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MS ENVIRONMENTAL BIOLOGY
CAPSTONE PROJECT

by

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A Project Presented in Partial Fulfillment
of the Requirements for the Degree
Masters of Science
in Environmental Biology

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CHAPTER 1. LITERATURE REVIEW

The Detrimental Effects of Ocean Acidification on Marine Ecosystems Due to the Shifts in Predator-Prey Dynamics of Fish

Introduction

Due to the combined histories of industrial and agricultural efforts, the release of anthropogenic carbon dioxide (CO_2) has caused atmospheric concentrations of CO_2 to increase from its pre-industrial average of 280 parts per million (ppm) to a monthly average of more than 400 ppm (National Oceanic and Atmospheric Administration, 2018). Further, ~50% of that increase has occurred in the last four decades (Feely et al., 2009). This rapid and marked increase of atmospheric CO_2 is a problem on its own, but it is also spawning a secondary and equally immense problem in our oceans. Of the anthropogenic CO_2 released into the atmosphere, 26% is absorbed by our oceans (Sabine et al., 2004). Ocean surface water CO_2 concentrations increase in proportion to the increased atmospheric CO_2 concentrations of that same region from year to year (Feely et al., 2009). Thus, as we continue to pollute the air, we also proportionally pollute our oceans.

Although it is seemingly beneficial for the ocean to absorb CO_2 to reduce the atmospheric concentration of the most abundant greenhouse gas, this leads to dissolved CO_2 in the water. Dissolved CO_2 in water becomes carbonic acid, H_2CO_3, which then dissociates to form a single proton, H^+, and a bicarbonate ion, HCO_3^-. The increase of protons in the ocean then lowers the seawater pH. This process is known as ocean acidification (OA). In the pre-industrial past, the amount of dissolved CO_2 in the ocean was about 10.5×10^6 mol/m^2 and the average pH of the ocean was around 8.2 (Sabine et al., 2004). Currently, the amount of dissolved CO_2 is close to 13.7×10^6 mol/ m^2, and still rising and the pH of the ocean is about 8.1 (Sabine et al., 2004;

National Oceanic and Atmospheric Administration, 2018). The dramatic increase in dissolved CO_2 happened rapidly, within the last 100 years (NOAA, 2018). Although the difference between 8.2 and 8.1 would be slight in a small body of water, this difference is substantial in the ocean. Ocean pH will likely decrease another 0.3-0.4 units by 2100, approaching 7.7 (Sabine et al., 2004; Feely et al., 2009). This prediction of such drastically altered future ocean conditions will presumably be detrimental to the organisms that inhabit it because they have evolved in water of higher pH.

Ocean acidification has the potential to change the abundance and distribution of individual marine species and considerably alter entire ecosystems (Fabry et al., 2008). The survival of certain marine populations depends on each species' ability to either adjust to or withstand these extreme conditions. However, fish species will not only be directly impacted by OA-driven changes to their own population, but also indirectly impacted by the species with which they interact. Changes in environmental conditions, such as OA, can alter animal behavior, as well as interactions with other species (Barry et al., 2011). Predator-prey dynamics, in particular, play a major role in shaping many biological communities. One of the first behavioral changes in a species' behavior when adjusting to lower pH could be predator avoidance (e.g., inability to recognize predatory cues, inability to signal to conspecifics about predator presence, etc.) because of the neurological pathways that control this behavior (Munday et al., 2009; Dixson et al., 2010; Barry et al., 2011; Lonnstedt et al., 2013). On the other hand, OA also diminishes the ability of predatory fish to detect their prey or even causes them to avoid their prey altogether (Cripps et al., 2011; Pistevos et al., 2015). Many ecosystems rely heavily upon a top predator; so, if the predator can no longer detect prey, then the entire ecosystem could experience changes across trophic levels in a trophic cascade (Nagelkerken & Munday, 2016;

Rosa et al., 2017). An improved understanding of the ecosystem-level impacts of OA requires a shift from single-species studies to studies of the long-term impacts on populations, multispecies dynamics, and communities in a variety of ecosystems (Barry et al., 2011). Despite the numerous studies that have observed negative impacts of ocean acidification on predator-prey dynamics, far fewer have investigated the ramifications for ecosystem processes. This research is important for ecologists and marine biologists in order to best understand how these behavioral changes might occur and how to mitigate the problems they cause. Fish populations and marine ecosystems in general already suffer significant negative impacts from overfishing (Sissenwine et al., 2014; Le Pape et al., 2017), so, any additional threats will exacerbate the dilemma fish face. This review explores the current understanding of the effects of OA on: (i) predator and prey fish species, (ii) predator-prey dynamics between these fish species, and (iii) how these altered dynamics affect ecosystems as a whole. Most of the studies reviewed were conducted in Australia, which is the most extensively studied marine ecosystem. This review will also compare and contrast the findings of these Australia-based studies with those undertaken in other parts of the world.

Antipredator Sensory Impairment of Fish due to OA

Predator avoidance, an innate behavior in fish, increases an individual's fitness and ensures the persistence of an entire species (Houston et al., 1993). In predator-prey interactions, fish swim away from a threat rapidly (Domenici & Blake, 1997). Several studies have indicated that OA degrades the $GABA_A$ receptor in fish, an inhibitory neurotransmitter receptor in the brain, thereby compromising their innate behavior for avoiding predators (Chivers et al., 2014; Chung et al., 2014; Knight, 2014; Esbaugh, 2018). These innate behaviors include recognizing the predator's presence and swimming away, hiding, and/or signaling to other conspecifics. OA reduces the ability of prey to: (i) detect predators, (ii) recognize predators from other prey when detected, (iii) escape predators, and (iv) signal to others of a predator’s presence.

Ocean acidification can damage the sense of sight (vision), smell (olfaction), and sound (aurally) in prey fish species (Munday et al., 2009; Dixson et al., 2010; Simpson et al., 2011; Lonnstedt et al., 2013; Ferrari et al., 2017). In laboratories in the Great Barrier Reef, larval fish have lost the ability to differentiate predators through olfactory and visual cues. Under natural conditions simulated in a tank, clownfish larvae (*Amphiprion percula*) showed the innate ability to both detect predators and to differentiate between predator and non-predator cues (Munday et al., 2009; Dixson et al., 2010). However, when reared under experimentally manipulated conditions to mimic OA, larvae in the settlement-stage could no longer differentiate predators from non-predators (Dixson et al., 2010). In fact, the larvae in the settlement-stage not only lost their ability to detect predators but also were attracted to the predator’s olfactory cues (Munday et al., 2009; Dixson et al., 2010).

In another case, fish species lost both the ability to signal (i.e., bobbing behavior) and their ability to recognize those signal cues, from other conspecifics. In natural conditions simulated in a tank, ambon damselfish (*Pomacentrus amboinensis*) have demonstrated the innate ability to react to predator presence when they see other conspecifics bobbing to indicate when a predator is present (Lonnstedt et al., 2013). The damselfish were then exposed to: (i) olfactory cues only and (ii) visual cues only (Lonnstedt et al., 2013). Under experimentally manipulated conditions to mimic OA, the damselfish completely lacked response to olfactory cues of predatory dottyback (*Pseudochromis fuscus*) but responded to the visual presence of dottyback fish by escaping (Ferrari et al., 2012; Lonnstedt et al., 2013; Ferrari et al., 2017). However, despite being presented with both types of predatory cues, the damselfish performed no antipredator signaling behavior (Ferrari et al., 2012; Lonnstedt et al., 2013). This lack of bobbing behavior suggests that even if the fish can detect the shape visually, they fail to signal conspecifics that predators are present (Lonnstedt et al. 2013). This dramatic loss, and in some cases even reversal, in predator-avoidance behavior likely leads to the probability of more frequent predator encounters and decreased survival rate of predator encounters not only of individuals but also others who are not warned.

In order to maintain predator avoidance behavior, responses to visual stimuli partially compensate for the loss of response to olfactory cues (Lonnstedt et al. 2013; Ferrari et al., 2017). Visual perception may intensify to compensate for the loss of other senses and response abilities. Despite having the innate ability to avoid predators through auditory cues, clownfish larvae reared in experimentally manipulated elevated CO_2 levels (which increased acidity) did not avoid simulated predatory noises (Simpson et al., 2011). This has the potential to diminish juvenile clownfish survival (Simpson et al., 2011). Without the ability to sense predators through olfactory cues or noise avoidance, prey fish may suffer from increased predation. Although recognition of visual cues may be enhanced to compensate for these losses, it is detrimental for these fish species to lose response to both olfactory and auditory cues.

While many of the published studies were conducted in Australia, OA affects—and will continue to affect—oceans globally although the changes to ocean pH will not be evenly distributed. Thus, studies conducted in other parts of the world are vital to a complete understanding of the global effects of OA. Recently, the number of global studies about fish species has increased and some of these studies add substantial evidence in support of conclusions drawn from Australian research sites. For example, the first report of OA effects on Indian Ocean fish was only just published recently (Wang et al., 2017), whereas the issue of OA has been known about and investigated since at least 2004, and possibly earlier (Sabine et al., 2004). This group received a gift of ten male and ten female Indian Ocean medaka (*Oryzias melastigma*) from State Key Laboratory of Marine Environmental Science (Xiamen University, Xiamen, China). Their population of medaka were inbred for ten filial generations and used as a model species for fish from coastal waters. Medaka have demonstrated the innate escape behavior to predatory cues, but when exposed to experimentally manipulated acidified ocean conditions, the medaka demonstrated escape behavior less frequently (Wang et al., 2017). Larval medaka also exhibited a high rate of abnormalities in their eye size and position as well as in their brain morphology (Wang et al., 2017). These abnormalities impair their visual recognition and disrupts their ability to respond to stimuli (Wang et al., 2017).

Additionally, the freshwater (and occasionally saltwater) rainbow trout (*Onchorhynchus mykiss*) in Canada innately avoid longear sunfish (*Lepomis megalotis*) and pumpkinseed sunfish (*Lepomis gibbosus*). Under experimentally acidified conditions, the trout failed to recognize the pumpkinseed sunfish, which they had been pre-conditioned to recognize through olfaction (Brown et al., 2012). Furthermore, under the same acidified conditions, the trout demonstrated no change in foraging behavior in response to predatory longear sunfish cues (Brown et al., 2012). If trout cannot recognize a predator approaching as it forages, it is highly probable that the sunfish will hunt the trout successfully. Despite being presented with predatory cues of both congeners, the rainbow trout were unable to detect either as a predator.

Studies done at marine CO_2 seeps in Papua New Guinea demonstrate compelling evidence in support of laboratory-manipulated findings (Munday et al., 2014). The use of a natural laboratory puts many of the other manipulated studies into context and validates their relevance. The investigators in Papua New Guinea examined whether juvenile reef fishes in a natural habitat exhibit behavioral abnormalities that align with those found in manipulated experiments, most of which were done by these same investigators. Fish from CO_2 seeps were attracted to predator odor (Munday et al., 2014), which aligns with previous findings (Munday et al., 2009; Dixson et al., 2010; Lonnstedt et al., 2013). These findings strongly imply that manipulated experiments align with real-world consequences of OA.

Although the results of some studies outside Australia are consistent with those from Australia, one other does not so clearly coincide. Juvenile Atlantic cod (*Gadus morhua*), which inherently avoid brown trout (*Salmo trutta*) through olfactory cues, still actively avoided predator cues after exposure to acidified conditions (Jutfelt & Hedgarde, 2013). Despite the majority of findings showing reduced antipredator response in OA conditions, juvenile Arctic cod demonstrated no change before and after the prolonged exposure to highly acidified ocean conditions. Further studies are needed to either support these findings or conclude that they are an outlier.

As studies outside of Australian sites continually emerge, there is still an obvious deficit of investigations about these various fish communities and their ecosystems. This approach has allowed better insight into how OA affects the multiple stages of development of prey fish physiology and behavior due to changes in brain function. While the research on predator avoidance and response behavior is extensive, it still lacks global context (i.e., research in other parts of the world). Although many experiments in Australia have indicated the impaired ability of prey fish to detect their predators through sensory cues, other studies are needed to show the response to OA of their predatory counterparts.

Sensory Impairment of Predatory Fish due to OA

Just as avoiding predators is innate in prey fish, so is predation by those very predators.

Although OA harms predatory fish in similar fashion to their prey, fewer investigators discuss predators to the same extent as their prey counterparts. This is ironic considering the massive role that predators have in maintaining food webs and marine ecosystems. Changes in the behavior of predators could lead to detrimental trophic cascades throughout the food web. Many predators in the Great Barrier Reef have already shown diminished foraging and hunting behaviors due to the effects of OA.

Predatory dottyback innately prefer the olfactory cues of injured prey, but after exposure to experimentally manipulated acidified conditions, their preference shifts to avoidance (Cripps et al., 2011). Thus, despite higher activity levels (i.e., any activity other than feeding) in the high CO_2 treatment than in the mid-CO_2 treatment, the feeding activity of the predatory dottyback was lower (Cripps et al., 2011). This indicates that predicted future ocean conditions may slow the response of predatory dottyback, and subsequently harm their ability to hunt.

Among predatory fish, sharks are typically top (“apex”) predators in coral reefs, making their behavior a vital contribution to maintaining top-down control on the food web (Nagelkerken & Munday, 2016; Rosa et al., 2017). After exposure to elevated CO_2 levels and warming, Australian Port Jackson sharks (*Heterodontus portusjacksoni*) fail to hunt their prey at a higher rate and take four times longer to locate prey through olfaction (Pistevos et al., 2015). Because the sharks were unable to locate prey, they ate less and therefore, were smaller in size than those in the control conditions (Pistevos et al., 2015). Unlike the other literature in this review, this study manipulated both CO_2 levels and warming to investigate the interacting effects of rising temperature and OA. Elevated CO_2 conditions inhibited the olfactory and visual behavioral responses that contribute to the effective hunting behavior of the Port Jackson sharks (Pistevos et al., 2015; Rosa et al., 2017). The hindered ability of sharks to hunt and act as top-down predators could cause trophic cascades in this ecosystem through the proliferation of lesser elasmobranch species that comprise the shark's typical prey (e.g., skates, rays, etc.) (Myers et al., 2007).

Many of these studies have focused exclusively on benthic sharks that use buccal pumping as means of ventilation; more studies should also include pelagic sharks that depend on ram ventilation. Sharks that pump water through their mouth and over their gills rely on buccal pumping for ventilation. Most species that use buccal pumping spend most of their time on the bottom of the ocean floor. As sharks evolved into more active predators, their morphology evolved to allow them to take in water as they continually swim. Ram ventilation rams water into the mouth and lets it flow out through the gill slits. Many sharks can alternate between these two methods. The differing methods of ventilating acidified water conditions could have differing effects on the physiological growth of embryos in pregnant sharks (Rosa et al., 2017). Additionally, investigators should strive to include different climate zones (e.g., polar), habitats (e.g., open ocean), ontogeny (i.e., how larvae physiologically differ from one another due to OA), and ecosystems other than coral reefs in this research (Barry et al., 2011; Rosa et al., 2017). This is important in order to best understand the broader impact of OA on apex predators in marine ecosystems.

Similar to studies about antipredator behavior, few studies are done outside of Australia, some in support and some in contradiction. For instance, smooth dogfish sharks (*Mustelus canis*) from the North Atlantic Ocean responded to high CO_2 conditions similarly to fish in studies from Australia (Dixson et al., 2015). The smooth dogfish sharks, which rely on buccal pumping, exhibited less attraction to olfactory cues of prey after exposure to elevated CO_2 levels for 3-4 days (Dixson et al., 2015). These sharks also attacked food less aggressively even when olfactory cues were present (Dixson et al., 2015). OA impairs the ability of smooth dogfish to recognize and track their prey based on olfactory cues, indicating that OA renders this predator's top-down control less effective. The absence of an effective top-down predator could lead to major ecosystem degradation because of the release of mesopredators (Myers et al., 2007).

Some research from outside Australia directly contradicts previous findings in Australia. Ocellated wrasse (*Symphodus ocellatus*) from CO_2 seeps in the Mediterranean Sea greatly tolerated elevated CO_2 levels (Cattano et al., 2017). Their prey detection behavior exhibited this tolerance, possibly due to prior exposure to high-risk predation levels (Cattano et al., 2017). The ocellated wrasse retained their ability to recognize prey in acidified conditions despite previous studies that have demonstrated the loss of prey recognition. Additionally, juvenile blacksmith (*Chromis punctipinnis*) that reside in kelp forests in the northeastern Pacific Ocean also tolerated acidified conditions (Kwan et al., 2017). This could also be explained by their exposure to oscillating acidified conditions. As the seasons change, the capabilities of trees to capture and store CO_2 also changes; the oscillation of acidified ocean conditions reflect these changes. As trees capture more atmospheric CO_2, the ocean dissolves proportionally less CO_2. Rather than a genetic predisposition to lower pH, these oscillations have rendered the blacksmith more tolerant because they are not chronically exposed to acidified environments. However, the blacksmiths also demonstrated behavior differences between seasons, confirming that temperature plays a role in these changes (Kwan et al., 2017). Warming only proves to exacerbate any negative impacts caused by OA.

These differing findings about fish indicate that different fish species will have a variety of behavioral responses to OA. Moreover, the response of predators to OA outside of the Australian marine ecosystem cannot be predicted solely from Australian research. However, both require further investigation for a more comprehensive understanding of how predatory fish adjust their behavior in response to OA. Further, the repercussions on whole ecosystems cannot be derived from studying predator and prey separately, but from studying them in conjunction with one another.

OA Impairment of Predator-Prey Dynamics

Many studies that examine the effects of OA on fish exclusively focus on a single species' response, considering them independent of their prey and/or predator species. More research needs to be done on the dynamics between predator and prey and how their interactions affect the ecosystem as a whole. In some studies, prey behavior appeared to be more strongly impacted than the predators that also suffered from reduced prey detection when comparing their respective success rates. In experimentally manipulated elevated CO_2 conditions, predatory dottyback had reduced prey detection and capture ability while damselfish failed to recognize predator olfactory cues (Ferrari et al., 2011). When observing both the dottyback and the damselfish, prey detection and capture was more successful than predator avoidance despite the inhibited abilities of both (Ferrari et al., 2011). Further study should be done to discuss these dynamics between these predator and prey fish species to determine the overall effects in the ecosystem.

OA Indirectly Causes Trophic Cascades

In order to fully understand the effects of predator-prey fish dynamics on ecosystems, these smaller interactions between species must be further examined. Many of these interactions and dynamics can be broken down as result of trophic cascades. Elevated CO_2 levels and warming often result in smaller larvae and juvenile recruits (Ferrari et al., 2011), which typically results in smaller adult fishes as well. If a specialist predator must eat the smaller prey in acidified conditions, that predator might compensate for the lack of energetic value by increasing its consumption rate (Gaylord et al., 2015). OA may also increase or decrease the energetic needs or metabolic rates of predators (Couturier et al., 2013; Rosa et al., 2014; Cattano et al., 2018); changes such as these have broader implications for the ecosystem at large (e.g., higher rates of predation resulting in prey population declines) (Gaylord et al., 2015; Cattano et al., 2018). Additionally, if predators need more fish than before, their inhibited hunting abilities due to OA may prevent them from meeting the demands of these higher metabolic rates (Gaylord et al., 2015). This could ultimately lead to a decline in their own populations because of the decrease in size and recruitment of juveniles.

Sometimes, cascades can be caused by the loss of a keystone species. For example, the juvenile coral trout (*Plectropomus leopardus*), a piscivorous reef fish that is also one of the most important fisheries species on the Great Barrier Reef, became attracted to the odor of potential predators (Munday et al., 2013). This could potentially cause the loss of this keystone species, leading to a variety of ways that the ecosystem could collapse (Munday et al., 2013).

However, the most notable trophic cascades are those that affect every trophic level, which are commonly triggered by the decline of top-down predators, such as sharks. The threats to sharks are grimmer simply because there are already fewer of them than their prey and any decreases in their population make it harder to recruit juveniles. There is a significant deficit in the investigation of the relationship between OA, top predatory fish, and trophic levels. However, despite this deficiency of research, there is a multitude of studies and substantial evidence that discusses the implications of the loss of apex predators due to overfishing. Their loss due to overfishing does not equate to their inability to hunt effectively due to OA, but their effects on the ecosystem could be comparable depending on how OA changes their hunting abilities.

In the case of overharvesting of large predatory sharks in coastal oceans, released rays, skates, and smaller sharks overtake ecosystems and decimate mollusk populations. Due to the absence of top-down predation from sharks, the populations of rays, skates, and smaller sharks (i.e., “mesopredators”) have fewer causes of death and hence become over-populated (Myers et al., 2007). These lesser elasmobranchs eat mollusk populations at a non-sustainable rate (leading to destabilizing the ecosystem and driving local extinction) (Myers et al., 2007). If OA disrupts the ability of prey detection and hunting of sharks, then it effectively eliminates the top-down effects of those apex predators, resulting in trophic cascades. In order to develop an approach to the problem of OA, we must further explore these indirect species dynamics.

Although the observational study done in CO_2 seeps in Papua New Guinea found that fish were attracted to predator cues in high CO_2 conditions, they also found that fish diversity and community structure differed minimally between CO_2 seeps and nearby control reefs (Munday et al., 2014). Despite their predictions, attraction to predator cues did not appear to affect the overall ecosystem dynamics. They acknowledged that the differences in abundances of some fishes could be driven by the different coral community aspects of CO_2 seeps rather than by the direct effects of high CO_2 (Munday et al., 2014). Perhaps this means that fish species will adapt in time to be tolerant or could even thrive in these predicted CO_2 conditions. On top of their response to OA, these species must rapidly adapt to the warming temperatures of our oceans as well because these two conditions will act synergistically.

Conclusion and Future Directions

Rising levels of CO_2 in the ocean could detrimentally change the predator-prey dynamics among fish, which could drastically change ecosystems in general. It has been examined how

OA can impair physiology and consequently the survival behavior of fish species. When exposed to predicted future OA at different development stages, some species lose the ability to distinguish between the olfactory cues of predators and non-predators, regardless of global location (Munday et al., 2009; Dixson et al., 2010; Brown et al., 2012; Lonnstedt, et al., 2013; Wang et al., 2017), while others showed the loss of auditory discrimination (Simpson et al., 2011). However, the deterioration of response to olfactory and auditory cues has the potential to be compensated for by an increased response to visual cues (Lonnstedt et al., 2013). Predatory fish species also suffer from impaired hunting behavior (Dixson et al., 2015; Pistevos et al., 2015). Due to exposure to acidified conditions, the predatory dottyback responds to olfactory cues from prey at a slower rate (Cripps et al., 2011). The potential for trophic cascades cannot be ignored when top-down predators such as sharks are effectively absent in many of these ecosystems (Myers et al., 2007; Gaylord et al., 2015). The ramifications of loss of predators, especially apex predators, is severely lacking in this realm of study.

Ultimately, the interactions between predator and prey change the overall ecosystem, via top-down trophic cascades in food webs. Few researchers have investigated this relationship, and in order to find a solution to these predicted problems, more studies need to revolve around this “big picture” idea (Barry et al., 2011). Many studies are done exclusively in the Great Barrier Reef, as many of these observed changes radiate from Australia and its unique ecosystems, but more work needs to be done globally. The research outside of Australia is less comprehensive but altogether contributes context. At times, they can even provide further support for previous findings. It is important to bridge the gap between Australia and the rest of our oceans in order to best understand how to approach a solution for this predicted global problem. Although many studies outside of Australian sites are coming to light, there is still an obvious lack of investigations about the interactions between predatory fish and their prey. Understanding these relationships is foundational to the ultimate understanding of how ocean acidification is affecting species interactions, which can lead to larger shifts in the ecosystems themselves. It is vital to continue examining these effects because fish populations and marine ecosystems suffer from overharvesting already (Sissenwine, et al., 2014; Le Pape et al., 2017). Their plight worsens with every additional threat.

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CHAPTER 2. GRANT PROPOSAL

Synergistic Effects of Acidification and Warming on the Behavior and Survival of Green Sunfish (*Lepomis cyanellus*)

Abstract

Acidification and warming threaten the health of freshwater ecosystems. Few ecologists have investigated the synergistic effects of acidification and warming on freshwater fish, such as the green sunfish (*Lepomis cyanellus*). If freshwater fish respond to these pressures the same way that marine fish do, green sunfish could lose the ability to avoid predators and successfully recruit individuals to future generations. Together climate change and freshwater acidification may threaten the ability of green sunfish to persist. I propose to measure these synergistic effects on egg hatching, juvenile survivorship, antipredator behavior in two-factorial manipulated experiments. I will expose fertilized green sunfish eggs from Coot Lake to lower pH and increased temperature and measure their hatching rate and juvenile survival. I will then quantify the effect of these pressures on antipredator escape response of adult sunfish when exposed to catfish olfactory cues. Using these factorial designs, I will also determine whether acidification and warming synergistically or additively influence the recruitment & behavior of green sunfish. Because green sunfish are distributed widely in North America, the results of this proposed research will not only help understand how a commercially important fish in Boulder County may respond to changing water quality, but will also be used to project how other freshwater fish may respond to the joint threats of acidification and warming.

Literature Review

Surface water CO_2 concentrations in freshwater ecosystems have increased in proportion to annual increases in regional atmospheric CO_2 (Sullivan et al., 1999; Feely et al., 2009). Thus, as humans pollute the air, they also proportionally pollute waterways. Ecologists have long studied how atmospheric CO_2-driven acidification and warming affect our oceans (Gaylord et al., 2015), but far fewer have investigated its effects on freshwater bodies (Harris et al., 2010; Leduc et al., 2013). Although atmospheric changes primarily drive decreased pH and higher temperatures in the oceans (Feely et al., 2009), changes both on the landscape (mining and loss of shade) and in the atmosphere (acid rain) drive these changes in freshwater systems (Schindler, 1988; Dunford et al., 2012). Because the total volume of water in freshwater bodies is smaller than the total ocean volume, freshwaters are more sensitive to these anthropogenic changes. Freshwater pH levels have decreased at three times the rate of oceans (NOAA, 2018; Weiss et al., 2018), while freshwater temperatures have risen almost twice as much as the oceans have due to their shallower depths (Feuchtmayr et al., 2009). Both warming and acidification in these waterways have significant effects on the fish populations that inhabit them.

In isolation, both acidification and warming impair sensory functions and escape behavior in fish, and cause decreased fecundity in ocean fish (Walther et al., 2002; Pörtner & Peck, 2010; Couturier et al., 2013). Acidification and warming similarly impair freshwater fish populations (Feuchtmayr et al., 2009). Acidification in some lakes and streams have resulted in massive declines of commercially valuable fish species (Schofield, 1976; Muniz, 1984). Lower pH prevents both eggs and larvae from developing, leading to recruitment failure and ultimately population extirpation (Schofield, 1976; Sayer et al., 1993; Ou et al., 2015). Warming also impairs the escape behavior of freshwater fish (Feuchtmayr et al., 2009; Pörtner & Peck, 2010). These pH- and temperature-driven declines have important implications for sustainable management of sunfish populations in freshwater systems given that the magnitude of climate change is greater than predicted and continues to worsen (Dudgeon et al., 2006).

However, acidification and warming do not act independently of each other. Their synergism compounds impairment to homing abilities, predator avoidance, and juvenile biomass in marine fish (Nagelkerken & Munday, 2016), but changes in marine fish populations cannot be proxy to changes in freshwater fish populations. Studies of acidification and warming are primarily conducted in the ocean, but few in comparison consider these synergistic effects on streams, rivers and lakes (Kroeker et al., 2013; Nagelkerken & Munday, 2016). Fewer studies still have investigated the effects of warming and acidification on fish populations, such as green sunfish (*Lepomis cyanellus*), in different regions. Measuring their negative response to warming and acidification will support sustainable management of other freshwater fish populations by using them as proxy. Increased temperature and acidification of freshwater bodies in Boulder County could hinder the ability of fish to avoid predators and recruit successfully. Boulder County can use these population projections to determine the best fishing regulations.

Objective and Anticipated Value

The aim of this study is to investigate the impacts of acidification and warming on green sunfish (*Lepomis cyanellus*) population persistence in Boulder County Parks and Open Space (BCPOS) streams. Because the green sunfish is a common species and geographically widespread (Shaklee et al., 1977), it is ideal for understanding the response of other fish populations. The growing threat of climate change and acidification exacerbate the established threats of overfishing, flow modification, and water pollution on these freshwater fish populations (Dudgeon et al., 2006). I propose to measure the synergistic effects of acidification and warming on egg and juvenile survivorship and antipredator behavior in manipulated experiments. To widely disseminate the results of this localized study and encourage additional research on this global issue, this research will be published in academic journals. Additionally, the proposed research will include projected water quality in Boulder County, which will inform adaptive management plans for freshwater systems.

Question 1*:* Do freshwater acidification and warming individually influence green sunfish recruitment (i.e., fecundity and juvenile survivorship)?

Hypothesis & Prediction 1: Lower pH and higher temperature will each hinder the development of the eggs and larvae. I expect to see lower hatching success in both warmer and acidified treatments then the control (Jarrett et al., 1993; Sayer et al., 1993; Feuchtmayr et al., 2009).

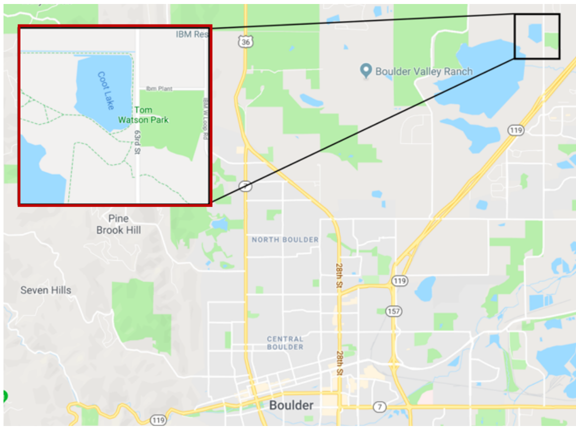

Figure 1. Coot Lake, Boulder County, CO

Question 2*:* Do freshwater acidification and warming individually alter green sunfish antipredator behavior?

Hypothesis & Prediction 2*:* Freshwater acidification and warming impair the olfactory nerves of green sunfish that control their ability to detect cues from predators. I expect that the green sunfish will fail to detect and avoid their predators in warmer and acidified conditions.

Question 3*:* How do freshwater acidification and warming act synergistically to impair the recruitment and antipredator behavior of green sunfish?

Hypothesis & Prediction 3*:* Acidification and warming interact synergistically on green sunfish populations. The increase in temperature predisposes fish to the effects of lower pH (Munday et al., 2009; Nagelkerken & Munday, 2016). I expect the lowest hatching success, lowest juvenile survival, and most reduced antipredator behavior than predicted in the synergism treatment than predicted from the additive effects of temperature on acidification (Kroeker et al., 2013).

Methods

Study Design 1*: Determining the individual and synergistic effects of acidification and warming* *on eggs and larvae. (See **Questions 1** and 3)*

I will collect green sunfish from Coot Lake in Boulder County and, in the lab (Jarrett et al., 1993), measure hatching success and juvenile survivorship of their fertilized eggs under the following conditions: ambient, elevated temperature, acidification, and both elevated temperature and acidification (3 replicates per treatment) (Fig. 1; Fig. 2) (Kroeker et al., 2013). At the time of collection, I will record the pH and temperature of the water in Coot Lake, and set the elevated conditions of the treatments (temp: Ambient + 2.0°C; pH: Ambient – 0.4) (Fig. 2). I will measure hatching rate of ~50 fertilized eggs (over 2 days) in petri dishes and juvenile survival of those hatched eggs (over 8 days after hatching) in holding tanks.

***Study Design 2**: Determining the individual and synergistic effects of acidification and warming on the antipredator behavior of green sunfish. (See **Questions 2** and 3)*

I will collect adult green sunfish and adult catfish from Coot Lake and, in a lab-based study, evaluate the effects of treatments in Design 1 on antipredator behavior. I will place the catfish in a holding tank of ambient water conditions for 24 hours, then expose the sunfish to the catfish olfactory cues from concentrated "fish juice" (Hemmings, 1966). I will observe antipredator behavior and assess their escape response (Brooker & Dixson, 2016).

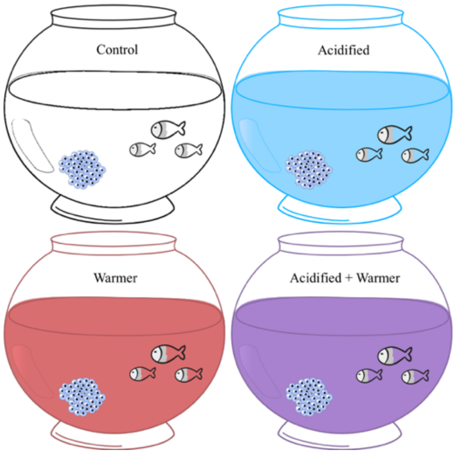

Figure 2. Experimental Design

***Data Analysis**:* I will use two 2-way factorial ANOVAs with an interaction to test the effects of temperature and acidification on (i) hatching success and juvenile survivorship, and (ii) antipredator behavior (Munday et al., 2009). If there are significant effects, Newman-Keuls multiple comparison tests will compare means between warmer, acidified, synergistic, and ambient treatments (Munday et al., 2009).

Project Requirements, Logistics, Timeline and Negative Impacts

The proposed research will be conducted from March through October 2019 (Table 1). I will gain IACUC approval from Regis University. I will submit a detailed map of proposed locations to BCPOS and Colorado Parks & Wildlife to obtain permission to sample of *L. cyanellus* on BCPOS property. I also need to obtain a scientific collecting permit in order to catch and release any captured *L. cyanellus*. By using catch and release, the impact on collected individuals will be minimal. Water acidity and temperature collection will also have minimal impact to BCPOS lands. I am requesting $9,716 for equipment and consumables (Table 2).

Table 1. Anticipated Timeline

Dates in 2019	**Activities**	**Deliverables**
March	• Obtain IACUC approval from Regis U	• IACUC approval
mid-March – April	• Discuss sampling locations with BCPOS • Obtain catch and release permits for green sunfish from CPW	• BCPOS approval • Permits
May – mid-June	• Collect ~1,000 eggs • Measure water acidity and temperature at Coot Lake • Prepare tanks and petri dishes	• Raw data from surveys
June – July	• Perform lab-based experiments • Begin writing report	• Raw data from experiments
August – Sept	• Analyze data & Finish draft of report	• Report draft
Oct	• Finish final draft of report	• Final report

Table 2. Anticipated Budget

Item	**Justification**	**Cost, unit (Source)**	**Quantity**	**Total Cost**
Tanks	Determine juvenile survivorship and adult antipredator behavior	$100 (Regis)	12	$0
pH meter	Measure pH of water	$15 (Regis)	1	$0
Digital thermometer	Measure temperature of water	$20 (Regis)	1	$0
Glass jar	Contain predatory olfactory cues	$3 (Regis)	5	$0
Small net	Catch green sunfish	$10 (Regis)	2	$0
Sulfuric acid (H_2SO_4)	Alter pH for experiments	$42/2.5L (Fisher Scientific)	2	$84
Gas	1 trip to site from Regis & back	$0.535 / mile (IRS)	40mi	$22
Petri dishes	Determine egg hatching success	$5/20pk (USA Scientific)	2	$10
Stipend	Conducting field data collection, laboratory work, data analysis, and report writing	$300 / week	32 weeks	$9,600
Total Proposal Request				$9,716

Regis University will be donating use of all equipment necessary.

Qualifications of Researcher (see Attached Resume)

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CHAPTER 3. JOURNAL MANUSCRIPT

Meta-Analysis of Sylvatic Plague Prevention Methods in Prairie Dog Populations

Abstract

Sylvatic plague has devastated prairie dog (*Cynomys* spp.) populations by more than 96%. However, prairie dogs enhance habitat heterogeneity and mammalian diversity of grasslands in central North America, so saving them from this plague is necessary in preserving grassland ecosystems. There are three main methods of preventing plague: vaccine, insecticide baiting, and insecticide dusting. I hypothesize that vaccination will be the most effective method of preventing massive population decline due to sylvatic plague because it provides lifetime immunity to unaffected prairie dogs, whereas insecticides such as fipronil and deltamethrin only provide effective treatment for a maximum of one year. In order to quantify the efficacy of each method, I conducted a meta-analysis of 20 studies over the past 40 years. According to the analysis, vaccines provide the most protection against plague in comparison to dusting and baiting. Although vaccines most effectively protect against plague, there is a trade-off between the methods; vaccines require more effort and money while dusting and baiting are most cost-efficient but less effective against plague. Providing the best protection against plague in prairie dogs is important for the continued restoration and conservation of short-grass prairies and grasslands. Therefore, refuges that have populations of high priority should vaccinate their prairie dogs.

Introduction

Prairie dogs (*Cynomys* spp.) play a vital role in maintaining the short grass prairies of central North America (Miller et al., 1994; Quirk, 2006; Baker et al., 2013). These ecosystem engineers aerate the soil, preventing the encroachment of shrubs or other tall vegetation, thus preserving native grasslands. Their presence and behavior enhance habitat heterogeneity and mammalian diversity (Miller et al., 1994; Antolin et al., 2002). Their burrows provide important habitat infrastructure to other species, such as mountain plovers (*Charadrius montanus*), golden-mantled ground squirrels (*Callospermophilus lateralis*), and burrowing owls (*Athene cunicularia*) (Miller et al., 1994; Antolin et al., 2002). American bison (*Bison bison*), pronghorn (*Antilocapra americana*), and mule deer (*Odocoileus hemionus*) preferentially graze on the same land inhabited by prairie dogs (Krueger, 1986; Ceballos et al., 1999; Fahnestock & Detling, 2002). Further, a number of predators, including badgers (*Taxidea taxus*), coyotes (*Canis latrans*), ferruginous hawks (*Buteo regalis*), and the endangered black-footed ferrets (*Mustela nigripes*), depend on prairie dog populations for their diet (Cully, 1989; Antolin et al., 2002; Cook et al., 2003). Because of their contributions to the land and their support of other various grassland species, prairie dog populations significantly contribute to maintaining ecosystem integrity in central North America.

Historically prairie dogs ranged from southern Saskatchewan to Chihuahua, Mexico, with many American states hosting significant populations (Milne-Laux & Sweitzer, 2006; Facka et al., 2008). Before the 1900s, prairie dog populations went well beyond the hundreds of millions (Facka et al., 2008), but their populations have declined more than 96% and their range has decreased more than 75% since then (Barnes, 1993; Milne-Laux & Sweitzer, 2006). The decline of prairie dogs has contributed to the loss of other species in grassland ecosystems (Cully, 1989; Barnes, 1993; Antolin et al., 2002). For example, a decline in a prairie dog population typically leads to a subsequent collapse of black-footed ferret populations, a specialized predator of prairie dogs (Barnes, 1993; Antolin et al., 2002). Conserving prairie dog populations in their native habitats is important for preserving short-grass prairie ecosystems across the western United

States.

These huge population declines are due, in large part, to sylvatic plague (*Yersinia pestis*). Sylvatic plague has killed more than 99% of prairie dogs in many individual colonies (Cully, 1989). Sylvatic plague was first discovered in prairie dogs in the late 1890s, but has been present long before then. The fatal pathogen, *Y. pestis*, has caused human plagues in the past, most notably the Black Death in Europe (years 1347-1353). More is known about its effects on humans and other mammals, such as prairie dogs, than its origins. Fleas infected with *Y. pestis* transmit the plague to the prairie dogs, who act as amplifying hosts of the bacterium. When a prairie dog becomes infected, the level of pathogen becomes high enough that uninfected mosquitos can become infectious from biting prairie dogs. Amplifying hosts further contribute to the spread of the disease within their own species (Barnes, 1993). Once the epizootic disease infects even one prairie dog, the chances of losing the entire colony rise to 90-100% if not treated immediately (Collins et al., 1984; Seery et al., 2003). Even with active population and colony control, the loss to sylvatic plague is upwards of 80% at any given time (Collins et al., 1984; Seery et al., 2003). In order to prevent further population loss, recent management plans to prevent plague on government-owned lands include burrow dusting with insecticide, fipronil baiting, and vaccination (Antolin et al., 2002; Seery et al., 2003; Mencher et al., 2004; Yoder & Miller, 2010; Abbott et al., 2012; Jones et al., 2012; Cárdenas-Canales et al., 2017; Poché et al., 2017; Rocke et al., 2017).

To prevent plague, government land managers commonly dust prairie dog burrows with insecticide to exterminate flea populations directly (Barnes et al., 1972; Beard et al., 1992; Seery et al., 2003). Most agencies now use deltamethrin as the chosen insecticide but previous management plans also used carbaryl or permethrin. Fipronil is also an insecticide, but not typically used in dust. Many land managers coat oats in fipronil and distribute them at all active burrows (Collins et al., 1984; Poché et al., 2017). After ingesting the oats, the insecticide collects in the oils of the skin and hair follicles, causing death in fleas that rest on the prairie dogs. Dusting and baiting both require intense labor, making burrow dusting and oat distribution afford about the same effort. Furthermore, managers must re-administer both treatments every year, resulting in higher cumulative effort than a one-time treatment per prairie dog such as a vaccine. Sylvatic plague vaccine (SPV) is a relatively new plague prevention method (Abbott et al., 2012; Rocke et al., 2017). Managers typically administer SPV orally via oats, and then test its efficacy through drawn prairie dog blood. Biologists then test prairie dog blood for seroconversion (Mencher et al., 2004; Yoder & Miller, 2010; Abbott et al., 2012; Cárdenas-Canales et al., 2017). When effective, this method provides lifetime immunity to any treated prairie dogs. Although government-owned land managers have implemented these prevention methods over many years, prairie dog populations have not returned to their pre-1900s numbers (Milne-Laux & Sweitzer, 2006). An assortment of biological factors (e.g., inconsistent implementation, failed vaccine, inadequate dusting method) and anthropogenic factors (e.g., industrial agricultures, real estate development) may contribute to this dilemma, but the most effective approach to preventing population declines from sylvatic plague has not yet been determined.

To determine the effectiveness of each method, I assess which preventative measure most effectively works against plague in prairie dog colonies across the continental United States. I hypothesize that vaccination will be the most effective method of controlling sylvatic plague because it provides lifetime immunity to unaffected prairie dogs, whereas insecticides such as fipronil and deltamethrin only provide effective treatment for a maximum of one year. If lifetime immunity, provided by vaccines, is more effective than immediate control of fleas, via insecticides, I predict, that on average, vaccinated prairie dogs across all studies will have fewer cases of plague and, hence, higher survival rates against plague compared to the control treatment (Yoder & Miller, 2010; Cárdenas-Canales et al., 2017). To quantify the efficacy of each method, I conduct a meta-analysis of multiple studies over the past 40 years.

Methods

Literature Search

To identify literature that has assessed plague prevention, I searched the following databases: Academic Search Premier, Biological Science Database, CQ Researcher, Environment Complete, ScienceDirect, and Wiley Online Library. I used Boolean logic to search in these databases to include all related pieces of literature, using phrases "prairie dog" AND plague AND (vaccine OR dust* OR bait*). I read through the abstracts of 56 papers to identify relevant research for this study. Upon further screening, I only included studies that had a control in their trials. In total, I found four studies about fipronil, eight for dusting, and eight for vaccines (Table 1).

Table 1. Literature included in meta-analysis

Literature	**Method**
Eads et al., 2019	Baiting
Jachowski et al., 2011	Baiting
Jachowski et al., 2012	Baiting
Poché et al., 2017	Baiting
Barnes et al., 1972	Dusting
Beard et al., 1992	Dusting
Biggins et al., 2010	Dusting
Eads et al., 2018	Dusting
Hoogland et al., 2004	Dusting
Hoogland et al., 2018	Dusting
Seery et al., 2003	Dusting
Tripp et al., 2016	Dusting
Cárdenas-Canales et al., 2017	Vaccine
Mencher et al., 2004	Vaccine
Rocke et al., 2010	Vaccine
Rocke et al., 2015	Vaccine
Rocke et al., 2017	Vaccine
Tripp et al., 2015	Vaccine
Tripp et al., 2017	Vaccine
Yoder & Miller, 2011	Vaccine

Data Extraction and Analysis

I compared the effects of each method (i.e., dusting, baiting, vaccine) to determine if one worked significantly better than the others against plague. I determined the mean of the treatment group, the mean of the control group, and the sample size and standard deviation of each. I used these to calculate the bias correction factor (*J*) and the pooled standard deviation (*s*). To standardize different measures of effect, I used Hedges' *d* as a common measure of effect size. Using the 'metafor'-package in the R program (Version 3.5.2), I conducted a cumulative meta-analysis of the difference in effect between the methods (Viechtbauer, 2010; Rosenberg et al., 2013).

Results

Vaccination significantly controlled plague significantly better than both baiting (p<0.001) and dusting (p<0.001) (Figure 1). On average, vaccinations controlled plague -1.52 (95% CI: -1.25 – -1.79) more than in the control groups (p<0.001). Vaccine effectively helped prairie dog populations survive against plague 1.52 more times than the other two methods. Dusting also controlled significantly better than baiting (p<0.001).

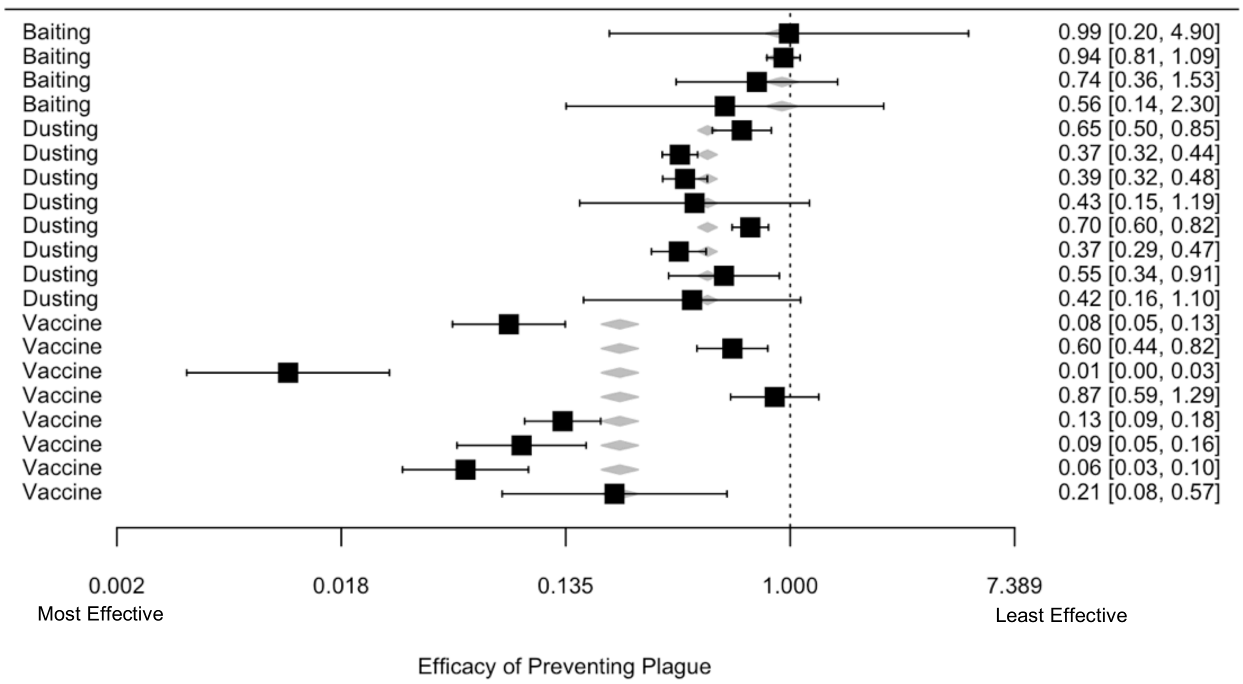

Figure 1. Forest plot showing the effects of each study, represented by its method of preventing sylvatic plague. The figure shows the relative ability of each method in controlling the plague in the treated versus the control group with corresponding 95% confidence intervals in the individual studies and based on a fixed-effects model. Vaccines have the more negative the effect, therefore, it is more effective at controlling plague than dusting and baiting.

Discussion

This meta-analysis supported that vaccines protect against plague better than dusting or baiting (Figure 1). This supports the original hypothesis, and further exhibits that for every two prairie dogs that baiting or dusting can protect, the vaccine can protect three (Figure 1). Five acres of land can typically have 40-50 individuals. At RMANWR, which is about 12,500 acres, there could be as many as 300,000 prairie dogs, assuming that the prairie dogs occupy at least half the land. With a population this large, the land managers must choose the best method of protecting their prairie dogs from plague, but they must also consider three main factors when choosing a prevention method: effort, effectiveness, and cost. Effort is the amount of work required to administer the treatment to prairie dogs. Effectiveness is the extent of protection against plague and supporting population survival. Cost includes the price of the treatment itself as well as the laborer(s) it entails.

Dusting and baiting both require administration to every active burrow within the area. These methods will exterminate flea populations directly (Barnes et al., 1972; Collins et al., 1984; Beard et al., 1992; Seery et al., 2003; Poché et al., 2017), whereas vaccine enhances the immunology of the prairie dogs themselves. Managers typically administer vaccine orally via oats, similar to baiting insecticide, but vaccination also requires a subsequent test of its efficacy through drawn prairie dog blood (Mencher et al., 2004; Yoder & Miller, 2010; Abbott et al., 2012; Cárdenas-Canales et al., 2017). Additionally, managers must re-administer all three treatments every year: dusting and baiting because that is the maximum amount of time it provides protection and vaccine because they annually breed through the spring months (March-May) (Hoogland, 1997; Tripp et al., 2017). Because of the extra step after administration, there is more effort in using vaccines as treatment compared to dusting and baiting.

Although dusting and baiting require less effort than vaccines, they are also less effective than vaccines against plague (Figure 1). This could be due to a plethora of field errors, starting from making the treatment to its administration onto the burrows themselves. Additionally, the difference in effects could also be due to fleas becoming desensitized to treatment (Rust, 2016). In the case of successful treatment, population increase would mean more intensive treatment. In this case, the compounded effort of blood testing for seroconversion could far outweigh its benefits. However, despite the extra effort required of vaccines, its effectiveness still makes it the better option over the other two. However, there is one other factor to consider in this choice.

Vaccines are the best method of preventing their population declines due to plague, but vaccines are also costlier than either of the other two methods (Abbott et al., 2012). Dusting and baiting cost $50/acre while vaccines cost $300/acre (administration + post-testing). After the initial vaccine administration, testing would happen less often, bringing down the cost of vaccine to $100-125/acre. Laborers are an additional cost, but the help of volunteers to administer the vaccine may alleviate the cost for the vaccine itself. However, this may not be enough to make vaccines worth investing in. There is a trade-off between the methods: vaccines are best against plague but require more effort and money, while dusting and baiting are more affordable but less effective against plague. Long-term, however, the less effective aspect of dusting and baiting could lead to their more intensive use or even the reintroduction of more prairie dog colonies, eventually costing at least another $5million. In recent years, there have been developments of drones that could apply baits to 5,000 acres in just 10 minutes (Keirn, 2017; Kreiger & Matchett, 2019). These drones would substantially reduce the cost of labor if the method is successful. In 10 years, baiting and dusting could each cost $5.5m, taking into account that fleas will likely become grow less sensitive to the treatment (Rust, 2016), while vaccine $7.5m. In 20 years, baiting and dusting could cost \$10.1m + \$5m for potential reintroduction, vaccine \$13.8m. In 30 years, baiting and dusting could cost \$24.1m, vaccine \$20.6m. Ultimately, vaccines would cost the least of plague prevention methods.

Rocky Mountain Arsenal NWR has recently started implementing a management plan involving all three methods in different areas of the refuge, potentially collecting data for comparison. Many land managers employ one method at a time or control for differing factors (i.e., age, sex, season, etc.) (Beard et al., 1992; Seery et al., 2003; Mencher et al., 2004; Yoder & Miller, 2010; Poché et al., 2017). This meta-analysis is the first to compare all these studies to one another. However, it may be limited by the fact that there were only four papers regarding baiting, whereas there were at least eight regarding both dusting and vaccines. Before my study, there was no literature comparing these methods to one another. Future work could focus on studying rebounding prairie dog colonies that have suffered from anthropogenic stressors (e.g., road proximity, construction, domestic pets) or sylvatic plague (Avila-Flores, 2009; Shannon et al., 2014). Many times, anthropogenic stressors can lead to higher exposure risk to infectious disease such as plague (Daszak, 2001). Determining how these colonies respond after treatment could be very useful for further management plans.

Despite the limitations of this analysis, it is instructive to understand the differences between these methods. When even one prairie dog is infected by sylvatic plague, the entire colony is at risk of extirpation. Managers could use this knowledge to make more informed decisions about which method to employ for their land and their prairie dogs. Because of their importance to other grassland species and their contribution to preserving short grass prairies in central North America (Krueger, 1986; Cully, 1989; Miller et al., 1994; Ceballos et al., 1999; Antolin et al., 2002; Fahnestock & Detling, 2002; Cook et al., 2003; Quirk, 2006; Baker et al., 2013), prairie dog colonies must be protected from this plague. Managers should keep effort, effectiveness and cost in mind when choosing between the three methods, but vaccine should be the primary option. For refuges such as RMANWR, the prairie dogs are important for maintaining other species and the grasslands themselves, making their colonies a higher priority. For lower priority colonies, baiting and dusting are viable options against the plague.

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CHAPTER 4. STAKEHOLDER ANALYSIS

Stakeholder Values of Treating Prairie Dogs Against Sylvatic Plague

Background

Prairie dogs (*Cynomys* spp.) play a vital role in maintaining the short grass prairies of central North America (Miller et al., 1994; Quirk, 2006; Baker et al., 2013). Prairie dogs support many grassland species through their burrowing behavior, including burrowing owls (*Athene cunicularia*), American bison (*Bison bison*), mule deer (*Odocoileus hemionus*), and the endangered black-footed ferrets (*Mustela nigripes*) (Krueger, 1986; Cully, 1989; Miller et al., 1994; Ceballos et al., 1999; Antolin et al., 2002; Fahnestock & Detling, 2002; Cook et al., 2003). By aerating the soil, prairie dogs are maintaining the mixed and short grass prairies, which prevents woody plants from establishing in the grasslands and encroaching into the prairies. The loss of prairie dogs commonly leads to the establishment and encroachment of trees and woody shrubs into mixed and short grass prairies (Bonham & Lerwick, 1976; Weltzin et al., 1997; Dulamsuren et al., 2008; Van Auken, 2009). Protecting these ecosystem engineers are important for maintaining grassland ecosystem integrity.

Historically prairie dogs ranged from southern Saskatchewan to Chihuahua, Mexico, across many American states (Milne-Laux & Sweitzer, 2006; Facka et al., 2008). Before the 1900s, prairie dog populations went well beyond the hundreds of millions (Facka et al., 2008), but their populations have declined more than 96% and their range has decreased more than 75% since then (Barnes, 1993; Milne-Laux & Sweitzer, 2006). Sylvatic plague has contributed massively to their huge population declines, killing more than 99% of prairie dogs in many individual colonies (Cully, 1989). Once the epizootic disease infects even one prairie dog, the chances of losing the entire colony rise to 90-100% if not treated immediately (Collins et al., 1984; Seery et al., 2003). Even with active population and colony control, the loss to sylvatic plague is upwards of 80% at any given time (Collins et al., 1984; Seery et al., 2003). In order to prevent further population loss, recent management plans on government-owned lands to prevent plague include burrow dusting with insecticide, baiting with fipronil (another insecticide), and vaccination (Seery et al., 2003; Yoder & Miller, 2010; Jones et al., 2012; Cárdenas-Canales et al., 2017; Poché et al., 2017). As they provide lifetime immunity against infection, vaccines are more effective method against sylvatic plague (Aquino, Unpublished data).

Most of the year, Rocky Mountain Arsenal National Wildlife Refuge faces the problem of losing their prairie dog communities due to sylvatic plague, which would lead to other species declines and potential loss of its grasslands (Barnes, 1993; Antolin et al., 2002). In order to maintain their current prairie dog populations, the managers should treat their colonies with vaccine to prevent massive population decline due to plague. The treatments only last a year and they need to be reapplied seasonally to newborn prairie dogs, to each individual burrow or each individual prairie dog. It is extremely labor intensive and may not be worth the cost of testing.

Stakeholders

The main stakeholders are the Rocky Mountain Arsenal National Wildlife Refuge managers. They want to preserve the population of prairie dogs because they help maintain the grasslands for the general public and to support the native and endangered black-footed ferret population, which makes up 1/3 of the nation's entire population (U.S. Fish and Wildlife Service, 2015). As prairie dog specialists, black-footed ferrets require large prairie dog populations. Preserving prairie dogs provides a healthier and more stable grassland ecosystem. In order to protect their population, they have had volunteers who spend days distributing fipronil oats to all active burrows. Vaccines are costly, but the funding is available through the budget. However, there is a lack of funding for extra laborers to distribute them and having volunteers is the most cost-effective route.

Another stakeholder would be black-footed ferret conservationists such as the Defenders of Wildlife. Much like the RMANWR managers, they want to preserve the population of prairie dogs in order to support the black-footed ferret population at the refuge. Further, a number of predators depend on prairie dog populations for their diet, including black-footed ferrets (Cully, 1989; Antolin et al., 2002; Cook et al., 2003). Because black-footed ferrets are prairie dog specialists, they rely on a sustainable and thriving prairie dog community. Defenders of Wildlife are also interested in the conservation of endangered wildlife species for their donors.

The Commerce City Parks and Open Space also want to preserve the prairie dogs at the RMANWR because they want to keep the refuge a grassland. They support the "Northwest Greenway," which is a greenway, composed of grasslands and open trails, used mainly for recreational purposes. To remain a fun and beautiful location for families and potential tourists to visit, the grasslands need to be preserved. Similar to the RMANWR, Commerce City Parks and Open Space wants to keep their grasslands green and thriving, which rely on prairie dog activities.

Along that same vein, there are many local visitors and hikers who visit the refuge to hike the land and/or see the megafauna (i.e. bison and mule deer). The refuge has nearly 1,000 visitors each week; these visitors would want to maintain the land as it is. To preserve the landscape and healthy bison and mule deer populations, prairie dogs must continue to aerate the soil and consequently maintain the grasslands of our nation as U.S. citizens. Additionally, prairie dogs are very charismatic and cute fauna. Many of these visitors would want to save them from suffering deathly plague.

Birders could fall on either side of the argument in preserving prairie dogs. Birders from groups such as the Rocky Mountain Bird Observatory or the Denver Field Ornithologists want to protect prairie dog populations to support bird populations at the refuge for a couple of reasons (VerCauteren et al., 2001; England, 2003). Ferruginous hawks, red-tailed hawks, bald eagles, and golden eagles all prey upon prairie dogs for their diet while burrowing owls use inactive prairie dog burrows for shelter (Miller et al., 1994; Antolin et al., 2002; Cook et al., 2003). To support the raptor population at the refuge, birders are at odds with independent shooters who target the prairie dogs because the shooters are removing the important prey and ecosystem engineers of these birds (England, 2003).

Local gardeners are less obvious stakeholders. There is a community currently being built right next to the refuge. Prairie dogs ruin and ravage small gardens for food. Prairie dogs in urban areas such as Commerce City often manage to eat from local gardens. They can easily go through wire fencing and get as far as two miles away from their burrows to forage food (Dowle & Deane, 2009). Commerce City has begun construction of a new neighborhood along the perimeter of the refuge (plumbing is currently being installed), which renders the problem between local gardeners and prairie dogs more prevalent. Any of these new residents who will have a garden will also likely prefer to have smaller prairie dog communities in the surrounding area.

Local farmers would not want to spend money in order to preserve prairie dogs. Although prairie dogs are important, they view them as pests that cause problems rather than prevent or solve them. For example, horses commonly break their legs when they step into prairie dog burrows, which means they have to be put down (Work, 2016). Additionally, they view their preservation as costlier than its benefits are worth, rendering it unnecessary and gratuitous. Not only is treatment costly, it needs to be reapplied quite often. The treatments only last a year and they need to be reapplied seasonally for newborn prairie dogs to each individual burrow or each individual prairie dog. It is extremely labor intensive, and may not be worth all the effort. In support of their view, they could cite the fact that even as management preserves part of the prairie dog population, they also kill a portion of the population to better control them and reduce the probability of spreading plague.

Recommendation

RMANWR should vaccinate their prairie dogs to prevent massive population declines in the case that they do contract sylvatic plague. The population has been nearly extirpated in the past due to this plague (Tripp et al., 2016), which threatens the existence of their prairie and grassland habitats. The high population currently residing at RMANWR raises the chances of contracting the plague. This plague could cause the complete extirpation of this community at RMANWR, leading to the encroachment of trees into the grassland and to the decline of 1/3 of the nation's endangered black-footed ferret species (Bonham & Lerwick, 1976; Miller et al., 1994; Weltzin et al., 1997; Quirk, 2006; Dulamsuren et al., 2008; Van Auken, 2009; Baker et al., 2013). Maintaining this population is important for multiple reasons, despite any belief that they are pests or costlier than they are worth. Vaccine is costly but it prevents sylvatic plague better than both baiting and dusting insecticides (Aquino, Unpublished data). To reduce its costliness, the refuge volunteers can distribute vaccine via oats. To mitigate the conflict between stakeholders, the refuge should concurrently control the populations (via direct shooting) from March to May (i.e., their mating season and subsequent gestation period) (Soulsbury & White, 2015). This would keep the population lower and more manageable. This recommendation is the best method to keep this grassland as it is without the threat of tree and shrub encroachment.

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