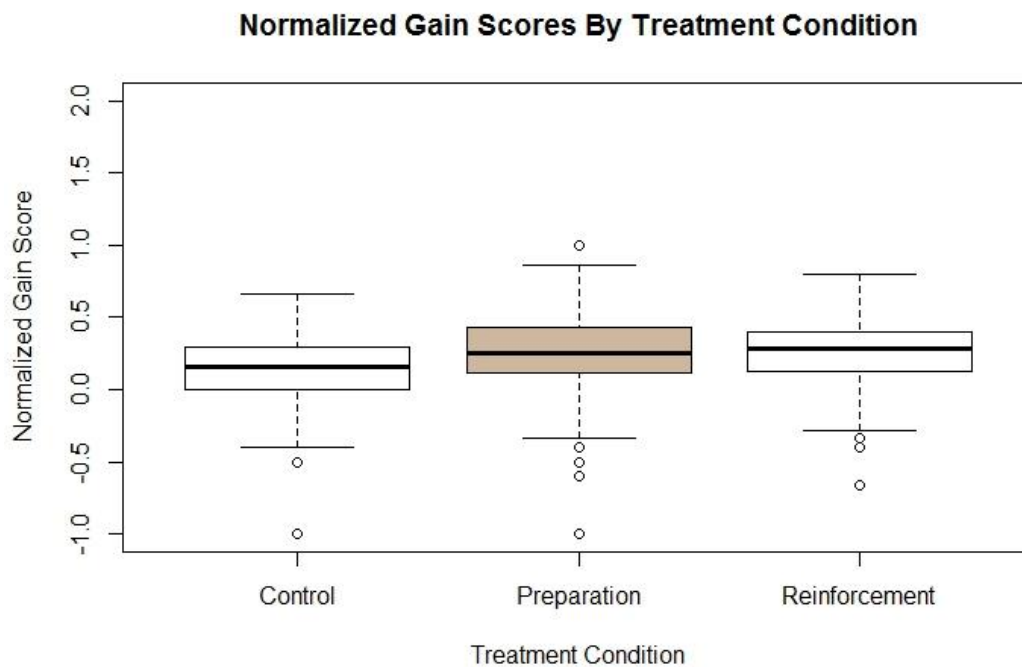


Figure 3.8 – Descriptive statistics for normalized gain score by treatment condition (Translation).

Table 3.6- Descriptive Statistics for Comparison of Means (Translation)			
	Control	Preparation	Reinforcement
Min	-1.00	-1.00	-0.67
1st Qu.	0.00	0.11	0.13
Median	0.15	0.25	0.29
Mean	0.14	0.22	0.25
3rd Qu.	0.29	0.43	0.40
Max	0.67	1.00	0.80
SD	0.28	0.30	0.25

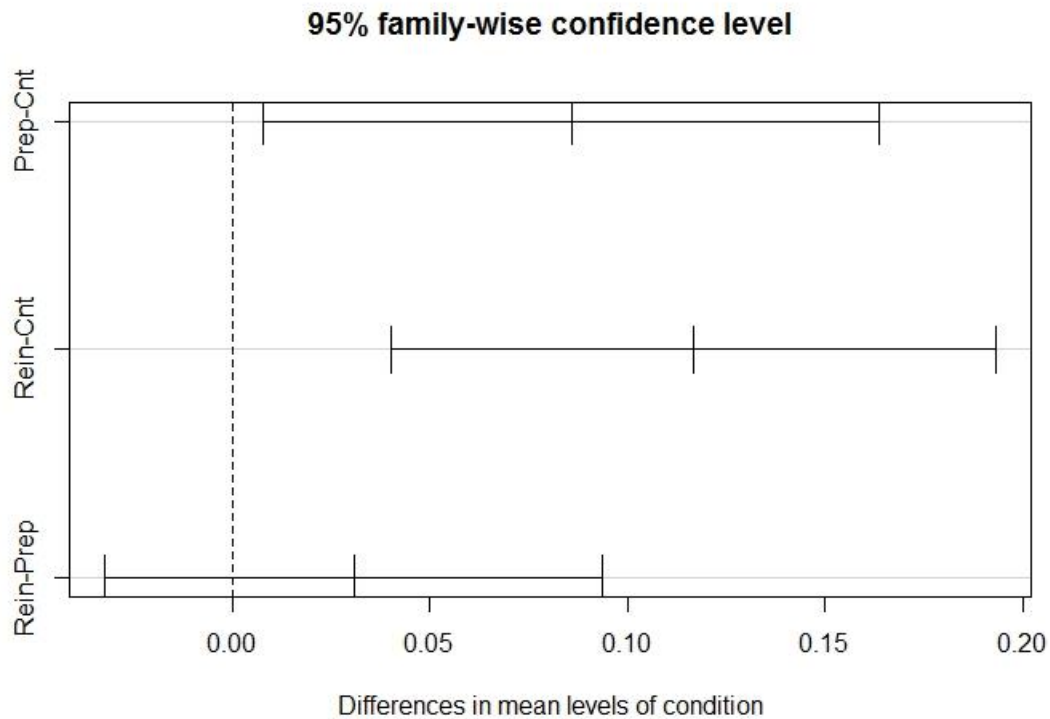


Figure 3.9 – 95% confidence intervals for comparison of means between treatment groups (Translation) (Rein. = Reinforcement Group, Prep. = Preparation Group, Cnt. = Control Group).

3.4 Discussion of Learning with Virtual Cell Animations as Preparation and Reinforcement

With undergraduate biology instruction continually evolving to meet the needs of a changing student population, investigation into the way that students interact with course materials outside of the classroom has become paramount. As a part of these efforts, we investigate learning gains when students interact with dynamic animations as either pre-class preparation or post-class reinforcement as compared to a control group that did not view the animations. Experiments conducted on three topics that have been suggested to be a common source of misconceptions amongst introductory biology students show no difference between students in the preparation and

reinforcement treatment groups (ATP: $p = 0.87$, mRNA : $p = 0.61$, Translation: $p = 0.45$). However, both treatment groups showed higher outcomes on the follow-up assessments than the non-treatment control group. These outcomes suggest the strength of student/content interactions may not lie in the timing or fashion of the interaction but simply in the fact that they were participating in the learning process outside of the classroom. In a traditional lecture-centered classroom environment that is prevalent in the typical undergraduate biology classroom (Eagan et al., 2014), these findings could provide insight into the best practices for student/content interactions outside of the classroom. Previous research has suggested a role of various instructional methods in both concept introduction and reinforcement (Demirci, 2010; Lee, 2014; Malik et al., 2014; Persky, 2015). While each of these methods has demonstrated their own merits, results of our study show the benefits of dynamic animation in both the preparation and reinforcement settings. The use of dynamic animation to provide achievement gains across multiple difficult topics could give both instructors and students alike a means of improving conceptual comprehension. The dynamic animations designed as part of the Virtual Cell Animation Collection, examined here, could serve as a centralized database of resources that can be accessed by instructors and students alike to aide in introductory biology instruction.

Results also show that the effects of animation mediated interactions were independent of the possible extraneous variables tested. With many introductory biology courses consisting of a large number of demographically different students, this could provide confidence that the resources developed by the Virtual Cell Animation

project can aide instruction despite class makeup. Such reliability is important for the widespread dissemination of any multimedia learning tool.

Limitations

The quasi-experimental design of this study could introduce a number of possible confounding variables. We however attempted to limit the impact of these confounding aspects by using random selection of classroom section, and the random assignment of classroom sections to treatments. Future research into student/content interactions outside of the classroom could benefit from a completely randomized experimental design. While this design would require smaller sample sizes and a less realistic learning environment than what is seen here, it could provide further insight into the learning process. Together with the current study, this design could aid in making more powerful conclusions concerning the use of dynamic animations outside of the classroom.

In addition, we feel that it could be important to investigate each of these methods of student/content interaction in an active learning-centered flipped classroom. While previous studies have shown the importance of preparation in such a classroom environment (Gross et al., 2015), it would be interesting to see the achievement outcomes when an emphasis is placed on animation as part of reinforcement in a flipped environment.

3.5 Conclusions of Learning with Virtual Cell Animations as Preparation and Reinforcement Outside of the Classroom

With recent reports focusing on student/content interactions outside of the classroom an emphasis has been placed on the development of quality resources to facilitate these interactions (Steve Olson & Riordan, 2012). In an attempt to examine the use of multimedia resources developed by the Virtual Cell Animation Collection as a mediator of such external interactions, we investigate the use of dynamic animation as either preparation prior to classroom instruction or reinforcement following classroom instruction. Using three introductory biology concepts that have been previously identified as common sources of misconception amongst undergraduate students; we look at the use of dynamic animation outside of the classroom. Results show a significant contribution of preparation and reinforcement treatments in introductory ATP synthesis ($p < 0.001$), mRNA processing ($p < 0.001$), and translation ($p < 0.05$) concept instruction. With the recent push for redesigning many classes to accentuate a flipped design (McLaughlin et al., 2014; O'Flaherty & Phillips, 2015), we hypothesized that when directly compared, animations used in a pre-class preparation fashion would lead to greater learning outcomes than those used as post class reinforcement. However, the results of this aim show no significant difference in the outcomes of any of the three topics examined. It is interesting that while both preparation and reinforcement treatments resulted in significantly greater learning outcomes than the non-treatment control group, there was no difference between the treatments. This could suggest that it is not the specific type of student/content interaction that instructors propagate outside of the classroom that is important but instead simply the

fact that such interactions are occurring at all. Results of this study can provide introductory biology instructors with the pedagogical freedom of assigning multiple types of outside of the classroom student/content interactions. Additionally, results provide empirical evidence suggesting the ability of dynamic animations produced by the Virtual Cell Animation Collection to mediate such interactions. Ultimately, the results of this study could promote understanding of introductory biology concepts answering the call of multiple recent reports on the need for improvement in undergraduate STEM education (Brewer & Smith, 2011; Steve Olson & Riordan, 2012)

Chapter 4

Virtual Cell Animations as Part of Stand-Alone Online Learning Modules

Calls for reform in undergraduate STEM education have placed an emphasis on student interaction with course content outside of the classroom (Brewer & Smith, 2011; Steve Olson & Riordan, 2012). While a previous aim of this project investigated the learning outcomes of various types of these interactions, this aspect of our study focuses mainly on the resources developed for student/content interactions either prior to classroom instruction or completely independent of the classroom itself. This focus stems from the recent push for online learning and the adoption of “flipped” course designs by some instructors (Berrett, 2012; DeLozier & Rhodes, 2016; Galway, Corbett, Takaro, Tairyan, & Frank, 2014). Assignments typically associated with such settings can vary greatly but there has been evidence for the use of online learning modules in such an environment (Khalil et al., 2010). In addition to this preparatory role of pre-class instruction, a number of institutions have moved some content instruction to an online environment that is completely independent of a physical classroom (Lim, 2007; Shin & Lee, 2009). This instructional design is often technology dependent and relies on the use of recorded lectures or learning modules. With a recent trend towards the use of

Note: section 4-1 has been adapted from Goff, E., Reindl, K., Johnson, C., McClean, P., Offerdahl, E., Schroeder, N., White, A. (2017, in press). Efficacy of a Meiosis Learning Module Developed for the Virtual Cell Animation Collection. *CBE – Life Science Education*.

technology in instruction (Martin & Carr, 2015), we focus the final aim of this study specifically on one of these methods, stand-alone online learning modules, as part of instruction.

Online learning modules have been shown to be beneficial in a number of different studies (Khalil et al., 2010; Serrat et al., 2014; Stelzer et al., 2009), however, these benefits seem to rely on the strict adherence multimedia guidelines in the developmental stages (Hatsidimitris, 2012; Huang, 2005). While following these guidelines can aide instructors in delivering content effectively, the developmental process could prove difficult for those without training in multimedia design. With this in mind, we have set out to research the efficacy of stand-alone learning modules developed for instruction in undergraduate introductory biology courses by individuals well versed in the field. These multimedia resources have been developed using animations from the Virtual Cell Animation Collection (McClellan et al., 2005; Reindl et al., 2015) and are focused on the delivery of often difficult content outside of the traditional classroom setting. As a part of this study we aim to use these resources to answer the question, “to what extent do Virtual Cell online learning modules aide in instruction of introductory biology concepts compared to a traditional classroom lecture?” The developmental strategies of the online learning modules and their embedded animations examined here follow the published guidelines of effective multimedia design (Mayer & Moreno, 2002; Mayer & Pilegard, 2014; McClellan et al., 2005). As a result, we hypothesize that students who interact with online learning modules on introductory biology topics will outperform students who only participate in

a traditional class lecture prior to a concept assessment. Results of this investigation will provide introductory biology instructors with empirical evidence of the efficacy of online learning modules developed using animations produced as part of the Virtual Cell Animation Collection. This evidence would provide confidence in the use of these resources as part of introductory biology instruction and would allow for proper preparation prior to classroom instruction on certain introductory concepts.

As part of this study, we focus on the introduction of two key concepts presented as part of introductory biology instruction; meiosis and cellular respiration. Students entering their undergraduate studies are typically assumed to have had some surface level introduction to both of these topics as part of their high school education (“Next Generation Science Standards,” 2015, “Standards - South Carolina Department of Education,” 2015). However, both of these topics have also been previously shown to be a source of major misconceptions in introductory biology (Capa et al., 2001; Kalas, O’Neill, Pollock, & Birol, 2013; Quinn, Pegg, & Panizzon, 2009; Songer & Mintzes, 1994). Despite these difficulties, published online learning modules such as those presented here are rarely found in the literature. To begin to rectify this, we have developed stand-alone learning modules that students can interact with outside of the classroom as a means of concept delivery. The investigation into the efficacy of these resources and a review of the development process are outlined below.

4.1 Efficacy of a Meiosis Learning Module Developed for the Virtual Cell Animation Collection

Abstract

Recent reports calling for change in undergraduate biology education have resulted in the redesign of many introductory biology courses. Reports on one common change to course structure, the active learning environment, have placed an emphasis on student preparation, noting that the positive outcomes of active learning in the classroom depend greatly on how well the student prepares prior to class. As a possible preparatory resource, we test the efficacy of a learning module developed for the Virtual Cell Animation Collection. This module presents the concepts of meiosis in an interactive, dynamic environment that has previously been shown to facilitate learning in introductory biology students. Participants ($n = 534$) were enrolled in an introductory biology course and were presented the concepts of meiosis in one of two treatments: the interactive learning module or a traditional lecture session. Analysis of student achievement show that students who viewed the learning module as their only means of conceptual presentation scored significantly higher ($d = 0.40$, $p < 0.001$) than students who only attended a traditional lecture on the topic. Our results show the animation-based learning module effectively conveyed meiosis conceptual understanding, which suggests that it may facilitate student learning outside of the classroom. Moreover, these results have implications for instructors seeking to expand their arsenal of tools for “flipping” undergraduate biology courses.

Introduction

Recent reports calling for reform in undergraduate biology education (Brewer & Smith, 2011; Olson & Riordan, 2012) have identified the active engagement of students in the learning process as a key factor in improving students' conceptual understanding. Indeed, the implementation of active learning strategies has consistently been shown to increase student achievement and concept retention in the classroom setting. The results of a recent meta-analysis ($n = 225$) found that STEM students in traditional classrooms had a 55% higher failure rate than those in an active learning settings (Freeman et al., 2014). In addition, active learning classrooms were found to provide almost a half standard deviation improvement in learning outcomes ($Z = 9.78$, $p < 0.001$). In response, university instructors are increasingly redesigning courses to introduce students to content outside of class, thereby freeing up in-class time for active learning (Gross et al., 2015; Jensen et al., 2015).

Recent research indicates that not all active learning classrooms are created equal; proper pre-class preparation is critical for successful implementation of active learning strategies. For example, Andrews et al. (2011) examined active learning and student achievement at 77 institutions nationwide, yet they found no significant differences in basic introductory biology learning outcomes between classes that used active learning strategies and those that used traditional techniques. The authors also noted that reported success in active learning could be a result of well-trained instructors effectively preparing their students prior to (i.e., outside of) class. Similarly,

“highly structured” course designs, where student pre-class preparation requires them to interact intimately with the content outside of the classroom, have demonstrated significant learning gains in active learning classrooms (Freeman, Haak, & Wenderoth, 2011b; Gross et al., 2015; Haak et al., 2011). Collectively, these findings underscore the importance of characterizing the types of out-of-class learning experiences that can provide appropriate levels of preparation for students to benefit from active learning pedagogies in class.

If proper preparation is the key to increasing achievement in the active learning classroom, it becomes imperative that we bridge the gap between how students are introduced to content outside of the classroom and how they interact with it during face-to-face meeting times. Identifying and characterizing the diverse ways in which students learn outside of the classroom will allow us to provide students with learning opportunities that provide the solid base of understanding needed to achieve the goals of in-class, active learning activities. Instructors have commonly required students to complete textbook readings or pre-class worksheets as preparatory activities (Freeman et al., 2011b; Haak et al., 2011; Moravec et al., 2010). While the benefits of these methods are shown in a highly structured classroom setting with proper guidance from the instructor (Freeman et al., 2011b; Moravec et al., 2010), it has been noted that not all students are equally motivated to read before class (Aagaard et al., 2014; Boekaerts, 2001; Marek & Christopher, 2011). In addition, simply assigning textbook readings without holding students accountable has been shown to likely result in poor participation rates (Aagaard et al., 2014; Vafeas, 2013). One increasingly popular

alternative to textbook and writing assignments is the use of online multimedia learning resources outside of the classroom (Crampton et al., 2012; Fung, 2015; Pierce & Fox, 2012; Zappe et al., 2009).

Well-developed multimedia resources provide instructors one option for students to process conceptual information in a short period of time (Kraidy, 2002; McClean et al., 2005; O'Day, 2010). By leveraging effective multimedia learning materials, instructors can provide students with effective instruction before class, thereby allowing for classroom time to be used for active learning activities rather than traditional lecture (DeLozier & Rhodes, 2016; Gross et al., 2015; Jensen et al., 2015).

Within the realm of molecular and cellular biology, one such collection of materials -

The Virtual Cell (VCell) Animation Collection- has been widely available since 2004.

These animations outline the basic introductory concepts of a variety of molecular and cellular biology topics (Reindl et al., 2015). Recently, these animations have been incorporated into online learning modules that can be implemented throughout an undergraduate biology course or as a stand-alone learning tool available to students.

Learning modules can augment a hybrid or flipped classroom setting by providing instructors a means of structured online content presentation that can be implemented outside of the classroom. In addition, these learning modules aim to answer the call (Brewer & Smith, 2011; Olson & Riordan, 2012) to engage STEM students outside of the classroom while preparing them for in-class active learning activities.

The VCell Animation team has completed the production of two online learning modules focused on concepts generally covered in introductory biology: biological energy flow and meiosis. One additional module has also been developed for upper-level cell biology covering the concepts of insulin signaling. The guiding principle of this effort was to develop stand-alone learning tools that provide instructors a reliable resource to deliver biology concepts to students outside of the classroom. In this study we aimed to investigate the effectiveness of one of these learning modules (meiosis) in the introductory biology course (Biol101) at a large public university in the southeast United States. We focused our efforts on the comparison of this online learning module to a traditional classroom lecture in order to determine if the two approaches were similarly effective at reinforcing the introductory concepts of meiosis to students. Our choice of traditional lecture as a control group was based on reports that instructors in STEM fields are, on average, more resistant than non-STEM instructors to adopting flipped class methods (Eagan et al., 2014; Kuiper et al., 2015). This study aims to answer the question, to what extent does the VCell meiosis online learning module reinforce meiosis concepts compared to a traditional classroom lecture? The online module in this study is designed to be a personal, self-paced interactive learning experience. We feel that the distinct interactive environment of the online learning module provides an experience that cannot be accommodated in a traditional lecture setting. As a result, we hypothesized that the online learning module would perform at a level equal to or better than that of a traditional classroom lecture. If our hypothesis was correct, we would show that students exposed to the basic concepts of meiosis through the meiosis

online learning module are at minimum equivalently prepared with the conceptual understanding of meiosis as compared to if they were presented by lecture alone. In addition we would be able to provide evidence that meiosis concept presentation via the learning module is on a level at least equivalent to the traditional lecture style that some STEM instructors have been hesitant to relinquish. Empirical evidence demonstrating the efficacy of learning modules at teaching meiosis concepts might create an entry point for traditional lecturers into the foray of active learning; instructors could assign the module before lecture thereby freeing up time for more student-centered activities targeting resilient meiosis misconceptions.

Student Understanding of the Concepts of Meiosis

The topic of meiosis is a common source of misunderstanding amongst many undergraduate introductory biology students (C. R. Brown, 1990; Kindfield, 1991, 1994; Newman, Catavero, & Wright, 2012). The K-12 science framework outlined by the National Academies Press (National Research Council, 2012) suggests that by completion of grade twelve, students should have an understanding of the cell cycle, sexual reproduction, DNA replication, chromosomal structure, and genetic variability. The process of connecting these underlying concepts is a critical component of understanding the mechanisms involved in meiosis. However evidence suggests that many undergraduate introductory biology students do not make these connections (Kalas et al., 2013; Newman et al., 2012). For example, undergraduate students commonly misrepresent chromosomes throughout the stages of meiosis, including

inaccurate depictions of sister chromatids and improper interactions between chromosomes (Dikmenli, 2010; Kindfield, 1991, 1994; Newman et al., 2012). However, even if we assume all students enter their undergraduate studies equipped with all of the prior conceptual understanding outlined in the K-12 standards, not all instructional resources meant to help connect underlying concepts and convey deeper understanding are equally effective (Tversky et al., 2002). One example of this is the comparison of external representations depicting biological processes as part of instruction. When directly comparing dynamic representations to their static counterparts, one meta-analysis (Höffler & Leutner, 2007) shows that students who were presented information using dynamic representations of biological concepts have higher learning outcomes ($d = 0.37$). With these positive learning outcomes in other realms of science education, we focus on using these resources as an instructional aide for teaching meiosis as well. By developing a dynamic, interactive learning module, our goal is to provide students with a visual guide that promotes the connection of concepts and ultimately a deeper understanding of topic of meiosis.

The Virtual Cell Animation Collection

Recent studies on the use of dynamic, animated multimedia have emphasized their ability to promote learning in the science classroom (Cook, 2012; Eilam & Gilbert, 2014; Kozma, Chin, Russell, & Marx, 2009; McElhaney et al., 2015). Dynamic representations of scientific processes are suggested to provide learners with cognitive assistance allowing them to process information more efficiently resulting in the

formation of more accurate mental models (Höffler & Leutner, 2011; Williamson & Abraham, 1995). These benefits are especially evident when students are presented concepts associated with the small, non-observable facets of molecular biology (Barber & Stark, 2014; Jenkinson & McGill, 2012; Marbach-Ad, Rotbain, & Stavy, 2008; McClean et al., 2005; Ryoo & Linn, 2012). It should be noted that not all forms of multimedia are created equally, and that dynamic representations have not always shown to be superior to their static counterparts (Tversky et al., 2002). Optimization of dynamic representations can be achieved through the applications of multimedia design principles (Mayer et al., 2003; O'day, 2006; O'Day, 2010), and following best practices for classroom implementation (Hill et al., 2015; Pierce & Fox, 2012).

The development of multimedia resources for use in an educational setting is not an uncommon practice in undergraduate education; however, finding empirically tested, free-to-use options can prove difficult. The current leaders in educational multimedia are well-funded textbook publishing companies. These companies typically produce resources that coordinate with the concepts presented in their publications that can be passed along to teachers for incorporation as they see fit (O'Day, 2010). Many of these packages are well developed and present concepts in a way that promotes learning for many students (Speckler, 2014), but they are limited in their accessibility. Typically, these publisher-produced resources are only made available to institutions that have adopted their textbook and students who have either paid for the book itself or have paid for access to their website (McGraw-Hill Connect, 2015). While

this practice can be a profitable business model, it provides little benefit to students who do not have access to these features.

In addition to publisher driven content, a second category of educational multimedia consists of free-to-use videos and animations that are often posted to internet sites such as YouTube or course-focused webpages as part of a learning management system. These resources are typically produced by either the instructor or a group of students in hopes of promoting better understanding of certain concepts. While many of these productions may be effective, there are a large number that introduce concepts inadequately (Azer, 2012; Raikos & Waidyasekara, 2014) which could potentially confuse the student by introducing misconceptions.

The VCell Animation Collection (NSF awards: 0086142, 0618766, and 0918955) addresses these concerns. The VCell team applied research-based principles of multimedia instructional design (Mayer, 2009; Mayer & Moreno, 2002) to develop a series of high quality animations and learning modules. In addition, all of these resources are free-to-use and openly accessible to both the teacher and the student. The VCell team included an expert group of cellular and molecular biology researchers in order to assure the validity of information within the videos, while following research-based design principles helping to maintain a low cognitive strain on the viewer (McClellan et al., 2005; Reindl et al., 2015). The VCell Animation Collection currently consists of a catalog of 24 animations depicting concepts of molecular and cellular biology. The collection is housed on the project's website

(<http://vcell.ndsu.edu/animations/>) and each animation is readily available for either streaming or downloading. The appeal of the VCell animations to students and educators can be exemplified through those who have completed the optional registration process. Currently, there are approximately 23,000 registered users from over 150 countries. In addition to the project website, the VCell Animation Collection also has a YouTube site (<http://www.youtube.com/user/ndsuvirtualcell>) currently boasting approximately 44,000 subscribers and over 12,000,000 viewings. The team has also developed a free Apple iOS application (<http://itunes.apple.com/us/app/virtual-cell-animations/id427893931?mt=8>) that has been downloaded approximately 175,000 times to date.

With such widespread appeal of VCell animations, the VCell development team has recently focused on using the animations as part of online learning modules geared towards presenting difficult biological concepts to students in fashion that is both effective in conveying the information and accessible in a setting independent of a lecture hall and instructor. It is the goal of these learning modules to provide effective resources that instructors can use to present concepts to students outside of the classroom, thereby allowing time in class to be devoted to active learning and other teaching strategies that require students to exhibit higher level thinking. In order to assess the module's ability to effectively convey the relevant information, the VCell learning modules had to be developed using current research on module design and multimedia learning (Mayer & Moreno, 2002; Mayer & Pilegard, 2014), as well as be rigorously tested in a classroom environment (Reindl et al., 2015). Details of

development strategies and how this study aimed to investigate the effectiveness of the module in a classroom environment are outlined below.

Module Development

Research has shown that with proper classroom implementation, online learning modules can promote both greater conceptual understanding and retention as compared to traditional methods of instruction (Florida, 2012; Hill et al., 2015; Khalil et al., 2010; Lancellotti et al., 2016; Stelzer et al., 2009). Development of learning modules with an attention to the cognitive load of the content presented can provide students with information in appropriately sized chunks that they can process and retain at their own pace (Ayres & Paas, 2007; Hatsidimitris, 2012; Khalil, Paas, Johnson, & Payer, 2005). In order to develop online learning modules that effectively convey the biological concepts needed for introductory level biology students, the VCell Animation team followed published multimedia design principles (Mayer & Moreno, 2002; Mayer & Pilegard, 2014) throughout the design process.

In accordance to the segmentation principle of multimedia learning, conceptual information presented by VCell learning modules are divided into three to four brief segments (Mayer, 2009). At the beginning of each segment, an onscreen narrator provides the learner with a set of key points that they should focus on in a subsequent animation clip. The learner is given prompts as to what will be shown and what key concepts they should grasp from the animation (Fig. 4.1). These prompts follow the pre-training principle by providing guidance and introducing key ideas, which should reduce

the cognitive strain on learners as they progress through the segments of the learning module (Clark & Mayer, 2011; Mayer & Pilegard, 2014). Following the presentation of these concept prompts, each module segment has an embedded animation from the VCell Animation Collection that presents the biological concepts that are the focus of that module. The development of these animations as a part of the VCell Animation project follows a strict adherence to the seven principles of multimedia learning presented by Mayer and Moreno (2002), adding strength to their design and aiming to increase their effectiveness. Research has also demonstrated that the integration of “thought questions” before and follow-up questions after periods of concept introduction further strengthen student learning and provide a means of formative assessment (Hegarty, 1992, 2004; Huang, 2005; Weston & Barker, 2001). To address this, the VCell modules also provide a series of thought questions at the beginning of each segment (Fig.4.1). These questions provide further structure, focus the student’s attention on important ideas, and prompt higher level thinking while viewing the animations. Following the viewing of the animation clips for a particular section, students are then asked to answer a number of follow up questions on what they have viewed (Fig. 4.2). Students are given immediate feedback on their answers and can be allowed to re-watch the previous animation before progressing if they feel that it is necessary to understand the concepts. To conclude the module, students are given another group of summative questions meant to provide feedback on all of the concepts within the module. The goal of these cumulative questions is to bring together concepts presented in each segment of the module and provide feedback to the learner

to help correct misconceptions that they might have. The experiment described below was designed to test the efficacy of the VCell learning module on the subject of meiosis.

Methods

Participants and Treatment Groups

In order to investigate the effectiveness of an online meiosis learning module as a stand-alone learning tool, we conducted an experiment using participants enrolled in the introductory biology course at a large public university in the southeast United States. Study participants ($n = 534$) self-enrolled in one of four sections of an introductory biology course (Biol101) offered in the fall of 2015. Classroom sections were randomly assigned to one of two treatments. The “online learning module” group ($n = 131$) consisted of two class sections that interacted only with the online meiosis learning module. The “traditional lecture” group ($n = 403$) consisted of two class sections that received instruction on meiosis in a traditional lecture setting. Instructors were aware of their participation in the experiment, however they were asked to make no changes to their typical instructional style. Students assigned to this treatment attended classroom lecture as normal and were not given access to the learning module until the end of the experimental period. Variation in treatment group size was due to uncontrollable variability in student enrollment between course sections, ranging between 68 and 271. Such variation in course section size is common at this university, and instructors typically do not vary teaching strategies between sections.

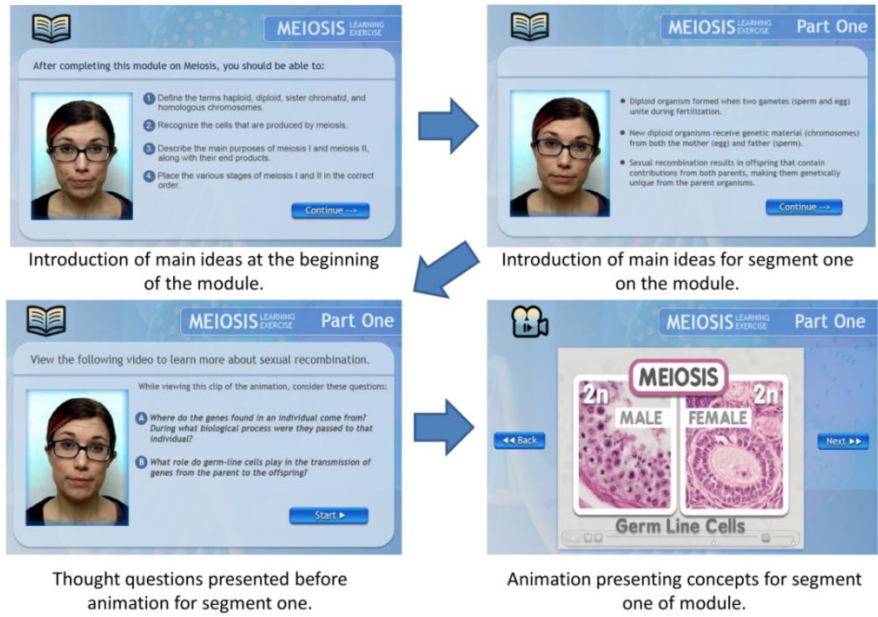


Figure 4.1 – Progression outline for online meiosis learning module

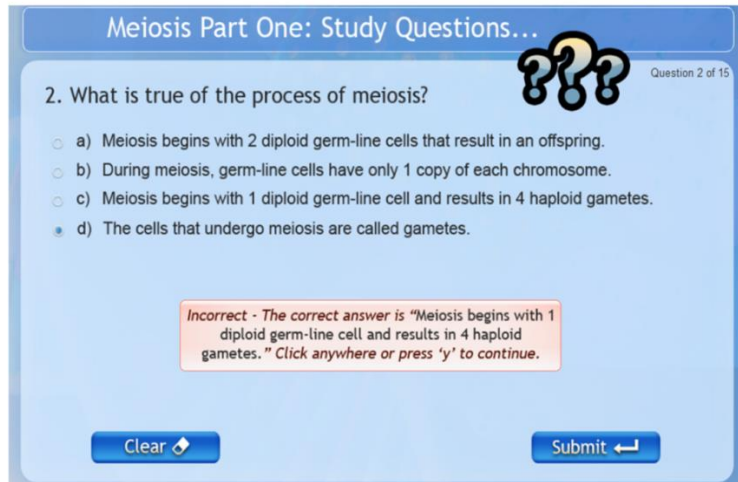


Figure 4.2 – Embedded student self-assessment with feedback upon incorrect response.

Assessment and Measures

Student conceptual understanding was assessed using instruments chosen by the research team for this project. Length of the assessment instruments were

purposely designed to remain relatively short so as to prevent interfering with instructor's course design while simultaneously maximizing student participation. Student participation in both the pre and post-test was 70% for the module group and 76% for the traditional group.

The pretest consisted of twenty five questions that focused on students' basic understanding of a variety of basic biological concepts. Ten questions focused on basic understanding of meiosis and were used to identify treatment outcomes (Appendix H), and five questions covered basic demographic information. In this study, we were only concerned with the ten meiosis questions and the demographic information. The meiosis pretest assessment consisted of five questions from validated concept inventories produced by the Q4B (Questions for Biology) team at the University of British Columbia (Q4B Concept Inventories, 2015; Kalas et al., 2013) and five additional slightly modified questions from the Campbell Biology textbook (Reece et al., 2014) . This textbook was chosen as it was used as the primary text for students in the introductory biology course in this study and represents a large market share of biology texts used nationwide. Questions selected for this instrument from the Q4B team correspond to numbers 2, 7, 12, 14 and 15 on the meiosis concept inventory. Per request of the Q4B project, access to these materials can be granted by contacting the team directly (Kalas et al., 2013). Modification was conducted to make questions more appropriate for introductory learners and consisted of removing confusing phrasing and images that were more representative of upper-level biology course concepts. In order to evaluate student improvement after treatment, the posttest contained the same

meiosis concept questions as the pretest. Cronbach's alpha was used as a measure of internal consistency of the assessment based on the presented sample (Pretest $\alpha = 0.55$; Posttest $\alpha = 0.57$).

While it is likely that introductory biology students have learned about the process of meiosis in high school, studies have shown that they may still harbor misconceptions (Kalas et al., 2013). Common misconceptions include an inability to decipher the number of DNA molecules present in a cell (Kindfield, 1991), misidentification of chromosomal elements and their interaction (Kindfield, 1991; Newman et al., 2012), and misunderstanding of the stages and timing of the cell cycle (C. R. Brown, 1990; Dikmenli, 2010). The assessment instrument implemented in this study directly measures student understanding related to each of these identified misconceptions.

As part of the pretest assessment, students were also asked the following question: "I learn best when information is presented in a visually stimulating (ie: animations/video) fashion". On a five point Likert scale, answers ranged from "Strongly Agree" to "Strongly Disagree". Our follow-up analysis focused on students that self-identified as one of the two possible extremes as these students are most likely sure of their personal preference to multimedia learning techniques. Additional demographic data was obtained from the University registrar (gender, ethnicity, year in school, major, and SAT score) and matched to student performance on the assessment instrument.

Experimental Procedures

At the beginning of the semester, all participants were given the pretest designed to assess the students' baseline understanding of the concepts to be introduced throughout the semester (Fig. 4.3). During the ninth week of the semester, students from both treatment groups were presented the topic of meiosis in their introductory biology course. The module group was assigned the meiosis online learning module as an out of class activity that was to be completed by the student entirely through the Blackboard learning management system. After completing the learning module, students were then directed to complete the posttest that measured students understanding of the presented meiosis concepts. Students were not allowed to revisit the module once it was completed. Students in the traditional treatment attended classroom lecture as normal and were not given access to the learning module until after the experimental period. After classroom instruction, students in the traditional treatment immediately completed the posttest via the Blackboard LMS.

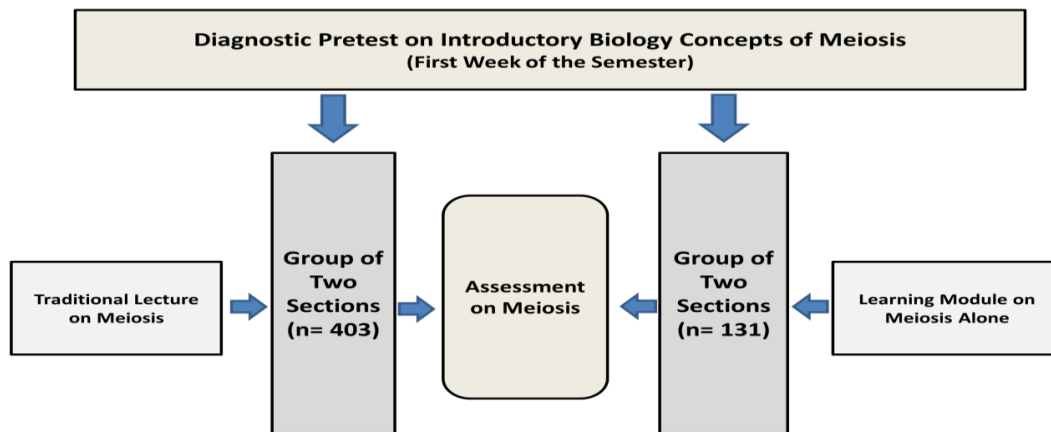


Figure 4.3 - Experimental design assessing the effectiveness of meiosis learning module developed from VCell animations as a stand-alone tool in introductory biology.

All students in this study were assumed to have a previous exposure to the basic concepts of DNA and the cell cycle as well as a general introduction to the process of meiosis as part of their high school education (Kalas et al., 2013; NAP, 2012). Throughout the module development process, assumptions of prior conceptual understanding allowed several aspects of meiosis to be introduced only as review. As one example of this, students are assumed to have a basic understanding of terminology as it relates to meiosis. Terms are introduced throughout as a review often accompanied with an onscreen visual and/or text. If students are familiar with the terms they can flow seamlessly to the next aspect of instruction without wasting time on a more detailed explanation. As a result, the module is focused more on the division events of meiosis without additional extraneous information that may add to the cognitive strain placed on the student (Chandler & Sweller, 1991). Module development was also informed by literature on common meiosis misconceptions. For example, the development team used variation in color and size when creating their depictions of chromosomes because students have difficulty identifying homologous chromosomes (Kindfield, 1991; Newman et al., 2012). These design aspects, along with narration and dynamic onscreen movement allows learners to follow the progression of chromosomal separation throughout meiosis. The added layer of guidance provided by the dynamic nature of the imbedded animation allows students to form more accurate mental models of the processes of meiosis (Williamson & Abraham, 1995). These design elements could also aide students in avoiding misconceptions associated with DNA

count (Kindfield, 1991) and cell cycle progression (C. R. Brown, 1990; Dikmenli, 2010) as well.

The instructors involved in the traditional lecture aspect of the research design were aware of the study being conducted and operated under the same assumptions of prior conceptual knowledge as did the module development team. Instructors did however have the ability to re-address any previous concepts as part of the lecture as they saw fit. Knowledge of misconceptions commonly associated with meiosis was determined by an instructor's own understanding of the literature or learning outcomes from previous semesters. Conceptually, lectures included the same meiosis concepts as were presented in the online learning module. This includes sexual reproduction, ploidy, chromosomal arrangement, cell cycle progression, cell division events, and resulting genetic variability. Content delivery styles however did have some intrinsic differences. The meiosis learning module was developed to be an interactive, personal experience where students observe processes as they happen on screen and then apply their knowledge to directed questions. Progression occurs on the student's own time and they have the ability to review the material multiple times if needed. The traditional lecture group met in a large presentation hall where information was presented as part of projected PowerPoint slides accompanied by instruction from the class professor. Progression generally occurs as dictated by the instructor, and professors tend to vary in their tone and general delivery styles. In addition, student-teacher interaction varies depending on classroom dynamics and student attitude. We attempted to account for aspects by analyzing for a section effect as described below. Ultimately, while the

concepts presented between the two experimental groups were the same, the method in which they were presented was indeed different.

Statistical Analysis

For each aspect of student achievement, descriptive statistics were compiled and inferential analysis run comparing treatment groups using the R statistical programming package (The R project for Statistical Computing, 2015). Normalized gain scores [$G = (\text{post score \%} - \text{prescore \%}) / (100 - \text{prescore \%})$] were calculated for each student that completed all aspects of the study (Hake, 1998). Multiple linear regression analysis was used to investigate the effect of possible explanatory variables on normalized gain scores. In addition to linear regression, we looked at individual demographic variables and analyzed treatment results across each factor. Using independent *t*-tests, we calculated *p*-values comparing treatment groups and calculated 95% confidence intervals for improvement differences between treatments. Cohen's *d*, a mean difference effect size, was reported when significant results were found. Two-way ANOVA was used to investigate possible interactions between treatment conditions and demographic variables.

Data Representation with Beanplots

In order to present our results in the most effective and representative manner, we implemented the use of beanplots as a graphic display of our data. As a variation on a more traditional boxplot, beanplots provide the viewer with additional information regarding the distribution throughout the sample (Kampstra et al., 2008). In viewing

beanplots, distributions are depicted by the width of the plot, with wider plots representing a larger distribution for a specific value. In addition, the mean value of a sample is noted by a bold line within the plot itself. Specifically, results from this study were depicted using asymmetrical beanplots. This allowed for a more direct comparison of the target groups outlined above and a more accurate representation of the data presented as part of our results. For our figures, p -values were also added above comparison groups to identify possible significance.

Results

Analysis of pretest scores between the traditional lecture group ($M = 3.69$, $sd = 1.72$), and the module group ($M = 3.48$, $sd = 1.48$) showed low recollection of concepts relating to meiosis. Student achievement was measured using normalized gain scores calculated from pre/posttest performance for each condition. Students who interacted with the learning module showed significantly higher normalized gain scores than students in the traditional lecture group ($t(317.03) = 4.42$, $p < 0.001$, $d = 0.40$) (Fig. 4.4, Table 4.1). Descriptive statistics relating to individual posttest items show that the learning module group had a higher percentage of students that answered correctly than the traditional group on all questions except for one.

Variable Analysis Using Linear Regression Modeling

In order to investigate the treatment outcomes across additional possible contributing variables we analyzed our data using linear regression modeling. Creation of a predictive model for student normalized gain scores originally included the

following factors: multimedia preference, class section, year in school, gender, ethnicity, pretest score, SAT total score and treatment condition (module/traditional). Our resulting linear regression equation was:

$$X_G = \beta_0 + \beta_1 * X_{\text{multimedia}} + \beta_2 * X_{\text{section}} + \beta_3 * X_{\text{year}} + \beta_4 * X_{\text{gender}} + \beta_5 * X_{\text{ethnicity}} + \beta_6 * X_{\text{pretest}} + \beta_7 * X_{\text{SAT}} + \beta_8 * X_{\text{treatment}} + \epsilon \quad (\text{Eq. 1})$$

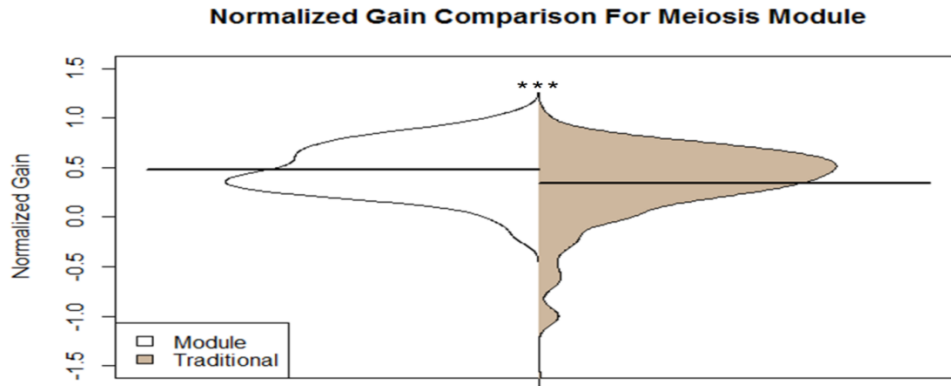


Figure 4.4 - Normalized gain score comparison of meiosis learning module and traditional lecture treatment. (***) $p < 0.001$

Table 4.1- Normalized Gain Score Meiosis Learning Module		
	Module	Traditional
Min	-0.20	-1.67
1st Quart	0.29	0.20
Median	0.43	0.43
Mean	0.47	0.34
3rd Quart	0.67	0.60
Max	1.00	1.00
Std. Dev.	0.26	0.38
95 % CI	0.07 > μ > 0.19	

Regression analysis of equation 1 shows a significant contribution from factors that suggest a prior knowledge, a college preparedness (pretest score and SAT score) component, and the treatment condition that the student received. The remaining factors examined in our model did not show significant contributions to normalized gain scores. We therefore created a more parsimonious model by removing variables with low correlation to student normalized gain score: multimedia preference ($r = 0.03$), year in school ($r = 0.002$), gender ($r = 0.03$), and ethnicity ($r = 0.02$) (Eq.2). Regression analysis again shows a significant contribution of treatment condition ($t(412) = 3.28, p = 0.001, d = 0.32$) to student achievement (Table 4.2).

$$X_G = \beta_0 + \beta_1 * X_{\text{pretest}} + \beta_2 * X_{\text{SAT}} + \beta_3 * X_{\text{treatment}} + \epsilon \quad (\text{Eq. 2})$$

Table 4.2 - Estimated Regression Coefficient for Linear Regression Equation 2 ($R^2 = 0.20, F = 33.6$)			
	Estimated Regression Coefficient	SE	p Value from t Test
Intercept (β_0)	0.07	0.15	0.66
Pretest Score (β_1)	-0.08	0.01	2.0 e -16 ****
SAT Total Score (β_2)	0.0005	0.0001	0.0001 ***
Treatment Condition (β_3)	0.12	0.04	0.001 **
* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.0001$			

Analysis of Possible Section Effect

Due to the variability in both instructor and enrollment numbers across sections, we also used linear regression to test for a possible section effect on assessment scores

across our study population. Initial analysis into section effect using equation 1 resulted in no significant effect of student section on normalized gain score ($t(406) = -0.56, p = 0.58$). Additionally, we refined our testing to account for section effect within treatment groups. Using Eq. 2, we substituted treatment condition for section within the specified condition to give us two models; one for the learning module group and one model for the traditional lecture group (Eq. 3, 4). Neither treatment group showed a significant effect due to the section in which students' received their designated treatment (learning module: ($t(99) = -0.21, p = 0.84$), traditional lecture: ($t(309) = -0.61, p = 0.54$)) (Table 4.3, 4.4).

Online Learning Module Group: $X_G = \beta_0 + \beta_1 * X_{pretest} + \beta_2 * X_{SAT} + \beta_3 * X_{section} + \epsilon$ (Eq. 3)

Traditional Lecture Group: $X_G = \beta_0 + \beta_1 * X_{pretest} + \beta_2 * X_{SAT} + \beta_3 * X_{section} + \epsilon$ (Eq. 4)

Table 4.3 - Estimated Regression Coefficient for Linear Regression Equation 3 (Learning Module Group)			
	Estimated Regression Coefficient	SE	p Value from t Test
Intercept (β_0)	-0.05	0.27	0.85
Pretest Score (β_1)	-0.05	0.02	0.009 *
SAT Total Score (β_2)	0.001	0.0002	0.0006 ***
Student Section (β_3)	-0.01	0.05	0.84
* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.0001$			

Table 4.4 - Estimated Regression Coefficient for Linear Regression Equation 4 (Traditional Lecture Group)			
	Estimated Regression Coefficient	SE	p Value from t Test
Intercept (β_0)	0.19	0.29	0.34
Pretest Score (β_1)	-0.09	0.01	2.27 e -14 ****
SAT Total Score (β_2)	0.001	0.0002	0.006 **
Student Section (β_3)	-0.02	0.04	0.54
* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.0001$			

Linear regression modeling resulted in no significant explanatory effects from the student demographic variables of multimedia preference, gender, year in school, or ethnicity. However with a moderate coefficient of determination ($R^2 = 0.20$), we decided to stratify student outcomes based on treatment condition across each of these factors. Additional analysis allows us to make inferences on the effects of treatment conditions within the spectrum of the individual variable, thus providing further evidence to answer the research question proposed for this study.

Self-Identification of Multimedia Learning Levels

Students in the learning module treatment that self-identified as multimedia learners (“Strongly Agree” selectors) show significantly higher normalized gain score ($t(127.18) = 2.63, p = 0.01, d = 0.39$) when compared to the traditional lecture treatment (Fig. 4.5, Table 4.5). Additionally, self-identified non-multimedia learners (“Strongly Disagree” selectors) show no significant difference in normalized gain score ($t(5.04) = -0.12, p = 0.91$) when comparing module and traditional lecture treatments (Fig.4.5). Two-way ANOVA also shows no significant interaction between treatment condition and multimedia learning preference ($F(1, 527) = 0.45, p = 0.50$). However, we do note that the total number of students ($n = 11$) in the “Strongly Disagree” category could affect the generalizability of our results. This is in contradiction to the disproportionately large number of “Strongly Agree” students ($n = 171$). This dichotomy in multimedia learning preference could also explain why despite contradictory results across learning

preference, linear regression still showed no effect on assessment outcome based on this factor as a whole ($t(406) = -1.41, p = 0.12$).

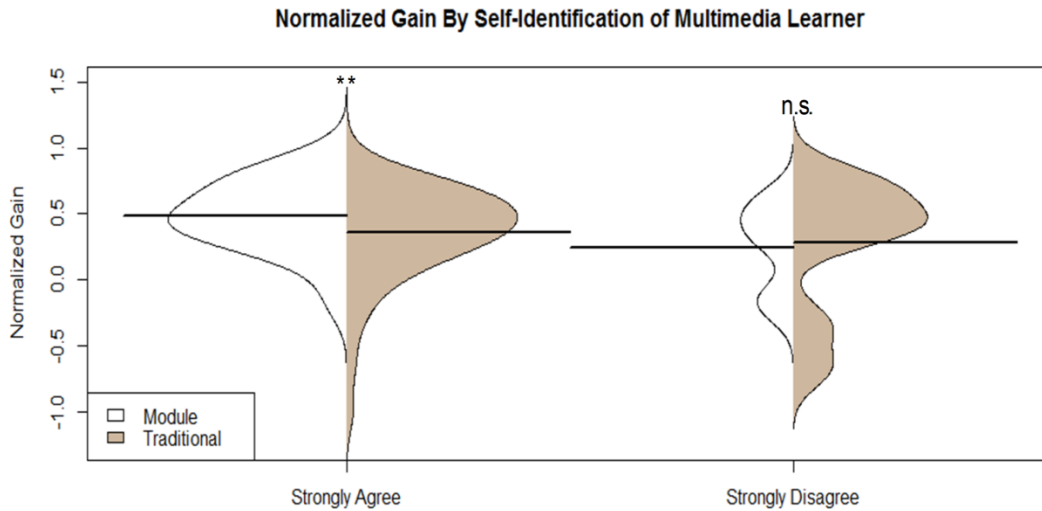


Figure 4.5 - Normalized gain score comparison of treatment by self-identification of multimedia learner. (** $p < 0.01$, ns = not significant)

	Table 4.5- Normalized Gain Score Meiosis Learning Module			
	Multimedia Learner (Strongly Agree)		Multimedia Learner (Strongly Disagree)	
	Module	Traditional	Module	Traditional
Min	-0.20	-1.00	-0.17	-0.67
1st Qu.	0.32	0.22	0.08	0.00
Median	0.50	0.43	0.33	0.42
Mean	0.49	0.36	0.25	0.28
3rd Qu.	0.67	0.60	0.45	0.61
Max	1.00	1.00	0.57	0.78
SD	0.27	0.39	0.38	0.51
95% CI	0.03 > μ > 0.23		-0.75 > μ > 0.67	

Performance as Influenced by Student Gender, Ethnicity, and Year in School

Demographic information was used to examine module performance based on student gender, ethnicity, and year in school. Analysis of assessment performance stratified across student gender (Fig. 4.6, Table 4.6) showed significantly higher normalized gain scores by students in the module treatment group than those in the traditional group for both males ($t(96.68) = 3.05, p = 0.003, d = 0.51$) and females ($t(215.03) = 3.39, p < 0.001, d = 0.37$). Additionally, two-way ANOVA suggests no significant interaction between treatment condition and student gender ($F(1, 527) = 0.40, p = 0.53$).

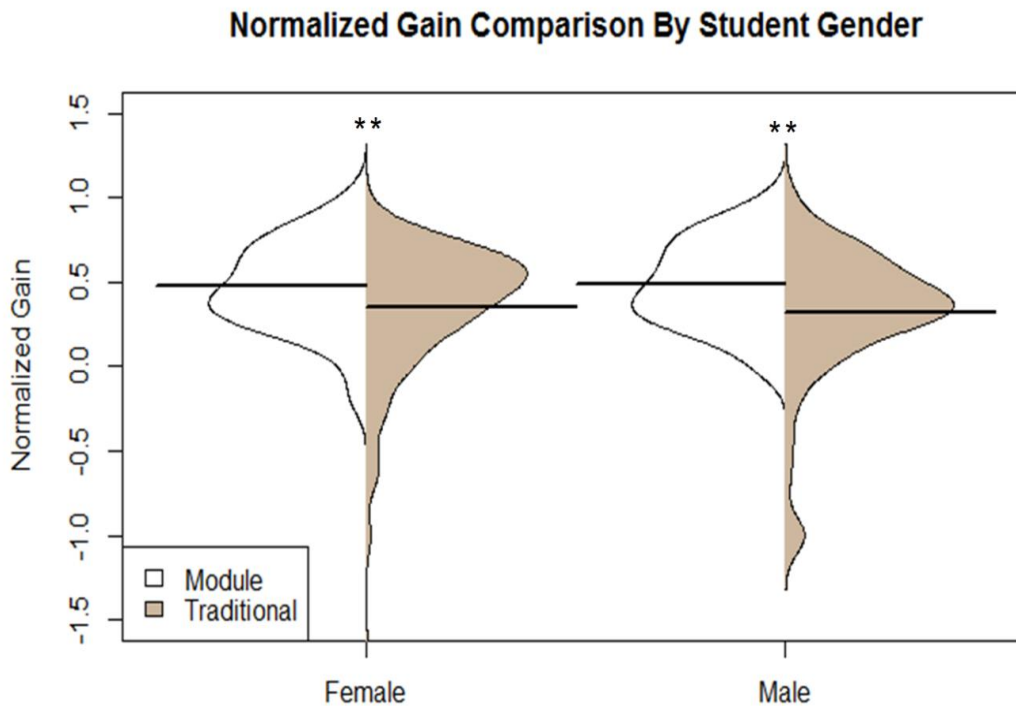


Figure 4.6 - Normalized gain score comparison of treatment by student gender. (** $p < 0.01$)

Table 4.6- Normalized Gain Score Meiosis Learning Module				
	Female		Male	
	Module	Traditional	Module	Traditional
Min	-0.20	-1.67	0.00	-1.00
1st Qu.	0.29	0.20	0.29	0.20
Median	0.43	0.44	0.50	0.35
Mean	0.47	0.35	0.49	0.32
3rd Qu.	0.67	0.60	0.67	0.57
Max	1.00	1.00	1.00	1.00
SD	0.27	0.37	0.25	0.40
95% CI	0.05 > μ > 0.19		0.06 > μ > 0.28	

Stratification by treatment condition as a function of student ethnicity was not possible for this study due to the disproportionate distribution in the ethnicity breakdown (White: 81%, African American: 9%, Asian: 7%, Hispanic: 2%, Other: 1%). It should however be noted that results from linear regression above show no significant effect on normalized gain score based on student ethnicity ($t(406) = 1.32, p = 0.18$).

In regards to students' year in school, due to the introductory status of this course there was a disproportionately small sample number of senior level students enrolled in the class. In order to account for this, we grouped class data into two categories; underclassmen (consisting of freshmen and sophomores), and upperclassmen (consisting of juniors and seniors). Analysis of student performance shows significantly higher normalized gain scores ($t(281.33) = 4.51, p < 0.001, d = 0.33$) for underclassmen that interacted with the online learning module as compared to those whose received instruction in the traditional lecture treatment (Fig. 4.7, Table 4.7). Upperclassmen results show no significant difference in normalized gain score

($t(24.23) = 0.35, p = 0.73$) between treatment groups. Two-way ANOVA also suggest no interaction between treatment condition and student year in school ($F(1, 527) = 0.04, p = 0.70$). It should be noted that the total number of students constituting the upperclassmen group was still small ($n = 32$) which could affect the generalizability of inferences pertaining to significance in the upperclassman comparisons. As was seen previously with learning preference, this small sample size could also possibly explain why, despite differences across categories, linear regression analysis showed no significant contribution of year in school to assessment scores ($t(406) = 0.51, p = 0.19$).

Discussion

In this study we set out to investigate the effectiveness of a learning module that incorporated a meiosis animation developed by the VCell Animation Collection team. Our results show that students who were presented the concepts associated with meiosis by means of a stand-alone learning module performed significantly higher ($p < 0.001, d = 0.40$) on an assignment designed to assess understanding of meiosis than students who received instruction solely in a traditional lecture setting. The module implementation strategies in our experimental design allowed the learning module to be tested as a true out-of-class concept presentation that could act as preparation prior to a classroom meeting. The significantly higher achievement seen in students who were presented information in the learning module condition provides preliminary evidence that the learning module can adequately present students to concepts in settings other than the classroom itself.

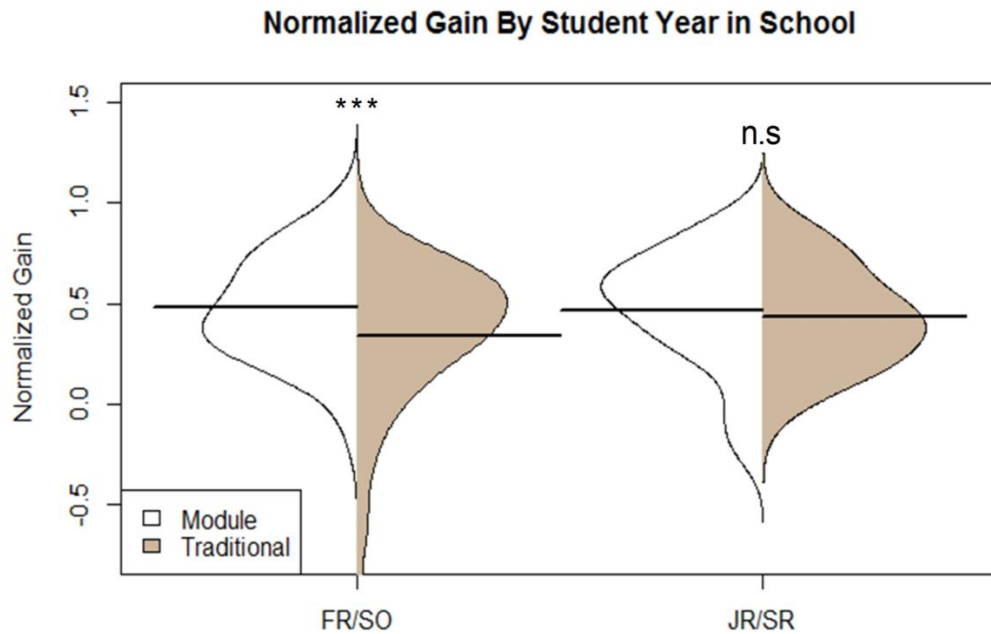


Figure 4.7 - Normalized gain score comparison of treatment by student year in school. (***) $p < 0.001$, *ns* = not significant)

	Table 4.7- Normalized Gain Score Meiosis Learning Module			
	Underclassmen (FR/SO)		Upperclassmen (JR/SR)	
	Module	Traditional	Module	Traditional
Min	-0.20	-1.67	-0.20	0.00
1st Qu.	0.29	0.20	0.31	0.25
Median	0.43	0.43	0.54	0.43
Mean	0.48	0.37	0.47	0.43
3rd Qu.	0.67	0.60	0.67	0.60
Max	1.00	1.00	0.86	0.86
SD	0.26	0.39	0.30	0.24
95% CI	0.08 > μ > 0.20		-0.17 > μ > 0.24	

Descriptive statistics relating to individual posttest items show that the learning module treatment group had a higher percentage of students answering correctly than the traditional lecture group for every question except for one. This question was one of four on the assessment that addressed chromosomal structure, and the learning module group showed a higher percentage of correct responses of the remaining three questions on the concept. In addition, a relatively low percentage (<50%) of students in both groups answered questions number one and nine correctly. Interestingly, both of the questions seem to address the concept of DNA amount through the stages of meiosis which has previously been shown to be a common misconception amongst introductory students (Kindfield, 1991). While we feel that the depth the assessment instrument used in this study does not allow us to make generalizable statements as they relate to specific concepts, a focus on such conceptual understand may be future direction for studies on the interactive learning module tested here.

Contributing Variable Analysis Using Linear Regression Modeling

The experimental conditions of this study did not allow us to randomly assign individual participants to specific treatments. As a result we acknowledge that it can be difficult to determine if the outcomes are truly due to the intervention being tested or the variation in student characteristics within the study (Theobald & Freeman, 2014). In order to help account for this, we created a linear regression model to predict student outcomes on the meiosis assessment. Our model (*Eq. 1*) shows no significant contribution to student scores due to the demographic or multimedia preference

variables that were investigated. The model did however point to prior knowledge (pre-test score) and SAT scores as possible contributors to student normalized gain scores. The relationship between prior knowledge and posttest scores makes sense as students who are more familiar with the material prior to instruction are more likely to achieve consistent scores on assessments after instruction. In regards to SAT scores, while they have been suggested as a possible predictor of freshman college success (Hannon, 2014), their contribution to student meiosis scores in our sample was extremely minimal ($\beta_{SAT} = 0.0005$). This suggests that despite their minor influence within our sample, SAT scores may have little to no contribution to learning outcomes on the topic of meiosis. Of most importance to our study however was the analysis of the contribution of treatment condition (module vs. traditional lecture) to student outcomes. Linear regression analysis showed a significant effect of treatment condition on assessment scores ($\beta = 0.12, p = 0.001$). This suggests that the manner in which meiosis concepts were presented to students in our study did play a significant role in the outcome of their meiosis assessment. Using linear regression, we were also able to show that within treatment conditions there was no significant effect on assessment score due to the section in which the students enrolled (Tables 4.3, 4.4). Regression modeling allows us to show the outcomes demonstrated in this experiment were most explained by the instructional treatment that participants received rather than the other possible contributing variables investigated. In order to provide further support for this, we also decided to stratify student assessment scores across the individual factors investigated in regression analysis. Stratification provided us with a more in depth view of treatment

effects within specific demographic categories, thus furthering the conclusion that the differences in learning outcomes observed can be attributed to the treatment condition.

Self-identification of Multimedia Learning Preference

Previous studies have investigated the possible link between preferred student learning styles and the effective use of multimedia learning tools on a variety of different concepts. While the results have been rather mixed (Carlson, 1991; Ross & Lukow, 2012), we attempted to account for the variability in preference for multimedia learning in our sample population. Previous studies have used a variety of instruments (Kolb, 1984; Ross & Lukow, 2012) to assess student learning styles; however, in our investigation we decided upon a more simplistic approach, allowing students to self-identify their level of multimedia preference. The participants in this study were asked to answer on a Likert scale how well they believe that they learn using multimedia resources such as animation and video. From this data, we selected the subset of students that chose one of the two extremes: strongly agree or strongly disagree. Students that self-identify as having either a strong preference or strong opposition to multimedia resources are thought to be more likely to have specific and memorable previous experiences with multimedia learning tools that could skew their achievement on the meiosis learning module. Our results show that students self-identifying as having a strong preference to multimedia learning resources scored significantly higher when they used the learning module rather than attended a traditional lecture setting ($d = 0.39, p = 0.01$). This outcome is no surprise considering these students already show

a preference to this type of learning. It is, however, of note that there was no significant difference ($p = 0.91$) between treatment groups when students identify strong opposition to multimedia learning tools. This would suggest that even among students who self-identify as being opposed to multimedia learning, the learning outcomes are equally high. However, as noted previously, the low sample number in the “Strongly Disagree” category could challenge any inferences made on this group. Even if we redesigned the analysis to include both those selected “Disagree” and “Strongly Disagree”, the sample size ($n = 28$) would still be disproportionate compared to the “Strongly Agree” group ($n = 171$). We also did not feel comfortable with grouping these two categories together since they could represent wide variation in students’ perception of multimedia learning. As a whole, linear regression still showed no effect on assessment outcome based on this variable. These findings are consistent with recent studies reporting that defined “learning styles” such as these do not effect student learning outcomes (Rohrer & Pashler, 2012). In a large introductory classroom where students from a myriad of educational backgrounds come together, results such as these are important. With interactive multimedia learning tools that, at a minimum, perform equivalently to a traditional lecture setting such as these, instructors can use the learning module investigated here with confidence that they can effectively convey the material needed to a diverse cross-section of students.

Demographic Variation

Recent studies have focused on the call for both a greater overall persistence in scientific majors as well as an increase in students enlisting in STEM majors (Brewer & Smith, 2011; Olson & Riordan, 2012). In order to achieve these reform goals, it is imperative that learning take place across the demographic spectrum that is seen in today's college lecture hall. To ensure this we set out to investigate the performance of VCell learning modules across multiple demographic variables. Our original plan was to analyze the results of the treatment groups across student major, year in school, ethnicity and gender in hopes of investigating achievement in a large classroom setting consisting of students with diverse backgrounds. However, with the introductory status of the course that was used in this investigation, the number of non-STEM major students enrolled in the study was too small ($n = 13$) for us to effectively analyze any treatment effect across student major. Disproportionate distribution also prevented stratification of treatment conditions across student ethnicity. Regression analysis however did not show any significant contribution of ethnicity to assessment outcome for the study presented here.

In demographic factors that we were able to investigate, we did see that when looking at module versus traditional lecture treatment by year in school, underclassmen performed significantly higher on the meiosis assessment when they received instruction solely from the learning module than from a traditional lecture setting ($d = 0.33, p < 0.001$). Upperclassmen, by contrast, show no significant difference in scores

across treatment ($p = 0.73$). It should be noted that the number of upperclassmen enrolled in this course was also rather low ($n = 32$) which could affect the results seen here. This could explain why regression analysis showed no significant contribution of year in school to student assessment outcome. In addition to student year in school, analysis of our results stratified by student gender also showed a significantly higher outcome for students in the module group regardless of gender, suggesting gender uniformity in module performance. Module performance for both males and females in this study again provides instructors confidence in assigning this stand-alone learning tool regardless of their class makeup.

Ultimately, achievement scores on the meiosis assessment in the learning module group were either higher than or on par with those of the traditional lecture treatment across the demographic conditions tested here. These results suggest that the achievement outcomes attained after learning from this learning module are consistent across the demographic variables investigated in this study. This again provides instructors preliminary evidence that this learning module can be used to prepare students with concepts in a setting outside of the lecture hall.

Limitations and Further Investigation

Dissemination of empirically tested learning modules that convey concepts to students despite differences in demographics or learning preference can provide instructors with powerful resources for implementation in a hybrid learning environment. These resources would provide students the preparation that is needed to

reap the benefits of an active learning-centered, flipped classroom environment (Freeman et al., 2014; Gross et al., 2015). While the results from our investigation show that student achievement was significantly higher for the learning module treatment group, we have yet to investigate its effectiveness in an actual flipped classroom setting. In the future, we plan to expand our research on learning modules to a variety of classroom environments including those of flipped format. These studies will focus on the effectiveness of VCell learning modules as compared to other methods of outside instruction such as reading assignments and recorded lectures. We would also like to expand the conclusions that can be made from the results of our future studies, and therefore would redesign our assessment instrument to examine specific concepts in more depth. This would allow us to make stronger conclusions on conceptual understanding of specific aspects of meiosis when learning with interactive modules, and lead to greater understanding of the strengths and weaknesses of the multimedia resources we've developed. This information can then be used to guide revisions to the modules or delineate more specifically when the module(s) may be most effective.

Additionally, we acknowledge that the quasi-experimental design in this study does have limitations. Further investigation using a true experimental design with participant randomization in a controlled environment would reduce the number of extraneous variables seen in this study and would add strength to our inferences. Future projects on the effectiveness of VCell online learning modules plan to include this level of experimentation. We also are creating new learning modules for use in undergraduate biology instruction that will require further investigation. From this

research, we aim to develop and test an entire collection of learning modules for use in introductory level undergraduate biology. This collection would serve as research-tested instructional tools by which instructors at any university can convey basic conceptual understanding to their students, thereby opening classroom time for active learning activities. By making these resources available to institutions nationwide we can provide additional learning resources that reinforce science learning as a whole in an effort to assist with STEM education reform.

Conclusions

The goal in the production of learning modules by the VCell Animation team is to provide high quality online resources designed to convey biological concepts across variation in student demographics and course design. One such course design in which effective learning modules may prove most beneficial is the flipped model of active learning classrooms that have led to higher student achievement in multiple studies (Freeman et al., 2014; Gross et al., 2015; Haak et al., 2011). However it has been noted that in order to achieve the greatest learning outcomes, students must be properly and adequately prepared prior to the class period (Andrews et al., 2011; Gregory, 2009). The results of our investigation show that students using a stand-alone learning module on the topic of meiosis achieved significantly higher outcomes on a meiosis assessment than students that received instruction in a traditional lecture setting alone. We believe that the dynamic, interactive nature of the learning module presented here provides students with cognitive assistance that may promote conceptual understanding. This

together with the ability to provide a one-on-one interaction with the material could aid the module in providing an alternative yet effective environment for students to reinforce ideas about meiosis. These results demonstrate the potential impact of online learning modules. However, we note that additional research is needed to investigate what features modules should have to further improve student learning, if modules appropriately prepare students for active learning activities in class, and how modules can be designed to most effectively prepare students for in class, active learning activities.

4.2 Continuation of Findings (Meiosis Instruction)

In order to obtain a representative cross section of the student enrollment of one entire year at the institution where this study was conducted, the experiments outlined previously in this chapter were again conducted during the following spring semester. Following the same experimental procedure, three additional introductory biology sections were randomly assigned them to treatment conditions (1 section received the traditional lecture treatment and 2 sections received the online module treatment). This provided data on 7 total sections (3 sections receiving the traditional lecture treatment and 4 sections received the online module treatment), spanning one full school year, and representing a total of 658 total introductory biology students. Using the same instrument designed to assess understanding of concepts related to meiosis described previously (Appendix H), student achievement was calculated as normalized gain scores and analyzed for effect of treatment conditions. Results over the

course of one full school year and statistical analysis of possible extraneous contributors to assessment scores are presented below.

Results

Normalized gain scores were calculated across two semesters of study in order to assess student achievement on the topic of meiosis (Hake, 1998). Comparison of treatment groups shows that students who interacted with the online learning module ($n = 184$) on the topic of meiosis have higher normalized gain scores on a concept assessment as compared to those who received the traditional lecture treatment ($n = 474$) (Fig. 4.8/Table 4.8). Further analysis shows that students who interacted with the online learning module ($M = 0.46, SD = 0.26$) showed significantly higher learning gains ($t(483.52) = -4.50, p < 0.001, d = 0.37$) than students who participated in the traditional lecture treatment ($M = 0.34, SD = 0.38$).

Analysis of Selected Extraneous Variables

Using the data from one full year of experimental instruction, further investigation was conducted using analysis of covariance to examine possible extraneous contributors to student achievement. Identical extraneous variables were analyzed as were previously outlined in the most succinct equation for meiosis extraneous variable analysis (Eq. 2). Instead of using linear regression as was reported in the previous publication, we focused on the use of analysis of covariance to examine these variables in a method consistent with the rest of this report. Analysis shows a significant contribution of pretest ($F(1, 476) = 100.66, p < 0.001$) and total SAT score

($F(1, 476) = 51.30, p < 0.001$) on student achievement (Table 4.9). Each of these aspects has been associated as an approximate proxy for prior knowledge and suggests its possible role in learning with online modules. In addition, results again show a significant influence of treatment group on assessment scores ($F(1, 476) = 7.82, p < 0.001$).

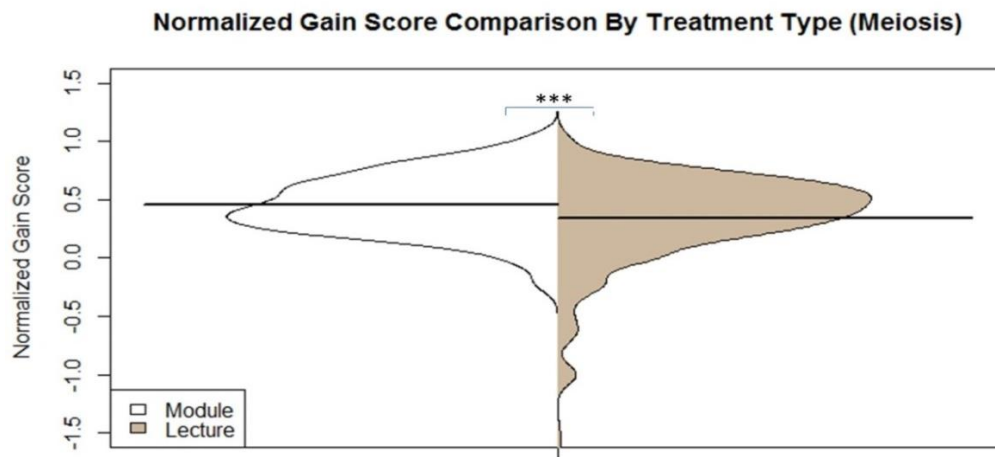


Figure 4.8- Mean normalized gains scores on the topic of meiosis by treatment type. (***) $p < 0.001$

Table 4.8 - Descriptive Statistics for Meiosis Instruction		
Normalized Gain Score		
	Module	Traditional
Min	-0.25	-1.67
1st Quart	0.28	0.20
Median	0.43	0.40
Mean	0.46	0.34
3rd Quart	0.64	0.57
Max	1.00	1.00
Std. Dev.	0.26	0.38
95 % CI	0.17 > μ > 0.07	

Table 4.9 - Analysis of Variance Table for Possible Extraneous Variables (Meiosis)					
Variable	df	Sum Sq	Mean Sq	F Value	p-Value
Treatment Condition	1	21.56	21.56	7.82	< 0.01 **
Pretest Score	1	100.66	100.66	36.48	< 0.001 ***
Total SAT Score	1	51.30	51.30	18.59	< 0.001 ***
Residuals	476	1313.38	2.76		
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$					

Analysis of data for possible section effect

To account for a possible effect of student section subsections were again created defined by treatment condition and one-way analysis of variance was conducted on the effects of student section on normalized gain score. Student section showed no significant contribution to normalized gain scores for neither the online module treatment group ($F(3, 180) = 0.89, p = 0.45$) nor the traditional lecture treatment group ($F(2, 471) = 0.71, p = 0.49$).

4.3 Extensions of Research (Cellular Respiration Instruction)

Much like the previously noted misconceptions related to the concept of meiosis (Kalas et al., 2013; Quinn et al., 2009), cellular respiration has also been a common source of difficulty for introductory biology students (Capa et al., 2001; Songer & Mintzes, 1994). Previous research has shown that introductory biology students harbor many misconceptions concerning cellular respiration that are shown to persist even after instruction (Songer & Mintzes, 1994). Among these are the role of oxygen, the importance of biological gradients, and the role of digested food in the production of

chemical energy (C. W. Anderson, Sheldon, & Dubay, 1990; Capa et al., 2001; Haslam & Treagust, 1987). It has also been noted that instruction on the topic of cellular respiration is not only shown to be difficult for students, but for instructors as well (Igelsrud, 1989; Songer & Mintzes, 1994). In an attempt to address these difficulties in cellular respiration instruction, we have developed an online learning module on the topic that can be used as a means of concept introduction in a stand-alone manner that students can interact with on their own time and in their own environment. Details of the development process of this and other learning modules are outlined in the publication posted previously in this chapter. In addition, the developmental storyboard for this online learning module on the topic of cellular respiration is included as part of the supplemental material (Appendix I).

We attempt to further understanding of the implementation of online learning modules developed using animations produced by the Virtual Cell Animation Collection by extending our research to the topic of cellular respiration. This extension of the research again aims to answer the question, “to what extent do Virtual Cell online learning modules aide in instruction of introductory biology concepts compared to a traditional classroom lecture?” Due to the strict adherence to the published guidelines on multimedia development, we again hypothesize that students who interact with online learning modules on introductory biology topics prior a traditional lecture on the topic will outperform students who do not interact with these materials on a concept assessment. Results of this investigation will further the body of empirical evidence on

the efficacy of online learning modules developed using animations from the Virtual Cell Animation Collection.

Methods

Participants and Treatment Groups

In order to investigate the effectiveness of an online learning module as a stand-alone learning tool, the investigation outlined previously continued with extensions to the topic of cellular respiration. Study participants (n = 629) self-enrolled in one of four sections of an introductory biology course. Classroom sections were again randomly assigned to one of two treatments. The “online learning module” group (n = 341) consisted of two class sections that interacted only with the online cellular respiration learning module. The “traditional lecture” group (n = 288) consisted of two class sections that received instruction on cellular respiration in a traditional lecture setting.

Assessment and Measures

The assessment instrument consisted of twenty five questions that focused on students’ basic understanding of a variety of basic biological concepts. Ten questions focused on general biology concepts, ten questions focused on basic understanding of cellular respiration and were used to identify treatment outcomes (Appendix J), and five questions covered basic demographic information. In this study, we were only concerned with the ten cellular respiration questions and the demographic information. Weighted Bloom’s Index was calculated and found to be 46.67, suggesting a middle-

order of cognitive skill level (Freeman et al., 2011a). In order to evaluate student improvement after treatment, the posttest contained the same concept questions as the pretest. Cronbach's alpha was used as a measure of internal consistency of the assessment based on the presented sample (Pretest $\alpha = 0.54$; Posttest $\alpha = 0.57$).

Student preference for multimedia learning was gathered using the following question with a five-point Likert scale: "I learn best when information is presented in a visually stimulating (ie: animations/video) fashion."

Experimental Procedures

Experimental procedures were similar to those outlined previously in the investigation of meiosis learning. At the beginning of the semester, all participants were given the pretest designed to assess the students' baseline understanding of the concepts to be introduced throughout the semester (Fig. 4.9). At the midpoint of the semester students from both treatment groups were presented the topic of cellular respiration. The module group was assigned the cellular respiration online learning module as an out of class activity that was to be completed by the student entirely through the Blackboard learning management system. After completing the learning module, students were then directed to complete the posttest that measured students understanding of the presented concepts. Students in the traditional treatment attended classroom lecture as normal and were not given access to the learning module until after the experimental period. After classroom instruction, students in the

traditional treatment immediately completed the posttest via the Blackboard learning management system.

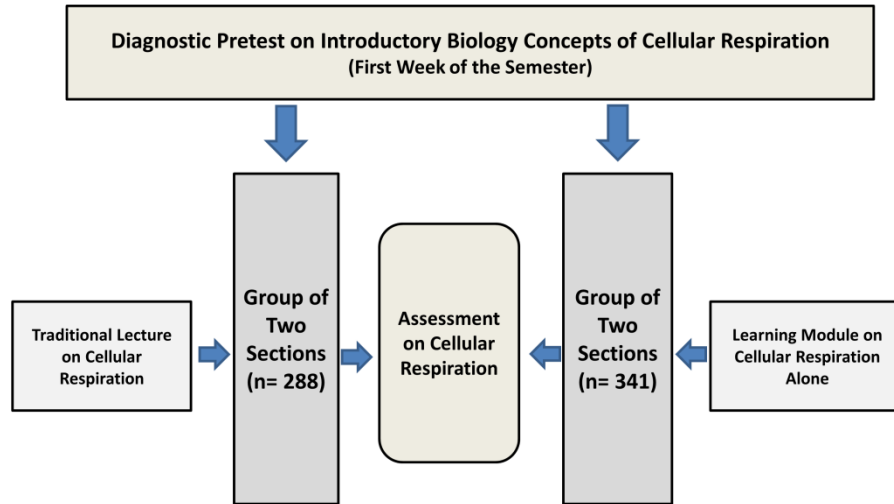


Figure 4.9 - Experimental design assessing the effectiveness of cellular respiration learning module developed from VCell animations as a stand-alone tool in introductory biology.

Statistical Analysis

For each aspect of student achievement, descriptive statistics were compiled and inferential analysis comparing treatment groups was conducted. Normalized gain scores were calculated for each student that completed all aspects of the study (Hake, 1998). Analysis of covariance was used to investigate the effect of treatment as well as possible explanatory variables on student outcomes. Cohen's *d* was reported when significant results were found.

Results

Comparison of treatment groups shows that students who interacted with the online learning module ($n = 341$) on the topic of cellular respiration have higher normalized gain scores on a concept assessment as compared to those who received the traditional lecture treatment ($n = 288$) (Fig. 4.10/Table 4.10). Further analysis shows that students who interacted with the online learning module ($M = 0.56$, $SD = 0.39$) have significantly higher learning gains ($t(437.77) = 7.15$, $p < 0.001$, $d = 0.59$) than students who participated in the traditional lecture treatment ($M = 0.23$, $SD = 0.69$)

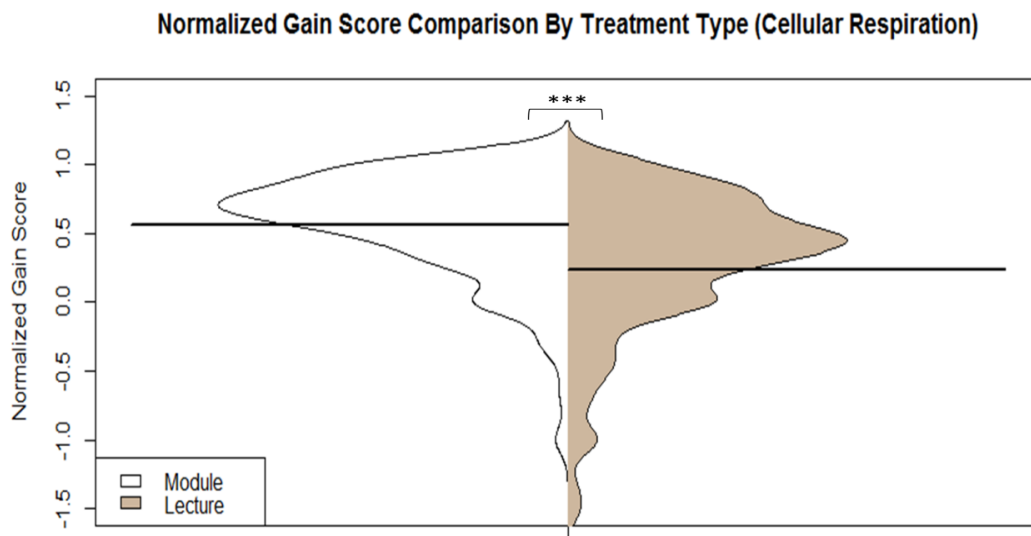


Figure 4.10.- Mean normalized gains scores on the topic of respiration by treatment type. (***) $p < 0.001$)

Table 4.10 - Descriptive Statistics for Cellular Respiration Instruction		
Normalized Gain Score		
	Module	Traditional
Min	-1.00	-4.00
1st Quart	0.33	0.00
Median	0.66	0.40
Mean	0.56	0.23
3rd Quart	0.83	0.67
Max	1.00	1.00
Std. Dev.	0.39	0.69
95 % CI	0.42 > μ > 0.23	

Analysis of Selected Extraneous Variables

Identical extraneous variables were selected as were originally identified in the previous aspect of this study aim. Student year in school was classified as either underclassman (freshman/sophomore) or upperclassman (junior/senior). In addition, student ethnicity was classified as either white or underrepresented minority (URM). Analysis of covariance again shows a significant contribution of pretest ($F(1, 275) = 5.80$, $p = 0.02$) from the extraneous variables tested (Table 4.11). In addition, results again show a significant influence of treatment group on assessment scores ($F(1, 275) = 43.34$, $p < 0.001$).

Table 4.11 - Analysis of Covariance Table for Possible Extraneous Variables (Cellular Respiration)					
Variable	df	Sum Sq	Mean Sq	F Value	p-Value
Treatment Condition	1	132.50	132.50	43.43	< 0.001 ***
Pretest Score	1	17.73	17.73	5.80	0.02 *
Previously Enrollment	1	0.75	0.75	0.25	0.62
Total SAT Score	1	7.00	7.00	2.29	0.13
ACT Composite Score	1	2.37	2.37	0.77	0.38
Multimedia Learning Preference	4	14.59	3.65	1.19	0.31
Year in School	1	0.00	0.00	0.00	0.97
Student Gender	1	0.56	0.56	0.18	0.67
Student Ethnicity	1	8.27	8.27	2.71	0.10
Residuals	275	840.68	3.06		
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$					

Analysis of data for possible section effect

To account for the possible effect of student section on learning outcomes, subsections were created defined by treatment condition and one-way analysis of variance was conducted on the effects of student section on normalized gain score. Student section showed no significant contribution to normalized gain scores for either the online module treatment group ($F(1, 339) = 0.36, p = 0.55$) or traditional lecture treatment group ($F(1, 286) = 0.77, p = 0.38$).

4.4 Discussion of Learning with Stand-alone Online Learning Modules Developed for the Virtual Cell Animation Collection

Recent reports on the state of STEM education have called for reform in a number of different facets. Among these is an emphasis on increasing student interaction with course materials outside of the classroom (Brewer & Smith, 2011; Steve Olson & Riordan, 2012). As one possible resource to meet these needs, we examined the use of online learning modules produced using animations from the Virtual Cell Animation Collection. These learning modules follow strict guidelines as part of their development that have been shown to increase learning gains when incorporated into multimedia resources (Mayer & Moreno, 2002; Mayer & Pilegard, 2014; O'Day, 2010), which may speak to their efficacy in conveying conceptual understanding. Results show that students who interacted with the stand-alone online learning module of the topic of meiosis have higher learning gains than students who only attended a traditional lecture on the topic ($p < 0.001$, $d = 0.37$). Likewise, students who interacted with the stand-alone learning module on the topic of cellular respiration also show higher learning gains than students who attended a traditional lecture on respiration ($p < 0.001$, $d = 0.59$). With previous studies suggesting a prevalence of misconceptions in instruction on both of these topics (Capa et al., 2001; Quinn et al., 2009), these results could have implications for alternative instruction strategies in introductory biology. One setting where these findings may be beneficial is a flipped classroom environment where preparation prior to instruction is key (Gross et al., 2015). Stand-alone learning modules that outperform a traditional lecture setting could provide instructors with trustworthy resources that can be used to present concepts outside of the physical

classroom. This could free up classroom time for other instructional strategies that have been shown increase conceptual understanding; such as active learning (Freeman et al., 2014). Properly developed online learning modules that can deliver content in a fashion equivalent to a traditional lecture could also provide students with an effective means of concept review. Such trusted review materials could serve as a meaningful refresher in upper-level classes that typically build upon introductory concepts. Reinforcement of these basic concepts could provide students a firmer foundation which may promote deeper learning of upper-level concepts. Both of these aspects of online learning modules could have important implications on the learning process in undergraduate biology.

Results of this study also show that the educational benefits of the stand-alone online learning modules tested here were not affected by many of the common extraneous variables that have been previously shown to influence learning with multimedia resources. On the topic of meiosis, only student pretest score and total SAT score were shown to have a significant contribution to student achievement. Pretest scores are often associated with a student's level of prior conceptual understanding. This prior knowledge has also previously been shown to be associated with achievement when interacting with multimedia (L. ChanLin, 2001; Yarden & Yarden, 2010). Considering students often enter introductory biology with various levels of prior introduction on the topic of meiosis (Kalas et al., 2013; Quinn et al., 2009), this may affect the way that students interact with multimedia resources. Students with higher levels of prior knowledge could be more apt to extract deeper conceptual aspects of the

online learning module due to their previous levels of understanding than those who exhibit lower levels of prior knowledge (Yarden & Yarden, 2010). However, total SAT score may affect student achievement on a different level. SAT, and other college entrance exams, have been previously suggested as a predictor for student success at the university level (Hannon, 2014). As part of this predictive success however, it has also been suggested that SAT scores may be directly influenced by a student's cognitive processing ability (Frey & Detterman, 2004). Cognitive processing, like prior knowledge, has also been shown to influence performance with multimedia resources (Chandler & Sweller, 1991; Hegarty, 1992; Wheeler & Wischusen, 2014). The effect of total SAT score on student-outcome in the study presented here may be more of an influence of cognitive processing than that of any predictive qualities of the exam.

Investigation into online learning modules on the topic of cellular respiration also show a possible contribution by student pretest score however the association between student outcome and SAT score is not present on this topic. Much like meiosis, students often enter their undergraduate students with varying levels of prior understanding on the topic of cellular respiration. Due to previous reports on the contribution of prior knowledge to successful interactions with multimedia (L.-J. ChanLin, 1998; Yarden & Yarden, 2010), we again believe that students with higher levels of prior knowledge may be extracting more conceptually from the online learning module due to their previous levels of understanding than those who exhibit lower levels of prior knowledge.

Limitations

While the results of the investigation presented here show the benefits to learning afforded by stand-alone online learning modules as compared to a traditional lecture setting, it would be interesting to see if these benefits are also exhibited when compared to an active learning-centered flipped classroom environment. Research has previously shown that an active learning-centered environment can outperform a traditional classroom setting (Freeman et al., 2014), we would therefore hypothesize that such a comparison would lead to more comparable learning gains. However, we note that such an active learning-centered classroom environment is not typical in most undergraduate institutions.

We also acknowledge that the quasi-experimental design in this study does have limitations and suggest that smaller scale studies may be beneficial. The size and scale of the experiments here present a more realistic view of the undergraduate population at the institution of our study and more appropriately answer the research questions proposed, however qualitative data from smaller scale studies in the future could aid in the development of additional learning modules.

4.5 Conclusions on Learning with Stand-Alone Online Learning Modules Developed for the Virtual Cell Animation Collection

In response to the recent emphasis on effective content interaction outside of the classroom we aimed to answer the question, “to what extent do Virtual Cell online learning modules aid in instruction of introductory biology concepts compared to a traditional classroom lecture?” Using stand-alone online learning modules produced in

accordance with research-supported guidelines of design (McClellan et al., 2005; Reindl et al., 2015), we investigated student/content interactions on two topics that have previously been the source of difficulty for introductory biology students; meiosis and cellular respiration. Results show that in regards to instruction on both meiosis ($p < 0.01$) and cellular respiration ($p < 0.001$), students who interacted only with an online learning module had significantly higher normalized gain scores than those who were introduced to the chosen topics as part of a traditional classroom lecture. Analysis of possible extraneous variables shows pretest scores and total SAT score as possible contributors for the topic of meiosis and only pretest scores as a possible contributor for the topic of cellular respiration. Results of this study provide empirical evidence for the use of online learning modules as a stand-alone form of concept introduction for introductory biology students. Such evidence can provide instructors confidence in these resources and support the use of such materials to complement alternative instruction strategies in the introductory biology classroom.

Chapter 5

Discussion, Suggestions for Further Research, and Conclusions on Learning with Resources from the Virtual Cell Animation Collection in Introductory Biology

5.1 Discussion on Learning with Resources from the Virtual Cell Animation Collection in Introductory Biology

Recent reports on reform in undergraduate STEM education have placed a focus on student-centered learning that includes multiple modes of instruction to accompany traditional lecture strategies (Brewer & Smith, 2011). One of the more innovative of these modes that has recently risen in popularity to meet these calls is the use of multimedia resources as part of course instruction. Reports outlining such resources have shown that the use of multimedia to aid instruction can be beneficial in the learning process for many students in a number of different environments (Asthana, 2008; Mayer, 2009; Mayer & Sims, 1994; Rhodes et al., 2014). However, these benefits are not universal and some studies suggest that inclusion of some types of multimedia as part of instruction can have little, if any, benefit to the learning process (Azer, 2012; Raikos & Waidyasekara, 2014; Tversky et al., 2002). With the persistence of such contradictions, there is an emphasis on the development of multimedia resources with a focus on research-based principles of design (Clark & Mayer, 2011; Hatsidimitris, 2012; Huang, 2005; Mayer et al., 2003; O'Day, 2010). One collection of multimedia resources that has followed these design elements in a meticulous fashion throughout their development is the Virtual Cell Animation Collection (Reindl et al., 2015). Preliminary,

small-scale research into the efficacy of these resources has shown that increased interaction with Virtual Cell animations leads to higher learning gains in biology students as compared a control group (McClellan et al., 2005). As a part of the research presented here, we aimed to further the investigation into the use of multimedia resources produced by the Virtual Cell Animation Collection by expansion to a much larger scale population of students, as well as research into the specific implementation practices that can commonly be seen in the undergraduate biology classroom. We focus on the use of these resources in the introductory biology classroom as it is often a student's first exposure to undergraduate biology instruction and has been emphasized as a focal point of reform by many recent reports (Brewer & Smith, 2011; Steve Olson & Riordan, 2012). Results from this study aim to provide empirical evidence of instructional "best practices" using Virtual Cell multimedia resources as part of introductory biology instruction. Such evidence provides confidence for those introductory biology instructors looking to find effective means of content delivery in an ever-evolving STEM education environment. In addition, results presented here provides guidelines for the implementation of research-based practices that promote increased student/content interaction outside of the classroom and the "flipping" of instruction that has been shown to be beneficial in the learning process (Brewer & Smith, 2011; DeLozier & Rhodes, 2016). Together, this strengthens the pedagogical practices of many undergraduate biology instructors and thereby lead to more knowledgeable biology students who are ultimately better prepared to meet the needs for the future STEM workforce (Steve Olson & Riordan, 2012).

Aim One: Virtual Cell Animations as a Part of Classroom Instruction in Introductory Biology

The current literature on the use of static versus dynamic images as part of introductory biology instruction has shown to be somewhat inconclusive (Ardac & Akaygun, 2005; Tversky et al., 2002). In an attempt to provide evidence for the use of one form of imagery over another to augment classroom instruction, we focused on two introductory concepts that are a common source of difficulty in undergraduate biology; photosynthesis (Södervik et al., 2015) and mitosis (Ozcan et al., 2012). Results of experimentation over two semesters of introductory biology show that students who were presented content with classroom lectures that included dynamic animations showed higher normalized gain scores on assessments on both the topic of photosynthesis ($t(153.4) = 2.90, p < 0.01, d = 0.40$), and mitosis ($t(229.23) = 4.71, p < 0.001, d = 0.59$) as compared to those who were presented lectures augmented only with static graphics. Previous research on the use of dynamic animations has shown that students can form more accurate mental models when animations are used to present such difficult sequential processes (O'Day, 2010; Williamson & Abraham, 1995). Many of the misconceptions connected with the topics in this aim have been associated with students' difficulty interpreting detailed step-wise mechanisms (Ozcan et al., 2012; Parker et al., 2012; Södervik et al., 2015). Results of our investigation suggest the ability of animations produced as part of the Virtual Cell Animation Collection to help students make the mental connections required to understand these difficult concepts. Our experimental design addresses the previously outlined experimental variables that have been noted as possible contributors to unclear findings in the literature (Tversky et al.,

2002). As a result, these findings on the efficacy of Virtual Cell Animations as part of introductory biology classroom instruction support their ability to aide in instruction on the introductory concepts tested. This ability to help in the formation of accurate base representations could provide learners with a stronger foundation on which to build their knowledge while matriculating through their program of study. A stronger foundation could allow students to experience the learning benefits throughout their coursework and possibly lead to an increase in retention of students in STEM majors and a more knowledgeable workforce upon graduation.

In order to show that the achievement gains outlined as part of this aim are independent of other extraneous variables, we used both experimental and statistical means as part of our design. Experimentally, we attempted to control as many of factors that arise from a pseudo-experimental approach as possible by randomizing sections to treatment and controlling for instructional influence throughout the experiment. Despite these attempts, we do recognize that these effects could still be somewhat evident, as is shown by a section effect in one branch of the mitosis experiment. To adjust for such effects, and to analyze for the contribution of possible others, statistical control using analysis of covariance was implemented. As part of this, we controlled for extraneous contributors to students achievement that have been previously associated with variability in multimedia learning (L. ChanLin, 2001; Hegarty & Kriz, 2008; Ruiz-Primo et al., 2011; Wong et al., 2015). Our findings show no significant effect of the extraneous variables tested in the photosynthesis aspect, and a significant effect of only total SAT scores on student posttest score in the mitosis aspect of this study. We note

that SAT score has previously been suggested to be a predictor of academic success for undergraduate students (Hannon, 2014); however there has been little evidence that these scores correlate to the ability to interact with forms of multimedia. In addition, we note that not all students enrolled in introductory biology enter their undergraduate institution with SAT scores and scores from the other common predictive examination, ACT composite score, did not show a significant effect in our study. As a result, we acknowledge that SAT may play a small role in the ability for students to learn certain introductory biology concepts; however we feel that these effects are likely small and could be represent a variation in student cognitive ability rather than their preparedness (Frey & Detterman, 2004).

Results of the first aim of this study suggest the ability of dynamic animation that is implemented as part of instruction to increase achievement when learning difficult introductory biology concepts. These benefits are possibly due to the compensatory effects of animations on topics where students are required to interpret and understand step-wise mechanisms (Höffler & Leutner, 2011). In addition, the Virtual Cell development team's adherence to research-based guidelines for multimedia design could play a role the effectiveness of their implementation. The results reported as part of this study could give instructors confidence in these resources leading to their implementation, and ultimately increased achievement on difficult concepts for students enrolled in undergraduate introductory biology.

Aim Two- Virtual Cell Animations as Part of Instruction Outside of the Classroom

Calls for reform in STEM education have emphasized the need for increased effective student/content interaction with course content outside of the physical classroom (Brewer & Smith, 2011; Steve Olson & Riordan, 2012). These external interactions have traditionally focused on homework assignments that serve as reinforcement of concepts that were previously presented as part of classroom instruction (Anliker et al., 1997; Planchard et al., 2015). However, recent pedagogical innovations have emphasized the role of preparatory activities prior to class meetings as a means of student/content interaction outside of the classroom (DeLozier & Rhodes, 2016; Gross et al., 2015). While the benefits of each format of external interaction has been shown individually, the direct comparison of each form has been limited. Focusing on three topics, ATP synthesis, mRNA processing, and protein synthesis that have previously been shown to be common sources of misconception in introductory biology students we focus on this comparison by using dynamic molecular animations created by the Virtual Cell Animation project to mediate out of class student/content interaction.

Results on all three of the topics tested here show that when compared to a non-treatment control group both the preparation treatment group (ATP synthesis: $p < 0.001$, mRNA Processing: $p = 0.001$, translation: $p < 0.05$) and the reinforcement treatment group ($p < 0.001$, mRNA Processing: $p < 0.001$, translation: $p < 0.001$) performed significantly higher a follow-up assessment. Considering previous literature

suggesting a benefit of both types of student/content interaction outside of the classroom, these findings were not necessarily surprising. However, when directly compared, the effects of preparatory and reinforcement activities using Virtual Cell animations on student achievement showed no significant difference (ATP synthesis: $p = 0.87$, mRNA Processing: $p = 0.61$, translation: $p = 0.48$). This would suggest that it is possible that it is not the timing of student/content interaction that is important but instead the fact that students interact with the content outside of the classroom at all. With timing of interaction being of less importance it could provide introductory biology instructors freedom in their instructional approach as long as they facilitate some level of external student/content interaction. We note that while these results were shown in a traditional biology classroom where a lecture-centered content delivery style typically dominates, the outcome of the comparison between preparatory and reinforcement activities may differ in a “flipped classroom” environment where active learning strategies are prominent. We chose the traditional lecture environment due to its prominence in biology instruction both nationwide (Eagan et al., 2014) and at the institution where this study was conducted. In addition, with the importance of pre-class preparation in the flipped classroom environment (Gross et al., 2015) we felt that using such an instructional setting would bias the findings towards the preparation treatment. Ultimately, we show as part of this study that there was no significant difference in student achievement when dynamic animations were used to facilitate student/content interactions outside of the physical classroom.

The extraneous variables that were examined as part of this aim were selected due to their previously noted contribution to learning outcomes when using multimedia resources. Each of the topics investigated here show no significant contribution to learning outcomes by the extraneous variables tested. Considering the previous evidence suggesting their possible effect on multimedia learning this could be seen as somewhat curious. However, we note that in the experimental design used in this investigation that animation was not the only form of contact between the students and the content material. The dynamic animations were instead used as an external resource that was used outside of the physical classroom to augment an instructor's lecture. This aspect may allow students to avoid the contributions of potential extraneous variables and focus solely on the learning gains that accompany the dynamic animations. Such outcomes could prove beneficial when instructors are planning for the use of dynamic animations as part of introductory biology instruction.

Investigation into the use of dynamic animations produced as part of the Virtual Cell Animation Collection to augment traditional instruction on three introductory topics as either preparation or reinforcement shows that both treatment groups significantly outperform a non-treatment control group that did not view animations outside of the physical classroom. Additionally, it was shown that comparison of treatment groups for each of the topics investigated here that there was no significant difference in learning outcomes when animation was used as preparation when compared to when they were used as reinforcement. The results of this study support the use of dynamic animations to facilitate student/content interactions outside of the physical introductory biology

classroom. These findings could provide introductory instructors empirical evidence supporting the use of dynamic animations produced by the Virtual Cell Animation Collection as part of instruction. In addition, these findings support the use of such multimedia resources as both preparation and reinforcement.

Aim Three: Virtual Cell Animations as Part of Stand-Alone Online Learning Modules

Recent reports on STEM education have emphasized student interactions with course content both inside and outside of the physical classroom (Brewer & Smith, 2011; Steve Olson & Riordan, 2012). Additionally, many undergraduate courses are being moved out of the traditional lecture hall and are being relocated to a completely online environment (Jacobs, 2014). This transition has placed an emphasis on the proper development and implementation of resources to mediate these interactions. To account for this, we investigate the use of stand-alone online learning modules developed by the Virtual Cell Animation Collection to promote learning in introductory undergraduate biology (Reindl et al., 2015). Learning modules created using this collection of resources were developed according to research-based guidelines for multimedia design and aim to introduce students to course content in a stand-alone manner outside of the physical classroom (Clark & Mayer, 2011; Mayer, 2014; Mayer et al., 2003). To investigate the efficacy of these online learning modules, a focus was placed on the introductory concepts of meiosis and cellular respiration. Both of these topics have been previously been associated with misconceptions common to many introductory biology students nationwide (Capa et al., 2001; Kalas et al., 2013; Quinn et

al., 2009; Songer & Mintzes, 1994). Results of our study show that students who interacted only interacted with stand-alone online learning modules on the topic of meiosis ($t(483.52) = -4.50, p < 0.001, d = 0.37$) and cellular respiration ($t(437.77) = 7.15, p < 0.001, d = 0.59$) had significantly higher learning gains than students who only attended a traditional lecture on these topics. These outcomes suggest the ability of these online learning modules to communicate conceptual knowledge to students in an environment independent of the physical classroom. With an effective method of conveying concepts outside of the classroom, instructors could adopt alternative instructional strategies that have previously been shown to promote learning in introductory biology (DeLozier & Rhodes, 2016; Galway et al., 2014).

In an attempt to account for the possible contribution of extraneous variables on student learning with online learning modules, analysis of covariance was implemented. Statistical analysis shows a contribution of prior knowledge ($p < 0.001$) and total SAT score ($p < 0.001$) on learning on the topic of meiosis and only prior knowledge ($p = 0.02$) on learning on the topic of cellular respiration. Prior knowledge has been previously noted to contribute to students' ability to interact with multimedia resources (L. ChanLin, 2001; Jensen, Kummer, & Banjoko, 2013), so its contribution in this study is not a surprise. Like many topics, students typically enter their introductory biology courses with a high degree of variability in their previous exposure to both meiosis and cellular respiration (Capa et al., 2001; Kalas et al., 2013; Quinn et al., 2009; Yarden & Yarden, 2010). This variation could allow students who have a higher degree of previous exposure to concepts to focus on the more specific details presented in an

online lesson while their more novice counterparts cannot (L. ChanLin, 2001; Yarden & Yarden, 2010). As a result, instructors who wish to implement the online learning resources examined here should be aware of these effects of prior knowledge. The contribution of total SAT score to learning gains with the meiosis learning module was interpreted as an expression of student cognitive ability (Frey & Detterman, 2004). Like prior knowledge, cognitive ability has also been previously linked to learning with multimedia resources (Ayres & Paas, 2007), therefore this contribution to learning outcomes is not a surprise. We do however note that SAT scores were not reported for all students in this study and that the other common college entrance exams, the ACT, showed no effect on learning gains. We therefore hesitate to make any broad statements of contribution in this study. Future investigations should note the possible relationship and account for this in extensions on this research.

Results of this final aim of our investigation of multimedia resources developed by the Virtual Cell Animation team suggest the ability of stand-alone online learning modules to facilitate learning on the topics of meiosis and cellular respiration in an environment independent of the traditional classroom setting. Such a resource could allow instructors to move the core introduction of concepts away from the physical classroom, thus freeing out time for the adoption of alternative teaching strategies such as active learning (Freeman et al., 2007). Additionally, resources that allow students to interact with core concepts in an environment of their choosing could provide a means of reinforcement for more advanced students looking to revisit introductory topics (Wenner, Burn, & Baer, 2011). Each of these strategies for the adoption of online

learning modules could feasibly promote a deeper learning of the foundational concepts that are critical building blocks of more complex biological idea. The formation of a firmer foundation through the use these resources could help in both student matriculation as well as career preparation; thereby answering calls for improvement to undergraduate STEM education.

5.2 Limitations and Suggestions for Further Research

The design of the experiments outlined here was chosen in order to investigate learning outcomes of the implementation of multimedia resources produced by the Virtual Cell Animation Collection in a large introductory biology course that is common in many undergraduate environments. Testing these resources in such an environment provides a more realistic view of the use of their use in the typical undergraduate classroom, which adds to the usefulness of the conclusions presented here. Due to the lack of a practical way to randomize such a large number of students to treatment groups we implemented a quasi-experimental approach with a randomization of sections to specific treatments. As we have stated previously, we acknowledge the potential weaknesses of such a quasi-experimental design and have attempted to limit the contribution of extraneous variables both experimentally and statistically. However, future investigation into the use of Virtual Cell resources could benefit from a smaller scale, completely randomized experimental design. Such a design could provide insight into not only the efficacy of multimedia resources as part of instruction but the driving force behinds such learning gains as well. Together with the current study, the results of

such a randomized study could aid in making more powerful conclusions concerning the use of animations outside of the classroom.

In addition to these smaller scale studies, further investigation into the use of multimedia resources as part of introductory biology instruction could include data on content retention over a time. Studies have previously suggested that students who view animations as a part of introductory biology instruction show greater concept retention over time than students who do not view animations (O'day, 2007). While it is outside of the scope of this current research, it would be of interest to investigate such aspects of concept retention when students are presented with experimental treatments outlined as part of this study. Based on the previous studies focused on retention, we would hypothesize that the use of multimedia resources would again promote greater content retention compared to traditional methods. Findings from such research could further support the efficacy of Virtual Cell resources as a part introductory biology instruction.

While the findings presented here are the results of experimental methods used to examine Virtual Cell resources in a traditional lecture style biology class, we acknowledge that further investigation of their benefits as part of an active learning class may be useful. Reports show that in the STEM fields, instruction is still dominated by the traditional lecture format (Eagan et al., 2014), an aspect of pedagogy that is mirrored at the institution of where this research was conducted. In addition, Andrews et al. (2011) shows that the learning benefits of an active learning classroom may

depend on the training of the instructor. However, as STEM instruction gradually migrates to the use of more active learning techniques in the classroom, empirical evidence on the use of the multimedia resources examined here in an active learning centered classroom may become increasingly beneficial.

Finally, while we feel that the research presented as part of this study investigated each aim in the aspect of introductory biology concepts that are common sources of misconception, we acknowledge that results may be topic sensitive. Many biological concepts involved step-wise mechanisms that are often difficult to interpret for introductory students; however, this is not the case with all topics. In addition, many students enter their undergraduate studies with varying degrees of background on biological topics as part of their secondary education. As a result, further investigation using a variety of different topics could therefore provide insight into which areas benefit the most from the use of multimedia resources. These insights could then be used to help develop an instructional “best practice” guide for Virtual Cell resources in the undergraduate introductory biology classroom.

5.3 Conclusions of Learning with Resources from the Virtual Cell Animation Collection in Introductory Biology

Multiple national reports have called for reform in undergraduate STEM education which has placed an emphasis on the development and the experimental investigation of pedagogical best practices in STEM instruction (Brewer & Smith, 2011; Steve Olson & Riordan, 2012). With the ever-evolving environment of many college classrooms, many of these best practices have incorporated multimedia resources to

mediate various aspects of instruction. While the efficacy of these resources at introducing concepts has been shown to vary, the literature on production of educational multimedia provides a number of evidence-based guidelines for production of instructional multimedia resources (Mayer, 2009; Mayer & Pilegard, 2014). In order to further the investigation into the use of multimedia in the introductory biology classroom we focus on one specific collection of molecular animations, the Virtual Cell Animation Collection (Reindl et al., 2015), that was developed with strict adherence to the previously outlined guidelines for multimedia development. Using seven different cellular and molecular biology concepts that have been previously noted as a common source of misconception amongst introductory students, we investigate the role of multimedia in three different aspects of introductory instruction: as part of a classroom lecture, outside of the classroom as either pre-class preparation or post-class reinforcement, and as a stand-alone online learning module.

Results of investigation comparing educational imagery as part of instruction on two introductory topics show that students who viewed dynamic animations as part of instruction exhibited higher learning outcomes than those that viewed static images as part of in-class instruction. These results provide insight into a somewhat murky literature base on the use of imagery in the classroom (Tversky et al., 2002). With the step-wise nature of many introductory biology concepts, the dynamic representation of mechanisms in an animated form could provide a cognitive aide to students in the learning of these often difficult topics. Results from this study provide evidence for the

use of dynamic animation in biology instruction and could lead to the production instructional best practice incorporating animation into classroom lectures.

As a method of mediating student/content interaction outside of the classroom, we also looked animations as a means of either pre-class preparation or post-class concept reinforcement. While the merits of each practice has been examined individually (Bowman et al., 2014; Gross et al., 2015), their direct comparison has been insufficiently investigated. Results of our study show that while both preparation and reinforcement treatment groups have significantly higher learning outcomes than a non-treatment control group in three common introductory biology concepts. However, with all concepts tested the results show no significant differences in learning outcomes when the two treatment groups were directly compared. This could suggest that despite the recent push for course redesigns to accent a “flipped classroom “environment, the timing of student/content interactions may not be as important as the fact that there is simply some type of outside of the classroom interaction. These outcomes could provide instructors pedagogical freedom in the development of assignments for students outside of the classroom while simultaneously providing evidence for the use of animation to mediate these interactions.

The use of online learning modules to deliver instructional content outside of the classroom has become increasingly prominent with the push for online instruction and classroom restructuring (Khalil et al., 2010; Phillips, 2015). The results of the development and implementation of two stand-alone learning modules on the topics of

meiosis and cellular respiration show their ability to promote significantly greater learning outcomes than traditional, lecture-based instruction on the topic. These outcomes could provide introductory biology instructor confidence in such resources which could free up class time for the proven strategies of active learning and scientific learning (Freeman et al., 2007). Additionally, stand-alone modules could provide an effective means of review of course material for those who may be lacking in certain topics. These results aim to promote the widespread adoption of these stand-alone modules as a resource for instruction in introductory biology.

With an ever-evolving instructional environment of many introductory STEM courses, the need for effective, evidence-supported resources to propagate learning has become paramount. By investigating the use of dynamic animations developed as part of the Virtual Cell Animation Collection, we show the ability to multimedia resources to promote learning on multiple difficult introductory biology concepts. In an instructional world that is often dominated by either unsupported or over-priced multimedia packages, the development of an effective, free-to-use collection of resources can be extremely useful. We provide support of such a collection as part of this study and aim to provide resources to promote a deeper understanding of introductory biology concepts. Results of this study provide evidence for resources that answer the call for a more effective and innovative environment in undergraduate STEM education.

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Appendix A: Photosynthesis Assessment Instrument

1. During what stage of photosynthesis is O₂ produced?
 - a. cyclic photophosphorylation
 - b. the light reactions involving photosystems I and II**
 - c. carbon fixation
 - d. the Krebs cycle
2. During what stage of photosynthesis are ATP and NADPH converted to ADP + Pi and NADP+?
 - a. the light reactions
 - b. the dark reactions**
 - c. both of the above
 - d. none of the above
3. In the light reactions, when light strikes the pigments what is the immediate result?
 - a. excited electrons are passed to electron acceptors**
 - b. electrons are fused to form ATP
 - c. glucose is produced
 - d. carbon fixation occurs
4. The dark reaction in photosynthesis is limited by
 - a. CO₂, and light**
 - b. CO₂, light, and water
 - c. water, temperature, and CO₂
 - d. oxygen, water, and temperature
5. The oxygen that is released as O₂ during photosynthesis came from _____ molecules.
 - a. carbon dioxide

- b. **water**
 - c. glucose
 - d. chlorophyll
6. The pigment molecules responsible for photosynthesis are located in the
- a. cytoplasm of the cell
 - b. stroma of the chloroplast
 - c. **thylakoid membrane of the chloroplast**
 - d. all of the above
7. Which of the following is the source of the carbon in sugar produced during photosynthesis?
- a. **carbon dioxide**
 - b. water
 - c. rubisco
 - d. ATP
8. Which of the following occurs in the stroma of the chloroplast?
- a. light dependent reaction
 - b. electron transport chain
 - c. **calvin cycle (aka- the dark reactions)**
 - d. photosynthesis
9. Which of the following statements about photosynthesis is true?
- a. the light reactions can occur only in the light, the dark reactions only in the dark
 - b. photorespiration is more efficient at producing glucose than is photosynthesis
 - c. **the light reactions produce the energy-rich compounds that are used to run the dark reactions**
 - d. all of the above are true
10. Which of the following statements accurately describes the relationship between photosynthesis and cellular respiration?
- a. photosynthesis occurs only in autotrophs; cellular respiration occurs only in heterotrophs

- b. photosynthesis uses solar energy to convert inorganics to energy-rich organics; respiration breaks down energy-rich organics to synthesize ATP**
- c. photosynthesis involves the oxidation of glucose; respiration involves the reduction of CO₂
- d. photosynthesis and cellular respiration occur in separate, specialized organelles; the two processes cannot occur in the same cell at the same time

Appendix B: Photosynthesis Lecture Review

Please view the taped lectures assigned to you making note of if the presenter sufficiently presented the biological concepts listed below. These concepts are selected as the main ideas to be introduced as part of the topic of photosynthesis. Please select either yes or no depending on your opinion as to if the concept was sufficiently conveyed by the presenter. The final question is meant for you to provide any feedback on the presenter's lecture style. In this section please note any major differences in delivery, emphasis, etc that may be evident in the lectures provided to you. Thank you in advance for your participation.

The lecture viewed included: STILL IMAGES / ANIMATION

Photosynthesis Concepts

1. There is a distinct difference between the manner in which autotrophs and heterotrophs obtain their organic compounds. YES/NO
2. The main photosynthetic organelle of a plant cell is the chloroplast. The chloroplast contains pigment containing thylakoid membranes as well as non-pigment containing stroma. YES/NO
3. The light-dependent reactions occur in the thylakoid membranes and use light excited electrons to split water molecules and produce ATP, NADPH and O₂. This reaction requires exposure to light waves to occur. YES/NO
4. The light-independent reactions occur in the stroma and use the high energy compounds ATP and NADPH to convert atmospheric CO₂ into organic compounds This reactions is independent of sunlight however it does not require darkness to occur. YES/NO

5. The connection between cellular respiration and photosynthesis allows both autotrophs and heterotrophs to function in organic compound production in the environment. YES/NO

6. Please note any differences in lecture style that you may have noticed in the taped lectures provided. (Optional)

Appendix C: Mitosis Assessment Instrument

1. Mitosis _____
 - a. Is how cells grow bigger
 - b. Is how cells reproduce and tissues grow**
 - c. Is how cells enlarge
 - d. Is how cells prepare for reproduction
2. The sister Chromatids split completely in which stage? _____
 - a. **anaphase**
 - b. interphase
 - c. telophase
 - d. prophase
3. The four stages of mitosis in their correct order are: - _____
 - a. Prophase, metaphase, anaphase, telophase**
 - b. Prophase, telophase, anaphase, metaphase
 - c. Anaphase, prophase, metaphase, telophase
 - d. Telophase, prophase, anaphase, metaphase
4. During metaphase _____
 - a. the chromosomes duplicate
 - b. the spindle fibers align chromosomes at the center of the cell**
 - c. the cell membrane closes around the new cells
 - d. the chromatids split at the Centromere
5. In which stage do the nuclear envelopes form around the separate sets of chromosomes?
 - a. Anaphase
 - b. Interphase
 - c. Telophase**
 - d. Prophase
6. During interphase _____
 - a. the cell grows
 - b. chromosomes start to duplicate
 - c. the cell prepares for mitosis
 - d. all of the above**
7. What structure inside the cell helps pull the chromatids apart? - _____
 - a. Centromere
 - b. Spindle fiber**
 - c. Nucleus
 - d. membrane

8. The genetic information for an organism is found _____
- a. **In the cell nucleus**
 - b. In the cytoplasm
 - c. In the cell membrane
 - d. In the golgi body
9. Mitosis makes _____
- a. 4 unique cells
 - b. 2 unique cells
 - c. 4 identical cells
 - d. 2 identical cells**
10. What does the cell create during the s stage of the cell cycle?
- a. more organelles
 - b. a copy of DNA**
 - c. 2 daughter cells
 - d. greater surface area

Appendix D: Mitosis Lecture Review

Please view the taped lectures assigned to you making note of if the presenter sufficiently presented the biological concepts listed below. These concepts are selected as the main ideas to be introduced as part of the topic of mitosis. Please select either yes or no depending on your opinion as to if the concept was sufficiently conveyed by the presenter. The final question is meant for you to provide any feedback on the presenter's lecture style. In this section please note any major differences in delivery, emphasis, etc that may be evident in the lectures provided to you. Thank you in advance for your participation.

The lecture viewed included: STILL IMAGES / ANIMATION

Mitosis Concepts

1. Mitosis functions in living things to repair/replace dead and damaged cells, and to aid in growth and development of the organism. YES/NO
2. The cell cycle is comprised of interphase (made up of G1, S, and G2 stages) followed by a period of cell division known as m-phase (made up of mitosis and cytokinesis). Interphase is the main component of the life cycle of the cell and is followed by a shorter period of division (m-phase). YES/NO
3. Cells within an organism can progress through the cell cycle and different rates based on their function within the organism. YES/NO
4. Mitosis is comprised of five stages (prophase, prometaphase, metaphase, anaphase, and telophase) that function to divide genetic material between two identical daughter cells. YES/NO
5. Plant cells require the formation of a cell plate in order to separate daughter cells at the end of mitosis due to the presence of the plant cell wall. YES/NO
6. Please note any differences in lecture style that you may have noticed in the taped lectures provided. (Optional)

Appendix E: ATP Synthesis Assessment Instrument

1. The initial source of energy for oxidative phosphorylation is _____.
 - a. substrate-level phosphorylation
 - b. kinetic energy that is released as hydrogen ions diffuse down their concentration gradient**
 - c. NADH and FADH₂
 - d. ATP
 - e. ATP synthase
2. Where are protons pumped during chemiosmosis in aerobic respiration?
 - a. Out of the mitochondria into the cytoplasm
 - b. Out of the membrane of the cell into the extracellular matrix
 - c. Out of the mitochondrial matrix and into the outer compartment of the mitochondria**
 - d. Out of the cytoplasm of the cell and into the mitochondrial matrix.
 - e. Out of the nucleus and into the mitochondria
3. ATP that is produced from substrate-level phosphorylation requires _____.
 - a. Cytochrome C
 - b. An input of extraneous energy
 - c. A concentration gradient of protons
 - d. a high-energy phosphate group that is transferred directly to ADP**
 - e. all of the above are needed
4. In a concentration gradient across a membrane, particles will move until
 - a. all particles have settled on the side that originally contained a higher concentration.
 - b. all particles have settled on the side that originally contained a lower concentration.
 - c. both sides of the membrane have equal concentration then stop completely.
 - d. both sides of the membrane have equal concentration then continue minimal movement back and forth across the membrane.**

- e. none of the above.
5. In liver cells, the inner mitochondrial membranes are about five times the area of the outer mitochondrial membranes. What purpose must this serve?
- It provides for an increased rate of the citric acid cycle
 - It provides for an increased rate of glycolysis.
 - It provides liver cells to survive with less mitochondria than other cell types
 - It increases the surface for substrate-level phosphorylation.
 - It increases the surface for oxidative phosphorylation.**
6. In a mitochondrion, if the matrix ATP concentration is high, and the intermembrane space proton concentration is too low to generate sufficient proton-motive force, then
- ATP synthase will increase the rate of ATP synthesis.
 - ATP synthase will stop working.
 - ATP synthase will hydrolyze ATP and pump protons into the intermembrane space.**
 - ATP synthase will hydrolyze ATP and pump protons into the matrix.
 - None of the above will occur
7. Oxygen diffuses from the blood cells down its concentration gradient. As cells become more active and oxidative phosphorylation increases in the cell, which of the following occurs?
- The concentration gradient for oxygen decreases and oxygen movement into the cell decreases.**
 - The concentration gradient for oxygen increases and oxygen movement into the cell decreases.
 - The concentration gradient for oxygen decreases and oxygen movement into the cell increases.
 - The concentration gradient for oxygen increases and oxygen movement into the cell increases.
 - The concentration gradient for oxygen and its rate of movement into the cell do not change
8. During chemiosmosis
- Energy is generated by coupling exergonic reactions with other exergonic reactions

- b. Energy is generated because H⁺ ions move freely across mitochondrial membranes
 - c. ATP is synthesized when H⁺ ions move through a protein port provided by ATP synthase**
 - d. A concentration gradient is generated when large numbers of H⁺ ions are passively transported from the matrix of the mitochondrion to the mitochondrion's intermembrane space
 - e. H⁺ ions serve as the final electron acceptor
9. A mutant protist is found in which some mitochondria lack an inner mitochondrial membrane. Which of the following pathways would be completely disrupted in these mitochondria
- a. Glycolysis
 - b. Oxidative phosphorylation**
 - c. Alcoholic fermentation
 - d. The Krebs cycle
 - e. The Krebs cycle and glycolysis
10. Which of the following statements is false in regards to the inner mitochondrial membrane?
- a. The inner mitochondrial membrane has multiple electron carriers associated with it.
 - b. There is a proton gradient associated with the inner mitochondrial membrane.
 - c. Chemiosmosis is associated with the inner mitochondrial membrane.
 - d. The inner mitochondrial membrane plays a role in the production of pyruvate**
 - e. ATP synthase is associated with the inner mitochondrial membrane

Appendix F: mRNA Processing Assessment Instrument

1. Select which of the following statements below that is false in regards to RNA splicing events.
 - a. RNA splicing removes introns
 - b. RNA splicing is mediated by the spliceosome
 - c. RNA splicing always occurs in the nucleus
 - d. All of the above statements are true**
2. In mRNA processing, which of the following is added to the 3' end of a mRNA molecule?
 - a. Approximately 250 Uracil molecules
 - b. a poly-A tail**
 - c. a methylated guanine
 - d. all of the above are added to the 3' end of a mRNA molecule
3. Which of the following is added to the 5' end of messenger RNA?
 - a. a methylated guanine**
 - b. an adenylated adenine
 - c. an aminated cytosine
 - d. a hydroxylated thymidine
4. The poly A tail of a mRNA molecule allows for:
 - a. Passage of the mRNA out of the nucleus**
 - b. Splicing of the coding sequences on the RNA molecule
 - c. Formation of a protein
 - d. Proper folding of mRNA molecules
5. The stages of RNA processing result in:
 - a. formation of a protein
 - b. Formation of a copy of a DNA molecule
 - c. Formation of a mRNA molecule that can leave the nucleus**
 - d. Formation of precursor mRNA that remains in the nucleus

6. During the process of splicing, the RNA segments joined to one another by spliceosomes are _____.
a. The 5'cap and Poly (A) tail
b. Coding sequences on the RNA molecule
c. snRNPs
d. non-coding sequences on the RNA molecule
7. Spliceosomes are composed of _____.
a. snRNPs and other proteins
b. polymerases and ligases
c. the RNA transcript and protein
d. snRNPs and snurps
8. Introns are
a. coding sequences found only on the RNA molecule
b. coding sequences found only on the DNA molecule
c. non-coding sequences that can remain a part of mRNA molecules with no effect on the resulting protein
d. non-coding sequences that are typically removed during the processing events
9. Blocking of the stages of RNA processing would result in:
a. Production of proteins with non-coding information
b. Misfolding of proteins
c. Inability of the mRNA to leave the nucleus
d. Production of proteins that are larger than expected
10. Why is the mRNA not equal in length to the DNA it was transcribed from?
a. the mRNA was longer because it has a Poly A tail
b. The mRNA was longer because it contains only introns
c. The DNA was shorter because it does not have the Methylated cap
d. The mRNA was shorter because of Intron splicing

Appendix G: Translation Assessment Instrument

Questions 1-8 are excluded by request of Q4B Concept Inventory (Kalas et al., 2013)

1. Which of the following molecules are the products of translation?
 - a. DNA
 - b. amino acids
 - c. messenger RNAs
 - d. proteins**
 - e. cells
 - f. chromosomes

2. In which of the following processes does a nucleic acid exhibit catalytic activity?
 - a. DNA synthesis
 - b. RNA synthesis
 - c. Protein synthesis**
 - d. Meiosis

Appendix H: Meiosis Assessment Instrument

Questions 1-5 are excluded by request of Q4B Concept Inventory (Kalas et al., 2013).

6. _____ most closely resembles events of mitosis except that the cells are _____.
- interphase, diploid
 - meiosis II, diploid
 - meiosis I, haploid
 - meiosis II, haploid**
7. One of the earliest events that distinguishes meiosis occurs in prophase I and involves:
- Condensation of chromosomes
 - Loss of the nuclear membrane
 - Movement of chromosomes towards the metaphase plate
 - Pairing of homologous chromosomes**
8. The process of meiosis produces four cells with nonidentical chromosomes. The event that produces distinctive chromosomes occurs during:
- telophase I
 - prophase I**
 - metaphase II
 - prophase II
9. In a eukaryotic cell, DNA replication results in an increase in the
- Amount of DNA in that cell**
 - Number of chromosomes in that cell
 - Number of spindle fibers in that cell
 - Ploidy of that cell (e.g. from 2n to 4n)

10. Which of the following is unique to mitosis and not a part of meiosis?
- a. homologous chromosomes pair forming tetrads
 - b. homologous chromosomes cross over
 - c. chromatids are separated during anaphase**
 - d. homologous chromosomes behave independently

Appendix I: Cellular Respiration Module Storyboard

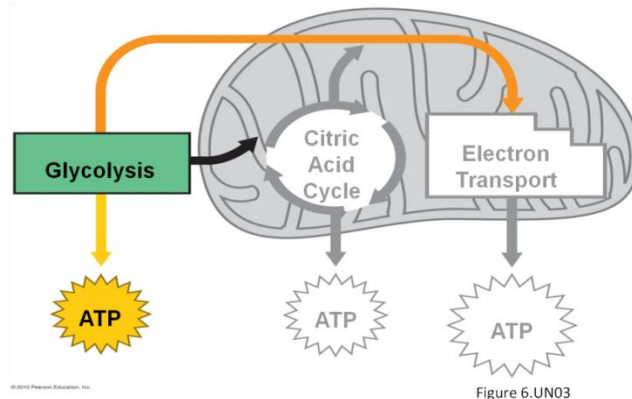
Slide One: Cellular Respiration: An Overview

Spoken narration with still image outlining these points

The purpose of this learning module is to present the process of cellular respiration as a series of coordinated enzyme-catalyzed reactions that capture free energy from simple carbohydrates.

- Cellular respiration integrates three individual stages: Glycolysis, the Citric Acid Cycle, and the Electron Transport Chain (coupled with chemiosmosis).
- During these stages, the chemical energy stored in glucose molecules is transformed into high energy phosphate bonds in ATP molecules that are then available for cellular work.
- The overall reaction for cellular respiration is: Glucose + Oxygen -> Carbon Dioxide + Water + Energy (ATP)

Highlight each stage as it is mentioned in the description



Slide Two: (Stage One: Glycolysis)

Spoken narration with an image outlining these points on Glycolysis

- The first stage of cellular respiration is glycolysis. This process occurs in the cytosol of the cell.
- During glycolysis, the bonds in glucose molecules are rearranged to produce two pyruvate molecules, NADH, and ATP.

- The pyruvate and NADH formed during glycolysis may be transported from the cytosol to mitochondria, where they are used in subsequent phases of cellular respiration.
- The end products of glycolysis are: Pyruvate, ATP, and NADH

Slide Three: (Stage One: Glycolysis)

Spoken narration with glycolysis images while presenting these thought questions. Start it with something along the lines of, “while watching the following animation, consider the following thought questions”as in the other modules.

- Where in a cell does glycolysis occur?
- What is the function of ATP in the preparatory (first) phase of glycolysis?
- What products of glycolysis can be immediately used and what products travel to the next stage of cellular respiration?

Slide Four: (Stage One: Glycolysis Animation)

Glycolysis (overview) animation: 00:10 – 0:48

Slide Five: (Stage One: Glycolysis Questions) (incorrect answers give feedback and correct answer)

- 1.) The molecule that most commonly begins the series of reactions that make up glycolysis is a(n) _____.
 - a. ATP molecule
 - b. NADH molecule
 - c. Glucose molecule
 - d. Oxygen molecule

Slide Six: (Stage One: Glycolysis Animation)

Glycolysis (overview) animation: 0:48 – 1:21

Slide Seven: (Stage One: Glycolysis Questions) (incorrect answers give feedback and correct answer)

- 1.) Glycolysis requires energy to begin the process of converting glucose into pyruvate. What is the source of this initial energy investment?
 - a. ATP molecules
 - b. NADH molecules
 - c. Glucose
 - d. Oxygen

Slide Eight: (Stage One: Glycolysis Animation)

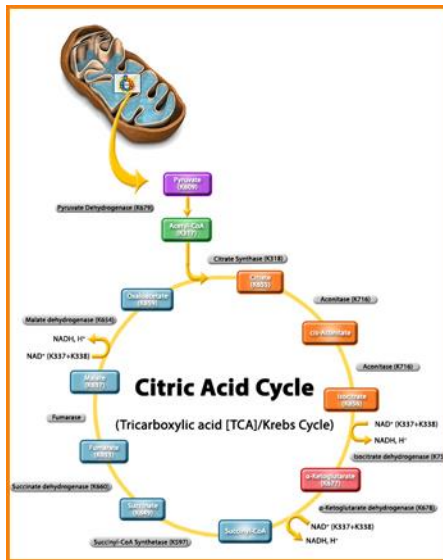
Glycolysis (overview) animation: 1:23- 2:40

Slide Nine: (Stage One: Glycolysis Questions) (incorrect answers give feedback and correct answer)

- 1.) What is the function of the NADH molecule produced during glycolysis?
 - a. It serves as an electron carrier
 - b. It helps produce ATP at a later step in cellular respiration
 - c. Both of the above
 - d. None of the above
- 2.) Which of the following products of glycolysis continues on to later steps of cellular respiration?
 - a. Pyruvate
 - b. NADH
 - c. Both of the above
 - d. None of the above

Slide Ten: (Stage Two: Citric Acid Cycle)

Spoken narration with image of the citric acid cycle outlining these points on the Citric Acid Cycle.



- Upon completion of glycolysis, pyruvate is transported into the mitochondria where it is converted to acetyl-CoA. This process is known as pyruvate oxidation.
- The acetyl-CoA (not pyruvate) then enters the citric acid cycle where ATP is produced, electrons are captured by electron carriers (NADH and FADH₂), and carbon dioxide (CO₂) is released.
- The Citric Acid Cycle is also commonly known as the Krebs's Cycle

Slide Eleven: (Stage Two: Citric Acid Cycle)

Spoken narration with CAC image while presenting these thought questions. Start with, “while watching the following animation, consider the following thought questions” ...as in the other modules.

- What is the function of pyruvate oxidation?
- What is the fate of the products of the citric acid cycle?

Slide Twelve: (Stage Two: Citric Acid Cycle Animation)

Citric Acid Cycle (overview) animation: 00:10 – 0:55

Slide Thirteen: (Stage Two: Citric Acid Cycle Questions)

- 1.) The Citric Acid Cycle occurs in the _____.
 - a. Cytosol
 - b. Mitochondrial matrix
 - c. Outer mitochondrial membrane
 - d. Plasma membrane

Slide Fourteen: (Stage Two: Citric Acid Cycle Animation)

Citric Acid Cycle (overview) animation: 0:55 – 1:50

Slide Fifteen: (Stage Two: Citric Acid Cycle Questions)

- 1.) The electrons released during the citric acid cycle are used to make what electron carriers?
 - a. H₂O
 - b. NADH
 - c. FADH₂
 - d. All the above
 - e. B + C
- 2.) Progression through the citric acid cycle results in the loss of carbon molecules that ultimately form _____.
 - a. pyruvate
 - b. carbon dioxide
 - c. glucose
 - d. citric acid

Slide Sixteen: (Stage Two: Citric Acid Cycle Animation)

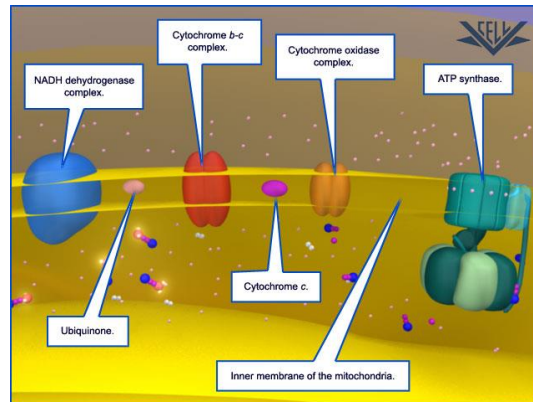
Citric Acid Cycle (overview) animation: 1:50 – 2:26

Slide Seventeen: (Stage Two: Citric Acid Cycle Questions)

- 1.) Pyruvate oxidation converts pyruvate produced in glycolysis into _____.
 - a. Acetyl-CoA
 - b. Citric acid
 - c. Glucose
 - d. Acetate

Slide Eighteen: (Stage Three: Electron Transport Chain and Chemiosmosis)

Spoken narration with image of ETC outlining these points on the ETC



- The next stage of aerobic respiration is the electron transport chain.
- The function of the electron transport chain is to form a concentration gradient that is then used to produce ATP during chemiosmosis
- NADH and FADH₂ formed during glycolysis and the citric acid cycle, are transported to the inner mitochondrial membrane for use in the electron transport chain.
- As electrons from these carriers move in a step-wise fashion through the electron transport chain, free energy is used to pump hydrogen ions across the membrane into the intermembrane space. This pumping of hydrogen ions creates a concentration gradient across the inner mitochondrial membrane.
- The electrons ultimately react with an oxygen molecule resulting in the production of water.

Slide Nineteen: (Stage Three: Electron Transport Chain and Chemiosmosis)

Spoken narration with ETC image presenting these thought questions. Start with, “while watching the following animation, consider the following thought questions”....as in the other modules.

- How does the movement of electrons through the electron transport chain create a concentration gradient across the inner mitochondrial membrane?
- What role does this concentration gradient play in the production of ATP?
- What role does oxygen play in the movement of electrons through the electron transport chain?

Slide Twenty: (Stage Three: Electron Transport Chain and Chemiosmosis)

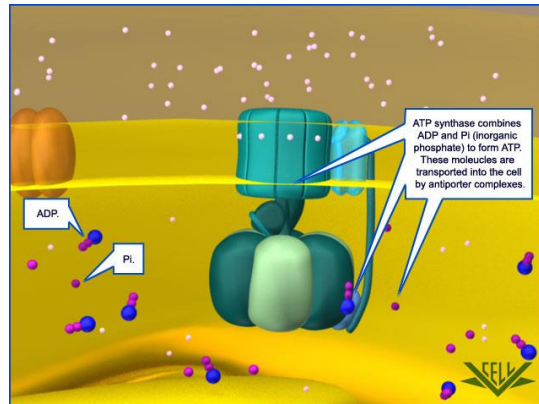
Electron transport chain animation: 1:38 – 3:02

Slide Twenty one: (Stage Three: Electron Transport Chain and Chemiosmosis Questions)

- 1.) The movement of electrons through the electron transport chain provides free energy for the pumping of _____ across the inner mitochondrial membrane.
 - a. Hydrogen ions (protons)
 - b. Oxygen molecules
 - c. ATP
 - d. Electrons
- 2.) The final electron acceptor at the end of the electron transport chain is _____.
 - e. Hydrogen
 - f. ATP
 - g. Oxygen
 - h. NADH

Slide Twenty two: (Stage Three: Electron Transport Chain and Chemiosmosis)

Spoken narration with image outlining these points on chemiosmosis



- A hydrogen ion concentration gradient exists between the inner and outer mitochondrial membrane.
- Potential energy from this concentration gradient is used to produce ATP.
- The passage of hydrogen ions from one side of the inner mitochondrial membrane to the other is known as chemiosmosis. The movement of these ions allows an enzyme (ATP synthase) to join together ADP and inorganic phosphate to produce ATP.

Slide Twenty three: (Stage Three: Electron Transport Chain and Chemiosmosis)

Spoken narration with talking head presenting these thought questions.

- Why is chemiosmosis dependent on the actions of the electron transport chain?
- Where did the electrons that pass through the ATP synthase molecule originate?

Slide Twenty four: (Stage Three: Electron Transport Chain and Chemiosmosis)
ATP synthase (gradients) animation: 0:41 – 1:27

Slide Twenty five: (Stage Three: Electron Transport Chain and Chemiosmosis Questions)

- 1.) Concentration gradients are formed when there is a higher concentration of a molecule on one side of a biological membrane than another.
 - a. True
 - b. False

Slide Twenty six: (Stage Three: Electron Transport Chain and Chemiosmosis)
ATP synthase (gradients) animation: 1:25 – 2:39

Slide Twenty seven: (Stage Three: Electron Transport Chain and Chemiosmosis Questions)

- 1.) ATP synthase uses the movement of _____ across the mitochondrial matrix to produce ATP molecules.
 - a. Hydrogen ions (protons)
 - b. Pyruvate
 - c. NADH
 - d. None of the above
- 2.) Blocking the formation of a hydrogen ion gradient across the inner mitochondrial membrane would have what effect on ATP production?
 - a. An increase in the ATP production
 - b. A decrease in the ATP production
 - c. No effect on ATP production
 - d. You cannot tell from the given information

Slide Twenty eight: (Cellular Respiration Summary)

Spoken narration with the same image from slide one outlining these points as a summary

- Cellular respiration involves three stages (Glycolysis, Citric Acid Cycle, and Electron Transport Chain) that function together to capture free energy for use in cellular work.
- Throughout cellular respiration energy is transformed from chemical energy stored in the bonds of glucose, to high energy

phosphate bonds found in ATP molecules. These high energy phosphate bonds represent a more usable form of energy than those seen in the glucose molecule.

Slide Twenty nine: (Cellular Respiration Summary Questions)

- 1.) Energy initially stored in the bonds of glucose molecules ultimately can be found in the bonds of what molecule?
 - a. ATP
 - b. CO_2
 - c. NADH
 - d. ATP synthase
- 2.) The NADH molecules produced during glycolysis can ultimately aide in production of ATP during chemiosmosis.
 - a. True
 - b. False
- 3.) The formation of a hydrogen ion concentration across the inner mitochondrial membrane is possible due to the properties of _____.
 - a. Potential energy from a concentration gradient
 - b. NADH and FADH_2 donating electrons from other processes in cellular respiration
 - c. The Electron Transport Chain
 - d. All of the above
- 4.) If the transport of pyruvate to the mitochondria were blocked, what would be the resulting effect on the products of cellular respiration?
 - a. ATP production would decrease
 - b. NADH production would decrease
 - c. CO_2 levels would increase
 - d. ATP production would increase

Appendix J: Cellular Respiration Assessment Instrument

1. In the presence of oxygen, cells oxidize glucose completely to carbon dioxide and water according to the chemical equation: $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$

In the process, about 35 molecules of ATP are generated per molecule of glucose oxidized, so that some of the energy released by oxidation is recovered as usable chemical energy.

The principal role of O_2 in this process is to:

- a. **accept electrons released by glucose oxidation, forming H_2O .**
 - b. supply the oxygen for CO_2 production.
 - c. react with glucose to cleave it into smaller fragments for further oxidation.
 - d. participate as a reactant in generation of ATP from ADP and Pi.
2. Glycolysis requires an initial investment of energy to begin the process of breaking down simple sugars. Where does this energy come from?
 - a. NADH molecules
 - b. ATP molecules**
 - c. Water
 - d. Oxygen
 3. NADH and $FADH_2$ both function to:
 - a. serve as a means of immediate energy
 - b. remove waste produced in the stages of cellular respiration
 - c. transport electrons to the electron transport chain**
 - d. directly produce energy in the form of ATP
 4. The Citric Acid Cycle begins with what molecule?
 - a. pyruvate
 - b. NADH
 - c. ATP
 - d. Acetyl- CoA**

5. Carbon atoms are released during the Citric Acid Cycle in the form of _____?
- a. NADH
 - b. $C_6H_{12}O_6$
 - c. CO_2**
 - d. Carbon Monoxide
6. If a cell was incapable of producing NADH and $FADH_2$, what would be the ultimate result?
- a. Increase in the production of CO_2
 - b. Increase in the production of ATP
 - c. Decrease in the production of ATP**
 - d. Decrease in the production of CO_2
7. Concentration gradients formed as part of the electron transport chain are a direct result of an uneven distribution of what ion?
- a. Hydrogen**
 - b. Oxygen
 - c. Glucose
 - d. Nitrogen
8. If a cell were incapable of forming a concentration gradient across the inner mitochondrial membrane which of the following would no longer function?
- a. Glycolysis
 - b. Pyruvate oxidation
 - c. Citric Acid Cycle
 - d. ATP synthase**
9. Which stage of in cellular respiration would still occur if the cell had no oxygen present?
- a. Glycolysis**
 - b. Pyruvate Oxidation
 - c. Citric Acid Cycle
 - d. Electron Transport Chain
10. Which form of energy is not represented in the stages of cellular respiration?
- a. Chemical energy stored in the covalent bonds of sugars
 - b. Chemical energy stored in the high energy phosphate bonds of ATP

c. Potential energy from uneven distribution of hydrogen ions across a membrane

d. All of the above forms are represented

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