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MS Environmental Biology Capstone Project

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MS ENVIRONMENTAL BIOLOGY
CAPSTONE PROJECT

by

Catherine M. Shapiro

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

REGIS UNIVERSITY
May, 2022

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CHAPTER 1: LITERATURE REVIEW

Comparing the Ecological Effects of Artificial Light at Night to Different Types of Freshwater and Marine Ecosystems.

Introduction

Artificial light at night (ALAN) is a major source of pollution to waterways. Since 2012, it is estimated that artificial light is experienced by 80% of the human population and is growing at a rate of 2.2% per year (O'Connor et al., 2019). Many studies have focused on how light affects terrestrial ecosystems, but scientists only recently started to study how artificial light affects aquatic ecosystems (Davies et al., 2014). Artificial light can originate from both permanent and temporary sources in aquatic habitats. Permanent sources include terrestrial settlements (areas such as towns and cities) and oil rigging operations. Temporary sources of light include shipping and fisheries operations (Davies et al., 2014). These artificial light sources can increase the intensity of light in neighboring harbors, estuaries, and river systems. In freshwater ecosystems, artificial light enters waterways from human settlements, passing automobiles, and the recreational lighting of waterways. In marine ecosystems, artificial light may originate from shipping, oil rigging, and fisheries operations, as well as human settlements. An abundance of ALAN interferes with the natural (solar and lunar) lighting of these ecosystems and the processes their inhabitant's use.

Increasing the intensity of light in aquatic ecosystems can disrupt biological processes in aquatic organisms. Aquatic organisms are adapted to function in both daylight and lunar light. Many aquatic organisms rely on cues from natural lighting to initiate biological processes such as reproduction, migration, and dispersal (Schligler et al., 2021). Pelagic fish use natural light to determine depth, shrimp and krill use natural light for diel vertical migration-the process of migrating laterally within the water column, and Trinidadian guppies use natural light to determine when it is safe to emerge from cover (Kurvers et al., 2018; Davies et al., 2014). ALAN has the potential to disrupt key biological processes that may in turn be detrimental to the ecosystems in which these organisms reside. Important biological processes that may be disrupted due to ALAN include growth, reproductive success, and predation rates.

This paper will highlight the effects ALAN has on fish in different types of marine and freshwater ecosystems. The ecosystems that will be examined are temperate marine, tropical marine, freshwater temperate, and freshwater marine. The basis for analysis will focus on reproductive success, growth, and predation rates of fish species in these aquatic ecosystems. Fish are a foundation species in aquatic ecosystems, therefor if ALAN impacts the survival of fish, larger ecosystem impacts will follow.

ALAN with Reproductive Success

Reproductive success is a fundamental aspect of a healthy population in an ecosystem. Most adult teleost fish and larvae rely on lunar cues to begin the reproductive processes such as spawning and egg hatching (O'Connor et al., 2019). Many fish synchronize with lunar phases to release gametes, often to relieve pressure from predation and seasonal variance of the tides (Takemura et al., 2004). Since many fish have developed reliance on light cycles to determine reproductive times, ALAN has the potential to disrupt reproduction by disturbing the natural light cycle (Schligler et al., 2021). For example, Fobert et. al (2019) found that the eggs of the Ocellaris clownfish (*Amphipiron ocellaris)* do not hatch under the influence of ALAN. However, ALAN does not influence the frequency of spawning or fertilization in adult *A. ocellaris* (Fobert et al., 2019). Eggs from *A. ocellaris* are shown to have specific cues for hatching, which ALAN masks. *A. ocellaris* is not the only species of Indo-pacific reef fish that relies on lighting cues for reproductive behaviors (Fobert et al., 2019). Reef fishes in the families Pomacentridae, Amphiprionidae, Apogonidae, and Labridae also use lunar cues to initiate reproductive processes (Takemura et al., 2004). Because so many fish rely on lighting cues, the presence of ALAN may have severe effects on the population dynamics of coral reefs.

Like marine fishes, freshwater fishes also use natural light for reproductive cues. Most studies of the reproductive success of freshwater fish in European temperate habitats focus on hormones and gene expression to determine if ALAN has detrimental effects. When exposed to ALAN, Eurasian perch *(Perca fluviatilis)* and roach (*Rutilus rutilus)* show reduced sex steroids and gonadotropins, which are important indicators of the beginning of a spawning session (Brüning et al., 2018). With decreased expression of hormones, reproduction is suppressed, which could have a profound effect on freshwater fish populations. Artificial light also influences reproduction in tropical freshwater species. In a study exposing Zebrafish (*Danio rerio),* a popular laboratory fish originally found in Indian rivers, to constant ALAN, Khan et. al. (2018) found the development of ovarian tumors in response to light treatments. Tumors in the ovaries of fish can inhibit successful reproduction, driving reproduction rates down. Like the effects shown in marine fish, artificial light also has negative effects in the reproductive success of freshwater fishes.

ALAN with Growth and Development

Larval recruitment is a key factor in the growth process of marine reef fish. Larval recruitment characterizes the process in which pelagic larvae transition to reef-associated juveniles, also referred to as settlement and metamorphoses (Holzer et al., 2017). Settlement is a crucial factor in determining growth and population size of marine tropical reef species because it is the process in which larvae decide which area is the most beneficial to metamorphosize. The settlement process is based on natural lighting cues (Besson et al., 2017). There is a brief period between this process and metamorphosis, and, in tropical reef fishes, it is usually less than 24 hours (O'Connor et al., 2019). With the addition of artificial light at night, the settlement process of marine larvae is disrupted at a crucial point in their development and has the potential to alter growth and reef dynamics (O'Connor et al., 2019).

The presence of ALAN plays a role in habitat avoidance and growth in larval recruits of tropical marine reef fish in the Indo-Pacific. It has detrimental effects on species fitness and post settlement survival in larvae (O'Connor et al., 2019), which alters the growth of these fish and the population dynamics of reef ecosystems. In the larval recruitment of Convict tang larvae (*Acanthurus triostegus),* ALAN is thought to alter the preference of settlement sites, with larvae preferring to settle and metamorphosize in places without ALAN (O'Connor et al., 2019). Larvae that settled in an ALAN environment had lower levels of thyroid hormone, T3, suggesting that fish that settle with artificial light may have impaired development (O'Connor et al., 2019). A preference for darker habitats can also alter the distribution of reef fish across various reefs, as larvae typically settle away from the reef their parental units spawned, altering the processes certain fish contribute to reefs (O'Connor et al., 2019). Additionally, Orange Fin Anemone fish (*Amphiprion chrysopterus)* juveniles had high mortality when exposed to ALAN in the first month due to predation, and an overall decline in growth (Schligler et al., 2021).

Artificial light at night may have different effects on freshwater fish due to differences in the larval recruitment stage, with the survivorship of freshwater larvae being 44 times higher than that of marine larvae (Houde, 1994). A study in North American temperate habitats prolonged the photoperiod of brook trout (*Salvelinus fontinalis)* with artificial light and found that growth increased (Lundova et al., 2019). Exposure to a longer photoperiod delayed the gonadal development in these fishes, allowing for growth without the energy depleting effects of sexual maturation (Lundova et al., 2019). In another case, the physiology of the Western mosquitofish (*Gambusia affinis)* had no correlation with ALAN and growth rate. This may be due to the tolerant nature of the western mosquitofish (Miner et al., 2021). *Gambusia affinis* is typically found in urban settings, so it is possible this fish has evolved to be more tolerant of ALAN. The findings in these two studies suggest that freshwater fish are affected less by ALAN than marine fish.

ALAN with Predation

ALAN can alter food webs and predation rates in aquatic ecosystems. Many fish species, both freshwater and marine, as well as aquatic invertebrates are known to engage in phototactic behavior, meaning they are attracted to light (Barker & Cowan, 2018). Both adult fish and fish in the larval recruitment stage both exhibit this phenomenon (Mueller & Neuhauss, 2010), to which ALAN has the capacity to modify. Numerous species of fish are also visual predators. If fish are both attracted to light and visual predators, there should be an increase in predation in areas affected by ALAN. In near shore, temperate marine habitats, predation of various marine sessile invertebrates increased on nights when ALAN was implemented due to increased visibility in otherwise dark or naturally lit ecosystems (Bolton et al., 2017). ALAN also increases the presence of loose group dwelling fish, which are attracted to light sources by phototactic behavior (Bolton et al., 2017). This, in turn, increases the presence of larger predatory fish allowing predation at times when, in naturally lit areas, there is typically less activity (Becker et al., 2013). Increased predation also limits the availability of resources, further altering fish communities because fish will migrate to more plentiful habitats (Bolton et al., 2017). This can cause detrimental effects on the food web, potentially altering it to an unnatural top-down system (Becker et al., 2013). Predation rates under the influence of ALAN have the capacity to create a trophic cascade, altering the structure of marine communities and degrading the health of the ecosystem.

Predation in freshwater ecosystems exposed to ALAN also increases. In North American temperate habitats, the presence of ALAN caused an increase in the predation of *M. dolomieu* eggs due to a decline in the energy bank of the parent fish guarding the eggs (Foster et al., 2016). Since the parent fish spent more time guarding instead of resting at night, efficiency of nest guarding behavior was reduced, causing an increase in egg predation. An elevation in the predation of eggs has detrimental effects on the population of *M. dolomieu*. Trinidadian guppies (*Poecilia reticulata*) were also found to be influenced by the presence of ALAN. In a laboratory experiment, guppies who were subjected to ALAN emerged from refuges quicker and spent more time in open spaces (Kurvers et al., 2018). This altered behavior in the guppies has the potential to increase their predation since they are spending more time out of a refuge. Fish foraging with ALAN in European temperate freshwater ecosystems is also modified. ALAN enhanced the predation of Eurasian perch (*Perca fluviatilis*) on the invertebrates *Gammarus fossarum* in freshwater environments (Czarnecka et al., 2019). Eurasian perch are visual predators, and the increased light allowed for elevated foraging success. This can reduce the invertebrate population, decreasing the availability of resources and therefor reducing the population of the perch (Czarnecka et al., 2019). All these effects from artificial light at night can alter food webs in a detrimental manner.

Discussion

In both freshwater and marine environments, reproductive success of fishes is hindered by ALAN. In the studies conducted of reproductive success, all found at least some connections between ALAN and negative impacts on fish reproduction. Though, the disadvantages varied between ecosystem types, which may be explained by the focus of the studies that have been conducted thus far. These differences may be due to the difficulty of reef fish reproduction in captive settings. In marine fishes, more focus was on the circadian rhythms of reef fish and egg hatching in the Indo-Pacific (Fobert et al., 2019; Schligler et al., 2021), whereas in freshwater fishes, researchers looked more at the levels of hormone expression in the fishes in temperate and tropical settings (Brüning et al., 2018; Khan et al., 2018). While the conclusion that ALAN has negative effects on reproductive success in fishes is valid, it will be beneficial to research this effect in several types of ecosystems. The effects artificial light at night has on temperate marine and tropical freshwater ecosystems have comparatively few studies than those of Indo-Pacific reefs and European freshwater temperate ecosystems, highlighting the need for more research in these areas.

While there is evidence that ALAN has an injurious effect on the growth and larval recruitment of marine Indo-Pacific reef fish, the opposite was found for the growth of North American freshwater temperate fish. Freshwater temperate fish tended to either have increased growth or no effect due to the presence of artificial light at night (Lundova et al., 2019; Miner et al., 2021). This is the opposite of the marine reef fish and shows a clear difference in the growth processes between the two ecosystems. Because of the limited research on how ALAN affects the recruitment stage in freshwater fishes, further research is needed before definitive conclusions can be drawn.

The predation rates in both temperate marine and temperate freshwater ecosystems increased due to ALAN. This can be explained by the increase in visibility at night due to illumination from ALAN and fishes phototactic behavior (Czarnecka et al., 2019; Bolton et al., 2017). Since many fish are visual predators, increasing the visibility allows these organisms to hunt for longer periods of time where, in natural ecosystems, predation is typically decreased at night (Becker et al., 2013). It is also important to note that some species of fishes are nocturnal, so unnatural light at night may impact their feeding strategies. There were comparatively few studies on the effects of fish-on-fish predation, with most research conducted on fish-invertebrate interactions. Though both temperate marine and temperate freshwater ecosystems saw an increase in the predation of sessile invertebrate species, more research is necessary to fully understand how ALAN impacts this aspect of ecosystem dynamics.

Conclusion

With the present amount of research, ALAN seems to have the most negative effects on marine reef ecosystems in the Indo-Pacific and temperate freshwater ecosystems in North America and Europe. The Indo-Pacific is a sensitive area due to its popularity as a shipping hub, allowing for an increase in light disruption from shipping vessels, as well as countless implemented oil rigging and fishing operations (Bhaskar, 2021; Chevron Affairs, n.d.). Temperate freshwater ecosystems are especially affected due to proximity to human settlements and the connectivity humans have created inland, allowing for light disruption from automobiles and city lights. Mitigation for these areas should be implemented as marine protected areas and reserves with the absence of artificial light.

There are significant gaps in the available research for all ecosystem types. Within tropical marine ecosystems, there seems to only be studies in the Indo-Pacific region, leaving questions about the Caribbean and South African reef systems. There are sparse numbers of studies on tropical freshwater ecosystems, with the only information on the effects of ALAN on fish being in zebrafish (*Danio rerio*) and Trinidadian guppies (*Poecilia reticulata).* There are a small number of studies in temperate marine habitats, which mostly focus on Eurasian perch (*Perca fluviatilis*). Because of the low number of studies, and the bulk of the research skewed towards tropical marine ecosystems, more research is needed to fully understand the effects.

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CHAPTER 2: GRANT PROPOSAL

The Effects of Artificial Light at Night on Pumpkinseed Sunfish (*Lepomis gibbosus*) Growth.

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Section 1. Abstract

Abstract:

The effect of artificial light at night on our aquatic ecosystems is a growing concern within the scientific community. Understanding how artificial light affects the biological processes of fishes is of utmost importance as they play vital roles in aquatic ecosystem dynamics. Studies have shown that artificial light has negative implications for reproductive success and predation of fishes, though, there have been few studies on how it affects growth. Thus far, the studies that have focused on growth in fishes indicate that the effect may be species specific, with varying results between species. I plan to add to the existing literature on artificial light at night by exploring its effects on meso-predators, specifically Pumpkinseed sunfish (*Lepomis gibbosus*), a hardy species that provide many ecosystem services. By testing these fish under laboratory conditions, I aim to identify the response of fish growth to artificial light at night. Measurements will be used to determine the growth rate of light-affected pumpkinseeds. Hormone samples of the fish will allow the growth responses to be assessed. This research will contribute to the current body of knowledge of the effects of artificial light at night and allow future researchers to further expand the knowledge of its adverse effects on aquatic ecosystems.

Section 2. Anticipated Value, Literature Review, Objectives, Hypothesis

Anticipated Value:

Artificial light is increasing globally by 2.2% per year and is known to have impacts on the physiology of fishes. Previous studies have focused on how artificial light affects terrestrial ecosystems, but relatively little research has been conducted on how it affects aquatic ecosystems. Artificial light adversely affects fish reproduction and predation rates, however, few studies focus on the physiological growth of freshwater fishes in a standard genotype. Most studies conducted thus far on the growth of fish affected by artificial light focus only on large popular game species or smaller laboratory species. The findings in this study will provide a better interpretation of the overall effects artificial light has on ecosystems and will contribute to better preservation of the diversity of lakes and streams. Many people worldwide depend on fish as sustenance, so understanding the effects of artificial light on our aquatic ecosystems is crucial if we want to keep using this resource.

Literature Review:

Artificial light at night (ALAN) is a major source of pollution to our waterways. It is estimated that 70% of the human population lives within 5km of a water source (Kummu et al., 2011) and 80% of the population experiences artificial light at night. With the present amount of data, around 4.3 billion people worldwide rely on fish as their main source of protein (*FAO - News Article*, 2014). With our current reliance on fish, and a 2.2% increase of light entering our aquatic ecosystems per year, it is essential to determine the impacts artificial light has on the biological and ecosystem processes that occur in our waterways (O’Connor et al., 2019).

Artificial light can originate from both permanent and temporary sources in aquatic habitats. Permanent sources include areas such as towns and cities and temporary sources of light include shipping and fisheries operations (Davies et al., 2014). These artificial light sources can increase the intensity of light in neighboring lakes, reservoirs, and river systems, as well as the intensity of light in the atmosphere, which in turn increases the light entering aquatic ecosystems. An abundance of ALAN interferes with the natural (solar and lunar) lighting of affected ecosystems and the processes their inhabitant's use.

Increasing the intensity of light in aquatic ecosystems can disrupt biological processes in aquatic organisms. Fish are adapted to function in both daylight and lunar light and rely on cues from natural lighting to initiate biological processes such as reproduction, migration, and dispersal (Schligler et al., 2021). Artificial light also influences the growth rates of fishes, with many marine fish showing a decrease in size when exposed to large amounts of artificial light. However, this phenomenon is not thoroughly studied with freshwater fish. Most current studies on the effects ALAN have on growth focus only on popular game and laboratory fish and conclude that the impacts are species specific and have varying results (Lundova et al., 2019; Miner et al., 2021). Studying fish species other than popular game fish will provide researchers with a better understanding of the influence artificial light at night has on lesser-known fish species that provide important ecological services.

ALAN has shown neutral effects on the growth rate of some freshwater fishes. A study prolonging the photoperiod of brook trout (*Salvelinus fontinalis*) with artificial light found that growth increased (Lundova et al., 2019). Sexual maturation in *S. fontinalis* is taxing towards the energy bank of the individual, which reduces somatic growth. Exposure to a longer photoperiod delayed the gonadal development in these fishes, allowing for growth without the energy depleting effects of sexual maturation (Lundova et al., 2019). *Salvelinus fontinalis* benefited from increased growth due to ALAN though it prolonged the time to maturation, which delays reproduction. While increased growth can be positive, these effects negatively impact this species' population dynamics, and the ecosystem services they provide. In a small non-game species, the physiology of the Western mosquitofish (*Gambusia affinis)* was found to have no correlation with ALAN and growth rate. This may be due to the highly tolerant nature of the western mosquitofish (Miner et al., 2021). Western mosquitofish are often found in urban settings and give live birth rather than laying eggs, so it is possible this fish has evolved to be more tolerant of environmental pollutants including ALAN. The findings in these two studies suggest that the growth of freshwater fish in ALAN conditions varies and the effects may be species specific.

Pumpkinseed sunfish (*Lepomis gibbosus*) are popular fish species that typically subsist in the littoral zone of lakes and reservoirs, often where artificial light has a profound presence (Fuller, 2004). Pumpkinseeds play vital roles in their ecosystems as meso-predators, as they maintain aquatic macroinvertebrate populations and are prey for bird species and large predatory fish (Downs, et al., 2002; Paulson and Hatch, 2002). By studying this meso-predator under the influence of ALAN, researchers can better understand the role this phenomenon plays on the food chain.

Studies on the growth of freshwater fishes in the presence of artificial light at night are lacking. There is not enough literature on growth in freshwater fish exposed to ALAN to determine if artificial light causes ecological effects such as trophic cascade and altered life dynamics. Understanding how artificial light affects the growth of fish is important for mitigation and creating healthy ecosystem dynamics so that humans can still use these important natural resources.

Objectives:

This study will investigate whether increased artificial light at night alters growth in pumpkinseed sunfish. Light intensity will be quantified at the Chicago-Lake Michigan interface (CLM) due to its high intensity lighting in proximity to an aquatic ecosystem. CLM light intensity will determine the upper range of light pollution entering ecosystems and allows evaluation of the growth response of pumpkinseeds. Analyzing these effects provides future researchers with information regarding potential alterations to the life dynamics of pumpkinseed fish.

Hypotheses:

The proposed project will test the hypothesis that urban light pollution negatively affects fish growth. Through this hypothesis, I predict that juvenile pumpkinseed sunfish exposed to Chicago light regimes will have slower growth and increased time to maturity than in other light regimes.

Section 3. Methods

Methods:

Study Species:

Pumpkinseed Sunfish, an abundant freshwater fish found in many freshwater ecosystems across North America, will be the focal species in my study. They are deep-bodied and laterally compressed fish, with body coloration of orange, green, yellow, or blue, with dense spots of reddish copper or gold and a crimson spot on the opercular flap. Pumpkinseeds typically range from 152-203 mm in length and 171-286 g in mass (Smith, 1979). They are primarily found in the littoral zone of waterbodies with dense vegetation, rock structures and submerged logs, and inhabit territories of 0.23 to 1.12 hectares, (Downs, et al., 2002; Fish and Savitz, 1983). Pumpkinseeds are diurnal and exhibit most behaviors during the daytime, while resting at night (Downs, et al., 2002).

Study Site:

This study will be conducted in the aquatic lab at Regis University, Denver, CO.

Quantifying Light Intensity:

Following Jechow & Hölker (2019), light intensity entering the ecosystem will be measured using a luxmeter at midnight on the surface water at three points in the CLM. One at Oak Street Beach, closest to the city, one northeast of New Buffalo, farthest from the Chicago city limits, and one at the Indiana Dunes National Park, which is between the two (Appendix 1). Sampling points will be chosen randomly within each site and averaged to determine the mean light intensity in each site. These measurements will provide a basis for the upper, mid, and lower ranges of artificial light entering ecosystems.

Housing:

We will obtain 24 captive bred juvenile pumpkinseeds from Zimmerman's Fish supply. Individuals will be grouped in twos, one male and one female, and randomly placed into 12, 55-gallon, aerated treatment tanks with waterflow from natural water quality parameters. Water temperature will be maintained at a range of 21-24° C and oxygen levels will be maintained above 95% saturation for the duration of the study. Each tank will have a natural substrate and fish will be fed standard fish pellets.

Treatment:

I will use four different light treatments on separate tanks housing two individuals each. Treatment 1 will be the control tanks, with LED lights matching the natural day and night light intensities. Treatment 2 will be the low light intensity tanks, with LED lights matching the natural light during the day and the light intensity measured from St. Joseph Beach at night. Treatment 3 will be the intermediate light intensity tanks, with LED lights matching natural day light, and the light intensity measured from the Indiana Dunes at night. Treatment 4 will be the high light intensity tanks, with LED lights matching natural day light, and the light intensity measured from Oak Street Beach at night. Blackout separators will be employed between each treatment tank to minimize contamination from varying treatments. Each light treatment will be replicated 3 times.

Growth Measurements:

Individual fish will be taken weekly from treatment tanks and placed in holding tanks to obtain standard length, girth, and weight. In addition, following Kidd et al. (2010) and Tacon et al. (2000), blood samples will be taken from the tail veins of each fish and plasma separated using standard centrifuge techniques. Plasma samples are diluted 1:30 by respective assay buffers and analyzed at 405 nm using a Beckman Coulter DTX 880 Multimode Detector, to determine levels of growth hormone (GH), prolactin (PRL), and somatolactin (SL).

Data Analysis:

One-way ANOVAs will be conducted to determine the variations between light treatments and the measured growth and hormone levels between each sex of juvenile pumpkinseeds. If assumptions are not met for the ANOVA, data will be logarithmic transformed. If variations are present, post-hoc Tukey HSD test will be employed to further analyze the differences. Multiple linear regressions will also be conducted to determine relationships between light intensities and growth, light intensities and hormone expression, and hormone expression and growth between treatment groups.

Project Requirements:

To conduct this study, I will need laboratory space at Regis University and necessary equipment to run experiments. I will also need approval from IACUC.

Potential Negative Impacts:

Negative impacts will be minimal. There is potential for fish mortality during this study. At the conclusion of this study, remaining individuals will be euthanized by standard MS-222 techniques.

Project Schedule:

Date:	**May 2022**	**June 2022 to Feb 2023**	**March 2023**	**April 2023**
Activity:	Lab set up, light quantification, and 2-week acclimation period	Data collection	Data analysis	Draft/edit/ complete report
Product:	Light intensity measurements and functioning lab.	Growth measurements and analysis of hormone levels.	Have information for the results section.	Final Report.

Section 4. Budget

Budget:

Item	**Justification**	**Cost per Unit**	**Quantity**	**Total**
Aqueon Standard 55g Tank	Housing for study subjects	$150	12	1,800
Juvenile fish via Zimmerman's fish	Study subjects	$6	24	$144
AS803 Lux Light Meter	To obtain light intensity measurements	$21.95	1	$22
Roundtrip flight to Chicago	To obtain light intensity measurements	$150	1	$150
Beckman Coulter DTX 880 Multimode Detector	To obtain measures of growth hormones	$2,200	1	$2,200
22ga hypodermic needles 100ct	To obtain blood samples	$11	2	$22
Purina Aquamax Pondfish 4000 Pond Fish Feed	To feed study subjects	$40	1	$40
CaribSea Eco Complete Black Planted Aquarium Substrate	For substrate in tanks	$20	12	$240
50-Watt Equivalent MR16 Dimmable LED Light Bulb Glass	For light treatments	$8	12	$96
Simple Deluxe clamp light with aluminum reflector 12 pk	For light treatments	$85	1	$85
Eclipse blackout curtain	To reduce contamination from other treatments	$7	12	$84
SuperDeal Electric laboratory centrifuge	For separating plasma in blood samples	$86	1	$86
Work Compensation	For researchers	$3,000	1	$3,000
Total Resource Expenditures				$7,969

Common aquarium equipment such as heaters, air pumps and stones, airline tubing, and light timers and dimmers are available in the Regis University aquatic lab.

Section 5. Qualifications of Researchers

CATHERINE M. SHAPIRO
Denver, CO, 80211 · (708)522-3493 · kshap49@gmail.com

Education

Regis University, Denver, CO

M.S. Environmental Biology. Expected graduation in early May 2022.

College of Charleston, Charleston, SC

Graduated in 2020 with B.S Marine Biology and a minor in Environmental and Sustainable Studies.

Work and Internship Experience:

Data Analysis Intern
Bureau of Land Management · January 2022-April 2022

Providing statistical analysis using the frequentist method to determine the probability of occupancy and the detection probability of Pinyon Jays at the Royal Gorge Field Office, CO. Creating models in ArcGIS that describe the analysis across the study area.

Aquarist Intern
South Carolina Aquarium, Charleston, SC· November 2020 – May 2021

Trained and prepared cownose stingrays for medical examinations. Provided husbandry to elasmobranchs in the Great Ocean tank and Shallows exhibits. Assisted in medical procedures such as stingray hysterectomies and teleost necropsies. Prepared food for exhibits, maintenance of exhibits, and care of exhibit inhabitants.

Naturalist Guide
Coastal Expeditions, Charleston, SC · April 2018 – November 2020

Conducted kayak tours in local marine estuaries in Charleston, SC. Interpreted biological processes and wildlife for clients. Utilized in depth knowledge of marine ecosystem dynamics. Ensured client safety on the water. Provided maintenance to marine watercraft.

Research Experience and Presentations:

Grassland Ecology Research Associate
Denver Mountain Parks and Highlands Ranch, Denver, CO · August 2021-May 2022

Assisted biologists in creating baseline data on plant communities within the parks. Included the use of quadrat sampling, GPS, and plant identification. Analyzed how grazing intensity affects plant communities and soil composition using R. Prepared a management plan for both Denver Mountain Parks and Highlands Ranch. Conducted through Regis University.

Elephant Behavior Research
Denver Zoo, Denver, CO · August 2021-December 2021

Studied bull elephants in Denver Zoo to determine if stereotypic behaviors increased and if resting decreased when the elephants were in a hormonal period. Included coding behaviors on ZooMonitor and analyzing the data with R. Results were presented to professors, colleagues, and students at Regis University.

Stream Assessment
City of Lewisville, CO · August 2021-May 2021

Collected data on fish and macroinvertebrate populations in Coal Creek through kick nets and seines. Analyzed data with R to determine how aquatic communities responded to wildfire. Prepared and presented results to the City of Lewisville.

Hollings Marine Laboratory
Research Assistant, Charleston, SC · January 2020 – March 2020 (interrupted - COVID19)

Assisted in laboratory research regarding symbiosis of microbial communities on marine sponges. Performed laboratory techniques such as pipetting and centrifuging. Prepared and analyzed samples and pertinent article information.

Volunteer:

South Carolina Aquarium
Dive Volunteer · August 2016 – November 2020

Scuba diving and food preparation for the Great Ocean Tank exhibit. Upheld all dive safety measures, cleaned rockwork, vacuumed tank floor, participated in underwater educational shows, fed tank inhabitants (Teleosts, sharks, loggerhead turtle), and provided animal observations to the husbandry team.

Certificates

PADI Advanced Open Water scuba · American Canoe Association Level 2 Kayak Instructor · CITI Program: Investigators, Staff, and Students; Wildlife Research.

Skills

R/RStudio· GIS · NEPA · Colorado plant ID · Wetland Delineations · Grant Writing · Report Writing · Ecological Modeling · Technical/Scientific Writing · Stakeholder Analyses · Data Visualization · Field Research · Laboratory Techniques · ZooMonitor · Communication Skills · Public Speaking · Marine Organism Identification · Customer Service · Utilizing Dichotomous Keys · Literature Review · Wildlife Education · Microsoft Office · G-Suite · Square · Spanish

Relevant Course Work

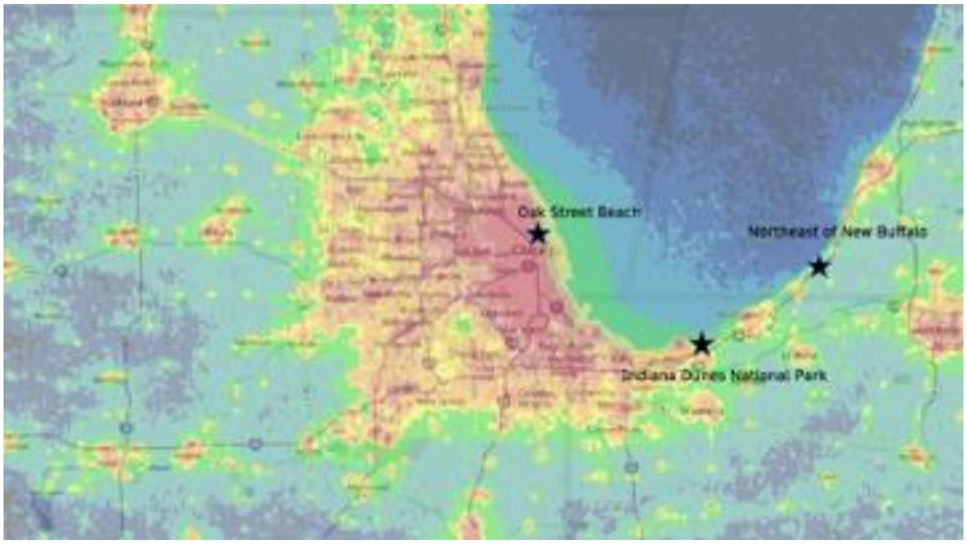
Regis University, Denver, CO:

· Biostatistics and Research Design · Advanced Ecological Modelling · Wetlands Delineation · Ecological Applications to GIS · Advanced Ecology · Advanced Field Ecology· Aquatic Ecology and Bioassessment · Advanced Behavioral Ecology · NEPA

College of Charleston, Charleston, SC:

· Biology of Fishes · Invertebrate Zoology · General Ecology · Oceanography · Analytical Chemistry · Physics 1&2

Appendix:



Appendix 1: Light Intensity Map: Chicago-Lake Michigan Interface light intensity sampling locations.

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CHAPTER 3: JOURNAL MANUSCRIPT

Impacts of Pinyon-Juniper Woodland Management on Pinyon Jay Occupancy.

Abstract

Pinyon-juniper forests are a wide-spread habitat type throughout the western United States. Despite the extensiveness and wide scale expansion of this habitat type throughout the 20th century, there is a long-term decline in the Pinyon Jay, a mutualistic species of pinyon pines. Current research suggests that changes to pinyon-juniper woodlands through mechanical thinning practices are a factor in this decline. This study aims to uncover the relationship of Pinyon Jay occupancy to mechanical thinning treatments and landscape features in Colorado. Fifty-two sites were sampled in the land managed by the Royal Gorge Field Office of the Bureau of Land Management, and these sites varied whether they had or had not experienced mechanical thinning treatments. Pinyon Jays were surveyed using the point-count method, and landscape data was extracted remotely through ArcGIS. I modeled the probability of occupancy of Pinyon Jays and flock sizes with varying landscape features using logistic and Poisson regressions. My results suggest that there is a higher probability of Pinyon Jays occupying sites without thinning treatment that are lower in elevation with less terrain ruggedness. Additionally, flock size was a more responsive variable when compared to occupancy, with larger flock sizes occurring in sites without thinning treatment at lower elevations and terrain ruggedness. There is not a clear answer for recovering Pinyon Jay populations but understanding their relationship with their natural environment with human influences can help managers make well-informed decisions about land management.

Introduction

Pinyon-Juniper woodland ecosystems are one of the most widespread habitat types in the western United States, encompassing nearly 40 million hectares (Magee et al., 2019). These woodlands typically are found in a range of arid climates and transition into savannas, grasslands, or brush-dominated vegetation zones (Finch & Tainter, 1995). Pinyon-juniper woodlands consist of a variety of pinyon pine (*Pinus* spp.) and juniper (*Juniperus* spp.) tree species, and the composition of species depends on the woodland's location within the western United States (Finch & Tainter, 1995). These woodland ecosystems also provide critical habitat to at least 70 species of birds and 48 species of mammals, some of which are obligate to this specific habitat type (Finch & Tainter, 1995). With the prevalence of these key habitats across the western United States, management is necessary to ensure species longevity as well as preserve the ecosystem services they provide to humans.

The widespread nature of pinyon-juniper woodland ecosystems has led to intensive management (Magee et al., 2019). Historically, pinyon-juniper woodlands were managed to increase forage for livestock using methods such as chaining or bulldozing to remove stands of trees; however, this is no longer a primary motivation for land managers (Magee et al., 2019). Presently, management practices are mostly employed to mitigate fire risk, or to benefit ecological conditions for species of concern, such as the Greater Sage-Grouse (Boone et al., 2018; Johnson et al., 2018). These management measures include removing dense stands of pinyon-juniper through burning, chemical treatments, or mastication (Ernst-Brock et al., 2019; Magee et al., 2019). Thinning treatments can also increase the amount of available forage, which may benefit species such as Wild Turkey (*Meleagris gallopavo*) and mule deer (*Odocoileus hemionus*) (Bombaci & Pejchar, 2016; Bergman et al., 2014). While mechanical thinning is used to benefit several woodland species, it may have negative effects on pinyon-obligate species (Johnson et al., 2018); Magee et al., 2019). Currently, there is limited research on how thinning treatments impact woodland and shrubland species over the long term.

Thinning treatments in pinyon-juniper woodland can have unintended consequences, especially on pinyon-obligate avian occupancy. Pinyon-juniper woodlands provide critical nesting habitat for more breeding bird species than any other type of terrestrial habitat in the western US (Magee et al., 2019). Thinning treatments reduce the viable habitat for bird species that utilize closed canopy systems such as the Black-throated Gray Warbler, Plumbeous Vireo, and Hermit Thrush, which rely on high and dense canopy cover (Magee et al., 2019). However, species that are not associated with canopy systems either experience no significant effect or benefit from thinning treatments (Bombaci & Pejchar, 2016). Avian species that utilize closed canopy systems in pinyon-juniper woodlands often rely on dense canopies for nesting, as well as the bark and foliage for foraging (O'Meara et al., 1981). Thinning treatments reduce canopy density and alter the surrounding plant communities leading to a reduction in avian occupancy (O'Meara et al., 1981). Regardless of treatment method, bird species richness and diversity are lower in treated areas than in non-treated areas (Crow & van Riper III, 2011; O'Meara et al., 1981). Existing thinning protocols especially fail to account for impacts on pinyon-specialist wildlife, such as the Pinyon Jay (*Gymnorhinus cyanocephalus*) (Boone et al., 2018; Johnson et al., 2018).

Pinyon Jays are a highly social corvid, widely known for their mutualistic relationship with pinyon pines (Boone et al., 2018; Boone et al., 2021). The bulk of the Pinyon Jay's distribution lies south of the intermountain region and extends into the southwest, with the highest abundance in eastern Nevada and central New Mexico (Boone et al., 2018). The jays forage for and cache the seeds of the single-leaf piñon (*Pinus monophylla*) and two-needle piñon (*P. edulis*) for sustenance in winter months, relying on them as their primary food source (Boone et al., 2018). A flock of Pinyon Jays can harvest and cache millions of seeds, making them the pinyon pine's primary long-distance disperser within the birds' range (Johnson et al., 2018). Pinyon Jays also rely on pinyon-juniper woodland for nesting sites, nesting colonially and cooperatively in traditional nesting grounds (Johnson et al., 2018). Yet, the Pinyon Jay is one of the most rapidly declining bird species in the western United States. This species is declining at a rate of ~3.6% per year since 1966, despite a large-scale regional expansion of pinyon-juniper woodlands throughout the 20th century (Johnson et al., 2018; Magee et al., 2019).

Despite the rapid decline of Pinyon Jays, there is still minimal knowledge of the cause of these population reductions, though changes to pinyon-juniper woodlands are a leading hypothesis among researchers (Boone et al., 2021). There is especially little region-specific information on jay populations and the effects of woodland management practices within Colorado, which contain a large amount of pinyon-juniper woodland dominated by *Juniperus scopulorum, J. monosperma,* and *Pinus edulis* (M. Rustand, personal communication; Miller & Tausch, 2001). The Bureau of Land Management (BLM) lands in Colorado are subject to intensive mechanical management due to increased fire mitigation efforts, and much of the land has not been surveyed for Pinyon Jay occupancy (M. Rustand, personal communication). It is important to determine how the management of woodlands influences the Pinyon Jay as there are minimal conservation efforts currently in place and, without the jays, the future of pinyon-juniper woodlands is unclear.

The goal of this study is to analyze the results from the first year of long-term Pinyon Jay occupancy surveys conducted by the Royal Gorge Field Office (RGFO) on Colorado BLM land.

From these surveys, I will determine the probability of occupancy of Pinyon Jays, and how the probability is affected by thinning treatments within the BLM land. I will also investigate landscape features that may influence the probability of occupancy and how flock sizes vary across study sites in response to landscape features. Based on the findings in previous research, I hypothesize that thinning treatments will have a negative effect on the occupancy and flock sizes of Pinyon Jays in pinyon-juniper woodlands. The information from these analyses will be of the utmost importance in determining future conservation efforts for the declining Pinyon Jay and will lay groundwork for the future analyses of this on-going project.

Methods

Site Selection and Study Area:

Employees of the Bureau of Land Management collected field data in pinyon-juniper Woodlands managed by the Royal Gorge Field Office in 2021. They selected sampling sites using the LANDFIRE (LANDFIRE, 2008) existing vegetation type data through ArcGIS to remotely delineate pinyon-juniper woodlands within the RGFO. BLM employees applied a 2x2 km cell grid on the RGFO area and identified cells that contained at least 1 km^2 of pinyon-juniper woodlands and 1 km^2 of BLM land as potential sampling sites. Following the identification of the potential grid cells, an equal-probability stratified spatially balanced (generalized random-tessellation stratified or GRTS) sample was drawn from the grid. There were two strata for sampling: cells that had some level of mechanical thinning treatments and cells that did not. A total of 50 sites were extracted for sampling, with 25 cells from each stratum and 5 extra cells if other sites were inaccessible. BLM employees determined the number of sampling sites through the following formula from Mackenzie et al. (2017):

$$Var\left(\hat{\psi}\right)=\frac{\psi}{s}\left[\left(1-\psi\right)+\frac{\left(1-p^{*}\right)}{p^{*}-Kp\left(1-p\right)^{K-1}}\right]$$

where $p^* = 1 - 1 (1 – p)^K$, or the probability of detecting pinyon jays at least once during K surveys of an occupied site. K represents the number of planned surveys, p is the detection probability, and Ψ is the probability of occupancy. The product of this analysis yielded that a sample size of 50 with 2 repeated visits each is adequate for addressing the studies objectives.

Habitat Sampling:

Sampling for habitat characteristics of sites occurred remotely through ArcGIS software. BLM employees extracted elevation and mean terrain ruggedness index values for each site from digital elevation models and LANDFIRE raster data using the extract function from the statistical R package *raster* (R Core Team, 2021; Hijmans, 2022).

Pinyon Jay Surveying:

Employees at the BLM collected data for Pinyon Jay occupancy. Pinyon Jay occupancy surveys began at sunrise and ended at 10:30 am when Pinyon Jay activity is highest. BLM employees visited each site twice between March 1st 2021, and May 14th 2021, each with a different observer than the previous survey. Observers subjectively established observation points prior to sampling with 4 observation points per site, each at a minimum of 500 meters apart to ensure adequate detectability and safe access on variable topography. Once at the observation point, observers sampled visually and auditorily for 3 minutes, and then played an audio recording of Pinyon Jay calls for another 3 minutes, for a total of 6 minutes per sample point. Observers recorded the presence or absence of Pinyon Jays and flock size was estimated visually for each site surveyed. Observers also recorded wind speed on the Beaufort scale, temperature, and weather at each site at the completion of the sampling period.

Statistical Analysis:

I conducted data analysis using logistic and Poisson regressions through the R statistical program (R Core Team, 2021). To answer the question of how Pinyon Jay occupancy is influenced by thinning treatments, I fit two generalized linear models. Occupancy is the general presence and activity of an organism in some habitat, which can be broken down into Pinyon Jay presence and flock size. I first fit a logistic regression with Pinyon Jay presence as a binomial response and treatment status as the predictor to distinguish sites that had some level of mechanical thinning treatment and sites that did not. Then, I fit a Poisson regression with flock size as the response and treatment as the predictor. Because the data was over dispersed, I fit this model using quasi-likelihood.

To answer the question of how the probability of occupancy varies in Pinyon Jays within the BLM RGFO, I fit a logistic regression with Pinyon Jay presence as a binomial response variable, and through model selection from the MuMIn package in R (Bartoń, 2020), the predictor variables resulted in a combination of 3 landscape variables: elevation, terrain ruggedness and treatment status. Predictor variables were chosen based on the lowest AIC values for the model overall. I then used the best fitting model and applied it to the predict () function in R to yield the odds of finding a Pinyon Jay within each site and converted them to probabilities.

To determine the detection probability of Pinyon Jays within the BLM RGFO, I fit a single season occupancy model following Mackenzie et al. 2002 using the R package unmarked. Within the double right hand side formula in this model, I held detection constant and used the variables that had the most influence over occupancy as determined by model selection from the binomial (presence/absence) probability of occupancy model. The output from the detection probability model was then converted to probability.

Additionally, I fit Poisson regressions to similarly model flock size as a function of landscape variables. Pinyon Jay flock size was the response variable, and through model selection from the MuMIn package in R (Bartoń, 2020), the predictor variables resulted in a combination of the landscape variables elevation, terrain roughness and treatment status. Predictor variables were chosen based on the lowest AIC values for the model overall. Due to data overdispersion, I fit this model using quasi-likelihood.

Results

From March 1st to May 15th, 2021, a total of 800 Pinyon Jays were detected throughout the Colorado BLM lands administered by the RGFO. Pinyon Jays occupied a total of 17 out of 52 sample sites, with 9 sites having some degree of mechanical thinning treatment and 8 sites without mechanical thinning treatments.

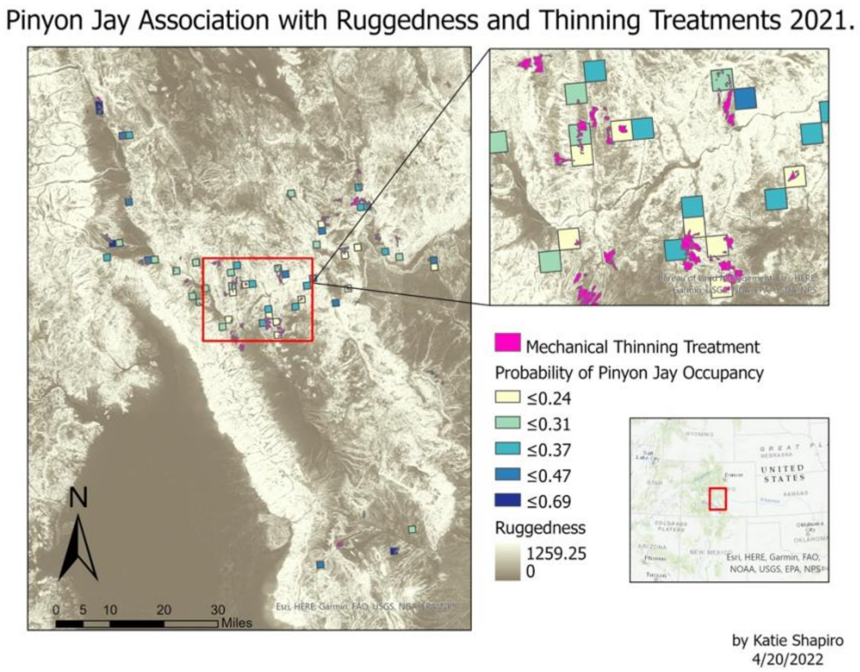
Occupancy Modelling

The probability of Pinyon Jay presence in treated sites was 0.33 (95% CI: 0.18-0.53) and 0.32 in untreated sites (95% CI: 0.20-0.46). The odds of Pinyon Jay occupancy were 6.25% greater in sites with treatment than in untreated sites, although, this difference was not significant (p-value: 0.884, 95% CI: 22% decrease-114% increase).

Model selection for the occupancy of Pinyon Jays determined two comparably good logistic models with a delta AIC value of 0.12 between them. The two best models were averaged and included elevation, treatment status, mean terrain ruggedness, and an interaction of mean ruggedness and treatment status (Table 1). After accounting for an interaction between mean ruggedness and treatment type, for every 1 unit increase in mean ruggedness the probability of Pinyon Jay occupancy decreased insignificantly by 0.38 in treated sites (p-value: 0.069, 95% CI: 0.46 decrease- 0.56 increase) and increased by 0.56 in untreated sites (p-value: 0.512, 95% CI: 0.28 decrease-0.63 increase) (Figure 1). The interactive effect of mean ruggedness and treatment on Pinyon Jay occupancy was 70% weaker in treated sites vs. untreated sites (p-value: 0.048, 95% CI: 0.9%-90.9%). After accounting for ruggedness, as elevation increased by 100 m in both treated and untreated sites, there was an insignificant 0.52 increase in the probability of Pinyon Jay occupancy (p-value: 0.459, 95% CI: 0.9% decrease-91% increase) (Figure 2). Overall, there was no significant effect of Pinyon Jay occupancy in untreated areas at greater terrain ruggedness than in treated areas.

Table 1: Summary of binomial regression with landscape and treatment as predictors of occupancy. Bold p-values indicate significance. All values are shown on the untransformed natural log scale.

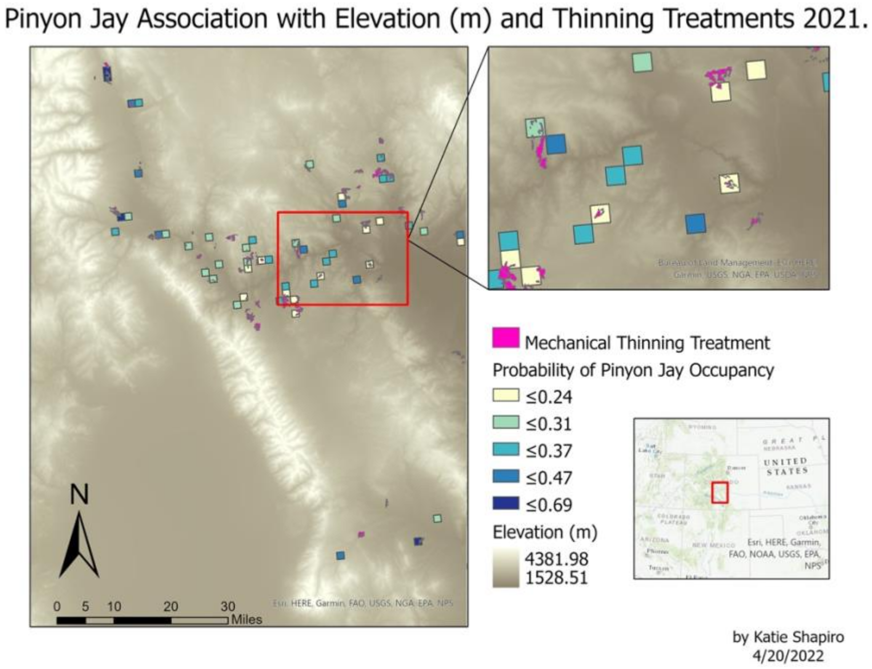
	Estimate	Adjusted S.E	Z-value	P
Untreated (intercept)	-3.116	2.659	1.172	0.241
Treated (relative to untreated)	2.270	1.249	1.818	0.069
Mean Ruggedness	0.251	0.382	0.656	0.512
Elevation (m)	0.0008	0.001	0.740	0.459
Mean Ruggedness * Treated	-1.203	0.609	1.975	**0.048***



Pinyon Jay Association with Ruggedness and Thinning Treatments 2021.

by Katie Shapiro
4/20/2022

Figure 1: Probability of occupancy with terrain ruggedness index as a factor for each sampling site, determined by the averaged logistic model. Darker colored boxes represent higher probability of Pinyon Jay occupancy, and darker colored ruggedness values represent lower ruggedness. Pink polygons represent areas which have had mechanical thinning treatments.



Pinyon Jay Association with Elevation (m) and Thinning Treatments 2021.

by Katie Shapiro
4/20/2022

Figure 2: Probability of occupancy with elevation (m) as a factor for each sampling site, determined by the averaged logistic model. Darker colored boxes represent higher probability of Pinyon Jay occupancy, and darker colored elevation values represent lower elevation. Pink polygons represent areas which have had mechanical thinning treatments.

Detection Probability

After holding detection constant and using elevation and an interaction between ruggedness and treatment status, the detection probability for Pinyon Jays within the sampled BLM land was 0.81 (p-value: 0.02, 95% CI: 0.57-0.94).

Flock Size Modelling

Untreated sites had a median flock size of 27.35 Pinyon Jays (95% CI: 15.94-43.11), and treated sites had a median flock size of 21 Pinyon Jays (95% CI: 10.08- 44.45). Although flock sizes in treated sites were 22% smaller than flock sizes in untreated sites, this effect was not significant (p-value: 0.515, 95% CI: 37% decrease-63% increase) (Figure 3).

Model selection for flock size determined a Poisson regression model that included mean ruggedness, elevation, and an interaction between elevation and treatment status as predictors (Table 2). After accounting for an interaction between elevation and treatment type, for every 100m increase in elevation in treated sites, median flock size significantly decreased by 44.1% (p-value:0.018, 95% CI: 23.3%-60%) (Figure 4). For every 100m increase in elevation in untreated sites, median flock size significantly decreased by 15.3% (p-value:0.031, 95% CI: 2.8%-27%) (Figure 4). When holding elevation constant, Pinyon Jay flock size differs significantly by 34% between treated and untreated sites (p-value: 0.018, 95% CI: 9.5%-53%). As mean ruggedness increased in both treated and untreated sites, there was a 43.1% decrease in median flock size (p-value:0.003, 95% CI: 39%- 78%) (Figure 5). Overall, there tends to be significantly larger flocks in untreated sites at lower elevations with less mean terrain ruggedness.

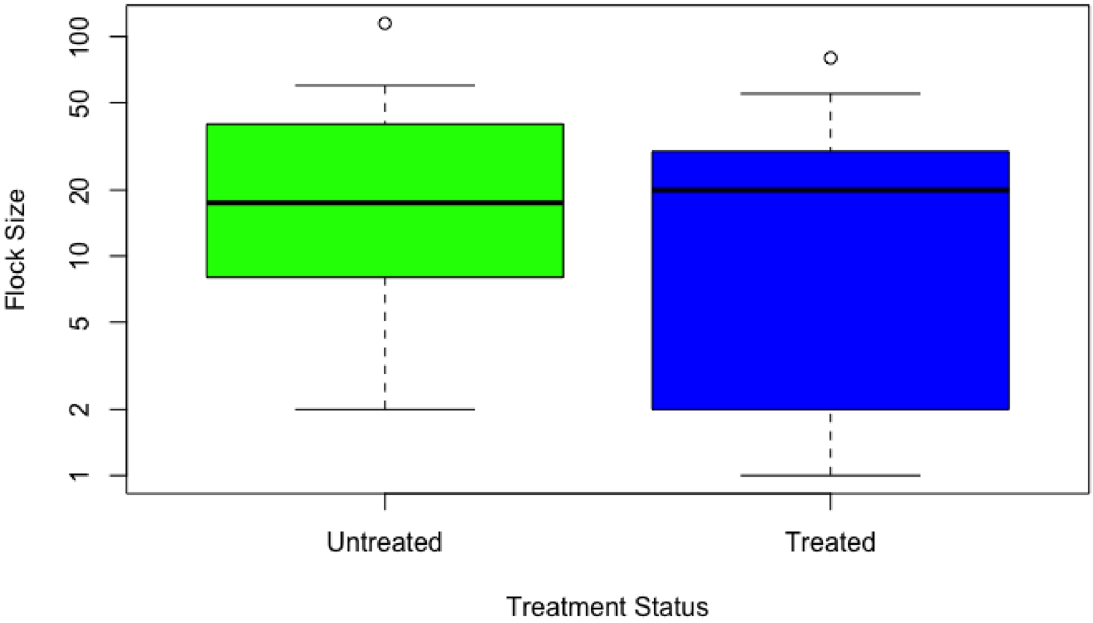


Figure 3: Comparison of Pinyon Jay flock size and pinyon-juniper woodland treatment status. Blue represents treated sites, and green represents untreated sites. There were no significant relationships between Pinyon Jay flock sizes and pinyon-juniper woodland treatments. Error bars represent the 25th and 75th percentiles, and points represent flocks outside of those percentiles.

Table 2: Summary of Poisson regression with landscape and treatment as predictors of flock size. Bold p-values indicate significance. All values are shown on the untransformed natural log scale.

	Estimate	S.E.	T-value	P
Untreated (intercept)	8.021	1.602	5.007	**2.72e-05***
Treated (relative to untreated)	9.331	3.705	2.518	**0.018***
Elevation (m)	-0.002	0.001	-2.267	**0.031***
Mean Ruggedness	-0.565	0.175	-3.227	**0.003***
Elevation (m)*Treated	-0.004	0.002	-2.517	**0.018***

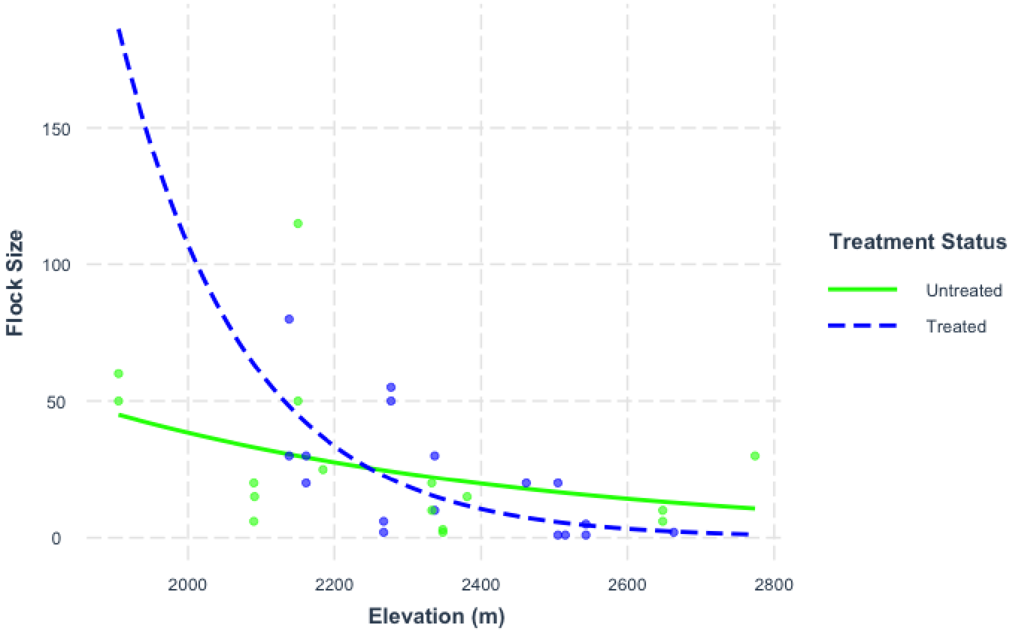


Figure 4: Poisson regression of the relationship between flock size, and the interaction of elevation and treatment status. Blue points represent flock sizes along the elevation gradient in treated sites and green points represent flock sizes along the elevation gradient in untreated sites. The blue line signifies the relationship between flock size and elevation in treated sites and the green line signifies the relationship between flock size and elevation in untreated sites.

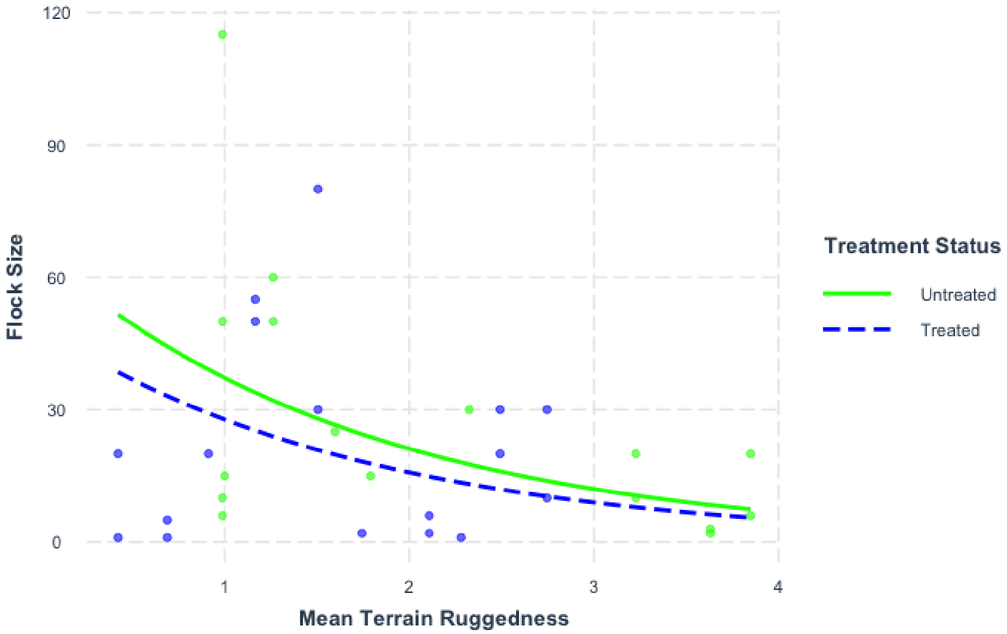


Figure 5: Poisson regression of the relationship between flock size, mean terrain ruggedness, and treatment status. Blue points represent flock sizes along the mean ruggedness index in treated sites and green points represent flock sizes along the mean ruggedness index in untreated sites. The blue line signifies the relationship between flock size and mean ruggedness in treated sites and the green line signifies the relationship between flock size and mean ruggedness in untreated sites.

Discussion:

The goal of this study was to determine the probability of occupancy of Pinyon Jays in the sampled BLM areas and how differing landscape features and management history influence this probability. BLM employees collected survey data from a variety of sites within the BLM lands administered by RFGO. I analyzed the survey data using GLMs to model effects of mechanical thinning treatments and landscape features on Pinyon Jay presence and flock size. My results indicate that mechanical thinning treatment by itself is not a strong predictor, but treatment status accompanied by elevation and terrain ruggedness play a significant role in influencing Pinyon Jay occupancy as demonstrated by the high detection probability. Additionally, I found that Pinyon Jays are more likely to occupy untreated sites at lower elevation with less terrain ruggedness. Further, flock size responded more strongly to these variables than the occupancy of Pinyon Jays at any particular site.

Comparing Pinyon Jay presence data and flock size data to mechanical treatment status, respectively, suggested that treatment status alone was not as strong of a predictor for Pinyon Jay occupancy than a combination of landscape features. When using presence data, there was higher occupancy in treated sites, which did not support my hypothesis. However, this can be attributed to the location of the mechanically treated sites. The mechanically treated sites were mostly located in lower elevations with less terrain ruggedness, as it is easier for mechanical thinning crews to access those areas over higher-elevation, rougher areas (M. Rustand, personal communication). Previous studies have found that Pinyon Jays typically reside in lower elevation areas where there is less terrain ruggedness, which the findings of this study support in the absence of treatment status (Boone et al., 2021). Alternatively, the results from the flock size analysis indicated that Pinyon Jays formed larger flocks within untreated sites, where mechanical thinning had not taken place. This suggests that mechanical thinning influenced the size of the flocks that were occupying the sites. However, these results for both the presence and flock size analyses were insignificant, indicating that while there was an effect from thinning treatments, they were not sufficient on their own to accurately define the probability of occupancy.

Introducing landscape features into the models in addition to treatment status yielded more meaningful results than using models based on just the treatment status, allowing identification of significant differences that otherwise would have been obscured in the simpler model. In the presence model, there was a significant difference in the interaction between terrain ruggedness and treatment status, showing that treatment and ruggedness combined influenced the occupancy of Pinyon Jays (Table 1, Figure 2). While the presence of Pinyon Jays wasn't significantly altered by thinning treatments and landscape features, the size of the flock was, suggesting that there is a biologically important difference when using abundance data compared to presence data. When comparing flock size to landscape features, the model yielded all significant results showing that larger flocks were more associated with lower elevations and less terrain ruggedness (Table 2). Previous research revealed that there are less observations of a Pinyon Jay by itself, potentially due to Pinyon Jays occurring more frequently in flocks of 50 to 500 individuals (Johnson et al., 2010; Marzluff & Balda, 1989; Novak et al., 2021). The presence of larger flocks can indicate a better environment for nesting Pinyon Jays, to which this study revealed that larger flock sizes tended to occupy untreated sites rather than treated sites, even though most treated sites were at low elevations with less terrain ruggedness (Figure 4, Figure 5). However, it is important to note that breeding individuals were not specifically assessed in this study. While both presence and abundance data can assess occupancy, abundance data offered a more descriptive look into the relationship between Pinyon Jays and their environment in this study. The results yielded from the flock size models supported the hypothesis that mechanical thinning treatments have a negative effect on Pinyon Jay occupancy.

The findings from previous research support the results of this study that determined that Pinyon Jays use pinyon-juniper woodlands selectively at lower elevations with less ruggedness (Boone et al., 2021; Novak et al., 2021). This can be attributed to the way that Pinyon Jays use pinyon-juniper woodlands. Multiple studies have found that Pinyon Jays typically nested in areas with high tree cover and woody debris (Boone et al., 2021; Johnson et al., 2015; Johnson et al., 2018). Pinyon Jays are colonial breeders, so larger flock sizes, or colonies, may typically have breeding individuals, relating to this study's finding that larger colonies occupied untreated sites, which would have denser tree cover for nesting than treated sites (Johnson et al., 2018). Additionally, Boone et al. (2021) found that Pinyon Jays generally foraged in older tree stands with woody debris and a wide range of tree cover. This is a characteristic of sites without mechanical thinning and is consistent with my results. Yet, Boone et al. (2021) and Johnson et al. (2010) also found that Pinyon Jays use open woodland stands for caching seeds. Open woodland areas allow for the Pinyon Jay to more easily cache and retrieve seeds (Johnson et al., 2010). This behavior may account for some of the insignificant results of the presence models because the sites that had mechanical thinning treatments were more open, showing that Pinyon Jays use both treated and untreated sites for seed caching, and foraging and breeding, respectively.

The findings of this study have important management implications for pinyon-juniper woodlands. Previous studies have found that there is a strong relationship between pinyon-specialist avian species and the presence of mature trees (Johnson et al. 2018; Crow & van Riper III, 2011). Concentrating thinning treatments in younger stands may benefit species such as the Pinyon Jay that rely on mature woodlands for nesting and foraging, because older stands produce the most cones which the Pinyon Jays harvest (Johnson et al. 2015). Additionally, limiting thinning treatments to small sections of woodlands can increase structural diversity, allowing for an increase in avian species richness, while still reducing the fuel load of the woodlands (Crow & van Riper III, 2011). These thinning treatments should not be conducted within nesting sites and should be closer to roads and other areas with human disturbance attributes (Johnson et al. 2015). Specifically, how to address this ongoing problem is not immediately clear, but action much be taken to prevent further losses to pinyon-juniper specialist populations, as there are distinct interactions between mechanical thinning treatments and pinyon-specialist species. There are many valid reasons for mechanically thinning woodlands, as it reduces fuel load and can create better habitat for other species of concern (Bombaci & Pejchar, 2016; Boone et al., 2018; Johnson et al., 2018). However, as shown from this study and other previous studies, there is a clear negative effect of thinning treatments on the Pinyon Jay and other avian pinyon-juniper specialists (Boone et al., 2018; Crow & van Riper III, 2011; Johnson et al., 2018; Magee et al., 2019; O'Meara et al., 1981). Overall, continuing to monitor avian populations in tandem with varying fuel reduction treatments will allow managers to make conscious decisions regarding pinyon-specialist species that can subsequently allow for recovery of the declining Pinyon Jay.

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CHAPTER 4: STAKEHOLDER ANALYSIS

Horseshoe Crabs: A Matter of Life and Death for Both Red Knots and Humans

Introduction:

The Mid-Atlantic has long been a hub for horseshoe crab (*Limulus polyphemus*) harvesting for bait and biomedical use in the United States (Read, 2022). For years, the Atlantic States Marine Fisheries Commission (ASFMC) relied on a wildlife modelling software called the Adaptive Stochastic Dynamic Programming (ASDP) to determine the optimal harvesting level of horseshoe crabs to maintain population levels. The ASDP allowed for the harvest of male horseshoe crabs only, but this model did not consider uncertainty in the life histories of horseshoe crabs. In 2022, the ASFMC revised the wildlife modelling software using an Approximate Dynamic Programming (ADP) approach to incorporate the uncertainty in life histories of horseshoe crabs and an endangered avian species called the Red Knot (*Calidris canutus*). The new ADP approach to determining optimal harvesting levels of horseshoe crabs maximizes the average total reward for the system and now allows for the take of female horseshoe crabs, as well as males. These revisions to the horseshoe crab wildlife modelling software have raised concerns about the sustainability of the horseshoe crab population, and importantly, the conservation of the Red Knots. Since 2008, federal and state agencies have worked to restore the populations of both horseshoe crabs and Red Knots. The Red Knots stop in the Mid-Atlantic to renourish themselves on horseshoe crab eggs each year while on a 9,000-mile migration route (Read, 2022). The introduction of these management changes may further limit the Red Knots' access to horseshoe crab eggs. Additionally, pressures from the biomedical industry, which relies on a compound in horseshoe crab blood called Limulus amebocyte lysate (LAL) for medical manufacturing, place further pressure on the populations of horseshoe crabs. This is especially relevant because LAL has been instrumental in manufacturing and researching COVID-19 vaccines.

A solution is needed to relieve the pressures on the horseshoe crab and Red Knot populations, while maintaining the supply of LAL to the biomedical industry. There is currently a substitute for LAL called recombinant Factor C (rFC) that is as effective as *Limulus* blood products. Yet, rFC has not been adopted into pharmaceutical manufacturing methods in the United States due to hesitations from the US Pharmacopeia. Therefore, this report recommends adopting rFC in the US manufacturing methods, slowly incorporating it into pharmaceutical manufacturing, and editing the wildlife modelling software to allow for male horseshoe crab harvest only.

Background:

Biomedical Use

Pharmaceutical manufacturers have relied on Limulus amebocyte lysate (LAL) found in horseshoe crab blood to test for endotoxins in new medications, sterilize new vaccines, and prevent contamination in biomedical devices since its approval in 1977 (US. FDA, 2019). Currently, it is estimated that around 500,000 Atlantic horseshoe crabs *(Limulus polyphemus)* are harvested along the Atlantic coast per year to make LAL (Maloney et al., 2018). The horseshoe crabs are harvested alive, partially drained of their blood to manufacture LAL, and released alive into the wild (Figure 1). Although they are reintroduced back into the wild, conservation groups estimate that 15%-30% of crabs die after reintroduction (Maloney et al., 2018). Companies have attempted to breed horseshoe crabs in captivity specifically for biomedical use, however, it is nearly impossible to get horseshoe crabs to procreate in captivity (Funkhouser, 2011). Population analyses for both the horseshoe crab and the Red Knot show that the biomedical industries dependence on LAL is not ecologically sustainable (Gauvry, 2015; Maloney et al., 2018). Additionally, the decimation of Asian horseshoe crab *(Carcinoscorpius rotundicauda)* populations to manufacture TAL (a compound comparable to LAL but from this species) may cause the world to become entirely dependent on LAL, further placing pressure on the Atlantic horseshoe crab population (Gauvry, 2015). The synthetic substitute recombinant Factor C (rFC) is an extremely promising solution to decrease the industry's reliance on horseshoe crabs and is approved in an estimated 60 countries, but not yet in the United States.

Figure 1: Horseshoe crab bleeding lab for biomedical use. Source: envirobites.org.

The US Pharmacopeia

The US Pharmacopeia is a non-profit scientific organization that sets quality and safety standards for medical manufacturing. Pharmaceutical manufacturers that sell their products with or without approval from the US Food and Drug Administration's (FDA) must follow the standards set by the US pharmacopeia, which by law are enforceable by the FDA (US Pharmacopeial Convention, 2015). However, the US pharmacopeia is reluctant to set the quality of rFC equal to the quality of LAL despite the numerous peer-reviewed studies that validate the effectiveness of rFC (Bolden et al., 2020). Endotoxins in biomedical manufacturing are a serious health concern, and pharmaceutical manufacturers have been exceedingly cautious about transitioning from LAL to new detection technologies (Maloney et al., 2018). Additionally, endotoxin testing using LAL has highly regulated testing methods, which pushes pharmaceutical manufacturers to follow the well-established methods rather than adopting new ones (Maloney et al., 2018). The methods to test with rFC have not been incorporated into the general worldwide Pharmacopeia, making companies that want to use it perform their own validation to prove that the performance of the rFC tests match the LAL tests. (Gorman, 2020; Maloney et al., 2018). This extra-validation is an extremely burdensome process that plays a role in pharmaceutical manufacturers' reluctance to move away from traditional testing methods such as LAL (Jimenez, 2021). Historically, rFC was under a patent from a single manufacturer, making pharmaceutical manufacturers reluctant to use it because they did not want to rely on only one manufacturer for such a crucial factor in biomedical testing (Maloney et al., 2018). However, since 2018 the patent has been lifted and there are multiple suppliers of rFC, making it a more available and cost-effective option to replace LDL in the United States (Maloney et al., 2018). Ultimately, once the US Pharmacopeia decides that rFC testing is equivalent to LAL testing, it can be used widely without the burdensome process of extra validation, making it easier for pharmaceutical manufacturers to adopt it into their products (Jimenez, 2021).

Horseshoe Crab Populations

Atlantic horseshoe crabs are an integral part of the marine ecosystem, as well as the US economy. Other than biomedical use, horseshoe crabs are harvested as bait and to make fertilizer (Smith et al., 2017). Horseshoe crabs are often thought of as the best bait to catch eel, whelk, and conch, which drives commercial fishermen to continue to harvest them. Due to an increase in the need for bait in the whelk fisheries, the horseshoe crab populations were decimated in the 1990's from overfishing (Chesler, 2021; Watson et al., 2018). Before 1990, an estimated 45,000 horseshoe crab eggs per 11 square feet could be found along the Atlantic coastline after each year's breeding season (Botton et al., 1994). According to the New Jersey Division of Fish and Wildlife, the egg numbers have since decreased to about 5,000 to 10,000 per 11 square feet per year (Chesler, 2021). The 1990 overfishing of horseshoe crab populations spurred the ASMFC to introduce the previous wildlife modelling software (ASDP) for male only horseshoe crab harvesting in 1998, but the populations have yet to recover to pre-1990 abundance (Smith et al., 2017) (Figure 2). The lack of recovery for Atlantic horseshoe crab populations is in part due to overfishing and biomedical harvest, but also from habitat loss, climate change, bycatch, and water pollution (Smith et al., 2017). Because of these multiple threats, Atlantic horseshoe crabs are currently classified under the IUCN Red List as vulnerable (IUCN, 2020).

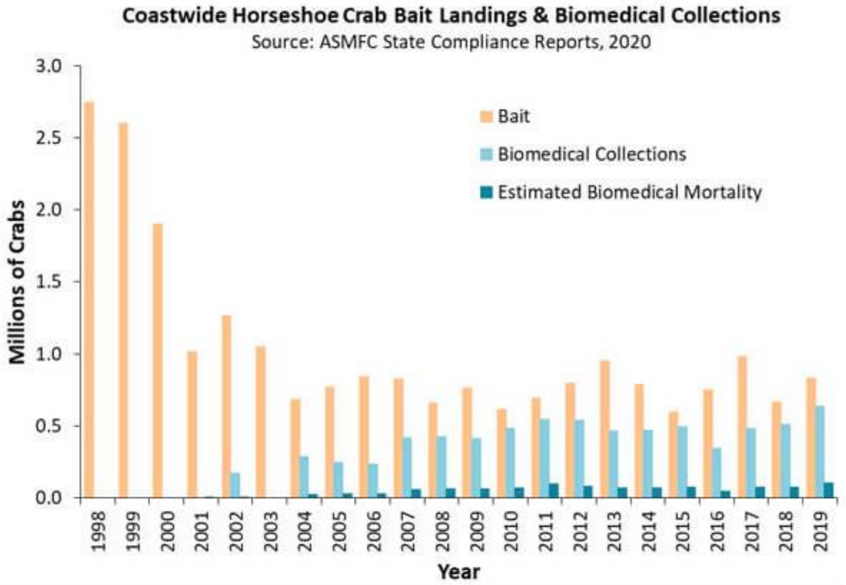

Figure 2: Horseshoe crab harvesting estimates from 1998 to 2019. Source: pda.org.

Red Knot Populations

Red Knots migrate annually from the southern tip of South America to their breeding grounds in the Arctic (Niles et al., 2009). During this 9,000-mile migration, the Red Knots make a final rest stop along the US Atlantic coast, specifically in Delaware Bay, before a direct flight to the arctic. Once in Delaware Bay, the Red Knots fuel up for the rest of their extensive journey on the eggs of horseshoe crabs. This final stop is crucial to ensure the birds have enough energy for the remainder of the migration, as well as successful breeding (Baker et al., 2004). The overharvest of horseshoe crabs in the 1990's significantly decreased the amount of breeding horseshoe crabs, and thus decreased the number of eggs available to the Red Knots (Niles et al., 2009). The reduction of eggs led to a 78% decrease in Red Knot abundance from 1985 to 2008 (Baker et al., 2004; Niles et al., 2009). In 2014, the Red Knot was classified endangered under the Endangered Species Act and is still currently listed (*U.S. Fish & Wildlife Service*, 2014).

Stakeholders:

The Biomedical Field

There are competing interests within the biomedical industry regarding endotoxin testing with LAL and rFC. On one hand, pharmaceutical manufacturers need a method for endotoxin testing as it is critical in developing safe vaccines and is necessary for maintaining public health, especially with the current trends of COVID-19 and vaccine demand (Gauvry, 2015; Maloney et al., 2018). Especially with the controversy in the US Pharmacopeia over whether the substitute rFC is as effective as LAL, researchers, vaccine developers, and medical professionals want to keep using the natural option until the synthetic is fully validated for use (Gorman, 2020). The controversy around rFC places them in favor of the new wildlife modelling software (ADP) in which female horseshoe crabs can be harvested. However, they also want a sustainable source of endotoxin testing materials. They are inclined to support rFC and alter the new wildlife modelling software (ADP) to include male only harvests to ensure enough LAL/rFC for future biomedical production (Gorman, 2020). However, manufacturers of LAL are opposed to the introduction of rFC methods to the US Pharmacopeia and would support the use of the new wildlife modelling software (ADP) with male and female harvest (Jimenez, 2021). Leading US LAL manufacturers such as Charles River Laboratories, Lonza, and Associates of Cape Cod say they need to see more evidence that rFC is as effective as LAL (Charles River, 2018). The US Pharmacopeia relies heavily on the insights of these three companies as they have had a monopoly on endotoxin testing methods in the US over the past few decades (Gorman, 2020; Jimenez, 2021). Multiple non-profit organizations and scientific publications have called out the US Pharmacopeia, saying that the need for more data is a stalling tactic formed by these companies who want to maintain their monopoly in endotoxin testing (Gorman, 2020; Jimenez, 2021; Maloney et al., 2018). Since there are many peer-reviewed articles that vouch for the effectiveness of rFC, one of the main barriers between adoption of its methods are the LAL manufacturers (Gorman, 2020; Jimenez, 2021).

Conservation Organizations

Mid-Atlantic environmental organizations such as the Delaware Audubon, the New Jersey Audubon, Defenders of Wildlife, and Earthjustice have voiced concerns over the new wildlife modelling software (ADP) with male and female harvests. They want to preserve the integrity of the ecosystem and support endangered and threatened species. Approval of the revised wildlife modelling software (ADP) will further limit the Red Knot birds access horseshoe crab eggs, for which they use as energy to make it through their migration route. They are concerned about the unintended consequences of increased horseshoe crab harvesting which can lead to the extinction of both the Atlantic horseshoe crab and the Red Knot bird. They are opposed to the revisions and support the approval and use of rFC (Read, 2022).

Fisheries

Commercial horseshoe crab harvesters, anglers, and fisheries support the revisions for more crab harvesting. These revisions will allow them to make more money and support their livelihood. Use of rFC, rather than LAL, will decrease the amount of horseshoe crabs needed for harvesting, thus increasing the horseshoe crab populations. An increase in horseshoe crab populations can result in less strict regulations on the harvesting of crabs in the future, benefiting anglers and their livelihoods (Gorman, 2020).

Proposed solution:

To protect the horseshoe crab population, as well as aid in the conservation efforts of the Red Knots, I propose the US Pharmacopeia adopt rFC testing methods without further validation procedures to make it easier for manufacturer use and alter the new wildlife modelling software to only include male horseshoe crab harvesting. The solution allows the biomedical industry to continue endotoxin testing in a sustainable way, ensuring safety and reliability in future medical manufacturing.

Pushing leaders in the biomedical industry to use rFC can allow for the incorporation of rFC methods into the general US Pharmacopeia, which makes it easier for pharmaceutical manufacturers to transition to different endotoxin testing technologies (Maloney et al., 2018). To push biomedical manufacturers to use rFC, a campaign educating the public about the horseshoe crab harvesting issue and the use of rFC should be conducted. If the public knows about the current issues regarding vaccine manufacturing and LAL, they may be more inclined to push the leaders of the US Pharmacopeia to act. Additionally, a goal of reducing LDL use by 50% in five years should be considered by pharmaceutical manufacturers and can be achieved by incorporating rFC testing into lower stake biomedical supplies until it is fully approved for use in the US. This will make the transition from LAL to rFC more manageable as pharmaceutical manufacturers will already have an idea of the methods for rFC testing. Approving rFC should satisfy the biomedical industries need for a sustainable source of endotoxin testing material while maintaining the safety standards for medical manufacturing.

Once rFC is adopted for use, the Atlantic States Marine Fisheries Commission should revert the new wildlife modelling software (ADP) to male only harvesting and combine it with the previous software's (ASDP) derivative regulations. With decreased harvesting from the biomedical industry and stricter guiding regulations from the new modelling software (ADP), horseshoe crab populations will have a greater chance of full recovery and will aid the conservation efforts of the Red Knot, satisfying conservation groups. With a greater chance of population recovery for the horseshoe crab, the Red Knots will have a greater abundance of horseshoe crab eggs to subsist them on their long migration route, aiding in their conservation efforts. Moreover, fisheries and anglers will still be able to harvest horseshoe crabs, maintaining their market and livelihoods.

Conclusion:

We cannot stop the harvesting of horseshoe crabs without a proper substitute to make vaccines and medications. Horseshoe crabs would benefit from a moratorium on harvesting, but public health would be impacted. Additionally, we cannot allow horseshoe crabs to go extinct because of their role in the marine ecosystem and food web. The best solution for this issue is to approve the use of rFC for widespread use in biomedical manufacturing and return to the harvesting regulations based on the previous wildlife modelling software (ASDP). This solution relieves the pressures on the Horseshoe crab population, benefits the red knot birds, allows anglers to maintain their livelihoods, and allows the biomedical industry to continue making vaccines and medications safely and effectively. Actions for approval should happen swiftly, as the current harvesting rate is not ecologically sustainable and is projected to increase in the next two decades (Gauvry, 2015). We have the means to replace LAL, and we should do so before the horseshoe crab population reaches a point of no return.

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