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Dear reader,

Welcome to the Anne Arundel Community College Journal of Emerging Scholarship! This publication is dedicated to showcasing the exceptional research of our undergraduate students.

Undergraduate research provides students with invaluable opportunities to engage in real-world problem-solving, critical thinking, and creativity. Through research projects, students can explore their academic interests, gain practical skills, and develop the confidence to tackle complex challenges, while also preparing for future academic and professional endeavors.

In this journal, you will find a diverse range of research articles from our undergraduate students. Each manuscript has undergone a rigorous review process by our esteemed reviewers and research librarians. We are grateful for their time and expertise in ensuring the quality and relevance of the articles.

We hope that this journal inspires and motivates more students to pursue research and provides a platform for the dissemination of their findings. We would also like to thank our faculty mentors who have supported and guided our students throughout their research journey.

Sincerely, The 2022–2023 Editorial Board 2022-2023 EDITORIAL BOARD

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The Effectiveness of a System Without Soil

ABSTRACT

Commercial aquaponic systems produce food through a symbiotic relationship between plants and aquatic animals. We constructed a small aquaponic system to determine if commercial aquaponics could be replicated on a smaller, educational scale. This system used the waste from Nile tilapia to grow a combination of lettuce and tomatoes by cycling water between the fish and plant tanks. The system proved effective in growing both tilapia and food plants. The plants were evidently able to gain proper nutrients from the aquatic animal byproducts and were able to remove potentially toxic nitrogen wastes from the animal tank.

INTRODUCTION

There is a critical need for sustainable methods of producing food for an ever-growing human population that is approaching eight billion. Aquaponics, where animals and plant are grown together, could be an important part of the solution (Goddek et al. 2019). Our goal was to determine if we could create, for educational purposes, a small-scale aquaponics system modeled after commercialized aquaponics systems that would be effective in growing both tilapia and food plants. A balanced aquaponic system promotes plant growth by utilizing the waste products of an aquatic animal species as fertilizer while utilizing plants to remove toxic nitrogen wastes in order to promote growth of the animals (Espinal and Matulić 2019).

aquaponics sustainability nitrogen waste food production tilapia

KEY WORDS

FACULTY MENTOR

Paul J. Bushmann, Ph.D. Professor, Department of Biology

MATERIALS AND METHODS

We paired Nile tilapia (*Oreochromis niloticus*), a fish commonly raised and harvested as a food source, with lettuce and tomatoes. We introduced 25 tilapia fingerlings (Live Aquaponics, Pomona Park, FL), each approximately 4–5 cm in length, to a 946 liter tank of fresh water. The lettuce and tomato plants were planted in a 1.2 m. x 1.2 m. 284 liter reservoir that was placed above the tilapia tank. The system was constructed this way, shown in Figure 1, due to space constraints as well as convenience of directional water flow.

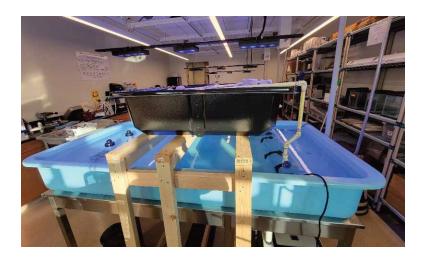


FIGURE 1 Constructed small-scale aquaponic system.

We planted lettuce and tomato seeds in cubes of rock wool, an inert substrate commonly used in hydroponics systems for starting seeds. We used approximately 30 rock wool cubes each for the lettuce and tomato seeds, as shown in Figure 2. Two seeds of each plant were placed in a single rock wool cube. The planted rock wool cubes were then saturated with water to begin the germination process. The rock wool was kept saturated through the entire germination process.

Once the seeds germinated and grew to be approximately 1 cm tall, they were transplanted into slatted, plastic pots containing gravel. The pots were then placed in the 284 liter reservoir. The reservoir was filled with a porous, ceramic pebble substrate (brand



FIGURE 2 Lettuce seeds (left) and tomato seeds (right) germinating in rock wool substrates.

FIGURE 3

Lettuce (left) and tomato (right) pots planted in a ceramic, pellet substrate. name GROW!T) and then filled with water. The pots were placed randomly throughout the reservoir. They were pushed into the substrate so that the seedling was level with the substrate surface. The pot bottom extended down into the water, so that water was pulled up to the seedling by capillary action.

Water in the plant reservoir flowed down into the tilapia tank via a PVC standpipe. The water exiting the tilapia tank drained through two standpipes at one end of the tank and ran through filter socks and a biofilter before being pumped back into the tilapia tank at the opposite end. Two pumps near the tilapia tank inflow pumped water back into the plant reservoir. Our goal was to utilize a recirculating wet/dry filtration system remove solid waste while utilizing the plant reservoir to remove nitrogen compounds.

After the plants were placed in the reservoir, a subset of 10 lettuce and 10 tomato pots were labeled, with five of each plant placed on either side of the reservoir, as shown in Figure 3. For



each numbered pot, two specimens were measured for 1) height at the apical meristem, and 2) the full plant height including leaves. These measurements were taken on a weekly basis to later calculate the rate of growth for both the lettuce and tomato plants.

The tilapia were fed a commercial tilapia pelleted food (Purina AquaMax 300, Lakeway Tilapia, Rutledge, TN) three times per week. We qualitatively observed the growth of the tilapia, but did not quantitatively measure growth. We conducted twice weekly nutrient level tests on the tilapia tank water to evaluate the ammonia, nitrite, nitrate, and pH levels using a RedSea Marine Care Test Kit. The system was also equipped with a digital monitoring system, APEX Fusion, that constantly measured the pH and temperature of the tilapia tank.

RESULTS

Fish

Three tilapia fingerlings died soon after introduction, but the remainder grew throughout the experiment and appeared to be healthy (Figure 4).



FIGURE 4 Tilapia fingerlings at week one of the project (left), and tilapia at week 17 (right).



Plants

Both the lettuce and tomatoes grew quickly, and appeared healthy and dark green throughout the experiment (Figure 5). There did not appear to be any nutrient deficiencies observable by leaf color or growth rate.

Figure 6 shows mean full plant heights for lettuce and tomatoes over the course of the experiment. The lettuce (6A) grew most quickly between days 25 and 45, and mostly stopped vegetative

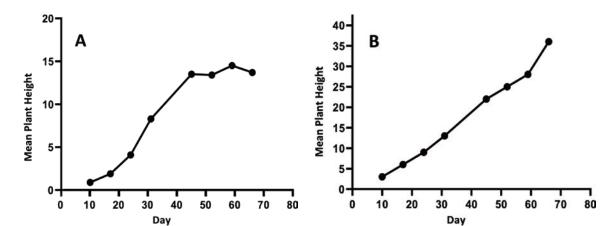


FIGURE 6

FIGURE 5

Aquaponic system in early weeks (left), and after three months growth (right).

Mean full plant height for measured lettuce (6A) and tomatoes (6B). growth by day 50. The tomatoes (6B) continued growth throughout the experiment and were still showing rapid vegetative growth at the end of the experiment.

Nutrients

By day 15, while the plants and the tilapia were small, the ammonia levels were at their highest reading at 1–2 ppm, as shown in Figure 7A. About a week after ammonia levels were at their highest, we saw a spike in the nitrite and nitrate levels, as shown in Figures 7B and 7C. By day 25, ammonia levels dropped to about 0.2 ppm and remained there for the duration of the experiment. By day 45, nitrite levels had fallen to zero and nitrates were low. Nitrites remained very low for the remainder of the experiment. Nitrates were measurable but reduced, compared with their earlier levels.

DISCUSSION

The results of the experiment are promising because we successfully produced both fish and plants in a sustainable manner. All organisms grew in size and appeared healthy, with little mortality loss. We were able to successfully scale a commercial aquaponic system down to an educational size without losing function.

The system showed an early ammonia peak due to fish waste. Nitrifying bacteria, present in the biofilter, converted ammonia into nitrite and less toxic nitrate. The rapidly growing plants then absorbed all three forms of nitrogen from the water to build proteins for growth. Nitrate levels falling demonstrated that the plants

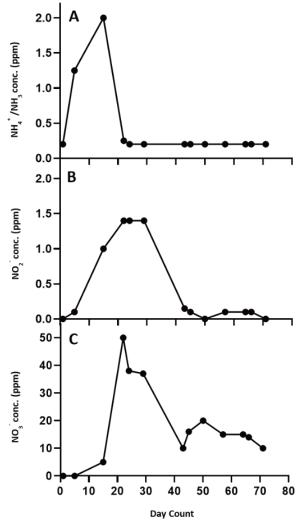


FIGURE 7

System ammonium/ammonia (7A), nitrite (7B), and nitrate (7C) concentrations (ppm) over the course of the experiment. were having an effect, as only plants can remove nitrate from the system. The system was thus able to keep nitrogen wastes low without the need for water changes.

Some challenges we faced in the first few months of the project included fish survivorship, uneven growth among plant cultures, and different rates of maturity of our chosen crops. Not all of the original 25 tilapia fish survived, so we may need to research factors that we can modify to improve survivorship. The random placement of the plants in the reservoir allowed for the underdevelopment of some plants while others flourished. This may have been due to uneven lighting or uneven water flow. We may need to place them in a more thoughtfully designed pattern or rotate the placement of the plants. The lettuce grew to maturity before the tomato plants, making one crop ready to harvest as opposed to both at the same time. It would be helpful to separate the two types of plants to make for a smoother harvest. We also faced challenges in discovering which wavelengths of light to use and how far away from the plants the lights should be placed.

The health and sustainability of an aquaponics system depends on many factors, including which specific variety of seeds are used, which aquatic animal species is chosen, which plant crops are chosen, and what level of filtration is used. The chosen plants have to work symbiotically with the chosen aquatic animals so that a favorable equilibrium of nutrients being absorbed versus being expelled by each organism is achieved.

To further our understanding of sustainable, symbiotic aquaponic systems, we could replicate our project with other known farm raised fish such as carp, catfish, or salmon. We could also replicate our project with different plant crops that require various levels of nutrients to determine which aquatic species produces the most sustainable level of nutrient for that species of plant crop. We could also replicate our project with two tanks, one being an aquaponic system with plants and fish, the other being an aquaculture system of just fish to demonstrate that the plants are in fact what removes the nitrates from the system. Without the plants, results should show ever increasing and toxic nitrate levels.

REFERENCES

- Espinal, C. A. and D. Matulić. 2019. Recirculating Aquaculture Technologies. pp. 35–76 In: Goddek, S, A. Joyce, B. Kotzen and G. Burnell (eds.) Aquaponics Food Production Systems. Springer Nature Switzerland AG. 619 pp.
- Goddek, S. A. Joyce, B. Kotzen and M. Dos-Santos. 2019. Aquaponics and Global Food Challenges. pp. 5–18 In: Goddek, S, A. Joyce, B. Kotzen and G. Burnell (eds.) Aquaponics Food Production Systems. Springer Nature Switzerland AG. 619 pp.

Consultation Centers: Bridging the Information Gap between Policy-Makers and the Public

ABSTRACT

Development and welfare policies in countries with high rates of poverty and in wealthier countries are built on the use of incentives, which can cause unpredictability in economists' models and the inefficient use of aid. Economists and policy-makers, on the one hand, may make policies based on inaccurate assumptions about public response; similarly, the aid recipient can make uninformed choices that are detrimental to their long-term welfare. Through examination of various developing countries, this paper highlights the widespread flaws in this relationship between policy-maker and the public and proposes a framework built on "consultation centers," enabling data to flow more freely between consultant and consultee. The proposed consultation centers would be a place where complex concepts can be communicated between consultant and the consultee through the use of common references such as currency, time, and difficulty. Examination of Citizens Advice in Scotland and inefficient aid distribution in Anne Arundel County will reveal an opportunity for the implementation of consultation centers here in Maryland. This paper concludes that, in theory, consultation centers promote rationality from consultants and consultees by easing the spread of

KEY WORDS welfare policies efficiency adverse incentives incomplete information

FACULTY MENTOR

Uzma Qureshi, Ph.D. Professor, Economics School of Business and Law information, thus creating a more equal relationship between economists and impoverished communities. A side-benefit of the proposed framework would be to return some of the welfare work back to the community level, and perhaps reduce the political polarization about welfare policies based on adverse incentives for impoverished communities.

INTRODUCTION

Nobel Prize winning economists Esther Duflo, Abhjit Banerjee, and Michael Kremer have changed the field of development economics by focusing on the micro aspect of incentives. In many cases, simple incentives like subsidies, taxes, and loans are much less efficient than they are purported to be. In their book Poor Economics, Duflo and Banerjee claim many of the citizens in Udaipur India, living on an income of less than 99 cents per day are severely undernourished, yet "most people living with less than 99 cents a day do not seem to act as if they are starving" (Banerjee and Duflo 23). Duflo and Banerjee then go on to cite "the typical poor household could spend up to 30 percent more on food," and, instead, they spend their income on festivals, tea, and alcohol (Banerjee and Duflo 24). This may seem counterintuitive considering undernourishment "can cut productivity by up to 20 percent," and this can lead into what economists call a nutrition poverty trap (Poor Workplace Nutrition). This same phenomenon can be found in Nicaragua, where around 57% of urban individuals living below the economic poverty line own a radio for entertainment and 21% own a television, while they are considered to be starving (Banerjee and Duflo 71). In China, where subsidizing fortified wheat noodles and rice only incentivized people experiencing poverty to "[consume] less of those two items and [eat] more shrimp and meat," which are more costly and less nutritious (Banerjee and Duflo 24). Seemingly, people with low income around the world are making all the wrong decisions. This is because, in many cases, there is a divide in communication between economists and the public.

PROBLEM STATEMENT

First, economists, at times, make unfounded assumptions due to a lack of adequate information. The New York Times best seller *Freakonomics* is riddled with such examples, the most compelling of which is an analysis of Israeli day care centers. Day care centers were attempting to decrease the number of parents who picked up their children late. Economists responded with a fine against late pick-ups (Dubner.) Dubner then went on to describe the results of the fine by saying, "few would have predicted what we found: introducing a financial penalty for showing up late actually caused parents to do just that. Parents stopped showing up on time entirely" (Dubner). This example is quite similar to the subsidization of fortified goods in China. Both examples include economists attempting to manipulate the actions of the public through financial incentives with the assumption that a price change uniformly results in a desired change in action from the public. According to Dubner, the fine for late pick-ups gave an option for parents to pay off the guilt of picking up their children late (Dubner). In this scenario, the increased cost actually incentivized the public to consume more of a good because the economists who had designed the policy did not have sufficient information about the various factors that influence parental decisions (the option to avoid guilt). Similarly, one can assume there may have been multiple factors other than price affecting the decisions of the starving individuals in Nicaragua, Guatemala, India, and China. For example, one reason for the consumption of tea, alcohol, radio entertainment, and expensive meat over cheaper nutritious food could be that those non-subsidized goods enabled the community to socialize with one another (e.g., drinking tea while watching soccer on the tv). This can create a more tightly knit social structure, which is vital for survival in low-income communities, since friends may act as an informal insurance policy (Banerjee and Duflo 230). Another possible explanation is, contrary to the assumption of traditional economists, some people living in poverty may value cultural enrichment and social interaction over financial stability. The last explanation could be that low-income individuals have a shortened planning horizon. All these explanations are possible and not mutually exclusive. However, the identification of these explanations is difficult without bridging the divide in communication between impoverished communities and economists.

Second, at times, members of impoverished communities make unfounded assumptions due to a lack of adequate information. For example, many Egyptians living under the poverty line assume an increase in iron intake in their diets is unnecessary and unattainable. In Egypt, nearly 40% of the population has iron deficiency anemia and 50% of women are iron deficient (Brouzes et al.1; Tawfik et al. 32). The high prevalence of anemia in women is especially dangerous since it is linked to premature birth, low infant weight, and higher risk of infant mortality (Kanu, et al.). Additionally, stunted growth, lower learning capacity, higher health costs, and decreased work capacity are documented in Egypt due to hereditary anemia (Abu-Ouf and Mohammed 146; Alanwar et al. 9). However, despite the costs of iron deficiency, many Egyptians still lack knowledge of the benefits of proper nutrition. According to American University in Cairo, Egyptians with low income "often perceive stunting as something hereditary and not associated with poor diet" (Alanwar et al.13). This demographic's lack of awareness of the iron deficiency problem makes it so "at the family level, improvement of malnutrition cannot be achieved without exploring the existing level of knowledge among others regarding healthy diet" (Salem et al. 2948). Considering multiple organizations subsidize fortified Baladi bread and iron-fortified biscuits, the Egyptian impoverished community may be lacking adequate knowledge needed to take advantage of economists'

subsidization programs (Elhakim 261; Mohamed et al. 794). One cannot efficiently aid impoverished communities with conventional subsidization policies if the members of these communities are not aware of the benefits of said aid; thus, development organizations must bridge the divide of knowledge between economists and low-income communities.

CONSULTATION CENTER GENERAL DESCRIPTION

A "consultation center" is an original concept I have developed as a platform in which economists and low-income communities can have a dialog about issues and jointly create solutions. Economists have a unique bird's eye view of the situation through their ability to conduct surveys, focus groups, and interviews. Individuals with low income have a unique and vital perspective on poverty. In a consultation center, one may enter and schedule an appointment with a relevant expert, whether that be an economist specializing in the financial aspect of domestic abuse, nutrition, education, or housing. The consultation center will then promote a dialog on available options for common decisions, incorporating a survivor-centered approach. According to UNFPA, a survivor-centered approach, usually used by domestic abuse services, emphasizes that "all actions taken should be guided by respect for the choices, wishes, rights and dignity of the survivor. The role of helpers is to facilitate recovery and provide resources to aid the survivor" (Minimum Standards). The consultant at the consultation center will lay out data and explain it to the best of their ability; and if the client refuses a certain option recommended by the consultant, then the consultant may ask for a reason but must respect the client's decision to refuse. After refusal, the consultant should provide secondary and tertiary options to discuss with the client. For example, in an Egyptian consultation center, consultants will lay out the data on eating subsidized fortified Baladi bread using common references. Curtis Ulmer, the author of "Teaching the Disadvantaged Adult," when teaching math, recommends using a coin chart to teach more abstract concepts like percentages and decimals (Ulmer 71). Money is used as a reference for unknown concepts. The consultation center will incorporate a similar method, where each decision will be quantified into money and hours of rest. After more time in the field and with more research, a third reference may be added: difficulty. For example, if the decision is to buy fortified Baladi bread, then the references could be a monetary increase in wage minus the cost of Baladi bread, an hourly increase in available time (decrease in fatigue from anemia increases productivity, which increases available time), and the difficulty of finding or buying the product (1-3 easy)4-7 moderate 8-10 hard). This format is loosely similar to management consultancy programs, such as the Women's Resource Center in Azerbaijan, which has made substantial progress. The Women's Resource Center in Azerbaijan has successfully given "women spaces to meet, network, exchange ideas, forge partnerships, attract capital, gain confidence, and grow professionally" (Fathi and Gulara). However, many other management consultancy programs frequently adopt a west-rest mentality (Frenkel and Yehouda). This mentality results in a clear and unproductive cultural divide between consultant and client. Consultancy programs have tried to avoid this deficiency with training on understanding other cultures, but to an extent this may deepen the divide rather than bridge it. According to a Bhababian point of view the "West's attempt to shape the other in its own (not quite precise) image, and the historically situated continuous encounter between the West and its others throughout the colonial and neo-colonial era, constitute a third space, which is not entirely governed by the laws of either the ruler or the ruled." (Frenkel and Yehouda). However, consultation centers may avoid inadvertently creating this third place with UNFPA's survivor-centered approach and a focus on local data rather than national. First, for all advice given to clients

at a consultation center, the use of local data will be given priority over national to keep advice relevant. Second, since the consultant will have full faith in the client's ability to make sense of the data being presented to them, there would be reduced incidence of a cultural clash between consultant and consultee. Instead, both can learn from each other.

The survivor-centered approach and a focus on local data is also useful domestically, since imprecise images of an "internal other" can create inefficiency in the U.S. For example, many wealthy individuals believe one's work ethic and choices are the root cause of poverty, creating an image of people in poverty who are lazy or undeserving of wealth (Menasce Horowitz et al.; Lichter and Martha 20; Introduction to Sociology). This conception of people living in poverty manifests itself in policies that have work requirements for eligibility such as TANF, SNAP, and Medicaid. However, "overall, the increase in total earnings from TANF's work requirements is about equal to the reduction in benefits," and "work requirements in SNAP and Medicaid have reduced benefits more than they have increased people's earnings" (Work Requirements; Hahn and Simms). Work requirements, in most cases, create a difficult-to-navigate bureaucracy that discourages many eligible individuals from applying to assistance programs (Work Requirements). In some cases, the lack of aid while searching for a job can make finding employment more difficult, resulting in lower employment, as seen in the case of Medicaid, which will be discussed later in more detail (Work Requirements).

A more specific example of these processes is TANF, the work requirements of which increased employment among its beneficiaries; however, income was not affected (Work Requirements). Concluding that TANF work requirements successfully decreased government payments without any harm to income is tempting but possibly inaccurate. The average income of total TANF

beneficiaries before work requirements did not change significantly, but this is only due to the wide variance in their incomes. Individual incomes changed dramatically, with a vast increase in the number of people falling into deep poverty offset by a similar number of people lifted above the poverty line (Work Requirements). One is tempted to conclude that people thrown into deep poverty deserved it since they chose not to work, but again, this analysis is inaccurate. According to the Urban Institute, members of low-income communities usually work when they are able without the incentive of work requirements (Hahn and Simms). The increase in employment after TANF's work requirements were added could possibly indicate beneficiaries became employed at a suboptimal time, if we assume that they would have already been employed if their circumstances had allowed it. Possibly, single parent beneficiaries of TANF had to suffer more stress and/or sacrifice crucial time with their children to meet TANF requirements. Those who lost their TANF coverage after work requirements were implemented most likely could not make such sacrifices and were punished for their circumstances rather than their merit. TANF work requirements decreased federal monetary costs, but, likely, at the cost of income polarization, mental wellbeing, and child development. On the other hand, one cannot minimize the importance of gainful employment psychologically, economically or socially on people's personal lives and on the communities that they live in. However, how best to harness this potential benefit requires a deeper understanding of the decision-making process of those in need, so that policy-makers can offer choices where the individual freely chooses gainful employment as opposed to being pushed into it by one-size-fit-all requirements.

The effects of SNAP and Medicaid work requirements provide clearer evidence of the need for policy improvement. Effects on employment were not significant for SNAP and were negative for Medicaid (Work Requirements). However, both SNAP and Medicaid exhibited a net loss of income after work requirements were implemented (Work Requirements). A large portion of beneficiaries remained unemployed even after work requirements were implemented and lost their aid, thus greatly decreasing their income (Work Requirements). Many claimed the requirements were too onerous to meet even considering the consequences (Work Requirements). Clearly, punishing those living in poverty for being unemployed does not consistently raise employment.

THE FOUNDATION FOR IMPLEMENTATION

To begin setting up consultation centers, a similar framework to Citizens Advice centers in the United Kingdom can be used. Citizens Advice hires experts to consult with clients for free and allows qualified volunteers to help at their leisure (Volunteer in a Citizens Advice Bureau). The focus for Citizens' Advice when it was first established in 1939 was to help citizens get access to welfare programs (History of Citizens Advice Service). However, Citizens Advice now provides aid for money management, employment, and bureaucracy navigation (History of Citizens Advice Service). Relevant experts in regional welfare programs and financial literacy help clients understand any questions they may have about banking, aid, and health. Citizens Advice also records all problems that clients produce in a central location for data analysis. With this data, research on the needs of consumers is made much easier. Creating a Citizens Advice center will provide a good foundation for the development of consultation centers while simultaneously providing a community with effective aid.

Citizens Advice in Scotland has been extremely successful in providing opportunities for low-income citizens. For example, Citizens Advice Scotland had provided nearly 75,000 individual pieces of advice in 2021 (Ekton). According to an independent report from Europe Economics, Citizens Advice Scotland provided up to \pounds 148 million worth of community gain to Scotland during the year 2019/20 (Citizens Advice Worth). This figure "includes aspects of preventative spend[ing]. For example, the savings generated for health services by people avoiding negative outcomes associated with choosing between heating their homes and feeding their families, as well as the impact on the economy of money being unlocked for people" (Ekton). Two of their largest programs are the Money Talk Team (MTT) and the Armed Services Advice Project (ASAP). MTT provides help with issues in income and debt for the average Scottish citizen. According to Tanya Gersiova, a Citizens Advice project coordinator, "one of the barriers to talking about money is the complexity of information. The benefits system, for example, is complicated, which is why many people switch off and end up not claiming what they're entitled to. The MTT aims to cut through all the complexity and help you make the right choices" (Gersiova). MTT puts a focus on the client and tries to bridge the information divide between aid provider and recipient. This is a strategy I would adapt for consultation centers. Gersiova then mentions a woman who stopped at a Citizens Advice center and asked for help navigating child care aid (Gersiova). She said, "I knew what was going to happen to my body. I just hadn't quite realized what having a child was going to do to our finances" (Gersiova). She is now a part of the vast community of individuals who have been guided through complex aid bureaucracies by Citizens Advice. MTT alone has helped over 55,000 clients and has put an extra 50 million pounds in client's pockets (Gersiova). In addition, ASAP provides financial advice to members of the armed forces and, "through receiving benefits to which they are entitled, debt written off or rescheduled or charitable grants received, [ASAP] client financial gain recorded over the year to date was $f_{2,154,512.82}$, which amounts to a community gain of $\pounds 3.91$ for every $\pounds 1$ of funding for the 2021/22 year" (ASAP Annual Report). In their annual report, ASAP tells the story of a widowed ex-army client who had come to ASAP

distraught and anxious. He had been his wife's caregiver, but when she passed, he lost his entitlement to pension credit (ASAP Annual Report). At 65 years old he could not find a job to meet his increased bills and was dipping into his savings to get by (ASAP Annual Report). He had stopped meeting with friends and family because of the cost of travel (ASAP Annual Report). ASAP helped him claim his military benefits, giving him over 10,500 pounds (ASAP Annual Report). After ASAP's aid, he was able to meet with loved ones and "reported that his quality of life was significantly improved as well as a sense of security and independence" (ASAP Annual Report).

Citizens Advice centers can also create a foundation for consultation centers. Although Citizens Advice focuses on helping individuals navigate the bureaucracy and gain financial literacy instead of attempting to translate the costs and benefits of dayto-day decisions with common references, it can help provide the research required to build a consultation center. Citizens Advice centers gather data on the lives of low-income individuals to figure out where aid needs to be directed. For example, ASAP in 2022 identified trends in client questions like: "support needs relating to regulated fuel (oil, gas) increased over the last year, while issues relating to charities, both food bank and non-food bank, both increased" and "issues relating to Adult Disability Payment are now within the top 10 advice issues, while information about services has dropped out of the list altogether" (ASAP Annual Report). Highlighting the areas that need the most aid can create a more efficient allocation of resources for all welfare programs. Consultation centers, similar to the Citizens Advice model described above, would be embedded in communities and hence provide access to community specific conditions, constraints, and work and consumption patterns. Citizens Advice, for example, has found that many British consumers have been overpaying for utilities by a cumulative 24.1 billion pounds from 2004 to 2019

(Monopoly Money). This type of research can help economists quantify some of the benefits and harms of common decisions, which can then be shared with the community.

The consultation center proposal goes beyond the scope of the Citizens Advice model. While sharing the attributes of community-level engagement and increased access to information and guidance regarding community access to existing welfare policies, the consultation center envisions a re-thinking of welfare policies in light of the information gathered from this community-level engagement of policy-makers. Consultation centers will go beyond explaining existing aid programs to clients or redirecting aid programs to new needs. Consultation centers will take a strong lead in understanding low-income individuals' constraints, objectives, and decision-making processes that might benefit them in their day-to-day choices, whether or not they relate directly to existing welfare programs. Suppose an individual is debating the use of subsidized housing. In that case, a Citizens Advice center may provide methods of application, legal advice, and an explanation of basic terminology (ex. tenancy deposits, holding deposits, etc.) (Housing; How Much It Costs.) However, there are many other considerations to make in such a scenario. For example, the time and energy costs of moving; the loss of community (a possible loss of informal insurance as mentioned prior), available jobs, the local availability of quality goods and services, and many other implicit factors that are not explicitly related to the subsidized housing unit. And this might not be an exhaustive list. A consultation center will open up a two-way information exchange between users and makers of welfare policies, where information is not just provided to community members, but it is actively received from the community in order to better inform future policies.

IMPLEMENTATION IN ANNE ARUNDEL COUNTY

Over the last few years, Anne Arundel County public schools

implemented a free lunch and breakfast program much like many other school districts in the U.S. Across the country, 95% of counties out of 62 districts in 31 states reported that free lunch programs like this significantly reduced child hunger (Large School District Report). However, this program is losing funding quickly as Covid-19 becomes more manageable. As reporter Wong states, advocates of the free food program are worrying "families that became financially insecure during the pandemic might not know they now need to apply for free meals" (Wong). Furthermore, a 2016 study reports Anne Arundel County already has a low participation rate (51%) in food supplement programs within those who are eligible (Maryland Poverty Profiles). A consultation center can provide multiple benefits in this context. First, why is the participation rate low? Is it because of lack of information or some other constraint or consumer choice? Second, if it is low because of lack of information and ability to navigate the system, then information and guidance can be provided on how to access the benefit. Third, if it is not lack of information but some other constraint that makes the participation rate low, then that is useful information to be relayed back to policy-makers. And finally, at a deeper level, what are some of the root causes that keeps families in need of this program? How much of a turnover is in the recipients of the program? How does the program affect other choices that recipient families make?

Anne Arundel County has also had a long-standing problem with domestic violence aid awareness. From 2016 to June 2018, domestic violence helpline calls had been increasing, reaching up to 2,204 in one year (Brown). However, this is only a fraction of the total population of individuals who are victims of domestic violence, since these are only the people who knew about the helpline. The YWCA, the county's provider of domestic violence aid, states that one of their most common complaints is that "no one knows about them" (Brown). Communication of domestic violence aid is so lacking that, according to a 2018 report "Several victims said they got the best information from the bathroom wall in the local hospital" (Brown). Domestic violence aid is extremely important for the livelihood of domestic violence victims and deserves more outreach than a hospital bathroom can provide. Consultation centers can provide locations of shelters and contact for reliable counseling all in one center. Since the local community consultation center will be the place to obtain information on all available welfare programs, there will be cost savings in that each program will not have to fund its own advertising program. Funding for advertising can be pooled for consultation centers, so consultation centers can direct individuals to whichever aid program fits their needs best using multiple platforms: an in-person meeting with a consultant, phone calls, and a website. This way, vital programs like YWCA receive adequate advertising even if their individual budgets cannot handle mass advertising.

Next, consultation centers can use their research to quantify pressing decisions. For example, consultation centers can find the average cost of leaving one's home to live in a shelter and which methods of employment are most accessible and efficient in mitigating those costs. Consultation centers help beneficiaries visualize options with statistics and models and direct them to more aid programs. Consultants will not and cannot determine whether the costs for a decision outweigh the benefits or vice versa, but that is not their purpose. A consultation center consultant's job is to lay out understandable data in front of the client so they can engage in mutual discourse with a relevant expert and can conduct their own cost-benefit analysis with better knowledge of all the variables.

CONCLUSION

By enabling low-income individuals to make more informed decisions and enabling economists to better understand low-income individuals' struggles and goals, consultation centers embedded in communities, will create a more efficient environment for traditional development policies. This proposal for consultation centers is not promoting a development policy in which policy-makers should suddenly halt all attempts to influence choices made by the people living with inadequate income via subsidies, taxes, or other welfare programs. It is simply making an argument for bringing the analysis to the community level and opening a two-way road to better information that can simultaneously inform policy-makers and aid recipients for making better policies and better choices, respectively. A county-level pilot project, for example, would be a wonderful way to explore the possible benefits of a consultation center. Connecting this pilot project to an institution of higher learning, like a community college, can be instrumental in further connecting the community to the college. Such a consultation center would benefit from the faculty's subject-matter expertise in the various social and health sciences, arts and humanities, business and law while also creating avenues for information to flow back to the larger academic community and better inform welfare policy.

WORKS CITED

- Abu-Ouf, Noran M., and Mohammed M. Jan. "The Impact of Maternal Iron Deficiency and Iron Deficiency Anemia on Child's Health." *Saudi Med Journal*, vol. 36, no.
 2, Feb. 2015, pp. 146–49, *PubMed*, DOI: 10.15537/smj.2015.2.10289. PMID: 25719576; PMCID: PMC4375689.
- Alanwar, Abeer, et al. "Tackling Stunting and Anemia in Egypt." AUC Knowledge Fountain, American University in Cairo, 2018, https://fount.aucegypt.edu/ studenttxt/67?utm_source=fount.aucegypt.edu%2Fstudenttxt%2F67&utm_ medium=PDF&utm_campaign=PDFCoverPages.
- "ASAP Annual Report." Armed Service Advice Project, Citizens Advice Scotland, Sept. 2022, https://www.cas.org.uk/system/files/publications/20221101_asap_report_oct_ 2021-_sept_2022.pdf.
- Banerjee, Abhijit V., and Esther Duflo. Poor Economics: Rethinking Poverty and the Ways to End It. Random House India, 2013.

- Brouzes, Chloé, et al. "Urban Egyptian Women Aged 19–30 Years Display Nutrition Transition-like Dietary Patterns, with High Energy and Sodium Intakes, and Insufficient Iron, Vitamin D, and Folate Intakes." *Current Developments in Nutrition*, Oxford University Press, 6 Jan. 2020, https://www.ncbi.nlm.nih.gov/pmc/articles/ PMC6964731/pdf/nzz143.pdf.
- Brown, Pamela. "Eliminating Racism, Empowering Women: An In-depth look at Domestic Violence in Anne Arundel County October, 2018." Partnership for Children, Youth and Families, Anne Arundel County, October 2018, https://www.aacounty.org/ boards-and-commissions/partnership-for-children-youth-families/forms-and-publications/2018-needs-assesment-domestic-violence.pdf.
- "Citizens Advice Worth up to £245m to Scotland." Citizens Advice Scotland, 2020, https:// www.cas.org.uk/news/citizens-advice-worth-%C2%A3245m-scotland#:~:text=The%20Citizens%20Advice%20network%20is%20worth%20up%20to%20 %C2%A3245,new%20analysis%20published%20today%20reveals.
- Dubner, Stephen J. "What Makes People Do What They Do?" Freakonomics, 23 Oct. 2013, https://freakonomics.com/2013/10/what-makes-people-do-what-they-do/.
- Introduction to Sociology: Understanding and Changing the Social World. U of Minnesota, 8 Apr. 2016, https://pressbooks.howardcc.edu/soci101/chapter/8-4-economicinequality-and-poverty-in-the-united-states/.
- Ekton, George. "Building Community Wealth through Advocacy and Advice." *Citizens Advice Scotland*, 14 Nov. 2022, https://www.cas.org.uk/news/buildingcommunity-wealth-through-advocacy-and-advice.
- Elhakim, Nadine. "Fortifying Baladi Bread in Egypt: Reaching More than 50 Million People through the Subsidy Program." Food and Nutrition Bulletin, PubMed, Dec. 2012, S260-271, DOI: 10.1177/15648265120334S302.
- Fathi, Leyla, and Gulara Humbatova. "Women Resource Centres in Azerbaijan Stand Strong through the Covid-19 Pandemic." United Nations Sustainable Development Group, United Nations, 7 May 2020, https://unsdg.un.org/latest/blog/womenresource-centres-azerbaijan-stand-strong-through-covid-19-pandemic.
- Frenkel, Michal, and Yehouda Shenhav. "Management Consulting in Developing and Emerging Economies: Towards A Postcolonial Perspective." *The Oxford Handbook of Management Consulting*. E-book ed., Oxford University Press, 29 March 2012 https:// www.researchgate.net/publication/272085150_Management_Consulting_in_

Developing_and_Emerging_Economies_A_postcolonial_Approach.

- Gersiova, Tanya. "It's Good to Talk about Money." Citizens Advice Scotland, 10 Dec. 2022, https://www.cas.org.uk/news/its-good-talk-about-money.
- Hahn, Heather, and Margaret Simms. "Poverty Results from Structural Barriers, Not Personal Choices. Safety Net Programs Should Reflect That Fact." Urban Institute, 16 Feb. 2021, https://www.urban.org/urban-wire/poverty-results-structural-barriers-not-personal-choices-safety-net-programs-should-reflect-fact.
- "History of the Citizens Advice Service." *Citizens Advice*, 2023, https://www.citizensadvice. org.uk/about-us/about-usl/history-of-the-citizens-advice-service/.
- "Housing." Citizens Advice, 2023, https://www.citizensadvice.org.uk/housing/.
- "How Much It Costs to Rent." *Citizens Advice*, 2023, https://www.citizensadvice.org.uk/ housing/renting-privately/private-renting/how-much-it-costs-to-rent/.
- Kanu, Florence A., et al. Anemia Among Pregnant Women Participating in the Special Supplemental Nutrition Program for Women, Infants, and Children — United States, 2008–2018. United States, Center for Disease Control, 24 June 2022, http://dx.doi.org/10.15585/ mmwr.mm7125a1.
- "Large School District Report 2022." Food Research & Action Center, 24 May 2022, https:// frac.org/large-school-district-report-2022.
- Lichter, Daniel T., and Martha L. Crowley. "Poverty in America: Beyond Welfare Reform" *Population Bulletin*, June 2002, vol. 57, no. 2, Population Reference Bureau, https:// www.prb.org/wp-content/uploads/2021/02/06052002_57.2PovertyInAmerica.pdf.
- "Maryland Poverty Profiles." Maryland Poverty Profiles, Maryland Alliance for the Poor, 2018, https://mda.maryland.gov/about_mda/Documents/SNAB/Maryland-Poverty-Profiles_2018_1-15-2018_T.pdf.
- Menasce Horowitz, Juliana, et al. "What Americans See as Contributors to Economic Inequality." *Pew Research Center*, 17 Aug. 2020, https://www.pewresearch.org/ social-trends/2020/01/09/what-americans-see-as-contributors-to-economicinequality/.
- Mohamed, S., et al. "Evaluation of the Level of Micronutrients in Fortified Foods in Alexandria, Egypt." *Eastern Mediterranian Health Journal*, 2010, vol. 16, no. 7, pp. 794– 800, World Health Organization, https://applications.emro.who.int/emhj/V16/ 07/16_7_2010_0793_0800.pdf?ua=1.

"Monopoly Money - How Consumers Overpaid by Billions." Citizens Advice Bureau,

May 2019, https://www.citizensadvice.org.uk/Global/CitizensAdvice/campaigns/ Citizens%20Advice%20Briefing%20-%20Monopoly%20Money%20-%20 May%202019.pdf .

- "Minimum Standards for Prevention and Response to Gender-Based Violence in Emergencies." UNFPA, Nov. 2015, https://unfpa.org/sites/default/files/pub-pdf/ GBVIE.Minimum.Standards.Publication.FINAL_.ENG_.pdf.
- "Poor Workplace Nutrition Hits Workers' Health and Productivity, Says New ILO Report." International Labor Organization, United Nations, 15 Sept. 2005, https://www.ilo.org/ global/about-the-ilo/newsroom/news/WCMS_005175/lang--en/index.htm#: ~:text=The%20world%20is%20facing%20a,up%20to%2020%20per%20cent.
- Salem, Marwa, et al. "Improvement of Healthy Diet Related Knowledge among a Sample of Egyptian Women in Three Upper Egypt Governorates Using a Community Based Intervention." *Open Access Maced J Med Sci*, 30 Aug. 2019,vol. 7, issue 17, pp. 2947–52, PubMed, DOI: 10.3889/oamjms.2019.748. PMID: 31844462; PMCID: PMC6901867.
- Tawfik, Afak, et al. "Anemia and Iron Deficiency Anemia in Egypt." IOSR Journal of Pharmacy, Apr. 2015, vol. 5, no. 4, pp. 30–34, http://www.iosrphr.org/papers/v5i4/ F054030034.pdf.
- Ulmer, Curtis. *Teaching the Disadvantaged Adult*. E-book, National Association for Public Continuing and Adult Education, Eric, 1969. https://eric.ed.gov/?id=ED030045.
- "Volunteer in a Citizens Advice Bureau." *Citizens Advice Scotland*, 2023, https://www.cas. org.uk/about-us/volunteer-citizens-advice-bureau.
- Wong, Alia. "Are School Lunches Free This Year? What to Know Now That Pandemic-Era Meal Program Is Ending." USA Today, 1 Sept. 2022, https://www.usatoday.com/ story/news/education/2022/09/01/school-lunch-free-us-what-know-enduniversal-meals/10340417002/.
- Work Requirements and Work Supports for Recipients of Means-Tested Benefits. United States, Congress, Congressional Budget Office, June 2022, https://www.cbo.gov/publication/ 58199.

Tracking Horseshoe Crab Populations with DNA Barcoding

ABSTRACT

The horseshoe crab (HSC), Limulus polyphemus, has existed for millions of years and has undergone little structural change, giving them the name, "living fossil". They are important as a food source for shore birds, as bait for the fishing industry, and as the source of an important component for contamination screening of medical products. A number of long-term spawning surveys have been conducted along the east coast of the United States, such as tagging to track individuals over multiple seasons to determine if they return to the same spawning sites. Genetic studies of HSC sequences have been conducted to identify variations like single nucleotide polymorphisms (SNP) to understand population structure and gene flow. Studies involving HSCs from very distant locations on the east coast of the United States suggest that there is limited gene flow between regions a great distance apart. This work focuses on HSCs in the Chesapeake Bay collected from five locations, and two from the mouth of the Delaware Bay. There are indications that there is considerable genetic diversity at each site, suggesting that mating is not limited to an isolated population, and the identification of haplotypes at sites very distant from each other does suggest significant gene flow between populations. It is important to understand how much genetic diversity, or lack thereof, there is in HSC population along the Atlantic coast. This has implications for regional management and ensuring a healthy and diverse population.

KEY WORDS horseshoe crab mitochondrial DNA sequence haplotype

FACULTY MENTORS

variant

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INTRODUCTION

Horseshoe crabs (HSCs) have long been a vital part of estuarine ecosystems, with fossil records dating to the Ordovician period (more than 400 million years before modern humans). Their lack of adaptation throughout history gives them the status of "living fossils" (Botton 2009). HSC shells serve as a substrate for epibionts such as barnacles (Botton 2009), and their eggs provide a vital protein source for migrating shorebirds such as Red Knots and Sanderlings (Botton 2009). Moreover, the unique properties of HSC blood have helped advance the biomedical industry, with its role in endotoxin testing (Krisfalusi-Gannon et al. 2018). Annually, several hundred thousand HSCs are collected and bled before they are returned to the water. From the blood limulus amebocyte lysate (LAL) is purified and used to test medical devices and vaccines for endotoxins produced by bacteria that could cause illness or death in humans. However, with a high commercial demand for HSC blood and their use as bait in the commercial fishing industry, HSC populations are under pressure. The ecological consequences of their diminishing numbers remain an important, yet little understood, scientific question.

HSCs are divided into four known species: Carcinoscorpius rotundicauda, Limulus polyphemus, Tachypleus trientatus, and Tachypleus gigas (Sarmiento et al. 2021). This study focuses on Limulus polyphemus, also known as the Atlantic horseshoe crab, which is found along the east coast of the United States from Maine and in the Gulf of Mexico. While the spawning behavior of HSCs during summer months has been extensively studied, there is less known about the migration patterns of HSCs during the remainder of the year (Smith et al. 2002). Several studies have tracked HSC movements using physical tagging and radio-tracking. One study tracked 24 females over a short period of time and found that they spawned multiple times and were likely to return to the same beach multiple nights (Brousseau et al. 2004). This study did not track HSCs over multiple seasons.

DNA sequence analysis is another method that has been employed to compare target DNA sequences in samples collected from individuals at different spawning sites. Sequence differences unique for specific locations can indicate site preference for spawning and limited gene flow, suggesting decreased genetic mixing between populations and regional genetic patterns (King and Eackles 2004). DNA analyses have targeted differences in restriction endonuclease patterns (Saunders et al. 1986) or copy number differences in about two dozen microsatellite sites from samples obtained from geographically distant areas along the eastern shore of the United States (King and Eackles 2004; Johnson 2016). A long-term survey of HSCs in the Chesapeake Bay has been conducted by Dr. Paul Bushmann (unpublished data). Dr. Bushmann's team has been recording HSC spawning numbers annually since 2004, and limited tracking studies have suggested that there is not significant migration of HSCs between beaches from year-to-year (unpublished data). In 2018 his group began collecting blood samples from a subset of the HSCs for DNA sequence analysis. Over the next several years, blood samples were also collected from additional locations along the Chesapeake and Delaware Bays.

Finding DNA haplotype patterns can provide insight into horseshoe crabs migration between locations. A haplotype in this study refers to a unique combination of single polymorphisms within a section of mitochondrial DNA. To better understand genetic diversity of HSC populations in the Chesapeake Bay and Delaware Bay, a section of mitochondrial DNA was targeted for sequence comparison between individuals collected from seven locations. Analysis of mitochondrial DNA is a common method for studying genetic diversity among and between geographically separated populations (Aini et al. 2021; Zhan et al. 2021). In several previous studies the protein-encoding, adaptive loci, *cytb* and *cox1*, were targeted to study genetic diversity and population structure. However, non-coding, neutral sequences are predicted to be more likely than coding regions to accumulate polymorphisms over time and result in higher genetic diversity while requiring a smaller sample size (Holderegger et al. 2006). Therefore, in this study, a non-coding section of *Limulus polyphemus* mitochondrial DNA was targeted, but the limited size of that locus prompted the targeting of a larger segment that also encompasses small tRNA encoding genes, and a portion of a 12s rRNA-encoding gene. Sequence comparisons were used to identify sequence differences and variants grouped according to location of sample collection. The hypothesis is that high genetic diversity within populations and low sequence differentiation between populations suggest that HSCs do not favor a specific spawning location, but demonstrate significant migration and gene flow.

RESEARCH METHODS

Sample Collection

Between 2018 and 2022, HSC blood samples were collected during the HSC mating season, March through July, at night during full moon high tides. HSC mating is most active at this period of time and provided the researchers the best opportunity to collect samples. Beaches were scanned and the date, time, location and sex were recorded in the anecdata.org app. Wearing gloves to handle the crabs, a sterile syringe was used to withdraw approximately 1 mL of blood which was transferred to a sterile 1.5 mL Eppendorf tube, sealed and stored on ice for transport to the lab. Samples were stored in a -80°F freezer until processed.

Genomic DNA Purification

HSC blood samples were thawed at room temperature. Genomic DNA was isolated with a Zymo Quick-DNA Miniprep Plus kit (Genesee Scientific). In a labeled 1.5 mL Eppendorf tube, cell lysis reagents, proteinase K (10 mg/mL), and 10 ul of blood were

mixed by pipetting up and down and then incubated at 55°C for 25 minutes. Genomic Binding Buffer was added and mixed with a vortex mixer. Samples were then transferred to a spin column in a collection tube, centrifuged, and flow-through discarded. The column was washed twice, transferred to a clean collection tube, and DNA was eluted in sterile, molecular grade water. After a final centrifuge, the prepared samples were stored at -20°C. After DNA isolation, the DNA concentration was determined spectrophotometrically (1 unit at 260 nm= 50 ug/mL). A 260/280 ratio was also calculated.

Amplification of Mitochondrial Target Sequence

Primers targeted a 992 bp mitochondrial sequence from position 12754 bp to 13745 bp including the non-coding region 13229 bp to 13576 bp (numbering based on accession number NC_003057). The primers used in this study were srRNA-R1 (5' ACGAGGAACCATCGATTACAGAGC) (Lavrov et al. 2000) and HCisREV (5' GGTTATGAGCCCAATAGC) (This study). The reaction contained 2 ul genomic DNA, 1X buffer, 0.5 mM dNTPs, 0.2 uM forward primers and reverse primers, and Q5 DNA polymerase (New England Biolabs). Amplification was performed at 98°C for 10 min then through thirty cycles at 98°C (1 min), 64°C (1 min), 72°C (1 min), then a final extension of 72°C (10 min). Additional primers were utilized to amplify larger regions of the mitochondria and some of those sequences were used in alignments to confirm sequence correctness (data not shown).

Agarose Gel Analysis of PCR Products

Samples were analyzed on a 1.5% agarose gel and stained with ethidium bromide. Sample lanes that contained an amplification product of approximately 990 bp were selected for further sequence analysis. Samples were then sent to Genewiz (Azenta) for sequence determination. Each sample was sequenced on both strands using the same primers utilized in the amplification.

Sequencing and DNA Subway Manipulation

The forward and reverse sequences obtained from Genewiz (genewiz.com) were uploaded into DNA Subway Blueline as FASTA files (dnasubway.cyverse.org). The sequences were paired, aligned and trimmed to obtain the maximum stretch of sequence agreement on both strands. The sequences were then saved as FASTA files for further alignment to other samples.

Sequencing Alignment, Haplotype Identification, and Diversity Analysis

Within the Unipro UGENE genome analysis suite, trimmed sequences were compared to Genbank accession numbers NC_003057 and JX983598. In many cases more than one DNA sequence had been determined from a single blood sample, so duplicates were checked for agreement and extra copies removed. Sequences were aligned with ClustalW, a multipurpose sequence alignment tool (SFi). All sequences from samples collected in this study were found to contain over twenty nucleotide differences when aligned with NC_003057.1, but only one difference when aligned with JX983598. Consequently, in analyses, the JX983598 sequence was designated as the reference haplotype to which sequences from this study were compared. Novel haplotypes, variants, were identified by additional nucleotide differences beyond the one common to all samples. Variants with the same sequence were clustered and given a common name.

Aligned sequences were trimmed to be of equal length, and saved as Mega files for upload and analysis with DNA Sequence Polymorphism (DnaSP) software version 6 (Rosaz et al. 2017). Genetic diversity was measured with haplotype number and haplotype diversity and Wright's fixation index (FST). While HSCs are diploid for nuclear chromosomal DNA, they are haploid for mitochondrial DNA. The pairwise F-statistic (FST) and AMOVA were calculated in Arlequin version 3.5, a software package for population genetic data analysis (Excoffier and Lischer, 2010).

RESULTS

Sample Collection

Between 2018 and 2022, samples of HSC blood were collected from a subset of surveyed crabs at sites on the Chesapeake and Delaware Bays (Figure 1). Table 1 shows the date, location, and number of blood samples collected between 2018-2022 from seven different locations along the Chesapeake and Delaware Bays. In general, higher numbers indicate more HSC abundance at the time of sampling. However, on several occasions no HSC sightings were made during the expected breeding period at a given study area, resulting in no collection for a particular field survey (these Figures are not captured in Table 1). On other occasions blood was only collected from a portion of sighted HSCs due to limited time and supplies. As a consequence, the numbers in Table 1 provide only a rough estimate of HSC abundance at the various locations during the years studied. The most frequent HSC sample location was Cove Point, which had samples taken four of the five years in the study. Bay Ridge, Cape May, and Terrapin Park only had samples recorded during one season.



FIGURE 1

Map of Collection Locations. Stars give the approximate location of sampling sites, and the abbreviations for sites are in parentheses.

Year (Number of Samples)	Site Name	Number Samples Collected	Number Samples Sequenced
2018 (N=61)	Bay Ridge (BR)	20	15
	Cove Point (CP)	21	17
	Flag Pond (FP)	20	12
2019 (N=19)	Cove Point (CP)	1	1
	Flag Pond (FP)	18	9
2020 (N=14)	Cape Henlopen (CH)	14	6
2021 (N=26)	Cove Point (CP)	2	2
	Cape May (CM)	10	7
	Sandy Point (SP)	10	5
	Terrapin Park (TP)	4	2
2022 (N=23)	Cape Henlopen (CH)	12	5
	Cove Point (CP)	6	5
	Sandy Point (SP)	5	5
TOTALS		143	92

TABLE 1 Sample Collection by Year and Location.

DNA Isolation and Polymerase Chain Reaction

Genomic DNA was isolated using a Zymo DNA mini-prep kit according to the manufacturer's instructions with several modifications. To prevent DNA degradation by endogenous nucleases, a smaller than recommended volume of HSC blood was utilized (10 ul). In addition, HSC blood tends to coagulate due to the presence of limulus amebocyte lysate (LAL), making samples very viscous. Care was taken to include some portion of liquid and coagulated material in each preparation.

After purification, DNA samples of approximately 0.1 mL were obtained. The concentration and quality of samples were determined spectrophotometrically and by visualization on 1.5% agarose gels (Figure 2). The target sequence results in a 992 bp amplification product. PCR samples of 992 bp were sequenced (Genewiz). If a sample did not produce an amplification product, the reaction was repeated. If two rounds of amplification were

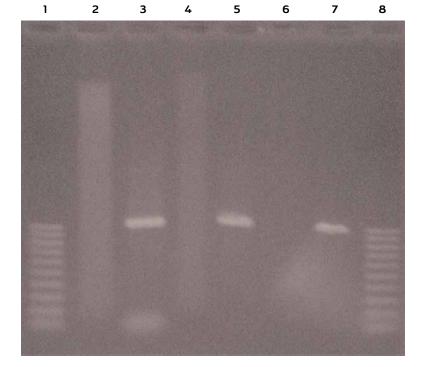


FIGURE 2

Agarose gel of Samples. Lanes 1 and 8 contain a marker. The band closest to the wells is a 1000 bp fragment. Lanes 2, 4, and 6 contain genomic DNA isolated from CP12, CP19 and FP9, respectively. Lanes 3, 5 and 7 contain amplified DNA products from CP12, CP19 and FP9, respectively. Note: the small band in lane 3 of less than 100 bp is primer-dimer, an artifact that does not affect results.

unsuccessful, the DNA sample was discarded and a fresh genomic DNA sample was prepared.

The success of genomic preparation and amplification were not solely based on the calculated purity, as determined by spectrophotometric analysis and agarose gel electrophoresis. Figure 2 shows the same volume of three genomic preparations run alongside the amplification results from those genomic samples. Although lane 6 does not appear to contain any discernible DNA, amplification of the target was successful (Figure 2, lane 7). Approximately 10% of HSC genomic DNA preparations were unsuccessful at least twice, and the basis of this failure is unclear. This is an area of further investigation.

DNA Sequence Trimming and Alignment

Amplification products of the expected size were sequenced on both strands using forward and reverse PCR primers (Genewiz). Sequences obtained from the vendor were obtained as text files and electropherograms to visualize the quality of the sequence obtained. The forward and reverse strand sequences were converted to FASTA files and uploaded into DNA subway Blue Line for analysis (dnasubway.cyverse.org). Within the DNA Subway Blue Line the forward and reverse sequences from each DNA template were aligned, compared, and trimmed to the portion of sequence with the highest confidence, where both strands were in agreement.

The trimmed sequences were saved as FASTA files and uploaded into Ugene for alignment and comparison (Okonechnikov et al. 2011). Sequences obtained in this study were compared to two previously published L. polyphemus mitochondrial DNA sequences, NC_003057 (Lavrov et al. 2000) and JX983598 (Sarmiento et al. 2021). While the exact location and date of collection for the two reported sequences are not specified in references, the NC_003057 was collected decades before the JX983598 sample (Lavrov et al. 2000; Sarmiento et al. 2021), and the NC_003057 sample was collected along the southern portion of the U.S. eastern coastline, while JX983598 was collected along the New Hampshire coastline.

As illustrated in Figure 3, the two reported mitochondrial sequences contain a number of differences in the region of interest. In alignments it was found that sequences in this study are significantly more similar to JX983598, with only one difference between JX983598 and all sequences in this study. Therefore, JX983598 was used as the reference haplotype against

NC_003057	12754	ACGAGGACCATCGATTACAGAGCAGATTCCTCTGAACAGCTTAAAGCACCGCCAAATTTT	12813
JX983598	8445	ACGAGGACCATCGATTACAGAGCAGATTCCTCTGAACAGCTTAAAGCACCGCCAAATTTT	8386
NC_003057	12814	TTAGGTTTCATGATCAACAATTACTACCCTAATTTCCTTTACACCTTAAAATAACAGGGT	12873
JX983598	8385	TTAGGTTTCATGATCAACAATTACTACCCTAATTTCCTTTACACCCTTAAAATAACAGGGT	8326
NC_003057	12874	ATCTAATCCTGGTCTATAATCAAAGCTTCaaaaaacaaaacTAACCTATTATCCCCAA	12933
JX983598	8325	ATCTAATCCTGGTCTATAATCAAAGCTTCAAAAAAAAAA	8266
NC_003057	12934	статалалаттстасстттсаладатала аталаталадабатталалаастсаталтттала	12993
JX983598	8265	CTATAAAAAATTCTACCTCTCAAGATAAATACAATAGGTATTAAAAAACTCATAATTTTAAA	8206
NC_003057	12994	AATCAAAAAGTTTAACTAAGGTCCTCTGTCTAACCGCGGTAGCTGGCACAATTTTAACCG	13053
JX983598	8205	AATCAAAAAAATTTAACTAAGGTCCTCTGTCTAACCGCGGTAGCTGGCACAATTTTAACCG	8146
NC_003057	13054	ACCTAACTTATAAATTACCGAACCTCACCCTAATGAGTTATTCAGAACTAAATACTACGa	13113
JX983598	8145	ACCTAACTTATAAATTACCGAACCTCACCCTAATGAGTTATTCAGAATTAAATACTACAA	8086
NC_003057	13114	aaaaacaacaaaaatciaattaaattgtcatasaaatctaagtatgtttatattattat	13173
JX983598	8085	AAAAAAACAACAAAAAATTAAATTAAATTGTCAAAAGAAATCTAAGTATGTTTATATTATTCAT	8026
NC_003057	13174	ACAGTTAAAGAAAAATCAAAATCAAAATTTAATGTTCTTTTTATATATCTCGGTAAAATT	13233
JX983598	8025	ACAGTTAAAGAAAAATCAAAATCAAAATTTAATGTTCTTTTTATCTATACTCCGGTAAAATT	7966
NC_003057	13234	CTTATTATTTCTACtttttttACATTGTAGaaataatattatattattatatgaaatta	13293
JX983598	7965	CTTATTATTTCTACTTTTTTACATCGTAGAAATAATATTATTATTATTATTATGAAATTA	7906
NC_003057	13294	taagactaaaaattttaaaaaatctatttccaagtaaaaatttttattta	13353
JX983598	7905	TAAGACTAAAAATTTTAAAAAAATCTATTTCCAAGTAAAAATTTTTATTTA	7846
NC_003057	13354	aataaatGTATACCCCCCATTATGCTTAAATTGTCTTAAGATATTTTATAATTTACTTA	13413
JX983598	7845	AATAAATGTATACCCCCCATTATGCTTAAATTGTCTTAATATATTTTATAATT	7786
NC_003057	13414	TTCTTCATGTGGTTTATTTATTCTACCAAATGATAATCAGGATCATAGTTTAGTATACTT	13473
JX983598	7785	TTCTTTA GTGGTTTATTTATTCTACCAAATGATAATCAGGATCCTAGTTTAGTTT	7726
NC_003057	13474	TAATCGCCCATTTAAGATAGAATAATTTATAAGTGTTCATTAGATAATAGATGAGATATT	13533
JX983598	7725	TAATCGCCCATTTAAGATAGAATAATTTATAAGTGTTCATTAGATAATAAATGAGATATT	7666
NC_003057	13534	TCTTTGAAAAAGTATAATAGTGTAATTACTCAACATTCAAAAAGGTGAAGTGCCTGATTA	13593
JX983598	7665	TCTTTGAAAAAGTACAATAGTGTAATTACTCAATA	7606
NC_003057	13594	AAGGATTATTTTGATGGAATAAATCAAATAGGGAAACCCTATCTCCACCTAATTTTAATG	13653
JX983598	7605	AAGGATTATTTTGATGGAATAAA	7546
NC_003057	13654	GTTTGAACCATCCCTTTTGAAATCAAAATTCAACGTGCCGTACACCAAAAATTAGAAAAG	13713
JX983598	7545	GTTCGAACCATCCCCTTTGAAATCAAAATTCAACGTGCCGTACACCAAAAATTAGAAAAG	7486
NC_003057	13714	TAAGCTAATTTAAGCTATTGGGCTCATAACCC 13745	
JX983598	7485	TAAGCTAATTTAAGCTATTGGGCTCATAACCC 7454	

FIGURE 3

Alignment of L. polyphemus mitochondrial sequences. Accession numbers NC_003057 and JX983598 were aligned in the region of the mitochondrial DNA that was targeted by primers. The underlined sequence illustrates the non-coding region. The red boxes indicate sites that differ between the two published mitochondrial DNA sequences. Twenty-one of those positions in JX983598 agree with the samples collected and analyzed in this study. The red arrow indicates position 7731 in JX983598 is an A, but is a T in samples in this study.

Haplotype Designation ^{a,b}	Number of Samples with Variant Sequence	JX983598 Position	JX983598 Sequence	Variant Sequence
MIT03	1	7693	С	Т
MIT04	3	7720	С	Т
MIT05	5	7737	А	G
MIT06	6	7738	С	Т
MIT07	1	7778	А	С
MIT08	1	7676	Т	С
МІТ09	1	8040	С	Т
MIT10	1	8037	А	G
MIT11	2	8101 8126	C C	T T
MIT12	4	8126	С	Т

TABLE 2

Haplotype Specifics. ^aHaplotypes MIT01 and MIT02 are not not included. Although changes were found in those samples, they require verification with additional sequence runs. ^bAll sequences collected over the course of this study contain a single allele difference when compared to JX983598 at position 7731. The JX983598 sequence contains an A at the position while all samples from this study contain a T at the position.

which samples in this study were compared and which was used for variant position numbers were identified.

Once JX983598 was chosen as the reference sequence, each trimmed sequence was uploaded into Ugene and aligned with ClustalW (Thompson et al. 1994). Differences between the reference and individual sequences collected during this study were noted and used to identify and name haplotypes (Table 2). All samples with the same allele sequence were grouped. Table 2 shows the position of each variant difference, with the nucleotide found in the variant and the sequence in JX983598. With the exception of haplotype MIT11 (samples CM3A and FP30), containing two

Site Name	Number of Samples Sequenced	Number of Variant Samples	Number of Variant Haplotypes	Haplotype Diversity
Bay Ridge (BR)	15	3	2	0.3846
Cape Henlopen (CH)	11	1	1	0.1818
Cape May (CM)	7	3	1	0.6667
Cove Point (CP)	25	10	5	0.6482
Flag Pond (FP)	21	7	7	0.6140
Sandy Point (SP)	11	0	0	0.0000
Terrapin Park (TP)	2	1	1	1.0000



Sample Variants by Location.

polymorphisms compared to JX983598, each variant haplotype contains one nucleotide difference in the region of interest.

Frequency of Variants in Study Samples

Table 3 shows the frequency of nucleotide variance at the seven sample locations along the Chesapeake and Delaware Bays during the study period (2018–2022). A total of 25 of the 92 sequenced samples were found to contain SNPs. With the exception of Sandy Point, SNPs were identified in at least one sample at every location. Cove Point and Flag Pond both have the highest number of samples collected and sequenced.

Genetic Diversity and Population Structure

DnaSP 6 software requires sequences of equal length, so the lengths of sequence were trimmed to 472 nucleotides, including the entire non-coding region, 347 nucleotides, plus 125 nucleotides that encode short tRNAs and a portion of the 12S rRNA gene. To maximize the length analyzed 9 sequences needed to be extended with data that has not been confirmed in multiple sequencing runs. In no case did the extensions involve any of the SNP sites. Cape May, Cove Point, and Flag Pond demonstrated relatively high haplotype diversity, while Sandy Point, Cape Henlopen, and Bay Ridge demonstrated relatively low haplotype diversity (Table 3). All had low nucleotide diversity, with all values below 0.0022 (data not shown).

Haplotype	Sample Name	Date Collected	Variants Per Site
MIT03	FP13	6/26/18	FP-1
MIT04	BR7	6/25/18	BR-1
	CP18	6/29/18	CP-1
	FP18	6/26/18	FP-1
MIT05	CP8	5/27/18	CP-2
	BR2	6/25/18	BR-2
	BR14	6/25/18	FP-1
	CP17	6/26/18	
	FP33	2019	
MIT06	CP14	6/26/18	CP-5
	CP12	6/26/18	FP-1
	CP13	6/26/18	
	CP15	6/26/18	
	CP24	6/26/18	
	FP27	6/26/18	
MIT07 ^a	CP10	6/27/18	CP-1
MIT08	CP27	6/15/22	CP-1
MIT09	FP24	6/26/18	FP-1
MIT10	FP20	6/26/18	FP-1
MIT11	CM3	6/7/21	FP-1
	FP30	2019 ^b	CM-1
MIT12	CH8	7/17/20	CH-1
	CM1	6/7/21	CM-2
	CM2	6/7/21	TP-1
	TP2	6/10/21	

TABLE 4

Variants by Location and Date. ^aNeeds confirmation because the same sample has another sequence run that does not contain the allele difference. ^bThe exact collection date was not recorded. Table 4 shows more specifics for each variant including the date they were collected and the number of variants per each location of collection. Flag Pond had the greatest number of samples belonging to different haplotypes, with seven different types. Five out of the ten Cove Point variants belong to MIT06. Five haplotypes were identified at multiple locations, and five were observed at only one location.

	BR	СН	СМ	СР	FP	SP	TP
BR	-						
СН	.036	-					
СМ	.196ª	.111	-				
СР	.039	.083	.185ª	-			
FP	0 ^b	0 ^b	.089	.008	-		
SP	.051	.000	.250	.100	0	-	
ТР	.015	0 ^b	0 ^b	.047	0 ^b	0	-

TABLE 5

Pairwise F_{ST} between HSC populations. ${}^{\mathbf{a}}F_{ST}$ value shows significance (p<0.05). ${}^{\mathbf{b}}F_{ST}$ negative values that are recorded as 0.

Pairwise F_{ST} values were calculated in Arlequin (Excoffier et al. 2010). Values were relatively low, and negative F_{ST} values are effectively considered the same as zero, so they were reported as such (table 5). A zero value for F_{ST} suggests that there is no genetic subdivision between the two populations compared. Only two comparisons, Cape May to Bay Ridge and Cove Point to Cape May reached significance.

Across the seven locations, F_{ST} was calculated to be 0.0546,

where a value less than 0.05 indicates little overall differentiation, and 0.05 indicates a moderate differentiation between the populations at the seven sites (Del Carpio et al. 2011). The AMOVA results also showed that most variation was found within each population rather than between populations (table 6).

Source of Variation	Degrees of Freedom	Percent Variation	F _{ST}	<i>p</i> -value
Among Populations ^a	6	5.46	0.0546	0.022 (+/-0.005)
Within Populations	81	94.54		

TABLE 6

Analysis of Molecular Variation (AMOVA). ^aPopulations: BR, CH, CM, CP, FP, SP, TP.

DISCUSSION

HSC spawning behavior involves coming up onto accessible beaches during high tide, with the highest numbers spawning during full and new moons during the months of June and July. When the HSCs emerge for spawning they are visible for surveying to track population sizes at each location. There are a number of completed and on-going surveys of HSCs along the east coast of the United States (myfwc.com, delawarebayhscsurvey.org). However, after the crabs leave the beach and enter the water, there is much less known about their activities and movement.

In the absence of feasible methods to track large numbers of HSCs between spawning seasons, finding DNA variation patterns will offer insight into how and if the HSCs migrate between locations. Analysis of mitochondrial DNA is a common method for studying genetic diversity among and between geographically separated populations (Aini et al. 2021; Zhan et al. 2021). In several previous studies the genes *cytb* and *cox1*, have been utilized to analyze genetic diversity and population structure. Both *cytb* and *cox1* are protein-encoding genes, so they are expected to be under selective pressure, potentially decreasing the mutation rate. These loci are also referred to as adaptive sites. Such sites are evolutionarily important, considering that mutations that are deleterious would be expected to be selected against and not accumulate in the population (Holderegger at al 2016). For the purposes of studying gene flow, however, non-coding, neutral loci are useful and may require a smaller sample size.

HSC mitochondrial DNA, however, is very efficient and has only small stretches of non-coding DNA. The largest, of approximately 347 nucleotides, is bordered on both sides by several tRNA-encoding genes and a 12S rRNA gene. To increase the size of the targeted sequence in this study to increase the chance locating SNPs, these RNA encoding genes were included in the amplified targets. While the inclusion of both neutral and adaptive loci in the study may be predicted to complicate analysis of gene flow statistics, mutation percentage was 1.7% in non-coding and 4.0% in coding. This contradicts the assumption that coding regions accumulate mutations more slowly.

While the amplified product in this study was 991 bp, after alignment of the complementary strands and trimming of uncertain nucleotides at the ends, the sequence aligned and analyzed in DnaSP6 and Arlequin was 472 bp. This included the entire non-coding 347 bp region and 125 nucleotides that encode short tRNAs and a portion of the 12S rRNA gene. Analysis of diversity and population structure was possible with our data, with the goal to eventually have confidence in sequences over 600 bp and with five reads across each the target from each HSC collected. For example, in the areas adjacent to the 472 bp analyzed in this study, several samples contained additional potential SNPs. However, there were not sufficient samples or sequence repeats to include in the haplotype classification.

Although 150 samples have been collected and close to 100 individual HSC sequences have been analyzed, this project is still

in an early stage of development. Reaching confidence in statistical analysis of single polymorphisms in relatively short sequences, in this case about 450 nucleotides, requires a large sample size and multiple DNA sequence repeats for confirmation. Additional analysis of samples previously collected and collection of additional samples are underway. Even with the limited data set, a number of statistical analyses were possible. There are many genomic DNA analytical packages available, more than can be conveniently listed here, but many were inappropriate for this study. Some analyze much larger DNA targets, up to and including entire genome sequences, were developed for use with diploid autosomal chromosomes, or require very large data sets. DnaSP 6 and Arlequin were chosen to calculate haplotype diversity (Hd), nucleotide diversity, genetic differentiation (FST), and AMOVA, and they were chosen to match earlier studies analyzing DNA targets of less than 1000 bp and fewer than about 500 samples (Aini et al. 2021; Zhan et al. 2021).

Our results suggest that there is more migration occurring than would be predicted from HSCs returning annually to the same beach, and that there is significant gene flow among the locations in the Chesapeake Bay, as indicated by relatively high genetic diversity values at many sites, insignificant FST values in pairwise analyses and an overall FST that suggests only moderate genetic differentiation among all of the sites.

Of note, in the two pairwise analyses that reached significance, suggesting regional differences between populations spawning at each site, the comparison involved a site on Chesapeake Bay compared to Cape May, at the mouth of the Delaware Bay (Table 5). This differentiation was not observed between any of the sites on Chesapeake Bay when compared to Cape Henlopen, also on the Delaware Bay, although Cape Henlopen is on the southern shore of Delaware Bay, closer to the Chesapeake Bay.

In addition, Cape Henlopen displayed relatively low genetic

diversity, and Sandy Point didn't show any diversity across 11 samples collected two years apart. The basis for the low diversity at those two locations is unclear and highlights the need for additional sample collection and analysis.

To increase confidence in the results already obtained and to gain additional information, more samples need to be collected, more of the collected samples need to be processed, more targets need to be amplified with more replicates, and the length of the target DNA needs to be increased. All of these goals are in progress along with utilizing other statistical analysis measures. For example, with a larger number of samples from both bays, regional genetic differentiation analysis can be performed comparing samples from three regions, northern Chesapeake Bay, southern Chesapeake Bay, and mouth of Delaware Bay. Finally, the amount of migration from the eastern to the western shore of the Chesapeake Bay might be analyzed with collection of more samples at Terrapin park on the western shore and inclusion of new sites along the western shore.

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REFERENCES

- Aini NK, Wardiatno Y, Effendi H, Madduppa H. 2021. High genetic diversity and mixing of coastal horseshoe crabs (*Tachypleus gigas*) across major habitats in Sundaland, Indonesia. PeerJ. 9:e11739. https://doi.org/10.7717/peerj.11739.
- Botton ML.2009. The ecological importance of horseshoe crabs in estuarine and coastal communities: a review and speculative summary. In: Tanacredi JT, Botton ML, Smith D, editors. Biology and conservation of horseshoe crabs. Boston (MA): Springer; p. 45–63. https://doi.org/10.1007/978-0-387-89959-6_3.

- Brousseau JL, Sclafani M, Smith DR, Carter DB. 2004. Acoustic-tracking and radio-tracking of horseshoe crabs to assess spawning behavior and subtidal habitat use in Delaware Bay. N Am J Fish Manag. 24(4):1376–1384. https://doi.org/10.1577/15 48-8675(2004)24%3C1376:AAROHC%3E2.0.CO;2.
- Del Carpio PD, Basnet RK, De Vos RCH, Maliepaard C, Visser R, Bonnema G. 2011. The patterns of population differentiation in a *Brassica rapa* core collection. Theor Appl Genet. 122:1105–1118. https://doi.org/10.1007/s00122-010-1516-1.
- Excoffier L, Lischer HE. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour. 10(3):564–7. https://doi.org/10.1111/j.1755-0998.2010.02847.x.
- Johnson KT. 2016. Population genetic analysis of Atlantic horseshoe crabs (*Limulus poly-phemus*) in Coastal Massachusetts [master's thesis]. Amherst (MA): University of Massachusetts. 55 p. https://doi.org/10.7275/7940684.
- Holderegger R, Kamm U, Gugerli F. 2006. Adaptive vs. neutral genetic diversity: implications for landscape genetics. Landsc Ecol. 21:797–807. https://doi.org/10.1007/ s10980-005-5245-9.
- King TL, Eackles MS. 2004. Microsatellite DNA markers for the study of horseshoe crab (*Limulus polyphemus*) population structure. Mol Ecol Notes. 4:394–396. https://doi. org/10.1111/j.1471-8286.2004.00663.x.
- Krisfalusi-Gannon J, Ali W, Dellinger K, Robertson L, Brady TE, Goddard MK, Tinker-Kulberg R, Kepley CL, Dellinger AL. 2018. The role of horseshoe crabs in the biomedical industry and recent trends impacting species sustainability. Front Mar Sci. 5:185. https://doi.org/10.3389/fmars.2018.00185.
- Lavrov DV, Boore JL, Brown WM. 2000. The complete mitochondrial DNA sequence of the horseshoe crab *Limulus polyphemus*. Mol Biol Evol. 17(5):813–24. https://doi. org/10.1093/oxfordjournals.molbev.a026360.
- Okonechnikov K, Golosova O, Fursov M. 2012. Unipro UGENE: a unified bioinformatics toolkit. Bioinformatics. 28:1166–1167. https://doi.org/10.1093/bioinformatics/ bts091.
- Rozas J, Ferrer-Mata A, Sánchez-DelBarrio JC, Guirao-Rico S, Librado P, Ramos-Onsins SE, Sánchez-Gracia A. 2017. DnaSP 6: DNA sequence polymorphism analysis of large data sets. Mol Biol Evol. 34(12):3299–3302. https://doi.org/10.1093/ molbev/msx248.

- Sarmiento ME, Chin KL, Lau NS, Aziah I, Norazmi MN, Acosta A, Ismail N, Yaacob NS. 2021. Mitochondrial DNA sequence of the horseshoe crab *Tachypleus gigas*. Mitochondrial DNA B Resour. 6(6):1710–1714. https://doi.org/10.1080/238023 59.2021.1930213.
- Saunders NC, Kessler LG, Avise JC. 1986. Genetic variation and geographic differentiation in mitochondrial DNA of the horseshoe crab, *Limulus polyphemus*. Genetics. 112(3):613–27. https://doi.org/10.1093/genetics/112.3.613.
- Smith DR, Pooler PS, Swan BL, Michels SF, Hall WR, Himchak PJ, Millard MJ. 2002. Spatial and temporal distribution of horseshoe crab (*Limulus polyphemus*) spawning in Delaware Bay: Implications for monitoring. Estuaries. 25:115–125. https://doi. org/10.1007/BF02696055.
- Thompson JD, Higgins DG, Gibson TJ. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22(22):4673–80. https://doi.org/10.1093/nar/22.22.4673.
- Zhan HX, Hao ZP, Tang R, Zhu LN, Zhao JJ, Hou SM. 2021. High genetic diversity and strong genetic structure of *Strongyllodes variegatus* populations in oilseed rape production areas of China. BMC Ecol Evol. 21:18. https://doi.org/10.1186/ s12862-021-01752-6.

MARGARET C. NESTER

Horseshoe Crab Aquaculture as a Model to Study Behaviors and Population Stability

ABSTRACT

Horseshoe Crabs (HSC), Limulus Polyphemus, are arthropods that have existed for millions of years. These animals play an important role in the ecosystem as they are a food source for a variety of species, including sharks, sea turtles, and bony fish. Additionally, their eggs provide a crucial food source for migratory shorebirds and their blood contains a compound Limulus Amebocyte Lysate (LAL) that is used in biomedical research to test injectable drugs for bacterial contamination. HSCs have undergone very little structural change throughout evolution, but are now exposed to a variety of factors including pollution and changing water chemistry due to human activity and climate change. A number of studies have tracked spawning behaviors in nature, but there is still much to learn about other behaviors and how they respond to various environmental changes. The ability to conduct studies in a controlled environment is an important tool in studying these ancient organisms. In this study, HSCs have been successfully established and maintained in laboratory culture, a stable diet established and initial feeding studies have been conducted.

INTRODUCTION

The Atlantic Horseshoe Crab (*Limulus Polyphemus*) is considered a

KEY WORDS

feeding behavior

limulus

aquaculture

FACULTY MENTORS

Tammy Domanski, Ph.D. Professor, Biology Department and Director, Environmental Center

Owen Keys, B.S. Chesapeake Conservation Corps keystone species, playing an important role both economically and ecologically (Botton 2002). They are necessary bait for a number of eel and whelk fisheries, the source of an endotoxin testing agent with its blue blood, and a food source for a number of migrating birds that feed on the HSC eggs (Krisfalusi-Gannon et al. 2018). Annual surveys are performed in many areas to monitor populations and inform local management programs (Brockman et al. 2011; Estes et al. 2021). However, field surveys have limitations. While surveys check size and health of captured HSCs, they can't study the response to specific condition changes or observe individual HSCs over time.

Additionally, there are already indications that the HSC population is at risk. The Delaware Bay, the location of the largest population of HSC spawning in the world, has seen an order of magnitude decrease in HSC egg availability for migrating birds since the 1990s (Smith et al. 2022). The population in the Chesapeake Bay is small compared to the Delaware Bay, and the exact count is uncertain. Maryland Department of Natural Resources sponsors a public HSC spotting website, but there is not a reported annual count. Additionally, there are two reporting databases within the Anecdata, a free platform for community science (anecdata.org).

HSCs have been on the International Union for Conservation of Nature's (IUCN) vulnerable list since 2016, and are a depleted population as of 2022 (Smith et al. 2022). Harvesting in the Mid-Atlantic Region is regulated by the Atlantic States Marine Fisheries Commission, which has recently revised the framework to respond to concerns for Calidris canudus conservation, migrating birds that rely on HSC eggs during migration (ASMFC 2021).

In addition, bleeding HSCs for LAL results in an estimated mortality ranging from 8% to over 30% (Hurton and Berkson 2006; Hamilton et al. 2020). The LAL harvest procedure incorporates multiple stressors, several of which have been shown to alter both the behavior and physiology of HSCs (Anderson 2013).

Concerns about HSC population size have prompted increased research into their ecology, in part to better understand foraging behavior and the impact of changes in food sources and feeding sites due to anthropogenic influences (Lee 2010). Adult HSCs feed on a wide range of invertebrates during their spring spawning migration in Delaware Bay (Botton 1984). This can include many species of marine worms, bivalves and crustaceans. Study of HSC food selection and feeding behaviors have highlighted some of the methods they use for feeding. HSCs use chela, the last pair of walking legs, and chelicerae, non-walking legs, to direct food into their mouths and typically feed on sources no larger than their mouths (Razali et al. 2020). Having multiple eyes, vision might be expected to be important for HSC detection of prey. However, researchers have explored the visual processes of horseshoe crabs and found that in general their vision is poor (Berkson and Shuster 1999; Zaldivar-Rae et al. 2009; Carmichael and Brush 2012) and does not appear to be used in foraging for food (Barlow 2001; Saunders et al. 2010). While multiple eyes and photoreceptor cells are present, there is no reported use for the ommatidia in foraging for food (Razali et al. 2020). Several studies have indicated that chemoreception is essential in foraging and that HSCs may only respond to chemicals in a solution, including solutions of particular amino acids (Hayes and Barber 1982; Razali et al. 2020). HSCs, like many other crustaceans, are environmental food generalists, but that does not necessarily mean that they do not have food preferences, as they will show preference when food is abundant and varied (Carmichael et al. 2009).

Initial studies had two goals in mind, a desire to learn about this population's feeding behaviors and a desire to understand their care requirements. These goals further research because establishing a baseline for care and basic survival behaviors in captivity is necessary for hypothesis-driven studies. While there are some other studies on behavior that are referenced within this work, understanding behavioral of this particular population in this particular aquaculture setting is necessary as a comparison if there are adverse responses in future studies that significantly change their environment.

RESEARCH METHODS

Aquaculture Tank Preparation and Maintenance

Preparation of 2, 200-gallon tanks included cleaning the tanks, filling the tank with purified water, and adding 25 mummichogs as a seed population. Salt was added to a final concentration of 30 ppt, and bacterial colonization with Microbacter (Brightwell Aquatics). To avoid accumulation of toxic ammonia, a protein skimmer was added to each tank (Octo Classic 150-INT). Tank conditions are monitored and logged daily including pH, temperature, nitrate/ nitrite and ammonia levels.

Two sock filters per tank are utilized to collect impurities that are caught in the system. The socks must be cleaned weekly with extensive rinsing and soaking in a 10% bleach solution followed by additional extensive rinsing. The tank also runs on a UV lamp pump. Water is pushed through a pipe which is flooded with UV light, inhibiting bacterial and algal growth.

Once the tank conditions stabilized, ten juvenile HSCs were obtained (Dynasty Marine Associates; Marathon, FL) and added to one of the tanks, and the mummichogs were moved to a separate tank. Play sand was thoroughly rinsed and autoclaved before adding to several areas in the tank, first in shallow trays, then in piles in several areas of the tank.

Water levels are monitored continuously and tap water is added to maintain level and salinity. Water changes occur on an as needed basis. Generally, thirty gallons of water are drained and thirty gallons of new clean saltwater is added. This equates to about a fifteen percent water change. Tank cleaning involves scrubbing, siphoning, and water replacement.

HSC Care, Feeding and Health Assessments

The HSCs are fed a diet of pre-cut squid, sea clams, bait shrimp, and shiners (Anglers, MD). The ingredients are blended together into a chunky paste that is then frozen into 2 g cubes. The HSCs are fed at least 12 g of food on each weekday. On days when feeding studies are conducted, they are not fed until the study is conducted.

Each HSC was marked with its own unique color of nail polish for identification and tracking (Figure 2). These marks were made between the compound eye and the lower edge of the shell. Marks were made on both the left and right side of the HSC. As the markings wear off, or are lost as a crab molts, they are replaced.

The HSC's are assessed on a weekly basis to keep accurate data regarding growth patterns. The carapace width, HSC length and weight are measured weekly (Table 1). Carapace width is at the area where the carapace comes to a point between the hinge and the abdomen when looking from a dorsal view. The total length is recorded and is measured simply from the tip of the carapace to the tip of the tail. Lastly, the weight is recorded placing the HSC on a tared balance and subtracting excess water weight.

Timed Feeding Assessment

A cube of frozen food (2 g) was placed in a circular, clear, 20 cm diameter glass culture dish containing approximately 1 L of water taken from the HSC tank. The cube was slightly broken up, and a HSC was placed directly next to the food. The HSC behavior was recorded and the time it took for the crab to finish the food was measured. Each HSC was given a maximum of 30 minutes to eat before being placed back into the regular tank. The HSCs could be removed prior to the 20-minute mark if they had either finished the food or they were completely uninterested in food. The bowl was emptied and thoroughly rinsed before addition of fresh tank water for each of the HSCs. This assay was performed twice with one week separating the two trials.

Response to Food Availability

A 76 cm x 32 cm clear, rectangular glass tank was cleaned and rinsed thoroughly. Approximately 10 liters of salt water was added. In this experiment, the water in the tank was not from the tank housing the HSCs but was prepared fresh. This was to avoid removing an excessive amount of water from the HSC tank in a short period of time and to mitigate potential for food particles being in the water. A 2 g chunk of food and a rock of similar size and color were simultaneously placed at either end of the tank. The rock was placed in the tank to determine if the HSCs were using the sense of smell or if they were using visual input to find the food source. Once both sank to the bottom, a HSC was placed at the center of the tank, facing away from the handler. HSC behavior was recorded for approximately 30 minutes and the time to find and feed was also measured. Notes were taken regarding their activity level and a graded scale was utilized to score their attraction to the food source.

RESULTS

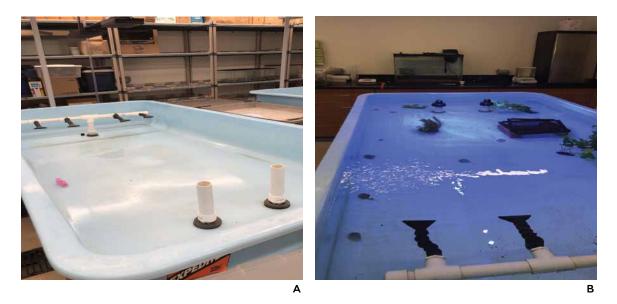
Establishment of HSCs in Aquaculture

Two aquaculture tanks were prepared for HSCs including cleaning, filling with water of the appropriate salinity (25–30 ppt), pre-inoculation with beneficial bacteria, and addition of mummichogs as indicators of water health. The salinity was raised over the course of a week so that the mummichogs that were assisting with the cycling of the tank could survive the changes in salinity and slowly adapt to the new set parameters (Table 1). Once optimal conditions were established and the mummichog population reached an equilibrium several weeks after the predicted

	Florida Condition	Center Conditions
Salinity (ppt)	30	25-30
Temperature (°C)	24-28	20

TABLE 1 Environmental Conditions Comparison.

initial death of approximately 30% of the introduced population, 10 HSCs were introduced into one of two tanks (Figure 1). To prevent competition, the mummichogs were placed in a separate tank. After about 6 weeks they were moved into another tank so that an algae problem could be fixed. They have remained in that tank since.



Monitoring Health

To track individual HSCs it was necessary to tag each with something relatively waterproof, non-invasive and unique to each. Nail polish was found to fit all of these requirements, so each HSC is marked on both sides of the carapace with a specific color (Figure 2a). The polish is applied while the HSC is taken out of the tank for health assessments or if one of the spots has worn off. FIGURE 1 Aquaculture Tank before preparation (a) and after (b).



FIGURE 2

Horseshoe crabs labeling and assessment. (a) HSCs are marked with individual colors on each side of the carapace. (b) HSC length, width and mass are measured weekly. The HSCs are assessed weekly, recording weight, carapace width and total length (Table 1). This is done to have an accurate assessment of their overall health and to track the rate of weight gain and growth. This data provides a window into the growth pattern of the HSCs.

DISTAN⁵ 01

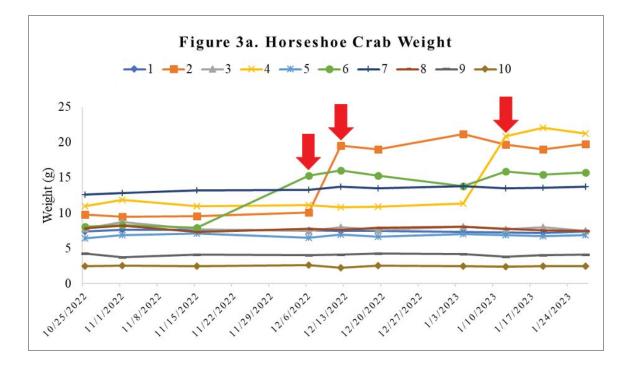
в

Over the first three months of assessment, which began within two weeks of the HSCs arriving, most parameters have remained stable for each HSC. When the juveniles arrived, they varied significantly in size and weight (Table 3). The only significant changes in any of the parameters occurred in the period following molting.

HSC #	HSC Color	Date	Weight (g)	Carapace Width (cm)	Total Length (cm)
1	Red	10/25/22	7.38	3.8	7.6
2	Green	10/25/22	9.8	4.2	8.8
3	Yellow	10/25/22	7.8	3.8	7.8
4	Purple	10/25/22	10.97	4.3	8.6
5	Blue	10/25/22	6.41	3.6	7
6	Olive	10/25/22	8.06	3.5	7.9
7	Black	10/25/22	12.63	4.5	10
8	White	10/25/22	7.84	3.5	8
9	Silver	10/25/22	4.29	3.4	6.5
10	Colorless	10/25/22	2.48	2.5	5.1

TABLE 2

Health Assessment Parameters. This Table shows the first data set recorded.



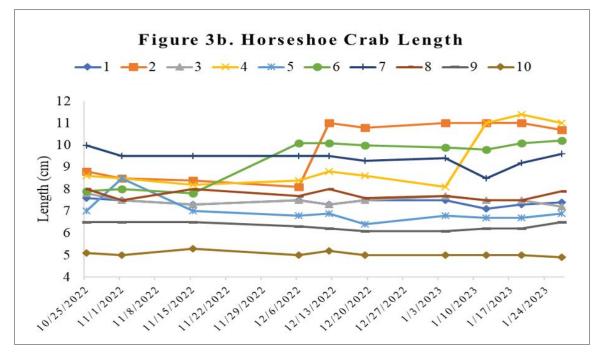


FIGURE 3

HSC measurements over time. (a) Weight measured over time. (b) Each number represents a specific HSC. The arrows indicate the significant weight increase after a HSC molted.

Response to Food Availability

Initial observations of feeding behavior were performed by adding food to the tank in several chunks distributed randomly, ensuring that the food sank. Initially, frozen minnows were irregularly cut into small chunks less than about 0.5 cm wide (as recommended

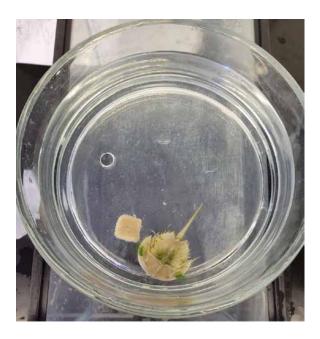


FIGURE 4

Timed feeding study. Each HSC was placed directly next to the 2 g cube of frozen food and observed from all sides, including the bottom to observe feeding behavior. by vendor). Casual observation suggested that HSCs were feeding but quantitative analysis was not performed to ensure that each HSC was attracted to, or ingesting food. There was also concern that the small size of the juvenile HSCs prevented them from being able to break up the fish chunks.

At approximately week 3, the HSCs were switched to a prepared, complex frozen fish meal (Fish Frenzy, LRS). However, the specific composition of the food was not provided on the label, and it was still unknown if all HSCs were eating a significant amount. After some research, a defined, blended food

source composed of pre-cut squid, 12 frozen minnows, 5 big shrimp and soft quahog clams was developed. The ingredients were blended and frozen, with small, irregularly sized chunks being used for daily feeding. This appeared to make it easier for the HSCs to break up and ingest.

A timed feeding experiment was designed to provide each HSC with a defined mass of food, separate from the other crabs (Figure 4). This assay was performed twice and in each trial the feeding behavior of each HSC was assigned a grade according to a scale developed for the assessment (Table 3). The HSCs were assigned a number from 0–5 reflecting their activity level and level of feeding. Of note, HSC4 (purple) did not eat and moved very little in both trials when compared with the other HSCs. HSC4 then molted four days after the second timed feeding assessment

HSC Number	HSC Color	Behavior Score ^a 12/19/2022	Behavior Score 1/4/2023
1	Red	3	4
2	Green	4	5
3	Yellow	2	4
4	Purple ^b	0	0
5	Blue	1	2
6	Olive	4	3
7	Black	2	3
8	White	2	3
9	Silver	1	2
10	Colorless	2	0

TABLE 3

Summary of Timed Feeding Experiments. ^aScores were assigned according to the following scale: 0-no movement, no feeding; 1-small amount of movement, eats very little; 2-ate up to half of food available, movement normal; 3-ate all food, movement normal; 4-ate all food, movement increased; 5-ate all food within first 5 minutes, hectic movement. bHSC molted four days after feeding trial 2.

was performed (Figure 3).

The timed feeding assessment established that most of the HSCs eat when presented with food in a small enclosed area. However, it was not clear how the HSCs find food when it is in their vicinity in a larger enclosure. Therefore, the next assessment tested the attraction of each HSC to the presence of food in the area, but not directly next to the HSC. In this study a 2 g chunk of food, and a rock of similar size and appearance were introduced to each side of each HSC, each approximately 0.5 m away from the HSC (Figure 5).

The behavior of each HSC was graded on a scale developed for this study (Table 4). Initial observation of the first two HSCs

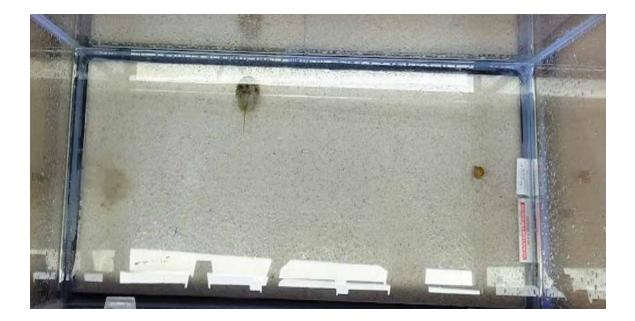


FIGURE 5

Behavior in the Presence of a Food Source. This image depicts an overhead view of the behavioral analysis tank set up.

placed in the tank, numbers 1 and 2, suggested that they were attracted to the food, due to their movement toward the side of the tank where the food was placed. However, neither ate the food. To eliminate distraction from outside of the tank (such as another small fish tank next to the feeding tank with active bubbling), for the third HSC the rock and food were switched. In that case the HSC moved toward the rock, in the direction of the other fish tank on the lab bench. This suggested that the HSCs might be responding to movement, not to the food provided. To prevent outside distractions, cardboard was used to shield each side (Figure 5). From that point on in the experiment, the responses were similar for most HSCs, with 50% of the subjects appearing to randomly find the food. Only 20% immediately found the food, but then ignored it and, the other 30% immediately located and ate the food.

HSC Number	HSC Color	Behavior Score ^a 1/13/2023
1	Red	4 ^b
2	Green	5 ^b
3	Yellow	4 ^c
4	Purple	3
5	Blue	3
6	Olive	3
7	Black	5
8	White	3
9	Silver	3
10	Colorless	5

TABLE 4

Response to Food Availability. ^aScores were assigned according to the following scale: 0-no movement, ignored food; 1-movement away from food, never found food; 2-movement away from food, appeared to randomly find food but did not eat; 3-movement away from food, appeared to randomly find food and ate all of the food; 4-movement toward food, but did not go to or eat food; 5movement toward food, found and ate food. ^bTesting tank did not yet have cardboard blocking the sides of the tank walls. ^cHSC was in the tank when the tank was moved, may have affected its behavior.

DISCUSSION AND FUTURE STUDIES

The first goal of this study, establishing a healthy population of HSCs has been accomplished, but additional parameters to assess health will be required moving forward. The ten HSCs shipped overnight from Florida and placed in the AACC aquaculture facility have survived and remained healthy, although the initial measurements only track size (Table 2 and Figure 3). One additional indicator of health status is molting, and three of ten have molted in the first three months at AACC. In the health assessment that was conducted it was very difficult to accurately gauge overall health from the current data collected. HSC weight only increases after molting, and in this study their mass nearly doubled soon after molting. Other measures of overall health might include measuring blood glucose levels or tracking heart rate, which could provide a real-time measure of health.

Blood glucose concentration in hemolymph is a common parameter measured to evaluate response to stress (Principe et al. 2019), and there are some reports about average glucose levels and other blood chemistry in HSCs (Smith et al. 2002; Arnold et al. 2021). Initial analyses with a point-of-care testing instrument (VQPet H) have resulted in inconsistent results (data not shown). There are conflicting reports on the validity of point-ofcare instruments so comparison to a standard glucose oxidase tube reaction will be performed (Allender et al. 2010; Principe et al. 2019). Other blood chemistry parameters are also being considered for testing.

Measuring heart rate is another parameter being considered for study. HSC heart rate is approximately 32 beats per minute. However, measuring heart rate in HSCs may require invasive placement of sensors that are not feasible for regular monitoring (Kodirov et al. 2018).

The second goal of the study, defining baseline feeding behavior in a captive population has resulted in some interesting results but will require more specific testing. Initial behavior observations were performed as the HSCs acclimated to their new environment in the aquaculture tank, and as several different food sources were tested. The vendor that the HSCs were obtained from reported feeding chopped minnows, but the HSCs did not seem to be attracted to that food when they arrived, and they appeared to have difficulty with any pieces of significant size (over 0.2 cm approximately). When the food source was switched to a pre-blended frozen mix containing a mix of a number of marine organisms, feeding improved. However, it was unclear if the HSCs were at all attracted to food or randomly found it during normal activity, which prompted the initial behavior studies that were performed. While the first feeding studies revealed that all HSCs will feed on the in-lab prepared food mix, there are still questions to be assessed including if the crabs are attracted to food or are opportunistic and only feed when they come in contact with food, if there are specific preferences for nutrient types and if there is competition for food among the crabs.

There was no indication from the second feeding study

performed that there is an immediate attraction to food in the HSC vicinity (Table 4), so additional studies utilizing foods in solution, more careful acclimatization to a feeding tank before introduction to food, and other variations will be tested. However, there were some observations of note. HSC4, marked with purple nail polish, did not move or eat during the second feeding study. This lack of activity was unusual when compared with the other HSCs. A pale appearance around the edges of the carapace along with some dark discoloration visible in and around the compound eyes suggested that it was ready molt. As predicted, this particular HSC did molt and increase its mass nearly 100% within a couple days of molting. These observations may prove indicative of a HSC preparing to molt, an important developmental step. It was also observed many times that the newly molted HSC remained fairly still for approximately 24 hours post-molt. Identifying a HSC preparing to molt will enable more careful monitoring of the process to learn more about how some key physiological parameters change during the stress of molting.

To better understand their movements and responses to changing stimuli, tracking of individual HSCs with video and infra-red light is in the early stages of implementation. It is difficult to identify patterns in movement from observation alone, and it is impossible to observe the crabs continuously. For example, in all three molt events, molting occurred overnight and was not directly observed if we have the proper camera in place there would be a video containing what it looks like when they molt and any potenital different behaviors. In addition, if there are complex patterns in HSC movements that are difficult to visually identify, data points that track position over time can be statistically analyzed to find patterns. There is precedent for this type of tracking (Dubofsky et al. 2013).

While this study is early in development, there has already been significant progress in establishing a healthy population of HSCs. Some of their most basic behaviors and responses, or lack of response, to certain stimuli have been observed and recorded. From these baseline data, additional studies to ascertain potential attraction to and competition for food will be assessed. Using behaviors studied under optimal conditions for comparison, the goal is to assess the response to adverse changes associated with anthropogenic effects like changes in food quantity, the presence of microplastics in their environment and changes in availability and quality of feeding areas.

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REFERENCES

- Allender MC, Schumacher J, George R, Milam J, Odoi A. 2010. The effects of shortand long-term hypoxia on hemolymph gas values in the American Horseshoe Crab (*Limulus polyphemus*) using a point-of-care analyzer. J Zoo Wildl Med. 41(2):193–200. https://doi.org/10.1638/2008-0175R2.1.
- Anderson RL, Watson WH, Chabot CC. 2013. Sublethal behavioral and physiological effects of the biomedical bleeding process on the American horseshoe crab, *Limulus Polyphemus*. Biol Bull. 225(3):137–151. https://doi.org/10.1086/BBLv225n3p137.
- Arnold JE, Hadfield, CA, Clayton LA, Cray C, Jones D, Payton, M. 2021. Development of methodology and reference intervals for the analysis of the free-ranging Atlantic horseshoe crab *Linulus polyphemus* hemolymph. Vet Clin Pathol. 50(2):259–272. https://doi.org/10.1111/vcp.12983.
- Barlow RB, Hitt JM, Dodge FA. 2001. Limulus vision in the marine environment. Biol Bull. 200(2):169–76. https://doi.org/10.2307/1543311.
- Botton, ML. 1984. Diet and food preferences of the adult horseshoe crab *Limulus polyphemus* in Delaware Bay, New Jersey, USA. Mar Biol. 81:199–207.

- Botton ML. 2009. The ecological importance of horseshoe crabs in estuarine and coastal communities: a review and speculative summary. In: Tanacredi JT, Botton ML, Smith D, editors. Biology and conservation of horseshoe crabs. Boston (MA): Springer; p. 45–63. https://doi.org/10.1007/978-0-387-89959-6_3.
- Brockmann JH, Johnson SL. 2011. Long-Term study of spawning activity in a Florida Gulf Coast population of HSCs (Limulus polyphemus). Estuaries Coasts. 34(5):1049–1057. https://doi.org/10.1007/s12237-011-9419-1.
- Carmichael RH, Rutecki D, Annett B, Gaines E, Valiela I. 2009. Diet composition of juvenile horseshoe crabs: implications for growth and survival of natural and cultured stocks. In: Tanacredi JT, Button ML, Smith DR, editors. Biology and conservation of horseshoe crabs. Boston: Springer. p. 521–534. https://doi.org/10.1007/978-0-387-89959-6_33.
- Chen Y, Robinson K, Nol E, Bopp J. 2021. Revision to the adaptive resource plan framework and peer review report. Atlantic States Marine Fisheries Commission (ASMFC) Washington DC. http://asmfc.org/uploads/file/63d41dc32021ARM_Framework RevisionAndPeerReviewReport_Jan2022.pdf.
- Dubofsky EA, Simpson SD, Chabot CC and Watson WH III. 2013. Patterns of activity expressed by juvenile horseshoe crabs. Biol Bull. 225:42–49. https://doi.org/10.1086/ BBLv225n1p42.
- Estes MG, Carmichael RH, Chen X, Carter SC. 2021. Environmental factors and occurrence of HSCs in the North Central Gulf of Mexico. PLOS One. 16(1):e0243478. https://doi.org/10.1371/journal.pone.0243478.
- Hamilton KL, Burnett LE, KG Kalisperis, Rachel EG, Fowler, AE. 2020. Physiological impacts of time in holding ponds, biomedical bleeding, and recovery on the Atlantic HSC, *Limulus Polyphemus*. Comp Biochem Physiol A Mol Integr Physiol. 239:110554. https://doi.org/10.1016/j.cbpa.2019.110554.
- Hayes WF, Barber SB. 1982. Peripheral synapses in *Limulus* chemoreceptors. Comp Biochem Physiol A Comp Physiol. 72(2):287–293. https://doi.org/10.1016/ 0300-9629(82)90221-3.
- Kodirov SA, Psyrakis D, Brachmann J, Zhuravlev VL. 2018. Limulus and heart rhythm. J Exp Zool A Ecol Integr Physiol. 331(1):61–79. https://doi.org/10.1136/bmj. 2.3481.544.

Krisfalusi-Gannon J, Ali W, Dellinger K, Robertson L, Brady TE, Goddard MK,

Tinker-Kulberg R, Kepley CL, Dellinger AL. 2018. The role of horseshoe crabs in the biomedical industry and recent trends impacting species sustainability. Front Mar Sci. 5:185. https://doi.org/10.3389/fmars.2018.00185.

- Lee WJ. 2010. Intensive use of an intertidal mudflat by foraging adult American Horseshoe Crabs Limulus polyphemus in the Great Bay Estuary, New Hampshire Curr Zool. 56(5): 611–617. https://doi.org/10.1093/czoolo/56.5.611.
- Razak MRM, Kassim Z. 2018. Feeding mechanism of adult tropical horseshoe crabs Tachypleus gigas toward feeds' conditions. ASM Sci J. 11(2):76–85.
- Razali FN. 2020. Updating a New trend in Horseshoe crab feeding behavior in captivity: towards a healthy practice of horseshoe crab rearing. Aquac Aquat Conserv Legis. 13(3):1394–1409.
- Schreibman MP, Zarnoch CB. 2009. Aquaculture methods and early growth of juvenile horseshoe crabs (*Limulus polyphemus*) In: Tanacredi JT, Botton ML, Smith D, editors. Biology and conservation of horseshoe crabs. Boston (MA). Springer. p501–511. https://doi.org/10.1007/978-0-387-89959-6_31.
- Smith JAM, Dey A, Williams K, Diehl T, Feigin S, Niles LJ. 2022. Horseshoe crab egg availability for shorebirds in Delaware Bay: dramatic reduction after unregulated horseshoe crab harvest and limited recovery after 20 years of management. Aquatic Conserv. 32(12):1913–1925. https://doi.org/10.1002/aqc.3887.
- Smith SA and Berkson J. 2005. Laboratory culture and maintenance of the horseshoe crab (*Limulus polyphemus*). Lab Anim. 34(7):27–35. https://doi.org.10.1038/laban0705-27.
- Smith SA, Berkson JM, Barratt RA. 2002. Horseshoe crab (*Limulus polyphemus*) hemolymph biochemical and immunological parameters. In: International Association for Aquatic Animals Medicine Conference Proceedings. https://www.vin.com/apputil/ project/defaultadv1.aspx?pid=11257&catid=&id=3864817&meta=&authorid=.

U.S. Military Propaganda in Film and Television

ABSTRACT

The U.S. has long used film and television to justify its military actions abroad. Films like Top Gun: Maverick and Zero Dark Thirty operate to entertain and bolster war-hungry and nationalistic narratives through negative depictions of enemy states and positive depictions of U.S. military actions. The Department of Defense directly supplies airplanes, soldiers, information, and military bases to film directors in exchange for control over the final film's script. Through both subtle and drastic changes made to many blockbuster films, a patriotic pro-war sentiment has emerged as a common theme amongst these film franchises. News media companies echo the same patriotism by exploiting the fears of the public such as threat of nuclear war or terrorism. Together, these industries provide a particularly brutal image of global politics to the American consumer. This essay seeks to answer the question: How does the U.S. Military utilize film and televised news media as a tool for propagandizing the American public?

INTRODUCTION

The United States is often viewed by its residents as exemplary of freedom: freedom of the press, freedom of speech, freedom of religion, etc. (Saad, 2013). Its citizens tend to refer to other states' governments (especially those of the U.S.'s enemy states) as enforcing extreme totalitarianism and restricting most, if not all, dissenting opinions from its populace. Analysis of 150 articles from CNN, the New York Times, and FOX News show that the word

KEY WORDS

propaganda film television military United States

FACULTY MENTOR

Darius M. Cureton, Ph.D. Assistant Professor, English and Transfer Studies "oligarch" is almost exclusively reserved for referring to Slavic billionaires. In contrast, U.S. billionaires are referred to as "businessmen" (Macleod, 2019). Inversely to the belief that the U.S. welcomes (or even allows for) political dissent, the U.S. government has subtly kept opposition on a tight leash since its inception. One particularly insidious way this is completed is in the cooperation between media entities and the Department of Defense to justify and manufacture consent for U.S. military action.

This collaboration, referred to as the Military-Entertainment complex, operates as a quid pro-quo for both media and military parties, as film crews gain access to authentic military equipment and resources while the Department of Defense gains complete control over said film's script (DoD assistance to non-government, entertainment-oriented media productions, 2015). The effects of the Military-Entertainment complex can be seen in almost any American theater. Upon arriving at one of these theaters, one will likely see advertisements for gritty war films like *Top Gun: Maverick*

FIGURE 1.1

Top Gun Maverick Global Premiere



Note. From Top Gun Maverick Global Premiere [Photograph], by O. O. McCoy, May 2022, Wikimedia Commons (https:// commons.wikimedia.org/wiki/File:Top_Gun_Maveric_ Global_Premiere_May_2022.jpg).

and patriotic superhero movies like *Captain Marvel* (Fig. 1.1). These blockbuster films become avenues through which the Department of Defense can justify and minimize U.S. wrongdoing and amplify—or outright fabricate the wrongdoings of other nations, as seen in examples discussed later in this study.

One may be tempted to believe that the Military-Entertainment complex is a purely modern invention that came about with the creation of the motion picture. While the Military-Entertainment complex evolved significantly and rapidly after the invention of the motion picture, it is ultimately a continuation of a previously established pattern of the promotion of U.S. interests through popular media.

The burning of Norfolk is one of the earliest examples of the U.S. Military fabricating events to advance its interests. Initially, the prospect of separation from the British Empire was an idea viewed with indifference by the U.S. working class (Broadwater, 2008). American members of the middle and upper class were frustrated with Britain's recent tightening of imperial trade laws, which American colonists were notorious for evading (Guo, 2016; *British reforms and colonial resistance, 1763–1766*, 2023).

When Lord Dunmore, the royal governor of Virginia, removed fifteen and a half barrels of gunpowder from Williamsburg's magazine and threatened to enlist enslaved peoples and indentured servants, promising their freedom, he greatly exacerbated these frustrations (Broadwater, 2008). When Lord Dunmore (Fig. 1.2) launched an attack at Great Bridge, he was defeated and was forced to surrender Norfolk (Broadwater, 2008). When Norfolk was surrendered to the Virginian army, they promptly burned the city and blamed it on the British forces (Broadwater, 2008). This shift of blame was conducted through the publication

FIGURE 1.2 John Murray, 4th Earl of Dunmore



Note. From John Murray, 4th Earl of Dunmore [Painting], by Reynolds, J., 1765, Scottish National Portrait Gallery, Scotland (https://www.nationalgalleries.org/ art-andartists/8802/john-murray-4th-earl-dunmore).

of newspapers, such as the London Chronicle's March 5th , 1775, issue, where the article claims, "Letters ... from Virginia mention that the town of Norfolk has been bombarded by the King's ships & a great part of it burnt down." (Hughes, 2022). American involvement in the burning of Norfolk was not acknowledged until 1777 (Broadwater, 2008). By the end of the American Revolution, middle-class citizens benefited the most, and slavery became even more entrenched in the states (Broadwater, 2008).

From the preceding account of the inception of the American Revolution and the burning of Norfolk, it is evident that the wealthy have a vested interest in swaying public opinion to achieve profitable ends. This strategy, of course, did not cease to be after the American victory against the British Empire. To explore the world of modern propaganda, we will first need to understand Noam Chomsky's Propaganda Model.

In his seminal work *Manufacturing Consent*, Noam Chomsky outlines how the wealthy control public opinion to generate support for war, invasion, and genocide abroad as well as disapproval of revolutionary action and dissent in enemy states. He proposes a Propaganda Model which can be explained as a gradual reduction of "raw news" through five filters: financial ownership, advertising, sourcing news, flak, and anti-communism, although, according to the authors, contemporary filters such as islamophobia are replacing anti-communism (Herman & Chomsky, 2002).

Financial ownership acts as a gate between the working class and the wealthy. The costs of maintaining a publication or producing a blockbuster film are too high for the typical person to afford. Thus, media power pools into the hands of the wealthy and are robbed from the working class. This allows narratives that support the profit motives of media conglomerates, most of whom have a significant stake in weapons contracting, to saturate popular narratives regarding overseas conflicts to keep climates as profitable as possible for said conglomerates (Herman & Chomsky, 2002).

Advertisement serves as the second filter in the propaganda model. Advertisers are essential to the media business, as they subsidize production costs for media companies. Without advertisers, media companies would have to significantly raise the price of their product(s). Thus, advertisers effectively decide if a media outlet can operate. Because of many media companies' membership to a larger conglomerate, many times conglomerates that are heavily involved in the war, energy, and oil industries, advertisers will fund media outlets that keep a "favorable climate" for their parent companies to continue to extract resources from the third world (Herman & Chomsky, 2002).

The third filter of the Chomsky propaganda model is sourcing. Media outlets usually get information from government officials and corporate spokespeople, as these officials are seen as reliable experts. These experts effectively control what news is reported and who receives "raw news" to publish. Thus, if a reporter or media outlet offends one of the conglomerates or governments they represent, that outlet no longer receives fresh news directly from "reliable" sources.

When news reports criticizing the aforementioned governments and conglomerates gain traction, the fourth filter of the propaganda model, flak, comes into play. Flak is the attack on critical reporting by other outlets or entities to minimize criticism. One can see this occur when reports of climate change are accused of fear mongering and radicalism.

Although dissolved in 2001, the Global Climate Coalition (GCC, pictured in Fig. 1.3.) is an excellent microcosm for the practice of delegitimizing dissent. The GCC had representatives of companies such as ExxonMobil, Ford Motor Company, Shell, and Texaco within its ranks. This lobbying organization discredited climate change reports by launching a U.S. campaign that advised against policy that reduced greenhouse gas emissions in response to global warming treaty talks in Kyoto. (Vidal, 2005) This campaign was ultimately successful, as President George Bush sought advice from ExxonMobil, influencing his decision not to sign on to the treaty (Vidal, 2005).

The fifth and final filter of the propaganda model in the 1980s, when *Manufacturing Consent* was initially published, was

FIGURE 1.3

Environmental activists protest Shell's membership in the Global Climate Coalition



Note. From Environmental activists protest Shell's membership in the Global Climate Coalition at an annual shareholder meeting [Photograph], by Cheskin, D., 1997, Getty Images (https://grist.org/accountability/how-the-globalclimatecoalition-derailed-climate-action/)

anti-communism. In American propaganda, The Soviet Union and Communism represented an existential threat to freedom of speech, democracy, and American values (Le, 2017). The U.S. and the Soviet Union viewed the Cold war as an ideological battle; thus, the war was fought by shifting public discourse against the opposing state (Ward et al., 2019). This battle of ideas spurred President Harry Truman to launch The Campaign of Truth, an "intensified program of propaganda ... in order to combat enemy lies" (Propaganda - Cold War, 2022). In the U.S., propaganda shifted "to hard-hitting propaganda in its most apparent form" (Propaganda - Cold War, 2022). This propaganda included cartoons with villainous depictions of both communists and the Soviet Union, as well as sensational news reporting.

The Psychological Strategy Board (PSB) was created by Truman and oversaw propaganda campaigns targeting numerous countries in Europe, Asia, and the Middle East (*Propaganda – Cold War, 2022*). These campaigns were outlined in National Security Council Document 10/2, including the following measures: "black propaganda, psychological warfare, subversion, assistance to underground resistance movements, paramilitary operations, and economic warfare." (*Propaganda – Cold War, 2022*). During this time, propaganda became synonymous with the Cold War and psychological warfare. However, this perception would later also become synonymous with the Soviet Union for reasons which will be covered later in this paper.

Since the attack on the Twin Towers in 2001, Antiterrorism has slowly begun to replace anti-communism as the fifth filter.

News Media and films alike push fear mongering narratives about "Islamic extremists" and even American citizens of Muslim and/ or of Middle Eastern descent. Anti-muslim media is almost everywhere these days and can be seen in the whitewashing of U.S. war crimes in the latest Call of Duty and in Zero Dark Thirty, where the horrific torture of Abu Ahmed is framed as a net moral positive (MacLeod, 2022; Bigelow, 2012). Right-wing news anchors and highly reported politicians such as Tucker Carlson and Ron DeSantis (who was recently found out to have actively participated in the torture of Guantanamo Bay inmates. This torture included practices like sleep deprivation, noise pollution, and forcefeeding.) regularly speak of Muslim people and people of Middle Eastern descent as if they are dangerous threats to the U.S. (Empire Files, 2022; Simpson, 2019). These alarmist narratives are not exclusive to the American right, however. Self-identified progressive voices see Islam as antithetical to American society and weaponize atheism to delegitimize the spiritual aspects of Islam (Beydoun, 2018). The use of these filters in U.S. media frames the Military and its actions as being "for the greater good" at best and heroic at worst. The U.S. Military, through the use of media, can justify its past actions and manufacture consent for future actions. Public consent for Military actions, particularly in the Middle East, dramatically serves the interests of defense contractors.

LITERATURE REVIEW

Film Propaganda in WWI

When the first World War broke out in 1914, both cinematographers and world governments realized the powerful reach film could achieve as propaganda (The Great War, 2016). There was a significant demand for actual war footage distribution, as the sheer scope of the war affected the entirety of the U.S. Initially, the public regarded the little actual footage of the war as "disappointing" (The Great War, 2016). This is because it was nearly impossible to film combat footage during the war. Most fighting was done in adverse conditions, such as at night, at dusk, and while raining. Cameras were also cumbersome and non-portable, which made it extremely dangerous for camera operators to crawl out of the trenches even when they had an opportunity to film live combat (The Great War, 2016). Even when cinematographers successfully managed to successfully film combat, the footage would likely be censored in the final cut. It became widely understood that combat footage was extremely unlikely to reach the public, so filmmakers decided to reenact military conflicts for the camera instead (The Great War, 2016). Staged pro-war films, such as *The Battle of Somme* and *Heart of the World* became huge successes, as they satisfied public demand for frontline footage.

The Battle of Somme was produced by Britain's War Information Bureau, the Wellington house. The Wellington house remains infamous in public perception of propaganda, as its expressed goal was to target neutral states (specifically the United States) and convince them to join the war in support of Britain (World-Information, 2007). The Wellington House produced large-scale propaganda efforts in almost every medium, creating everything from recruitment posters to political cartoons (The Great War, 2016). Although Wellington House was not a U.S. propaganda apparatus, it greatly contributed to public perceptions of propaganda, eventually leading to the "stabbed in the back" myth gaining traction in Germany and additionally causing the U.S. to hesitate from joining World War II, as many Americans believed they had been deceived into joining the war effort by the British (The Great War, 2016). This perception also irreversibly changed how propaganda and psychological warfare were viewed by the public. Soon after the declaration of the United States stating they would join the war, the Committee on Public Information was established in 1917 and it operated as the United States' propaganda arm, producing movies, pamphlets, cartoons, and more

with the purpose of mobilizing public opinion behind World War I (Neumann, 2017).

Film Propaganda in WWII

World War II was a time of extreme utilization of propaganda (film or otherwise), as the propaganda apparatuses of world governments had matured during World War I and the creation of film propaganda had become increasingly profitable. American propaganda for the war, according to Krista Richmond in her thesis, "The Beginning of a Beautiful Friendship: World War II Propaganda in Feature Films From 1939-1943", had a major theme of "good Americans" and "bad Germans". Richmond screened six feature films released from 1939 to 1943 for propaganda messages relating to World War II. These films were The Wizard of Oz (1939), Gone with the Wind (1939), The Grapes of Wrath (1940), Citizen Kane (1941), The Maltese Falcon (1941), and Casablanca (1943). According to Richmond, more than one-third of propaganda messages were categorized as Pro-American or Pro-Democracy (Richmond, 2003). Anti-German films also contained almost a third of these messages (Richmond, 2003). Richmond concluded that film was a valuable tool for government propaganda during the war.

Anti-Japanese sentiment was also a major theme in American propaganda films because many Americans were either unfamiliar with or willfully ignorant to the Japanese people's way of life. The attack on Pearl Harbor was still very recent, and due to cultural and geographic differences, Japan and its people were seen by many Americans to be representative of "subhuman beasts associated with a primitive world" (Gianos, 1998 as cited in Richmond, 2003). Richmond states that Japan, as depicted in film, was seen mostly along racial lines, and despite the Office of War Administration wrestling with filmmakers to soften their depiction of Japan, many studios did not, as Japan was not a major market for American films (Richmond, 2003).

In contrast to depictions of Japanese people, the Italian people were not seen as uniformly evil and were instead used for comic relief. Richmond states that depictions of Germans were more complicated. American filmmakers made significant efforts to distinguish the average "good" German citizen from German Nazis, effectively shifting Anti-German propaganda to Anti-Nazi propaganda (Richmond, 2003).

Due to the massive propaganda campaign created by Britain's propaganda arm, which was discussed in the previous section, many Americans believed the British were attempting to lure the U.S. into war (Richmond, 2003). This led to American filmmakers attempting to "democratize" Britain in public perception to increase Americans' enthusiasm for the war effort (Richmond, 2003). This could be because Hitler and the Nazi party were expressly inspired by British and American atrocities and propaganda (Snyder, 2017). Adolf Hitler was particularly inspired by American concepts such as Manifest Destiny, America's treatment of Indigenous people and Black people, and Britain's World War I propaganda.

Racist depictions of the Japanese and their culture were not the only anti-Japanese propaganda churned out by U.S. media in the second World War, however. One day after the U.S. dropped "Little Boy," one of the country's recently developed atomic bombs, Brigadier General Thomas F. Farrell received orders from the War Department to "engage in a propaganda campaign against the Japanese Empire in connection with the new weapon and its use against Hiroshima" (*The atomic bombings of Hiroshima and Nagasaki*, 2022). General Farrell then dropped 16,000,000 propaganda leaflets in Japanese cities and implemented regular broadcasting of U.S. propaganda via Radio Saipan (*The atomic bombings of Hiroshima and Nagasaki*, 2022). One of the leaflets distributed in Japan reads: "If the war continues the result will be destruction of the homeland of Japan. This is an obvious fact. The longer the war continues, the greater will be the work in reconstructing the nation after the war, and the nation's resources will be forever impoverished. It is an easy matter to sacrifice one's life for the nation. But true loyalty is to put an end to the war and to work toward reconstructing the nation." (*Propaganda and warning leaflet dropped on Japan*, 2022).

It should be noted that Japan was already heading toward surrender before the bombing of Hiroshima and Nagasaki. The question was not if Japan was going to surrender, but if it would surrender to Russia or to the United States (TeleSUR, 2016). Japanese leaders knew that if they were to surrender to Russia, the Japanese Empire would not be able to continue. American leaders also knew this and did not want Japan, a strategic spot in the region, to be "lost" to Communism. The bombing of Hiroshima and Nagasaki served to send a message to Russian leaders that "if they did not go along with U.S. plans in Europe and the Pacific, this is the fate that awaited [Russia]." (TeleSUR, 2016).

It is in Hollywood narratives of the bombings carried out on Nagasaki and Hiroshima that one can see the U.S.'s nascent Military-Entertainment Complex begin to emerge. *The Beginning or the End* is an American propaganda film produced by the War Department echoing the U.S's official narrative regarding the atomic bomb: "The bomb was clearly necessary to end the war with Japan and save American lives—and we needed to build new and bigger weapons to protect us from the Soviets." (Mitchell, 2014). Early versions of the film's script were originally skeptical of Truman's decision to drop the bombs on Japan and provided "stark realism" that showed the true effects Fat Man and Little Boy had on the Japanese people (Mitchell, 2014). Original plans for the film called for "ghost-like" depictions of the wreckage and even zoom-in shots on the burned faces of Japanese infants. The purpose of the film, according to Greg Mitchell, an American author who has written twelve non-fiction novels on U.S. politics and history, initially, was supposed to reflect the regrets of the scientists who worked on the bombs. The stark change in theme occurred when the director of the Manhattan Project, General Leslie Groves, secured the right of script approval for the film and a hefty \$10,000 fee (Mitchell, 2014). Mitchell lists the fabrications and suppressions of evidence featured in the film:

- In the revised script, no victims were shown, and the footage showing victims was replaced with shots featuring destroyed landscapes.
- All doubts regarding the morality of the bombing "disappeared" and the bombing was instead presented as "justifiable and admirable".
- The revised script lightened the effects of atomic fallout.
- The film completely fabricated a Japanese nuclear facility in Hiroshima.
- The film fabricated the existence of a scientist who prevented a chain reaction from the bomb that would have killed 40,000 people on a Pacific Island. The character prevents this by exposing himself to a lethal amount of radiation and says the following before his death: "God has not shown us a new way to destroy ourselves. Atomic energy is the hand he has extended to lift us from the ruins of war and lighten the burdens of peace." (Mitchell, 2014)

These are only a few fabrications present in the script. The sentiments and narratives shown in the film effectively created the long-lasting justifications of the bombings that are still echoed today.

Anti-North Korean Film and Television Propaganda

Korea, during World War II, was occupied by Japan, after World War II, the country was split horizontally along its 38th peninsula. Russia occupied the northern part of Korea, while the U.S. occupied the south (The Cold War, 2020). After Russian and U.S. troops had left North and South Korea, respectively, Korea split into two states. Each of these states held separate elections due to North Korean and Russian concerns over the fairness of an UN-administered election, and each respective "backer" of the states solidified the power of the elected president in the region (The Cold War, 2020). The Democratic Republic of Korea (a.k.a. North Korea) became a state in the September of 1948, only a few weeks after The Republic of Korea (a.k.a. South Korea) became an independent state in August. Each state wanted to "take" the rest of Korea. The U.S. supported South Korea, while China supported North Korea, as Russia did not want to face the U.S. directly (The Cold War, 2020). The war ended after Chinese forces pushed back UN forces, causing a conclusion to the war and establishing the front line as the "de-facto" boundary between North and South Korea (Millett, 2022).

During the Korean Civil War, the U.S. dropped 635,000 tons of bombs and 32,557 tons of napalm on Northern Korea (TeleSUR English, 2017). Three million Korean citizens were killed during the war, with the lion's share of deaths belonging to that of North Korean citizens (TeleSUR English, 2017). Due to North Korea's general resilience in the face of the war, propaganda against the state continues into the present day. (TeleSUR English, 2017).

Criticisms of North Korea in film often address concerns about the country's nuclear capabilities and its people's quality of life. While criticisms of North Korea are by no means uniform, feature films like *The Interview* (as well as its reception) reflect the public's perception of the country as a hostile state. In a 2019 study for the Pew Research Center, Christine Huang found that 58% of Americans view North Korea's nuclear program as a major threat to their country. This number is just above the 26-Country median of 56% (Huang, 2019).

The Interview, a 2014 film produced by Seth Rogan and Evan Goldberg, is a particularly poignant example of these anxieties bubbling over. The film follows the story of two journalists tasked with killing the North Korean President Kim Jong-Un. As Sony Pictures prepared for the film's release, a hacker group known as the Guardians of Peace leaked thousands of files stolen from Sony's servers (Cheddar, 2019). The Guardians of Peace insisted that Sony not release *The Interview* in any form, threatening gifting a "Christmas present" to theaters which showed the film (Cheddar, 2019). This hack prompted a major controversy surrounding Sony Pictures, as the company quickly shut down all promotion of the film and cut parts of the film (Cheddar, 2019). The film was later released on Christmas Day without incident (Cheddar, 2019). The North Korean government has maintained it's statement that it was not involved with the hack (Cheddar, 2019).

The FBI credits Park Jin Hyok, a North Korean Citizen and employee of Chosun Expo for the hack (Office of Public Affairs, 2018). The FBI stated that, according to the charges brought against Park Jin Hyok, Chosun Expo is a company affiliated with Lab 110, a component of North Korea's military intelligence (Office of Public Affairs, 2018). Sony employees and *The Interview*'s producers have doubts about the FBI's quick attribution to the North Korean government (Siegel, 2019). Steve Mosko, the former television head for Sony Pictures, stated that he never believed the hack was ever about *The Interview* (Siegel, 2019).

Seth Rogen recalls his uncertainty regarding the FBI's North

Korea narrative, stating that there were no breaches or attempted breaches made to his data or his co-director's data (Siegel, 2019). An additional hole in the FBI's narrative appeared when Max Popov, a Russian hacker and FBI informant, claimed that an associate presented him with a cache of the leaked documents, dated before and after the hack (Siegel, 2019). Popov sent the cache to Jeffery Carr, a researcher and founder of Taia Global Inc. (*Jeffrey Caruso*, 2023). Although the FBI has not changed its narrative, former FBI agent E.J. Hilbert stated that "With any attack, and very likely with the Sony attack, they thought they had full containment, and they probably did not, and therefore information could have continued to have been extracted by the bad guys from various methodologies," (Siegel, 2019).

As evidenced in the Sony Pictures data breach controversy, fears of North Korean hostility are commonly held by members of the American government and public. Both the portrayal of North Korea as hostile in *The Interview* and real life events which surround the film are reflective of anxiety surrounding the country.

Film and Television Propaganda during the Vietnam War

Vietnam was colonized by the French along with Laos and Cambodia from 1887 to 1954 (*Indochina: Region, Asia*, 2020). Under French rule, forces were authorized to dispel native Vietnamese dissent by any means necessary. This order even went as far as to galvanize the French to criminalize the act of identifying oneself as Vietnamese (Luna Oi!, 2020). The French also looted the country of its artifacts, many of which are still unreturned and on display in French museums. During World War II, the Japanese Empire invaded Vietnam and the country was under both French and Japanese regimes. Between 1940–1945, 2 million Vietnamese people died of starvation.

After the Japanese surrendered in the wrapping up of World War II, Vietnamese President Ho Chi Minh declared Vietnam independent (Walbert, 2022). In a strategic move, Ho Chi Minh surrendered to the French in 1945 to allow the other forces to exit Vietnam, leaving the Vietnamese army with only one enemy to combat (Luna Oi!, 2020; Walbert, 2022). The First Indochina War began later in 1946 when the Viet Minh began fighting against French colonial rule (Walbert, 2022). After the Viet Minh successfully defeated French forces in 1954, France signed the Geneva accord which forced Viet Minh troops to retreat north and forces in support of French rule to the south (Walbert, 2022). South Vietnam later declared themselves independent and appointed Ngo Dinh Diem through a rigged election (Walbert, 2022). Diem was executed by the South Vietnamese military with the tacit approval of the U.S. (Walbert, 2022).

On August 2nd of 1964, North Vietnamese troops allegedly fired upon the U.S.S. Maddox, galvanizing U.S. President Lyndon Johnson to effectively declare war on North Vietnam without a declaration from Congress, signing into effect the Gulf of Tonkin Resolution, which allowed the U.S. to take any measures necessary to prevent armed attacks against U.S. forces and any future aggression against the U.S. (Walbert, 2022). By the next year, the U.S. began operation "Rolling Thunder"—a sustained bombing campaign against North Vietnam that lasted three years (Walbert, 2022). On March 16th of 1968, the U.S. slaughtered hundreds of Vietnamese civilians in the Mai Lai massacre (Walbert, 2022). This caused U.S. citizens to turn their opinions against the war, stopping Lyndon Johnson from running for re-election, and a slow pulling out of Vietnam to take place over the following years.

Anti-Vietnam propaganda usually contained themes of evil and depraved Viet Minh fighters and horror stories about American prisoners of war. *The Green Berets*, directed by John Wayne, Ray Kellogg, and Mervyn Leroy, was created by John Wayne, who cited frustrations with the Anti-War movement in the U.S. as his inspiration for the film (*The green berets* [Review of the film *The green berets*, 1968], 2022; Rico, 2018). The film was backed by the Pentagon and President Lyndon Johnson and was "made with the specific intention of countering existing opinions about the war" (Rico, 2018). The film features a simple plot: an anti-war journalist travels to Vietnam after being lectured by an army official about the ideological battle against Communism, said journalist witnesses U.S. forces conducting humanitarian aid as opposed to the Viet Minh committing brutal acts of violence, and the journalist sees the error of his ways and changes his opinion on the conflict (Rico, 2018). There is no mention of the use of Agent Orange, an extremely harmful chemical sprayed over Vietnam as a part of the U.S.'s Herbicidal Warfare Program, or of operation "Rolling Thunder." Pro-War films of this kind omit the atrocities committed by the U.S. to trick its citizens into believing the war was justifiable.

Film and Televised Propaganda Post-9/11

Afghanistan, strictly as a landmass, sits near both oil-rich nations like Iran and the former-Soviet republics of Turkmenistan, Uzbekistan, Tajikistan, and Kyrgyzstan and key challengers to U.S. Hegemony Russia, India, and China (Empire Files, 2020). In 2010, Afghanistan itself was found to have almost \$1 trillion in untapped mineral deposits by the U.S. (Risen, 2010). The deposits include materials such as iron, copper, cobalt, gold, and lithium (Risen, 2010). An internal Pentagon memo stated that Afghanistan could become the "Saudi Arabia of lithium" (Risen, 2010).

With the onslaught of Afghanistan's initial occupation by the British Empire in 1838, the massive cultural growth and prosperity the nation had previously enjoyed for over three thousand years was sent sharply backward (Empire Files, 2020). The British Empire subjugated Afghanistan's people for nearly eighty years until the nation won its independence in 1919 (Empire Files, 2020). The revolution was followed initially by the formation of a feudal monarchy until the Democratic People's Party of Afghanistan was established in 1965 (Empire Files, 2020). The party implemented land reform, equality for women, legalization of trade unions, and a literacy campaigns (Empire Files, 2020). The CIA organized the ex-landlord class and conservative religious sectors into militias with the intent of overthrowing the newly independent and democratic state (Empire Files, 2020). In response, the Soviet Union sent troops to support the Democratic People's Party of Afghanistan (Empire Files, 2020). The troops sent by the Soviet Union were able to withstand the Mujahideen insurgency (Empire Files, 2020). The US launched Project Cyclone with the goal of forcing the Soviet Union into a quagmire (Empire Files, 2020).

The U.S. funded the Mujahideen even further. Journalist Ahmed Rashid states that 35,000 Muslim radicals from forty separate Islamic countries joined the Mujahideen from 1982-1992, and that the creation of the CIA-desired war required the recruitment of Arab, Egyptian, and Pakistani Extremists, concluding "the fundamentalism that emerged in Afghanistan, is a CIA construct" (Empire Files, 2020). Those who comprised the Mujahideen later formed the Taliban, which gained control over Afghanistan in 1996 (Empire Files, 2020; Taliban: Political and religious faction, Afghanistan, 2018). The U.S. initially welcomed the Taliban victory warmly. Marty Miller, the former VP of the Union Oil Company of California (UNOCAL), secretly invited Taliban leaders to UNOCAL's headquarters in Texas (Empire Files, 2020; Unocal and Bridas woo the Taliban for oil pipeline project, 2022). Miller stated that because the Taliban "didn't have a clue about the oil and gas business" the goal was to establish trust (Buvarp, 2015). Relations between UNOCAL and the Taliban eventually crumbled, leaving the U.S. searching for other ways to gain access to Afghanistan's land.

News and film media often portray the U.S.'s goals in the invasion of Afghanistan as clear: to capture and kill Osama Bin Laden and to overthrow the Taliban. Furthermore, mass media attempts to show the U.S. as effective and driven towards this cause. George Bush himself stated, "if they want [the U.S.] to stop our military operations, they just got to meet my conditions." (Empire Files, 2021). However, George Bush denied the Taliban's offer to capture and extradite Osama Bin Laden, along with their offer of total surrender to U.S. forces (Empire Files, 2015; Empire Files, 2020). The war quickly turned into a quagmire, with generals like Major General Mark Carleton-Smith of the British army stating that the Afghanistan war was "neither feasible nor supportable" and that the "American strategy [was] doomed to fail" (Empire Files, 2015). Even Taliban Foreign Ministry official Sayed Hashemi expressed frustration at the vagueness of the U.S.'s war effort in a public statement, stating, "... we do not know what to do now. We have been given no counter proposals [By the U.S.]. And now the perception ... is that maybe the United States is always looking for a Boogeyman." (Empire Files, 2020).

The United States officially withdrew from Afghanistan on August 30th, 2021 (Kiely & Farley, 2021). This withdrawal arose, initially, from former president Donald Trump's agreement with the Taliban to pull out U.S. troops by May 1st, 2021 (A historical timeline of Afghanistan, 2014). Although Trump called off the talks in September of 2019 after a U.S. soldier was killed in a Taliban attack (A historical timeline of Afghanistan, 2014). Days before current U.S. president Joe Biden was inaugurated into office, the Trump administration announced plans to cut U.S. troops in Afghanistan in half (A historical timeline of Afghanistan, 2014). In April of 2021, Biden announced plans to complete the U.S. withdrawal from Afghanistan by September 11th, 2021 (A historical timeline of Afghanistan, 2014). On August 15th of 2021, the Afghanistan government fell to the Taliban, causing the Biden administration, despite being warned against freezing Afghanistan's assets, to freeze the \$7 billion in reserves Da Afghanistan Bank (DAB) held at the Federal Reserve

Bank of New York (*A historical timeline of Afghanistan*, 2014; Nichols, 2021; Goldbaum et al., 2022). This move was a catastrophic blow to a country already facing severe political and economic turmoil. On September 14th, 2022, both the U.S. and Swiss governments unveiled a project whose goal is to selectively disperse only half of the frozen \$7 Billion to "U.S.-Approved organizations" (Byrd, 2022; Office of the Spokesperson , 2022; AJ+, 2022). It was decided by the administration that the other half of the \$7 Billion would go into a compensation fund for 9/11 victims, leading Al Jazeera Journalist Sana Saeed to call the decision "further punishment for a crime that Afghanistan never committed but has paid for for two decades." (AJ+, 2022).

Media pressures in favor of the U.S. invasion of Afghanistan were immense in the months after 9/11. Sana Saeed, the host of Al Jazeera's series *Backspace*, calls U.S. coverage of Afghanistan: "an exorbitantly expensive spectacle of salvation and dehumanization using the body of the Afghan woman as a front.", using Oprah Winfrey's removal of a Burqa from an Afghan activist, after the reading of "Under the Burqa" by Eve Ensler at the 2001 *Vagina Monologues* gala event as an example of this spectacle (AJ+, 2022). She explains that "U.S. media has held hands tightly with U.S. foreign policy, helping justify an invasion born in large part out of the fog of vengeance following the 9/11 attacks." (AJ+, 2022).

Saeed argues that American journalists tend to paint the U.S. as a "reluctant savior," rather than a force which occupied Afghanistan for twenty years, cited an increasingly vague goal for doing so, and promptly left the nation in turmoil during its withdrawal (AJ+, 2022). Saeed further posits that one of the most significant vectors by which U.S. mass media does this is through the hyperfocus on violence against Afghan women, without evaluating the role American militarism plays in said violence (AJ+, 2022). She states that the western public places the blame

for violence against Afghan women on culture and argues that this perception paints Afghan culture as brutal and unfair (AJ+, 2022). Saeed then states that harsh U.S. economic sanctions used against Afghanistan since 1999 severely impact Afghan women's health care (particularly maternal health care) and overall physical and mental wellbeing of Afghan women (AJ+, 2022).

Saeed argues that another vector through which U.S. mass media propagandizes its audience towards militaristic beliefs is through the portrayal of American troops as empathetic "goodguys," citing news stories from entities like Insider and Reuters which dispersed emotional stories about American soldiers holding Afghan babies (AJ+, 2022). Norah O'Donnell, a reporter for CBS, stated on an August 2021 broadcast that "The American military is the greatest in the world, not only because of its superior force, but because of its humanity." (Hollar, 2021).

Regarding the freezing of DAB's \$7 Billion in assets, AJ+ points out that most U.S. coverage of the event fails to connect the dots between Afghanistan's devastating famine and the frozen assets (AJ+, 2022). Saeed states that the blame for this famine is not placed on the U.S. government, but rather is placed on the Taliban (AJ+, 2022). Thus, Saeed argues, that the U.S. implemented a policy of mass starvation. (AJ+, 2022)

In terms of fictional media, particularly post-9/11 U.S. action films, Leon Thomas, creator of the YouTube video-essay channel *Renegade Cut*, argues that the DoD and CIA want Hollywood productions to portray the military as always knowing best and having the people's best interest at heart (Renegade Cut, 2019). This is most effectively done through action movies, as action movies both often necessitate use of expensive military equipment and tend to be very well received (Renegade Cut, 2019). Thomas argues that 9/11 action films like *Man of Steel* (which was funded by the DoD), where Superman brutally kills the in-universe equivalent to the 9/11 attackers, provide a catharsis for American audiences (Renegade Cut, 2019).

In his video-essay Post-9/11 Action Movies, Thomas shows his audience the nationalist and militaristic symbolism and arguments present in the American action film Olympus Has Fallen. Thomas argues that Olympus Has Fallen implicitly appeals to the "American psyche" by using imagery of U.S. monuments crashing down and attacks orchestrated by suicide bombers (Renegade Cut, 2019). In the film, practices like indiscriminate slaughter and torture are portrayed as effective, despite their real-life unreliability (Renegade Cut, 2019). Thomas states that the goal of catharsis Olympus Has Fallen seeks to exude provides a "conclusion" to Americans "tired of the forever-war," which Thomas notes, is impossible in a war against a concept (Renegade Cut, 2019).

METHODS

In pursuance of gaining more information on how the U.S. military justifies its actions through film and televised media, two data collection methods were selected. To pursue quantitative information, meaning information which can be measured mathematically, on the subject, I conducted an internet-based survey of fifty-eight participants. A survey is defined by Lynee Gaillet and Michelle Eble, authors of *Primary Research and Writing: People, Places, Spaces*, as a collection of data from people who willingly partake in a collection of questions for a study (Gaillet & Eble, 2016). I conducted this survey through Google Forms and disseminated the survey through word-of-mouth and promotion through both personal social media accounts and paid promotion campaigns on TikTok, Instagram, Reddit, and Facebook. My goal for the survey was to ascertain general public opinion on the events of both previous and current American-involved conflicts.

To gain qualitative (descriptive) information, I interviewed Professor A, a Director of Graduate Studies and Associate professor at a research university in Indiana and expert in the cultural history of video games as well as the connection between media and war, and Professor B, a professor of political science, sociology, and cultural anthropology at a community college in the state of Maryland. Professor B has expertise in international relations and the impact and development of communications technology, as well as its use by state actors. Interviews are defined by Gaillet & Eble as being primary-research tools for gaining information from knowledgeable sources and incorporating alternative perspectives on any given topic (2016). From these interviews, my goal was to gain insight into how the Military-Entertainment Complex operates as well as its effects on the public psyche.

RESULTS & DISCUSSION

After the survey had been completed, the data was analyzed carefully. Google Forms was used both to aggregate the data and to analyze the results. By the completion time of the survey, I received fifty-eight total results. I asked these fifty-eight participants fifty-two questions each. Of the emergent patterns drawn from responses to these questions, a few were notable.

QUANTITATIVE DATA

The response to the questions "Have you heard of or are familiar with the North Korean president Kim Jong-un?" and "Have you heard of or are familiar with the South Korean president Yoon Suk-yeol?" were very slanted. 100% of participants (58) were familiar with Kim Jong-un, but just 31% (17) of participants were familiar with Yoon Suk-yeol. I believe the tendency of most people (who are overwhelmingly American) to know of North Korea's president in contrast to only 31% (17) knowing of South Korea's can be interpreted as an effect of anti-North Korean sentiment and alarmism in U.S. mass media. This contrast can also be seen when measuring familiarity with historical North and South Korean leaders, respectively. When asked if they were familiar with the first North Korean President Kim Il-sung, 71.4% (42) of respondents stated that they knew of Kim Il-sung. When asked if they were familiar with the first South Korean President Syngman Rhee, 87.9% (50) of respondents stated that they were not familiar with the leader.

Another intersection of these responses can be seen amongst responses to the questions "How would you rate your perception of the Democratic People's Republic of Korea (North Korea)?" and "How would you rate your perception of the Republic of Korea (South Korea)?." Of the 50 who chose to answer this question, self-reported perceptions of North Korea were overwhelmingly negative, with 31.6% (18) of respondents reporting a negative perception of North Korea and 29.8% (17) of respondents reporting a very negative perception of North Korea. Only 7.1% (4) of respondents reported a positive or very positive perception of North Korea, while 19.3% (11) of respondents were undecided. In contrast, perception of South Korea was much more mixed. 36.8% (21) of participants reported either a positive or very positive perception of South Korea, another 36.8% (21) of respondents were unsure, and only 12.3% (6) reported a negative perception of the country. From these results discussed so far, we can see that despite most people not knowing of South Korea's past or present leadership, opinion against North Korea and its leadership (both modern and historical) are overwhelming.

I also found it interesting that 54.4% (31) of respondents were unfamiliar with Operation Rolling Thunder, a sustained aerial bombing campaign carried out by the U.S. against the Democratic Republic of Vietnam. In a similar vein, 38.6% (22) of respondents were unfamiliar with U.S. use of Agent Orange in Vietnam, although it should be noted that extreme negative opinion of this use was held by 45.6% (26) of respondents, which may point to increased fame of the action than shown in the existing data. This unfamiliarity with U.S. campaigns and war crimes shows the success of U.S. propaganda in suppressing alternative coverage and criticism regarding such actions in the public sphere.

QUALITATIVE DATA

Professor A Interview

I began my interview with Professor A by explaining the scope of my research and listening to his ideas on the subject at large. Professor A explained, firstly, that the U.S. Military has a long history of working with "cultural producers" Smith 28 of "all manner of entertainment" prior to the invention of the video game. Professor A gave an example of this by pointing to the Office of War Information's collaboration with Hollywood studios during World War II and "positive relationships" between comic book producers and the U.S. Military.

Professor A emphasized that the U.S. Military, although sometimes engaging in brazen collaboration with video game producers, largely remains indirectly involved with said producers. Rather, Professor A stated that most working relationships amongst cultural producers and the U.S. Military are "born of convenience" rather than being akin to clandestine projects to propagandize the American public. In essence, positive relationships between producers and the U.S. Military have the shared goal of seeking profit.

When pro-U.S. militainment is created, the U.S. government's job: "to make the military more effective" and to "give people a generally better image of the armed forces" is completed, resulting in increased recruitment, which eventually becomes profit for transnational defense contractors and increased funding for the U.S. Military. As a result of creating this propaganda, producers reap the rewards of exploiting public insecurities and fervor regarding American war efforts to sell their product. These insecurities are exploited by video game producers through the inclusion of real-world enemy states. This inclusion, whether its "North Korea, ... a named enemy in Middle East like Iran, ... an unnamed Arab country, ... something like Russia, or a kind of post-Soviet rogue state" serves to create relevant enemies which "tap into ... an existing cultural mythology that is easily sellable."

When asking Professor A about the large-scale consolidation of media companies and how it effects the output of said company's subsidiaries, he responded by stating "it really comes down to an issue ... of agenda setting and framing ... So, having ... [a] consolidated media market does not tell you what you can say, but typically limits the things about which you can talk." Professor A says that although there are "certainly internet-based media outlets where [one] can get contrarian views ... that push against dominant narratives" he also states that "if your nightly news show ... is supported by ads, not offending the [consumers of] these ads is ... front of mind." Professor A concluded from this question that the "hyper-monopolization" of the entertainment and information industries necessitated a "less diverse range of voices" and marginalization of opposing voices.

Although the question of consolidation was my last planned question for the interview, I wanted to know what Professor A thought of the recent addition to the *Call of Duty* franchise and how *Call of Duty: Modern Warfare II* promotes a pro-U.S. narrative. Professor A answered this question in detail by stating that in the game's narratives, the player takes on the role of a "central agent of change in [*Call of Duty*'s] universe." This agent that the player takes control of does not have to "deal with that world after the G.I.s have come and gone". Even in the original *Call of Duty: Modern Warfare*, when players experience "interstitial moments … where [the player] plays as the civilian … and become a victim of a larger conflict", these brief moments of experience only serve to reinforce the larger narrative "that [the player] need[s] to be the one exercising … marshal action. [The player] need[s] to be the one using … state-sponsored violence. Yes, what [the player is] doing is gruesome, but ... if [the player does not] act, the results are ... much worse" as evidenced in moments seen from a civilian perspective.

Professor A explained, further, that militainment is "ultimately not all that curious" and "boils down" geopolitics to a "cartoonish" degree, rather than intentionally training players to exercise military violence. He stated, "... it does leave you with a kind of pat ideological answer ... that is easy to hold onto. But in fact, we know that the world is far more complicated than that. But ... presenting those truths in ... entertainment is deeply unsettling and ... does not sell a lot of products."

Professor B Interview

I began my interview with Professor B by asking his name, occupation, and expertise as background for the rest of the interview. Professor B answered by stating that he teaches political science, sociology, and cultural anthropology at a community college in the state of Maryland. He also stated that he completed his master's in international relations and is working on his PhD in an "interdisciplinary program that looks at the impact of communications technology, the development of communications technology, and its utility of virtue in the way that it's exercised by state actors."

Next, I asked Professor B how the Department of Defense works with producers to promote a pro-U.S. narrative in film. While he outlined specific instances of direct government collaboration with film producers such as the Creole Commission, Professor B stated that "there's something more fundamental going on." Like Professor A, Professor B emphasized that both media producers and the U.S. government have the shared interest of profit maximization. He stated, "the interest, ... [of] the military industrial complex, ... coincides with the quest for profit on the part of privatized businesses." Professor B then identified stories which inspire or reflect "sensationalism, conflict, [and] violence" are profitable because "that's the type of information people are addicted to."

Professor B stated that this sensationalist pro-military entertainment "reflects favorably ... in the armed forces and it is conducive to munition sales." He pointed out that this connection is a coincidence "that both [American political] parties are happy with" and that said coincidence "has probably been more influential on the public psyche ... than has been any direct sort of over the table deals between the entertainment industry and the Pentagon."

I then asked Professor B why so many televised news reports & films have a clear slant against states with opposing interests to the U.S. like Russia, China, and North Korea. Professor B recounted experiences during the "early ... months of the invasion of Iraq" where he viewed media pundits outright stating favor towards the U.S. He also stated that in the moments after 9/11, the public was "very patriotic and perhaps out for blood ... [they were] mourning in both a national sense and a personal sense," which made them "easy targets" for propagandizing. Professor B further explained that delivery of news is not explicitly "planned" to be propagandizing in nature, but rather media that is propagandizing tends to be very lucrative for both media companies and those who have financial stake in the war machine. He stated, "You see a clear confluence of interest between media, elites, corporate elites, and political elites, and they know that they all have their role to play, and they all more or less want to see the same thing happen because that's how they got there in the first place."

Finally, I asked Professor B how intense media consolidation effects these conglomerates' subsidiaries' film and news reporting output. Professor B began by explaining that major companies like Verizon and Comcast cannot merge because it would mean the end of market competition within that sector and may lead to the further "breaking-up" of the two companies for the market's sake. He explained that companies, whether they are in the information and entertainment industry or the food industry, are tasked with creating the illusion of options. These factors result in a much smaller window of opinion.

However, Professor B also stated that propaganda in the form of news reporting is becoming obsolete, as the advent of participatory and social media are changing the media landscape. Although Professor B explained that it may be good that media is becoming more participatory (and thus more democratic), he argues that this advent "brings with it a slew of new problems." Professor B observed that "Incoming generations now have very little idea of how to differentiate between academic sources ... and some rubbish on the internet" and that "trolling" and comment-section vitriol "injects a sort of toxicity into media that wouldn't be there in traditional forms of media."

CONCLUSIONS

From this research, a few overarching conclusions can be reached. The first of these conclusions being the apparent pervasiveness of indirect influence driven by the shared interest of profit maximization on the media industry by the U.S. Military. This arrangement, referred to as the Military-Entertainment Complex, benefits both the U.S. military (including its defense contractors) and mass media conglomerates. The arrangement benefits the U.S. military in that it increases recruitment, funding, and munition sales. Media corporations' benefit from this arrangement through rapid audience growth, and thus profit, caused by sensationalist news reporting. The second of these conclusions is that through the Military-Entertainment Complex, narratives that revise, justify, or outright erase American war crimes are created. These works can be created either with direct assistance and influence from the U.S. Department of Defense or can be created as a result of shared interests between media conglomerates, defense contractors, and the U.S. Government. The third and final conclusion that can be drawn from the research is that specifically in the post-9/11 world, video game and media companies take advantage of national and personal grief experienced by the American population in the wake of 9/11 to manufacture consent for invasion. This can be clearly seen in movies like *Olympus Has Fallen* and *Man of Steel*.

RECOMMENDATIONS

Regarding future research, I believe it may be beneficial to investigate the ties between media, the interests of transnational corporations, and the interests of the U.S. Government. This research could provide examples of shared interests between media entities, government officials, and the transnational capitalist class. I would also recommend further quantitative research to ascertain the public's opinions on various conflicts and how those opinions connect with media reporting.

LIMITATIONS

Participants in my survey tended to be overwhelmingly white and college-aged, which may have affected some of the answers respondents gave. The sample size of my research was also small and cannot be extrapolated to the American public at large, so repetition of this study with a larger sample size could be beneficial to gaining knowledge on this subject.

Generally, this study has shown that the U.S. Military justifies its actions through film and television, both through direct influence and loose connection of actors and interests. This study has also concluded that this influence on media and news reporting affects the public opinion of the Military and its conflicts, thus manufacturing consent for war. Further investigation of the Military-Entertainment Complex is recommended to ascertain how this justification and manufacturing of consent operates in the modern era, especially with the advent of social media.

REFERENCES

- AJ+. (2022). How the U.S. media betrayed Afghanistan [Video]. YouTube. https://www.youtube. com/watch?v=1K7pGfJOLRw
- The atomic bombings of Hiroshima and Nagasaki. (2022). Atomic Archive. https://www. atomicarchive.com/resources/documents/med/med_chp2.html
- Beydoun, K. A. (2018, May 26). US liberal Islamophobia is rising and more insidious than rightwing bigotry. *The Guardian*. https://www.theguardian.com/commentisfree/ 2018/may/26/us-liberal-islamophobia-rising-more-insidious
- Bigelow, K. (Director). (2012). Zero dark thirty [Film]. Sony Pictures.
- The British propaganda campaign in World War I. (2007). World-Information Institute. http://world-information.org/wio/infostructure/100437611661/100438658408? opmode=contents
- British reforms and colonial resistance, 1763–1766. (2023). Library of Congress. https:// www.loc.gov/classroom-materials/united-states-history-primary-source-timeline/ american-revolution-1763-1783/british-reforms-1763-1766
- Broadwater, J. (2008). A rich man's revolution, a poor man's fight for independence [Review of book *The politics of war: Race, class, and conflict in revolutionary Virginia*, by M. A. McDonnell]. *Reviews in American History*, 36(3), 321–328.

- Byrd, W. (2022, September 28). U.S. to move Afghanistan's frozen central bank reserves to New Swiss fund. United States Institute of Peace. https://www.usip.org/publications/2022/09/ us-move-afghanistans-frozen-central-bank-reserves-new-swiss-fund
- Cheddar. (2019, October 15). The story behind the hack that wreaked havoc on Hollywood The Vault [Video]. YouTube. https://www.youtube.com/watch?v=RDUgziJUKhw
- Cheskin, D. (1997, May 14). Environmental activists protest Shell's membership in the Global Climate Coalition at an annual shareholder meeting [Photograph]. Getty Images. https://www.gettyimages.in/detail/news-photo/shareholders-inpetro-chemical-company-shell-arrive-for-news-photo/829918740
- The Cold War. (2020). Korean War 1950–1953 The Cold War documentary. [Video] YouTube. https://youtu.be/ViVGj58kt34
- DoD assistance to non-government, entertainment-oriented media productions. (2015). Federal Register, 80(153), 47834-47850. https://www.govinfo.gov/content/pkg/FR-2015-08-10/pdf/2015-19279.pdf

Buvarp, T. (Director). (2015). Taliban oil [Film]. Fenris Films.

- Empire Files. (2015, September 12). 9/11 and the belligerent empire [Video]. YouTube. https:// youtu.be/xf-_gVroG_k
- Empire Files. (2020, June 26). Afghanistan war exposed: An imperial conspiracy [Video]. YouTube. https://youtu.be/dUPkeQBkCVU.
- Empire Files. (2021, September 11). Never forget: Lessons of the post-9/11 warpath [Video]. YouTube. https://youtu.be/BKCVN4hmrmo
- Empire Files. (2022, November 19). Ron DeSantis's military secrets: Torture & war crimes [Video]. YouTube. https://youtu.be/FFonj600fTI
- Gaillet, L., & Eble, M. (2016). Primary research and writing: People, places, and spaces. Routledge.
- The green berets [Review of the film The green berets, 1968]. (2022). IMDb. https://www. imdb.com/title/tt0063035
- Goldbaum, C., Padshah, S., & Shah, T. (2022, February 13). Biden's decision on frozen funds stokes anger among Afghans. *The New York Times*. https://www.nytimes. com/2022/02/13/world/asia/afghanistan-funds-biden.html
- The Great War. (2016, September 9). Reaching the masses Propaganda film during World War 1: The Great War special [Video]. YouTube. https://www.youtube.com/watch?v= LxgpEUL23AY&ab_channel=TheGreatWar
- Guo, J. (2016, October 20). The unexpected answer to the biggest mystery of the American Revolution. *The Washington Post.* https://www.washingtonpost.com/ news/wonk/wp/2016/10/20/what-we-can-learn-from-one-of-the-biggest-mysteries-and-misunderstandings-of-the-american-revolution
- Herman, E. S., & Chomsky, N. (2002). Manufacturing consent: The political economy of the mass media. Pantheon Books.
- A historical timeline of Afghanistan. (2014, December 31). PBS NewsHour. https://www.pbs. org/newshour/politics/asia-jan-june11-timeline-afghanistan
- Hollar, J. (2021, September 24). Missing voices in broadcast coverage of Afghan withdrawal. FAIR. https://fair.org/home/missing-voices-in-broadcast-coverage-of-afghan-withdrawal
- Huang, C. (2019, February 25). Global concerns persist about North Korea's nuclear program before second Trump-Kim summit. Pew Research Center. https://www.pewresearch.org/ fact-tank/2019/02/25/global-concerns-persist-about-north-koreas-nuclear-program-before-second-trump-kim-summit
- Hughes, T. (2022). The burning of Norfolk. Timothy Hughes Rare and Early Newspapers: Authentic Original Newspapers for Sale. https://www.rarenewspapers.com/

view/540782

Indochina: Region, Asia. (2020). Encyclopædia Britannica. https://www.britannica.com/ place/Indochina.

Jeffrey Caruso. (2023). O'Reilly. https://www.oreilly.com/pub/au/3720

- Kiely, E., & Farley, R. (2021, August 17). Timeline of U.S. withdrawal from Afghanistan. FactCheck.org. https://www.factcheck.org/2021/08/timeline-of-u-s-withdrawalfrom-afghanistan
- Le, D. (2017, May 25). Propaganda campaigns during the Cold War [Submitted as coursework for PH241]. Stanford University. http://large.stanford.edu/courses/2017/ph241/le2
- Luna Oi! (2020, December 6). Vietnam War from a real north Vietnamese perspective: Armchair historian animated history debunked. YouTube. https://youtu.be/Di7BLBINFX0
- MacLeod, A. (2019, September 15). Russia has "oligarchs," the US has "businessmen." Common Dreams. https://www.commondreams.org/views/2019/09/15/russiahas-oligarchs-us-has-businessmen
- MacLeod, A. (2022, November 18). Call of Duty is a government PsyOp: These documents prove it. MintPress News. https://www.mintpressnews.com/call-of-duty-is-a-governmentpsyop-these-documents-prove-it/282781
- McCoy, O. O. (2022). Top Gun Maverick global premiere [Photograph]. Wikimedia Commons. https://commons.wikimedia.org/wiki/File:Top_Gun_Maveric_Global_Premiere_ May_2022.jpg
- Millett, A. R. (2022, August 24). Korean war, 1950–1953. Encyclopædia Britannica. https:// www.britannica.com/event/Korean-War
- Mitchell, G. (2014, March 9). How bombing Hiroshima got Hollywood makeover. WhoWhatWhy. https://whowhatwhy.org/uncategorized/bombing-hiroshima-gothollywood-makeover
- Neumann, C. E. (2017). Committee on public information. The First Amendment Encyclopedia. https://www.mtsu.edu/first-amendment/article/1179/committee-on-publicinformation
- Nichols, M. (2021, September 9). U.N. warns Afghanistan needs money to prevent total breakdown. Reuters.https://www.reuters.com/world/asia-pacific/un-warns-afghanistan-needsmoney-prevent-total-breakdown-2021-09-09
- North Korea "bans skinny jeans" in fight against capitalism. (2021). 9 News. https://www.9news. com.au/world/north-korea-bans-skinny-jeans-mullets-in-fight-against-capitalism/

06df6f55-236f-4647-8814-0add9b112626

- Office of Public Affairs. (2018, September 6). North Korean regime-backed programmer charged with conspiracy to conduct multiple cyber attacks and intrusions. U.S. Department of Justice. https://www.justice.gov/opa/pr/north-korean-regime-backed-programmercharged-conspiracy-conduct-multiple-cyber-attacks-and
- Office of the Spokesperson (2022, September 14). The United States and partners announce establishment of fund for the people of Afghanistan. U.S. Department of State. https://www. state.gov/the-united-states-and-partners-announce-establishment-of-fund-for-thepeople-of-afghanistan
- Propaganda Cold War. (2022). Encyclopedia of American Foreign Policy. https://www. americanforeignrelations.com/O-W/Propaganda-Cold-war.html
- Propaganda and warning leaflet dropped on Japan. (2022). American Experience. PBS. https:// www.pbs.org/wgbh/americanexperience/features/pacific-propaganda-leaflet
- Renegade Cut. (2019). Post-9/11 action movies [Video]. YouTube. https://www.youtube. com/watch?v=GwIeV6L8YiE
- Reynolds, J. (1765). John Murray, 4th Earl of Dunmore [Painting]. Scottish National Portrait Gallery, Scotland. https://www.nationalgalleries.org/art-andartists/8802/johnmurray-4th-earl-dunmore
- Richmond, K. (2003). "The beginning of a beautiful friendship": World War II propaganda in feature films from 1939–1943 [Masters thesis, University of Tennessee Knoxville]. https:// trace.tennessee.edu/utk_gradthes/5285
- Rico, J. (2018, December 26). 10 popular war films that were used as political propaganda. LiveAbout. https://www.liveabout.com/top-films-used-as-political-propaganda-3438686
- Risen, J. (2010, June 14). U.S. identifies vast mineral riches in Afghanistan. The New York Times. https://www.nytimes.com/2010/06/14/world/asia/14minerals.html
- Saad, L. (2013, January 7). Americans consider individual freedoms nation's top virtue. Gallup. https://news.gallup.com/poll/159716/americans-consider-individual-freedoms-nation-top-virtue.aspx
- Siegel, T. (2019, November 25). Five years later, who really hacked Sony? The Hollywood Reporter. https://www.hollywoodreporter.com/movies/movie-features/five-years-whohacked-sony-1257591
- Simpson, S. (2019, July 10). Muslim advocates to Fox News: Fire Tucker Carlson after latest anti-Muslim rant against Ilhan Omar. Muslim Advocates. https://muslimadvocates.org/

2019/07/muslim-advocates-to-fox-news-fire-tucker-carlson-after-latest-anti-muslim-rant-against-ilhan-omar

- Snyder, T. (2017, March 8). Hitler modeled his plan for global conquest after America's manifest destiny. Slate Magazine. https://slate.com/news-and politics/2017/03/nazigermanys-american-dream-hitler-modeled-his-concept-of-racial-struggle-andglobal-campaign-after-americas-conquest-of-native-americans.html
- Taliban: Political and religious faction, Afghanistan. (2018). Encyclopædia Britannica. https:// www.britannica.com/topic/Taliban
- TeleSUR. (2016, June 15). The empire files: Untold history of imperial Japan & the bomb Part 1 [Video]. YouTube. https://youtu.be/dUPkeQBkCVU
- TeleSUR English. (2017, May 10). Do North Koreans hate U.S.? [Video]. YouTube. https:// youtu.be/Z-TsG-fOViM
- Unocal and Bridas woo the Taliban for oil pipeline project. (2022). Worldpress. https://www. worldpress.org/specials/pp/pipeline_timeline.htm
- Vidal, J. (2005, June 8). Revealed: How oil giant influenced Bush. The Guardian. https://www. theguardian.com/news/2005/jun/08/usnews.climatechange
- Walbert, D. (2022). The Vietnam war: A timeline. ANCHOR: A North Carolina History Online Resource. https://www.ncpedia.org/anchor/vietnam-war-timeline
- Ward, M., Pierson, S., & Beyer, J. (2019). Formative battles: Cold war disinformation campaigns and mitigation strategies. Wilson Center. https://www.wilsoncenter.org/publication/ formative-battles-cold-war-disinformation-campaigns-and-mitigation-strategies

