Diceros bicornis longipes as a Tool to Test Species De-extinction using Somatic Cell Nuclear Transfer

EMILY LALONDE[1], HANNAH MAHONEY[1]
1. Integrated Science Program, Class of 2020, McMaster University

SUMMARY

The need for a solution to species extinction has become more crucial following the official extinction of the West African black rhinoceros in 2011 and the more recent extinction of the North African white rhinoceros on March 18th, 2018. This literature review explores the de-extinction of the West African Black rhinoceros through somatic cell nuclear transfer, commonly known as cloning. This idea was developed by analyzing the literature related to the several methods of de-extinction: back breeding, somatic cell nuclear transfer, and genetic engineering. Furthermore, the role of the West African Black rhinoceros within its ecosystem before and after extinction, as well as its genetic variation as a subspecies of the black rhinoceros, is discussed. Common concerns pertaining to de-extinction and cloning are also examined in order to justify potential future initiatives.

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INTRODUCTION

As of March 2014, the International Union for Conservation of Nature (IUCN) had assessed 71,576 terrestrial and fresh-water species; of this, they classified 860 as extinct or extinct in the wild, and 21,286 were deemed threatened with 4,286 described as critically threatened. These extinctions cannot simply be explained based on past extinctions in Earth's history: humans must be negatively affecting a large portion of species. This theory is further supported by comparing pre-human extinction rates established by the IUCN of 0.1 extinctions per million species years (E/MSY) to present extinction rates of approximately 100 E/MSY (Pimm et al., 2014).

Based on these findings, the prevention and solution to extinction seem to be of the utmost importance. A recent strategy is the concept of de-extinction which involves resurrecting extinct species using back-breeding, somatic cell nuclear transfer (SCNT), and genetic engineering. These techniques utilize current technologies and resources to represent a new field of scientific research. While de-extinction events have occurred and will be discussed in more depth throughout the review, no organism born from a de-extinction event has lived for more than a few days. This is expected to change, as the field of genetics is one of the fastest advancing scientific fields in the world (Eisenstein, 2015). The question then arises of how using de-extinction as a means of conservation and study will impact the environment of the cloned organisms; moreover, if there will be an impact on extant animals.

This review aims to use the literature surrounding the West African Black rhinoceros (WABR), a subspecies of the African black rhinoceros, as a qualitative tool to help hypothesize the effects of de-extinction, as the subspecies was declared extinct in 2011 (Moodley, 2017). By summarizing how the loss and reintroduction of the subspecies may impact its environment and the discussion around species de-extinction, the WABR is an excellent example that could be used to elucidate the de-extinction of other species that have been extinct for similar periods of time and eventually ones that have been absent for longer.

METHODS

Articles used in this systemic review were found using online journal databases including, but not limited to, Web of Science. The Web of Science database was used as the primary database as it includes articles from a variety of disciplines. This is essential for this literature review because a range of different topics are covered. For example, scientific topics such as the methodologies of de-extinction (e.g. back-breeding, SCNT, and genetic engineering) as well as ecological concepts surrounding the WABR and its reintroduction are discussed. However, subjects that are more related to social sciences are also reviewed. The topics discussed in Ethics and other Considerations, which delve into the ethics and other considerations of species de-extinction, fall more within this category.

Search terms were used consistently when searching all resources to ensure relevant articles were found. For example, in order to find scientific articles pertaining to SCNT, the search terms included: SCNT, cloning for de-extinction, and somatic cell nuclear transfer extinct species. All searches conducted involved using more than two search terms to ensure that a wide range of articles on the subject was included.

It was important to ensure that only relevant articles were used to create this literature review. Therefore, articles chosen had to abide by a list of inclusion criteria; however, the criteria varied between topics. For example, the age of the articles pertaining to species de-extinction did not matter as this section is meant to include all relevant instances of species de-extinction. Whereas, articles pertaining to the population decline and extinction of the WABR were published prior to 2007, as the species was declared extinct on March 18th, 2018. Moreover, information relating to rhinoceros had to be constrained to articles based on work in Africa, and more specifically West Africa. However, all articles had to be peer-reviewed and directly related to the topic.

TECHNOLOGIES OF DE-EXTINCTION

There are currently three techniques that are used and explored as solutions to species extinction. These techniques include back breeding, somatic cell nuclear transfer (SCNT), and genetic engineering. Their specific methodologies and applications are discussed in the following subsections.

BACK-BREEDING

The concept of back-breeding as a method of de-extinction was made popular by the Heck brothers. Between the 1920s and the 1930s, the brothers attempted to recreate the auroch (*Bos primigenius*), a breed of large wild cattle that was domesticated approximately 2,000 years ago and became extinct over 400 years ago (Stockstad, 2015). The brothers attempted to resurrect the auroch by cross-breeding different types of cattle and selecting the desired traits for their offspring. The result of such experiments found the Heck cattle to not be very morphologically similar to the auroch (van Vuure, 2002). Despite this, the experiments introduced the concept of selective breeding to recover distinct ancestral phenotypes within a population. While the new species may be similar to the extinct species phenotypically, the exact gene pool remains extinct with the original species (Price, 2006). Still, back-breeding provides an interesting means of filling previously empty ecological niches and allows for the new species and the ecosystem to re-evolve naturally (van Vuure, 2002).

SOMATIC CELL NUCLEAR TRANSFER

Unlike back-breeding, somatic cell nuclear transfer (SCNT) aims to create a replica that possess identical nuclear DNA to its donor. Cloning by SCNT (Figure 1) has been successfully accomplished in 23 mammalian species as of February 2018 (Lui et al., 2018).

The first mammalian example of SCNT was Dolly the Sheep in 1996. Sir Ian Wilmut of the Roslin

Institute accomplished this by using a mammary gland of a six-year-old female sheep in her third trimester of pregnancy. These experiments are significant because the results proved that a mammalian adult cell can be completely reprogrammed (Wilmut, 1997). Dolly the sheep was the product of a multistep process involving three female sheep. One provided the genetic material that was inserted into an unfertilized and enucleated oocyte provided by a different female. After undergoing fertilization, the oocyte became an embryo and was implanted into a third female surrogate (Campbell, 1996). Since the birth of Dolly the sheep, technical improvements and increased knowledge of cellular reprogramming have enhanced the efficiency of SCNT. Despite this, the overall ability of SCNT to produce a live offspring is only on the order of 1-3% (Booth et al., 2001).

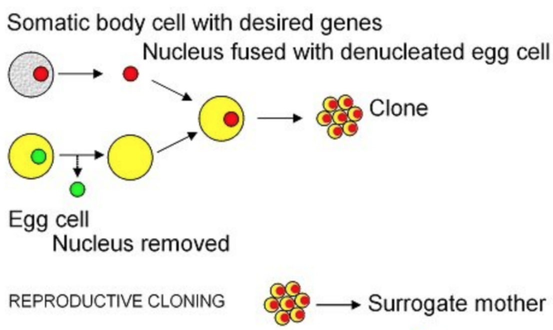

Figure 1: A nucleus from an adult somatic cell is injected into an enucleated oocyte (egg cell with the nucleus removed). Together they are reprogrammed. During reprogramming, the adult somatic cell becomes an undifferentiated stem cell which upon fertilization develops in the same manner as an embryo (Schorschski, 2007).

In terms of de-extinction, SCNT is more attractive than back-breeding because it holds the potential to truly resurrect an extinct species (on the nuclear level) rather than just produce a new species that possess similar traits. However, obtaining intact living cells is not simple with an extinct species as DNA within tissues begin to decay, damage, and fragment shortly after death (Lindahl, 1993).

GENETIC ENGINEERING

Genetic engineering utilizes advances in the fields of ancient DNA extraction and genome editing to combat extinction. These advances create living cells that possess extinct genes, which then can be used for SCNT (Yu & Bradley, 2001). Genetic engineering provides a means of de-extinction for animals that have been extinct for a long time, unlike the WABR. For this reason, it will not be discussed in great detail in this review.

THE WEST AFRICAN BLACK RHINOCEROS

The West African Black rhinoceros (*Diceros bicornis*) (WABR) is a subspecies of the African Black Rhinoceros species. They are distinguishable from the white rhinoceros by their hooked upper lip, which allows them to browse and feed on leaves from bushes and trees. Like all rhinos, the WABR is a megaherbivore and plays an important role in the health of its native environment (Cromsigt et al., 2014). Norman Owen-Smith, a highly regarded ecologist whose work pertaining to rhinoceroses will be referred to throughout this review, defined megaherbivores as plant-feeding animals that typically attain an adult body mass in excess of 1,000kg (Owen-Smith, 1987). With both male and female WABRs easily reaching weights up to 1,300kg, the species fits well into this definition.

The important role of megaherbivores can be summarized by the Keystone Herbivore Hypothesis, which states that large animals are necessary ecosystem engineers, opening up densely forested regions, and thus food resources, for smaller ungulates (Mills et al., 1993). The term 'keystone species' is poorly defined and broadly applied with possible applications to organisms on all trophic levels, but it is generally used to describe any species other organisms largely depend upon. The removal of such species results in drastic changes to the environment. The extinction of a keystone species like the WABR results in a trophic cascade, thus affecting the entire ecosystem. As a keystone herbivore, rhinos maintain the diverse grasslands in which other, smaller organisms depend upon (Cromsigt et al., 2014). Their diet aids in the reduction of woody plants and bushes, which allows grasses to grow in their place (Kotze and Zacharias, 1993). This benefits other animals such as small mammals and ungulates who depend on grasses for food and shelter, thereby increasing the biodiversity of the area. In 1987, Norman Owen-Smith conducted a study which used extant megaherbivores (rhinos, elephants, giraffes) to extrapolate information on the importance of Pleistocene Megaherbivores. The study suggested the removal of megaherbivores would be a mechanism for the loss of approximately half the mammalian genera within a shared ecosystem (Owen-Smith, 1987).

RHINO SUBSPECIES AND GENETIC VARIATION

In 1992, Norman Owen-Smith published *Megaherbivores: The Influence of Very Large Body Size on Ecology* which identified seven subspecies of black rhinoceros (Owen-Smith, 1987). While this is technically true, the intraspecific variation of black rhinos has been discussed by various authors and still remains unsettled. The most accepted scheme consists of seven subspecies but published work by Kes Hillman-Smith and Collin Grooves in 1994 argued the existence of an eighth subspecies due to geographical range overlap and the existence of small isolated populations (Hillman-Smith, 1994). Due to the extinction of three subspecies, *D.b. longipes, D.b. brucii, and D.b. bicronis,* and critical endangerment of *D.b. chobiensis* (possibly one surviving individual), confirmation of an eighth subspecies has been near impossible. Even with advances in genetic testing, only four subspecies have been tested for genetic differences and variations (Moodley, 2017).

In a study by M.K Swart and J. Ferguson in 2002, genetic relationships between the subspecies East *(D.b. michaeli),* West *(D.b. longipipes),* South-Centre *(D.b. minor),* and South-West *(D.b. bicornis) of* African Black rhinoceroses were determined (Moodley, 2017). F-Statistics in the study found significant difference between the populations, with no evidence of interbreeding, although the WABR *(D.b. longipes)* was determined to have the lowest genetic diversity of all subspecies tested. After years of population crashes due to poaching (refer to Somatic Cell Nuclear Transfer), the dangerously low genetic diversity was to be expected (Wilson and Peter, 1988). The WABR was also found to be the most genetically distinct of the black rhino subspecies (Harley et al., 2005; Moodley, 2017).

POPULATION DECLINE AND EXTINCTION OF THE WEST AFRICAN BLACK RHINOCEROS

Historically, the territory of the WABR extended through the savannah zones of Central-West Africa, an area which includes Tanzania, Zambia, Zimbabwe, and Mozambique (Moodley, 2017) (Figure 2). For much of the 1900's, the WABR had the highest population of all the subspecies with approximately 850,000 individuals (Cohn, 1988). During this time, the WABR population began to decline due to the high volume of trophy and big game hunting, but the populations began to rise again after conservative efforts were implemented in the 1930's (Kiffner, 2017). However, as the population increased conservation, actions were no longer strictly enforced, and the population once again declined due to poaching (Kiffner, 2017). Between 1970 and 1992, the WABR experienced a population decline of 96%. It was during this period that the rates of poaching started to increase more rapidly (Emslie, 2013).

By 1988, the population of the WABR was in the hundreds (Kiffner, 2017). While habitat loss and trophy hunting were still partly responsible for this decrease in population, they could not account for the speed in which it occurred. Poaching was the main reason for this unprecedented loss in the WABR population and it continues to be one of the greatest threats to megaherbivores and other animals globally (Tilman et al., 2017). A 2006 field study conducted by Isabelle and Jean-Francois Lagrot attempted to assess the population of what was believed to be the last existing WABR population in Cameroon (Lagrot et al., 2007). Over 46 field patrols were organized in the area situated roughly between Faro National Park on the western border and Bouba Ndjida National Park on the eastern border of the country, totaling over 2,500 km of patrol effort. Using historical data, information from a network of villagers, and cooperation with trophy-hunting guides, the fieldwork illustrated that no reliable sign of rhinoceros presence was found to attest to the survival of the WABR. The study concluded the extinction of the WABR five years before it was officially declared extinct by the IUCN.

WEST AFRICAN BLACK RHINOCEROS POST EXTINCTION

The removal of megaherbivores from an ecosystem is hypothesized to result in the loss of

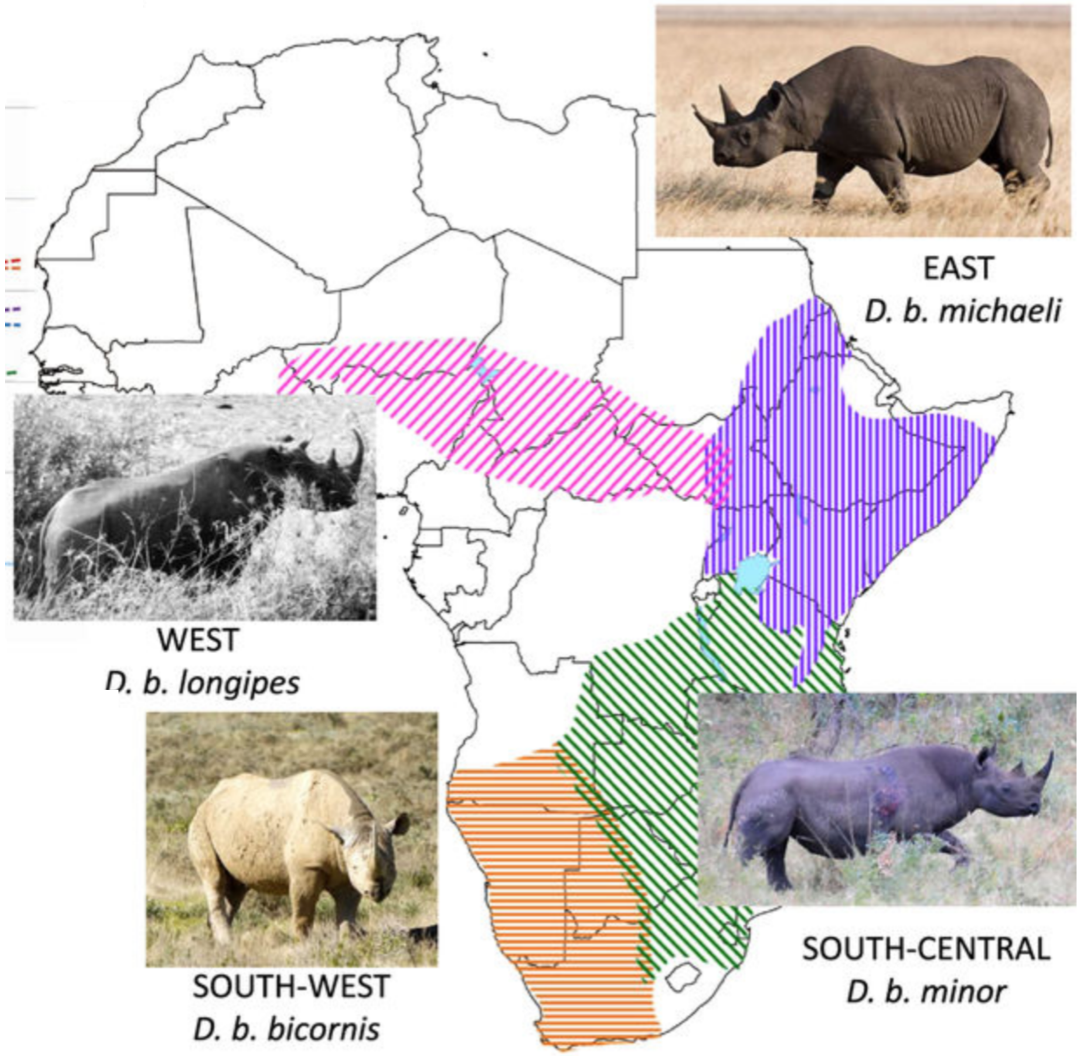

Figure 2: Historic range of four black rhino subspecies as determined for the study by Yoshan Moodley and his team. The historic habitat of the WABR (*D.b.longipes)* is shown in pink (Moodley et al., 2017).

approximately half of the mammalian genera sharing the ecosystem (Owen-Smith, 1987). While the WABR was important for reducing the population of woody plants in their habitats, and thus increasing the food availability to other smaller herbivores, they shared their habitat with other megaherbivores that played similar roles. The territory of savannah elephants overlaps in some areas with the historical territory of the WABR (Pimm et al., 2014). Savannah elephants are also known for pushing down woody trees to obtain fruits and leaves and to open space to reach wild grasses. In that sense, the loss of the WABR simply left an open niche for another megaherbivore to fill (Pimm et al., 2014). How fast the niche space is filled depends entirely on the competition between organisms for that position in the ecosystem, and whether another organism can fill it (Flannery, 2015). While the elephant and rhinoceros have been known to occupy similar niches, many elephant species are also at risk of extinction by poaching, and the niche left the WABR has yet to be entirely filled (Flannery, 2015).

Although its absence has minimally affected smaller herbivores, the WABR played an integral part of an ecological relationship with large predators and scavengers (Ripple et al., 2015). Megaherbivores not only facilitate the hunting success of large predators by opening up densely forested regions and making smaller prey more vulnerable, their carcasses also yield more nutrients to a wider suite of scavengers than those of smaller species. Large carnivores tend to consume less of large carcasses, thereby leaving more for other species (Ripple et al., 2015).

Megaherbivores are not immune to predation especially since their young are often in the preferred size range of predators. Overall, the WABR influenced the predator-prey dynamics either as an individual or by facilitating the hunting of other mammals. The loss of the WABR depleted the prey availability of large predators such as lions sharing the habitat (Ripple et al., 2015). Overhunting of large herbivores in West Africa has reduced the prey availability, which, at least in part, has caused regional lion populations to become critically endangered (Ripple et al., 2015).

POTENTIAL DE-EXTINCTION OF THE WEST AFRICAN BLACK RHINOCEROS

To successfully clone a WABR through SCNT, an available resource of genetic material and a sufficient surrogate mother and oocyte donor need to exist. Preferably, genetic material would come from sampling a WABR when the creature was still alive, and the material properly stored to avoid degradation (Harley et al., 2005). Fortunately, measures have been taken to preserve the genetic material taken from a live WABR; cell cultures collected from ear clippings of the rhino are stored in nitrogen gas at the University of Cape Town (Harley et al., 2005). While this genetic source likely offers the best quality DNA, other sources may also be utilized, such as stored WABR horns.

Apart from genetic material, SCNT requires a surrogate mother. An adequate surrogate mother for this process depends on the genetic relationship between the two species; the species of the mother and the species of the clone. Having a genetically similar species as a surrogate mother and/or oocyte donor is stated as ideal across literature defining SCNT (Folch et al., 2009). For extant species, this means surrogate mothers are from the same species, usually chosen from a pool of healthy female individuals. When attempting to accomplish de-extinction, there is no perfect surrogate mother. In the case of the bucardo the surrogate mother was a domesticated goat, a distant relative of the bucardo. Differences in uterine environments, prenatal development, and imperfect genetic material made successful pregnancies rare, with only two of 57 trials resulting in a live birth (Folch et al., 2009). Although the reborn bucardo went extinct again within hours, the birth suggests that streamlining techniques could make success rates significantly higher.

For the WABR, the ideal surrogate mother would be of the black rhino subspecies, whichever was the most closely genetically related. As of today, the WABR has been genetically compared to three other subspecies and determined to be the most genetically distinct subspecies (Moodley, 2017). This means there is no strong genetic relationship between the WABR and the East, South-West, and South-Centre African Black rhinoceroses. Even though it may be difficult to determine the subspecies of black rhino which is the closest relative to the WABR, using another subspecies would still be the best option as an oocyte donor and surrogate mother. The conservation status of the black rhinoceros species as a whole is considered critically endangered, except the South-Western subspecies (*Diceros bicornis occidentalis*) which is considered vulnerable (Grooves and Grubb, 2011). It would be redundant to use an individual from a critically endangered subspecies as a surrogate mother for a WABR de-extinction event because the process would take away valuable time in which the mother could produce offspring of her own subspecies. Based on the lack of known genetic relationships linking the black rhinoceros subspecies, it is reasonable to suggest a surrogate mother and oocyte donor be taken from the South-Western subspecies, as they are not as severely endangered.

Using cell cultures from ear clippings is the most feasible method of resurrecting the WABR because they possess DNA. Another approach would be to perform SCNT using the keratin within the horns of the WABR. Extracting DNA from keratinous materials requires the use of buffers (DTT, proteinase K, and detergent) and then the purification using methods involving organic materials or silica-column (Campos & Gilbert, 2012). This sort of technique would apply to prehistorically extinct species, such as the woolly mammoth.

INVASION

Invasion ecology interfaces with a variety of distinct sub-disciplines including ecology economics, environmental law, and epidemiology (Lockwood et al., 2013). This review has chosen to focus only on the ecological impacts pertaining to the biological invasion of non-native species. While all sub-disciplines of invasion ecology are important, this review aims to simplify the definition of invasion and use this to determine whether the de-extinction of an organism should be considered an invasion event.

Creating a general yet precise definition of invasive species has been a topic of discussion and controversy amongst conservationists and largely depends upon the viewpoint of the observer (Moutou & Pastoret, 2010). For the purpose of this review, the definition of invasiveness will follow the stage-based approach, a common methodology used to elucidate the parameters surrounding invasion of a species (Arim et al., 2006). Specifically, this review will define an invasive species as any species introduced to a non-native environment in which it is able to overcome environmental resistance and proliferate to a point in which it reduces the fitness of native species. As well, this review will define a non-native environment as an environment that may have once been a native location to a species, but post-extinction, the environment has changed significantly in the sense the species is no longer required to play an ecological role.

Environmental resistance, as mentioned by Charles S. Elton in his work *The Ecology of Invasion by Animals and Plants*, is the number of factors within an environment that restrict the biotic potential of an organism to proliferate (Elton, 2000). One of the main tenets of invasion biology is that most species introduced to a new environment do not survive. This is largely due to environmental resistance. Many species are highly adapted to the specific environment from which they evolved, and introduction to a new area in which they have not specialized or adapted to means they are unlikely to become an invader (Elton, 2000).

If an organism is brought back by a de-extinction process, the organism must be able to survive, whether as an invader or not, in the environment in which it is placed. For an organism that went extinct recently like the WABR, the organism should have no unforeseen ill effects after being reintroduced to their native habitat. The habitat has likely not changed in a way that would make it difficult to reintroduce. Like the reintroduction of wolves into Yellowstone National Park the reintroduction of a keystone species like the WABR will likely result in an increase to the health and biodiversity of the ecosystem.

But what about a case in which a long-extinct species is reintroduced into an environment that has drastically changed since their extinction? As will be discussed later, there has been a growing interest in the cloning of a woolly mammoth since the discovery of well-preserved specimen in Siberia (Palkopoulo, 2015). The woolly mammoth has been extinct for 10,000 thousand years and would be reintroduced into a completely different ecosystem compared to the one it left (Nogués-Bravo, 2008). Some extinct species may not have been invasive during their lifetime; however, they may severely disturb the dynamics of the present day.

Whether a de-extinction event is considered invasive remains unclear, as there has been limited research in this area. In the case of the WABR, not enough time has passed for its native ecosystem to change in a way that makes it ecologically redundant. Such as in the case of the Yellowstone wolves which will be discussed, the environment has not adapted to the absence of the WABR and reintroduction would likely increase the ecological health of the environment (refer to Genetic Engineering). Species redundancy, assuming natural extinctions, depends on the role a species played in an ecosystem (Gitay, 1996). If a species was one of many that played similar ecological roles, its extinction may not have a large impact on the surrounding environment, but since many modern extinctions involving species like the WABR do not occur naturally, it is reasonable to assume all recently extinct species are not ecologically redundant, and that reintroduction would not be invasive. Recently extinct, as defined by the IUCN, refers to any species that has gone extinct since 1500 CE but for the purpose of this review recently extinct will refer to any species that has gone extinct since 1760 (Feinstein, 1998). It was this time that the Industrial Revolution began, and human impact on animal ecosystems increased exponentially (Feinstein, 1998).

ETHICS AND OTHER CONSIDERATIONS

With any emerging innovation in science, there must be consideration for the potential ethical, ecological, societal, and economical issues that might arise from such a project. Within this literature review, both cloning and de-extinction can be subject to a lot of scrutiny for a multitude of reasons based on their fairly recent emergence. The following section aims to explore the considerations that must be made prior to implementing a reintroduction or de-extinction project. As well, common fears and concerns surrounding cloning and de-extinction will be discussed.

CONSIDERATIONS REGARDING SPECIES REINTRODUCTION AND DE-EXTINCTION

Historically, the motivation for species reintroduction has been to restore a species in an ecosystem and not to restore the ecosystem itself. However, reestablishing a specific species in a region where it had gone locally extinct will affect the dynamics of an ecosystem, especially if said species is a keystone species as in the case of the gray wolf (*Canis lupus*) to Yellowstone National Park. The eradication of the gray wolf began during the colonization of North America by Europeans because they were considered to be a danger to livestock (Ripple et al., 2013). By 1930, populations were completely eliminated from Montana, Idaho, and Wyoming (Bangs & Fritts, 1996). A recovery pact was set in 1974, in hopes of eventually removing the gray wolf from the Endangered Species Act in the Northern Rocky Mountains of the United States. However, wolf reintroduction was viewed as controversial because it was thought it would most likely significantly affect the human environment. Specifically, it was theorized to greatly disrupt agricultural and local practices. Therefore, a revision to the recovery pact was made in 1987 (finalised in 1994) entitled the Environmental Impact Statement 1987 (Bangs & Fritts, 1996). Throughout the winters of 1995 and 1996, wolves from British Columbia, Canada were introduced into the three states. Of particular interest are the wolves that were introduced into Yellowstone National Park and their impact on the environment.

As a result of the loss of the wolves, Yellowstone National Park witnessed an overall decline in its biodiversity. Due to the loss of the apex predator within this ecosystem, the local elk (*Cervus elaphus*) population proliferated, causing a trophic cascade and reducing vegetation and thus habitats and food resources for smaller organisms (Beschta & Ripple, 2010). After the reintroduction of the gray wolf, elk populations decreased again, allowing new plant growth. Changes such as increased height growth in willow, aspen, cottonwood, and other types of vegetation were observed (Beschta & Ripple, 2007). The recent rise in riparian vegetation helped to decrease the erosion of river channels, thus changing the flow of rivers and replenishing sources of wood, leaves, dissolved organic carbon, and nutrients that are required by aquatic ecosystems (Beschta & Ripple, 2010). Beavers, also previously locally extinct in the park, returned, and this encouraged the return of otters, muskrats, and reptiles (Beschta & Ripple, 2010). The return of the wolves to Yellowstone National Park is considered one of the most successful acts of conservative ecology in the 20th century (Beschta & Ripple, 2010).

The reintroduction of the gray wolf into Yellowstone National Park was a 65-year process that required a lot of deliberation. On a similar topic, a roundtable session in 2004 addressed some of the requirements for reintroduction of species that have gone locally extinct. Specifically, it considered the reintroduction of noble crayfish (*Astacus astacus*), back to their native habitat (Taugbøl, 2004). Noble crayfish were native to the Glomma and Halden waterways in Norway. In 1987 and 1989 the waterways were struck by crayfish plague (*Aphanomyces astaci*) and the populations underwent complete fatality. (Taugbøl, 2004). During the roundtable session, several scenarios were provided for the appropriate reintroduction of crayfish: (1) to restore a population that was recently lost, (2) to help return a native species to its historic range, and (3) to preserve genetic diversity of that species by creating new or confined populations. After considering these guidelines for reintroduction, it was also imperative to assess whether the reason for local extinction remains present- in this case crayfish plague. Reintroduction would not be justified nor successful if the threat still existed (Taugbøl, 2004). Reintroduction projects began in Norway in 1989 and 1995, and in 2001 the waterways were surveyed to track the progression of the project. The researchers found that density of the crayfish populations were much lower than densities observed pre-plague. However, they related this slow population growth with similar observations from other crayfish being introduced into a new environment in Norway (Taugbøl, 2004).

In terms of the reintroduction or de-extinction of the WABR, these examples provide insight into some of the considerations that must be investigated prior to any project of this sort. The research that was conducted before, during, and after the reintroduction of the gray wolf into Yellowstone National Park is a true success story for initiatives of this nature. It acts as an additional motivator for the reintroduction of the WABR, and as the WABR is also a keystone species, its successful reintroduction -like the wolves of Yellowstone- could benefit its ecosystem in a similar manner (refer to Genetic Engineering). Moreover, the roundtable session provides parameters that could be applied in determining whether the reintroduction of the WABR to its natural habitat is justified. In this case of de-extinction, it is important to assess whether the reason they no longer persist in their past environment remains a factor, just as the roundtable session considered the presence of crayfish plague in the rivers prior to reintroduction. If the illegal and legal practices of rhinoceros hunting persist, then the risk of history repeating itself would be inevitable. Additionally, it is important that the de-extinction of the WABR is not for exploitative purposes and in the best interest of the species and its ecosystem. This concept will be discussed further below. Despite current laws and restrictions regarding illegal rhinoceros hunting, poaching remains at a level of crisis in many countries. In 2017, the South African government reported that 1,028 rhinoceroses were illegally killed. While this figure is less than 2016 levels it is still very high compared to levels recorded in 2007 (Environmental Affairs, 2018). These statistics imply that history would in fact repeat itself, thus some solution would need to be reached before such a de-extinction project. It is important to note that these examples suggest that species reintroduction always benefits the ecosystem; however, this is not always the case.

ETHICS OF CLONING AND DE-EXTINCTION

The concept of cloning and de-extinction is a highly debated topic. Scientists wish to advance the field of cloning extinct animals for the sake of their research. Whereas, bioethicists, policy makers, and the media, strongly protest the idea (Fiester, 2005). Arguments against de-extinction are concerned with animal welfare, human health, environmental (refer to Genetic Engineering), political, and moral issues (Sherkow, 2013). With such concerns, the question arises as to whether opposition is based off of the fear of the unknown. The following subsection aims to provide enough context to form an opinion on the matter.

A major concern with de-extinction is animal welfare. The animals that are used as surrogates in SCNT may suffer due to complications during their pregnancies as well as during surgeries to implant embryos and to remove failed attempts (Carter, 2002). The offspring may die as high levels of genetic abnormalities and chronic diseases are common. Due to the risks of SCNT, The Animal Welfare Act (enacted in 1966) restricts such treatment (Animal Welfare Act, 1966). Moreover, it is imperative that there is a distinct reason for animal de-extinction and that it is not to serve as a public attraction, as is the case of zoos and other institutions that exploit animals (Sherkow, 2013).

The cloning of animals can also cause problems that hold the potential to eventually affect humans more directly. Of the utmost concern is the prospective cloning of humans or commercial cloning that may arise upon perfection of cloning techniques (Fiester, 2005). This idea sparks even more controversy than the concept of de-extinction. Furthermore, if cloning becomes common practice in the food industry, the livestock may be unsafe to consume due to unexpected results from genetic modification such as increase in allergens (Fiester, 2005). These issues do not particularly relate to the topics discussed in this paper, however, they help to provide context to the overall fears that surround cloning.

The moral issues regarding cloning and de-extinction are the most relevant to the project proposed in this paper. However, these concerns are the most ambiguous. They demand an answer that is on the same order of the divine power. It is somewhat inconceivable to imagine reversing the 'natural' course of world. Some questions include whether things happen for an ultimate reason, or what determines if such a cause is almighty enough. In terms of assessing the moral validity of such an initiative, there is no singular answer that encompasses every instance of cloning or de-extinction. It depends on the situation and a cost-benefit analysis should be conducted for each project (Fiester, 2005). With regards to animal and human welfare in the context of the cloning and de-extinction of the WABR, the benefits seem to outweigh the costs. This initiative would not support the exploitation of animals, their population and the entire ecosystem holds the potential of prospering, and humans are not directly affected.

CONCLUSION & FUTURE DIRECTIONS

Understanding the current literature pertaining to de-extinction is pivotal to making informed decisions on what will likely be a part of future conservation ecology and bioethics.

While still an emerging and controversial topic, the use of gene technology in species conservation and de-extinction is likely to be an unavoidable result of advancements in the field of genetics and conservation ecology. While no longer science-fiction, the de-extinction of animals may take years to perfect, but many groups are working together to make de-extinction a reality for a variety of species.

A group of Australian Scientists from the University of New South Wales in Sydney, Australia, have been focused on completing a successful de-extinction event since 2013 (Stone, 2013). The researchers have been attempting to revive the Australian gastric brooding frog *(Rheobatrachus silus)* using donor eggs from a distant relative and DNA from a specimen preserved since 1970. Using SCNT, the researchers have been successful in reviving the genome of the extinct frog by allowing the cells from SCNT to divide and form embryos. Although the embryos of the frog did not survive for more than a few days, they were all confirmed to contain the genetic material from the sample specimen (Stone, 2013). The team suggest fixing technical and methodological issues would make success more likely.

Reviving the woolly mammoth has also been a future target in the field of biotechnology and genetics. The goal has spanned multiple research teams, including a team from McMaster University. In 2015, a team of international researchers at McMaster sequenced the genome of two Siberian woolly mammoths (Palkopoulo et al., 2015). With multiple sources of woolly mammoth DNA available from a number of well preserved, frozen specimens found in glaciers and Arctic permafrost, researchers from Harvard suggest that a viable mammoth clone could be produced in as soon as two years (Shapiro & Seddon, 2016).

As the discussion on de-extinction turns away from 'if' and towards 'when', the argument of which organisms to bring back becomes more prevalent. While bringing back extinct mammals such as the woolly mammoth would be a huge advancement, future aspirations should not forget about recently extinct organisms and preserving the genetic diversity of a species not only as live individuals, but also as cultured genetic samples. The future of de-extinction has a multitude of implications for conservation and protection of critically endangered species and could be used to avoid cases such as those of the North African White rhinoceros *(Ceratotherium simum cottoni)*. Considered endangered since 1909, the North African White rhino subspecies was declared functionally extinct as of March 19th, 2018 after the death of the last male specimen (Gross 2018). Although two female North African White rhinos still exist, they were genetically related to the male and any further breeding attempts would result in inbred offspring. A genetic library of the species DNA could have aided in the production of genetically viable offspring, but with less than 500 individuals existing in 1975, the species had already bottlenecked to a point of genetic decline (Gross, 2018). While de-extinction should not be treated as a priority over conserving extant species and keeping them from becoming extinct, it will offer the possibility of introducing genetically healthy populations of once lost species back into the environment.

It is also suggested that more tangible and experimental research be conducted pertaining to the re-introduction of previously extinct species. Researchers would do well to accumulate data pertaining to the impacts and reintroduction of recently extinct organisms before the reintroduction of long extinct organisms such as the woolly mammoth or other species. There is limited experimental data that can be used to quantify the impacts of de-extinction on ecology and justify it at the same time. This is why the WABR would likely be an excellent choice as a de-extinction candidate. As a recently extinct mammal that would not be considered invasive after reintroduction, the WABR would help elucidate existing questions and concerns about de-extinction.

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