APPROACHING CHALLENGES IN ANIMAL CONSERVATION: UNDERSTANDING ELEPHANT REPRODUCTIVE BIOLOGY AND UTILIZING ANIMAL BEHAVIOR RESEARCH EXPERIENCE TO ENGAGE STUDENTS IN SCIENCE

By

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To Holly, who inspired me to be all that I am
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<td>AI</td>
<td>Artificial Insemination</td>
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<tr>
<td>Ac</td>
<td>Acrosome</td>
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<tr>
<td>ACT</td>
<td>Adaptive Control of Thought</td>
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<td>AN</td>
<td>Annulus</td>
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<td>ART</td>
<td>Assisted Reproductive Technologies</td>
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<td>ATP</td>
<td>Adenine TriPhosphate</td>
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<td>AV</td>
<td>Artificial Vagina</td>
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<td>AZA</td>
<td>Association of Zoos and Aquariums</td>
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<td>CASA</td>
<td>Computer-Assisted Sperm Analysis</td>
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<td>CBS</td>
<td>Cross Banded Structures</td>
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<td>CCTT</td>
<td>Cornell Critical Thinking Test</td>
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<td>CD</td>
<td>Central Doublets</td>
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<td>CITES</td>
<td>Convention of International Trade in Endangered Species of Wild Fauna and Flora</td>
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<tr>
<td>CITT</td>
<td>Center for Instructional Technology and Training</td>
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<tr>
<td>CLC</td>
<td>Cholesterol-Loaded Cyclodextrins</td>
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<td>CP</td>
<td>Connecting Piece</td>
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<td>CSRDE</td>
<td>Consortium for Student Retention Data Exchange</td>
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<tr>
<td>CURE</td>
<td>Classroom Undergraduate Research Experience</td>
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<td>DM</td>
<td>Dense Material</td>
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<td>EAZA</td>
<td>European Association of Zoos and Aquaria</td>
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<td>EE</td>
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<tr>
<td>EEP</td>
<td>European Endangered Species Programme</td>
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<td>FAc</td>
<td>Folded Acrosome</td>
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<td>FS</td>
<td>Fibrous Sheath</td>
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<td>HM</td>
<td>High Motility</td>
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<td>HTU</td>
<td>Horse Teaching Unit</td>
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<td>IBL</td>
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<td>IUCN</td>
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<td>LC</td>
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<td>Moderate Motility</td>
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<td>MP</td>
<td>Midpiece</td>
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<td>PC</td>
<td>Proximal Centriole</td>
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<td>Species Survival Plan</td>
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<tr>
<td>STEM</td>
<td>Science, Technology, Engineering, Mathematics</td>
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<td>SURE</td>
<td>Survey of Undergraduate Research Experience</td>
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<td>TM</td>
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<td>TEM</td>
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<td>ZPD</td>
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Abstract of Dissertation Presented to the Graduate School of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

APPROACHING CHALLENGES IN ANIMAL CONSERVATION: UNDERSTANDING ELEPHANT REPRODUCTIVE BIOLOGY AND UTILIZING ANIMAL BEHAVIOR RESEARCH EXPERIENCE TO ENGAGE STUDENTS IN SCIENCE

By

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Major: Animal Sciences

To address two current challenges in animal conservation, this dissertation examined approaches to increase student engagement in science and issues in reproductive biology affecting Asian elephants. The specific objectives were to: 1) identify the optimum course delivery platform for an undergraduate animal behavior research course; 2) assess student opinions and learning gains after participating in a research course; 3) review the literature to identify underlying causes of poor captive elephant semen quality; and 4) assess the morphometrics and ultrastructure of elephant semen samples of varying quality, and compare sperm biology between elephants and their closest relatives (manatee and hyrax). To address objectives one and two, an animal behavior course was created and offered at both the University of Florida and Santa Fe College. Students were required to plan, conduct, and report on an animal behavior research project of their own design. Traditional, online, and flipped teaching methods were compared by assessing student academic performance and critical thinking. To evaluate student opinions of the course, the Classroom-based Undergraduate Research Experience (CURE) survey was conducted. Science,
Technology, Engineering and Mathematics (STEM) retention was measured by assessing student majors before and one year after course enrollment. Overall teaching format influenced grades and critical thinking, and positive effects of the flipped format were seen at both institutions. High learning gains (above national average) and STEM retention (99.3%) were reported. These results suggested this undergraduate research course taught in the flipped method may be a good model to increase STEM retention. In regard to objectives three and four, a literature review revealed changes to management and husbandry, combined with new protocols for semen collection and assessment, may be needed to improve elephant semen quality. Morphometric data showed measurements of sperm cells were similar between high and low-quality samples and the ultrastructure of poor semen samples was similar to previously reported literature in the elephant. Initial comparisons of sperm biology between the manatee and the hyrax suggested elephants and manatees may be more closely related than the elephant and the hyrax, which could provide insight into new approaches to improve elephant reproductive success.
CHAPTER 1
INTRODUCTORY REMARKS

Many challenges to animal conservation exist. Two key examples of these challenges in education and biological sciences include student interest in studying science and the conservation of elephants that are bred and managed in captivity.

Student retention across Science, Technology, Engineering, and Mathematics (STEM) fields is declining, and the United States is falling behind other countries in science education (Lowell et al., 2009; Provasnik et al., 2009). Student engagement in science may depend on opportunities to apply key concepts early in their academic career. Therefore, there is a critical need to develop and deliver research experiences to a greater number of undergraduates early in their collegiate careers, while assessing which teaching method suits these students best. Students in the information age are radically changing how they think and learn and may prefer a more student centered and active learning approach (Prensky, 2001, 2005; McLaughlin et al., 2014). While many teaching approaches have been examined over the years, it is important in higher education to assess different methods to find approaches that are best suited for digital age learners. While traditional and online courses have been accepted for several years, the new and emerging flipped approach has been shown to improve student learning and satisfaction in some cases (McLaughlin et al., 2013; Mortensen and Nicholson, 2015).

In Chapter 3 of this dissertation, I evaluate the effects of teaching delivery formats on student learning and critical thinking of undergraduate students to help refine teaching methods for these students. A course entitled “Wild Discoveries” was created to help address this issue. Wild Discoveries is an undergraduate research-based
course where students explore animal behavior research and plan, conduct and assess a project of their own design. Measuring and analyzing animal behavior was chosen for the course because we believed it would be more accessible and engaging to students. In addition, the area of animal behavior and welfare is in need of increased public awareness (Mench, 2008). This course was taught at both the University of Florida and Santa Fe College. The course was offered in three different teaching formats: traditional, online and flipped, to discover which format was best suited to modern students and the effect on learning in a research-based class. Student's academic achievements and critical thinking scores were assessed to understand which teaching method fulfilled these criteria best.

Another large area of concern is the loss of students in STEM degrees. It has been suggested that this could be due to student confidence, motivation and interest (Crisp et al., 2009; Seymour and Hewitt, 1997). One way to increase learner interest is to engage students in research experiences, especially during their most informative and impactful years; for example, during their freshman and sophomore years of college (Lopatto, 2007). Students that have participated in undergraduate research show a higher chance of continuing in a STEM degree, but most aren’t aware of research opportunities or don’t have access or time to participate in them. Therefore, introducing these research options as a course may be a good approach to increase STEM interest (Russell et al., 2007).

In Chapter 4 of this dissertation, I report the effects of involvement in an undergraduate research-based course on STEM retention in first and second year students. The same Wild Discoveries course that is described in Chapter 3, was also
used for this objective, with the exception that it only focused on students from the University of Florida. Besides addressing course delivery format, another major goal of the course was to introduce freshman and sophomores to research early in their collegiate careers to encourage them to continue in a STEM degree. Comparisons of learning gains, opinions and attitudes about science and STEM retention were evaluated to analyze the effect of this course on students.

In addition to studying the challenges involved in educating and engaging modern students in the STEM fields, this dissertation focused on a key conservation crisis involving biological approaches for improving reproductive success in elephants. Asian elephants are considered endangered and African elephants are vulnerable (IUCN 2017). Elephants are housed in captivity in an attempt to create self-sustaining breeding programs to increase elephant populations and to increase public awareness about elephant conservation and status. A major hurdle impeding the success of these breeding programs is that semen samples are highly variable and inconsistent within and among bulls of both elephant species (Kiso et al., 2011; O’Brien et al., 2013a,b; Imrat et al., 2014). Without good semen quality, Assisted Reproductive Technologies (ART) cannot be applied to elephants, which may be the only way to increase elephant population. Therefore, in Chapter 5 of this dissertation, I review different factors that influence semen quality and identify potential paths to resolving this issue in captive elephant reproduction.

Studies on semen quality need to be addressed to further understand and pinpoint the differences and similarities between high and low-quality samples. Sperm morphometrics and ultrastructure comparisons could reveal important information to
help us understand the mystery of poor semen quality in captive elephants. Another approach involves using phylogenetic relationships to assess the differences in reproductive biology. By comparing sperm biology between the elephant’s (*Elephas maximus*) closest relatives: the manatee (*Trichechus manatus*) and the hyrax (*Procavia capensis*), we may get a better understanding of evolutionary relationships (Gage, 1998; Maree, 2011). Also, the comparative research between these three species and discoveries relating to variation in sperm quality could lead to improved protocols for enhancing semen quality in elephants. For these reasons, in Chapter 6 of this dissertation, I review the morphometrics and ultrastructure of high and low-quality elephant semen samples and further compare these traits to the manatee and hyrax.

In summary, this dissertation addresses two key issues important for animal conservation issues. Firstly, the involvement and engagement of the next generation of students in STEM is necessary to improve public awareness of current critical conversation challenges, such as poor semen quality in elephants. By exposing students to research earlier in their careers, we expect them to develop a deeper understanding and appreciation for the scientific method and that these positive experiences will lead to further research. With increased numbers of students engaged in STEM majors, the chance of future researchers discovering new approaches for improved breeding programs to save elephants from extinction also increases. Also, providing students with a unique opportunity to participate in research that is not normally available can help them to learn about the many different types and areas of research. The ultimate goal is that these experiences will help train and prepare
tomorrow’s science leaders to think critically and develop the research skills needed to solve critical conservation issues and possible alter the future of the elephants.
CHAPTER 2
EDUCATION: A REVIEW OF THE LITERATURE

Introduction to Education Research

There is a critical need to evaluate pedagogical delivery platforms best suited for undergraduates in the information age. The population of students entering the higher education system today are diverse and radically changing how they think and learn (Prensky, 2001; 2005). It is evident now more than ever, that a switch from the ‘industrial-age’ education system to the ‘information-age’ education system is needed (Watson and Reigluth, 2008). Educational teaching methods are constantly evolving, but the practical application of new teaching methods is a slow process. Furthermore, there is evidence that the United States is falling behind other countries in science education (Provasnik et al., 2009) creating a further need to refine teaching methods and increase the number of students in Science, Technology, Engineering and Mathematic (STEM) degrees.

This chapter will discuss the theoretical and conceptual framework that helped to guide the two education Chapters of this dissertation (Chapters 3 and 4). This review focuses on learning theories, types of learners, pedagogical approaches, education in the information age and STEM education.

Learning Theories

Teaching and learning theories are at the heart of education. In each classroom and teaching method used today, different learning theories are used to guide instruction. The five main learning theories that still exist in many teaching environments today and that will be discussed in detail below are: behavioral theory,
Behavioral Theory

In behavioral theories, learning is the process of forming associations between stimuli and response and features of the environment serve as cues for learners’ response (Schunk, 2012). For example, when reinforcement is applied, it strengthens a behavior and increases the future likelihood of the behavior when similar stimuli are present. John Watson was considered to be the founder and champion of modern behaviorism (Heidbreder, 1933). He believed that for psychology to become a science it required observable and measurable phenomenon and this type of phenomena was ‘behavior’ (Watson, 1924).

Thorndike’s Connectionism is a behavioral theory that has influenced inquiry-based learning and critical thinking today. Thorndike first described ‘trial and error’ learning where successful responses in behavior are established and unsuccessful ones are abandoned (Schunk, 2012). Testing of thoughts and ideas through trial and error is a large part of the scientific method used today. However, inquiry-based learning involves cognitive processes allowing learners to critically think about such situations. Thorndike stated that teachers should help students to form good habits and teach them how to apply educational content that they have learned (Thorndike, 1912).

Operant conditioning principles from Skinner’s Operant Conditioning theory are applied to many aspects of teaching and learning today (Schunk, 2012). Operant conditioning can also be viewed as ‘learning by doing’ (Lesgold, 2001). Skinner believed that instruction is more effective when: 1) Teachers use small steps when presenting material, 2) Active learning rather than passive is implicated, 3) Immediate
feedback is given to learners, and 4) Learners move through material at their own pace (Skinner, 1954; Schunk, 2012). He also believed that instruction involves shaping student behavior from an initial behavior to the desired behavior (Schunk, 2012).

Observing and measuring behavior is still used in the education system today to assess student learning. While one can apply aspects of behavioral theories to increase student learning and success, thought and information processes are also very important to student learning and success.

**Social Cognitive Learning**

The effect of the environment on learners’ outcomes is consistent with behavioral and social cognitive theories. However, Bandura believed that there was more to learning that just behavior and that reciprocal interactions among behaviors, environmental variables, and personal factors such as cognition and self-regulation shape the learning and performance of behaviors (Bandura, 2001; Zimmerman and Schunk, 2003). Learners process information within their environment through interactive (enactive) and observational (vicarious) learning. Self-regulation refers to personal agency and learners desire to self-regulate important life aspects with goals in mind (Zimmerman and Schunk, 2001). Modeling provides learners with information about consequences of actions and motivates observers to act accordingly (Schunk, 2008). Rosenthal and Zimmerman (1978) found that using modeling along with explanation is more effective than just explanation alone and cognitive modeling includes verbalization and demonstration of the thoughts and reasons for performing specific behaviors or actions (Meichenbaum, 1977).

Therefore, social cognitive theory focuses on gaining information and ideas from the social environment while involving cognitive processes. Teachers should be models
of all information in both their actions and their instruction and use verbalization and
demonstration to explain their thinking and encourage self-efficacy. Peers can further
serve as models and motivate other peers to act accordingly when they are successful
and perform well.

**Information Processing Theory**

Information processing theory encompasses all theoretical perspectives that deal
with the sequence and execution of cognitive events (Schunk, 2012). It focuses on how
people react to environmental events, encode the information they received and either
relate it to knowledge that already exists in their memory, or store new knowledge
(Shuell, 1986). Once information is stored in one’s memory, it can be retrieved as
needed. Unlike the behaviorist theory where learners respond to information that is
imposed on them, the information processing theory suggests that learners are active
seekers and processors of information (Mayer, 1996).

Atkinson and Shiffrin (1968; 1971) proposed a two-store (dual) memory model to
explain information processing. Information processing begins with an input (e.g.
visual). This input is briefly held in a sensory form until the sensory register transfers it
to short-term memory. Here information is either related to information in the long-term
memory, or it is encoded and rehearsed to a new memory, or in some cases forgotten.
Due to the short time frame that sensory memory holds stimuli (~1-3 seconds) it is
important that this information is encoded quickly (Miller, 1956). Maintaining attention
and captivating students is critical to encoding information and transferring it to long-
term memory. Concepts that are related to knowledge already known or stored, can aid
in information encoding and reorganizing of experiences through adding new ones or
changing old ones involved.
One alternative to the two-store model is the levels or depth of processing which suggests that memory is affected by the type of information processing rather than the location of it (Craik and Lockhart, 1972; Craik and Tulving, 1975). There are three different levels: physical (surface), acoustic (phonological sound), and semantic (meaning). The physical level is the most superficial with each level after representing a deeper type of processing. The deeper information is stored, the better the memory.

Activation level is another alternative that is similar to both of the aforementioned models. This view suggests that different memory structures don’t exist, but there is one memory with active or inactive states (Anderson, 1990). Active information can be accessed quickly and can be maintained in an active state through rehearsal. Rehearsal can also increase activation spread to activate inactive portions of the memory.

The Gestalt theories view learning as a cognitive phenomenon where experiences are reorganized into different perceptions of people, events or things (Koffka, 1922; 1926). Figure-ground relation, proximity, similarity, common direction, simplicity, and closure are types of principles that people use to organize their perceptions (Koffka, 1922; Kohler, 1959). However, this theory is no longer accepted because it does not explain how people perceive items as similar in the first place. Anderson’s adaptive control of thought (ACT) theory proposes that people store meanings rather than precise wordings and networks and common elements are required (Anderson, 1990).

Information processing theories illustrate the importance of organized, comprehensive material regardless of the type of teaching method selected. Connecting information to real world examples and understanding similarities and
differences between information is important. Paivio (1971) hypothesized a dual-coding theory where verbal associations and visual imagery were found to be two ways that a person could expand on their knowledge. Reinforcement and rehearsal help to increase information maintained in the memory and the more that knowledge in the long-term memory is encountered, the stronger it will be (Baddeley, 1998). Therefore, information processing theories lend a wealth of information to the design of instructions on how students store and retrieve information they have learned.

**Experiential Learning**

Several scholars, notably, John Dewey and Jean Piaget were important in the construction of the experiential learning theory (Kolb, 1984). There are six main portions to experiential learning: 1) Primary focus should be on the process of learning not the outcomes, 2) Learning is in fact relearning and refining ideas, 3) Learning requires moving back and forth between reflection and action, as conflict, differences and disagreement drive learning, 4) Learning is a holistic process including thinking, feeling, perceiving and behaving, 5) Synergetic transactions between people and the environment result in learning, and 6) Learning is the creation of knowledge. Kolb (2014) describes experiential learning as a learning cycle. It begins with learners partaking in a concrete experience. Next, they participate in reflective observation of their experience, leading to conceptualization of their experience. This conceptualization allows them to further understand what this experience means to them, further increasing their understanding. At this point the individual begins seeking more information about this experience or developing different ideas or opinions from the experience. Finally, learners enter active experimentation where they test their new ways of thinking.
The application of experiential learning is to teach learners how to manage and control learning through inventing their style, emphasizing the role of experience in the learning process (Kolb, 1984). Reflection is a key component of experiential learning allowing learners to reflect on their experiences and test them to their generalizations. Reconsidering experiences and converting them to new knowledge and ideas allows for transferrable knowledge to be created that can be applied in new contexts. Experiential learning is becoming highly recognized in higher education and is often included in applications of inquiry-based learning and constructivist theories.

**Constructivist Theories**

Constructivist theory is an epistemology about learning and knowledge and how it is constructed. It proposes that people are active learners who are able to construct their own knowledge (Geary, 1995). In constructivist teaching environments teachers shouldn’t control learners, instead they should be facilitators (Schunk, 2012). Learner knowledge is formed inside rather than outside and therefore constructions that are true to one person, are not necessarily true to anyone else (Cobb and Bowers, 1999).

Piaget was a founder of constructivist learning. According to Piaget’s Cognitive Development Theory, cognitive development depends on: 1) Biological maturation, 2) Experience in physical environments, 3) Experience in social environments, and 4) Equilibration (Schunk, 2012). The first three factors depend on the fourth factor, equilibrium. Equilibrium is the central factor behind cognitive development and refers to the motivation and biological drive to produce a state of equilibrium (Duncan, 1995). As reality is assimilated, information is understood, and structures are accommodated leading to change (Schunk, 2012). Piaget also showed that as children learn they pass through fixed sequences of stages: sensorimotor, preoperational, concrete operational
and formal operational. In these stages, concrete operational is characterized by high cognitive growth and after learning and experiencing this stage, people can drop into the concrete operational stage even when put in a new situation (Piaget, 1952; Wadsworth, 1996; Meece, 2002).

Vygotsky, was another influential individual in constructivism. He believed that humans could alter their environment for their own purposes and that social activity and interactions in the environment led to reorganizing mental structures (Vygotsky, 1978). His zone of proximal development (ZPD) showed the importance of scaffolding in children. He believed in helping children in the beginning and slowly backing off as they progressed until they become independent learners. Vygotsky postulated that learners’ interactions with the environment and the experiences that they bring to each situation greatly influences how they learn (Schunk, 2012).

In this day and age, many teachers are unprepared or unsure of how to teach in a constructivist environment (Elkind, 2004). While many are concerned about the lack of teacher direction in the classroom combined with a larger emphasize on deeper conceptual understanding rather than lower-level, basic skills, methods such as discovery learning, inquiry teaching, peer-assisted learning, discussions, debates and reflective teaching are acceptable ways to incorporate constructivist teaching in the classroom (Schunk, 2012). Discovery learning involves constructing and testing hypotheses and obtaining knowledge on your own, rather than through lectures from teachers (Bruner, 1961). Another form of discovery learning is inquiry teaching, which will be described in further detail later in this review. Peer-assisted learning encourages
student cooperation and employs active student learning that has been shown to lead to greater achievement gains than traditional instruction (Fuchs et al., 1997).

Reflective teaching is based on thoughtful decision-making that takes into account knowledge about students, context, psychological processes, motivation and learning and knowledge about oneself (Schunk 2012). Henderson (1996) listed four components of reflective teaching: 1) Teaching must be sensitive to the context, 2) Must include fluid, flexible planning, 3) Teachers must be knowledgeable and aware of why they do what they do and be keen observers of situations, and 4) Teachers must seek solutions to problems and find the best solution rather than settle for less than satisfactory. Overall, constructivist teaching challenges students to construct new knowledge and provides rich experiences that further encourage student learning. This learning theory is also very focused on a learner-centered teaching environment, moving away from a less desired teacher-centered environment in today’s education system.

**Learning Styles**

Each individual learns differently and has a specific learning style that applies to them. Learning styles are individual variations in how learners perceive, organize, process and remember information (Shipman and Shipman, 1985). The Gregorc Delineator explains the different mind styles that exist today (Gregorc, 1984). There are four main learning preferences: 1) Abstract sequential, 2) Abstract random, 3) Concrete sequential, and 4) Concrete random. Interestingly, most learners have some of every area in their learning styles, but one or two of the areas are stronger. Abstract sequential learners prefer intellectual and vicarious experiences and value logic and rational approaches to the world. Abstract random learners have a preference for
emotions and physically pleasing environments and are flexible. Concrete sequential learners have a preference for order, precision, and hands-on experiences. Finally, concrete random learners utilize creative and original problem-solving techniques and are very independent.

Another way of assessing individual learning styles was invented by Kolb (1971). He identified four different learning styles: 1) Diverging, 2) Assimilating, 3) Converging, and 4) Accommodating. Learners with a diverging style prefer group work, listening to others and receiving feedback. Assimilators prefer readings, lectures and models, with time to think on information. Converging learners prefer experimenting with new ideas, laboratory work and applying their work to real life, practical situations. Finally, the accommodating learner prefers to work with others, sets goals, tests different approaches to complete a project. Learners in each of these groups also differ in personality types, educational specialization, professional career, current jobs and adaptive competencies (Sternberg, 2014).

The two different ways to assess individual learning styles that were mentioned above show the wide range of learners that exist in the educational world. Different learning styles affect how well learners make decisions, or work with others, or how organized they are, and it also shows that some learners need auditory cues when learning and some visual (Kolb, 1984). In order to be a successful, ‘master teacher’, it is important to know your audience, know your students and know their preferred styles to ensure that your teaching allows everyone with different learning styles to understand (Schunk, 2012).
Learners also come with preconceptions about how the world works. Sometimes these preconceptions can be correct and sometimes they are instead misconceptions (Schunk, 2012). Preconceptions can affect how learners chose to learn or act to their learning environment. Knowing your learners’ preconceptions and making use of metacognition in your teaching style to allow learning through thinking, doing, smelling, touching, and tasting, creates learners that are aware of their own thought process (Matlin, 2009). Nussbaum and Novick (1982) suggested a three-stage model to help modify student beliefs. First, their preconceptions must be revealed and understood. Second, conceptual conflict with those conceptions is created and third, new or revised ideas about the phenomena or topic should be facilitated.

Educators need to create learning environments that are complementary to the many different learning styles of students. Different types of teaching such as lectures, experimentation, inquiry-based learning, group work, discussion, supplementary material, feedback, and presenting material in different forms (tactile, auditory and visual) can help address the learning needs of all students (Schunk, 2012). Lastly, different types of teaching and course delivery may help create a learning environment that is better suited for addressing the many different types of learner styles and needs.

**Pedagogical Approaches**

Discovering how people learn is a science. Therefore, creating a learning environment that increases student success is very important in the educational world. Within higher education, there are three main pedagogical approaches, or delivery formats, that exist today. These approaches consist of face-to-face format, online learning, and the new and emerging flipped classroom. For each pedagogical approach
discussed, specific components that are critical to the success of each delivery format will be addressed.

**Face-to-Face: Traditional Classroom**

Face-to-face learning involves the traditional classroom where students come to class and learn through listening to lectures and complete homework and readings on their own time. Important components for teaching in a face-to-face environment are: 1) Conceptual understanding, 2) Cooperative environment and 3) Setting high expectations.

Conceptual understanding is important in helping students to understand the importance of the lesson and its content. Connections, models and graphical learning are all ways to use conceptual understanding in the classroom. Connecting information to students’ life experiences can aid in student learning (Marzano et al., 2001). Organization, logical flow and meaningfulness are critical for information processing as students must firmly understand the importance of the material to become engaged and process and retain the information being taught (Marzano et al., 2011; Schunk, 2012).

Including graphic organizers in lectures is important to help students organize information into conceptual frameworks and grabs their attention through visualization (Marzano et al., 2001). Activities that allow students to connect information learned to new situations will help them learn at a faster rate, while increasing their information storage (Bransford et al., 1999). Therefore, this practice utilizes information processing and cognitive theories as well as some behaviorism theories through lecturing. Small parts of constructivist theories such as relating “big concepts” to previous experience are included in this practice, however it is not a full constructivist-learning environment as the teachers do more than just facilitate students’ thoughts and actions.
Cooperative learning is often misunderstood and misused (Koutselini, 2009). Putting students into groups constitutes “collaborative” learning. Cooperative learning involves five main points from Johnson and Johnson (1974): i) Positive Interdependence. All members must have resources to actively contribute and assignment role and boundaries must be made so member contributions are not redundant, ii) Individual accountability. All group members must be formally and informally assessed, to ensure that all individual effort has contributed to the final result, iii) Promotive interaction. All members must actively engage in group work, discussion, dialogue and conflicts, iv) Direct instruction to group learning skills. In order for promotive interactions to be successful, creation of thoughtful discussion and rules of handling conflicts need to be taught, and v) Ongoing group processing. Learners need to be taught to constantly critique and refine their processes.

A few examples of cooperative learning are, jigsaw technique (Aronson et al., 1978), group investigation (Sharan and Sharan, 1992) and learning together (Johnson and Johnson, 1999). This type of learning improves self-esteem, increases motivation and engagement and gives students less feelings of isolation (Johnson and Johnson, 1999; Marzano et al., 2001). This can be included in the traditional classroom by breaking up lectures with short cooperative processing often with focused discussion before or after the lecture and turn-to-your-partner discussions throughout. This can re-engage the students and includes all students in learning the material (Smith et al., 2005; Tomcho and Foels, 2012).

High expectations. These will improve student learning and create a thorough curriculum (Tileston, 2004). It is important for teachers to set expectations that are
challenging but obtainable and to provide them with the information and ideas to complete them. Modeling challenging expectations and how to go about processing and creating acceptable outcomes will enhance student learning. Teaching students how to control the outcome and reducing their beliefs that their ability to be successful is strongly related to past performances will help them achieve these expectations (Ryan and Deci, 2000). Therefore, tasks to help reinforce success such as teaching students to set goals and adhere to them and providing feedback can help students to achieve high expectations while increasing their learning and understanding of content (Marzano et al., 2001). Modeling good practices and successful outcomes is important as “success breeds success” (Schunk, 2012). Bandura and colleagues showed the importance of modeling, and not only observing, but also performing them themselves (Schunk, 2012). Therefore, demonstrating how to acquire skills and expectations with guided practice and checking their understanding with feedback, will lead to social cognitive development.

Online Learning Environment

Online learning environments take place completely online, with no instruction in a face-to-face classroom. In other words, it is a “virtual” classroom. Important components of an online learning environment are: 1) Self-regulation (self-monitoring, instruction and reinforcement), 2) Multimedia design (conducive to learning) and 3) Interactive communication tools.

In an online learning environment, self-regulation, self-monitoring, self-instruction and self-reinforcement are crucial aspects for success. Students need to learn to be self-regulated. They need to set goals and develop strategies to achieve their goals.
These goals should be monitored and reflected upon by themselves, peers and teachers (Zimmerman, 2000).

Metacognition and motivation are important for self-regulated learning. Scaffolding and design of the online course from the teacher guides and supports the student how to achieve understanding and self-direction (Vygotsky, 1978). Breaking down large goals into short-term goals can help students regulate their goals and produce higher self-efficacy. Features of self-regulation can be embedded into templates and tools in the online learning context (Meyer et al., 2010). Showing the cost-benefit ratio of using tools and technology, when to use these tools, and practicing the tool is important in convincing students that the tool helps them learn and leads to success. Self-assessments of learner attitudes and values can aid in their self-regulation. Self-efficacy in using the software and course design leads to higher learning and motivation (Pintrich, 2003). Therefore, clear feedback on competence and skill, challenging materials that allow for success, personal identification with content and its importance and utility can increase student efficacy and competency (Pintrich, 2003). Self-regulated learning can be shown through scaffolding the learning principles in phases to help lead the students to success: i) Goal setting and strategic planning phase (self-motivation, goal orientation and self-efficacy), ii) Performance phase including self-control, self-instruction and self-observation and iii) Self-reflective phase that includes self-evaluation and self-reaction all the while giving constructive feedback.

Dual processing of information from Paivio (1971) plays a large role in designing the best online environment for students. The design of multimedia should be an environment that is conducive to learning. Multimedia learning should be organized
around three core principles: firstly, that humans possess separate channels for processing visual and verbal material, secondly, this channel is limited, meaning that only a limited amount of information can be processed at one time and thirdly, deep learning requires active processing during learning such as organizing and integrating. As mentioned above, multimedia has a positive association between students’ self-efficacy and learning. Believing that personal efforts will lead to success is connected with social cognitive theories and motivation, observed through others online, teachers and feedback. Proper multimedia can foster the use of processing and cognitive capacity promoting interest, value of the task and motivation to identify goals and intrinsic value in learning.

Three different forms of interaction are found in distance and online learning environments. These include: student-student interaction, student-instructor interaction and student-content interaction (Moore, 1989). These types of interaction are all types of communication tools that are important to increasing and enhancing learning in the online environment. They can be found through discussion boards, email messaging, and video chatting. Student-content interaction refers to interacting with the subject matter that is available online and relating and applying it. Choosing the best technology tools that allow for this communication and collaboration is needed. Cooperation as mentioned in the face-to-face education setting, focusing on social constructivism and social cognition, should also be considered here and used in online learning. Highlighting where students can aid their own learning and the learning of others can lead to positive interactions, facilitation and encouragement to accomplish
goals. Finally explaining what participation is required and what contributes quality interactions needs to be thoroughly explained (Svinicki & McKeachie, 2009).

**Flipped Classroom**

A new and emerging pedagogical approach is the flipped classroom. The flipped classroom is defined as an educational setting where interactive group learning activities occur in the classroom and direct computer-based individual instruction takes place outside of the classroom (Bishop and Verleger, 2013). Components that are critical to the success of the flipped classroom are: 1) Teachers as facilitators, 2) Incorporating inquiry-based learning, and 3) Use of low-stakes assessments.

Facilitators. In the flipped learning environment teachers are considered facilitators. Moving from a “sage on the stage to a guide on the side” is an important part of increasing learning in students (King, 1993). Teachers should guide students to solve problems and encourage application of material. This style of learning is rooted in constructivism. In the flipped classroom, students have access to the information online and they learn how to apply it to life to obtain knowledge. Teachers should walk around activities in the classroom and facilitate ideas and inspiration, but not direct it or give away the answers; lead students to the answers. Online videos/lectures should not be longer than 15 minutes, as student attention decreases after approximately 10 minutes of learning and less information is presented (MacManaway, 1970). Therefore, only including the important information and the ideas/methods to interpret and related information in the real world can facilitate students’ learning by allowing them to stay focused and apply themselves. Being facilitators of information allows students to learn in their own way and at their own pace and time.
Incorporating inquiry-based learning. Engaging students in Bloom’s taxonomy through application, analysis and synthesis of instruction and information increases learning outcomes (Krathwohl, 2002). Students are required to produce outputs that go beyond information that was presented in class, increasing their engagement in the material (Chi, 2009). Marzano et al., (2001) categorized six activities that could increase problem-solving and inquiry-based learning in the classroom. These include, making predictions about system changes, problem solving through generation of hypothesis and possible solutions, investigating historical events and analyzing them, inventing new research to test, using the scientific method to solve it and draw conclusions (experimental) and using hypothetical situations to find the best solution. Through working together in groups Piaget’s theory of social constructivism and cognitive conflict and cooperative learning styles from Vygotsky’s ZPD allow for student-centered learning. Inquiry-based learning helps students develop flexible knowledge, effective problem-solving skills, self-directed learning, collaboration skills and intrinsic motivation all life skills that lead to higher success (Hmelo-Silver, 2004). Enriching and reinforcing information through examples and real-life situations can help with information processing, storage and retrieval. Inquiry-based teaching allows for “knowledge in action” rather than “knowledge out of context” as described by Applebee (1996).

Low-stake assessments. Examples are discussions and quizzes at the beginning of class that are used to ensure completion and compliance of before-class work, but with minimal percentage of the final course grade. These assessments can create discussions and confidence between students and teachers and frequent short assignments can increase information processing and retrieval. Wideen et al. (1997)
showed that high-stakes testing discouraged teachers from using strategies that promoted inquiry-based and active learning. Assessing students’ process and rationale is more important than their memorization, therefore why more grading is placed on goals and justifying answers and less on memorization (Thoron et al., 2011). There is correlation between achievement scores and reasoning and if more emphasis is placed on learners’ ability to use scientific reason and use factual evidence (inquiry-based learning), even high-stakes tests such as Lawson’s Classroom Test of Scientific Reasoning (LCTSR) will show higher student achievement (Lawson et al., 2000). Therefore, high-stake assessments can still be used in summation, but low-stakes assessments and quizzes during learning will teach students to become reason at a higher level without being afraid of the consequences and increases repetition of key information.

**Future of the Flipped Classroom**

All of the pedagogical approaches that were previously discussed are acceptable delivery platforms for learner instruction in higher education. Traditional and online delivery platforms have been well accepted now for several years in the education system. However, the flipped format is a new and emerging format that was only developed in 2007. Due to the infancy of this teaching method some academics have already incorporated this platform into their instruction, while others are still uncertain about embracing this flipped approach (Huber and Werner, 2016).

Transformation in pedagogical approaches may be required to increase student-centered learning, focusing on critical thinking, and inquiry-based learning. Abeysekra and Dawson (2015) report on the need for more research on the efficacy of the flipped classroom and qualitative work on student learning and experiences in the flipped
classroom. In a review on the flipped format, it is also noted that empirical data on student learning and achievement between teaching formats is limited and controversial (O’Flaherty and Phillips, 2015). Some results with the flipped method have shown to improve student satisfaction, engagement and course grades over the traditional format (Critz and Wright, 2013; McLaughlin et al., 2013; Mortensen and Nicholson, 2015). Others have reported negative student feedback related to a reduction of in-person lecture time in a flipped class (Wilson, 2013; Missildine et al., 2013). Finally, some studies have shown that there are no differences in student grades or achievement when a course was taught in different formats (Galway et al., 2014; Harrington et al., 2015; Morgan et al., 2015).

While more empirical research on the flipped classroom is needed, its investigation is highly warranted. As the needs of learners are shifting and there is a push for the education system to shift from a teacher-centered to a learner-centered approach, the flipped classroom may be the answer to address the needs of our 21st Century learners (Watson and Reigeluth, 2008; Huber and Werner, 2016).

**Education in the Information Age**

**Industrial Age Education**

The Industrial Age education system is a factory-model, which means that it focuses on compartmentalized learning and expects all students to learn at the same rate as their peers (Reigeluth, 1994). While society has shifted from Industrial Age to ‘Information Age’ the education system has not (Senge et al., 2000; Watson and Reigeluth, 2008). Changes in communication and technologies in the Information Age have made the education system today obsolete (Reigeluth, 1994). Students born from 1982 on are known as the ‘Millennial Generation’ and this Industrial Age education
system does not meet the needs of this new population of students entering our school systems today (How and Strauss, 2007).

**Millennials and Information Age Education**

The millennial generation, digital natives, net generation, and generation Y are all terms that describe the generation of students today (Jones et al., 2010). These students have grown up with digital technology in the Information Age. They are the most diverse generation of college students in gender, race and income, are a very busy group of students with a 37% decrease in unstructured time, are always connected and have a preference for experiential and engaging learning environments (Prensky, 2001; Jones et al., 2010; Phillips and Trainor, 2014). In 2002, the Center for Educational Statistics had predicted that by 2013, 75% of enrollment in higher education would be accounted for by digital natives. While some studies show that there is variation among students that lie within the next generation age band (Tapscott 1999; Prensky, 2001; Howe and Strauss, 1991; Oblinger and Oblinger, 2005) others argue that there is no empirical research to prove there is a ‘distinct generation’ of students (Helsper and Eynon, 2010; Jones et al., 2010). However, with regards to education, it is agreed upon by all these authors that educators should determine what technologies students have access to, what types of learning styles they prefer and to better understand today’s students to help them learn and become successful.

A learner-centered approach to instruction would also address new needs and instructional ideas to support all students. The American Psychological Association conducted research to identify learner-centered psychological principles from educational research that affected how people learn. They categorized 12 principles in four areas: 1) Cognitive and psychological principles, 2) Motivational and affective, 3)
Developmental and social, and 4) Individual differences that influence how people learn (American Psychological Association’s Board of Educational Affairs, 1997; Lambert and McCombs, 1998). The National Research Council (NRC) conducted a two-year study to learn what would make it possible for students to develop a deeper understanding of course material. They found that customization and personalization in instruction was important for each learner with self-regulated learners taking control of their learning and gaining a deeper understanding of the material (Bransford et al., 1999). A two-fold focus of learners and learning was described by McCombs and Whisler (1997) that stated that when students are involved in learner-centered instruction they are also included in decision-making processes and they are treated as co-creators of their own learning. Therefore, an Information Age education system that focuses on learner-centered education and meeting individual learners’ needs would help to increase the success of today’s students (Watson and Reigeluth, 2008).

A recent study by Sarkar et al. (2017) incorporated technology infused learning in their business course that allowed for flexibility, immediate feedback, collaborative learning, active learning and learning independence and choice. The results showed that students had increased academic performance and this learning environment assisted in their learning process. Finally, they showed that digital natives prefer a more experiential environment than a passive learning environment and a collaborative learning environment. These results further support the future success of an Information Age education system. Therefore, as digital technology continues to advance, instructional methods in higher education systems should also continue to evolve to meet student’s needs.
STEM Education

Another important factor indicating a time for change in our education system is highlighted in the alarming decline of students pursuing a STEM degree (Lowell et al., 2009). Furthermore, there is evidence that the United States is falling behind other countries in science education (Provasnik et al., 2009). Information from Science and Engineering Indicators Report (2016) showed that at the bachelor’s level, approximately half of the beginning bachelor students in STEM majors between 2003 and 2009, had left school completely (20%) or left STEM for another major (28%). Less than half of students who begin in a STEM undergraduate degree finish in one (Wilson et al., 2012). National estimates of STEM retention losses are found to be higher in women and ethnically diverse populations (PCAST, 2012). Current data suggest that if this trend continues, the workforce will lose over 1 million STEM graduates in the next decade (PCAST, 2012). Therefore, there is a critical need to address student interest in STEM.

Several reasons have been addressed to understand why undergraduates drop out of STEM degrees. Some reasons include: 1) Loss of confidence due to poor grades in early years, which is most likely related to, 2) Feeling overwhelmed and/or discouraged from gatekeeper courses (chemistry, biology, calculus) and content overload, 3) Low morale from the high competition and unwelcoming atmosphere of STEM courses, 4) Teaching style of STEM professors, and 5) Becoming “turned off” of science (Seymour and Hewitt, 1997; Crisp et al., 2009; PCAST, 2012). Therefore, it is critical to the future of STEM fields and for the United States to retain their historical pre-eminence in STEM to research ways to increase the retention and recruitment of students in STEM degrees (PCAST, 2012).
Increasing STEM Retention

Many different solutions to increasing STEM retention have been suggested and researched. Graham et al. (2013) addressed the importance of assessing motivation and confidence of learners, which are qualities required for persistence. Motivation and confidence are found to be important elements in the most successful STEM retention programs as these elements spur academic success and allow them to feel like a scientist (Seymour and Hewitt, 1997). One study found that students attending a Hispanic Serving Institution were well represented in STEM majors, as they were not discouraged from STEM based on their family income or standardized testing (Crisp et al., 2009). Therefore, these types of institutions may be important to provide an equal opportunity for Hispanic students. The involvement of students with both their instructors and peers early in their education through learning communities has also been assessed (Dagley et al. 2016). STEM learning communities can be virtual or physical structures that allow students to work, learn and receive support from one another. Dagley et al. (2016) found a 43% higher retention of students in STEM majors that participated in a learning community, in particular with women, African Americans and Hispanics.

While all of the aforementioned elements and programs have been influential in increasing STEM retention, two very important factors that inspire STEM students will be discussed in greater detail: 1) Research-based courses, and 2) Active learning environments.

Research-Based Courses

The benefits of undergraduate research experiences are well known. However, most undergraduates aren’t exposed to research experiences until later in their degrees
or not until after their undergraduate degrees (Russell et al., 2007). A study commissioned by the US National Science Foundation (NSF) surveyed students that were involved in NSF-funded research. One survey asked participants that were involved in undergraduate research, what affect the experience had on them and what factors favored positive outcomes. The results showed that 68% said their interest in a STEM career increased at least somewhat. It also revealed that students who hadn’t anticipated graduate studies to change their path towards a PhD (Russell, 2006). This study found that undergraduate students that were involved in research were students in their third and fourth years with high academic standing (Russell, 2006). When graduate students that were not involved in undergraduate research (47% of STEM students) were surveyed three of the most common reasons for this were: 1) They didn’t have time (37%), 2) They were not aware of the research opportunities that were available to them (28%), and 3) It never occurred to them to do research (19%).

Typically, if undergraduates want to be a part of research they need to seek out a lab or professor to do research with on top of their schooling. The National Science Board’s Science and Engineering Indicators report showed that the greatest drop out of science courses is between first and second years. Therefore, the integration of undergraduate research into coursework could target a greater number of students, including those that would not for various reasons take the research initiative. Furthermore, course-based undergraduate research experiences (CUREs) would introduce more students to the positive aspects of STEM and may further help them relate seemingly useless and overwhelming gatekeeper course material to real-life situations (Weaver et al., 2008).
In support of this, it has been shown that the earlier undergraduates are exposed to research, the more likely it is they will remain in a STEM degree (Lopatto, 2007; Weaver, 2008). Data from the Survey of Undergraduate Research Experiences (SURE) reported that 87% of students participating in an undergraduate research experience planned to continue in a STEM field (Lopatto, 2007). Thiry et al., (2011) summarized interviews from 62 graduating STEM majors to understand what experiences during their undergrad were most important. Overall, students reported greater gains if their coursework was supplemented with “real-work” experiences, and most of the students’ positive statements came from research experiences rather than from coursework. However, the colleges were limited in what research experiences they could provide and labs only provided a “taste” of research (Thiry et al., 2011). Other benefits of CUREs are that students are allowed to make their own discoveries and work with practicing scientists problem-solving and practicing collaborative work; all part of STEM careers (Auchincloss et al., 2014). Long-term effects of CUREs have been shown to increase the likelihood of graduation in a STEM major and within 6 years (Rodenbusch et al., 2016). Research not only engages students and stimulates curiosity but it also encourages self-confidence and allows students to become scientists themselves, rather than just watching science happen around them (Graham et al., 2013).

**Active and Inquiry-Based Learning**

Active learning has been suggested for teaching today’s students, with a focus on inquiry-based learning (IBL; Prince, 2004; McLaughlin et al., 2014). Active learning and IBL have been found to increase student engagement and learning and when application of knowledge and material is completed in class, teachers are available to be facilitators and aid in their learning (Prince, 2004; Watson and Reigeluth, 2008; Chi,
Furthermore, the Boyer Commission Report (1998) calls for including research-based learning in education, with a focus on IBL and experiences to increase the science literacy.

Inquiry is “the art and science of asking questions about the natural world and finding the answers to those questions” (Thoron, 2018). Therefore, IBL refers to using the inquiry process to acquire knowledge. Inquiry-based methods are starting to play a large role in science education allowing learners to increase understanding by placing science in context (NRC, 2000). Five distinct features of IBL are: 1) Learners are first engaged in science-oriented questions, 2) Learners address these questions through evidence and evaluation, 3) Learners indicate new explanations to create an answer to the question, 4) Learners consider alternatives and other rationalizations, and 5) Learners justify and communicate their personal explanation (Thoron, 2018).

One of the most influential individuals in the evolution of IBL was John Dewey, who believed in progressive education and highlighted the need to ‘learn by doing’ (Dewey, 1938). He also believed the connection between education and experience was essential to learning. Dewey (1938) also stated that there is continuity in inquiry meaning that conclusions from one inquiry will be carried on to the future without having to be re-examined. This is an important goal of IBL today, where learners form a deeper understanding, allowing them to build upon and transfer knowledge from old situations to new.

In STEM degrees or careers, the process of scientific inquiry is imperative. Therefore, when teaching any of these majors, it is important to include the scientific method and IBL. This is commonly referred to as “scientific inquiry” and uses the
scientific method to help learners develop critical thinking skills. The scientific method is “a pattern of thinking that emphasizes asking questions, developing hypotheses to answer the questions, and testing the hypotheses with data” (Eggen and Kauchak, 2011). Critical thinking is “an individual’s ability and inclination to make and assess conclusions based on evidence” (Eggen and Kauchak, 2011). Learners are often exposed to the process of the scientific method in readings and lectures, but rarely get experience with the process. However, learners should be able to critically think through any situation, use resources to help form their argument, assess both sides of the argument, and communicate to justify their findings. Therefore, IBL not only fosters critical thinking skills but also argumentative skills, cooperative skills and builds transferrable intellectual skills.

An important part of IBL is the student interaction and discussion that takes place. Groupwork is “a set of instructional strategies that emphasize student-student interaction to supplement other models” (Eggen and Kauchak, 2011). When student’s work through a scientific-inquiry together they learn how to work with others that have a different backgrounds or ways of thinking and learn to diplomatically resolve disagreements (NRC, 2000). This will also improve their argumentative skills through learning how to support different sides of a claim with proper evidence and justification. Groupwork is an important quality for learners to have in the field of science, as it teaches them how to communicate ideas and the importance of collaboration. Whether they are going to become an astronaut or try to save a species from extinction, collaboration will be key to their success. When groupwork is involved in a classroom, instructors must be careful to make sure that groups are on task, each student is
individually accountable for their own understanding and groups must depend on each other to accomplish the scientific process (Eggen and Kauchak, 2011). Accountability is the most important as it will help to keep them focused and every person in the group will understand that they have a role to play to be successful (Antil et al., 1998).

IBL also promotes student-to-teacher contact, which is important for facilitating learning. This facilitation is important to note, as the NRC (2000) describes IBL as “something that students do, not something that is done to them”. Teachers should be able to help students understand the problem when needed, entice group discussion, ask questions, make suggestions, link errors to misconceptions and ensure that learners are considering all the possibilities and can justify their answer. Hmelo-Silver et al., (2007) stated that while IBL requires the teacher to be a “facilitator”, it still requires guidance and scaffolding from the teacher in order to facilitate the learning. Therefore, in order for IBL to be effective, teachers must know their prior learners’ knowledge, their cognitive state and their ZPD so that the tasks at hand are within each learner’s capabilities. Facilitators must also remember to be prepared and have adequate resources ready for learners to access while working through their scientific-inquiry. It is not necessary to know all the answers in IBL, as often there is no set answer, but having adequate knowledge about the topic and how to aid learners through difficult situations is crucial. Therefore, the question can be asked of an instructor: “if you can’t work your way through a scientific-inquiry how do you expect your students to?” Instructing IBL can be a difficult task, but it has been shown that teachers are able to resolve dilemmas and find interventions through collaboration with peers and reflecting on the lesson (Marx et al., 1994). While speaking of the
importance of student collaboration, teacher collaboration is just as important. Asking advice from other teachers, watching other teachers instruct or receive feedback from previous students is very important to create a successful lesson, especially if it is a new concept that is being taught. This type of support is very important to IBL and allows for lessons and strategies to be constantly improved. Besides collaboration, effective questioning and reflection are also very important to the success of IBL.

Effective questioning is an educational tool that is highly important during facilitation in IBL. According to Bloom’s Taxonomy (1956), lower-order thinking questions are related to knowledge and comprehension whereas higher-level questions include application, analysis, synthesis and evaluation. While knowledge and comprehension are important, higher-level questions are important when encouraging students to think more deeply or more critically, solve problems, encourage discussions and stimulate students to seek information on their own (Goodwin et al., 1992). Learning to use higher-level questioning during scientific-inquiry can be difficult as facilitators need to help develop student interest and motivation, increase critical thinking skills, and concept relation (Dyer, 2008). Questions can be open or closed, general or directed, and the appropriate “wait time” for an answer as well as when to redirect a question, help a student answer his or her own question, and providing reinforcement and encouragement is crucial. Learning how to maximize student participation can also be a challenge to IBL. A few suggestions to maximize participation are: ask questions of the entire class, randomly select students so they all listen, ask the question first and then call on a student, give students an opportunity to
ask questions and ensure your nonverbal reactions complement your verbal responses (Goodwin et al., 1992).

Reflective practice is “the process of conducting a critical self-examination of your teaching” (Eggen and Kauchak, 2011). Overtime every professional will acquire more knowledge and experience and will become more efficient at teaching. The best way to assess your own teaching is through reflection and deciding what components worked and what didn’t. No one teaches a perfect lesson and asking questions such as “What can you adapt?” “What went over really well?” “Where did the learners need extra help?” “What could I do next time to improve that?” “Was I clear about what I expected?” can help you to constantly improve and develop (Eggen and Kauchak, 2011).

Inquiry-based learning, much like Piaget and Vygotsky’s theories, has multiple levels or stages of learning. These stages can be as simple as confirming a principle or piece of information when the results are already known (Level 0), to being presented the question with a prescribed procedure to identify the unknown results (Level 1). In the next level learners design their own process, but the question is still known (Level 2). Level 3 requires learners to formulate their own questions and procedures and analyze their results. Finally, in Level 4, learners can assess and critique the results of other experiments and make their own recommendations (Thoron, 2018). It is important to know where your learners are in their cognitive development, both at what stage, (Piaget) and whether they require a little more coaching or facilitating (Vygotsky). It is important that learners have been exposed to IBL and the processes (level 0) before they are exposed to a level 3 or 4. In the classroom, IBL should start small and build
up, so students can learn to self-regulate and guide themselves through the learning, building upon each level.

The final point that is worth mentioning when it comes to IBL is learner motivation. Students must be motivated to learn, especially in higher education and extension courses as it is up to them to succeed. Learners are highly motivated when they can connect the use of the material beyond the learning in the course, are involved in setting goals and planning activities and when they are successful (Barrick and Thoron, 2016a). In IBL, students are highly involved in every step of the process. However, ensuring that instructions and expectations are clear is important to increase active participation and self-motivation and not leave learners puzzled or confused. Feedback and acknowledgement is also critical to increasing student motivation. Behaviors that are reinforced are more likely to be learned and quick, timely explanations of why a response may be wrong, leads to further inquiry about the particular issue without a period of negative emotions or struggling from the student. Finally, instructor enthusiasm is key. Movement, gestures, and voice inflections can all influence teacher enthusiasm and therefore student motivation (Barrick and Thoron, 2016b). Topics that are more interesting or easier to teach or are of huge interest to the instructor may generate greater enthusiasm from the learners. Teachers that are looking for student motivation or loss of student attention may notice that they had more student attention/motivation from one lesson to the next and could reflect on the difference in their enthusiasm. During IBL, teacher enthusiasm is important when facilitating students to keep learners interested and feeling positive about their situation.
The more excited the facilitator is about the current inquiry the more excited the learners will be as well.

Overall, it is important to note that IBL has been shown to be successful in several studies. A study by Thoron and Myers (2011) showed students scored significantly higher on content knowledge with IBL over traditional subject-matter learning. Lynch et al., (2005) also found IBL increased engagement and mastery goal orientation in students that were disadvantaged and that all study students outperformed their peers in the traditional instruction. Hickey et al. (1999) showed significantly larger gains in science and biology classes with IBL instruction. These studies demonstrated as well as increasing student participation, critical thinking and reasoning, IBL can increase knowledge and understanding and may be better suited for learners with disadvantages. Therefore, even though creating a new IBL lesson, learning how to be a facilitator and use effective questioning and reflection while at the same time maintaining student motivation may seem overwhelming it is well worth the challenge as the evidence suggests.

Inquiry-based instruction allows a shift from a more traditional-based method where teachers distribute knowledge and dictate actions, while learners play a very inactive role; to a novel method where teachers become facilitators. Therefore, constructivist and experiential learning theories, mixed with new teaching platforms such as the flipped classroom, may further stimulate interest in STEM careers.
CHAPTER 3
IDENTIFYING THE OPTIMAL COURSE DELIVERY PLATFORM IN AN UNDERGRADUATE ANIMAL BEHAVIOR RESEARCH COURSE

Introduction

The population of students currently enrolled in post-secondary educational institutions is diverse and radically changing how they think and learn (Prensky, 2001, 2005). Traditional education systems have focused on compartmentalized learning where students are expected to learn at the same rate as their peers. Suggested ‘Information Age’ approaches focus on learner-centered education and meeting individual learner needs (Watson and Reigeluth, 2008). Active learning has been suggested for teaching today’s students, focusing on inquiry-based learning (Prince, 2004; McLaughlin et al., 2014).

Three primary teaching approaches exist today in higher education; traditional, online and the emerging flipped format, an active, learner-centered approach where students watch lectures online and participate in learning activities during class time. This approach has been shown to improve student satisfaction, engagement, and course grades over traditional approaches (Critz and Wright, 2013; McLaughlin et al., 2013; Mortensen and Nicholson, 2015). However, others have reported some negative student feedback related to reduced in-person lecture time in flipped classes (Wilson, 2013; Missildine et al., 2013), suggesting that success of different formats may depend on course subject.

There is evidence that the United States is falling behind other countries in science education (Provasnik et al., 2009), suggesting a need to refine teaching methods for inquiry- and research-based courses. Our objectives were to evaluate the effects of delivery formats of a research-based course on student learning outcomes.
and critical thinking, at both a state university and community college. We hypothesized that students participating in the flipped class would show improved outcomes and critical thinking, compared to a traditional lecture or asynchronous online-only format, and that effects of different delivery formats may differ between university and college-level student populations.

**Materials and Methods**

**Student Population**

This study investigated students’ learning achievements through a research-based course, “Wild Discoveries: Zooming into the Scientific Method”, offered at the University of Florida (Gainesville, FL, USA) and Santa Fe College (Gainesville, FL, USA). The course was a 1-credit elective offered for two years at both the University of Florida and Santa Fe College (from the Spring semester of 2016 until the Fall semester of 2017). At the University of Florida, the course was taught through the Department of Animal Sciences and offered to first and second year students interested in learning more about the scientific method.

There were a total of 178 students enrolled in the Wild Discoveries course from the University of Florida and a total of 58 students enrolled from Santa Fe College over the two years the course was offered. Student data collection also included gender. Data collection methods were approved through the University of Florida Institutional Review Board.

**Course Structure**

The Wild Discoveries course was a basic undergraduate research course designed to guide students through the scientific method while they conducted their own research projects from start to finish. Animal behavior was chosen as a focal topic for
this course, due to the large portion of animal science and zoo animal program students in our student population and their noted interests. Students were expected to plan, conduct and report on an animal behavior research project of their own design. Students assessed animal behavior of either domestic or exotic species via either live observation or online web cameras. Besides learning the scientific method, the course presented information on topics including basic principles of animal behavior and ethical issues such as plagiarism, and protocols for using animal and human subjects in research.

To investigate the influence of course delivery format, the course was offered according to three different delivery formats: traditional, online, or flipped. At the University of Florida, the course was offered in the traditional format once and the flipped and online formats twice. At Santa Fe College the course was offered in the traditional and flipped formats twice and the online format once. Student enrollment numbers by format and semester are described in Table 3-1. The delivery method alternated between formats in consecutive semesters at both schools, dictating how material and assessments were presented (Table 3-2). The same instructor taught the course in all formats at both schools. In the traditional course, class time consisted of a weekly 50-minute period on campus, with material delivered using PowerPoint lectures and quizzes. Assignments and group work were completed on the students’ own time outside of the class period. In the asynchronous online course, students were expected to watch video lectures, complete quizzes and assignments, and participate in group work online independently. The flipped course included both an asynchronous online component and an in-class face-to-face component. Students were expected to watch
online lectures and complete weekly online quizzes. Further, students were expected to complete minimal assignments and group work online. In addition to the online component, students were expected to attend a 50-minute face-to-face class period on campus each week. This class time was used to reinforce topics from the previous online lectures and answer any questions the students may have had from the weekly online material (i.e., quizzes, assignments, and group work). Students were also allowed to work on their homework assignments or discuss their research with group members during the face-to-face class time. The online lectures provided through the online and flipped delivery formats were prerecorded at the University of Florida Center for Instructional Technology and Training (CITT) using Mediasite (Sonic Foundry, Madison, WI).

Lectures and material were divided into 14 modules using the online Canvas system (E-learning) with learning objectives and reminders for students within each module. As well, each module included access to quizzes, assignments and group discussion boards.

**Student Assessments and Outcomes**

For each of these delivery formats, student assessments remained consistent. Course assessment was based on weekly quizzes, weekly assignments, a final written report, a poster presentation of their project, and class attendance. The final grade out of 540 points was calculated based on 120 points from quizzes and assignments, 100 points from the written report, 50 points from the poster presentation, and 150 points from attendance.

There were 12 weekly quizzes. Quiz questions consisted of multiple choice, true and false and matching. There were 12 weekly homework assignments, which included
practiced with animal behavior observations, creating and assessing ethograms, data analysis practice, and identifying science project misconducts. Drafts of each of the required components of the research report were submitted as well.

In each format, students were required to complete a semester long research project on the topic of animal behavior. They were divided into small groups of 4 to 5 students and were asked to complete the following task: develop a research plan, complete a literature review, develop a hypothesis, conduct animal behavior observations, analyze and interpret their findings, write a report on their project, and present their information in a poster format. Animal behavior research was conducted on their own time for each of the three formats.

Attendance was recorded for all delivery formats. For the traditional and flipped formats, weekly attendance was taken during class time. For the online format, attendance was measured through participation in weekly online discussions.

To evaluate the effect of course delivery format on critical thinking, students were evaluated voluntarily using the Cornell Critical Thinking Test (CCTT), level X. This was administered on the first day of class and the final day of class.

Finally, student course evaluations were obtained from the University of Florida students at the end of each semester. Students rated a series of criteria on a scale of 1 to 5, with 1 being poor and 5 being excellent. We summarized descriptive statistics for outcomes for three key criteria: Communication of ideas and information, stimulation of interest in course, and facilitation of learning.

**Statistical Analysis**

Student outcome data were summarized by student for each form of assessment (quizzes, assignments, report, and poster). Cornell Critical Thinking Test scores (pre
and post) were included for analysis if both pre- and post-test scores were completed by the student (complete data sets at University of Florida: 69 for flipped, 51 for traditional, 9 for online; at Santa Fe College: 16 for flipped, 8 for traditional, 11 for online). Data were analyzed separately by school.

The effect of course delivery format on student outcomes were analyzed using PROC MIXED in SAS (v. 9.4, SAS Institute Inc., Cary, NC). The model included the fixed effects of format and the random effect of student. For data subject to a significant effect of format, the Tukey-Kramer adjustment was used in testing for pairwise differences between formats. For analysis of data from University of Florida where enrollment was higher, gender and the interaction between gender and format were included in the model as fixed effects.

We evaluated the changes between pre and post-test CCTT scores within each teaching format using a paired t-test. We also evaluated the effect of format on changes in CCTT scores using the MIXED procedure of SAS, in a model that included format as a fixed effect and pre-test scores as a covariate.

All values reported are least squares means. Significance was declared at $P \leq 0.05$, and trends reported at $0.05 < P \leq 0.10$.

**Results**

The Wild Discoveries course was offered for 5 semesters at both the University of Florida and Santa Fe College from Spring 2016 to Fall 2017. Descriptive data for student attendance by format and semester is shown in Table 3-3.

Effects of course delivery format on student assessments are presented in Table 3-4. At the University of Florida, there were no differences between formats for quiz grades, but grades differed by gender, with female students ($n = 140$) having higher
grades than males (n= 38; 85.7 vs. 81.2 %; \( P = 0.032 \)), with no interaction between format and gender \((P= 0.31)\). Assignment grades from the University of Florida students differed across formats, with lower grades in the traditional format compared to the flipped format, and students in the online format having intermediate grades. Females students also had higher assignment grades (92.8 vs. 88.8 %; \( P = 0.02 \)), with no interaction between format and gender \((P = 0.99)\). Finally, there was a difference between formats for report grades at the University of Florida, with lower grades in the traditional format compared to the flipped and online formats, and no difference between the flipped and online formats. Report grades did not differ by gender \((P = 0.50)\). Poster grades were similar across formats, but were subject to a gender by format interaction \((P = 0.039)\): in the flipped format, female students (n = 62) have higher grades than males (n = 21; 95.1 vs. 91.6 %; \( P = 0.030 \)) and no effect of gender in other formats \((P > 0.96)\).

The Santa Fe College students had similar quiz grades across formats, but all other assessment grades differed by format (Table 3-4). Assignment, report, and poster grades were lower in the online format compared to the traditional and flipped formats, with no difference between traditional and flipped.

We evaluated both changes between pre and post-test CCTT scores within each format, and also compared the magnitude of change in CCTT score across formats. Changes in student critical thinking pre and post-test scores within formats are described in Table 3-5. At the University of Florida, CCTT scores increased within the flipped format (Table 3-5), whereas there was no difference between pre- and post-test scores within the traditional or online format. At Santa Fe College, there was no
significant difference within pre- and post-test scores within any format. We also found that the magnitude of change in CCTT scores tended to depend on format ($P = 0.058$); students enrolled in flipped format tended to have a greater increase in score compared to online format ($P = 0.060$) whereas the magnitude of change in the CCTT score did not differ ($P > 0.05$) between flipped and traditional or online and traditional.

The descriptive summaries of student course evaluations from the University of Florida students are shown in Table 3-6.

**Discussion**

The aim of this study was to evaluate the best delivery platform for an undergraduate animal behavior research-based course. As undergraduate students are changing in how they think and process information, it has been shown that a transformation in pedagogical approaches is needed across many different programs and majors (Prensky, 2001; Watson and Reigeluth, 2008; Benner et al., 2010; Velegol et al., 2015). This transformation includes more student-centered learning, focusing in on critical thinking, inquiry-based learning and application of information, versus passively relaying facts to students (Benner et al., 2010). Active learning and inquiry-based learning has been found to increase student engagement and learning and when application of knowledge and material is completed in class, teachers are available to be facilitators and aid in their learning (Prince, 2004; Watson and Reigeluth, 2008; Chi, 2009).

In addition to ongoing challenges of adapting course delivery platforms to meet the needs of today’s science-focused students, we need to involve undergraduates in research earlier in their collegiate career. Therefore, the focal course for this study was developed with the intended aim of involving undergraduate students in the scientific
method and having students conduct their own research. We proposed in completing a research project of their own design, students would be fully exposed to the scientific method.

A concern with the flipped format is the potential for low class attendance if lecture information can be found online (Foldnes, 2017). Class attendance is positively associated with academic achievement in both traditional lecture-based classes (Crede et al., 2010; Schneider and Preckel, 2017) and the flipped classroom (Foldnes, 2017). We found no difference in student attendance between formats, which is consistent with previous findings in flipped compared to traditional format classes (Deslauriers et al., 2011; McLaughlin et al., 2014; Mortensen and Nicholson, 2015).

The results from the University of Florida students show that while format may not matter for some assessments, it was significantly different in others. When there was a difference, in the case of assignment and report grades, the flipped and online formats led to higher grades than the traditional format. These results may suggest that these formats facilitate greater learning and are best suited for first and second year students in an early-based research course. Both online and flipped classes provided access to the same online lectures, which students could watch as many times as needed on their own time. This may partially explain why students performed better. Mortensen and Nicholson (2015) found that students in a flipped format class watched lectures multiple times (e.g. 1.9 views/student for the first lecture of the course). The flipped format also provided more time for students to interact with each other and the instructor during class meeting times. It has been shown that when students are
informed of the purpose of the flipped classroom and how it works and why it is important, students are more engaged (Betihavas et al., 2016).

Our findings are consistent with numerous other studies that have shown that courses taught in the flipped format have shown improved student grades and achievement compared to the same course taught in a traditional format (McLaughlin et al., 2014; Mortensen and Nicholson, 2015; Peterson, 2016). However, empirical data on student learning and achievement between teaching formats is limited (O'Flaherty and Phillips, 2015). Some studies have shown that there are no differences in student grades or achievement when a course was taught in different formats (Galway et al., 2014; Harrington et al., 2015; Morgan et al., 2015). However, there has been limited previous comparison between all three delivery methods: traditional, online and flipped.

A meta-analysis of K-12 education showed that blended learning, same as flipped format, was more effective than either face-to-face or online format (Means et al., 2009). In a statistical literacy course, Gundlach et al. (2015) compared the efficacy of the three delivery formats taught within a single semester and found that most results were very similar between formats and showed no differences. The only differences they found between formats was an increase in perceived easiness in traditional format versus the online format (Gundlach et al., 2015). Somenarain et al. (2010) evaluated different delivery formats in a medical terminology course and found no significant difference in course grades and student satisfaction between the online and flipped courses; however it is important to note that each format was delivered by a different instructor. To our knowledge, the present study is the first to evaluate the efficacy of different delivery methods in a basic research-based course. Further, the design of this
study allowed assessing different delivery methods over the course of multiple semesters, with consistency of instruction.

The format appeared to play a large role with the students from Santa Fe College, especially with regards to the online course. Overall, the online format resulted in the lowest grades in all categories. Feedback from students indicated the online format was the least preferred delivery mechanism. It is possible that this may reflect differences in expectations or previous experiences of students enrolled in smaller colleges. However, our limited enrollment of students in the online course offered at Sante Fe College restricts our ability to generalize these results. A study by Zavarella (2008), showed completion rates for a community college mathematics course were higher in traditional formats with 80% completion versus online courses with only 61% completion. This study reported 70% of students in the online section withdrew because of problems associated with the online component of the course. Another study on college-level students in an online course found that gender, academic readiness, and number of online courses enrolled had a significant effect on how well students did (Aragon and Johnson, 2008). Finally, Jaggars and Bailey (2010) completed a meta-analysis on the effectiveness of fully online courses for college students and suggest that online learning may have negative effects on academically underprepared students. Our results are in agreement with Aragon and Johnson (2008) and Jaggars and Bailey (2010) that college-level students may be unprepared for an online course.

Interestingly, we found some effects of gender on student grades at the University of Florida. For the most part, these findings were independent of format.
However, in the case of poster scores, female students received higher grades in the flipped format only. It is important to note that our study population involved a greater percentage of females than males (78.7% vs. 21.3%), which was representative of the gender demographics at the departmental level (86.3 % vs. 13.7 %, female vs. male enrollment in the Department of Animal Sciences across 2016-2017, UF IFAS (2018) Enrollment Data) which we expect may influence this result.

Finally, we found that there was a gain in critical thinking within the flipped format, but not within the traditional or online format from the University of Florida students. This is consistent with results from Mortensen and Nicholson (2015) who showed increased critical thinking from pre- to post-test scores in the flipped format. Further, the magnitude of change in the Cornell Critical Thinking Test tended to be greater in the flipped format compared to online, with the traditional format having intermediate outcomes. These results suggested students may not be engaging themselves in critical thinking and inquiry-based learning as much in an online course compared to a more formal classroom setting. Perhaps classroom interaction, whether traditional or flipped, is needed to encourage and inspire student’s critical thinking. Deruisseau, (2016) also found a greater gain in critical thinking in a flipped versus formal science course at a liberal arts college, based on questions that required higher-order thinking using Blooms Taxonomy. Other studies have demonstrated active learning can increase critical thinking (Prince, 2004; Watson and Reigeluth, 2008).

University course evaluations are often used to get feedback from the students on the course. Our descriptive summary of course evaluations suggest that all formats were numerically similar and ranked ‘Above Average’. Others have found no
differences between the traditional and flipped courses with regards to course evaluations (Simpson and Richards, 2015).

Overall, our results suggest that teaching format can influence student grades and critical thinking scores whether at a four-year University or College. Our results demonstrated at the University level the flipped and online formats led to higher grades than the traditional format. These results may suggest these formats better facilitate learning from this group of students. Furthermore, the results from the CCTT increased in the flipped format compared to the other formats, suggesting that some aspect of in-class learning may help to increase critical thinking. Overall, this study demonstrated that the flipped format of teaching may produce more desirable outcomes for students partaking in a research-based course.
Table 3-1. Total student enrollment numbers in each of the three delivery formats and the number of semesters each format was offered at the University of Florida and Santa Fe College.

<table>
<thead>
<tr>
<th></th>
<th>University of Florida&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Santa Fe College&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td># Semesters</td>
<td># Students</td>
</tr>
<tr>
<td>Traditional</td>
<td>1</td>
<td>55</td>
</tr>
<tr>
<td>Online</td>
<td>2</td>
<td>40</td>
</tr>
<tr>
<td>Flipped</td>
<td>2</td>
<td>83</td>
</tr>
<tr>
<td>Total</td>
<td>5</td>
<td>178</td>
</tr>
</tbody>
</table>

<sup>a</sup>Gender data for University of Florida students: traditional (n = 6 male, n = 49 female), online (n = 11 male, n = 29 female), flipped (n = 21 male, n = 62 female).

<sup>b</sup>Gender data for Santa Fe College students: traditional (n = 4 male, n = 25 female), online (n = 1 male, n = 11 female), flipped (n = 5 male, n = 12 female).
Table 3-2. Location of where student assessments were to be completed within each of the three different delivery platforms.

<table>
<thead>
<tr>
<th>Assessment</th>
<th>Traditional</th>
<th>Online</th>
<th>Flipped</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In Class</td>
<td>Online/Home</td>
<td>In Class</td>
</tr>
<tr>
<td>Lectures</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Quizzes</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Assignments</td>
<td>✓</td>
<td>✓</td>
<td>✓(^a)</td>
</tr>
<tr>
<td>Group Work</td>
<td>✓</td>
<td>✓</td>
<td>✓(^a)</td>
</tr>
</tbody>
</table>

\(^a\)In the flipped format, assignments and group work was completed both within class time and on students’ own time.
Table 3-3. Student attendance (mean ± SD) from each semester that Wild Discoveries was offered at the University of Florida and Santa Fe College.

<table>
<thead>
<tr>
<th>Semester</th>
<th>Traditional</th>
<th>Online</th>
<th>Flipped</th>
</tr>
</thead>
<tbody>
<tr>
<td>University of Florida (2016)</td>
<td>97.5 ± 2.1</td>
<td>94.3 ± 3.8</td>
<td>94.2 ± 3.2</td>
</tr>
<tr>
<td>University of Florida (2017)</td>
<td>---</td>
<td>98.4 ± 2.5</td>
<td>94.5 ± 4.2</td>
</tr>
<tr>
<td>Santa Fe College (2016)</td>
<td>93.3 ± 6.5</td>
<td>81.1 ± 11.7</td>
<td>98.1 ± 5.1</td>
</tr>
<tr>
<td>Santa Fe College (2017)</td>
<td>98.1 ± 6.2</td>
<td>---</td>
<td>97.2 ± 4.8</td>
</tr>
</tbody>
</table>
Table 3-4. Student grades (mean ± SE) for categories of assessments (quizzes, assignments, reports, and posters) in traditional, online and flipped class formats at the University of Florida and Santa Fe College.

<table>
<thead>
<tr>
<th>Format</th>
<th>Assessment</th>
<th>Traditional</th>
<th>Online</th>
<th>Flipped</th>
<th>SE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>University of Florida</td>
<td>Quizzes</td>
<td>84.3</td>
<td>84.0</td>
<td>82.1</td>
<td>2.2</td>
<td>0.59</td>
</tr>
<tr>
<td></td>
<td>Assignments</td>
<td>88.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>90.9&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>93.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.8</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>Report</td>
<td>89.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>96.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>95.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.79</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Poster</td>
<td>94.2</td>
<td>94.5</td>
<td>93.4</td>
<td>0.98</td>
<td>0.50</td>
</tr>
<tr>
<td>Santa Fe College</td>
<td>Quizzes</td>
<td>74.5</td>
<td>71.3</td>
<td>77.2</td>
<td>5.5</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>Assignments</td>
<td>95.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>61.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>91.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.5</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Report</td>
<td>84.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>88.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.0</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>Poster</td>
<td>91.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>92.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.3</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<sup>a,b</sup>Different superscripts denote significant differences between formats (<i>P</i> < 0.05) for each assessment.
Table 3-5. Cornell Critical Thinking Test (level X) pre- and post-test scores of students enrolled in traditional, online, and flipped format at the University of Florida and Santa Fe College.

<table>
<thead>
<tr>
<th>Assessment</th>
<th>Cornell Critical Thinking Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-test</td>
</tr>
<tr>
<td><strong>University of Florida</strong></td>
<td></td>
</tr>
<tr>
<td>Traditional</td>
<td>52.25</td>
</tr>
<tr>
<td>Online</td>
<td>43.89</td>
</tr>
<tr>
<td>Flipped</td>
<td>48.74</td>
</tr>
<tr>
<td><strong>Santa Fe College</strong></td>
<td></td>
</tr>
<tr>
<td>Traditional</td>
<td>47.75</td>
</tr>
<tr>
<td>Online</td>
<td>43.64</td>
</tr>
<tr>
<td>Flipped</td>
<td>47.13</td>
</tr>
</tbody>
</table>

Note: University of Florida students: n = 69 for flipped, 51 for traditional, 9 for online; Santa Fe College students: n = 16 for flipped, 8 for traditional, 11 for online.
Table 3-6. Faculty course evaluations (mean ± SD) for key criteria of interest, summarized across all semesters by format, from the University of Florida. Scores are based on a 1 to 5 scale. 1 = poor, 2 = below average, 3 = average, 4 = above average, 5 = excellent.

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Traditional</th>
<th>Online</th>
<th>Flipped</th>
</tr>
</thead>
<tbody>
<tr>
<td>Description of course objectives and assignments</td>
<td>4.61 ± 0.73</td>
<td>4.47 ± 0.92</td>
<td>4.81 ± 0.45</td>
</tr>
<tr>
<td>Communication of ideas and information</td>
<td>4.61 ± 0.77</td>
<td>4.62 ± 0.56</td>
<td>4.76 ± 0.48</td>
</tr>
<tr>
<td>Stimulation of interest in course</td>
<td>4.64 ± 0.72</td>
<td>4.62 ± 0.56</td>
<td>4.90 ± 0.32</td>
</tr>
<tr>
<td>Facilitation of learning</td>
<td>4.77 ± 0.60</td>
<td>4.62 ± 0.56</td>
<td>4.71 ± 0.60</td>
</tr>
<tr>
<td>Overall assessment of instructor</td>
<td>4.72 ± 0.70</td>
<td>4.69 ± 0.54</td>
<td>4.92 ± 0.29</td>
</tr>
</tbody>
</table>
CHAPTER 4
CONTRASTING LEARNING GAINS AND ATTITUDES IN SCIENCE OF STUDENTS PARTICIPATING IN AN EARLY RESEARCH-BASED EXPERIENCE

Introduction

The quantity of students pursuing a Science, Technology Engineering and Mathematics (STEM) degree is declining (Lowell et al., 2009). Less than half of students who begin in a STEM undergraduate degree complete their education in a STEM degree (Wilson et al., 2012). The loss of persistence and interest in STEM degrees may be a result of students feeling overwhelmed and discouraged by gatekeeper college courses causing them to drop out of a STEM degree (Crisp et al., 2009; Seymour & Hewitt, 1997). Current survey data suggested based on recent enrollment and retention rates, the workforce will lose over 1 million STEM graduates in the next decade (PCAST, 2012). Therefore, there is a critical need to address student confidence, motivation and interest in STEM.

One way to increase learner interest is to actively engage students in STEM disciplines through inquiry-based learning and undergraduate research experiences. Data from a Survey of Undergraduate Research Experience (SURE) reported that 87% of students participating in an undergraduate research experience planned to continue in a STEM field (Lopatto, 2007). The survey also demonstrated that research-based learning experiences led to greater positive gains in student perceptions of science, and thus led to greater STEM retention rates. Russell et al., (2007) also found that 68% of students claimed their interest in a STEM career increased “at least somewhat” as a result of undergraduate research experiences. However, students exposed to undergraduate research were mostly juniors and seniors with high grade point averages. Therefore, many educators argue that there is a critical need to develop and
deliver research experiences to a greater number of undergraduates earlier in their collegiate careers (Lopatto, 2007).

In most cases, students are unaware of research opportunities available to them or don't feel that they have time to participate in research experiences (Russell 2006). Weaver et al., (2008) suggested undergraduate research should be integrated into coursework, targeting a larger number of undergraduate students, reaching students who may not independently pursue research opportunities, and providing a base for further research experiences. Course-based Undergraduate Research Experiences (CUREs) can therefore provide undergraduates with active, authentic research opportunities to think and learn as scientists (Graham et al., 2013). Creating CUREs of student interest while ensuring the scientific method is fully understood remains challenging. Evidence suggests that CUREs can improve learners’ opinions and knowledge of science, allows them to acquire a deeper understanding of scientific research and can increase their interest in a science career (Lopatto et al., 2008). For example, Lopatto et al., (2008) described the effects of undergraduate research experiences for students enrolled in their Genomics Education Partnership course. They found students reported learning and professional gains and received high ratings on student’s construction of knowledge, realization that assertions need evidence, analyzing data, and scientific writing (Lopatto et al., 2008). Jordan et al., (2014) described the increased stimulation of student’s interest and persistence in STEM disciplines and positive academic achievement in the Science Education Alliance-Phage Hunters program. However, more widespread adoption of CUREs in different
areas of STEM to students earlier in their undergrad career may reach more students and allow for larger persistence and interest in STEM disciplines

We developed an animal behavior research-based CURE to expose undergraduate students in their freshman and sophomore years to the scientific method and research. Our objectives were to assess the effectiveness of the course on student opinions of the scientific method and STEM retention. We hypothesized that student opinions, attitudes about science and knowledge of the scientific method would be higher after partaking in the Wild Discoveries course.

**Materials and Methods**

**Student Population**

This study investigated students’ opinions of the scientific method and STEM retention after partaking in a research-based course, “Wild Discoveries: Zooming into the Scientific Method”, offered at the University of Florida (Gainesville, FL, USA). The course was offered campus wide for two years at the University of Florida (from the Spring semester of 2016 until the Fall semester of 2017) through the Department of Animal Sciences to freshman and sophomores interested in learning more about the scientific method and animal behavior research. A total of 176 students (Males: 38 and Females: 138) were enrolled in the Wild Discoveries course from the University of Florida over the two years of the study. Student ethnicity was also assessed either as Caucasian (n = 105) or Minority (n = 70). Minorities were identified as Asian American, Black/African American and Hispanic/Latino. Student demographics from the CURE survey are found in Table 4-1. Data collection methods were approved through the University of Florida Institutional Review Board.
Course Structure

The Wild Discoveries course was designed for undergraduate students to expose them to research earlier in their collegiate careers. The course mirrored the scientific method, while allowing students to conduct an animal behavior research project of their own design from start to finish.

Animal behavior was chosen as the focal topic for this course for a number of reasons. Within the Department of Animal Sciences, University of Florida, a large percent of students either wish to apply to veterinary school or other post-graduate work and have an interest in animal-related research. Importantly, behavior is utilized as a key metric to evaluate animal welfare (Dawkins, 2003). Increasing public awareness and concern for the welfare of animals in captivity is broadly affecting animal industries, resulting in increasing welfare auditing and changes in typical animal management (Mench, 2008). This suggests that skills in understanding and measuring animal behavior may be important for students graduating from animal science programs. Finally, we considered the study of animal behavior to be accessible and engaging for students who are new to research, and therefore appropriate for a basic research-based course.

The University of Florida has access to animal resources through the animal extension units as well as student access to animals at their sister college, Santa Fe College Teaching Zoo (Gainesville, FL) allowing for multiple research opportunities that were highly accessible to the students. At the University of Florida’s Horse Teaching Unit (HTU) and at the Santa Fe College Teaching Zoo online web-cameras were installed that allowed for students to perform remote online observations without having to step foot on site in person. This allowed a larger cohort of students to be involved in
the research experience at one time (increased enrollment), as well as participation from students not currently on campus at the University of Florida.

The course ran over a 15-week semester and was worth 1 credit. Over the two years the course was offered in three differently delivery formats (traditional, online and flipped) that rotated every semester. This paper is the second paper to the Wild Discoveries course. Background information on course formats and student assessments, learning gains and critical thinking scores can be found in our first paper (Arnold et al., 2018). This paper will focus in on student research experience and STEM retention gains.

Course content was separated into 15 modules using the online Canvas system (E-learning) with learning objectives and reminders for students within each module (Figure 4-1). Lectures were prerecorded at the University of Florida Center for Instructional Technology and Training (CITT) using Mediasite (Sonic Foundry, Madison, WI). The content and brief description of each module is shown in Table 4-2. While the majority of the course focused on the scientific method, important topics such as plagiarism and ethics were also addressed. Supplemental content provided external links, videos, extra readings and other resources to help enhance the information that was required in each module and to give students an opportunity to continue learning. Students were expected to complete readings, quizzes and assignments throughout the course (Arnold et al., 2018), but the main focus of the course was on a semester long animal behavior research project. The course modules and assignments were set up to teach students how to plan, conduct and report on research of their own design.
Students were divided into groups of 4 to 5 members and were expected to create their own animal behavior project using the animals available to them through the University of Florida extension units and Santa Fe College Teaching Zoo. Students were placed into groups during the second week of class and by the fourth week of class they had become acquainted with their peers, performed a small literature review and decided on a research topic. Student projects had to be approved by the instructor before students were allowed to proceed. In order to collect data, students had to learn to create their own ethogram to record animal behaviors (Figure 4-2) To ensure that students understood how to use an ethogram, an inter-observer reliability test with a “test” example was performed and students had to receive an accuracy score of 90% or higher to continue. Students had approximately 6 weeks to collect their data. During the 6 weeks of data collection students wrote their introduction, learned how to analyze data sets and interpret the results, and how to write and abstract and a discussion. In the middle of the data collection time period, a team progress report was submitted to ensure groups were on the right track and collecting data. At the end of their data collection period students had approximately 4 weeks to analyze and interpret their data and explain their findings in a research report. During this time a draft of the report was required for students to receive feedback from the instructor before the final project was due. Finally, a poster was created by each group and presented at a “Poster Symposium Evening” at the University of Florida.

CURE Survey

The Classroom Undergraduate Research Experiences (CURE) survey developed by David Lopatto was administered to the students at the beginning and end of the semester (Lopatto, 2009; Grinnell College). In the pre-course survey, questions include
demographics, reasons for taking the course, level of student experience in various course elements, attitudes about science, and student learning styles. The post-course survey parallels the pre-course survey questions, with slightly different questions (except for attitudes about science) and has more emphasis on estimates of learning gains in specific course elements and learning benefits and overall experiences of the course. Attitude about science is the only survey that is exactly parallel between the pre- and post-course surveys. For more information about these surveys please refer to Lopatto (2009).

Several different components of the CURE survey were analyzed. A pre-course survey entitled “Reasons for Taking Course” asked each student to rate 10 questions given on the importance (not important to very important) of their reasons for being enrolled in the course. In the pre-course CURE survey students were asked to assess their prior experience on each element listed on a 1 to 5 scale with 1 meaning no experience to 5 meaning much experience or a master. In the post-course CURE survey, the course elements question rated the gains that students may have made as a result of taking the course with 1 meaning no or very small gain and 5 meaning very large gain. The differences of means of post-course and pre-course course elements survey questions were used to represent the data.

The survey also gave the results of students nationwide who had completed the CURE survey, allowing us to compare our students’ responses to the national. The national data used was obtained from June 1st 2016 to May 25th 2017 from David Lopatto’s group. CURE survey results were compared pre- and post- course as well as
between students in Wild Discoveries course and the national average (Lopatto, 2009). In all cases means were used to represent the data.

**STEM Retention Data**

Student’s declared majors were collected at the beginning of each semester through the University of Florida’s student self-service system and one year after completion of the course. Students’ retention and recruitment in STEM majors were assessed. Data from students in this course were compared to University of Florida STEM retention rate (University of Florida, 2015).

**Results**

The Wild Discoveries course ran for five semesters at the University of Florida between 2016 - 2017 (see Arnold et al., 2018 for further information). Students chose a variety of different animal behavior research topics ranging from domestic to exotic species, with some students even branching out to use webcams at other zoos and locations around the United States (Table 4-3). Students were able to complete their data collection within the 6 weeks of allotted time and all students were able to successfully analyze, interpret and report on their data.

The top 5 reasons (very important) for “Reasons for Taking Course” were “to get hands on research experience, interested in the subject matter, to learn about science and the research process, to learn lab techniques and because the course and/or instructor is good” (Figure 4-3). Course elements gains comparing students in the Wild Discoveries course to the national average are shown in Figure 4-4. Specific elements that were expected of the Wild Discoveries course versus not are grouped in Figure 4-4. Overall students in the Wild Discoveries course scored higher than the national average.
Student learning gains were assessed on the post-course CURE survey only. It has been shown in previous years that courses with a research-like component yield means higher than courses with no research-like component. Wild Discoveries average gains were compared with the national average (Figure 4-5). Overall, Wild Discoveries students had higher gains than the national average for every question except learning laboratory techniques, which wasn’t emphasized in the Wild Discoveries course.

Attitudes towards science questions appeared on both the pre- and post-course CURE surveys. The differences of means of post-course and pre-course were used to represent the data in Figure 4-6. Five of the questions in this section are referred to as “engagement scores” according to the CURE survey. This engagement scores have correlated with higher reported learning gains and a greater likelihood to declare a science major. The five engagement score questions were “explaining science ideas to others has helped me understand the ideas better”, “I get personal satisfaction when I solve a scientific problem by figuring it out myself”, “the process of writing in science is helpful for understanding scientific ideas”, “I can do well in science courses” and “even if I forget the facts, I’ll still be able to use thinking skills learned in science”. Some questions should have been answered with strongly disagree while others should have been answers with strongly agree. Overall it appears that Wild Discoveries students had more acceptable answers than the national average.

Finally, the last component on the CURE survey that was measured was post-course survey for overall assessment (Table 4-4). Our results showed that the Wild Discoveries course received higher scores than the national average for all questions.
Retention rates of STEM students were measured in the Wild Discoveries course and compared to averages at the University of Florida (Figure 4-7). Overall there were 138 students in STEM majors (13 in non-STEM) during the semester that students took the Wild Discoveries course and after 1 year there were 137 students in STEM majors (8 in non-STEM), showing the loss of 1 student. Also, 1 year later, student recruitment into a STEM major was assessed. Overall there were 5 students that were recruited to a STEM major. Data from Fall 2017 has not been added into the STEM retention results as it has not been a full year since students took the course (will be in Fall 2018).

Lastly, student comments received through the University Faculty Course Evaluations were: “I loved this course! The information was interesting and it provided a cool way to learn about conducting research on animals” and “I loved this course. It really opened my eyes to the field of research and it got me excited about possible pursuing animal behavior research as a career. It also helped me learn about the research process and how to conduct research”.

Discussion

The aim of this study was to expose a greater number of freshman and sophomores to a research-based course in hopes to increase STEM retention and recruitment as well as students' opinions of the scientific method. The President’s Council of Advisors on Science and Technology (PCAST, 2012) has shown that less than half of students that enter US colleges in a STEM major continue in STEM to graduation. In order to increase the national STEM retention rate educational reform with proven retention strategies needs to take place. Offering research experiences to a greater number of undergraduate students early in their undergraduate career is
recommended to increase STEM retention (National Research Council (NRC), 2000; PCAST, 2012; Weaver et al., 2008; Boyer, 1998).

The Boyer Commission Report (1998) calls for including research-based learning in education, with a focus on inquiry-based learning and experiences to increase the science literacy. In most cases undergraduates are exposed to research experiences on their own time and most do not get an opportunity to be involved in undergraduate research experiences, are not interested, don’t have time to be involved or are not aware that research opportunities are available to them (Russell et al., 2007). Russell et al., (2007) found that 47% of STEM students have no research experiences and those that do are mainly juniors and seniors with high-grade averages. Course-based undergraduate research experiences (CUREs) allow more students to be engaged in the research process, rather than a select few that would be lucky through research in faculty laboratories. Other benefits of CUREs are that students are allowed to make their own discoveries and work with practicing scientists and problem-solve and practice collaborative work, all part of STEM careers (Auchincloss et al., 2014). Long-term effects of CUREs have been shown to increase the likelihood of graduation in a STEM major and within 6 years (Rodenbusch et al., 2016).

The Wild Discoveries course was created in response to the new widespread attention to increasing STEM retention through undergraduate research courses. The course led to high student assessments and grades (Arnold et al., 2018) and as shown in this paper, a high opinion of science and research and increased STEM retention. The hope is that this animal behavior research course may be a working example that other departments and institutions can model. This course allowed students to take
elements and frameworks from other introductory courses and apply it to STEM education research. Student’s opinions of science and research and of the Wild Discoveries course were analyzed through the Classroom-based Undergraduate Research Experiences (CURE) survey. This survey is often used to analyze CUREs (Auchincloss et al., 2014) and compares learning benefits between course experiences and undergraduate research experiences and assesses attitudes towards science (Lopatto, 2008). In our CURE survey results, all components were equal to or higher than the national averages, exhibiting the success of the course. When students were asked to rate reasons for taking the course, the highest reasons revolved around “learning more about science and research and gaining experience” than fulfilling a requirement. This allows us to see the importance of offering research-based courses to a greater cohort of students. Research not only engages students and stimulates curiosity but it also encourages self-confidence and allows students to become scientists themselves, rather than just watching science happen around them (Graham et al., 2013). Involvement in the science community and student collaboration found through undergraduate research experiences has also shown to be very important to increasing learners’ interest and retention in STEM courses (Dagley et al., 2015; Graham et al., 2013).

Course element gains from our CURE survey results were consistent with others, showing that students were able to gain experience from their undergraduate research course (Lopatto, 2007; Sarmah et al., 2016). Sarmah et al., (2016) showed that course element gains after taking a CURE course at Indiana University-Purdue University Indianapolis were comparable to the national average and in most cases were higher
than the national average, similar to our results. As expected, components of the course that are highly emphasized during the research show higher gains than those topics that aren’t emphasized in the CURE. Other topics that students are already exposed to on a regular basis results in very neutral results as they already know a lot about these topics but didn’t learn anything new from the course. For example, in our Wild Discoveries course, writing a research proposal and a project entirely of a student design were the highest gains, consistent with findings from Sarmah et al. (2016). These are unique experiences provided to the students through this research-based course. Other topics such as lab or project where instructor knows the outcome, revealed a very neutral response as students are exposed to these situations during biology, chemistry and other course with laboratory sections. Finally questions such as taking tests in class and working on problem sets, gave a negative result for our course as these were not components that were covered at all during our research course and therefore students would not have gained anything from these topics. Therefore, when designing an undergraduate research course, it is important to consider what topics will be emphasized versus not, as that will depict where student gains are found. Learning gains in the Wild Discoveries course were compared to national average and our students obtained higher reported gains. This data is consistent with Sarmah et al., (2016) biology CURE course and Jordan et al., (2014) SEA-PHAGES course, where their students had higher gains than the national average.

One of the most interesting results from our Wild Discoveries course was the result of the CURE survey question on attitudes about science. In this survey question students had to either agree or disagree with the question given to them. Interestingly,
our students appeared to have a better understanding of science and what is correct versus not, than the national average. This was depicted by specific questions such as “scientists know the results of an experiment before they start” where our students rated this question lower (disagreeing) than national average students who rated it a lot higher (agreeing). Perhaps the lectures on ethics, plagiarism and unexpected or negative results allowed students to obtain a better understanding of science and the scientific method. Within the attitudes about science question, five questions were highlighted that were related to student engagement, which was further related to higher learning gains and a greater chance of declaring a science major (Lopatto D: CURE Survey. 2009). In our results, four these five questions were in the top five highest rated questions, perhaps relating to our high learning gains and STEM retention. We have not found other published examples of results of student’s attitudes to science in other CURE courses, and our results suggest that research experience positively impacted these attitudes.

In addition to assessing student’s opinions of science, we evaluated the STEM retention of students enrolled in Wild Discoveries. The number of students in STEM is declining and the national retention estimates are around 40%, with 27% dropping out of their STEM major within the first year (CSRDE, 2013; PCAST, 2012). STEM retention rates were very high (99.3%) one year after participating in the Wild Discoveries course. In contrast, the overall STEM retention rate at the University of Florida indicates 38% of STEM students change out of a STEM major after their first year of University (University of Florida, 2015). The higher retention rates in the Wild Discoveries course may therefore show the importance of undergraduate research
experiences in increasing STEM retention. Furthermore, due to the high retention rate after their first year of college when most retention rates drop, this course may help prove the importance of earlier undergraduate research experiences. Jordan et al., (2014) showed that early engagement in a research-based course increases retention into the second year with an approximate retention rate of 93%.

Undergraduate research may offer students a positive experience in a STEM-related course when other STEM courses may be difficult and overwhelming and therefore discouraging. Seymour and Hewitt (1997) discuss the main reasons for undergraduates leaving the sciences, which include “discouragement/loss of confidence from low grades, overwhelming curriculum, content overload, competitive STEM culture and atmosphere of STEM courses, poor teaching in STEM and loss of interest in STEM”. Introductory level STEM courses, often referred to as “gatekeeper” courses, have been shown to discourage students from continuing in a STEM degree (Crisp et al., 2009; Gainen, 1995). Undergraduate research courses may help expose students to the positive sides of STEM and help them relate the gatekeeper course material to real-life situations. In support of this, the earlier undergraduates are exposed to research, the more likely it is they will remain in a STEM degree (Lopatto, 2007; Weaver, 2008). Thiry et al., (2011) summarized interviews from 62 graduating STEM majors to understand what experiences during their undergrad were most important. Overall, students reported greater gains if their coursework was supplemented with “real-work” experiences, and most of the students’ positive statements came from research experiences rather than from coursework. However, the colleges were limited
in what research experiences they could provide and labs only provided a “taste” of research (Thiry et al., 2011).

Overall, our study helps explain the importance of exposing undergraduates to research earlier in their STEM degrees. Student opinions and learning gains were positive and numerically greater than the national average of students enrolled in other CURE courses, showing the success of the Wild Discoveries course in teaching students the scientific method. Our high STEM retention rate and small recruitment of students into a STEM degree after one year or taking this course show the positive outcomes of exposing students to research early on. Early participation in research may not only inspire students to remain in STEM degrees but may lead students considering graduate degrees or continued research in their future careers.
Table 4-1. Student demographics of the population that participated in the Classroom-based Undergraduate Research Experiences (CURE) survey. Population demographics from the CURE survey completed nationwide are also provided (National Averages).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Wild Discoveries&lt;sup&gt;a&lt;/sup&gt;</th>
<th>National Average&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-Survey</td>
<td>Post-Survey</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>31</td>
<td>19</td>
</tr>
<tr>
<td>Female</td>
<td>131</td>
<td>104</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alaskan Native</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>American Indian</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Asian America</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Black/African American</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>Filipino</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Foreign National</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hawaiian</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hispanic/Latino</td>
<td>31</td>
<td>23</td>
</tr>
<tr>
<td>Pacific Islander</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>White</td>
<td>95</td>
<td>74</td>
</tr>
<tr>
<td>Two or more races</td>
<td>13</td>
<td>10</td>
</tr>
<tr>
<td>Other</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Declared Major</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>155</td>
<td>115</td>
</tr>
<tr>
<td>No</td>
<td>11</td>
<td>9</td>
</tr>
</tbody>
</table>

<sup>a</sup>CURE results from Wild Discoveries class (Pre-course: n = 166; Post-course: n =125)

<sup>b</sup>CURE results from National Averages (Pre-course: n =12,002; Post-course: n = 9824)
Table 4-2. Summary of the 15 modules included in the course and brief description of content in each module of the Wild Discoveries course taught at the University of Florida. Note the mirror of the course layout to the scientific method.

<table>
<thead>
<tr>
<th>Module #</th>
<th>Topics covered</th>
<th>Research Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Course introduction &amp; CURE pre-survey.</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Introduction to the scientific method.</td>
<td>Group Introduction</td>
</tr>
<tr>
<td>3</td>
<td>Background on animal behavior research.</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>How to conduct a literature review.</td>
<td>Search Literature</td>
</tr>
<tr>
<td>5</td>
<td>How to formulate a research plan.</td>
<td>Pick Topic in Group</td>
</tr>
<tr>
<td>6</td>
<td>How to collect animal behavior data.</td>
<td>Start to Collect Data</td>
</tr>
<tr>
<td>7</td>
<td>How to analyze data &amp; statistics</td>
<td>Collect Data</td>
</tr>
<tr>
<td>8</td>
<td>How to interpret results/statistics.</td>
<td>Collect Data</td>
</tr>
<tr>
<td>9</td>
<td>Reporting data &amp; scientific writing.</td>
<td>Collect Data</td>
</tr>
<tr>
<td>10</td>
<td>Learn specifics of poster presentations.</td>
<td>Collect Data</td>
</tr>
<tr>
<td>11</td>
<td>Learn about scientific writing and referencing.</td>
<td>Finish Collecting Data</td>
</tr>
<tr>
<td>12</td>
<td>Ethics in research are discussed.</td>
<td>Analyze Data</td>
</tr>
<tr>
<td>13</td>
<td>Animal and Human use protocols are discussed.</td>
<td>Analyze Data/ Report</td>
</tr>
<tr>
<td>14</td>
<td>Course Ends. Post CURE survey and final report.</td>
<td>Hand in Report</td>
</tr>
<tr>
<td>15</td>
<td>Poster Presentation Symposium</td>
<td>Present Research</td>
</tr>
</tbody>
</table>
Table 4-3. Examples of student research projects conducted in the Wild Discoveries course from 2016-2017 at the University of Florida. The type of observation completed, either live or online are shown, as well as the location where the observed animals were housed.

<table>
<thead>
<tr>
<th>Research project title</th>
<th>Observation</th>
<th>Animal location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effects of enrichment toys on the play behavior of captive sea otters</td>
<td>Live</td>
<td>Monterey Bay Aquarium</td>
</tr>
<tr>
<td>Vocal vs. non-vocal communication in horses</td>
<td>Online</td>
<td>Horse Teaching Unit</td>
</tr>
<tr>
<td>The effect of a novel object on the behavior of mares and two year-old horses</td>
<td>Live</td>
<td>Horse Teaching Unit</td>
</tr>
<tr>
<td>Physical communication trends of chimpanzees</td>
<td>Online</td>
<td>Houston Zoo</td>
</tr>
<tr>
<td>Mimicry in Asian small-clawed otters</td>
<td>Live</td>
<td>Santa Fe Teaching Zoo</td>
</tr>
<tr>
<td>Differences in the social behaviors of Asian and African elephants</td>
<td>Online</td>
<td>San Diego Zoo</td>
</tr>
<tr>
<td>Correlation between positive and negative: social behaviors of the Visayan warty pig and the Asian small-clawed otters</td>
<td>Live</td>
<td>Santa Fe Teaching Zoo</td>
</tr>
</tbody>
</table>


University of Florida, Gainesville, FL, USA

Houston Zoo, Houston, TX, USA; https://www.houstonzoo.org/meet-the-animals/chimpanzee-window-cam/

Santa Fe College, Gainesville, FL, USA

San Diego Zoo, San Diego, CA, USA; http://sdzsafaripark.org/elephant-cam
Table 4-4. Responses (mean ± SD) from post-course overall assessment survey, evaluated on a scale from 1 (strongly disagree) to 5 (strongly agree).

<table>
<thead>
<tr>
<th>Survey questions</th>
<th>Course</th>
<th>National Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>This course was a good way of learning about the subject</td>
<td>4.64 ± 0.63</td>
<td>4.12 ± 0.95</td>
</tr>
<tr>
<td>This course was a good way of learning about the process of scientific research</td>
<td>4.73 ± 0.60</td>
<td>4.20 ± 0.93</td>
</tr>
<tr>
<td>This course had a positive effect on my interest in science</td>
<td>4.59 ± 0.74</td>
<td>3.97 ± 1.10</td>
</tr>
<tr>
<td>I was able to ask questions in this class and get helpful responses</td>
<td>4.64 ± 0.66</td>
<td>4.17 ± 0.97</td>
</tr>
</tbody>
</table>
Topic 3: Animal Behavior Research

Objectives

By the end of this module students will have completed the following objectives:

- Apply the steps to observing and collecting animal behavior
- Differentiate amongst individual animal behaviors
- Assess individual animal behavior using an ethogram

To Do List

- Read the paper: Sterotypic Behavior of a Female Asiatic Elephant
- Watch lectures:
  - How to Observe and Collect Behavior Data (14:41) Lecture PDF
  - Creating and Using an Ethogram (16:52) Lecture PDF
- Complete Topic 3 Assignment: Conduct an Inter-Observable Reliability Test
- Submit your post to the Topic 3 Discussion Board
- Take Quiz 3

Supplemental Content

- Methods of Animal Behavior Research Overview (02:53)
- Website explaining how to use an ethogram
- Video on instructions when using an ethogram (02:30)
- Video of Using Comparative Species Approach to Investigate Paternal Behavior Responses (07:59)

Figure 4-1. Example of a student view of an online learning module of the Wild Discoveries course taught in Canvas (E-learning).
Figure 4-2. An example of a student ethogram and observation worksheet for horse behavior research.

<table>
<thead>
<tr>
<th>Observed Behavior</th>
<th>Code</th>
<th>Horse Ethogram Behavior Descriptions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Social Behaviors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aggression</td>
<td>AG</td>
<td>Aggressive posturing, threatening facial expressions, biting, kicking, fighting, high pitch vocalization</td>
</tr>
<tr>
<td>Affiliative</td>
<td>AFL</td>
<td>Friendly and positive behavior to establish or reinforce social bonds, such as grooming another, touching, nuzzling, licking, or lower pitch vocalizations</td>
</tr>
<tr>
<td>Submissive</td>
<td>SB</td>
<td>Entwist away, tail twinking, lowering of body, or shying away from more aggressive animal</td>
</tr>
<tr>
<td>Play</td>
<td>PL</td>
<td>Activity that has no apparent use or purpose, but activity used for a sense of pleasure</td>
</tr>
<tr>
<td>Sexual</td>
<td>SX</td>
<td>Male or female sexual display, sniffing of genitals or urine, mounting or copulating</td>
</tr>
<tr>
<td>Maintenance Behaviors</td>
<td></td>
<td>Can best be described as the daily type of behaviors that assist in keeping animals alive, healthy and happy</td>
</tr>
<tr>
<td>Alert</td>
<td>AL</td>
<td>Rigid stance with head, eyes and ears oriented towards object or other animal</td>
</tr>
<tr>
<td>Locomotion</td>
<td>LC</td>
<td>Movement of the whole body in a forward or backward motion (2 or more steps in any direction)</td>
</tr>
<tr>
<td>Eating</td>
<td>ET</td>
<td>Can be seen in grazing and browsing in herbivores, ingestion of specially prepared diets in captive animals or ingestion other animals in predators</td>
</tr>
<tr>
<td>Investigative</td>
<td>IN</td>
<td>Sniffing, licking, pawing at unknown object or within an environment</td>
</tr>
<tr>
<td>Grooming</td>
<td>GR</td>
<td>Skin and coat care, can be seen as rolling, shaking, or rubbing, licking, rubbing at coat/skin</td>
</tr>
<tr>
<td>Drinking</td>
<td>DR</td>
<td>Ingestion of water</td>
</tr>
<tr>
<td>Eliminative</td>
<td>EL</td>
<td>Defecation, urination or regurgitation of food</td>
</tr>
<tr>
<td>Standing</td>
<td>ST</td>
<td>Non-alert, no movement, inactive</td>
</tr>
<tr>
<td>Laying Down</td>
<td>LD</td>
<td>Rosting, inactive</td>
</tr>
<tr>
<td>Not Visible</td>
<td>NV</td>
<td>Animal not in sight of the observer</td>
</tr>
</tbody>
</table>
Figure 4-3. Responses to pre-course classroom-based undergraduate research experiences (CURE) survey question: “Reasons for Taking Course”. For the 10 questions listed, students chose the level of importance those criteria played in their decision to take the course (“not important”, “moderately important”, and “very important”). Data are ranked in order of the criteria with the greatest proportion of “very important” responses to the criteria considered least important.
Figure 4-4. Course element gains of students, based on pre and post-class CURE survey data, in Wild Discoveries (means ± 0.15) course compared to National Average (means ± 0.47). Pre-survey questions had students indicate, “What they knew” and post-survey questions asked, “what they had gained”, on a scale from 1 (no or very small knowledge/gain) to 5 (very large amount of knowledge/gain). Reported data are differences between post- and pre-course means. “CURE emphasized” elements were targeted in this course, “standard outcomes” were components of general laboratory courses and “Deemphasized” elements were de-emphasized in this course.
Figure 4-5. Learning gains from the post-course classroom-based undergraduate research experiences survey. Scale is 1 to 5, with 5 as the largest gain. Means (±1.03) of Wild Discoveries data are compared with national averages (±1.07).
Figure 4-6. Attitudes about Science from the pre- and post-course survey. Includes 22 questions on a 1 – 5 scale with 1 = strongly disagree to 5 = strongly agree. Differences between Wild Discoveries post-course and pre-course means (± 0.57) and national average (± 0.86) are used to represent the data. Five questions relating to student engagement in science are shown in bold italics.
Figure 4-7. Retention rates of STEM students in the Wild Discoveries course, one year after taking the course and at the University of Florida between 2016 and 2017. Students that remained within the same STEM major are shown in black bars and those that changed STEM majors, still within a STEM major are shown in grey for the Wild Discoveries students (this data was not received for University of Florida students).
CHAPTER 5
REPRODUCTIVE CHALLENGES IN ELEPHANT CONSERVATION: A REVIEW OF POOR SEMEN QUALITY IN CAPTIVE ELEPHANTS

Introduction

Elephant conservation is a critical issue, and a primary challenge is associated with the reproduction of elephants managed in captivity. Asian elephants are classified as ‘endangered’ and African elephants as ‘vulnerable’ according to the International Union for Conservation of Nature (IUCN 2017). While many Assisted Reproductive Technologies (ART) have been attempted in captive elephant populations, one roadblock always remains: few samples collected from captive elephants provide sufficient quality for research or for ART. Various studies have shown successful semen cryopreservation and artificial insemination in both Asian and African elephants, but these studies are often limited by low sperm quality samples and repeated collections to obtain a sufficient sample (Howard et al., 1984; Thongtip et al., 2004; Saragusty et al., 2005; Portas et al., 2007; Thongtip et al., 2008a; Saragusty et al., 2009b; O’Brien et al., 2013b; Imrat et al., 2014; Kiso et al., 2013; Arnold et al., 2017; Pinyopummin et al., 2017). Semen samples that are collected manually are highly variable and inconsistent, within and among bulls of both species. An even larger problem is that often only a select handful of captive bulls are trained for semen collection, and produce good quality semen during collections, which will eventually lead to a loss of genetic diversity in our captive population. This poses a great threat to captive breeding of elephants, which is already not self-sustaining (Olson and Wiese, 2000; Wiese, 2000; Brown, 2014). This review first provides a brief background of elephants and their reproductive status in captivity and then delivers an integrative synopsis of the results from various peer-reviewed elephant papers, highlighting
possible reasons for the poor semen quality in male elephants and possible suggestions for improving semen quality in the future.

**Status of African and Asian Elephants**


Both species of African elephants are considered ‘vulnerable’. There are approximately 415,428 African elephants remaining, with the largest population in Botswana, and although their numbers are slowly increasing, poaching is still a major threat as well as habitat loss and fragmentation (Thouless et al., 2016). There are approximately 1,000 African elephants in captivity globally (Brown, 2014). Asian elephants are considered ‘endangered’ as their population has decreased by 50% over the last three generations (60-75 years). There are approximately 38,500-52,500 Asian elephants remaining, with 50% of them found in India and approximately 16,000 in captivity worldwide (Baskaran et al., 2011; Sukumar, 2006). The major threats to Asian elephants are habitat loss, degradation and fragmentation, which also lead to human-elephant conflict and poaching (IUCN 2017; Karanth and DeFries, 2010). As only male elephants have tusks for poaching, it has been shown that poaching can really skew the sex ratio of males:females (1:6 to 1:122 from 1969 to 1989; IUCN 2017).

Today, elephants are housed in captivity to try to create self-sustaining breeding programs to increase the elephant population, to provide ‘insurance’ against
anthropomorphic catastrophe, and to educate and inform the public about elephant conservation and status (Conde et al., 2011). In North America, the Association of Zoos and Aquariums (AZA) accredits zoos if they meet their high standards of animal care and management, veterinary care, education, conservation, research and safety requirements. Also, zoos that are accredited by AZA agree to participate in Species Survival Plan (SSP), which manages threatened and endangered populations in North American AZA accredited facilities. In Europe, breeding of elephants is recorded in the European Endangered Species Programme (EEP), managed under the European Association of Zoos and Aquaria (EAZA). In North America, there are currently 252 living Asian elephants (199 females and 53 males; Keele, 2015) and there are 208 African elephants (171 females and 37 males; Olson, 2011).

In captivity, the current rate of reproduction is not self-sustaining for either species due to poor reproduction (Olson and Wiese, 2000; Wiese, 2000; Hermes et al., 2007; Brown, 2014). Poor reproduction rates in captivity have been attributed to a variety of factors, including the absence of males in many institutions and limited number of reproductively active bulls, historical lack of breeding programs, high infant mortality, and reproductive aging and acyclicity of females in captivity (Hildebrandt et al., 2000; Brown et al., 2004a; Olson, 2011; Keele, 2015). Due to female reproductive aging, birth of female calves is necessary. It used to be that there was a skewed sex ratio of males:females, with most calves born from artificial insemination resulting in males (Saragusty et al., 2009a). However, current studbook data shows that this may no longer be as significant as it once was (Brown, 2014). Therefore, to increase reproduction rates in captivity, sex-sorted semen may be needed to increase the
number of female elephants in captivity. Unfortunately, one of the largest factors affecting the reproduction of elephants in captivity is the number of non-reproducing male elephants, semen inconsistency, and poor semen quality, making ARTs very difficult to perform.

**The Importance of ART and Semen Collection in Elephants**

Assisted reproductive technologies such as artificial insemination (AI), cryopreservation, semen sexing, in vitro fertilization, embryo transfer, and genome resource banking could help increase elephant numbers and maintain genetic diversity. AI could allow for genetic material from captive elephants around the world as well as from wild populations to be used to increase captive breeding programs (Howard et al., 1984; Brown et al., 2004a; Hermes et al., 2013). As well as propagating the elephant population, insemination of captive females may also reduce possible injuries to females during natural mating and can reduce the number of zoos that need to house bull elephants (Jainudeen et al., 1971; Schmitt and Hildebrandt, 1998; Brown et al., 2004a; Thongtip et al., 2004). Many facilities prefer not to house bull elephants as they can be dangerous to handle due to their unpredictable temperament and musth (Howard et al., 1984; Hildebrandt et al., 1998). AI has been developed for elephants (Schmitt et al., 2001; Brown et al., 2004b) and, to date, there have been 42 elephants conceived from artificial insemination with fresh, chilled semen (O’Brien et al., 2013b). Semen cryopreservation has also been successfully performed for both Asian and African elephants. However, only one female and one male elephant have been born with frozen-thawed semen to date (Saragusty et al., 2016). The success rate of AI in elephants with chilled semen is much higher than the success rate of frozen-thawed semen. Thongtip et al. (2009) reported that two AI trials with chilled semen yielded one
elephant calf, whereas 55 AI trials with frozen semen yielded only one pregnancy that failed to make it to term (Thongtip et al., 2009). A few studies have successfully sex-sorted elephant semen, however further studies are required to improve sort rate and test on multiple bulls (Hermes et al., 2009; O’Brien et al., 2009). To date, in vitro fertilization and embryo culture have not been reported in elephants. Recently, Hermes et al. (2013) collected semen from wild bull African elephants and optimized a protocol to cryopreserve this semen and bank it to help manage population genetics in captivity.

Although a few ARTs have been successfully optimized for elephants, many studies report on the difficulty of retrieving ejaculates with good semen quality. An initial motility of 60% or greater is warranted for artificial insemination or cryopreservation in order to maintain motility ≥ 30% after freezing and thawing – typical “cut-off” values for semen analysis (Saragusty et al., 2005; 2016). There are many males in captivity in North America that have only one or no living offspring (69% of Asian elephants and 71% of African elephants (Keele, 2015; Olson, 2011), indicating the need to retrieve good quality semen from more bulls in captivity for genetic diversity. Kiso et al. (2011; 2012) reported that less than a third of ejaculates in Asian elephant bulls exhibited >60% motility and many are contaminated with urine. Many others have reported on poor semen quality and the intraindividual and interindivdual variability. Therefore, a current research hurdle is to address the different factors that could be responsible for semen quality issues, as applying reproductive technologies towards challenges of elephant conservation require good quality semen samples from bull elephants. This literature review will first introduce the different semen parameters that are commonly
assessed during semen collection and then address the various factors that may be contributing to poor semen quality in captive elephants.

**Semen Evaluation and Indicators of Good Quality Semen**

When assessing semen quality and composition, the following parameters may be evaluated: volume, color, agglutination, pH, osmolality, creatinine, and concentration. When evaluating sperm quality and integrity, parameters including sperm viability, motility (total, progressive and other kinematic parameters with computer-assisted sperm analysis (CASA)), morphology, acrosome-intact, DNA fragmentation, mitochondrial activity, transmission electron microscopy (TEM) and scanning electron microscopy (SEM) are also utilized. These parameters are not always measured and often there is inconsistency in what is and isn’t measured between elephant studies.

Arnold et al. (2017) showed that initial motility and acrosomal integrity were significant predictors for success of further processing (i.e. post-thaw) motility and acrosomal integrity in the Asian elephant. Imrat et al. (2013) demonstrated that semen volume, pH, sperm concentrations and percentages of motile, viable and morphologically abnormal sperm all affected the post-thaw quality of Asian elephant spermatozoa. In almost all studies a wide range of motility and acrosomal integrity both within and among bulls collected is found. Both Imrat et al. (2012) and Saragusty et al. (2009b) have suggested that specific protocols for Asian elephant semen cryopreservation may have to be adjusted to suit specific bull cryopreservation.

O’Brien et al. (2013b) looked at low, intermediate and high sperm motility groups and found that for Asian elephants, sperm concentration was lower for high quality ejaculates than low or intermediate, (noted that 6 out of the 7 ejaculates came from 1 male). Ejaculates of low and intermediate quality were similar in African and Asian
elephants except that in the intermediate group, African elephants motility was higher than Asian elephants. This goes hand in hand with results from Kiso et al., (2013) where significant differences were found between all traits except osmolality between good and bad samples. Good motility ejaculates appeared to be characterized by larger volume and lower concentration, therefore suggesting more sperm were diluted with larger volume of seminal plasma (Kiso et al., 2013). Both these studies suggest that lower concentration and higher volume may be a good indication of a good semen sample.

Since so many elephant samples are contaminated with urine, assessing the urine concentration of samples may be a necessary step to include in assessing semen quality (as well as pH, which not many people study, but has been related to semen quality). Urine analysis has been completed in a few studies by measuring creatinine and urine urea nitrogen levels (Kiso et al., 2013; O'Brien et al., 2013a,b) to identify urine contaminated samples that may not always be easy to spot. O'Brien et al., (2013a) showed that the creatinine concentration of non-contaminated semen was less than 11.0 μg mL⁻¹. Creatinine was higher in low-quality samples (found in all: 20/20), than high quality (none: 0/13) and in intermediate quality only 6/12 or 50% showed > 14 ug/mL. For all motility parameters, after 24 hours of chilled storage, parameters were higher for high quality sample group than both intermediate and low quality sample groups and although agglutination was low for all groups, the rating was higher for high and intermediate quality samples compared to low quality. High quality ejaculates had higher intact plasma membrane (vitality) and greater proportion of morphologically normal spermatozoa and less detached heads than intermediate and low and plasma
membrane and acrosome integrity were similar in high and intermediate but both were better than low.

O’Brien et al., (2013b) also observed that while urine contamination varied across groups, pH and osmolality did not. This data agrees with Kiso et al., (2013) about osmolality, however other studies have shown a difference in pH (Kiso et al., 2013; Imrat et al. 2013). Studies have shown a relationship of pH with the number of detached sperm heads as well as a negative relationship of pH to volume (Imrat et al., 2012). They suggest this is due to the secretions from the accessory glands as the prostate fluid is more acidic and the seminal vesicles are more alkaline. Mann (1964) described the negative effects of excessive alkalinity of semen on fertility and motility. This may show the need for antioxidants in semen extenders. Kiso et al., (2013) also found that higher pH was correlated with good motility ejaculates. They also did assess urine contamination to see if that was the result of different pH of the good versus bad samples, but there was no difference, suggesting urine contamination was not a factor at play.

Agglutination may also be a good indicator of sperm quality. Overtime, Jainudeen et al., (1971) found head-to-head agglutination occurred in motile samples similar to Landowski and Gill (1964). However, agglutination was not found in African elephant semen (Jones, 1973) and in another study with Asian elephants by Heath et al., (1983) they did not observe head-to-head agglutination in their study. Brown et al., (2004b) reports that there was evidence of head-to-head agglutination in most of their ejaculates collected but it was not quantified. In both studies by O’Brien et al., (2013a,b), agglutination of semen samples were scored. O’Brien et al., (2013a) showed
very little agglutination across all sperm quality groups and species (0.5 ± 0.6) but the agglutination was higher in the high and immediate quality samples over low-quality. O’Brien et al., (2013b) reported non-viable fractions that were not used with agglutination of 2.2 ± 0.6 and a viable fraction with agglutination of 0.5 ± 0.3. The agglutination score was rated on 0 = none, 5 = 100% showing head-head agglutination. A study on agglutination in sperm showed that agglutination is mostly found with sperm cells with intact acrosomes, however some single cells with intact acrosomes are also found (Senger and Saacke, 1976). They also found that single cells that are exposed to agglutination deteriorated quicker and there is rapid loss of acrosomes in single cells that are agglutinated compared to sample with no agglutination. However, because cells that are agglutinated together maintain structural integrity it is suggested that there may be a role of agglutination in sperm cells.

The structural integrity of a sperm cell is as important to the overall functionality and success of the sperm cell leading to fertilization as sperm motility. Specifically the plasma and acrosome membranes and functional mitochondria are needed in order for sperm cells to fertilize (Sa-Ardrit et al., 2006). Mammalian sperm needs to go through capacitation and acrosome reaction before reaching fertilization capability (Senger, 2012). Different acrosomal tests and stains as well as TEM and SEM are good ways to learn more about the acrosome and sperm structure. TEM specifically can provide information on the subcellular function of the sperm cell (Heath et al., 1983; Sa-Ardrit et al., 2006). Kitiyanant et al., (2000) wanted to study the acrosome reaction of elephant sperm to see if they could learn more about it, since it is a required step for fertilization both in vivo and in vitro. They looked at the effects of: 1) Heparin, 2) Penicillamine, 3)
Hypotaurine plus epinephine, 4) Caffeine, and 5) cAMP on acrosome reaction by placing 200 ul of spermatozoa (100 x 10^6 sp/ml) in each treatment. They were incubated for two hours in each of the media at 39°C, then they were washed and stained. Samples were also fixed for TEM to assess ultrastructural changes in the acrosomal region. If the plasma membrane was fused with the outer acrosomal membrane the sperm was evaluated as acrosome-reacted and if inner acrosomal membrane was exposed, apical vesiculation was present and equatorial segment was visible (Kitiyanant et al., 2000). The percent of acrosome-reacted sperm from highest to lowest was as follows: caffeine, penicillamine hypotaurine plus epinephine, cAMP and heparin, with the first two treatments exhibiting significantly acrosome-reacted sperm. The success of the acrosome-reaction is important to use in future studies of in vitro fertilization in the elephant. Sa-Ardrit et al., (2009) used calcium ionophore and heparin to induce the acrosome reaction in elephant sperm and measured it with FITC-PNA (marker of acrosome leakage) and propidium iodide (marking of membrane damage). In this study they found that the calcium ionophore was more effective than the heparin in inducing the acrosome reaction. This is most likely because calcium ionophore causes a rapid influx of calcium into the cells causing the acrosome reaction (Zhang et al., 1990). This is interesting for future work with in vitro studies.

The last semen parameter that will be discussed here is DNA fragmentation as DNA is essential for creating an embryo. Sperm DNA is not a stable state, but is very dynamic and the quality of DNA can be damaged through processing. Imrat et al., (2012) looked at the DNA stability of elephant spermatozoa initially and after many
different semen processing techniques. They found that loss of DNA occurred during incubation at 37°C before further processing even occurred.

O’Brien et al., (2013a) found that after 24 hours of chilled storage, the majority of elephants displayed a large degree of DNA damage and this accompanied a high number of detached heads. The high quality ejaculates showed high DNA integrity at 24 hours of storage with average of 12.3% of samples showing fragmented DNA, while intermediate showed 55% and low 67.9% DNA fragmentation and when flow cytometry was performed, high ejaculates had a greater resolution of X and Y sperm populations than intermediate or low. However, ejaculates of high quality (13/157) showed a lower incidence of DNA fragmentation and detached heads over storage for 48 hours. The high DNA fragmentation in low and intermediate ejaculates shows that this may be due to aged spermatozoa. Therefore, DNA fragmentation could be used as an indicator of poor sperm quality.

Factors Influencing Semen Quality

A systematic review of the literature of 37 peer-reviewed studies which reported on semen collection in captive and wild elephants yielded a range of factors worth considering to improve semen quality, including collection method, collection frequency, semen composition and seminal plasma, species differences, age effects, musth and season effects, and housing factors associated with natural and captive living. In Table 5-1, we provide a comprehensive summary of the key findings across these studies. Overall, it is interesting to note that many studies on captive elephants utilized a large number of males and a high number of ejaculates, yet only a low proportion of ejaculates were of suitable quality (i.e. >60% initial total motility). In most elephant papers reported in the literature, >60% is the “cut-off” point for samples that will be used
for artificial insemination or sperm cryopreservation and sex-sorting as motility will be lost during semen processing.

**Collection Method**

Several collection methods have been described in both Asian and African elephants. The first collection and observation of semen characteristics was completed by Landowski and Gill (1964) on semen opportunistically collected from the vagina immediately after copulation. Following this, Jainudeen et al. (1971) collected semen passively from a male Asian elephant in Ceylon for evaluation of sperm and seminal characteristics. When a bull elephant was not able to mount females due to leg issues, they were able to collect semen through penile protrusion when the elephants pectoral region was stroked (Jainudeen et al., 1971).

Semen can also be collected through the reproductive tract of culled animals. In 1973, semen was taken from wild bulls during a culling program in South Africa (Jones, 1973). Semen was collected from excurrent duct of the testis and diluted with medium to stimulate motility.

Another collection method for collection of live wild bulls is electroejaculation, which requires sedation. In some early attempts of electroejaculation of wild African bull elephants, Jones et al., (1973, 1975) collected semen with no sperm, followed by another ejaculate with motile sperm. Other attempts at electroejaculation in elephants were completed by Ruedi and Kupfer (1981) and Ruedi et al., (1983), who ejaculated 3 bulls, yielding only 1 sample containing motile sperm. Howard et al., (1984) used a regimented protocol for electroejaculation in wild African elephant bulls that consisted of immobilization with 10 mg etorphine hydrochloride intramuscularly (darted from helicopter) and 120 electrical stimulations at 10 to 30 V in three series and was much
more successful than previous attempts. To date there have been several successful
collections from wild bulls with electroejaculation (see Table 5-1).

One study by Heath et al., (1983) used a modified equine artificial vagina (AV)
with stimulation of the base of the penis via rectum to collect semen from three Asian
elephant bulls. This study showed higher volumes of semen and semen were found in
every sample collected, compared to samples retrieved by Jainudeen et al., (1971).
Kitiyanant et al., (2000) also used an AV for semen collection of an Asian elephant. The
elephant was trained to use the vagina for 6 months and successfully ejaculated into the
AV. Samples were collected weekly and nearly all attempts were successful. This
method also resulted in better quality semen with similar viscosity in each collection,
unlike with electroejaculation where viscosity varies. However, it does require hands-on
human contact with the bull elephant, whereas many bulls are managed with restricted-
contact (e.g. keepers handle bulls from outside the enclosure; Schmitt and Hildebrandt,
1998).

Manual manipulation, also referred to as rectal massage, is the semen collection
method most used today. It is well-described by Schmitt and Hildebrandt (1998) and is
the most common method used in captive elephants today. Previously, Heath et al.,
(1983) described stimulation of the root of the penis via rectum with the artificial vagina
and Price et al., (1986) used manual massage with a condom to collect semen from an
elephant. According to Schmitt and Hildebrandt (1998) manual manipulation involves
“rectal massage of the pelvic portion of the urethra, near the seminal colliculus” leading
to penile protrusion and erection. The penis is cleaned and a collection sleeve is placed
over the penis. During the rectal massage when there was detection of an ejaculatory
response, massage in the region of the ampulla was included and soon after a sperm-rich sample was collected (Schmitt and Hildebrandt, 1998). Ultrasounding the bull to verify size of ampulla and seminal vesicles before and after collection can help to verify emptying of the glands (Hildebrandt et al., 1998). Immediately after collection semen should be placed at 35°C. This collection method has been successful in many studies (see Table 5-1). Interestingly, Imrat et al., (2014) found that small ampulla diameter (max size of ampulla = 5 cm) correlated to lower sperm quality and a larger diameter correlated positively to motile, morphologically normal and membrane intact spermatozoa. No correlation to diameter and bulls age was found.

Rectal massage was developed for captive elephants, as there are several risks with electroejaculation, such as frequent anaesthesia and possible injuries during sedation and recovery in captive enclosures. Also, samples collected from manual manipulation are sperm-rich and include very little dilution from seminal plasma and other accessory glands (Schmitt and Hildebrandt, 1998), whereas semen samples collected using electroejaculation are often very gelatinous. It also requires no drugs or expensive equipment. Manual manipulation also has benefits over the use of an artificial vagina, as it does not require intensive training to collect semen from a bull; only a restraining device if the bull is in “restricted contact” (Schmitt and Hildebrandt, 1998).

There have been studies done on the use of “standing sedation” in elephants, which can be safely used for manual manipulation of captive bull elephants in restricted contact. Although this technique is not recommended for routine procedure as it could lead to risks associated with chemical restraint (respiratory compromise) it could be
used for elephants that are not used to the procedure (untrained), facilities that do not have a restraint chute, or bulls that may be in musth (slightly more aggressive than normal). Portas et al., (2007) discuss manual manipulation using sedation on an Asian elephant bull. First the elephant is placed in his restraining chute and sedative drugs, specifically xylazine hydrochloride, are given in the triceps muscles and once signs of sedation were noted, butorphanol tartrate was administered. At the end of the procedure reversal drugs were administered to bring the elephant out of sedation. Semen was collected very easily (within 10 minutes of start of rectal massage) and this may have been associated with the use of xylazine as a sedative agent (Portas et al., 2007). Lüders et al., (2016; 2017) also showed that standing sedation could be successfully used in African bull elephants with butorphanol and medetomidine, with or without hyaluronidase and reversal with atipamezole and naltrexone, and is reliable and safe for transrectal ultrasonography and semen collection. It is important to note that Asian elephants may be more sensitive to sedation drugs than African elephants and caution should be taken to properly address correct drug amounts according to each species (Lüders et al., 2016).

Only two types of the collection techniques mentioned above are still routinely used for semen collection in African and Asian elephants today. Electroejaculation is used for semen collection in wild elephants and manual manipulation is used for semen collection in captive elephants. While both methods appear to be well established, it has long been reported that semen quality from manual manipulation in the captive elephant population is variable and low. The common theme of poor quality semen from rectal massage can be easily seen in Table 5-1 with ≤50% of samples used in
various studies. Brown et al., (2004b) discusses how sperm quality was variable from bull elephants that it was a limiting factor for the artificial insemination that they were trying to perform. Poor semen quality and variability from manual manipulation may depend on other factors such as prevalence of musth, on captive bull management and collection techniques.

One factor that should be considered when it comes to the type of collection is the presence/absence of seminal plasma. Elephant accessory glands include: 1) Seminal vesicles, 2) Ampulla, 3) Prostrate, and 4) Bulbourethral glands (Hildebrandt et al., 2000). It is worth noting that accessory glands differ between mammals (Senger, 2012). Seminal plasma is a secretion from the accessory sex glands that provides a nutrient rich medium that is essential for sperm motility (Senger, 2012; Elzanaty et al., 2002). While manual manipulation is the preferred method to collect semen from captive elephants, the sample obtained is not a full ejaculate and is often void of most of the accessory gland secretions (Schmitt, 2006). Also, seminal plasma components can vary between individuals since manual rectal massage doesn’t allow for the same mix of seminal plasma in each ejaculate and can lead to a high risk of urine contamination in samples (Hildebrandt et al., 1998; Rofdriguez-Martinez et al. 2011; Imrat et al., 2013). Electroejaculation (as noted in the “wild elephant” part of Table 5-1) appears to be a very successful way to collect semen with good motility and all accessory glands are stimulated, but it requires sedation and it would not be in good welfare to collect regularly by this method in captivity due to the risks of frequent anesthesia (Howard et al., 1984).
Therefore the question remains: are there components in accessory glands that are crucial for semen quality in elephant ejaculates? It has been suggested that high levels of seminal plasma elements may affect semen quality (Hong et al., 1984). The roles of accessory gland secretions have not yet been completely confirmed in elephants. In some species seminal plasma is beneficial and in others it is not (negative in stallion, positive in bull; Senger, 2012). While some studies show that removing seminal plasma is best (Saragusty et al., 2009b; Kiso et al., 2012) some studies have left seminal plasma in their semen and still obtained acceptable results (Jainudeen et al., 1971; Thongtip et al., 2004, 2008a; Arnold et al., 2017). Jainudeen et al., (1971) looked at sperm-rich and sperm-free ejaculates from an Asian elephant collected opportunistically and found that adding sperm-free ejaculates to the sperm-rich ejaculated improved sperm motility, possibly showing the importance of seminal plasma for sperm motility. Jones (1973) also showed that semen from the excurrent distal duct were immotile until sperm were diluted. Heath et al., (1983) found increased motility after subsequent daily collections and suggested a reason for this possible increase in motility could be due to the addition of seminal plasma components added each day, just like seminal plasma helped to induce motility in both studies by Jainudeen et al., (1971) and Jones (1973).

Studies in cattle bulls (Bos) have shown that differences between collection methods do exist. In one study, transrectal massage of the ampullary region resulted in lower sperm motility and viability than sperm from electroejaculation (Palmer et al., 2005). However, electroejaculation gave lower sperm motility than semen collected from an AV after cryopreservation (Leon et al., 1991). However another study showed
that Bali bulls collected by rectal massage had higher motility, functionality, viability, intact acrosome and lower seminal plasma, total proteins than electroejaculation, which is different than the first study described (Sarsaifi et al., 2015). These studies indicate the differences between semen quality from different collection methods. Schmitt (2006) discusses how manual manipulation does not stimulate proper contributions from accessory glands and these variable contributions might reflect some of the variability that we see. Short et al., (1967) assessed elephant reproductive tracts post-mortem and found that elephant’s seminal vesicles produce a watery substance and the bulbourethral glands produce more of a viscous substance. Howard et al., (1986) found some variability in ejaculate viscosity during electroejaculation and it was suggested that this may be attributed to differences in accessory gland stimulation during electroejaculation (i.e. more bulbourethral fluid). From Table 5-1 it is clear that almost all semen collections collected through electroejaculation in wild African elephants is acceptable quality and can be used, compared with rectal massage where <50% of samples are typically good enough quality to use, which could relate to either collection method or environmental and social factors, discussed in more detail later on in this review.

**Frequency of Collection**

Abstinence and collection frequency is a factor that has been found to affect sperm parameters in humans. Avoiding abstinence improves the percentage of sperm motility, normal morphology, volume and total motile sperm counts with male factor infertility (Levitas et al., 2004). After only 2 days of abstinence, sperm initiate a process of quality degradation, for those patients with male factor infertility (Levitas et al., 2004). In stallions, daily or twice daily collection is recommended (Sieme et al., 2004). In bulls
and stallions, poor semen quality can be related to accumulation of spermatozoa in the ampullae, vas deferens, and cauda epididymis when not collected frequently (Barth, 2007; Pickett and Voss, 1973). These studies indicated that it requires approximately 1 week after beginning a period of frequent ejaculations to achieve good semen quality and that re-accumulation of senescent sperm would occur after approximately one month of not collecting.

Few studies in elephants have attempted frequent collections. Following challenges with no sperm motility in semen of Asian elephants collected via AV, Heath et al., (1983) eventually collected one bull on consecutive days, twice a day, and found an increase in both volume and progressive motility each day, and samples were finally used on the third day (115 ml and 40% motility). Schmitt and Hildebrandt (1998), collected samples from one elephant 3 times a week (sometimes more frequently if semen was being used for artificial insemination) for 12 months to assess volume and concentration of regular collections. They found varying volumes of semen collected (0 – 200 ml), but no specific correlation to number of times collected and semen quality, and no clear pattern in volumes collected. Thongtip et al., (2008a,b) found that when bull elephants were not collected for a length of time, poor quality semen would often be collected. Using chemical and physical restraint to manually collect Asian elephants, Portas et al., (2007) found low motility ranging from 0 – 25% and concentration ranging from 720 – 1610 x 10^6 sp/ml, and suggested that this may be attributed to long periods without collection and poor fertility (Type II non-breeder; Hildebrandt et al., 1998).

Imrat et al., (2014) examined the effects of the frequency of collection on sperm quality of Asian elephants. They collected bulls five times on alternating days for a 10-
day period after a long period of rest (at least one year). With more frequent collections, they found improved sperm morphology (fewer detached heads and head and mid-piece abnormalities). O’Brien et al., (2013a) suggested that detached heads were correlated with DNA damage in aged sperm and found that aged sperm may still be collected even with regular collections. However, Imrat et al., (2014) found only one bull showed improved semen motility (0% to 60%) and membrane integrity (5% to 75%), suggesting that response varied between bulls. Despite some improvement, membrane integrity and motility still remained at low values, suggesting that other factors influenced quality, or that a 10-day period of collection was not long enough. Therefore, it is suggested that perhaps longer studies of alternate day collection on male elephants should be examined (Imrat et al., 2014).

Attempts to Improve or Further Understand Semen Quality in Elephants

Calcium is an activator of the acrosome reaction and therefore the levels of calcium in elephant seminal plasma should be assessed as there is a possibility that the calcium concentration may affect elephant semen motility (Sivilaikul et al., 2010). They found that calcium was only negatively correlated with motility and not with volume, concentration, pH, dead sperm or morphology. The average level of seminal calcium was 9.21 ± 0.85 and it was higher in low-motile groups (11.17 ± 1.62) than the high-motile groups (5.91 ± 0.50). Also the moderate-motile groups had higher seminal calcium than the high-motile groups (9.67 ± 1.41). These authors suggested that calcium may be utilized by the sperm cells during motility and therefore the high-motile group has the lowest level of calcium in their seminal plasma. However, there could be other factors in the seminal plasma that could be correlated with semen quality, so more
information is still needed. They also found that sperm cells in the low-motile group had higher abnormal morphology and dead sperm.

Several studies have been conducted to try and figure out if altering or adding chemicals and/or media to semen samples can improve the quality of the semen sample. While Saragusty et al., (2005) and Swain and Miller (2000) assessed the effect of egg-yolk and lipids on sperm cells, this was to help sperm survive chilling and cryopreservation, not initial motility. Thongtip et al., (2008b) looked at the effect of pentoxifylline (PTX) on poor quality sperm cells and had the first study in regards to increasing poor semen samples in elephants. In humans, PTX has been used in vitro to increase testicular sperm motility in culture (Angelopoulos et al., 1999) and induce hyperactivation (Kay et al., 1993) and it has enhanced sperm motility in electroejaculated baboon semen (Cseh et al., 2000). Other studies have shown that PTX can inhibit reactive oxygen species production (Gavella et al., 1991) and enhance the acrosome reaction (Tesarik et al., 1992). It has been suggested that PTX is able to enhance sperm motility through increasing intracellular cAMP concentration and activation of cAMP-dependent kinases through inhibiting phosphodiesterase (Tash et al., 1986). Using the previous literature on dosage amounts and incubation time of PTX, Thongtip et al., (2008b) tested the effect of 0.5, 1 and 2 mg PTX/mL for 1 hour on elephant sperm motility and movement of poor quality (0 – 9% motility, n = 8) and low-motile (10 – 30% motility, n = 3) samples using a CASA system. Samples were checked at 15 and 30 minutes. They found that PTX did not significantly improve sperm motility characteristics in either group, but there was a tendency for it to maintain sperm
motility and movement in the low-quality group up to 30 minutes. Perhaps PTX acts quicker and sperm should be analyzed before 15 minutes.

Seminal plasma characteristics including chemistry and protein profiles of Asian elephants were compared between good quality samples (≥65%) to lower quality samples (≤10%) by Kiso et al., (2013). It was found that there were differences in concentrations of creatine phosphokinase, alanine aminotransferase, phosphorus, sodium, chloride, magnesium, and glucose, with them being higher in good samples (Kiso et al., 2013). This could help to explain sample inconsistency. There was also one protein: lactotransferrin that was found in 85% of ejaculates with good motility and wasn’t found in 90% of ejaculates with poor motility. It has been suggested that the role of lactotransferrin may be to act as a natural antioxidant.

In some species it has been shown that GLUT proteins which play a role in the metabolism of glucose and fructose may play a role in compromising sperm quality by affecting the plasma membrane (Sancho et al., 2007; Bucci et al., 2010). Specifically, GLUT3, which is a transporter that works well with low concentrations of glucose (as seminal plasma has low amounts of glucose and high fructose, was assessed (Rigau et al. 2001; Haber et al., 1993). A study by Sajapitak et al., (2016) showed that in elephant sperm, the correlation between the percent of GLUT3-expressing sperm and sperm motility % and live sperm was very high. They GLUT3 proteins were found at the principal and end piece of the sperm tail.

The effects of different types of seminal plasma on Asian elephant sperm quality were assessed (Pinyopummin et al., 2017). The authors looked at different types of seminal plasma including stallion (Equus), boar (Sus) and dog (Canis) due to the
difference in accessory glands and that they could get a semen sample from these species that was considered a complete natural ejaculation. The stallion is similar to the elephant and has the exact same accessory glands, the boar is missing the ampulla and the dog only has the prostate gland (Senger, 2012). They found that autologous seminal plasma (seminal plasma retrieved from the same bull elephant) helped preserve sperm motility and acrosome integrity, and that homologous seminal plasma (seminal plasma from a different bull elephant) was not any better than autologous. However, heterologous (stallion seminal plasma only) did provide higher motility and velocity compared to autologous and helped to preserve acrosome integrity. They also looked at the seminal plasma from four different stallions and observed the same parameters among each stallion. It did appear overall that elephants do well with seminal plasma as motility and acrosome integrity were positively affected by the presence of it. To see a homologous effect like what has been seen in the stallion (Morrell et al., 2014), perhaps seminal plasma from different types of collection methods should be tried, as perhaps the right amounts of seminal fluids are not being obtained from the elephant by the manual manipulation method.

Pinyopummin et al., (2018) completed another similar study addressing the effects of stallion seminal plasma on elephant sperm. They found that elephant sperm motility was affected by dilution and that restoration of seminal plasma with stallion seminal plasma could improve motility when it was highly diluted. This was addressed because when storing cooled elephant semen, semen is diluted with extender as it has been shown in various studies to increase sperm motility (Thongtip et al., 2004b; Saragusty et al., 2005;2009b; Kiso et al., 2011; O’Brien et al., 2013a,b). However, due
to the previous study that shows that elephant sperm may do well with the presence of seminal plasma (also see study by Januideen et al., 1971) they looked at the addition of seminal plasma back to extended semen. They found that Asian elephant semen motility and velocity were affected by dilution during cooled storage and that restoring the proportion of seminal plasma with elephant seminal plasma did not maintain motility, but the addition of stallion seminal plasma did improve the impaired motility. Often extender is added at a ratio of 1:1, however, Pinyopummin et al., (2018) found that 1:1 dilution had higher percentages of motility and slow swimming sperm than the 1:15 dilution. This most likely has to do with the seminal plasma proteins that are found in semen.

While many studies have addressed proteomics, lipidomics could also be crucial to helping improve semen quality. There is definitely a difference between Asian and African elephants with regards to sperm plasma membrane lipidomics. African elephants have a greater number of polyunsaturated fatty acids than Asian elephants and this plays a role in their capabilities to withstand freezing and difference in extender requirements (Swain and Miller, 2000; Kiso et al., 2011, 2012).

In some species, lipid peroxidation has been suggested to play a role in poor sperm quality as the fatty acids in the plasma membrane are susceptible to free radicals (Aitken et al., 1993). A study by Thongtipsiridech et al., (2011) showed that oxidative stress may play a role in poor semen quality in captive male elephants. Malondialdehyde (MDA) is a stable lipid peroxidation product that can be found in seminal plasma and can be correlated to being high when semen quality is low (Andreea and Stela, 2010). Different antioxidants in the seminal plasma may help to
protect sperm cells from antioxidants (Saleh and Agarwal, 2002). A significant negative correlation between MDA level and progressive motility (R=0.2131) and normal morphology (R=0.1685) was found in the Asian elephant. Low-motile groups (<40% progressive motility) had higher levels of MDA than high-motile groups (>40% progressive motility) and MDA concentrations were very different between bulls (Thongtipsiridech et al., 2011). Therefore MDA may be a good indicator of semen quality, with high MDA levels correlating to poor semen ejaculates. Therefore perhaps steps could be taken to enhance/prevent lipid oxidation.

Kiso et al., (2012) showed that membrane stabilizers, such as modifying the cholesterol of the plasma membrane through the use of cholesterol-loaded cyclodextrins (CLC) of elephant semen before cryopreservation may help to modify the sperm membrane to increase the survival rates after cryopreservation in elephants. This is because membranes with higher cholesterol:phospholipid molar rations are more tolerant to changes in temperature (Amann and Pickett, 1987). In the study by Kiso et al., (2012), they found that CLC concentrations greater than or equal to 1.5 mg were needed to increase membrane cholesterol concentrations and incubating them with CLC’s increased cryopreservation post-thaw parameters. They also showed the importance of adding glycerol at 4C and diluting samples in an egg-yolk based medium upon post-thaw instead of a basic culture medium (Kiso et al., 2012).

Lipid peroxidation in spermatozoa and seminal plasma in elephants has recently been conducted (Satitmanwiwat et al., 2017). The authors wanted to see the difference of lipid and protein oxidation on samples of different quality. They found that seminal plasma malondialdehyde (MDA) and seminal plasma protein carbonyls (oxidative stress
biomarkers) were higher in poor motility (≤20%) than good motility (>60%) samples. An important factor relating to poor semen quality in elephants has been related to oxidative stress from reactive oxygen species (Thongtip et al., 2008a; O’Brien et al., 2013b; Satitmanwiwat et al., 2017). Also it was suggested that elephant sperm might be affected by free radicals interacting with polyunsaturated fatty acids in the sperm plasma membrane (Thongtipsiridech et al., 2011). They found no relation of age to oxidative stress biomarkers. It is possible that the results of the study by Satitmanwiwat et al., (2017) correlate nicely with results from Kiso et al., (2013), who found that the presence of lactotransferrin was correlated with good sperm motility. Satitmanwiwat et al., (2017) suggests that lactotransferrin (possible antioxidant) may decrease the amount of free iron that could initiate the lipid or protein oxidation.

Saragusty et al., (2016) looked at what they could do with poor quality semen from wild African elephants. They wanted to look at the effect of density-gradient centrifugation on samples as wild animals often have low motility and a high amount of abnormal sperm cells (Kiso et al., 2012; Saragusty et al., 2016). As protocols are being developed, it is inevitable that some samples will be poor and due to the value of samples from wild elephants, it would be silly to discard these samples. Although in elephants from the literature it appears that initial motility is decent in elephants, some frozen semen has quality of <30% (the minimum required for artificial insemination), which makes it too low to be used in artificial insemination programs. In this study they did not take pre-freeze motility into consideration, only post-thaw parameters. They did find that there was a difference between the gradient centrifugation treatments tried, but not with regards to acrosome integrity, normal morphology or yield of motile
spermatozoa. Optiprep was the only treatment that did not enhance motility over the control, all others did. The best results were found with Percoll with 6/10 samples having greater than 30% motility and when incubated after thawing for 3 hours at 37C, percoll showed the lowest decrease in motility. Overall, percoll and isolate tests exhibited higher motility and kinetic variables.

**Species Differences**

Although African and Asian elephants are very closely related, there seems to be aspects where their sperm differ greatly (Saragusty et al., 2009). First, the morphometrics (specifically head and tail) of the sperm cell appear to be different between the African and Asian elephant (Jainudeen et al., 1971; Johnson and Buss, 1967; Heath et al., 1983; Gilmore et al., 1998). Some studies have shown that long-term storage and cryopreservation of sperm from African elephants has been successful, whereas these protocols applied to Asian elephant semen are unsuccessful (O’Brien et al., 1997; Swain and Miller, 2000; Wendy et al. 2011).

Swain and Miller (2000) found that Asian elephant sperm cells have different fatty acid compositions of their plasma membranes than African elephants. Plasma membrane structure plays a role in sperm physiology and cryopreservation of spermatozoa, therefore the differences found in the plasma membrane composition and their ability to interact with extenders and cryoprotectants may explain why some protocols work and some fail (Swain and Miller, 2000). Membranes that have a high amount of polyunsaturated fatty acids are more fluid than membranes with a high amount of saturated fatty acids. Swain and Miller (2000) found that African elephants had higher levels of docosahexaenoic acid (22:6, n-3; 68.13% African versus 42.88% in Asian) and docosapentaenoic acid (22:5, n-6) than Asian elephants who had higher
levels of myristic acid (14:0), arachidonic acid (20:4, n-6) and docosatetraenoic acid (22:4, n-6), suggesting that Asian elephants have lower levels of unsaturated, long-chain fatty acids (unsaturated:saturated = 2.0). Long-chain unsaturated fatty acids may be required to cryopreservation of elephant spermatozoa helping to maintain membrane stability at lower temperatures. Swain and Miller (2000) speculated that these differences could be due to nutrition, genetic-based differences in fatty acid metabolism and/or differences in the turnover rates of long-chain polyunsaturated fatty acids due to cryogenic-induction. Saragusty et al., (2005) showed the importance of a egg-yolk based extender where the lipids in the egg-yolk can protect the spermatozoa during the chilling process. Once again the less polyunsaturated fatty acids in the Asian elephant semen may be leading to the reduced freezing ability of the Asian elephant versus the African elephant.

Graham et al., (2004) looked at the differences of extenders on the long-term storage of Asian elephant spermatozoa. They found that egg yolk extenders led to a reduction in the decline of viability (17%) compared to those without egg yolk (32.6%) for up to 48 hours. Therefore they suggested that a tris-citric acid/fructose-based with 20% egg-yolk extender was successful in improving and maintaining sperm motility. It is possible that the egg-yolk helps to prevent cold-shock through incorporating themselves into the plasma membrane. Thongtip et al., (2004) looked at the cryopreservation of Asian elephants to try to determine more successful methods for the Asian elephant. They used two different semen extenders (TEST and HEPT), both which were used in the African elephant and both had 20% egg yolk as well as two cryoprotectants: DMSO and glycerol. Overall post-thaw motility and acrosomal integrity
was significantly higher with TEST + glycerol (i.e. EM1 Post-Thaw motility: TEST + glycerol = 42.0% versus HEPT + glycerol = 24.0, TEST + DMSO = 9.2 and HEPT + DMSO = 4.4%), suggesting this as a acceptable freezing medium for Asian elephant semen (Thongtip et al., 2004).

Finally, Kiso et al., (2011) looked at different extenders on long term liquid storage (24 hrs) of both Asian and African elephant semen to identify the optimal extenders for each species. They found that after 24 hours of storage that the African elephant semen exhibited greater sperm quality parameters than the Asian elephant. They also found that storage at 22ºC or 4ºC was better for both species than storage at 35ºC. It was also noted that all five extenders tried worked great for the African elephant, but only extenders with a source of lipoprotein worked well for the Asian elephant. The three best extenders, maintained a good initial motility at 12 hours in the Asian elephant (BIL = 45%, TES = 40% and INR = 38%) and at 24 hours (BIL = 27%, TES = 13% and INR = 14%). The other two extenders showed a greater loss in motility and by 24 hours motility was less than 5%. With African elephant no differences were found in sperm parameters between extenders. Therefore these results suggest that African elephant spermatozoa maintained motility longer than Asian elephant spermatozoa and for both species, lower than body temperatures improved sperm survival and although any extender works well for African elephants, a source of lipoprotein is required for Asian elephant semen (Kiso et al., 2011).

**Effect of Age**

There are various reports of age effects on semen quality across studies, with lower quality sample obtained from young and older bulls. Thongtip et al., (2008a) found that the highest quality semen was observed between ages 23 to 43 years of age,
with an age range of 10 to 72 years of age. They found that age significantly affected seminal parameters and hormone profiles, with bulls between 51 to 70 years of age having significantly lower progressive motility, viability and seminal plasma zinc concentrations than younger bulls (Thongtip et al., 2008a). Imrat et al., (2014) also found that older bulls (> 35 years) had lower intact sperm membranes and more detached heads. Sperm concentration was lower in younger bulls (10 to 19) than older bulls and bulls between 23 to 43 had the highest percentage of normal morphology. When first collecting from a young elephant (6.5 years old), Saragusty et al., (2005) found poor motility in samples collected from the first collection and found variable motility ranging from 5 to 60% motility, but that motility stayed above 20% after his third collection. O’Brien et al., (2013b) looked at semen quality and age between different quality groups and found that the average age in each group was similar (~20), but differences were only found for one bull (Age 9) during his first year of semen collection where he gave low-quality ejaculates. Like the study by Saragusty et al., (2005) during the 4-year collection he gave high quality ejaculates at 11 and 12 years of age.

Keeping all of the above in mind, Thongtipsiridech et al., (2011) suggested that the reason for slightly better samples than average in their study may have been due to the ages of the elephants used (19- 46). Thongtip et al., (2008a) also found that age related to testosterone and seminal plasma characteristics. Older bulls had higher circulating T3 and similar T4 to young bulls and although serum testosterone did not differ by age, seminal testosterone was highest in the young bulls. Age-associated decrease in sperm quality is most likely due to functional changes of testes and endocrine system (Thongtip et al., 2008a).
Effect of Musth and Season

Musth is unique to elephants and refers to an annual period of high testosterone, searching for females in estrous and increased aggressiveness (Eisenberg and Lockhart, 1972; Poole and Moss, 1981). Musth signals include elevated stature, increased aggression and testosterone levels, urine dribbling, temporal gland secretion, and penile protrusion, both found in both Asian and African elephants (Poole and Moss, 1981; Rasmussen et al., 1996).

Captive elephants typically come into musth after 20 years of age, however it has been recorded as young as 7, and is about 1-3 months in length, which is longer than wild bulls (Schulte, 2000; Brown, 2014). This could possibly be due to how they are housed or their nutrition. It could also be from the constant effect of hyper testosterone secretion (Brown et al., 2007) playing negative and cumulative effects on males. Semen has been collected from male elephants that are not in musth, suggesting that musth is not necessary for reproductive function in captive elephants (Jainudeen et al., 1971). Howard et al., (1984) described results of electroejaculation of wild African elephants that varied in the presence of temporal gland secretions and testosterone levels, with one bull having high testosterone levels of >25 ng/ml, resembling those of Asian elephants in musth. However, they found that high motility samples were collected from both bulls regardless of presence of musth indicators, further suggesting that reproductive function and temporal gland activity, or possibly musth, are independent (Howard et al., 1984). Kiso et al., (2013) found that daily variations in semen quality were found even when bulls were not in musth, suggesting that sperm quality is more influenced by factors other than testosterone and musth. Also in the wild, most matings happen when a bull is in musth and therefore dominance is also
related to musth (Poole, 1989). However, bulls can only mate for a short period of time when they are musth because prolonged elevated testosterone will eventually damage sperm quality (Hildebrandt et al., 2006).

A long-term study by Hall-Martin and Van Der Walt (1984) reported temporal gland secretions and other musth indicators only in the two highest ranking African males in the population with testosterone levels varying from not in musth versus in musth (3.48 – 9.47 versus 19.80 ng/ml respectively). The higher ranking male also had higher levels of testosterone when not in musth compared to the lower ranking male. Jainudeen et al. (1972) reported that testosterone levels were low in Asian elephants when bulls were not in musth, but were extremely high during musth (4.3 - 13.7 versus 29.6 – 65.4 ng/ml). Thongtip et al. (2008a) reported that semen and serum were not collected during the period of time that 4 of 13 Asian elephant bulls came into musth due to aggressive behavior (Thongtip et al., 2008a). However, they noticed that, progressive motility was higher in most males during the month before musth, and decreased the month after musth in half the males, suggesting a possible relationship between motility and musth.

Seasonality is a factor that can influence semen quality in animals that are considered seasonal breeders like the horse. Stallions are “long day breeders” and endocrine and germinative testicular functions are influenced by season (Cox et al., 1988; Johnson 1985). Seasonality has yet to be observed in elephants. The hyrax, one of the closest relatives to the elephant, is also a seasonal breeder. During annual breeding season, testis weights and plasma testosterone levels were approximately five times greater (Neaves, 1973). Female elephants are known to be polyestrous, but little
is known about male elephant reproduction (Brown, 2006). If females are polyestrous breeders, there is a good chance that males are too (meaning that they are not seasonal breeders), but studies should be done to assess if testosterone is higher at different times of the year. Acquiring histological samples of the testes would only be possible through samples from deceased animals, which makes that approach null. However endocrine and testosterone monitoring could be crucial to determine if they have a seasonal cycle that affects semen quality. However this possible seasonality factor does not explain why we may get good quality semen from a bull one day and poor quality the next. Musth occurs annually in sexually mature bulls in the wild, but musth is often irregular in captive bulls (Brown et al. 2007; Sukumar 2003). Knowing more about the biochemistry of musth or the semen that is produced during musth could tell us a lot about elephants, but this is difficult as bulls can be dangerous to collect semen from or work with during musth (Schmitt and Hildebrandt, 1998).

Diurnal variation in testosterone concentration has also been assessed in elephants. Howard et al., (1984) collected semen from nine adult African male elephants in Kruger National Park, SA. Four males were collected in the morning between 07:30 and 08:00 hr and 5 bulls were collected in the afternoon between 15:00 and 18:00 hr, Serial blood samples were collected before electroejaculation, immediately after each of the 3 series and in 15 min intervals up to 45 minutes after electroejaculation. In the morning group (07:30 – 08:00 hr), testosterone concentration ranged from 1.4 to 8.2 ng/ml, and in the afternoon group, 4 out of the 5 bulls had testosterone concentrations that were <0.9 ng/ml. One bull, assessed at 16:00 hr had very high testosterone concentrations with the highest at 25.6 ng/ml. Only in the bull
assessed at 16:00 hr did electroejaculation have any affect on testosterone concentrations. In the bull with the highest concentration of testosterone, testosterone concentrations declined throughout the electroejaculation process to a final of 12.2 ng/ml 30 minutes after semen collection was finished. No direct correlation was found with testosterone concentration in the bulls that had temporal secretion (Bull 1 = 0.4, Bull 2 = 5.3 and Bull 3 = 18.3 ng/ml), and testosterone was not correlated with sperm motility or concentration (Howard et al., 1984). Overall, there was very little temporal fluctuation in testosterone levels over the sampling period and the reason for the difference in testosterone levels in individual males in unknown, but may relate to diurnal variation (Howard et al., 1984).

In rams (*Ovis*), many seminal characteristics are altered by season (Parkinson and Follett, 1994). Therefore Thongtip et al., (2008a) hypothesized that elephant semen quality would be correlated with both circulating (serum testosterone) and seminal concentrations of thyroid hormone (T4 and T3). In this study, Seasonality was separated into three different seasons in this study with the rainy season from July to October, winter from November to February and summer from March to June. They did find that seminal and hormonal parameters were different between the two motility groups, but this was also influenced by season (Thongtip et al., 2008a). Seasonality only affected serum testosterone. Serum testosterone correlated with viability being higher in the rainy season and winter. It was suggested that the reason for lower motility in the summer months may be due to ambient temperature and the fact that spermatogenesis is a temperature-sensitive process and that morphology is not affected by temperature. This may be especially true for elephants with their intra-
abdominal testes. Serum testosterone was the only hormone that was affected by season and this was higher in the rainy season when nutrition was high. Serum testosterone has been associated in other studies with musth and nutrition (Cooper et al., 1990). Seminal plasma testosterone and T3 were associated with semen quality, being higher in the moderate-motile group, which makes sense as T3 is associated with sertoli and leydig cell functions which indirectly modulate testosterone (Maran, 2003). Meeker et al., (2007) also showed that testosterone concentration and motility may be associated. In the winter and rainy season, viability was higher than the summer season and during the rainy season, concentration was higher than in the summer and winter, but normal morphology was the lowest in the rainy season. Hermes et al., (2013) also collected semen in the spring (dry season) of 2009 and in the fall (rainy season) in 2010. They found no significant differences in motility between the two collections, but did find significant differences between acrosome integrity and morphology of sperm. It remains unknown however if the difference is due to the different bulls used (only 2 were the same between seasons) or if the difference was due to the season.

**Housing and Social Factors in Captivity**

Housing and social factors differ greatly between captive housing and elephants in the wild, which may have important implications for understanding of elephant reproduction. In the wild, males live with the matriarch female group until they are adolescents and then disperse and live in bachelor herds with males of their own age or, eventually, in solitary (Poole, 1989; Schulte, 2000). Mixed groups with adult male and females are only seen during breeding season, often associated with musth (Poole and Moss, 1981). Adolescent males of 10-20 years of age are the most sociable age
group and are found in large groups with close proximity to other elephants (Evans and Harris, 2008). Socialization in bachelor groups is an intense period of learning and development as adolescent males interact and spar with each other to establish themselves in the dominance hierarchy. Males of prime reproductive age ~35-40 are often solitary. Males of all ages preferred to have older males (>36 years of age) as their closest neighbor compared to males of the same age (Evans and Harris, 2008). The reason for this is unknown, however it is suggested that it provides them with opportunities to learn from more experienced older males (Evans and Harris, 2008).

Very little information is known about the adolescent life of males and their social groups. Sexual maturity in males occurs around age 17, however they rarely mate until they are in their 30s due to competition with other older males (Poole, 1994). Mating success increases after the male’s first musth around 29 years of age (Poole and Moss, 1981).

Elephants are polygynous mammals and males travel large distances to find females during the breeding season in the wild. Therefore, searching and attracting strategies are crucial in elephant reproductive behavior (Poole and Moss, 1989). Auditory stimuli via vocalizations between males and females are used to find mates. Females in estrous use louder, low frequency calls (Poole and Moss, 1989). Olfactory and tactile stimuli are used to assess feces and urine from both females and males to determine their reproductive status (Poole and Moss, 1989). Males can detect a preovulatory pheromone in female urine two and a half weeks before estrus occurs (Bagley et al., 2006; Poole and Moss, 1989; Rasmussen, 1998). Females can also use
There is strong competition between males for females, as females are only receptive for 3-6 days every 3-9 years when actively reproducing (Poole 1989; Poole and Moss, 1989). However, when females are not pregnant or lactating they may be receptive for 3-6 days every 3 – 4 months (Brown, 2014). Mate guarding is a reproductive strategy where male elephants remain in close proximity to a female elephant, moving with her or influencing where she moves, chasing off males, and preventing rival males from obtaining access to the female (Poole et al., 1989; Poole et al., 2011). Musth is said to be the factor that brings males and females together (Poole, 1994). Musth males will begin their search for females in estrous and females prefer males in musth and will search for and choose them (Moss, 1983; Poole, 1989; Poole and Moss, 1989; Poole, 1999; Sukumar, 1989). Musth males are highly motivated to find females as they spend less time eating and more time searching for a mate (Poole et al., 2011). Male elephants typically do not have access to females until they are 18-25 years of age, due to other dominant males and therefore any breeding of younger males is typically opportunistic (Chelliah and Sukumar, 2013). Larger, older, musth males will obtain successful copulations over younger males (Poole et al., 2011). Paternity success showed that males over 20 years of age sired ~60% of calves and males that were >35 years of age obtained mating during mid-estrus (Ramussen et al., 2007). Males and females will spend several days together before, during, and after mating (Moss and Poole, 1983; Poole and Moss, 1989). The mating process can take up to 15-20 days to complete when environmental conditions are good and begins with...
“love play” and courtship – touching, nudging, placing trunk on partner, sensory
investigation, mounting and finally copulation (Chelliah and Sukumar, 2015; Joshi et al.,
2009).

Captive housing of males places considerable constraints on the development
and expression of natural reproductive and social behaviors, having possible
implications for reproduction. The absence of male-female interaction is a key
difference between management of captive elephants and elephants in the wild, and
may influence quality of collected semen samples. In captivity, most facilities only
house females due to the challenges of housing males (Brown, 2014) and the few
facilities that house more than one adult male typically house them individually (Schulte,
2000). The absence of social bachelor groups in captivity and lack of socialization with
dominant males (>36 years old) could affect developing male social and reproductive
behavior. In most cases in captivity, bulls are managed in protected contact, housed
individually and are allowed visual/olfactory/auditory access to females, with some
having access to females at times (Kiso et al., 2011; O'Brien et al. 2013b). It is well
established in other species that atypical social experiences and social isolation in early
life can cause deficits in reproductive behavior and fertility. In elephants, differences
are seen in sexual development in captivity compared to the wild. Males become
sexually mature earlier in captivity (Age 6) than in the wild (Age 25-29), just like females
(Keele et al, 2010; Sukumar, 2003). Patterns of musth also differ; in the wild, musth
occurs annually, whereas musth in captivity is often irregular and can occur several
times a year, with some Asian elephants in a continual state of temporal drainage and
increased testosterone secretion (Brown et al., 2007). These physiological differences may relate to welfare and reproductive behavior of elephants in captivity.

Arnold et al., (2017) showed that of three bulls with “controlled” full access to females at times, two out of the three bulls showed adequate semen quality, suggesting that female contact may influence fertility. The presence of typical sensory stimuli associated naturally with mating, including auditory and olfactory signals, may contribute to male fertility. For example, exposure to preovulatory pheromone, which would normally trigger search and mate guarding behavior, may stimulate males. Therefore if that is the case, having male-female interactions with female elephants that are not cycling would not stimulate the males. Perhaps collecting urine from female elephants ~2.5 weeks before they ovulate and using this urine could entice males, especially if there was a visual stimulus of a female too.

Male-male interactions in the wild also play an important role in breeding, and may be important to consider in captivity. In the wild, dominant bull elephants mate guard the females and prevent rival bulls from obtaining access to an estrous female (Poole, 1989). Younger males are often allowed to be in the same area, but not other competitive bulls. Therefore, libido, sexual motivation and dominance of one bull is a large part of breeding in the wild. This has also been shown in bovine bulls, with subordinate bulls being inhibited by the presence of dominant bulls (Blockey, 1979). However, if multiple females are in estrous or if the dominant bull is removed, subordinates will still breed (Blockey, 1979). Therefore, perhaps having multiple elephant bulls in one facility may decrease the libido and therefore inhibit sperm
production of other bulls. Approaches to aligning captive housing with natural mate guarding behavior may include providing space between bulls.

**Future Directions**

Much research has been done in the past with elephants to try and find the best extenders and additives to increase the quality and maintain motility of these samples for further processing. A lot of work has been done on the cryopreservation of elephant semen that is in fact very successful (Thongtip et al., 2004; Saragusty et al., 2009; Kiso et al., 2012, 2013; Arnold et al., 2017), but the use of these reproductive technologies is limited to the initial semen quality. Therefore, it is crucial to take a step back to the basics and use some of the new and evolving technology such as ‘omics (e.g. proteomics, lipidomics, genomics, metabolomics) to increase our knowledge on the basics of spermatozoa and seminal plasma composition. There has been recent investigation by Kiso et al., (2012, 2013) of seminal plasma composition (lactotransferrin) and addition of cholesterol (CLCs) to semen samples but additional work is needed to address individual bull variability and understanding the role of possible missing components in seminal plasma. A few suggested future directions are discussed in the following paragraphs.

When it comes to collection methods we have little control over what we can change with our captive elephants. Rectal massage remains the only feasible collection method as most bulls are held in protected contact, limiting the use of the artificial vagina (not even including the training required to use it). Perhaps, it would be worth trying an AV on bulls that are not held in restricted contact to see if semen samples vary in quality between that and rectal massage and/or if adding seminal plasma components from AV collections (i.e. full ejaculates) to manually collected samples.
improves motility. Furthermore, although electroejaculation in captivity is frowned upon, as it requires sedation and continuous application of this is not healthy, if semen samples from manual collection and electroejaculation of captive bulls could be collected and compared, this could educate us on the differences in semen composition between the two collection methods. Currently we are unsure if consistent good quality samples from wild elephants are due to electroejaculation or just that they are wild elephants. If we could compare electroejaculated samples between captive and wild samples this might give us some insight towards this. If this were to be used, electroejaculation would be used as a research tool only and not as a routine collection procedure. If the results showed that components/seminal plasma were the main difference (not captive versus wild) we could use this valuable information to possibly increase the success of our manually collected samples. Possible avenues would be adding different fractions of electroejaculated semen to our manually collected semen to see if it improves the quality, and if so seminal plasma samples could be collected and frozen from wild elephants to be applied to captive elephant semen samples. Finally, the use of an oxytocin injection to males during collection, or oxytocin added to semen after collection could help to improve semen quality and ease of collection as suggested by O’Brien et al., (2013b).

Frequent collections in elephants as shown by Heath et al., (1983) and Imrat et al., (2014) show promising results that frequent collections could help to improve semen quality and as they and O’Brien et al., (2013b) suggest, the poor semen quality could be due to aged semen in the ampulla. While frequent collections have shown to improve motility in other species such as cattle (Barth, 2007), it is worth a try, but would
require a lot of time and effort to figure out the right frequency and time needed to obtain a good sample from each individual bull. It is important to remember that the bull should be assessed for irritation or uneasiness if the increased frequency of collections is conducted due to the rigorous collection method.

Since rectal massage is the main form of collection, it is important to note that low sperm parameters are “representative of the captive population” now. Imrat et al., (2012) reported on a study of five elephants with very low semen motility (~20%). The reviewers were worried about the low motility of these elephants (as they should be), so the authors compared the parameters of their 5 ejaculates with previous ones and found no difference (P>0.2). They therefore decided that since these parameters represent the captive population in Thailand that this was the best they were going to get and to work with. In another study, only bulls that were supposed to have good semen quality were used (>80% collection success and >40% progressive motility) and still sub-fertility and male variability were found (Thongtipsiridech et al., 2011). Therefore working on adding components such as CLC’s and lactotransferrin protein to bad samples to try and improve them is definitely going to be a step in the right direction (Kiso et al., 2012;2013).

Results from Pinyopummin et al., (2017, 2018) suggest that adding seminal plasma from species even closer related to the elephant than the stallion, such as the closest related species to the elephant: the manatee or hyrax may help to improve elephant semen quality. Perhaps the seminal plasma characteristics and proteomics between the three species should be addressed. Phylogenetic comparisons of reproduction and sperm biology are of importance anyways. Manatees and hyrax are
the elephant’s closest relatives and knowing more about the reproductive tracts and sperm biology of these species and comparing them can help us to prioritize and maximize conservation of same taxa (Kunter et al., 2010).

Data on the lipid composition of sperm plasma membrane and seminal plasma is very limited with only one study reporting the difference between African and Asian plasma membrane fatty acids (Swain and Miller, 2000). It would be interesting to look at the difference in the lipid composition of high versus low-quality ejaculates to see if the fatty acid profile differs. If so it may be possible to add a fatty acid into the extender that helps to increase the quality, or perhaps a dietary supplement that could be feed to the elephants to help increase quality. A review by Esmaeili et al. (2015) suggests that dietary supplementation of omega-3 PUFA may increase sperm parameters in humans with infertility issues.

It is known in humans that mental stress causes abnormality of spermiogram parameters. Excessive psychological stress can affect the L-arginine-nitric oxide (NO) pathway, with an increase in NO level and a decrease of arginase in the L-arginine-NO pathway (Eskiocak et al., 2006). O’Brien et al., (2013) found considerable DNA damage after storage, indicating that sperm ageing may be a contributor and Imrat et al., (2012) found that Asian elephant spermatozoa are more susceptible to DNA than other mammals. Perhaps this has to do with the amount of disulphide bonding and number of arginine-lysine residues in protamines that determines the stability. Therefore DNA fragmentation should be monitored more closely and compared when different factors are adjusted. As much as we try our hardest to house elephants in the most natural way we can with little stress and good welfare, perhaps there is still an underlying
problem, maybe even a social problem? It has also been shown in humans that obesity can lead to an increased likelihood of abnormal sperm parameters and elevated risk for subfertility among couples where the male partner is obese (Du Plessis et al., 2010). Perhaps we should try a new dietary regime for our captive male elephants to decrease obesity?

While zoos work with in situ and ex situ programs to try and assist in the conservation of species, more research needs to be conducted with wild species to try and bring genetics into our captive programs. Hildebrandt et al., (2012) collected semen from a wild African bull and used it to inseminate a female in captivity. This is a very important message, as not only does it allow us to collect good quality samples from bulls, it also allows us to enrich the gene pool of our captive elephants since there are very few males in captivity and we don’t need to bring bulls into captivity with ART. However, it is important to realize that it is expensive to travel to get semen, it needs to be cryopreserved due to travel distance, samples have to pass bacteriological and virological tests to show they are negative for disease (import health certificate is needed) and then you need successful artificial insemination (Hermes et al., 2013).

Natural versus captive breeding suggests that elephants require male-female interaction in order to produce good quality semen. Females and their pheromones stimulate them. If male-female interaction cannot be formed, pheromones and the visual stimulus of females is most likely important. It is also important that facilities have a female that is cycling to attract bulls like their wild counterparts. The presence of more than one bull housed in one facility may also lead to suppression of less dominant
bulls. Therefore, how multiple bulls are housed in captivity should be further assessed and semen quality monitored accordingly.

Finally, if it is a management or husbandry issue, nothing that we can do to the semen sample will be able to increase the semen quality unless we change these practices first. Even if this requires a lot of research and trial and error sessions to find the best solution for these problems, in the long run won’t increasing the reproductive success of captive elephants be increasing their welfare? There are still many areas to test before we say it isn’t possible. With addition of male-female interactions, postovulatory pheromones, seminal plasma addition and supplementing missing components we have a good chance of success! Along with these suggestions, a structured collection process of all males in captivity with protocols specific to each individual should be assessed to increase the semen quality and breeding success of captive elephants.

Summary

The future of elephants relies on our ability to successfully use ART in captive breeding programs today. In order to do this, the quality of semen from bull elephants needs to be improved. A first step to address semen quality in captive elephants may be to compare collection methods (AV versus rectal massage versus electroejaculation) and ejaculate composition between each sample using ‘omics technology. A next step would be to start creating individual bull “portfolios” to better understand high and low semen samples within bulls. Frequency of collections, such as alternate day collections for a month would be a good way to determine if semen quality could be enhanced for each individual bull. When bulls are being collected for the first time, they should be continually assessed for two years or until improved semen quality is revealed.
Ultrasounding the reproductive track before and after collection and measuring the size of ampulla may help to understand if semen is available and of good quality. For situations were a bull is difficult to collect, or to try collecting from a bull during musth, standing sedation such as xylazine hydrochloride & butorphanol tartrate could be administered. Collections from each individual bull before, during and after musth, would also help to reveal if motility and musth are positively correlated.

When assessing semen parameters the following list should be evaluated for both high and low quality ejaculates for each individual bull:

- **Motility** – CASA parameters and kinematics
- **Membrane integrity** – using a vitality stain
- **Volume:Concentration** – High:Low may indicate high semen quality
- **pH** (neutral pH may correlate with good quality)
- **Osmolality** (good to know for further processing)
- **Creatinine**; <14 ug/mL means no urine contamination
- **Morphology & Acrosome reaction stains**
- **DNA Fragmentation** – can be an indicator of aged semen

Running both proteomics and lipidomics of the seminal plasma and sperm cell from high and low-quality ejaculates will help uncover more similarities and differences between these samples than what we already know (i.e. MDA, calcium, lactotransferrin, fatty acids, etc). If these assessments are conducted on all individual bulls, further comparison between bulls may help understand what is missing from semen samples of bulls that never produce good quality semen. Adding supplements, antioxidants, lipoprotein extenders (for the Asian elephant) or seminal plasma from other species with
identical accessory glands to the elephant may help to improve semen motility. When poor samples are identified, density gradient centrifugation may be used to improve the semen quality.

Finally, recording bull age, type of access to females including: olfactory, auditory, tactile, visual and presence of cycling females, descriptions of housing including: housed alone or with others, number of bulls at the facility, diet, exercise, indoor/outdoor access, amount of space, and identification of musth may help to understand more of what may be influencing semen quality within and between bulls.
Table 5-1. Comprehensive overview of peer-reviewed elephant studies specifically relating to semen collection, semen processing and semen quality.

<table>
<thead>
<tr>
<th>References</th>
<th>Age(s)</th>
<th>Collection Method(^1)</th>
<th>Number of animals/ejaculates</th>
<th>Initial Motility(^2,3) (%)</th>
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<tr>
<td>(Jainudeen et al., 1971)</td>
<td>35</td>
<td>OP(^1)</td>
<td>1/7</td>
<td>PM: 50</td>
<td>9</td>
<td>8.1</td>
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<td>(Heath et al., 1983)</td>
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<td>AV</td>
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<td>115</td>
<td>-</td>
<td>84.9 - 310.5</td>
<td>33</td>
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<td>9 - 38</td>
<td>RM</td>
<td>6/-2</td>
<td>-</td>
<td>27.5</td>
<td>7.05</td>
<td>1.61 x 10(^9)</td>
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<td>(Kitiyanant et al., 2000)</td>
<td>11</td>
<td>AV</td>
<td>1/7</td>
<td>TM: 65</td>
<td>40 -73</td>
<td>-</td>
<td>1000 – 3000</td>
<td>67.3</td>
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<td>Bull 2</td>
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<td>1/10</td>
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<td>-</td>
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<td>4/-2</td>
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CAPTIVE ELEPHANTS

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<td>4/-</td>
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**CAPTIVE ELEPHANTS**

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CAPTIVE ELEPHANTS

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<td>(Kiso et al., 2011)</td>
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**WILD ELEPHANTS**

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<th>pH</th>
<th>Concentration</th>
<th>Morphology (% Normal)</th>
</tr>
</thead>
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<tr>
<td><strong>Loxodonta africana</strong></td>
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<tr>
<td>(Jones, 1973)</td>
<td>15 - 50</td>
<td>Culled</td>
<td>3/3</td>
<td>3/3</td>
<td>TM: 30 - 50%</td>
<td>-</td>
<td>-</td>
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<tr>
<td>(Jones et al., 1975)*</td>
<td>-</td>
<td>EE</td>
<td>2/2</td>
<td>1/1</td>
<td>TM: 60%</td>
<td>260</td>
<td>-</td>
<td>4.8 x 10^6</td>
</tr>
<tr>
<td>(Howard et al., 1984)</td>
<td>Adult</td>
<td>EE</td>
<td>9/9</td>
<td>8/8</td>
<td>TM: 70</td>
<td>93.3</td>
<td>7.4</td>
<td>2408 x 10^6</td>
</tr>
<tr>
<td>(Howard et al., 1986)*</td>
<td>Adult</td>
<td>EE</td>
<td>6/6</td>
<td>6/6</td>
<td>TM: 72.5</td>
<td>104.5</td>
<td>-</td>
<td>2185 x 10^6</td>
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<td><strong>First Year (1982)</strong></td>
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<td><strong>Second Year (1984)</strong></td>
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<tr>
<td>(Hildebrandt et al., 2012)</td>
<td>36</td>
<td>EE</td>
<td>1/1</td>
<td>1/1</td>
<td>TM: 89</td>
<td>144</td>
<td>-</td>
<td>765 x 10^6</td>
</tr>
<tr>
<td>(Hermes et al., 2013)</td>
<td>14 - 36</td>
<td>EE</td>
<td>7/9</td>
<td>7/9</td>
<td>TM: 86.4</td>
<td>61.1</td>
<td>-</td>
<td>781.3 x 10^6</td>
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<td><strong>First Year (Spring 2009)</strong></td>
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<td><strong>Second Year (Fall 2010)</strong></td>
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</table>

\(^1\)OP = opportunistic, AV = artificial vagina, RM = rectal massage, EE = electroejaculation

\(^2\)For all parameters in the table, only initial semen sample parameters are given under the “Motility (%)” heading

\(^3\)TM = Total Motility, PM = Progressive Motility, LM = Low Motility, MM = Moderate Motility, HM = High Motility

*Denotes samples where both good and bad samples were used (Typically urine contaminated ones are still not used in these studies)
CHAPTER 6
COMPARING SPERM WITHIN AND AMONG SPECIES TO ADDRESS THE
CONSERVATION OF ASIAN ELEPHANTS (*Elephas maximus*)

Introduction

Asian elephants (*Elephas maximus*) are considered endangered according to the
International Union for Conservation of Nature (IUCN) with only approximately 40-50,
000 Asian elephants remaining. The population of captive elephants is currently not
self-sustaining and the Association of Zoos and Aquariums (AZA) predicts that with the
current reproduction rates, in the next 50 years there may only be five elephants
remaining in captivity (Wiese, 2000). Studies show that many factors such as, single
sex housing, ovarian acyclicity, and reproductive tract pathologies may be leading to the
overall poor reproduction rates observed in captive populations. However, the lack of
good semen quality from captive bull elephants is a leading factor that further restricts
the use of assisted reproductive technologies (ART) in captive breeding programs
(Hildebrandt et al., 2000, Brown et al., 2004a; Kiso et al., 2011).

ART utilizes spermatozoa collected from the male to increase the chance of
fertilization and overcome poor reproduction rates. Spermatozoa are highly specialized
cells that are vital for fertilization and undergo intense and varied selections to become
more fertilization-competent (Fitzpatrick and Lupold, 2014). Their survival and rate of
successful fertilization is variable within and among individuals and species. In elephant
semen analysis, an initial motility of 60% or greater is needed for artificial insemination
or cryopreservation to maintain motility ≥30% after freezing and thawing (Thongtip et al.,
2008a,b; Kiso et al., 2013; O’Brien et al., 2013b; Saragusty et al., 2016; Arnold et al.,
2017). Due to the inconsistency and variability of semen quality in elephants, further
research on sperm characteristics from both high and low-quality ejaculates need to be
assessed to determine patterns or differences that may lead to advancing protocols and techniques that increase sperm quality. Sperm morphometrics may be useful for comparing the dimensions of fertile and sub-fertile bulls as previous studies have suggested that morphometry and semen quality may be correlated (Coetzee et al., 1999).

Transmission electron microscopy (TEM) of sperm cells can detect underlying ultrastructural normality and abnormalities of sperm cells, which may be important to understanding their functionality and reproductive issues (Mortimer, 1994). The mammalian spermatozoon consists of a head, neck, connecting piece and flagellar tail that can be further broken down into midpiece, principal piece and the end piece (Fawcett, 1975). The head of the sperm cell contains the nucleus, is dorso-ventrally flattened, pyriform in shape and the acrosome covers a large portion of the sperm head (Fawcett, 1975; Maree, 2011). The acrosome produces enzymes that are released during the acrosome reaction to penetrate the zona pellucida and access the ovum (Senger, 2012). Therefore, intact and present acrosomes are essential for successful fertilization and can be evaluated with TEM. The connecting piece and centriole can be found in the neck of the sperm cell. Within the sperm tail a central pair (doublet) of single microtubules are surrounded by nine double microtubules creating a 9+2 pattern. These doublets are surrounded by an additional bundle of dense fibers, which create the 9+9+2 pattern (Fawcett, 1975). The outer microtubule doublets are connected by radial spokes to the central pair of doublets and dynein arms connect each doublet. Fawcett (1970) concludes that the shape and cross-section of the outer dense fibers can differ between species, as well as the number of gyres in the mitochondrial helix.
For example, in many species outer dense fibers 1, 5 and 6 are larger than the other fibers (Fawcett, 1970; Cooper and Hausman, 2009). In the midpiece of the flagellum, the axoneme and outer dense fibers are surrounded by mitochondria, which wrap around the sperm cell in a helical form. In the principal piece, a fibrous sheath surrounds the axonemal-outer dense fiber complex instead of the mitochondria. In the principal piece of the tail, fibers 3 and 8 end near the beginning and become thickenings of the longitudinal columns of the fibrous sheath (Fawcett, 1970; Cooper and Hausman, 2009). The 9+9+2 pattern, enlargement of the midpiece and presence of fibrous sheath in the principal piece are key differences between mammalian and 'primitive spermatozoa' (Fawcett, 1970). Presence and structural integrity of the mitochondria is important because it produces adenine triphosphate (ATP), which is necessary for sperm cell motility and function (Ruiz-Pesini et al., 2007). Finally, a plasma membrane covers the entirety of the sperm cell, isolating the cytoplasm and mediating interactions that occur between the cell and its environments (Saacke, 2008). With TEM, the presence and number of mitochondria gyres and plasma membrane integrity can be determined. Therefore, TEM can help to observe the presence of abnormalities when comparing the structure of high and low-quality sperm cells.

Our first objective was to assess and compare the morphometrics of high and low-quality semen and to provide descriptions of the ultrastructure of samples with little to no motility (0 – 20%). We hypothesized that spermatozoa morphometrics would be different between high and low semen quality ejaculates with detrimental damage observed in the ultrastructure of the poor-quality samples.
Another important area of interest is to compare sperm traits across phylogenetically-related taxa to assess the relative conservation of these morphometric traits (Simmons and Fitzpatrick, 2012). Cummins and Woodall (1985) compared the sperm size of 284 species and found many variations in size between the species. Downing Meisner et al., (2005) found that the head morphology of closely related mammals (e.g. perissodactylans) was similar, but that distant species could be vary. Also, the acrosome is diverse in its size and shape between species and is typically distinct for a species or closely related species, especially in the apical portion of the cap (Austin, 1976). Therefore, determining more about the structure (e.g. acrosome coverage and number of mitochondrial gyres) and morphology (head shape and size) of sperm cells can help to determine species similarities and differences. This could lead to advancements in the indefinite preservation of sperm cells, and other ART, ensuring the planet’s biodiversity. Furthermore, it can lead to further information concerning how closely related species are.

The Paenungulata clade consists of Elephantidae, Sirenia and Hyracoidea. While it is known that these species are related, the trichotomy of these three species remains unknown and there has been major controversy over whether the manatee or the hyrax is more closely related to the elephant (Murphy et al., 2004). In the past it was suggested that the hyrax may be more closely related to the elephant, but recent information suggests that the elephant may have emerged secondarily from the water, implying that the elephant and the manatee may be more closely related (Gaeth et al., 1999; Shoshani and Tassy, 2005).
Therefore, comparing the sperm biology of these species could help to discover new protocols that increase sperm quality in elephants as well as lead to further information on the trichotomy of this species. The second objective was to compare the sperm biology of the Paenungulata clade. For this comparative study, the Asian elephant (*Elephas maximus*), the West Indian manatee (*Trichechus manatus*) and the Rock hyrax (*Procavia capensis*) from the Paenungulata clade were used to represent Elephantidae, Sirenia and Hyracoidea, respectively. We hypothesized that sperm biology would be more similar between the elephant and manatee than the hyrax.

**Materials and Methods**

Unless otherwise stated, all materials and stains were obtained from Sigma Chemicals Co. (St. Louis, MO, USA).

**Animals**

For objective 1, a total of 20 semen samples were collected from 7 Asian elephant bulls in the United States. Bull ages ranged from 6 to 35 years of age. All bulls were given free access to water and regular access to feed. Bulls were managed in protected contact systems and housed in individual enclosures with visual, olfactory, and/or controlled access to females.

For objective 2, a total of 5 samples from 5 Asian elephant bulls, 4 samples from 4 manatees and 6 samples from 6 hyraxes were utilized. The elephant samples used for this objective were a subset of the high-quality elephant ejaculates from objective 1. Manatee samples consisted of 1 fresh ejaculated semen sample from a manatee housed at Puerto Rico Manatee Conservation Center in Bayamon, PR and 3 post-mortem sperm samples opportunistically collected from deceased manatees (within 24-48 hours after death) collected by the Florida Fish and Wildlife Conservation
Commission Marine Mammal Pathobiology Laboratory (MMPL) in St. Petersburg Florida (Federal Fish and Wildlife Permit #MA067116-2). Hyrax samples were opportunistically collected from 6 culled animals in Carnarvon, South Africa at the time of death. These animals were culled as part of a program to keep the hyrax population down.

For both objectives, animal research protocols were approved by the University of Florida’s Institutional Animal Care and Use Committee.

Objective 1. Analysis of High and Low-Quality Semen Samples (Asian Elephant)

Semen collection

Elephant semen samples were collected using the rectal massage technique as previously described by Schmitt and Hildebrandt, (1998). An ultrasound was completed before and after to assess the size of the ampulla and seminal vesicles (Schmitt and Hildebrandt, 1998). Samples were collected into a modified palpation sleeve attached to an insulated 50-mL collection tube to minimize exposure to light and temperature. The 50 ml tubes were switched regularly to avoid urine contamination. Immediately after collection samples were placed in an incubator at 35°C. Samples with urine contamination were discarded. A total of 10 semen ejaculates were considered “high-quality” and 10 semen ejaculates were considered “low-quality”. Samples that were ≥60% initial total motility (tMOT) were deemed high-quality ejaculates (≥60% common cut-off for good quality samples and samples <60% were considered low-quality. For this study, all low-quality samples were ≤20% tMOT with almost all samples ≤5% tMOT.

Semen evaluation

Ejaculates were immediately evaluated subjectively for motility using a phase-contrast microscope. Briefly, a 10 µl aliquot was pre-warmed and %tMOT was assessed under light microscopy using the 40x objectives. If there was <20% tMOT,
then motility samples were further analyzed on a computer-aided sperm analysis (CASA) system. For motility on the CASA system, 2 µl of semen was placed in a Leja slide chamber (Leja Products, B.V., Nieuw Vennep, Amsterdam, The Netherlands) pre-warmed to 37°C and analyzed with an HTM-IVOS Version 12.2 Hamilton Thorne system (Hamilton-Thorne, Beverly, MA, USA). A total of 200 tMOT spermatozoa were assessed. Fractions of the ejaculate with similar tMOT were combined and volume, pH (pH strips), and concentration (hemocytometer) were recorded.

For morphometrics, high and low-quality ejaculates were stained with two different stains depending on the source of the samples. During this dissertation research only low-quality ejaculates were collected (n=10). However, 10 good samples were available from previous collections that had never been analyzed or reported in any other publication. High-quality samples were fixed in 4% paraformaldehyde and stored at 4°C until slides were stained. These samples were stained using a modified Coomassie blue technique, as described by Larson and Miller (1999). Briefly, spermatozoa were centrifuged (2000g) and washed twice with 0.5 mL of 0.1M ammonium acetate (pH 9.0). The final sperm pellet was resuspended in approximately 50 µl of 0.1M ammonium acetate and 15 µL of this cell suspension was placed onto a glass slide, smeared and air-dried. Sperm smears were immersed in Coomassie stain (0.22% (w/v) Coomassie blue G-250, 50% (v/v) methanol, 10% (v/v) acetic acid, 40% (v/v) water) for 2 min at room temperature. Stained slides were rinsed thoroughly with water to remove excess stain, air dried and coverslips were permanently sealed with DPX mounting medium. Low-quality samples were not fixed and were washed twice with PBS within 30 minutes of collection and 15 µl was smeared on a slide and air-dried.
Once dry the sample was stained with SpermBlue (Microptic, S.L., Barcelona, Spain) for 60 seconds and rinsed for 5 seconds with distilled water and slides were allowed to air dry and mounted with a coverslip using DPX mounting medium. Morphometric parameters were assessed using the Sperm Class Analyzer SCA® morphology module using bright field optics under the 40x objective according to the protocol by van der horst and Maree (2009). At least 200 spermatozoa were assessed on the system for sperm head length, head width, head perimeter and head surface area. Due to the difference in staining method used, 5 high-quality ejaculates were stained with both Coomassie blue and SpermBlue and 100 sperm cells per slide were analyzed and parameters were compared between the two stains.

Ultrastructural characteristics were assessed to determine possible abnormalities of the sperm cell of low-quality samples and compare these samples to previously reported literature on Asian elephant ultrastructure. A 50 µl aliquot of low-quality semen samples from 3 elephants (EM1, EM2 and EM4) were placed in 250 µl of 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer for fixation and kept at 4°C. This sample was later processed for transmission electron microscopy (TEM) at the ICBR Microscopy Core at the University of Florida (Gainesville, FL). The following ultrastructure characteristics were assessed: a) Head (presence of acrosome, damage to acrosomal region, and normality of acrosome), b) Connecting piece (head to neck), c) Flagellar: mid-piece and principle piece, and d) Presence and number of mitochondria.

**Objective 2. Comparison of Sperm Biology of Paenungulata Clade**

**Semen collection**

Asian elephant semen was collected by the same means as previously described for objective 1, but only 5 samples with ≥60% motility were used. The fresh manatee
semen sample was collected from a captive manatee that was trained for semen collection through manual stimulation of the penis. Post-mortem manatee semen samples were collected from the vas deferens and cauda epididymis of the reproductive tract from 3 animals within 24-48 hours of death. Hyrax sperm was collected opportunistically from the epididymis of 6 culled wild animals at the time of death. When semen was extracted from the epididymis and vas deferens in both the manatee and hyrax, an incision was made into the duct of the epididymis and vas deferens and semen was extracted with a pipette. Semen was mixed with a small amount of phosphate buffered saline (PBS) and further assessed.

**Semen evaluation**

Sperm morphometrics and ultrastructure (TEM) were compared between the three species to identify similarities and differences between them. Elephant samples were stained with Coomassie blue and manatee and hyrax samples were stained with SpermBlue. For both stains, the same staining protocols discussed in objective 1 were used for this objective. Samples were then analyzed on the SCA® system in the morphology module using bright field optics under the 40x objective according to the protocol by van der horst and Maree (2009). At least 200 spermatozoa were assessed on the system for sperm head length, head width, head perimeter and head surface area and compared between the three species.

A 50 µl aliquot of semen samples from each species were placed in 250 µl of 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer for fixation and kept at 4°C. Samples were later processed for transmission electron microscopy (TEM). Hyrax samples were processed in South Africa (Electron microscopy Unit, Tygerberg Hospital, Cape Town, South Africa) and manatee and elephant samples were processed at the
ICBR Microscopy Core at the University of Florida (Gainesville, FL). The following ultrastructure characteristics were compared between the three species: a) Head (normality and percent coverage of acrosome), b) Flagellar: mid-piece and principle piece (main focus on outer dense fiber structure), and c) Presence and number of mitochondria.

**Statistical Analysis**

For objective 1, elephant semen quality comparison (high versus low-quality samples) were summarized in two different ways. First, all elephants were summarized by sample (High-quality: n=10 and low-quality: n=10), and analyzed using the MIXED procedure of SAS (v. 9.4, SAS Institute Inc., Cary, NC), with quality as a fixed effect and elephant as a random effect. To further control for potential effects of elephant, a secondary analysis was performed only on data from three elephants that provided samples of both high and low-quality. Data were summarized by elephant and quality, and similarly tested within the MIXED procedure of SAS, with quality as a fixed and elephant as a random effect. To assess for effects of stain on outcome variables, data were summarized by elephants (n = 5) across cells and the effect of stain was assessed using a paired t-test (Proc ttest, v. 9.4, SAS Institute Inc., Cary, NC) with elephant as the experimental unit.

For objective 2, descriptive summaries of data (mean +/- SD) were used to observe possible similarities and differences among species.

**Results**

**Objective 1. Analysis of High and Low-Quality Semen Samples (Asian Elephant)**

Semen characteristics for high and low-quality ejaculates from Asian elephants are given in Table 6-1. Overall 10 ejaculates were deemed high-quality from 5 different
bulls and 10 low-quality from 5 different bulls. Three of the five bulls were the same in each category (EM1, EM2 and EM3 gave both high and low-quality ejaculates) and two bulls were different in high (EM4, EM5) and low-quality (EM6 and EM7) categories.

**Sperm morphometry**

Four sperm head parameters were measured in the morphometric analysis: sperm head surface area, head perimeter, head length and head width. Figure 6-1. depicts how morphometric sperm head analyses were conducted.

The average sperm head morphometric parameters (mean ± SE) for each elephant measured in the high and low-quality groups are given in Table 6-2. Overall there was no difference for any of the sperm head morphometric parameters assessed between high and low-quality ejaculates for all 7 elephants assessed (Table 6-3). There was also no difference between sperm head parameters when EM1, EM2 and EM3 were compared (3 elephants in both high and low-quality groups – Table 6-4).

The results from the comparison of Coomassie blue and SpermBlue stains showed that overall there was no difference between stains for most sperm head morphometric parameters, with the exception of head width (Table 6-5).

**Sperm ultrastructure**

Transmission electron microscopy showed that ultrastructural features were similar among each of the three low-quality elephant samples processed. The head, including acrosome, and the midpiece and principle piece were analyzed through TEM. TEM showed that the acrosome coverage was thicker over the anterior region of the sperm nucleus and narrower around the equatorial segment with lateral continuity between them (Figure 6-2A). In most cases the distinct separation between the acrosome and post acrosomal region can be seen. In all elephants, the plasma
membrane appeared to be missing from most sperm cells (Figure 6-2B). In approximately 50% of the samples acrosomes were intact, but abnormalities of the acrosome included acrosomal disruption, folded acrosomes and complete loss of acrosomes (Figure 6-3). Overall the acrosome appeared to cover approximately 75% of the sperm head. Nuclear craters were present in some of the sperm cells (Figure 6-2 to 6-4). The bottom of the nucleus formed a concave structure with a layer of dense material at the basal plate, which connects to the capitulum (Figure 6-4). The proximal centriole is positioned in the neck region of the sperm cell (Figure 6-4). Longitudinal columns with cross banded structures were located beneath the capitulum in the connecting piece running towards the midpiece (Figure 6-5). Dense material is present on both sides of the neck with association to the mitochondria (Figure 6-6.). In Figure 6-6B an absence of mitochondria can be seen, however this defect was rarely found. The mitochondrial gyres form a helix around the outer dense fibers of the midpiece (Figure 6-4 and 6-8). The midpiece contains approximately 50 mitochondrial gyres with two mitochondria per gyre resulting in approximately 100 total mitochondria (Figure 6-7). Along the tail the axoneme is surrounded by nine outer dense fibers that are associated with a pair of microtubule doublets (Figure 6-8). The size of the outer dense fibers varied with 1, 2, 5 and 6 appearing larger than the others in the midpiece region (Figure 6-9). The typical 9 + 2 axoneme structure of the nine outer microtubule doublets and central pair was observed. The point where the midpiece and principal piece of the tail connect is referred to as the annulus. At this point the mitochondrial gyres end and a fibrous sheath begins that surrounds the outer dense fibers (Figure 6-7 and 6-10). In the principle piece, outer dense fibers 3 and 8 (in line with the central doublets of the
axoneme) fuse with the fibrous sheath (Figure 6-8). Overall, very few abnormalities were seen in the ultrastructure of the low-quality sperm cells. Abnormalities that were seen consisted of nuclear craters, absence or disruption of acrosomes and absence of plasma membrane.

**Objective 2. Comparison of Sperm Biology of Paenungulata Clade**

**Sperm morphometry**

Four sperm head parameters were measured for the morphometric analysis: sperm head surface area, head perimeter, head length and head width for each species. These measurements were taken exactly as they were for the elephant in objective 1 and the results are shown in Table 6-6. Morphology of the elephant, manatee and hyrax can be seen in Figure 6-11. The hyrax sperm is notably smaller in length and perimeter and slightly different in shape in comparison to both the elephant and manatee sperm. Elephant and manatee sperm appear longer and paddle-like while hyrax sperm appears almost equivalent in both length and width. Overall, elephant and manatee morphometric characteristics were similar across all parameters.

**Sperm ultrastructure**

Similar patterns were seen in the ultrastructure of the sperm cells from the TEM micrographs of elephant, manatee and hyrax. In all species, approximately 75% of the sperm head was covered by the acrosome (Figure 6-12). In the elephant and manatee, the acrosome was thickest at the anterior part of the sperm head and became much thinner near the equatorial segment (Figure 6-12). In the hyrax, there was a slight decrease in acrosomal thickness at the equatorial segment but this difference was not as distinct as in the elephant and manatee spermatozoa (Figure 6-12). Another difference noticed was the presence of the spear shaped acrosome on the manatee
sperm cells, which was different from either elephant or hyrax. The elephant showed approximately 50 mitochondrial gyres in the midpiece and the manatee and the hyrax approximately 45 (Figure 6-13). Therefore, the midpiece of these three species appears to be very similar. In each of the three species, a very distinct annulus can be seen with the outer dense fibers were surrounded by mitochondrial gyres in the midpiece and a fibrous sheath in the principle piece (Figure 6-13). A unique similarity between the three species was seen in the size of the outer dense fibers in the flagellum (Figure 6-14). For all three species, outer dense fibers 1, 2, 5, and 6 were larger than the rest. The typical 9 + 2 axoneme structure of the nine outer microtubule doublets and central pair was observed in all three species (Figure 6-14).

**Discussion**

The large number of low-quality ejaculates collected from captive male elephants is a major problem in continuing to use ART to help sustain elephant populations today. There are many different possibilities for poor semen quality in captive elephants ranging from collection methods, presence/absence of accessory gland fluid (seminal plasma), and housing and husbandry of captive elephants. With this, assessing the differences in sperm quality between high and low-quality ejaculates provides a starting point for continued research working towards overcoming these reproductive problems.

In the first objective of this study, we analyzed the sperm morphometrics of high and low-quality ejaculates to assess any similarities and differences. No differences were found between high and low-quality samples showing that sperm size remains consistent between these samples and is, at least for this species, most likely not related to sperm quality. In the second aspect of this first objective, we assessed the ultrastructure of poor quality semen. Few abnormalities were found in the low-quality
samples, suggesting sperm structure may not be a contributing factor for decreased semen quality. It has already been noted that morphology (percent of normal sperm) is different between high and low-quality semen samples (Thongtip et al., 2008a; Kiso et al., 2013; Imrat et al., 2014;). While a high number of detached heads in each sample was visually evident under light microscopy, ultrastructural alterations, with the exception of the absence of the plasma membrane, were mostly absent.

The purpose of assessing the morphometrics of Asian elephant sperm cells was not to re-describe the size of sperm cells as previously done by Jainudeen et al., (1971), Heath et al., (1983) and Kitiyanant et al., (2000). Instead, there was a separate two-fold purpose. Firstly, to our knowledge, only one other group of researchers has attempted to assess the morphometrics of Asian elephant semen using a CASA system (Rittem et al., 2010). Secondly, the intent was to assess potential morphometric differences of spermatozoa between high and low-quality ejaculates to determine if size was an indication of sample quality. Sperm size and form have been shown to be related to sperm function and the ability to complete the acrosome reaction (Menkveld et al. 2003). In three studies by Jainudeen et al., (1971), Heath et al., (1983) and Kitiyanant et al., (2000) Asian elephant spermatozoa size was subjectively measured and the average head length and head width (length/width) was 7.5 µm/4.5 µm, 7.7 µm/4.7 µm, and 7.5 µm/4.3 µm, respectively. In these studies, they did not measure area or perimeter. The results for head length in the current study (7.71 µm) were very similar to those previously recorded, but head width (3.50 µm) was quite a bit smaller. Heath et al., (1983) found that measurements assessed with scanning electron microscopy (SEM) were smaller than those completed with differential interference contrast
microscopy and suggested that during SEM preparation cell shrinkage occurs due to critical point drying. However, it is interesting to note that measurements from Rittem et al., (2010) which were analyzed on a Hamilton-Thorne CASA system were very similar to our results with an average head length of 7.8 µm and head width of 3.8 µm. While our width measurements appear smaller than those previously reported, with the exception of Rittem et al., (2010), we did not find any difference between high and low-quality semen samples, suggesting that the difference between our results and previous results could be due to the two different methods of measuring, subjectively versus objectively, or perhaps due to the differences in semen collection methods in each of the studies.

Another possibility for the discrepancy in results could be due to the different staining methods used to prepare sperm cells for morphometric analysis. Maree et al. (2010) showed that different staining techniques changed the morphometric dimensions of the head of human spermatozoa. This difference was due to the osmotic relationship of the semen to the stains and fixatives used. Some stains caused the sperm heads to swell, while others caused them to shrink. In their study, SpermBlue was used for human semen (same stain as one of the ones we used) and was very accurate compared to fresh, unstained spermatozoa measurements. In the previous studies, Jainudeen et al., (1971) used eosin-nigrosin stain to measure sperm size, Kitiyanant et al., (2000) used a triple stain technique, Heath et al., (1983) used differential interference contrast microscopy requiring no stain and Rittem et al., (2010) used eosin and polychrome methylene blue. The differences in stains could cause the discrepancy in width between these results and ours, but the measurements by Heath et al., (1983)
should be accurate depending on the osmolarity of the fixative used. The only possible reasoning at this time for this difference is between subjective and objective assessments, especially since our results correlated well to Rittem et al., (2010). However, further studies assessing Asian elephant morphometrics and the effects of different staining methods and objective versus subjective analyses should be conducted. In the future it would be important to compare fresh semen to the CASA results to ensure that accurate evaluations of sperm head morphometry are achieved.

In our study, some of the sperm cells were stained with Coomassie blue and some with SpermBlue. In order to ensure that the two stains were not affecting the results, we analyzed sperm cells from the same sample with each stain and found differences only in sperm head width between stains. Therefore, further studies should be conducted on Asian elephant semen to test the osmolality of SpermBlue and Coomassie blue to ensure that it is compatible for these species. Additionally, it would be useful to test these results against those measured from fresh, unstained spermatozoa under phase contrast or differential interference contrast microscopy.

It is also interesting to note that a study by Luther (2016) on the morphometrics of African elephant spermatozoa using the same CASA system and SpermBlue stain that was used in the current study found an average head length of 6.8 µm and width of 3.3 µm. This suggests that there may be a large difference in sperm size between Asian and African elephants, which has been previously suggested (Jainudeen et al., 1971). Luther (2016) assessed sperm morphometrics during two different seasons and found no differences in sperm head measurements that were assessed in this current
study but did find differences in regularity and roughness. Therefore, future studies with Asian elephant spermatozoa should assess more head parameters.

The structural integrity of a sperm cell is important to their overall functionality and fertilization ability. Specifically, the acrosomal and plasma membranes along with functional mitochondrial are required (Sa-Ardrit et al., 2006). TEM of poor semen quality samples is important to assess as it provides information on the subcellular function of the sperm cell. A limited number of previous studies have assessed the ultrastructure of Asian elephant spermatozoa; however, these studies assessed ultrastructure after cryopreservation (Heath et al., 1983; S-Ardrit et al., 2006). Heath et al., (1983) reported that one third of their samples were ultrastructurally-intact with >50% having no acrosomal damage. Therefore, we wanted to assess the ultrastructure of the sperm cell without the effects of cryopreservation. Although our ultrastructural analysis was of spermatozoa from poor quality samples, very few abnormalities were seen. The largest abnormality encountered during ultrastructural analysis was the loss of the plasma membrane. During chilling and cryopreservation, the plasma membrane of sperm cells is often the primary site of structural injury (Drobins et al., 1993). Buhr et al., (1994) found that cooling disrupted membrane phospholipids which may be correlated with loss of motility (White, 1993). These results suggest that the plasma membrane may be a parameter to assess in correlation with poor-quality semen samples.

One possible explanation for the lack of plasma membrane in our samples may suggest that sperm cells have undergone the acrosome reaction. Kitiyanant et al., (2000) described acrosome reacted sperm cells through TEM appearing as vesiculation
and leakage of acrosomal content, with the presence of apical vesicles to be the critical sign of acrosome reaction. However, they did note that early stages of acrosome reaction were difficult to see and that this early stage may be characterized by random breakdown of plasma and outer acrosomal membrane and many sperm cells may not be positioned in a way to observe this. Through our analyses, little vesiculation or damage to the outer acrosome was observed. During the acrosome reaction, the apical portion of the sperm plasma membrane fuses with the underlying outer acrosomal membrane, exposing the inner membrane (Senger, 2012). However, during this process enzymes are released that digest a pathway through the zona pellucida of the oocyte in order to allow the sperm to penetrate and fertilize. Therefore, sperm cells without a plasma membrane and outer acrosomal membrane would be unable to fertilize. Furthermore, this lack of plasma membrane could suggest that components in the seminal plasma, or lack thereof, may be leading to premature acrosomal reaction or loss of plasma membrane.

Sa-Ardrit et al., (2006) assessed the ultrastructure of Asian elephant semen after cryopreservation of semen. They found that the cooling, freezing and thawing process may cause changes in composition and structure of the plasma membrane and that lesions in the acrosome may be caused by intracellular crystallization during these processes. Our results showed that 50% of the sperm was acrosome intact, even though they were considered poor-quality samples with <5% motility. These results are consistent with Sa-Ardrit et al., (2005) who showed that after cryopreservation the acrosome of normal spermatozoa remained intact. Acrosomal abnormalities observed in our study were in agreeance to those observed by Sa-Ardrit et al., (2006). Such
abnormalities included: loss of the plasma membranes, disruption or complete loss to the acrosome membranes. Heath et al., (1983) also found similar results in relation to the acrosome as we did in our study. They found that the acrosomal equatorial segment of the acrosome was much narrower than the main segment and that there was distinct lateral continuity between them.

In contrast to our study, Sa-Ardrit et al., (2006) observed instances of swollen mitochondria (most likely due to the ice crystallization), which was not observed in any of our analyzed samples. Within this objective, the mitochondria were of great interest for analysis as the mitochondria in the sperm flagellum are responsible for generating energy for sperm motility. Therefore, mitochondrial abnormalities would suggest a reason for low motility. However, as previously stated, few to no mitochondrial abnormalities were observed. Heath et al., (1983) noticed the presence of dense material associated with the proximal end of the mitochondrial helix, which was also noted in our study. In both our study and Heath et al., (1983) very few tail abnormalities were noted. This study was to our knowledge, the first to determine the number of mitochondria in the midpiece of Asian elephant sperm cells.

The results from objective 1 disagree with our hypothesis that sperm size is correlated to sperm quality for this species. While we noticed very few ultrastructural changes in our poor semen quality samples, high and low-quality samples should be analyzed and compared in the future to further confirm these results. Ultrastructural analysis would still be a key feature to assess when trying new techniques or protocols to ensure that they are not leading to subcellular effects on the spermatozoa. Additionally, future work assessing the seminal plasma, including proteomics and
lipidomics should be conducted. Furthermore, if osmolality and tonicity of the surrounding media (i.e. fixative and stain) can change sperm cell dimensions; changes in the seminal plasma could lead to differences to sperm cell morphometrics.

The second objective of our study investigated whether sperm characteristics are similar between phylogenetically-related species: the elephant, manatee and hyrax. Our preliminary data supports our hypothesis that the sperm size and morphology of the elephant and manatee are closer related than to the hyrax. However, based on these preliminary observations of the ultrastructure, it appears that all three species show similar structural features of the spermatozoa and therefore warrants further research.

Taxonomically related mammals often have similar life spans, are of similar size, live in similar environments and share common physiological organization (Bronson, 1989). However, it is clear that within this clade, these assumptions do not always hold true. Currently, very little is known about the trichotomy of the elephant, manatee and hyrax. Anatomically, all three species possess internal testes (Werdelin and Nilsonne, 1999), high number of thoracolumbar vertebrae and osteological features of the ankle (Seiffert, 2007; Tabuce et al., 2007), peculiar pattern of dental eruption (Gheerbrant et al., 2005) and their mobile proboscis (however this is not a synapomorphy (Whidden, 2002).

Other comparative aspects that are known between these three species are that they are all herbivores. Interestingly, manatees are the only herbivorous marine mammal and have no evolutionary relationship to other orders of marine mammals (Irvine, 1983). A common similarity between the manatee and the hyrax is that they both need to see warmth in order to regulate their body temperature. With regards to
the reproductive biology of the Paenungulata clade, both female elephants and manatees have bicornuate uterine structures with ovaries that lie near the kidneys and both have a zonary endothelialchorial placenta. Little is known or recorded about the female reproductive biology of the hyrax. Common to all three species is the presence of auxillary mammary glands, and the extremely long gestation of each species (Elephant = 22 months, manatee = 12-14 months, hyrax = 7-8 months). While the males of all three species have intra-abdominal testes, there is very little else known about male manatee and hyrax anatomy. Food availability may play a small role on the reproductive behavior of both elephants and manatees, but neither appear to be seasonal breeders (Poole, 1989; Larkin, 2000). However, the hyrax are seasonal breeders strongly influenced by photoperiod (long day breeders; Millar, 1972). Also, elephants and manatees typically only have one calf, while hyraxes have between 1-6 young (Millar, 1972). Finally, the reproductive strategies of these three species appear to be very different. Female elephants have a permanent social structure with a matriarch, with males living alone or in bachelor herds, while manatees have a semi-social structure with no long-term bonds. Very little is known about the hyrax reproductive strategies except that they live in colonies of one dominant male with several peripheral males and females, which is different (Millar, 1972; Larkin, 2000; Sukumar, 2003). While there appear to be many similarities between the three species, there are also many differences. However, overall there is enough data to suggest that these three species all belong to the Paenungulata clade; the question remains is “who is more closely related to whom?”
In past years it was suggested that the hyrax may be more closely related to the elephant, however recent data suggest that elephants may have secondarily gone back to life in the water before finally ending up on land (Shoshani and Tassy, 2005). Three main reasons for this theory are due to the presence of a trunk in elephants (possibly used as a snorkel) that is well developed even in the earliest fetus, nephrostomes, in the mesonephric kidneys at all stages of development (a feature of aquatic vertebrates and not found in other viviparous mammals) and the fused pleurae of the elephant (Gaeth et al., 1999; Shoshani and Tassy, 2005). While this is very intriguing, further research is needed to address the co-evolution of these three species.

Previous studies have shown that lengths of the sperm head, midpiece and tail may suggest co-evolution of species between these traits (Gage, 1998; Anderson et al., 2005). Further studies have shown that sperm morphometry parameters are an indication that co-evolution has taken place (Maree, 2011). To our knowledge, there are no studies that directly compare sperm morphometrics or ultrastructure of the elephant, manatee, and hyrax.

In this study, the similarities in all sperm head parameters between the elephant and manatee may suggest that these two species are more closely related than the hyrax due to the co-evolution of these sperm parameters, as seen in other species (Gage, 1998). One study by Miller et al., (2001) assessed manatee sperm ultrastructure and then discussed its similarities and differences to elephant and hyrax ultrastructure that had been recorded in the literature. Miller et al., (2001) concluded that the manatee had a similar acrosome to the elephant but that they had a distinct annulus and lacked dense bodies in the neck of the sperm. In this study we did not
notice any significant differences in the annulus of the sperm cells. Furthermore, dense sections of the neck with dense bodies appeared to be present in some of the TEM micrographs for the manatee but was not noted in the hyrax. However, in order to make conclusive statements on these facts, further research on these two areas of the sperm cell need to be addressed. Another important factor that could be leading to the discrepancies between this study and Miller et al., (2001) could be that semen was collected opportunistically from urine samples, whereas urine contamination was avoided in the current study. We did find the same results as Miller et al., (2001) of an identical acrosome between the elephant and the manatee, except for the small difference of the more ‘spear-shaped’ acrosome of the manatee. Miller et al., (2001) suggested that the similarities in the acrosome may be due to physiological effects that are associated with reproductive efficiency and success. While the hyrax acrosome showed some minor differences, it was fairly conserved as well. Bedford and Millar (1978) reported the presence of two lateral vacuoles within the nucleus of the hyrax sperm cells. While we did notice some vacuoles in a few cells we did not notice this in every sperm head. These vacuoles were not seen in the elephant or manatee sperm, only a few small nuclear craters randomly dispersed throughout the head from time to time.

Ultimately, data from this paper not only offers insight into two important parameters of sperm quality, but also on which male spermatozoa traits have been conserved across the Paenungulata clade. This information can provide a foundation for further research on optimizing sperm protocols and techniques between these three species.
Table 6-1. Average semen characteristics (mean ± SE) for high and low-quality Asian elephant ejaculates.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>High-quality (n = 10)</th>
<th>Low-quality (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>tMOT (%)a</td>
<td>82.4 ± 14.2</td>
<td>5.7 ± 6.0</td>
</tr>
<tr>
<td>Volume (ml)</td>
<td>38.2 ± 27.9</td>
<td>38 ± 20.3</td>
</tr>
<tr>
<td>pH</td>
<td>7.5 ± 0.5</td>
<td>6.59 ± 2.2</td>
</tr>
<tr>
<td>Concentration (x 10^6 sp/ml)</td>
<td>833.6 ± 661.9</td>
<td>2008.6 ± 1195.9</td>
</tr>
</tbody>
</table>

a Total motility (tMOT)
Table 6-2. Average sperm head morphometric parameters (mean ± SE) for high and low-quality ejaculates for each of the seven Asian elephants measured in this study.

<table>
<thead>
<tr>
<th></th>
<th>Head Area (µm²)</th>
<th>Head Perimeter (µm)</th>
<th>Head Length (µm)</th>
<th>Head Width (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>High-quality ejaculates</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EM1 (n=2)</td>
<td>26.46 ± 2.68</td>
<td>16.82 ± 0.80</td>
<td>7.86 ± 0.30</td>
<td>3.63 ± 0.20</td>
</tr>
<tr>
<td>EM2 (n=4)</td>
<td>24.88 ± 2.77</td>
<td>16.41 ± 0.91</td>
<td>7.86 ± 0.36</td>
<td>3.28 ± 0.23</td>
</tr>
<tr>
<td>EM3 (n=1)</td>
<td>23.69 ± 3.45</td>
<td>16.28 ± 1.02</td>
<td>7.78 ± 0.40</td>
<td>3.25 ± 0.24</td>
</tr>
<tr>
<td>EM4 (n=1)</td>
<td>26.02 ± 2.85</td>
<td>16.56 ± 0.81</td>
<td>7.84 ± 0.29</td>
<td>3.55 ± 0.14</td>
</tr>
<tr>
<td>EM5 (n=2)</td>
<td>24.28 ± 3.31</td>
<td>16.39 ± 1.07</td>
<td>7.94 ± 0.42</td>
<td>3.28 ± 0.21</td>
</tr>
<tr>
<td><strong>Low-quality ejaculates</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EM1 (n=6)</td>
<td>26.57 ± 2.30</td>
<td>16.56 ± 0.83</td>
<td>7.78 ± 0.31</td>
<td>3.57 ± 0.19</td>
</tr>
<tr>
<td>EM2 (n=1)</td>
<td>26.04 ± 2.23</td>
<td>16.64 ± 0.85</td>
<td>8.00 ± 0.29</td>
<td>3.39 ± 0.17</td>
</tr>
<tr>
<td>EM3 (n=1)</td>
<td>25.22 ± 2.35</td>
<td>16.37 ± 0.88</td>
<td>7.60 ± 0.37</td>
<td>3.48 ± 0.20</td>
</tr>
<tr>
<td>EM6 (n=1)</td>
<td>27.39 ± 2.25</td>
<td>16.69 ± 0.77</td>
<td>7.69 ± 0.28</td>
<td>3.75 ± 0.18</td>
</tr>
<tr>
<td>EM7 (n=1)</td>
<td>24.45 ± 2.99</td>
<td>15.65 ± 0.77</td>
<td>7.33 ± 0.26</td>
<td>3.45 ± 0.18</td>
</tr>
</tbody>
</table>
Table 6-3. Average sperm head morphometric data (mean ± SE) for all Asian elephants (EM1 – EM7) summarized by sample and effects of quality with elephant as a random effect.

<table>
<thead>
<tr>
<th></th>
<th>High-Quality</th>
<th>Low-Quality</th>
<th>SE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head Area (µm²)</td>
<td>25.90</td>
<td>25.07</td>
<td>0.47</td>
<td>0.1362</td>
</tr>
<tr>
<td>Head Perimeter (µm)</td>
<td>16.36</td>
<td>16.44</td>
<td>0.13</td>
<td>0.5212</td>
</tr>
<tr>
<td>Head Length (µm)</td>
<td>7.71</td>
<td>7.79</td>
<td>0.07</td>
<td>0.2019</td>
</tr>
<tr>
<td>Head Width (µm)</td>
<td>3.50</td>
<td>3.43</td>
<td>0.06</td>
<td>0.2276</td>
</tr>
</tbody>
</table>
Table 6-4. Average sperm head morphometric data (mean ± SE) of Asian elephant spermatozoa summarized by elephants EM1, EM2 and EM3 only, and effects of quality with elephant as a random effect.

<table>
<thead>
<tr>
<th></th>
<th>High-Quality</th>
<th>Low-Quality</th>
<th>SE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head Area (µm²)</td>
<td>25.94</td>
<td>25.01</td>
<td>0.63</td>
<td>0.1581</td>
</tr>
<tr>
<td>Head Perimeter (µm)</td>
<td>16.52</td>
<td>16.50</td>
<td>0.13</td>
<td>0.9134</td>
</tr>
<tr>
<td>Head Length (µm)</td>
<td>7.79</td>
<td>7.84</td>
<td>0.08</td>
<td>0.7044</td>
</tr>
<tr>
<td>Head Width (µm)</td>
<td>3.48</td>
<td>3.39</td>
<td>0.09</td>
<td>0.3795</td>
</tr>
</tbody>
</table>
Table 6-5. Comparison of average sperm head morphometrics (mean ± SD) between Coomassie blue and SpermBlue stains for Asian elephant sperm.

<table>
<thead>
<tr>
<th></th>
<th>Coomassie Blue</th>
<th>SpermBlue</th>
<th>t_4</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head Area (µm^2)</td>
<td>25.8 1.69</td>
<td>26.7 ± 0.74</td>
<td>1.44</td>
<td>0.22</td>
</tr>
<tr>
<td>Head Perimeter (µm)</td>
<td>26.7 0.74</td>
<td>25.8 1.69</td>
<td>0.24</td>
<td>0.83</td>
</tr>
<tr>
<td>Head Length (µm)</td>
<td>7.8 0.23</td>
<td>7.8 0.39</td>
<td>-0.19</td>
<td>0.86</td>
</tr>
<tr>
<td>Head Width (µm)</td>
<td>3.6 0.14</td>
<td>3.4 0.20</td>
<td>2.87</td>
<td>0.045</td>
</tr>
</tbody>
</table>
Table 6-6. Average sperm head morphometric parameters (mean ± SE) of elephant, manatee and hyrax sperm.

<table>
<thead>
<tr>
<th>Species</th>
<th>Head Area (µm²)</th>
<th>Perimeter (µm)</th>
<th>Length (µm)</th>
<th>Width (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elephant</td>
<td>26.10 ± 0.07</td>
<td>16.47 ± 0.01</td>
<td>7.74 ± 0.01</td>
<td>3.53 ± 0.01</td>
</tr>
<tr>
<td>Manatee</td>
<td>24.80 ± 0.10</td>
<td>15.77 ± 0.03</td>
<td>7.42 ± 0.01</td>
<td>3.45 ± 0.01</td>
</tr>
<tr>
<td>Hyrax</td>
<td>20.82 ± 0.13</td>
<td>12.99 ± 0.03</td>
<td>5.36 ± 0.02</td>
<td>3.75 ± 0.01</td>
</tr>
</tbody>
</table>
Figure 6-1. Computer-aided morphometric sperm head analysis of Asian elephant. Black lines represent head length and width and the yellow shaded area represents the acrosome in a cap-like shape.
Figure 6-2. Transmission electron microscopic image of Asian elephant sperm head showing A) The presence of a thick intact acrosome (Ac) around the anterior portion of the sperm head and narrow acrosome around the equatorial segment (*). The post acrosomal region (PAR) where the acrosome ends can be clearly seen. Nuclear craters (NC) can also be seen in the nucleus of the sperm cell. B) Transverse/oblique sections of several sperm heads. The nucleus (N) and acrosomes (Ac) can be clearly seen. Absence of plasma membrane around the sperm cells can also be noted.
Figure 6-3. Transmission electron microscopic view of a longitudinal section of Asian elephant sperm showing an acrosomal defect called folded acrosome (FAc) and cells missing an acrosome (MAc).
Figure 6-4. Transmission electron microscopic view of a longitudinal section of Asian elephant sperm showing the sperm nucleus (N) and presence of nuclear crater (NC), the dense material (DM) at the base of the sperm head, the proximal centriole (PC) located just below the head as well as the midpiece (MP) and mitochondrial gyres (MG) of the midpiece.
Figure 6-5. Transmission electron microscopy of Asian elephant spermatozoa showing cross banded structures (CBS) around the longitudinal columns (LC) in the connecting piece. Outer dense fibers (ODF) and mitochondria (M) can be seen in the midpiece.
Figure 6-6. Transmission electron microscopy of Asian elephant spermatozoa showing the dense material (DM) that is present on both sides of the neck with association to the mitochondria in both A) and B). In B) an absence of mitochondria is noticed (*).
Figure 6-7. Transmission electron microscopy of Asian elephant spermatozoa showing a longitudinal section revealing the full length of the midpiece (MP), mitochondria (M), connecting piece (CP) and annulus (AN) where the midpiece and principle piece connect.
Figure 6-8. Transmission electron microscopy of Asian elephant sperm showing the cross section of the midpiece (MP) and principle piece (PP). In the midpiece, the surrounding mitochondrial gyres (MG) can be seen as well as the outer dense fibers (ODF). In the principal piece the presence of the fibrous sheath (FS) and absence of the mitochondrial gyres is seen. Outer dense fibers 3 and 8 fused with the fibrous sheath in line with the central doublets of the axoneme.
Figure 6-9. Transmission electron microscopy of Asian elephant spermatozoa showing cross sections of the sperm midpiece. Dense fibers 1, 2, 5, and 6 are indicated. The paired doublets (D) associated with each dense fiber can be seen as well as the presence of the central doublets (CD) showing the typical 9 + 2 pattern.
Figure 6-10. Transmission electron microscopy of Asian elephant spermatozoa showing a longitudinal section of the midpiece (MP), mitochondria (M) in the midpiece and principle piece (PP). The annulus (AN – circled) marks the end of the midpiece and the beginning of the fibrous sheath (FS) of the principle piece.
Figure 6-11. Sperm morphology of A) elephant, B) manatee and C) hyrax under the 40x objective on the SCA® system.
Figure 6-12. Transmission electron microscopy of the acrosome coverage (Ac) of A) elephant, B) manatee and C) hyrax sperm cells. In all figures the area covered by the acrosome (Ac) is indicated as well as the post acrosomal region (PAc).
Figure 6-13. Transmission electron microscopy of midpiece (MP) of A) elephant (50 mitochondria tightly packed in a helix around the midpiece area), B) manatee (45 mitochondria tightly packed in a helix around the midpiece area) and C) hyrax (45 mitochondria tightly packed in a helix around the midpiece area) sperm cells. In all three species, a very distinct annulus (circled) is observed with the same pattern of a fibrous sheath surrounding the axoneme in the principle piece versus the mitochondrial gyres in the midpiece.
Figure 6-14. Transmission electron microscopy of a cross section of the midpiece for A) elephant, B) manatee, and beginning of principle piece in C) hyrax sperm cells. The size of outer dense fibers (1, 2, 5, and 6) appears to be consistent between the three species. In all three species the presence of the paired doublets (D) associated with the outer dense fibers (ODF) can be seen along with the central doublets (CD).
CHAPTER 7
CONCLUSION

This dissertation tackled two diverse but interconnected challenges facing animal conservation today. It is well understood that changes to the education system are needed to increase the number of students in STEM degrees. Also, it is important to construct a self-sustaining breeding program with captive elephants in order to increase the population and to help eliminate their risk of extinction. The two main goals of this dissertation were to: 1) assess the effects of different delivery formats of an undergraduate research course on student academic performance, critical thinking, opinions of science and STEM retention; and 2) to assess one of the biggest problems in our captive elephant breeding program: poor semen quality by analyzing sperm biology of high and low-quality ejaculates from elephants and to compare and contrast elephant sperm biology to that of the manatee and hyrax.

In Chapters 3 and 4 student learning gains, opinions about science and STEM retention were studied by providing different delivery formats of an animal behavior in an undergraduate research-based course. To our knowledge this is one of the first studies to assess the effects of three different delivery formats (traditional, online and flipped) in an undergraduate research-based course. Also, our study is the first to assess learning outcomes and critical thinking of students between three delivery formats in both University and College settings. Our results showed the importance of including undergraduate research experiences in courses offered at a University or College.

The research in Chapter 3 specifically looked at the effects of different teaching formats on student grades and critical thinking scores for both University and College
students. At the University of Florida, the flipped and online formats led to better student outcomes than the traditional format, suggesting that they better facilitate the learning of these students. At Santa Fe College, students performed better in the traditional and flipped formats than the online format. However, since the online format was only offered once at Santa Fe College, it is difficult to make decisive conclusions on this format for these students. Critical thinking is rarely used as an assessment in the literature review and is important for evaluating the effects of student research. After partaking in a research course and exposure to the scientific method we hope that students would have increased critical thinking. In this study we found that critical thinking scores were only increased in the flipped format at the University of Florida, further suggesting that the flipped format may be better for students who are participating in a research-based course.

Chapter 4 documents University student’s opinions of science, their learning gains, and STEM retention after having participated in a research-based course. Our research found that exposing undergraduates to research earlier in their STEM degrees increases their opinion of science, improves learning gains, and increases STEM retention. Student learning gains and opinions of the course were rated higher in all aspects in our Wild Discoveries course than the national average of students enrolled in other research-based courses, which emphasizes the success of the course. We believe that this course can act as a model for other institutions to use for the creation of feasible and successful research courses within their curriculums.

The results from Chapters 3 and 4 have several implications concerning higher education systems. Animal behavior was chosen as a focal topic due to the need to
make more people aware of animal behavior and welfare. The material taught in this course will help students understand several of the important factors that exist in captive animal programs. Informal feedback from students showed that learning about animal behavior was of great interest to all students, even those without an animal background. Hopefully this positive research experience will inspire more people to become involved in science and welfare and conservation of species.

Chapters 5 and 6 presented a review of the literature on poor semen quality, reported on the morphometrics of high and low-quality ejaculates and compared the sperm biology of elephants to their closest relatives. This was the first comprehensive review of the literature discussing the potential reasons for poor semen quality in captive elephants and future directions to overcome this issue. To our knowledge, this was also the first study to compare the morphometrics of high and low-quality ejaculates objectively using the CASA morphology module. Furthermore, this was one of the first studies to compare elephant, manatee and hyrax sperm morphometrics.

Chapter 5 is a comprehensive review of all literature to our knowledge that reports on semen collections in elephants. One common, well-known factor is that semen quality both within and between bulls is variable and inconsistent. This review showed the amount of collections that were collected from elephants in each study versus the amount that was used. Only a small number of these studies used the entire collected sample. For the most part <50% of samples were of good enough quality (≥60% motility) for use in further research and ART. Several factors were concluded from this review which may be causing the poor semen quality, for example: collection method, collection frequency, semen parameters, species differences, age, season and
housing. While some factors are not easy to change (e.g. collection method), a good starting approach would involve the creation of specific protocols for each bull elephant housed in captivity. These individual protocols could help to increase the success of semen quality in elephants by helping to establish patterns contributing to improved semen quality. Applying new “omics” technology targeting high and low-quality samples is of critical importance to assess semen parameters in elephants. For example, Kiso et al. (2013) showed the importance of including proteomics when she found a missing protein in low-quality samples. Discoveries made from these experiments, could lead to novel additives and dietary supplements that increase semen quality. Finally, different approaches to housing elephants in captivity may need to be evaluated as captive environments are very different from how elephants reside in the wild. While elephants that are housed in captivity are taken care of exceptionally well, our reproductive success rate with this species in captivity is low. Therefore, reproduction parameters with a large emphasis on elephant housing, husbandry and behavior should be addressed and compared with studies conducted on wild elephants.

This dissertation involved the assessment of two of the elephant’s closest relatives: the manatee and the hyrax. It important to assess the sperm biology of these species to further develop protocols and to also determine differences to improve our understanding of the phylogenetic relationship of these species. Therefore Chapter 6 was divided into two main objectives. Firstly we assessed the difference in elephant sperm morphometrics of high and low-quality ejaculates and studied the ultrastructure of low-quality samples. Secondly we conducted one of the first comparisons of the sperm biology of the Paenungulata clade. Surprisingly, morphometrics did not vary
between high and low Asian elephant ejaculates. Instead, it appears that while motility, morphology, acrosome intactness, and DNA damage do differ greatly between high and low-quality samples, sperm size does not appear to have a correlation with high or low-quality samples. Also, while according to sperm biology the elephant is more closely related to the manatee, there is still more research that needs to be completed for the trichotomy to be fully explained.

Limitations and Future Directions

There were several limitations to the work presented in this dissertation. While the Wild Discoveries course was very successful, and the students enjoyed it thoroughly, one of the semesters at both the University of Florida and at Santa Fe College did not have enough students enrolled for it to be offered. Due to this, the traditional course was only offered once at the University of Florida and at Santa Fe College the online course was only offered once. This means that while the data typically included two semesters of one teaching format for both institutions, at each one there was a format that was only offered once. This could have affected the data as student enrollment may have been different. However, the one traditional course offered at the University of Florida and the one online course at Santa Fe College had very high student enrollment, which allowed for student numbers to remain consistent to that of the other formats with two semesters each.

To assess student evaluations and opinions of the course in detail, a post-course survey relating specifically to the course would have helped to address more specific questions about the course then those that appeared in the CURE survey. This would help to gain insights to help improve the course. One future suggestion for the course would be to include both the CCTT and CURE surveys as a mandatory part of the
course to increase the number of students who completed both the pre- and post-tests/surveys. While STEM retention was measured in this course, it is important to note that most of the students that were enrolled in this course were already in a STEM discipline. This is because the course was offered in the Animal Sciences Department and targeted students in the animal biology, animal sciences and zoology programs. Our observations that few students dropped out of the STEM degree and few students switched into a STEM degree were likely affected by this enrollment.

Overall, due to a large student enrollment in the course at the University of Florida and low enrollment at Santa Fe College, we were unable to compare the University of Florida students and the Santa Fe College students directly to each other. However, it remains unclear whether this would even be necessary as there are two different populations of students at each school. It is also important to note that we considered students that were in their first and second year of education at the University of Florida as freshman and sophomore students. However, in the state of Florida students can take University level courses in high school or at community colleges prior to enrolling at a University. In some cases, students acquire enough credits to be eligible as juniors when then begin University. This may have given some students an advantage over others in our student population.

In the future, it would be beneficial to offer the Wild Discoveries course more widely, at different institutions and with different instructors. While this course chose to only have one instructor to reduce variation between instructors, it would be interesting to see the effect an instructor makes on the course outcomes.
The final point concerning the education data is that our version of the flipped method in the Wild Discoveries course is very different from what most educators would think of as a flipped course. Our flipped version remained constant by providing the lecture material online and committing class time to reinforcing concepts and material that was covered in the lecture. There were no games or activities that took place during this class time, which is unique compared to other common flipped courses. We chose not to have these activities as we wanted to keep the material and format as close to the traditional and online formats as possible. Therefore, there is a chance that if these components were added to our flipped classroom that the observed benefits would be more pronounced.

For the elephant reproduction studies, poor semen quality was a limitation. Due to the very low chance of getting high quality ejaculates from captive bulls, research had to be designed that could work with low-quality semen. While several poor quality samples were retrieved, it was not possible to retrieve high quality ejaculates from all of the same bulls. In order to conduct the comparison, semen samples from collections in the past which had not been assessed previously in any other study were used for the high-quality ejaculates. Ideally, comparisons would have been done with both high and low-quality ejaculates from all bulls used in the study. Also, the lack of high quality ejaculates during this PhD research resulted in the absence of high quality ejaculates that could be processed for ultrastructure comparison. However, two studies in the past have assessed elephant ultrastructure, so there was previous literature to compare these samples to. Finally, due to the limited number of bulls in captivity, the sample
size for this study was relatively low. However, 6-10 samples is typical for research done in exotic species, especially elephants.

For the comparative research between the elephant, manatee and hyrax it is important to note that all semen samples were collected by different techniques for each species. Due to the logistics of collecting semen from each of these species, it is very difficult and likely impossible to collect semen using the exact same method from these animals. Also, it is important to note that the number of samples from each species was not the same (5 elephants, 4 manatees, 6 hyrax) because semen from these exotic species is not readily available.

In the future, protocols and records should be created for each individual bull elephant for semen collection in captivity. These reports should include housing and access to females (specifically cycling females), calves sired, and complete records of basic sperm parameters of both high and low-quality ejaculates. This information could help us learn more about factors that may be causing poor semen quality in elephants. Proteomics and lipidomics of seminal plasma and sperm cells comparing high and low-quality ejaculates and between different collection methods will enhance our ability to determine differences between samples. Investigating sperm morphometrics between different fixatives and stains and comparing ejaculate morphometrics subjectively under the phase contrast microscope to the CASA system is needed to verify sperm measurements of the Asian elephant.

Finally, assessing sperm motility patterns, kinematics, sperm length and specific areas of the sperm cell through TEM to further analyze the sperm biology of the Paenungulata clade would give us more insight into the similarities and differences
between these species. Also, including more reproductive parameters such as seminal plasma composition and reproductive histology would also be improve the comparison of these three species.

**Concluding Remarks**

In summary, this dissertation reported: 1) the importance of the flipped format for information age students; 2) the need for undergraduate research experiences in the form of research courses; and 3) the importance of future research on the sperm biology of elephants to help determine the cause of poor quality semen collected from captive elephants. In the future, the flipped teaching method should be a strong consideration in higher education systems for undergraduate research courses. Further studies on elephant husbandry (between captive and wild elephants) and “omics” of high and low-quality ejaculates, could help identify major issues contributing to sperm quality. Furthermore, this dissertation addresses the importance of including research courses in higher education to increase the number of students interested in science. By having a larger number of students interested in science, we may increase the number of researchers working to overcome conversation challenges such as the extinction of Asian elephants. Overall, this dissertation provides insight on how to improve the current enrollment and retention of students in STEM and approaches for maintaining the future of elephants for generations to come.


King A. 1993. From sage on the stage to guide on the side. College Teaching. 41:30-35.


Luther, I. 2016. Semen characteristics of free-ranging African elephants (Loxodonta africana) and Southern white rhinoceros (Ceratotherium simum simum) using Computer-aided sperm analysis, electron microscopy and genomics as diagnostic tools. PhD Diss. University of the Western Cape, South Africa.


Maree, L. 2011. Sperm mitochondria: Species specificity and relationships to sperm morphometric features and sperm function in selected mammalian species. PhD Diss. University of the Western Cape, South Africa.


BIOGRAPHICAL SKETCH

Danielle grew up in the small country town of Fisherville in Canada. She spent her childhood playing outdoors and helping her parents raise a few beef cattle. Her interaction with her many pets increased her love for animals and she developed a desire to help all creatures big and small. Danielle attended the University of Guelph in 2006 and graduated with a Bachelor of Science in animal biology. As an undergraduate, she worked at a family-owned animal park in Cambridge, Ontario named the “African Lion Safari”. Danielle spent her summers as a tour guide, educating the public about the different species that were found in the park as well as addressing the importance of zoos and conservation. One summer, she had a chance to work in the Elephant Barn, uncovering the direction of her future almost instantly. She fell in love with the elephants and learned that there were still so many gaps in knowledge that needed to be addressed to help improve the reproduction of elephants.

With the groundwork set for a real passion studying animal reproduction, Danielle decided to go back to the University of Guelph and get her master’s degree looking at the cryopreservation of Asian elephant semen. It was during her studies here that she found a field-friendly method for freezing Asian elephant semen, which had potential for increasing the declining elephant population. Encouraged by this research and wanting to learn more about the reproduction of elephants in captivity, she began her Doctor of Philosophy at the University of Florida to find avenues for the improvement of elephant semen quality. Danielle was accepted into a program at the University of Florida where she would have the opportunity to not only study elephant reproduction, but to also study education and public outreach methods to improve knowledge of conservation issues.
Furthermore, her dissertation research has involved studying the effects of delivery platforms and STEM retention of students enrolled in a research-based course. Creating and analyzing an environment to engage students in animal behavior research and their future in a STEM degree has been inspiring. Danielle only hopes she can continue to inspire the lives of undergraduates to become more involved in research.

Her experiences during this program have been greatly influential, and have helped Danielle further understand the importance of education to help save endangered and threatened species. Danielle hopes to continue her research in elephant reproduction, while further educating the public about both zoos and conservation. Her ultimate goal is to work in a zoo or conservation-outreach program to continue advocating for research and education on animal reproduction, behavior, welfare and conservation.