Rhodes Journal of Biological Science

Published by the Students of the
Department of Biology at Rhodes College

VOLUME XXXVII SPRING 2022

About this Issue

Statement of Purpose

The Rhodes Journal of Biological Science is a student-edited publication that recognizes the scientific achievements of Rhodes students. Volume XXXVII marks the sixteenth year since Mark Stratton and Dr. David Kesler brought the journal back into regular publication in 2006. Founded as a scholarly forum for student research and scientific ideas, the journal aims to maintain and stimulate the tradition of independent study among Rhodes College students. We hope that in reading the journal, other students will be encouraged to pursue scientific investigations and research.

Acknowledgements

The editorial staff would like to thank Dr. Boyle of the Department of Biology for her support and guidance in preparing this publication.

Image Credits

The front and back cover and section dividers for this year's edition of the *Rhodes Biological Journal of Biological Sciences* were created by Mia Harris, an Environment, Ecology, & Animal Behavior Biology Major from Nashville, TN. The front cover and one of the subject dividers illustrate the subjects for one of the featured articles, which studied the Memphis Zoo elephants. The two remaining section dividers displays the subjects of one of the review articles, which focused on amphibian conservation, and one of the featured articles, which studied dusky gopher frogs. The back cover art is dedicated to Professor Robert Laport and showcases the leaves of one of his favorite plants, the American tulip poplar.

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Editorial Staff

Emma Root '22 (Senior Editor) is a Biology major from Barrington, IL. On campus, she serves as the president of the Rhodes College Equestrian Team and is a member of the Chi Omega sorority. She is a member of honor societies including Phi Beta Kappa and Omicron Delta Kappa, among others. Off campus, Emma has worked as an academic intern in the Memphis Zoo Research Department and enjoys going for long walks with her dog. Upon graduating, she will be matriculating to Cornell University, College of Veterinary Medicine, where she will study small animal primary care and surgery.

Madeline Yde '23 (Junior Editor) is a Biology and Environmental Science major from Racine, Wisconsin. She has been involved in research at the Memphis Zoo since Spring of 2020, studying African elephant behavior and working in the amphibian lab. Maddy is a member of the women's soccer team at Rhodes, and she is involved in clubs on campus such as Food Recovery Network and Morgan's Message as well as the Kappa Alpha Omicron and Tri-Beta honors societies. In addition to her interests in the sciences, Maddy hopes to have the opportunity to continue exploring studies in Spanish and Latin American and Latinx studies. After graduation, she intends to pursue veterinary medicine.

Khanh Ton '23 (Junior Editor) is an Environmental Sciences major from Hue, Vietnam. She is a GIS Lab Supervisor RSA and worked as a research fellow for the Memphis Zoo and the Overton Park in previous years. Her current interests lie in invasive plant ecology, terrestrial biogeochemistry, and their interactions. Khanh is a member of the Kappa Alpha Omicron, Beta Beta Beta, Omicron Delta Kappa, and Mortar Board honor societies. Outside of the classroom, she is involved with Food Recovery Network, Memphis Food Waste Task Force, and the Diversity, Equity, & Inclusion Committee of Delta Delta Delta. This summer, Khanh will work as a Coastal Science Intern at the Bald Head Island Conservancy in North Carolina while attending a virtual research program at Emory University.

Izzy Wolfarth '24 (Junior Editor) is a rising junior and English major on the pre-med track. At Rhodes, she is involved with the Rhodes prison education program, Kappa Delta, The Sou'wester, and RJBS. She is originally from New Orleans and enjoys researching and writing about new or interesting subjects that she can share and discuss with others! She hopes you can enjoy reading this year's edition of RJBS and learn something new!

Kathryn Franks '24 (Junior Editor) is majoring in Biochemistry and Molecular Biology with a minor in Environmental Sciences. Her campus involvement includes member of Tri Delta Sorority, where she holds a spot in its Conduct Committee and Philanthropy Committee, working as VP of Recruitment in the Panhellenic Exec Council, a fellow in Dr. Hill's mycology lab, member of Rhodes Biological Journals, and Rhodes Sustainability Coalition.

Initial Real-World Experience with Cenobamate in Adolescents and Adults: A Single Center Experience

Taylor Elliott[a,b]; Tracee Ridley-Pryor[b,c]; Andrew J. Gienapp[b,d]; James W. Wheless[b,c]

a. Rhodes College, Memphis, TN USA
b. Neuroscience Institute, Le Bonheur Children's Hospital, Memphis, TN, USA
c. Division of Pediatric Neurology, University of Tennessee Health Science Center, Memphis, TN USA
d. Department of Neurosurgery, University of Tennessee Health Science Center, Memphis, TN USA

Following approval by the U.S. Food and Drug Administration (FDA) in late 2019, cenobamate (Xcopri[R]) has been utilized to treat adults with focal seizures. Based on its robust efficacy from the phase 2 trials, cenobamate was used in adolescent and young adult patients whose seizures were not controlled with previously available options. This study expanded its real-world application to this cohort with focal epilepsy and a history of drug-related rash. A retrospective study of patients exposed to cenobamate (n=45) was conducted. Dosage and serum levels, efficacy, drug interactions, and adverse effects were evaluated. After gradually increasing cenobamate to clinical effect using the FDA approved dosing protocol, 60% (n=22) of patients were responders. Adolescents were treated with an average daily dose of 204.0 mg and adults with 223.4 mg of cenobamate. Adolescents and adults had serum levels of 20.5 µg/mL and 26.7 µg/mL, respectively. The side-effect profile observed was similar to that seen in the phase 2/3 registry trials. Importantly, patients with a prior history of rash to other medications or antiseizure medications (n=5) experienced no rashes related to cenobamate. This real-world study supports the findings of prior controlled studies regarding the efficacy of cenobamate as a treatment for focal seizures in adolescents and suggests that patients with a history of rash may benefit from this medication.

Keywords: *adolescent; cenobamate; drug interactions; epilepsy; focal seizures; young adult*

Abbreviations: *DRESS=drug reaction with eosinophilia and systemic symptoms; FDA= Food and Drug Administration.*

Introduction

According to the World Health Organization, an estimated 50 million people worldwide suffer from epilepsy, with focal seizures being the most common form.[1] This fact, coupled with the large number that have pharmacoresistant epilepsy, has fueled the ongoing search for new anti-seizure medications. Over the past 3 decades, ongoing research has yielded an extensive number of new antiseizure medications; however, many patients with epilepsy continue to experience seizures even while taking multiple medications.

Cenobamate (Xcopri[R]) is a novel drug residing in the alkyl-carbamate family that has been approved to treat partial seizures in adults. However, it is distinctly different from its familial counterparts because of its capacity to selectively decrease Na^+ currents.[2] Felbamate, another member of the alkyl-carbamate family, has limited use outside of treatment for refractory epilepsy. There is no information from the cenobamate registry studies on the utility of combining these two drugs.

The U.S. Food and Drug Administration (FDA) approved cenobamate in November 2019 to treat partial seizures in adults based upon studies beginning in 2013. Prior studies demonstrated robust efficacy in adults.[3-5] To date, cenobamate is the only medication approved by the FDA that did not require Phase 3 randomized-controlled trials for efficacy; approval was based on efficacy results seen in the Phase 2 trials and open-label safety data from the one Phase 3 study.[3]

Krauss et al. demonstrated the potency of cenobamate in decreasing focal seizures particularly as dosing levels increased from 100 to 400 mg per day.[6] Additionally, patients entering the trial had suffered from refractory focal seizures for over 20 years, yet 9% still achieved seizure freedom on 200 mg cenobamate and 14% on 400 mg a day. Patients taking cenobamate 200 mg a day saw a 55% decrease in median seizure frequency with minimal adverse effects.[6] Most common side effects among all patients, regardless of dose, seen in prior trials included somnolence, dizziness, and fatigue.[4] As seen in the Greene et al. study, overall serum levels for patients achieving 50% seizure reduction ranged from 5 µg/mL to 35 µg/mL.[7] Likewise, prior studies demonstrated the safety and efficacy of long-term treatment with cenobamate as evident in its high retention rate, with approximately 80% of patients maintaining treatment for more than 6 months.[8]

To date, no studies exist to determine the efficacy of cenobamate in children and adolescents, nor in subpopulations excluded from prior studies (i.e., patients with a history of rash). Given the robust efficacy seen in the clinical trials for adults with refractory focal seizures, this compound was used at a National Association of Epilepsy Center accredited level 4 comprehensive pediatric epilepsy program for adolescent and young adult patients whose focal seizures were not controlled with current treatments. Real-world data in adolescents and adults were reviewed to evaluate the effectiveness of cenobamate in these populations and to support use of this new medication.

Methods

Institutional review board approval was obtained from The University of Tennessee Health Science Center (#21-08125-XP). Approval to waive consent was obtained. We performed a retrospective chart review of all electronic medical records in the Le Bonheur Comprehensive Epilepsy Program to capture all patients currently treated with cenobamate. Records on all patients treated with cenobamate from November 2019 (US approval) to June 2021 were reviewed. Patients involved were either adolescent (12–17 years of age) or adult (>18 years). None of the adolescents or adults were reported to be sexually active, and females included in this study did not become pregnant during treatment. The adult members of this group are long-term patients in the comprehensive pediatric epilepsy program who have been followed since childhood and have been unable to transition to adult epilepsy care. Both the adolescent and adult patients were prescribed cenobamate by their treating physician after an assessment of treatment options for refractory focal seizures and a discussion of risks and benefits with patients and family members. Cenobamate was obtained clinically as payors would allow and dosed according to the FDA approved regimen. Cenobamate was not able to be obtained for all patients due to payor issues, typically in those below the official FDA approved age of 18 years.

Data extracted from electronic medical records included patient demographics (age, sex), prior total number of previously attempted antiseizure medications or device(s), current number of antiseizure medications prescribed at initiation of cenobamate, number of total antiseizure medications used at the last follow-up, history of rash as an adverse effect to medication usage, dosage level of clobazam prior to and during cenobamate treatment, final cenobamate dosage and serum levels, seizure frequency prior to and during treatment with full dose cenobamate, and treatment side effects. All these patients were well known to their epileptologist (JW), were routinely asked about their current seizure frequency and possible adverse events at every visit, and all treatments were updated. Patients were instructed to take 1 dose of cenobamate per day at night, and all serum levels were obtained during routine clinical practice, not necessarily 8 hours after dose. Assays were sent to LabCorp (Memphis, TN USA) and assayed by liquid chromatography; mass spectrometry techniques were applied to determine cenobamate serum levels (therapeutic range, 5–35 µg/mL). Antiseizure medications were classified as enzyme-inducing or not at the time cenobamate was added to the regimen.[9] Cenobamate was dosed according to the currently approved FDA protocol, with the final dose based on clinical response and decision by the treating physician as to tolerability and effectiveness in the patient (Table 1).

Table 1. FDA-approved cenobamate dosing[a]

Timing	Amount
Initial Dosage	
Week 1 and 2	12.5 mg once daily
Titration regimen	
Week 3 and 4	25 mg once daily
Week 5 and 6	50 mg once daily
Week 7 and 8	100 mg once daily
Week 9 and 10	150 mg once daily
Maximum dosage	
Week 11 and thereafter	200 mg once daily
Maximum dosage	
If needed, based on clinical response and tolerability, dose may be increased above 200 mg by increments of 50 mg once daily every 2-weeks to 400 mg.	400 mg once daily

[a]Dosing according to FDA approved protocol (https://www.xcopri.com).

Results

A search of medical records found 45 patients (26 females and 19 males) treated with cenobamate for focal seizures with impaired awareness. Ages of the patients in the sample ranged from 13–44 years; 28.9% (n=13) were between the ages of 12–17 years of age (adolescents), and 47.6% (n=21) were 18–25 years of age, 15.6% (n=7) were 26–30 years of age, and 8.9% (n=4) were 31 years of age or older. Of the 45 total patients, 15 had focal seizures that progressed to bilateral tonic-clonic activity. Patients treated with cenobamate had medication-resistant epilepsy, having been exposed to an average of 12 prior antiseizure medicines before pursuing treatment with cenobamate (range, 3–21). In addition, 82% (n=37) of this group had either prior epilepsy surgery (n=2), device treatment (n=17 with 1 device; n=2 with more than one device), or both prior surgery and device treatment (n=16). There were 28 patients who reported rash with any prescribed medication in the past, 5 of whom had a history of rash due to prior antiseizure medications. None of the patients had a history of Stevens-Johnson or drug reaction with eosinophilia and systemic symptoms (DRESS) (Table 2). There were 10 (37 %) female patients using contraceptives during treatment. None reported changes in their menstrual cycle, and no pregnancies occurred.

Table 2. Patient Characteristics

Characteristic	Value
Gender, n (%)	
Male	18 (40.00%)
Female	27 (60.00%)
Age, median (range)	
Adolescents (12-17)	13 (13–17)
Adults (18+)	32 (18–44)
Study Attributes, n (%)	
Patients with devices (VNS, RNS)	17 (37.78%)
Patients with prior epilepsy surgery	2 (4.44%)
Patients with both surgery and device	16 (35.56%)
Patients taking concomitant EIAEDs	26 (57.78%)
Patients taking no concomitant EIAEDs	19 (42.22%)
Patients with prior anti-seizure medication drug-associated rash	5 (11.11%)

At initiation of cenobamate, patients were taking an average of 3 antiseizure medications (range, 1–5), while at the last follow-up their anti-seizure burden was decreased by at least 1 (range, 1–3) medication in 49% of patients (n=22); 7 (16%) were able to have 2 anti-seizure medications withdrawn. The average duration of treatment to date is 10.8 months with a range of 2 to 15 months and mean of 9 months. There were 27 patients (60%) who were responders (i.e., they obtained at least a 50% decrease in the number of focal seizures); both adolescents and adults obtained this extent of seizure improvement (61.5% of adolescent patients, n=8 and 59.4% of adults, n=19) (Figure 1). After comparing baseline seizure frequency, 7 (16%) patients became seizure free during the time of the study, with an average follow-up of 10.6 months (range, 4–15 months), while on treatment with cenobamate. A total of 5 patients discontinued the medication: 3 due to a lack of improvement in seizure control, and 2 were interrupted because of insurance complications (n=1) and gastrointestinal surgery (n=1), rather than issues of efficacy.

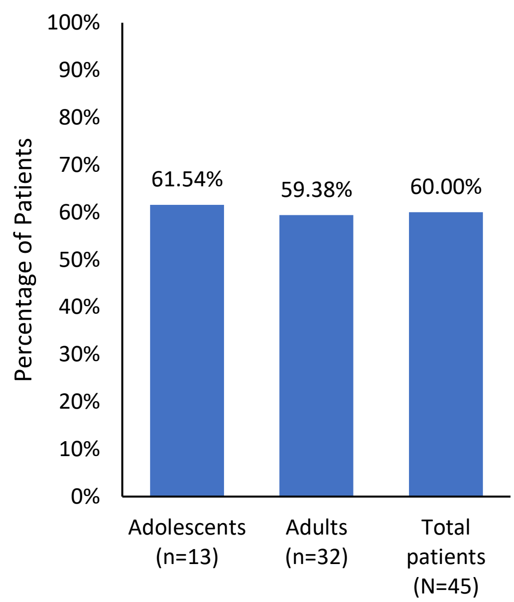

Figure 1. Percentage of Patients who Experienced at Least 50% Reduction in Seizures (Responder Rate)

The average daily dosage for cenobamate was 204.0 mg/day in adolescents (range, 50–300 mg/d) and 223.4 mg/day (range, 100–350 mg/d) in adults. The most common dose used in adolescents was 250 mg/d (n=5) and 250 mg/d in adults (n=9). There were 26 patients taking enzyme-inducing anti-seizure medications; the average cenobamate daily dose for these patients was 222.0 mg/d (range, 100–350 mg/d), and the average dose for the non-enzyme-inducing group was 215.8 mg/d (range, 50–350 mg/d). Serum levels in this study were determined using levels collected from 34 patients at the last follow-up appointment. Levels were not obtained for 11 patients. The average serum level for the enzyme-inducing group was 25.2 µg/mL (range, 8.5–46.6; prescribed cenobamate, 3.05 mg/kg/d); and 23.0 µg/mL (range, 9.8–44.3; prescribed cenobamate, 3.61 mg/kg/d) for the non-enzyme-inducing group (Table 3).

Lacosamide (n=19; 42.2%), cannabidiol (n=16; 35.6%), clobazam (n=13; 28.9%), and felbamate (n=10; 22.2%) were the most common medications taken concomitantly with cenobamate.

Concomitant administration of cenobamate with clobazam has been shown to increase the serum concentration of N-desmethylclobazam, resulting in increased dizziness, somnolence, and ataxia. In light of the drug-drug interaction between clobazam and cenobamate, clobazam doses were decreased based on clinical decision making to minimize these effects, most notably somnolence.[10] There were 13 patients taking clobazam at the time therapy was initiated with cenobamate, and they were prescribed doses of 5 to 60 mg of clobazam a day; 4 patients had significant somnolence and required adjustment of their cenobamate dose schedule and clobazam dose. Patients experiencing somnolence had an average daily clobazam dose of 22.5 mg a day (range, 5–40 mg/d) while those without side effects were taking an average of 30 mg a day (range, 10–60 mg/d). For these patients on clobazam and cenobamate who developed significant sedation, we typically paused at a lower cenobamate dose from the projected final cenobamate dose while the clobazam dose was lowered, as clinically allowed, waiting for the somnolence to improve. Also, all the patients who had sedation while taking clobazam and cenobamate had their total antiseizure medication burden decreased at last follow-up visit (n=2 by 1 medication; n=2 by 2 medications). For those on this combination of medications without somnolence, only 5 of the 9 patients had their total antiseizure medication burden lowered, 3 were unchanged, and it was increased in 1 patient.

No current studies have reported on the use of cenobamate and felbamate in combination. Of the 10 patients taking felbamate at cenobamate initiation, only 2 required adjustment to their felbamate dosage per clinical decision. Dosages of felbamate ranged from 2000 to 5400 mg a day with an average of 3350 mg a day at cenobamate initiation. Routine surveillance labs were reviewed for patients on felbamate, and they had not experienced any laboratory abnormalities on felbamate, even though all had been on therapy for over 1 year, or after beginning treatment with cenobamate.

Table 3. Study Findings – Cenobamate in Adolescents & Adults

Result	Value
Primary Findings	
Adolescent average dose (range)	204.0 mg/d (50–300 mg/d)
Adult average dose (range)	223.4 mg/d (100–350 mg/d)
EIAED group average dose (serum level)	222.0 mg/d (25.2 µg/mL)
Non-EIAED group average dose (serum level)	215.8 mg/d (23.0 µg/mL)
Patients decreasing medication burden by >1, n (%)	22 (48.89%)
Patients with >50% decrease in seizures, n (%)	27 (60.00%)
Adolescents with >50% decrease in seizures, n (%)	8 (61.54%)
Adults with >50% decrease in seizures, n (%)	19 (59.38%)
Patients attaining seizure freedom	7 (15.56%)
Other Findings	
Treatment duration average (range)	10.8 months (2–15 months)
Average follow-up (range)	10.6 months (4–15 months)
Patients taking lacosamide, n (%)	19 (42.22%)
Patients taking cannabidiol, n (%)	16 (35.56%)
Patients taking clobazam, n (%)	13 (28.89%)
Patients taking felbamate, n (%)	10 (22.22%)
Average number of prior anti-seizure medications (range)	12.2 (3–21)

Somnolence was the most frequently reported side effect. This was treated by decreasing the dose of cenobamate or other medications (n=8; 18%). No patient discontinued cenobamate due to adverse events.

Discussion

This study found that cenobamate was effective in treating focal seizures in adolescents and adults who had drug-resistant epilepsy. The slightly greater than 50% responder rate is especially impressive in this population with documented failure to a high number of prior antiseizure medications, and many had undergone epilepsy surgery and/or device therapy. In the phase 3 trial conducted by SK Life Sciences, common side effects reported were somnolence (28%), dizziness (28.1%), fatigue (16.6%), and headache (11.4%).[8] Somnolence was also the most common side-effect in this study. This could be ameliorated by dose adjustment of cenobamate or other medication, especially clobazam. Additionally, lowering their total drug burden of anti-seizure medication probably helped minimize the incidence of sedation. None of the study patients experienced new adverse effects. Importantly, patients with a prior history of rash to antiseizure medications or other prescription medicines (who were excluded from the FDA registry trials) experienced no rashes related to cenobamate.

Prior studies demonstrated that cenobamate interacts adversely with certain antiseizure medications, namely phenytoin, phenobarbital, and clobazam. To decrease the risk of adverse reactions, dosages of clobazam were reduced when cenobamate was initiated if the daily dose was greater than 30 mg. However, even knowing of this interaction, due to the long half-life of clobazam and its active metabolite, there were still difficulties with somnolence during this transition period to lower clobazam doses. This side-effect resolved over time with the adjustments in their clobazam dose. Interestingly, patients in this study prescribed higher doses of clobazam did not appear to experience more difficulty with somnolence, although this may be due to the relatively small difference in doses between the 2 groups (22.5 mg/day for those reporting somnolence, 30 mg a day for those not reporting somnolence). This side effect may be unavoidable for patients taking higher doses of clobazam at the time cenobamate is initiated, and it is worth counseling families that this will take several weeks to resolve if they are on this combination. No patients were on phenytoin or phenobarbital at cenobamate initiation.

This study matches with prior regulatory studies, suggesting that cenobamate is efficacious and safe to use in adolescents. Additionally, when the adolescents had similar serum levels as those seen in adults, they had similar efficacy. The strengths of this real-world study include the flexibility to adjust background medications, the speed of titration, and the dose of cenobamate to help improve tolerability of this medication, unlike the prior forced titration studies used in registry trials. This study suggests that cenobamate may be an effective medication to help control focal seizures in adolescents. The adult findings seem to mirror adult population studies (of achieving a seizure reduction of at least 50%). This promising finding supports the need for extrapolation studies of cenobamate for children and adolescents.

Limitations of this study include the fact that this is a single-center study with a small sample size and is retrospective in nature. All patients were well-known with baseline and follow-up seizure documentation, but not all cenobamate serum levels were able to be collected. Overall, the most substantial limitation of the study is that the adolescent cohort was only 29% of the total patient population (n=13). The benefit this small group received may not necessarily indicate that all adolescent-age patients with focal seizures will receive similar clinical benefit. Further studies of cenobamate in pediatric populations, using extrapolation techniques to achieve similar serum levels are needed.

Conclusion

This study provided evidence to support the efficacy from previous regulatory trials of cenobamate, extending it into the adolescent age group. No new adverse effects were reported. In addition, none of the patients with prior history of rash to any medication—or specifically to other anti-seizure medications—experienced a rash from cenobamate while using the approved dose titration, indicating that the medication can be used in this population. In this group of patients with refractory epilepsy, based on their number of prior antiseizure medications and surgical procedures, over 50% of the adolescent (12–17 years of age) and adult (18+) patients showed at least 50% seizure reduction following the addition of cenobamate to their prescribed medical regimen.

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Psychobiotics as a Potentially Useful Preventative Measure Against the Negative Consequences of Early Life Stress

Olivia Street, Department of Neuroscience, Rhodes College

The hypothalamic pituitary adrenal (HPA) axis is a key regulator of the body's response to environmental stressors; however, experiencing early life stress (ELS) leads to sustained activation of this axis and disrupts the development and epigenetic regulation of the cortical regions that coordinate it. As such, animals exposed to ELS have altered basal and stress-induced corticosterone levels, as well as increased anxiety-like and depressive behavior. These phenomena are reflected in humans, where those exposed to ELS have greater susceptibility to mood disorders. However, psychobiotics, which are beneficial probiotics, administered during early development have been effective in mitigating the adverse physiological and behavioral effects of ELS in rodents, and protecting against the transmission of altered behavior onto the offspring of ELS-exposed parents. Germ free (GF) animal models provide evidence for the connection between the microbiota and HPA-axis, as their lack of a microbiota results in altered development and function of the axis and related cortical areas. This effect is likely mediated by vagal afferents located in the gut that are responsive to changes in its composition. Psychobiotics take advantage of this connection by altering the composition of the gut microbiota to influence the development and function of the cortical regions that are affected by ELS. Therefore, psychobiotics effectively protect against adverse effects of ELS on those regions, resulting in typical physiological and behavioral functionality despite exposure to ELS.

Introduction

In today's world, stress is all around us: at our jobs, in our schools, and in our increasingly interconnected social worlds. Stress can sometimes even be present in a child's early development; This is called early life stress (ELS). The hypothalamic pituitary adrenal (HPA) axis is the body's primary way of responding to these stressors. In particular, the HPA axis receives inputs from cortical areas including the amygdala, hippocampus, and prefrontal cortex (PFC). If the HPA axis is activated by an external stressor, a hormonal signaling cascade begins, starting with the release of corticotropin which then releases the hormone (CRH) from the paraventricular nucleus (PVN) and ends with the release of glucocorticoids, cortisol in humans and corticosterone in rodents, from the adrenal cortex. Glucocorticoids then circulate throughout the body to prepare it to cope with the stressor, and bind to glucocorticoids receptors (GRs) in the hippocampus, prefrontal cortex (PFC) and other cortical areas to inhibit the HPA axis once it is no longer needed (Smith and Vale, 2006). However, experiencing early life stress (ELS) leads to chronic activation of this system, which exposes the developing brain to excess levels of CRH and glucocorticoids that disrupt its developmental processes and alter the epigenetic regulation of related cortical areas (Ivy et al., 2010; Nanicik et al., 2015; Sterrenburg et al., 2011; Murgatroyd and Spengler, 2011). These alterations negatively affect development, specifically the functionality of the HPA axis, and in turn increase susceptibility to anxiety-like and depressive behavior in rodents (Bravo et al., 2011) and mood disorders in humans (Fernando et al., 2012), which can be passed to offspring through epigenetic mechanisms (Coley et al., 2019).

Although detrimental, this process can be interrupted with the use of psychobiotics, which take advantage of the bidirectional relationship between the gut and brain to positively influence the development of stress-related cortical regions. The vagus nerve (VN) is the primary mode of afferent and efferent signaling along the axis, and likely underlies the ability of probiotics to influence neuronal processes, as vagal afferents located throughout the gut are responsive to probiotic-induced changes in the composition of the microbiota (Kim et al., 2020). As such, the administration of a probiotic can influence neuronal development and function through vagal-related modifications in immune activation (Moya-Perez et al., 2016). Preliminary animal models have demonstrated the effectiveness of psychobiotics in protecting against the adverse effects of ELS on HPA axis development and functionality, as well as in preventing ELS-associated behavioral effects from being passed to offspring of ELS-exposed rodents. Through this process, psychobiotics may provide a useful preventative measure against the negative effects of ELS on the HPA axis development and functionality for current and future generations by influencing vagal-mediated afferent signaling along the GBA to positively alter neuronal development and epigenetic regulation.

Vagal Pathways of GBA Communication

The GBA is a bidirectional system of communication between the brain and the gut. Thus, alterations in the microbiota of the gut mediate physiological and behavioral changes in the CNS (Sudo et al., 2004), while alterations in neural activity affect the function of the gut (Demaude et al., 2006) and composition of the microbiota (Bailey et al., 2011). As previously mentioned, the vagus nerve (cranial nerve X) has been established as a key neural pathway of afferent communication from the gut to the brain (Foster et al., 2017). This is demonstrated in Figure 1 below where the vagal afferents are located throughout the gut and are responsive to changes in the composition of the microbiota (Kim et al., 2020). This organization and the composition of the microbiota may underlie this mechanism of communication for the vagal afferents. (Bravo et al. (2011) Additionally, this composition demonstrates that oral administration of a L. rhamnosus strain, which altered the composition of the microbiota, reduces anxiety-like and depressive behavior, and reduces the expression of GABAAα2 receptor mRNA in the amygdala and other cortical areas of adult mice. Although, this seems successful, subdiaphragmatic vagotomy can block these behavioral and molecular changes from occurring, which indicates that the vagus nerve is necessary for afferent signaling along the GBA. To aid this issue, Bercik et al. found that the administration of oral antimicrobials (ATMs) altered the composition of the microbiota to increase both exploratory behavior and hippocampal expression of BDNF in adult mice, independent of whether the vagal nerve was intact (Bercik et al., 2011a). This discovery suggest that the effects of these ATMs are mediated by an alternative, non-vagal pathway. Taken together, these results indicate that there are both vagal and alternative, non-vagal pathways of afferent communication between the gut and brain.

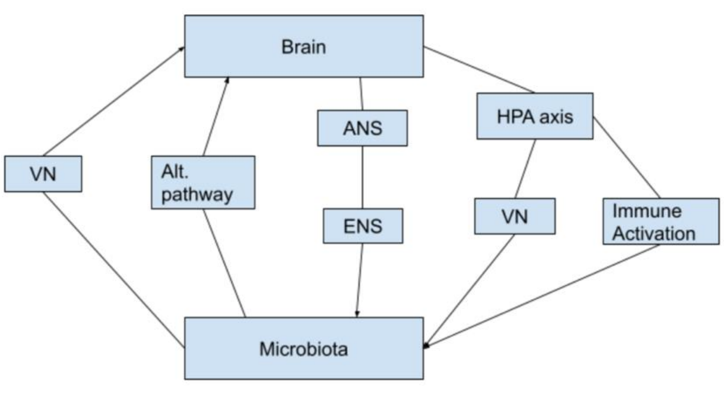

Figure 1. Describes the afferent (vagus nerve and alternative) and efferent (HPA axis-related vagal and immune activation, and the ANS to ENS connection) pathways of communication along the GBA, as discussed in the paper.

The vagus nerve also serves as a key efferent pathway of communication. Under non stressed conditions, the vagus nerve inhibits M1 proinflammatory macrophages in the small intestine (Yuan and Taché, 2017). Aside from this, HPA-related activation with the PVN inhibits the activity of the VN, meaning that the activation of the HPA axis indirectly has pro-inflammatory effects on the gut because the VN is no longer inhibiting it. In that way, continued inhibition of the VN by chronic stress may contribute to dysbiosis of gut microbiota, which is a reduction in the diversity of the species present in it. In line with this proposed mechanism, Bailey et al. found that social stress also induced dysbiosis, by decreasing the relative abundance of Bacteroides species, and activated the immune system, as indicated by increased interleukin-6 production (2011). Additionally, acute stress increases gut permeability (Overman et al., 2012), possibly through the activation of mast cells responsive to CRH (Cao et al., 2005) that is released as part of the HPA-axis mediated stress response (Dickerson and Kemeny, 2004). In parallel, the ANS influences the ENS, which coordinates intestinal functions such as permeability and motility, among others (Mayer et al., 2014). Overall, HPA axis-related vagal signaling and immune activation, and the relationship between the ANS and ENS work in tandem to regulate the brain's influence on the gut. Further research is needed to fully understand the mechanisms of action of both afferent and efferent GBA communication, although the vagus nerve plays a key role in both directions.

HPA Axis Development

As explained above, the vagus nerve connects the activity of the HPA axis to the GBA, in both afferent and efferent directions; however, the HPA axis has independent functionality as well. The HPA axis activates in response to a perceived stressor in the environment by preparing the body to effectively cope with the stressor and ultimately return to a state of homeostasis (Smith and Vale, 2006). The development of this system is critical to its' functionality in adulthood, and it is particularly sensitive to environmental input during both prenatal and postnatal periods of early development (van Ooers et al., 1998a). Therefore, exposure to early life stress (ELS) during early childhood can disrupt this development and affect epigenetic control of associated cortical regions, leading to alterations in both its basal and stress-induced activity, as well at the GR-mediated negative feedback loop. These physiological changes are mirrored by increased levels of anxiety-like and depressive behavior in rodents exposed to ELS (Moya-Pérez et al., 2017).

ELS in humans is often complex and involves both social and physical factors (Sheridan and McLaughlin, 2014), whereas animal models often use maternal-separation models to replicate the ELS condition (Moya-Perez, 2016). In both cases, ELS results in heightened activation of the HPA axis during a period in the lifespan in which its activation it otherwise low (Schmidt et al., 2006). This heightened level of activation exposes the cortical regions that are involved in the HPA axis process and creates excess levels of stress hormones that alter the development of said regions, as described in Figure 2. In particular, ELS is associated with increased CRH expression in the hippocampus resulting in changes in dendritic morphology (Ivy et al., 2010) and altered neurogenesis, differentiation, and survival of newborn cells (Nanicik et al., 2015).

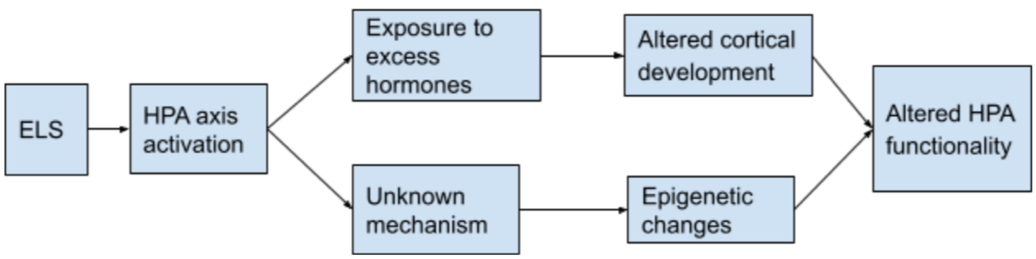

Figure 2. Describes how ELS leads to sustained HPA axis activation, which in turn alters the development and epigenetic regulation of associated cortical areas, resulting in altered HPA functionality.

Moreover, ELS induces changes in the epigenetic regulation of cortical regions in the HPA axis and the GR-mediated negative feedback loop. Although is not known if these changes are related to excess hormone exposure or not, Sterrenburg et al. (2011) found that chronic early life stress in rodents led to alterations in CRF gene methylation in the PVN and central amygdala (CeA) in a sex-dependent manner. In contrast, Roth et al. (2009) found that chronic early life stress reduced BDNF expression in the PFC. Aside from these two theories, epigenetic changes may also be responsible for the attenuated negative feedback regulation of the HPA axis found in ELS-exposed rodents and humans. This idea was tested by Weaver et al. (2004), who found that stress related behavior in maternal care, induced ELS in one's offspring and was associated with altered expression of a GR gene promoter in the hippocampus. Murgatroyd and Spengler (2011) further confirmed these results and found that altered transcriptional programming of the GR occurred through increased DNA methylation at a specific site in a promoter in response to ELS. Additionally, the DNA methylation changes that developed during early life persisted into adulthood. These results highlight one potential mechanism by which epigenetic changes regulate the attenuated GR feedback loop in the HPA axis after ELS. This pathway is notable because this type of epigenetic changes can persist throughout the lifetime and be passed onto future generations (Coley et al., 2019). These results show clear patterns of altered development of stress-related brain regions and epigenetic changes in response to ELS, for both current and future generations.

The proposed mechanisms work in tandem to alter the function of HPA axis in response to ELS and the functional consequences of this alteration include modifications in both basal and stress-induced activation of the axis (van Ooers et al., 1998), as well as an attenuated GR feedback loop (Murgatroyd and Spengler, 2011). These physiological changes are reflected in behavioral outcomes in animals, where they present as increased levels of anxiety-like and depressive behavior in rodents (Moya-Pérez et al., 2017), and in humans as increased susceptibility to mood disorders (Fernando et al., 2012). Therefore, the development of the HPA axis is critical to its later functionality, but ELS disrupts that process and results in adverse behavioral outcomes unless effective preventative measures are instilled to protect against any disruption.

That GBA and HPA Axis Connection

There is increasing evidence that the development of the HPA axis is influenced by afferent communication along the GBA, both vagal and immune-related, in response to changes in the composition of the microbiota. Germ-free (GF) models of animal development, in which the rodent is born and raised in a sterile environment and lacks a gut microbiota, have been useful in understanding this relationship. Sudo et al. (2004) demonstrated that GF mice had functional alterations in their HPA axis development, specifically resulting in an exaggerated response to stress, as measured by increased corticosterone levels and anxiety-like behavior compared to controls. When colonized by the microbiota of a typically developed mice at 6 weeks old, the exaggerated HPA response in mice was reversed; however, colonization at 14 weeks did not have an effect. This finding suggests that the effect of the microbiota on the HPA axis fits into the previously discussed window for its development. Luczynski et al. (2016) found that GF mice had altered volume and dendritic morphology in both the hippocampus and amygdala as compared to conventionally colonized mice, providing evidence that the microbiota also influences development of stress-related cortical areas.

As described in the Figure 3, The study of psychobiotics takes advantage of this relationship by using probiotics to positively influence the development of stress-related brain regions and protect against the functional effects of ELS on the HPA axis. Vagal afferents in the gut are responsive to these changes (Kim et al., 2020), and thus, likely underpin the effectiveness of psychobiotics. However, more research is needed to understand the exact mechanisms that partakes in the process of psychobiotics. Probiotics are specific strains, or combinations of strains, that are purposefully introduced to the gut microbiota to alter its composition. Certain strains have particular effects, and currently the Lactobacilli and bifidobacteria strains have been most researched in preclinical trials, with promising physiological and behavioral results.

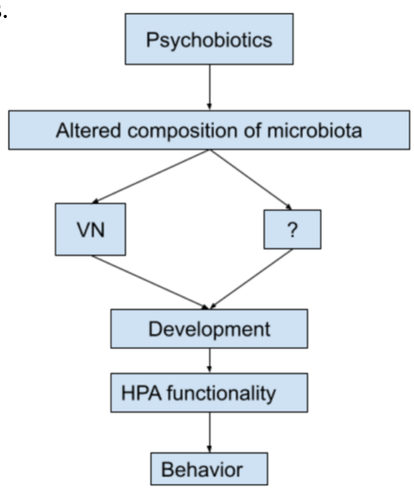

Figure 3. Demonstrates how psychobiotics alter the composition of the microbiota, in a way that the VN and other pathways are sensitive to, which affects the development of stress-related cortical areas and the axis itself to positively influence HPA function and behavioral outcomes, despite ELS.

rhamnosus was associated with reduced stress-induced corticosterone and anxiety or depression related behavior. Brave et al. (2011) also identified alterations in the expression of GABA receptor mRNA in the hippocampus, amygdala, prefrontal cortex, and other cortical areas, which may underlie those behavioral changes.

As previously noted, there may be a particular window for HPA axis development during which alterations in the composition of the microbiota can positively influence it. Moya-Pérez et al. (2017) investigated the effect of psychobiotics on the physiological stress response, immune activation, behavior, and diversity of the microbiome in mice exposed to ELS during early development. They divided male mice pups into four categories that were either subjected to maternal separation (MS) as a form of chronic stress or not, and supplemented with the probiotic B. pseudocatenulatum or placebo, from postnatal day 2 (P2) until P21. Then, on P41 they found that the MS-placebo fed mice had increases in basal and stress-induced corticosterone levels compared to the control. However, MS-probiotic mice displayed a reduced level of corticosterone in both conditions as compared to the MS-placebo mice. This result suggests that the administration of B. pseudocatenulatum may protect against negative alterations of ELS on HPA functionality. Using an elevated plus maze (EPM) as a behavioral test, they found that MS-placebo group demonstrated significantly more anxiety-like behavior than the control group on P41. The probiotic also reduced the levels of anxiety-like behavior of the MS-probiotic group to that of the non-MS mice. This result speaks to a potential application in humans, where the functional consequence of this psychobiotic on animal behavior would be reflected in decreased susceptibility to mood disorders in people (Fernando et al., 2012).

Moya-Pérez and colleagues investigated potential underlying mechanisms for these physiological and behavioral effects. They found that MS-placebo mice displayed intestinal dysbiosis, as shown through alterations in the relative abundance of several microbial species; however, this effect was not observed in the MS-probiotic group. The probiotic down-regulated MS-induced intestinal inflammation, as measured by lower levels of interferon gamma [IFN-y]) in the small intestine of MS-probiotic group relative to the MS-placebo group. These findings fit into the previously discussed evidence that the VN serves as the primary afferent along the GBA because it is sensitive to changes in the composition of the gut microbiota and may be related to immune activation (Kim et al., 2020). These results indicate that the administration of B. pseudocatenulatum protected mice from the negative effects of ELS on HPA axis' later functionality and behavior. The results from this study suggests altered composition of the microbiota and lessened immune inflammation as possible mechanisms by which this process occurred.

In addition to the positive impacts of psychobiotic administration observed during an animal's lifetime, Callagan and colleagues (2016) provide evidence that behavioral effects may also be heritable, possibly protecting future generations against the negative consequences of ELS on behavior. Callagan and colleagues (2016) exposed first generation male rats (F0) to MS and either fed them a probiotic mixture of Lactobacillus rhamnosus strains or placebo from P2 to P14 and mated them with non-stressed female rats. The researchers then raised the offspring pups (F1) under non-stressed conditions and gave the offspring of the non-probiotic F0 males either a probiotic treatment or none. A series of behavioral tasks were used to test the retention and extinction of aversive memories in the F1 generation. By comparing their results with previous literature, they demonstrated that persistent retention of aversive associations and relapse after extinction emerged earlier than normal in the offspring of MS fathers, demonstrating a generational effect of ELS.

However, probiotic treatment in the F0 generation prevented the transmission of MS effects on aversive learning to the F1 generation. Probiotic treatment of the F1 generation, but not F0, also eliminated the effects on aversive learning. These results suggest that probiotic treatment may be both an effective preventative measure and an efficient treatment of the epigenetic-mediated generational effects of ELS in rodents. Further research is needed to understand the underlying mechanisms of this process; however, it is possible that the administration of Lactobacillus rhamnosus prevented ELS-related changes in the epigenetic regulation of stress-related cortical areas from taking place in the F0 generation, which protected the F1 generation from experiencing the behavioral effects seen in the placebo group.

Conclusion

The connection between the gut and brain is increasingly understood to be a powerful tool that can be used to influence the development and function of the body, with potential applications to humans. Of course, psychobiotics is one potential application that takes advantage of the GBA to influence the development and function of the HPA axis in later life. This connection is particularly important because, as discussed in this paper, the development of the axis is critical to its functionality throughout our later lives. However, the development of the HPA axis can be disrupted by ELS resulting from induced and sustained activation of the axis in early life. To prevent the effects of negative exposure for the HPA axis, Moya-Pérez et al. (2017) demonstrated that psychobiotics administered during development are effective at preventing the adverse physiological and behavioral changes typically associated with ELS.

Additionally, Callagan et al. (2016) established that the heritability of ELS-related behaviors can be limited from one generation to the next. Overall, Vagal-mediated communication along the GBA is the mechanism most likely underpinning these results. In these studies, probiotic-induced changes in the composition of the gut microbiota are reflected in positively altered cortical development and epigenetic processes. The composition of the microbiota and the positively altered cortical development effectively counteracted ELS-related alterations in the same cortical areas that result from a functioning HPA axis and other behavioral outcomes. More research is needed to establish their potential applicability in humans; however, animal model studies suggest that psychobiotics would provide a similar and effective preventative measure against the negative consequences of ELS in humans.

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The Effect of Indoor vs. Outdoor Environments on the Growth Rates of Dusky Gopher Frog Tadpoles (*Lithobates sevosus)*

Madeline Yde, Sinlan Poo, and Allison Bogisich

Amphibian growth is generally characterized by three distinct life stages: embryonic, larval, and adult. The conditions of a tadpole's environment can affect their successful development. The intention of this experiment is to show whether there is a significant difference between growth rates of captive dusky gopher frog (Lithobates sevosus) tadpoles raised in indoor versus outdoor enclosures at the Memphis Zoo. Egg clutches were cared for in a lab setting for 10 days post-oviposition (DPO) before tadpoles were separated into indoor and outdoor enclosures. Tadpoles were measured at 26-27 DPO for total length (mm), tail length (mm), and body width (mm). Findings of this study demonstrated a significant difference in tadpole growth between enclosure settings. These results can be used to better inform the methodology for captive rearing of endangered amphibians that endure similar pressures as the dusky gopher frog. As humans further contribute to the decline of amphibian species via habitat degradation, it is imperative to gain a more complete understanding of how changes in temperature may affect their growth rate.

Introduction

Amphibian growth can be broadly defined by three distinct life stages: embryonic, larval, and adult. According to Gosner's staging table (Gosner 1960), the embryonic life stage includes stages 1-25; important developments from this period are the neural tube, gill plates and external gill filaments, muscular responses, adhesive organs, a transparent cornea and tail fin, and the operculum, among others. The larval stage, consisting of stages 26-41, includes critical morphological developments such as the hind limbs, foot paddles, toes, and tubercles that are necessary for adult amphibian life. Finally, the metamorphosis stages are stages 42-46, distinguished by the appearance of forelimbs, tail absorption, and changes to the mouth (Gosner 1960). These successive developments – especially at the larval stage– are crucial to amphibian survival.

However, a growing tadpole's environment can directly influence their development. As anthropogenic pollution and its resulting climate change heighten in severity, amphibian survival can become inhibited, as they are especially sensitive to environmental changes. The endangered dusky gopher frog (Lithobates sevosus), currently only present in populations in the state of Mississippi, relies on wetland habitat for larval growth. Wetlands are specifically susceptible to climate change because of its effect on the hydrological cycle (USFWS 2015). Additionally, amphibians, like dusky gopher frogs, are also influenced by climate change because of their permeable skin that amplifies their susceptibility. Because their skin permits greater percutaneous passage of substances, amphibians are more intensely affected by changes to gas concentrations and nutrient cycles in their habitat (Quaranta et al. 2009). It is therefore evident that amphibians, such as the dusky gopher frog, are at risk from climate change, and many of their populations are declining as a result (Wake et al. 2007). The Memphis Zoo spearheads a captive breeding program for the endangered dusky gopher frog, with a purpose to help contribute to a stable wild population (Reichling et al. 2022). This experiment intends to demonstrate whether there is a significant difference between growth rates of dusky gopher frog tadpoles raised in indoor versus outdoor enclosures. The results of this study will aim to improve the captive rearing procedures of amphibians. With amphibian species being increasingly threatened by habitat degradation from human development, it is critical to obtain a better understanding of how changes in temperature may affect their growth rate.

There have been anecdotal observations of a correlation between enclosure temperature and growth rates. Under natural settings, higher ambient temperatures have been associated with increased growth rates in amphibians (Li et al. 2020). It was therefore hypothesized that temperature would affect dusky gopher frog tadpole growth. It was predicted that dusky gopher frog tadpoles would exhibit a faster rate of development in indoor enclosures with higher ambient temperatures than outdoor enclosures with lower temperatures. The broader implications of this project include the potential for better recommendations for captive dusky gopher frog care as well as the application of specific findings on their temperature regulation to a wider group of amphibians. Since captive-release programs ultimately aim to contribute to the growth of wild populations, by improving the viable offspring in captivity, researchers can hope to increase the populations of these endangered species in their native habitats.

Methods

Six breeding pairs of dusky gopher frogs were closely monitored in an artificial pond at the Memphis Zoo. Four viable egg clutches were carefully obtained from the pond within 48 hours after they were laid. Each clutch was placed in a filtered water tank in a lab setting. At 10 days post-oviposition (DPO), tadpoles were moved from the lab into their respective indoor and outdoor enclosures. Two indoor and two outdoor enclosures (27.9 in x 17 in x 15.1 in) were monitored. Each indoor enclosure monitored for this experiment held 20 tadpoles, while each outdoor enclosure housed 25 tadpoles. Each individual enclosure was a part of a larger water system. Each indoor water system consisted of four enclosures, while each outdoor water system consisted of five enclosures. Each system had a water filtration system of 100 gallons. At 26-27 DPO, all tadpoles from the four enclosures monitored were measured for total length (mm), tail length (mm), and body width (mm) using Image J software (Figures 1 and 2) (Rasband 2018). Enclosures were tested once a week for ammonia (ppm), nitrate (ppm), and pH levels (Table 1). Additionally, daily temperature (ºF) recordings were taken via HOBO data loggers (Table 1).

The averages, standard deviation, and standard error were recorded for all measurements and conditions. A paired t-test was used to compare the morphological data between indoor enclosures and outdoor enclosures at 26-27 DPO (Figure 2) with an alpha valued of 0.05.

	Temperature (ºF)	Ammonia (ppm)	Nitrate (ppm)	pH
Indoor	74.29	0.08	9.50	7.45
Outdoor	60.13	0.06	7.46	5.60

Table 1. The average environmental conditions, including temperature (ºF), ammonia (ppm), nitrate (ppm), and pH levels for indoor and outdoor enclosures.

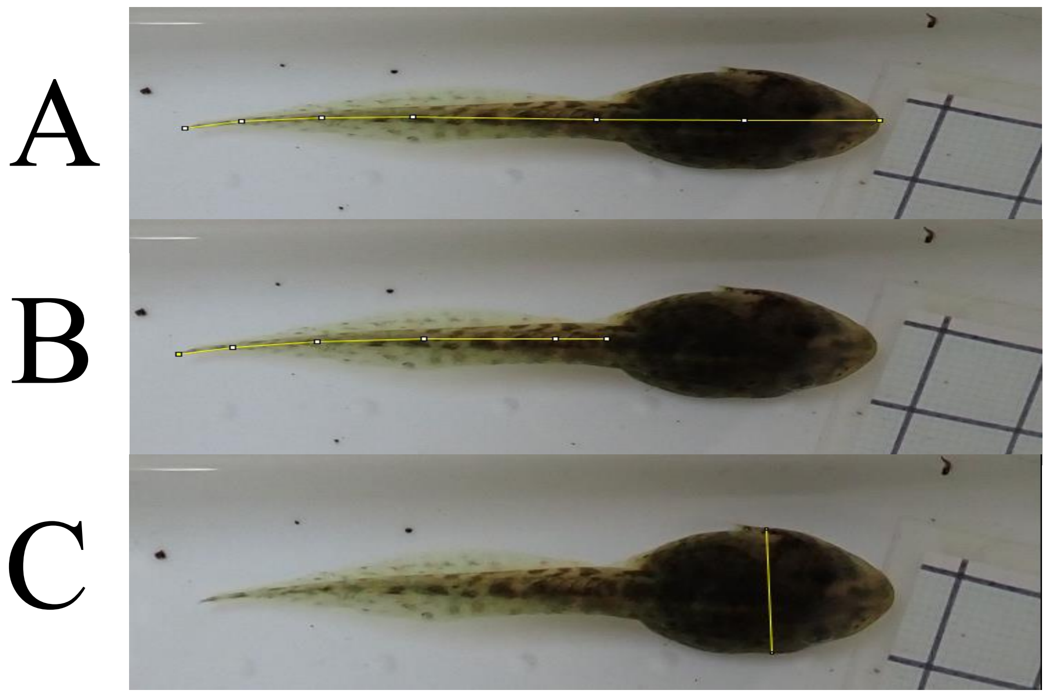

Figure 1. Demonstration of how tadpole measurements were taken through Image J of A) total length (mm), B) tail length (mm), and C) body width (mm). A scale of 1 mm was used.

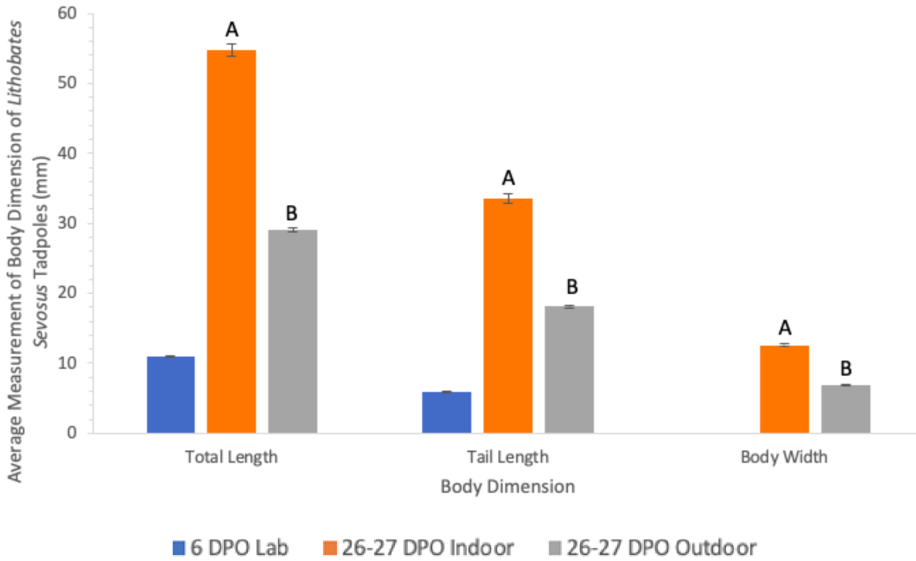

Figure 2. The average measurement (mm) of body dimension (mean + SE) of dusky gopher frog tadpoles (Lithobates sevosus) in indoor and outdoor enclosures at 26-27 DPO compared to the baseline indoor laboratory condition at 6 DPO.

Results

For the paired t-test, the value of A was compared to B for each respective body dimension. Tadpoles in indoor enclosures (total length: 54.77 $\pm$ 0.89, tail length: 33.53 $\pm$ 0.63, body width: 12.59 $\pm$ 0.19) grew as nearly twice fast as those in outdoor enclosures (total length: 28.88 $\pm$ 0.33, tail length: 18.02 $\pm$ 0.22, body width: 7.00 $\pm$ 0.10). The environmental conditions allowed for different results at the P=0.05 significance level (df=78 [for total length and tail length], 79 [for body width]; $P<0.001$).

The results for the effect of environment on the growth of dusky gopher frog tadpoles show that body dimension measurements were higher in indoor enclosure locations versus outdoor enclosure locations for total length (mm), tail length (mm), and body width (mm) at 26 DPO (Figure 2). On average, indoor total length was 24.89 mm longer than outdoor total length, indoor tail length was 15.51 mm longer than outdoor tail length, and indoor body width was 5.60 mm wider than outdoor body width Figure 2).

Discussion

The findings from this study support the hypothesis that temperature affects dusky gopher frog tadpole growth. There was a significant difference in the morphology of dusky gopher frog tadpoles between indoor and outdoor environments (Figure 2). This agrees with the previously published work that suggests an increase in growth rate in higher temperatures (Li et al. 2020). Amphibian size is important because a small size can negatively affect survivorship and reproduction, ultimately influencing population size (Poo et al. 2021). Population size matters due to the increased likelihood for inbreeding in smaller populations, which can reduce sperm quality, further lowering the viability of the population (Hinkson et al. 2020).

While temperature is assumed to be the primary factor influencing the results we observed, it possible that tadpole growth could have been affected by other variables not accounted for during this study. Possible confounding variables include ammonia (ppm), nitrate (ppm), and pH levels. Additionally, the difference between the stability of the indoor temperatures versus the fluctuating nature of the outdoor temperatures may also be a confounding variable. To avoid this in future experiments, the ammonia (ppm), nitrate (ppm), and pH levels could be more frequently recorded to notify research staff of the need to adjust the water in the enclosures to control these variables. Future experiments may be conducted on other amphibian species to corroborate the data from the current study and better understand general amphibian growth rate patterns. These data have strong implications for the comprehension of amphibian growth and development in captive breeding because they help demonstrate the optimal environmental conditions for dusky gopher frog tadpole growth. The results of this study will allow the Memphis Zoo and other researchers to increase the impact of the endangered dusky gopher frog program by maximizing larval growth through increased utilization of indoor enclosures, with carefully monitored temperature, ammonia, nitrate, and pH levels. Findings from this study on the impact of environment on size may therefore help build better captive conservation programs and thus contribute to biodiversity preservation.

Conclusion

In conclusion, the results support the hypothesis that temperature affects dusky gopher frog tadpole growth. Dusky gopher frog tadpoles removed from the lab setting exhibited higher growth rates when placed in an indoor enclosure than an outdoor enclosure. The results of this study may be useful in understanding amphibian growth and development in captivity. There is thus potential for increasing the capacity and success of captive breeding programs of the dusky gopher frog, as well as other amphibians, through emphasis on tadpole rearing in indoor enclosures instead of outdoor enclosures.

Acknowledgements

Thank you to the Memphis Zoo for permitting this project, to Dr. Sinlan Poo and Allison Bogisich for supervising the research, and to Dr. Sarah Boyle for enabling the opportunity.

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Agroecology: Sustainable Agriculture and Practices

Christina Karem, Rhodes College

Agroecology is the principle of applying ecosystem services to farming practices. This paper examines what agroecology is, how it differs from organic farming, and how business and economics play a huge role in its success. Additionally, the social movements driving agroecological practices and the feministic ideology behind such movements are analyzed. This paper explores how the European Union has attempted to implement agroecological-focused policy but lacks data to demonstrate the success of such practices. I will address how agroecology and agroforestry intersect policy, how aspects of feminism and social drivers influence policy, and practices in the real world today. I find that agroecology is inherently feminist, with an emphasis on creativity, a collaboration of knowledge, and that grassroots organizations by rural farmers drive change. However, the European Union has been limited in its attempts to upscale sustainable farming. The overall conclusion finds that agroecology is the next step in farming practices but suffers from a lack of monetary incentives.

Introduction

The need to integrate sustainable farming practices into modern agriculture is here, and one solution is agroecological farming. Agroecology is a farming approach influenced by natural ecosystem services. It combines local practices, ecological principles, and social aspects to agricultural systems, focusing on the interaction between plants, animals, humans, and the environment. The Food and Agricultural Organization (FAO, 2021) of the United Nations (UN) identified ten critical elements that make up agroecology: 1) diversity, 2) co-creation and sharing of knowledge, 3) synergies, 4) efficiency, 5) recycling, 6) resilience, 7) human and social values, 8) culture and food traditions, 9) responsible governance, and 10) circular and solidarity economy. These ten pillars represent a simple and holistic way to think about the realities of implementing agroecological practices. They are an analytical tool to facilitate decision-making by stakeholders and governments when planning, managing, and evaluating transitions to sustainable practices. While evaluation and large-scale implementation rely on monetary aid from governments, agroecology requires a bottom-up, horizontal learning experience from farmers working with sustainable practices hands-on (Anderson et al., 2019). Horizontal learning implies cooperation and communication in a non-hierarchical and anti-authoritarian way, urging contributions of knowledge from and to a general pool of learners (Anderson et al., 2019). There are three aspects of horizontal learning, 1) strengthening learning experiences, 2) building confidence and capacity, and 3) challenging hierarchy through prefigurative politics (Anderson et al., 2019). Wide-based and bottom-up learning encourages collaboration between stakeholders like farmers who may implement agroecology and the shared knowledge creates a sense of community between these stakeholders (Anderson et al., 2019).

To understand why agroecology is important in today's farming, we must understand a brief history of agriculture. Driver's explanation outlines the history of agriculture, and this paper breaks it down into a simpler and more concise timeline. The cultivation of crops and domestication of animals began around 11,000 BCE due to pressures from climate changes, increased population density, overhunting of wild animals, and cultivation of seeds. Interestingly, Driver suggests that the transition from hunter-gatherer societies to civilizations created a divide amongst leaders because civilizations created a space for a leader to rise, whereas hunter-gatherer societies were more based on communistic, shared resources. Jared Diamond's Guns, Germs, and Steel reiterates this argument through the idea that modernization of agriculture creates a division of labor, allowing those specialized outside of agriculture the ability to ascend (Bergot, 2004). Another step in agricultural advancement came from improvements in harvesting and irrigation, allowing wider areas to be cultivated, but depleting the land of nutrients too quickly (Driver, n.d.). The next major step was the technological advance in food production and transportation, like using refrigerated vehicles to transport produce greater distances. This allowed countries like the United States to provide food for countries across the world in times of low crop yield. The most recent advancement in agriculture is synthetic fertilizer and genetically modified organisms (GMOs), or the so-called Green Revolution. While fertilizers increased yield, synthetics began to pollute fields and water sources with an abundance of elements that were not used by plants. This causes an imbalance in ecosystem services, leading to the destruction of once fertile lands. In this brief history of agriculture, we see shifts to meet the needs of humans, but little respect for the environment. Agroecological and sustainable practices reconnect us to the earth and demonstrate a better understanding of the limited natural resources we have available. The driver helps us understand how progress created a more efficient, but long-term detrimental agricultural society reliant upon human influence rather than ecological principles.

As a leader in agroecology research, Gliessman (2021) asserts that our modern agricultural system is vulnerable, now more than ever. For the past 70 years, agriculture has been monoculture farms, with genetically similar crops, animals raised in confinement, high chemical use, and a lack of policy support for small and medium farms. Gliessman claims that agroecology is a way to combat these vulnerabilities because it incorporates both social and environmental components. Agroecology requires policy implementation, co-creation of knowledge and participation, and a basic understanding of ecology. It encourages the sustainable exploitation of ecosystem services. Ecosystem services are any positive benefit that wildlife or ecosystems provide to people (Costanza et al., 1997). These services include water regulation, nutrient balance in the soil, carbon sequestering, and air filtration. When ecosystem services are partnered with agricultural practices, the need for man-made sources that harm the environment, such as fertilizers and irrigation systems, is decreased. Natural systems are already in place for plants and animals, like nutrient recycling and the water cycle, so, by incorporating farming with ecology, we see an increase in ecological efficiency and a decrease in toxins. Agroecology is the science of developing the design and best practices of sustainable agriculture and community food systems (Kluson, 2019). These designs come from measuring how much is provided in a certain system, as not every area of the environment is suitable for food production (Kluson, 2019).

Agroecology differs from organic farming in many respects. Organic farming is not inherently agroecological farming because organic farming can, and is, done on a large, mono-cultured scale (Shulman, 2020). Organic farming on a large scale focuses solely on producing a singular organic crop. These organic farms do not use chemicals, but they still harm the environment through soil degradation, destruction of natural wildlands, and decrease of water sources (Shulman, 2020). Agroecology uses ecological systems to prevent the destruction of the environment while also avoiding chemical use. Organic farms are good for humans, but bad for the environment.

Agroforestry is a critical component of agroecology. Agroforestry incorporates the use of trees and shrubbery to shield farms from the sun, which allows farms to grow upwards rather than simply outwards. Agroforestry allows for the maximization of functional diversity, decrease in weed cover, and increased crop yield from the diversity of plants in a system (dos Santos, 2021). Agribusiness builds off agroforestry as well. Agribusiness relies on the environment for economic profit. It is not a reflection of societal success and is not always sustainable, but it is highly successful due to the multitude of resources available and required by our economy. Agribusiness boomed during the Green Revolution. The Green Revolution, between 1960 and 1970, was a rise in agricultural growth and management in developing countries, as well as crop breeding for hardy varieties (IFPRI, 2002). This was done to provide struggling countries with crops that could resist droughts and flooding and would grow faster and produce higher yields. Businesses like Monsanto took advantage of this new market, created crop varieties, and patented them. Taking advantage of struggling countries and nature became the norm, and agribusiness rose. There were no laws preventing the privatization of nature and these new varieties were in such demand that countries were willing to pay any price to save their citizens from hunger.

Policy could be the key to agroecology becoming successful worldwide. The UN has incorporated agroecology into its goals for future governments and sees it as a key to succeeding in the Sustainable Development Goals (United Nations, 2015). The UN created an award called the Future Policy Award that recognizes laws and policies that are making life better for the future. Policies recognized involve agroecology, promoting a sustainable, healthy, and organic lifestyle. This award demonstrates the importance of agroecology in supporting transitions to sustainable agriculture (Gliessman, 2019). It emphasizes that the need for policy is urgent and must be enacted globally.

Agroecology goes beyond natural science. It is an economic undertaking that requires funds and support to transition into, an environmental solution promoting a healthy use of the land around us, and a social movement requiring collaboration, participation, and co-creation. It considers the beliefs, values, and assumptions of the social, political, and economic life structures affecting agriculture and food systems (Kluson, 2019). Agroecology roots itself in the human tie to nature.

Agrobiodiversity is not a new field, but leaders in the field like Gliessman have brought more attention and education to the subject. This paper looks at social movements that drive agroecology and the policies and outcomes in various countries where agroecology has either been implemented or is planned to be utilized more widely. The questions this paper will attempt to address are:

1. How do agroecology and agroforestry intersect policy?
2. How do aspects of feminism influence the implementation of agroecology?
3. What are the social drivers behind agroecology as an agricultural movement?
4. In what ways is agroecology being practiced in the world today?

Discussion

Agroecology promotes, and benefits from, many social movements and relies heavily on bottom-up implementation. It calls for horizontal learning, grassroots movements, feministic ideology, and putting agroecology into practice. I will explore each of these topics in turn.

Bottom-up Involvement

Grassroots movements have been drivers of social change in a variety of subjects, from civil rights to war efforts to sustainability. Bottom-up involvement starts with the farmers; the people doing the work hands-on and dealing with the hardships and vulnerabilities of agroecological practices (Gliessman, 2020). Many studies follow grassroots movements and find that farmers are the ones who are willing to try these new systems. Hazard et al. (2018) states that it is the job of researchers to get into farmers' practices rather than farmers getting into researchers' practices. Researchers cannot demand that farmers conform to the needs of their projects like the typical research study's demand of asking for volunteers. In any case, it is better to naturally study the implementation of sustainable practices rather than force a practice onto a farm that is not ready. Successful implementation relies on local farming context valuing the transition to sustainability as well as mutual learning between researchers and farmers to understand the best ways to approach agroecology. The UN recognizes that farmer support is the way to make sustainable agriculture successful and implemented a multi-billion-dollar idea of repurposing agricultural support to transform food systems (Food Systems Summit, 2021). The idea is to hold prices for goods like crops, allowing farmers in small-scale farms to succeed in their transition to sustainable practices while remaining competitive against larger farms. By doing this, farmers who put in more money must charge higher prices to make a profit are not being outcompeted by farms that can charge less and sell more. The UN sees this as a way to move towards a healthier lifestyle for consumers and a nature-positive, greener option for agriculture.

Whilst subsidizing farmers is a great way to build a movement towards a sustainable system, many grassroots movements are the products of necessity rather than luxury. Seyfang and Haxeltine (2012) examine how civil society-based social movements and community initiatives aid in the transition to a low-carbon economy in the United Kingdom. This paper builds off Seyfang's original concept from a 2007 paper on community-led grassroots innovations that emphasizes predominately social innovations developed at the community level rather than the government level. This paper looks at the so-called "transition towns" and their attempts to move towards a more sustainable economy. The movement came from the realization that eventually oil would run out and reliance on this commodity would become dangerous to the survival of small towns (Seyfang and Haxeltine, 2021). The plan was to establish community-owned companies and promote the movement individually rather than relying on subsidies from the government and other stakeholders after non-renewable sources ran out. This type of transition allows smaller towns, business owners, and farmers to become more independent, as well as demonstrating that collaboration is not as hard as it sounds. These transition towns came from individuals, not a major organization of grassroots. This is important because it also shows how individuals yearn for sustainability in the economy, recognizing the detrimental effects traditional farming has on their health, wealth, and environment. The result of this "TT" was an organization and a website, Transition Network, that reaches across the world with transition towns popping up constantly. There are 1,081 groups and 25 hubs that provide assistance, guidance, and an escape from our traditional mono-cultured lifestyles (Transition Network, 2021).

Knickel et al. (2009) also writes about the idea that change comes from farmers, grassroots movements, and individuals. Knickel et al. looks at rural farmers as a source of innovation but sees a disconnect in the need to change and the willingness to change, likely due to structural issues and economics. As has been addressed, change comes from necessity, but willingness comes from partnership, economic incentives, and co-creation. They recognize the increasing multifunctionality of agriculture in rural areas and how that agricultural land can provide more than a few crops; it can take the idea of agroecology and build it to the epitome of the experience. The paper emphasizes the need for economic, environmental, and social dimensions of sustainability. Knickel et al. also goes on to discuss how critical the shared knowledge aspect of rural farming and agroecology is because creating a safety net and community of farmers experimenting will create a sense of trust in the system. When one farmer finds success, others will likely follow, but if that success is minimized or kept an industry secret, the point of transitioning to sustainable agriculture becomes null. Agricultural producers rely on research, extensions, education, and support systems to become successful. Their success leads to societal success when done sustainably.

However, as soon as we acknowledge that farmers' interests and societal interests may diverge, we must pose the question of whether and how innovation policies ought to respond to both farmers' and societal problems (Knickel et al., 2009). Policy change can come from social movements; one must show the government how important it is for laws to be made. The European Union is a notable case in terms of agroecological policy. They are using the ten elements of agroecology and the thirteen agroecological principles as a framework for translating agroecology into policy (Gliessman, 2021). Policy requires a coalition between producers and consumers with an understanding that sustainability is needed. Gliessman (2021) suggests that those who study agroecology are too complacent when it comes to expecting policy to fully embrace the concepts of agroecology and have it implemented correctly (2021). However, these grassroots collaborations and cohorts take policy and action into their own hands and demand change or at least make a change to show that it is possible. Reliance on the government is risky because the funding may not come through or change will be vetoed. Giraldo and McCune (2019) explain that with social movement participation in governance structures, social movements face risks when they allow themselves to become absorbed in collaborations with the state to build public policy for taking agroecology to scale (2019). This means that collaboration with the government right away for social movements can totally derail the campaign. The government is required to take all sides into consideration, and often finds lobbyists on the opposite sides of social movements tip the scale in opposition to the movements' idea for policy creation. The dominant class, which may not be rural areas or small to middle-scale farmers, find success, whereas these policy-driven social movements find failure. By encouraging rural areas to become sparks of innovation, like Transition Towns, there is a clear understanding that a sustainable future is possible. With this, governments can rely on methods used by rural towns, incentivize them to build further, and bring such action to larger farms through policy. The biggest hesitation of policy creation in this sense is the possibility of failure, but TTs show it can succeed. Rosset and Martínez-Torres (2012) also examine how rural farmers inspire movements of agroecology and use the idea of re-peasantry to explain an increase in agroecological movements. Rosset and Martínez-Torres cite van der Ploeg (2010), saying that peasants may pursue agroecology to the extent that it permits them to strengthen their resource base and become more autonomous in the economy while improving their conditions. This implies that these bottom-up movements are created by the lower class, who simply want to make their lives better, and by having this drive, they find that moving towards sustainable practices creates a better life in both a physical, economical, and environmental state. It is unlikely that farmers using sustainable agriculture would diverge from societal goals, but if this were to happen, correction through reassessment of the processes would need to happen from both an economic and civil viewpoint. The main goal of this transition is for everyone involved, from producers to consumers, to reap the benefits of sustainability in both an environmental and economic fashion. Missing this aspect puts the system at risk of collapse and can be corrected through policy changes and societal coercion.

Feminism in Agroecology

Feminism advocates for equality of rights regardless of gender. It demands that all people are treated fairly and given equal chances, education, and opportunities. Agroecology, as an emerging field, offers the chance for women to be at the center. Zaremba et al. (2021) suggests that agroecology is inherently feministic and that it is necessary to view it as such for a holistic and just food system transformation. They build off authors like Lopes and Jomalinis who claim that women bring a more creative and dynamic solution to the food crisis and have been at the center of food for longer than we realize. Society looks to centralize agroecology around our main producers, and most of these "gatherers" have been women. Zaremba et al. (2021) looks at feminism in agroecology to understand the underlying causes of inequality and disempowerment of all marginalized people by challenging patriarchal and colonial power structures. One of the Sustainable Development Goals posed by the United Nations in 2015 is to achieve gender equality, and agroecology is largely based on the SDGs. Therefore, we see how agroecology is rooted in feminism. Food security and agroecology are not just about sustainability but being able to reclaim one's rights to good and healthy food. Zaremba looks at the thirteen agroecological principles as created by the HPLE on Food and Nutrition (2019) through a feminist lens, explaining how women optimize outputs by limiting inputs whereas men are only looking to do the most efficient and easiest option, even if that means polluting. Examples such as recycling show that women are willing to use past products and return nutrients to the soil rather than burning or disposing of things in landfills. Another interesting aspect of feminism in agroecology is the history of gender roles affecting how women tend to the biodiversity of their surroundings. Prior to 1,000 years ago, men hunted and rarely interacted with plants, whereas women knew the importance of variety and conservation of necessary plants. This has led them to be "seed keepers" in parts of Africa and Asia (Zaremba, 2021). An assumption to be made is that women can feel more connected to the earth; the earth is typically referred to as "Mother", leading women to find a deeper connection than men. It also could be a reason that men are so quick to abuse and degrade her resources, expecting an abundance without any consequences. Another key factor from Zaremba's paper is the idea of shared knowledge among women. Men are traditionally more individualistic and lack the need to communicate and share success with others, except to brag potentially. On the other hand, women have relied on community and shared knowledge for generations. This connects back to the aspect of co-creation in agroecology; farmers must take the feminine trait of sharing knowledge so that any successes in sustainable agriculture are shared and praised. Men, being less likely to share success, do not have the innate desire to open up to those they may be "competing" against, but agroecology relies on sharing. The principle of co-creation is extremely feministic because it requires sharing of successful procedures and encouraging potential competitors to succeed as you have. Women are simply more open to this idea than men (Zaremba et al., 2021).

Zaremba makes a final, huge, claim in her paper, "As many activists have argued, agroecology is inherently grounded in feminism by its very nature as a movement that seeks to transform existing systems that are based in patriarchal, exploitative, and top-down structures—systems which have brought the world to the brink of ecological collapse while exacerbating social inequities". This statement exposes farming as a male-dominated field with little regard for ecology until we reach a point that women, who were repressed for so long, eventually literally took up tools and attempted to make a difference. Agroecology rooted in feminism demonstrates the need for equality, not just in humans but between humans, plants, animals, and the earth.

Mestmacher and Braun (2021) also study feminism, focusing on the regions of Biobío and Ñuble in Chile and the lower class, "peasant" women working the farms, and how state policy provides little to no help. They build off the idea of repeasantization by looking at how applying agroecology to small-scale farms leads to the scaling up of agroecology in the long run. Repeasantization is defined by Peterson (2011) as, "an agroecological transition on a field scale and as the strengthening of local peasant networks or [producer] and consumer networks and other actors, enabling an independent generation of knowledge and resources exchange". They found that women are on the front lines of agriculture in Latin America. For example, these two regions of Chile they looked at are considered hotbeds of agricultural feminism, with nearly 61% of farmworkers being women. The researchers wanted to know if feminist-based agroecology could sustain the livelihoods of these women and if the Chilean government impeded such abilities and the scaling up of agroecology. They suggest that women represent most food producers and preparers yet are exploited and profited off by a patriarchal society (Mestmacher and Braun, 2021). The women who attempt to make a livelihood for themselves through sustainable farming were found to have higher use of human labor rather than machine, use of natural fertilizers, composting, and cultivation of a variety of species instead of traditional, large-scale monoculture relative to current modern practices often presided over by men. Yet, these women who put so much work into their sustainable practices receive little demand for their products, so they do not have solidified channels to receive income. Women must be provided with the means and education to scale up processes while being supported by local communities.

Agroecology in Practice

Theoretically, agroecology sounds lucrative for the environment and the smartest choice for farming practices worldwide to preserve our food sources. Yet, we still see classic, monoculture farms dominate grocery stores and plates because there is no monetary incentive or aid for a transition. Although society may benefit, all costs are externalized to the farmers. The United States lacks innovation and drive to transition influential farms to agroecological practices, but the European Union is taking strides to attempt a reinvention of policy and practice.

France was a country early to address the problem of unsustainable agriculture. de Sartre et al. (2019) looks at French agriculture and their attempt to reclaim land through policy and action. This paper also introduces us to the EU's Common Agricultural Policy (CAP) which has organized agricultural production as its own specific economic and political sector oriented towards high productivity. This was also done in the United States during the Green Revolution. However, in the last ten years, the EU has transitioned agricultural policy to using agroecological principles and ecosystem services as means of production. de Sartre et al. (2019) concluded that the contrast between the agroecology and ecosystem services approaches tends to fade when we analyze how each of these instruments is put in place. Both lead towards the same results: sustainability in practice. The attempt to utilize land for more than looks or more than agriculture was a bold attempt that proved it could be successful. A variety of connections between departments, ideologies, and policies all led back to the main goal of sustainability. de Sartre (2019) also highlighted the importance of farmer-driven agroecology practices again, a common theme of the research.

Another big addition to agroecology in practice is the 2010 Guide to Best Practices released by Agrisud International. It is a 187-page document that is open access for farmers to read and educate themselves on standard agricultural practices, and then how to do so sustainably. This type of information allows rural farmers to implement sustainability on their own. One result of this document is a project based in Northern Laos called FORAE II with the goal of strengthening social and economic dynamics and promoting sustainable family farming (Agrisud International, 2018). The Guide to Best Practices gives families access to resources if they are not previously available through cultural knowledge or if the practice is new, like sustainable agriculture.

Summary

Agroecology is rising in Europe, inspiring more countries in Latin America and Africa to attempt to change their agricultural ways. There is little controversy that agroecology is the best way to farm for the future. It uses the earth to our advantage and takes little and gives back more. Ecosystem services already provide us with what we need, and we simply must find ways to capitalize on them. A major point of conjecture is how we can get the transition from traditional farming to sustainable farming. It requires money, government cooperation, time, economic downfall, trust, collaboration, and co-creation of knowledge. Everything comes down to money in the end; it is needed to transition to agroecological practices and is needed to survive off these practices. Without income, the point of farming is moot and will revert to harmful ways.

Something is missing. There is little data that gives true insight into whether agroecology is working. It is being used in countries around the world on a small scale, but simply being talked about, not measured, and demonstrated. The policies are there, the people are there, but the data are not. It is crucial to have to demonstrate that it works – or does not – and can be scaled up to larger farms. It will take longer, and data collection is a process, but it is a crucial part of the future of agroecology.

Future research must focus on collecting data and measuring the difference between monoculture and agroecology. The data will have to measure ecosystem services, food production, economic impact, and long-term viability will require government collaboration with rural farmers, but governments must work with the farmers, not farmers working with the government. It cannot be expected that farmers change their livelihoods to fit government schedules, but researchers and non-governmental agencies are helping them succeed. The future of this topic will also be implementing agroecology practices in more countries, like the United States, which is a huge producer of monoculture crops and uses chemicals and fertilizers to succeed. We cannot expect smaller countries to transition to sustainable practices without holding larger, more developed countries like the United States to the same standard. Larger countries also have the advantage of incentivizing this transition but standardizing international policy could even the playing field for countries that cannot afford expenditures on a new farming practice. Europe has taken strides, now it is the US's turn. Agroecology is the future of farming and the only way to reclaim the earth while providing enough food for our growing population without compromising our ability to feed ourselves in the future.

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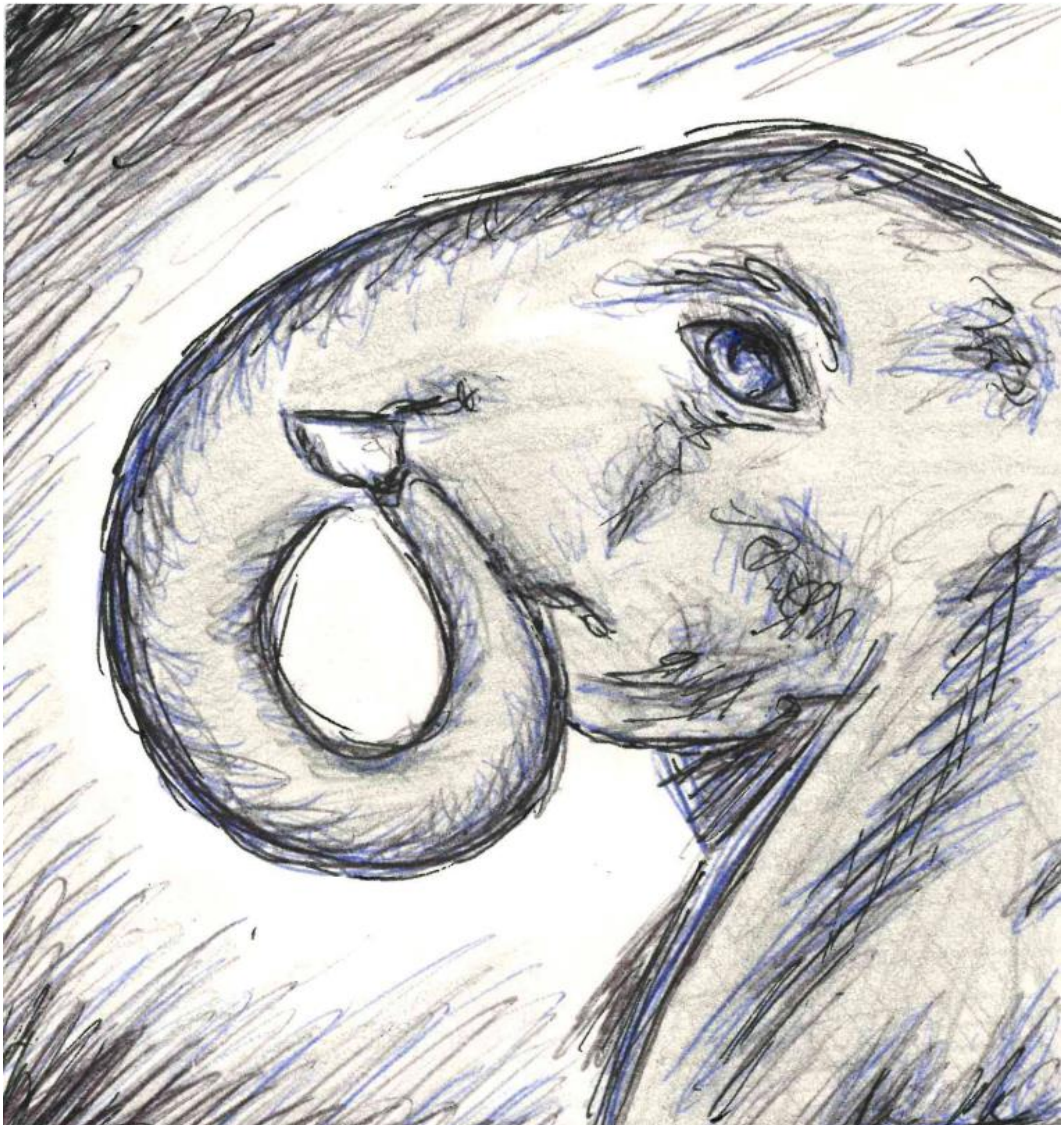

The Impact of Weather and Temperature on African Elephants *(Loxodonta africana)*

Mia Harris, Lauren Cordes, Joanne Hu, Jon Pulaski, Max Dixon, and Sarah Boyle

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Anthropogenic induced climate change is altering and intensifying global weather patterns. Global warming and extreme weather events have become more prominent, impacting behavioral rhythms for entire ecological communities. The behavioral patterns of African elephants (Loxodonta africana) are dependent on the health and quality of their habitat. The effect of weather conditions on animal behavior is even more important for captive animals that are possibly exposed to foreign climate conditions. We investigated the impact of weather conditions on the swaying behavior exhibited by Gina, Asali, Bambi, and Daisy at the Memphis Zoo. We hypothesized that sunnier, brighter days with higher temperatures induce higher levels of swaying behavior by the elephants. We used behavioral scan samplings at 2-minute intervals to quantify swaying behavior for each elephant. In total, we collected 287 hours of behavioral data during the summer of 2021. The results from this study can help elephant keepers revise husbandry routines and renovate the elephant exhibit in accordance with changing weather conditions in Memphis, potentially reducing the amount of time the elephants at the Memphis Zoo dedicate to swaying.

Introduction

Wild African elephants (Loxodonta africana) participate in a variety of behaviors including foraging for resources, such as food and water, and mating (Nkululeko Ngcobo et al., 2018). However, elephants in captivity have husbandry routines that provide them with food, making the need to forage less pronounced (Gruber et al., 2000). With the lack of stimuli due to the lack of foraging, elephants may resort to stereotypic behaviors to replace the species-typical behavior of foraging (Gruber et al., 2000). In elephants, stereotypic behaviors typically consist of repetitive pacing, rocking back and forth, as well as rhythmic head movements (Rees et al., 2004). Additionally, environmental conditions, temperature, and weather may impact the frequency of stereotypic behavior exhibited by captive elephants (Rees et al., 2004).

Climate change causes extreme fluctuations in weather, leaving terrestrial ecosystems and biodiversity vulnerable to severe changes (IPCC, 2019). With the growing prominence of climate change and harsh habitat, terrestrial animals in southern Africa, such as elephants, are presented with the challenge of regulating their body temperatures and adapting to an even hotter climate (Fuller et al., 2016). These rapid changes in local climate outpace the African elephant (Loxodonta africana) population's ability to adapt, these creatures are at a higher risk of extinction (Fuller et al., 2016).

The main question that we asked was: "How does temperature and weather affect the frequency of stereotypic behaviors in captive elephants?" We had three main hypotheses: Hypothesis 1: Temperature impacts the frequency of stereotypic behavior exhibited by the elephants. Prediction: As the temperature increases, the elephants will sway more frequently. Hypothesis 2: Weather conditions impact the frequency of stereotypic behavior exhibited by the elephants. Prediction: If the weather conditions are sunnier, then the elephants will increase the frequency of their stereotypic behaviors. Hypothesis 3: Weather and temperature influence each other. Prediction: Sunnier days will have higher temperatures.

Methods

We conducted our study in the African Veldt elephant exhibit in the Memphis Zoo. The exhibit was divided into three main regions, the left, center, and right exhibit areas. Each region had the capacity to be connected or disconnected from other regions via a metal gate or the chute. All three regions contained different modes of food enrichment. Concrete and wooden shelters were in the left and center exhibit areas, while a large elephant pool was in the center exhibit area.

We used behavioral samplings at 2-minute intervals to quantify swaying behavior for each elephant (Martin & Bateson, 2007). A grid was superimposed over the exhibit map to identify the locations of elephants, and an ethogram was used to define different elephant behaviors. In total, we collected 287 hours of behavioral data during the summer of 2021.

For our statistical analyses, we analyzed the individual results for each elephant separately. For Hypotheses 1 and 2, two Kruskal-Wallis tests were used to observe each elephant's swaying frequencies during different weather and temperature conditions. For Hypothesis 3, a Spearman's Correlation Rank test was used to analyze the relationship between weather and temperature.

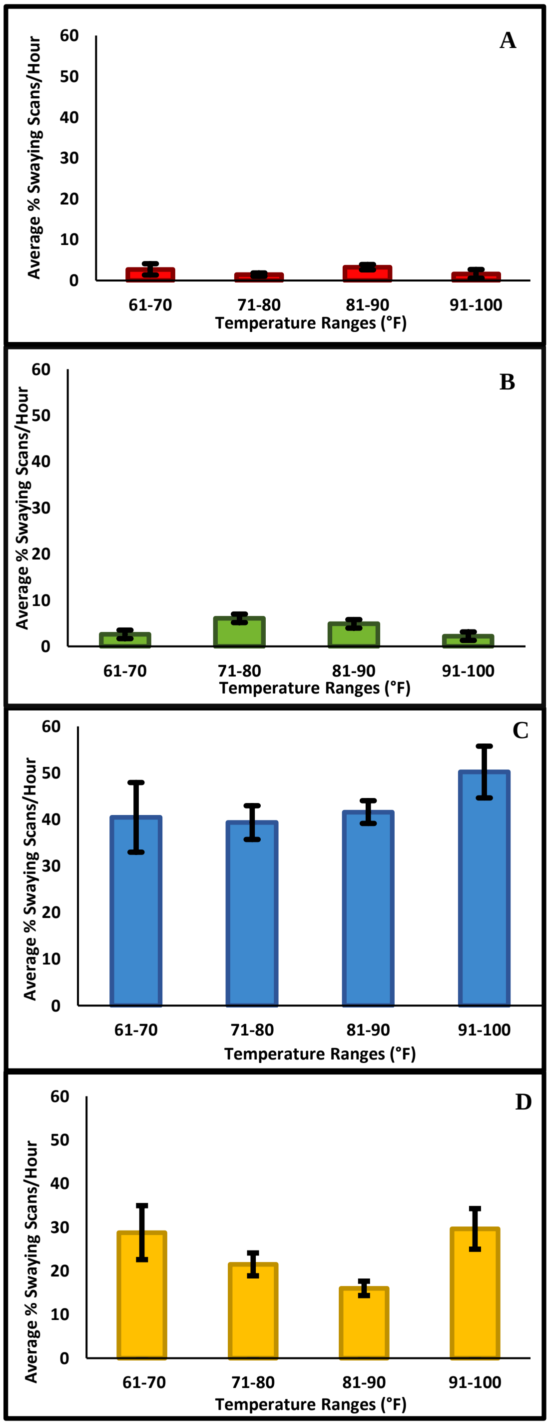

Figure 1. There was no difference in the percent of swaying scans per hour under different temperature conditions for Asali (A, $H_2 = 3.61$, $p = 0.31$), Gina (B, $H_2 = 0.91$, $p = 0.82$), and Bambi (C, $H_2 = 2.04$, $p = 0.56$). However, there was a difference for Daisy (D, $H_2 = 11.37$, $p = 0.01$), who swayed less during temperatures from 81-91°F than she did during temperatures from 91-100°F ($p = 0.025$).

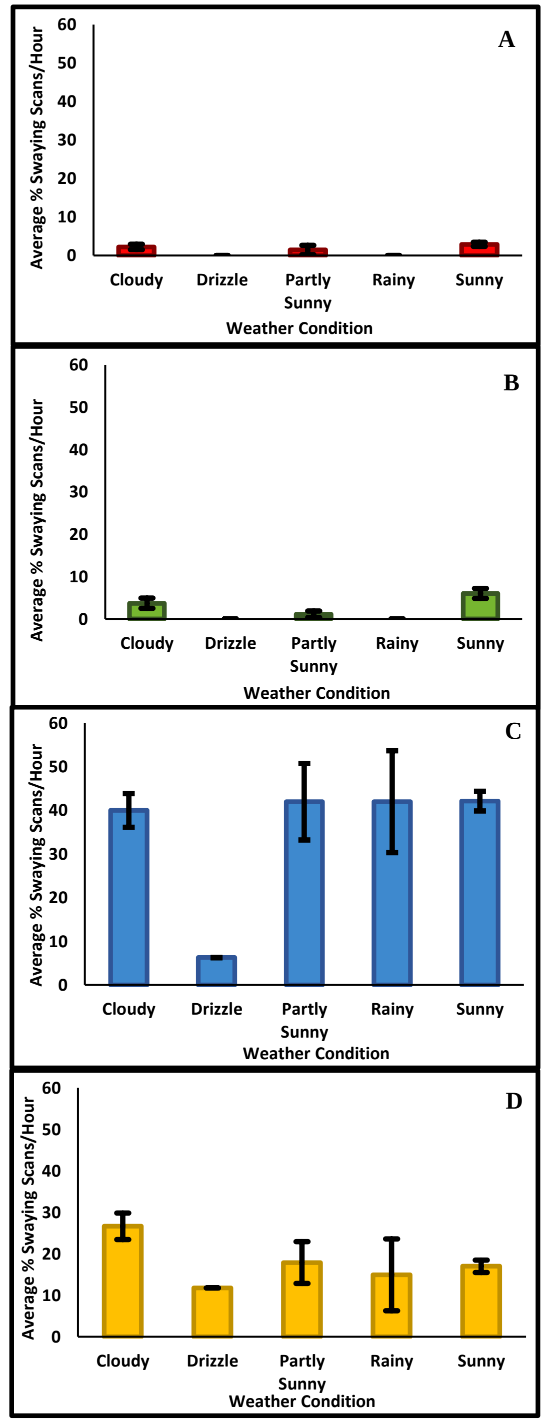

Figure 2. There was no difference in the percent of swaying scans per hour under different weather conditions for Asali (A, $H_2 = 5.20$, $p = 0.27$), Gina (B, $H_2 = 3.60$, $p = 0.46$), Bambi (C, $H_2 = 1.51$, $p = 0.82$), or Daisy (D, $H_2 = 6.72$, $p = 0.15$).

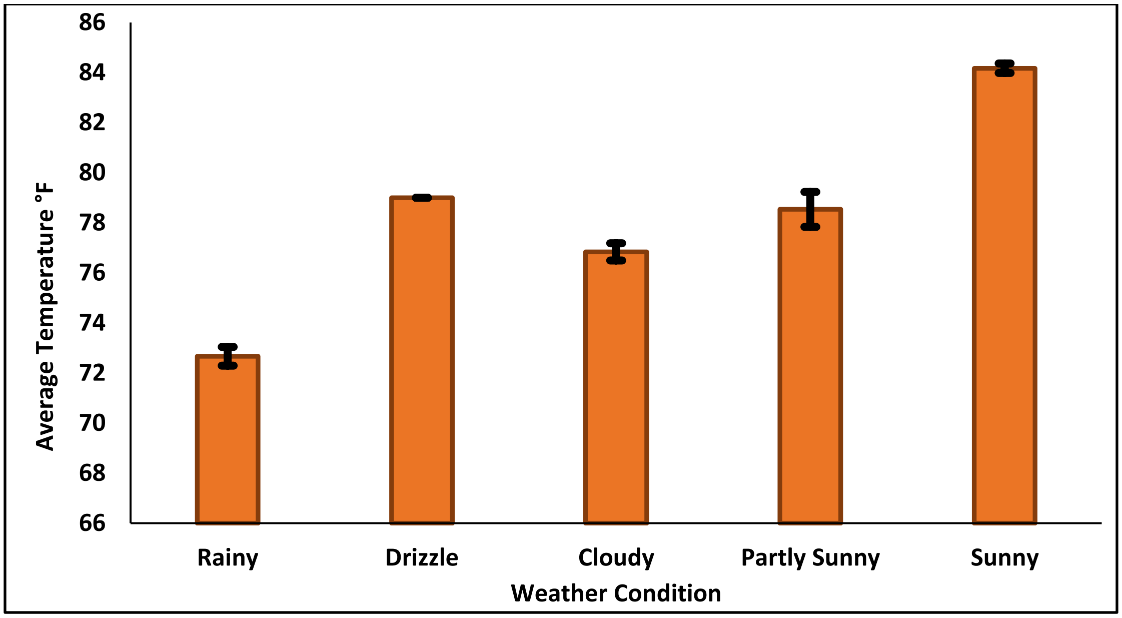

Figure 3. There was no correlation between average temperatures and the different weather conditions for May, June, and July (r_S = 0.700, n= 5, p = 0.233).

Results

There was no difference in the percent of swaying scans per hour under different temperature conditions for Asali (H_2 = 3.61, p = 0.31), Gina (H_2 = 0.91, p = 0.82), and Bambi (H_2 = 2.04, p = 0.56; Fig. 1). However, there was a difference for Daisy (H_2 = 11.37, p = 0.01), who swayed less during temperatures from 81-91°F than she did during temperatures from 91-100°F (p = 0.025). Overall, these data provide weak support for Hypothesis 1, that temperature impacts the frequency of stereotypic behavior exhibited by the elephants.

There was no difference in the percent of swaying scans per hour under different weather conditions for Asali (H_2 = 5.20, p = 0.27), Gina (H_2 = 3.60, p = 0.46), Bambi (H_2 = 1.51, p = 0.82), or Daisy (H_2 = 6.72, p = 0.15; Fig. 2). These data do not support Hypothesis 2, that weather conditions impact the frequency of stereotypic behavior exhibited by the elephants.

There was no correlation in the expected versus average temperature values for the different weather conditions (r_S= 0.700, n= 5, p = 0.233; Fig. 3). These data do not support Hypothesis 3, that there is a general relationship between weather and temperature for summer months.

Discussion

It is not possible to support a statement regarding the entirety of stereotypic behavior in captive African elephants. However, if our study was replicated with similar results, it may suggest that some individuals have a greater predisposition to stereotypic behavior under different climatic conditions; one may exhibit significantly longer and more frequent bouts of swaying than the rest of the herd in hotter temperatures (Figures 1 and 2).

Though our study was performed on captive African elephants and only found difference in behavior in correlation to temperature in one individual, it is interesting to note that similar studies support a correlation between stereotypic behavior and temperature in captive Asian elephants (Rees, 2003). Though Rees (2003) does not address weather in relation to swaying, our study’s findings for seasonal weather and temperature patterns suggest that further study on the topic is required for both animals (Figure 3).

This information is important for zookeepers and the husbandry routines they employ to care for their animals. During periods at the temperature in which the individual displays greater stereotypic behavior, individualized enrichment programs, brief quarantines in a holding area, or further temperature regulation may help to moderate the length and frequency of bouts of stereotypic behavior.

Acknowledgements

We would like to thank the Memphis Zoo and Rhodes College for providing with the opportunity to observe and study elephants over an extended period. We would also like to extend our gratitude to the elephant keepers and Memphis Zoo volunteer coordinators for continuously integrating Rhodes College students into their routine, schedules, and career. Finally, we would like to thank Professor Sarah Boyle for her unwavering encouragement and assistance during this journey.

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Food Waste Along the Food Supply Chain

Sophie Enda, Rhodes College

Food waste is a global issue that accounts for a third of all greenhouse gas emissions. By looking at different sectors of the food supply chain and consumer behaviors, this paper explores areas of the greatest concern regarding food waste. With over 40% of food produced being wasted before reaching consumers, more emissions are created by less-than-ideal disposal methods. By addressing food waste, money will be saved, emissions will be lowered, and fewer people will face hunger. This literature review found that altering consumptive behaviors and educating people on proper food storage, will drastically lower food waste.

Introduction

For most of human history, people have hunted and gathered their food or practiced subsistence farming. It was not until the Neolithic Revolution that people transitioned to the agricultural system that would be able to support higher population densities. Now, half of all habitable land is used for agriculture (Ritchie & Roser, 2020). As the global population continues to grow, production needs to keep up. Food requires an exorbitant amount of energy and resources during production and distribution (Williams et al., 2012). This allocation of resources such as land, energy, and water illustrate how important it is to mitigate the food waste problem now (Göbel et al., 2015). The global food system is one of the largest contributors of greenhouse gases with food production accounting for a quarter of worldwide emissions, as depicted in figure 1 (Brown, 2016). Most wasted food will end up in landfills where it will then be converted into methane, carbon dioxide, and other greenhouse gasses by anaerobic digestion (Melikoglu et al., 2013).

Unfortunately, approximately 40 percent of the United States food supply will be wasted, 17 percent of food loss occurs during harvest, 6 percent in handling and storage, 9 percent in processing, 7 percent in retail and distribution, and 61 percent in the consumption stage (Reich & Foley, 2014). No single actor can be blamed for food waste. The top three contributors to food waste are meat, fruit and vegetables, and bread and bakery products. Additionally, the environmental impact of food waste increases further up the supply chain – for example a potato discarded at a supermarket has a higher environmental impact compared to if it was discarded at the farm.

In this paper, I will focus on food waste in developed countries. In wealthier countries, there will be better access to food and, of course, that means we can afford to waste more food. The amount of food waste produced globally is more than enough to feed all people affected by hunger (Melikoglu et al., 2013). By addressing food waste, we can begin to understand how to better alleviate food insecurity and the environmental impacts brought on by our current agricultural practices and consumer behaviors. There is no one size fits all solution for food waste, and it is important to fully understand each step of the food chain if we are to combat this waste. Minimizing food waste will have positive effects on economic, social, ecological, and health-related fields.

To mitigate food waste, countries have established programs and goals to help face this challenge. For example, the European Commission created its Roadmap to a Resource Efficient Europe to reduce resource input into the food chain by 20 percent and cut food waste in half (Göbel et al., 2015). A big obstacle in achieving this goal is the structural modern lifestyle so engrained in our consumption behaviors, such as not eating food based on their imperfect appearance.

What makes decreasing food waste so difficult is that the food supply chain operates unlike any other supply chain (Gunders, 2017). The complexity of logistic processes, manufacturing and processing, distribution and consumption of food is much more regulated in order to maintain quality and safety (Gunders, 2017). I plan on discussing four major areas of concern regarding the environmental impacts of food waste – production, households, restaurants and supermarkets, and landfills.

Discussion

The United States, Japan, and United Kingdom are the top wasters of food, as seen in table 1 (Melikoglu et al., 2013). In America, the food supply is the most varied and abundant in the world (Gunders, 2017). With the high availability of food, waste seems inevitable. Looking specifically at the United States, it is estimated that 77 billion pounds of edible food is wasted from restaurants, convenience stores, and supermarkets – this equates to nearly 30 billion dollars being thrown away (Parfitt et al., 2010). It is important to recognize that people in developed countries have the luxury to waste food, which is not a universal trend. In the United Kingdom, the Waste and Resources Action Program (WRAP 2013), found that a third of food sold in the UK ends up as waste, even though half of it is still considered edible. In this paper, I will investigate the different players that lead to food waste along the food supply chain.

Food Waste in Production

Food waste begins at the farm. A case study found that on average, 65 percent of the unharvested crop was considered to be edible, but did not meet market standards (Johnson et al., 2018). When these crops are wasted, so is the water, cropland and fertilizer that went into growing the product. The farmer also experiences monetary losses by not being able to sell the product as well as money put into labor and equipment.

In 2016 alone, 9.2 billion kilograms of food were lost at the farm level as reported by the Rethink Food Waste through Economics and Data (ReFED 2016). As for fruits and vegetables in North America, 20 percent will be lost at the farm level in the field and packaging facilities. Food waste on the farming level can also be caused by unknown demands. Farmers must guess how much to grow based on previous demands, but those fluctuate leaving behind excess food. Additionally, labor shortages and costs are another factor that some produce is never harvested (Gunders, 2017).

In 1974, the first World Food conference zeroed in on the reduction of post-harvest losses being the answer to alleviating world hunger (Parfitt et al., 2010). For example, an estimated 88 million tons of food is lost or wasted in the European supply chain annually (Scherhaufer et al., 2018). A major reason food is wasted at the production level is because of cosmetic appearances (de Hooge et al., 2018).

Cosmetic specifications, include the product's appearance, weight, shape, and size but not its quality or safety (de Hooge et al., 2018). These 'imperfect' products will have trouble moving up the food supply chain and thus are discarded. This waste is not only environmentally problematic, but it poses economic problems for farmers. Post-harvest losses are completely avoidable; however, a large proportion of the food produced on a farm will never leave. For example, a citrus packer reported that 20 to 50 percent of the produce is unmarketable but is considered edible.

It is also important to note how food can be packaged for distribution. Most items seen on supermarket shelves have some type of plastic wrapping. Even though reducing plastic use will help lower greenhouse gas emissions, more greenhouse gasses are produced from food wasted compared to the plastic packaging (Gooch et al., 2010) Ironically, plastic packaging actually reduces food waste (Silvenius et al., 2014a). By using plastic packaging, food is protected during transportation and handling.

Food Waste in Supermarkets and Restaurants

Supermarkets are near the end of the food supply chain. Since large quantities of food are located in several physical locations, targeting supermarket food waste is a good strategy. Furthermore, variables such as national and regional legislation make is difficult to accurately make precise food waste estimates. A major reason food is wasted in supermarkets is due to perishable items having a short shelf life. This is further intensified by the fluctuating demand of certain items which makes predicting how much food a supermarket should order difficult (Lebersorger & Schneider, 2014). The USDA estimates a total loss of $15 billion annually in unsold fruits and vegetables alone (Gunders, 2017).

Factors leading to a greater risk of products being wasted include low turnover, short shelf life, and the minimum order size being large (Eriksson et al., 2016). Additionally, larger chain supermarkets waste more food compared to smaller grocery retailers because they have to deal with higher volumes of customers that demand top quality, fresh products (Filimonau & Gherbin, 2017).
Studies have found that the greatest wasted mass of perishable foods in supermarkets is bread and fresh fruit and vegetables (Lebersorger & Schneider, 2014). For example, in the UK, bananas are the most wasted fruit, accounting for 20 percent of the total fruit wasted (Filimonau & Gherbin, 2017).

In 2014, the Food Waste Reduction Alliance (FWRA) found that 37 percent of food waste occurs in retail stores and food services (Sakaguchi et al., 2018). In the restaurant sector, 4-10 percent of purchased food will be wasted before reaching customers. Food waste comes in many forms in the restaurant industry such as improper storage, inappropriate serving sizes, plate waste from the client, overproduction, and overstocking (Ofei & Mikkelsen, 2011) Additionally, a study conducted by the US Department of Agriculture (USDA) found that 21 percent of food available in restaurants will not be consumed. If food businesses addressed food waste as a serious problem, approximately $ 1.3 billion would be saved each year (Sakaguchi et al., 2018).

Food Waste in Households

Food waste in the home has many consequences including social, economic, and environmental costs. In regards to the social impact, food waste contributes to an increase of global food prices, creating a barrier for those most needing food (Graham-Rowe et al., 2014). The economic impact results from purchasing food, not eating, and then discarding it, which costs the average American family approximately $600 - $1000 a year (Graham-Rowe et al., 2014). In terms of weight, the average household in the UK, Norway, and Sweden wastes 4 kg of food every week (Williams et al., 2012).

Households waste food for many reasons, one being packaging. Approximately 20 – 25 percent of household food waste is related to the packaging – sizes too large for consumer's needs, too difficult to empty, or past its expiration date (Silvenius et al., 2014b). Additionally, lack of knowledge on how to store food properly contributes to food waste in the household (Silvenius et al., 2014a). Another reason for household food waste stems from people cooking, preparing, and serving more food than is consumed (Williams et al., 2012). In an effort to minimize household food waste, changes to packages must be made. Offering alternative sizes or making the packaging resealable are methods to combat household food waste.

Food Waste in Landfills

The last part of the food supply chain is the landfill. Food waste is the largest component of solid waste reaching landfills (Gunders, 2017). Management of food waste is essential in reducing the environmental impacts caused by landfills. In the United States alone, it is estimated that 97 percent of food waste is buried in landfills (Levis et al., 2010). By diverting food away from landfills, greenhouse gas emissions will lower. The biggest benefit of reducing food waste in landfills is methane avoidance (Brown, 2016). It is estimated that only 3 percent of food waste is composted (Gunders, 2017). Because some food waste is inevitable, the development of large-scale composting systems would help reduce the negative environmental impacts of food being broken down.

Summary

To effectively mitigate food waste, it is essential to critically examine the entirety of the food supply chain. If we were able to reduce food waste by just 15 percent, there would be enough food to feed more than 25 million Americans every year (Gunders, 2017). By redirecting food to composting facilities and away from landfills, methane emissions will be reduced, nutrients recycled, as well as raising awareness for how much food is being wasted because more attention will be brought to how much food is actually being wasted. Moving forward, we must revise the aesthetic standards of produce, practice farm level food recovery, shop more consciously, donate excess food, and educate consumers. These tactics will allow us to move towards a future with reduced food waste.

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All roads lead to… crushed crabs

Khanh Ton

It's hard to imagine a world without roads. They lead you to your everyday workplace, the Trader Joe's where your favorite frozen dumplings are calling your name, and your off-the-grid yearly vacation in the countryside. They also bring you the postcards from Italy where your friend is having a time and the takeout for your cozy nights in.

But the Atlantic ghost crabs would have to disagree with that.

Having a large home range of about 300 meters, these crabs could run fast, dig fast, and camouflage to blend with the sand. Combined with their nocturnal habits, they don't have many predators. Though described as "occult, secretive alien from the ancient depths," these crabs spend most of their time on land and only come into the water to keep their gills wet and lay larvae during the spawning season. It is, however, within these rare instances of traversing across the sandy shore that they find themselves in trouble.

An Atlantic ghost crab blending with the sand (credit: Julie Feinstein)

Like a lot of biology students in Brazil, Dr. Leonardo Lopes Costa (Universidade Estadual do Norte Fluminense Darcy Ribeiro) took a liking to marine biology in his undergraduate years. While his friends were studying popular topics like tropical ecology or plastic pollution, he dived into something people would not have thought to cause an environmental disaster: roads, and how they separate wildlife habitats.

Since 2012, he has studied and become an expert in coastal road ecology, which explores how roads and urbanization along the coast impact the wildlife community. During this time, he was finding crushed crabs where there were tire tracks on the beach and was curious about whether there was any connection between crab mortality and coastal traffic. He assembled a team and, together, they walked the beach.

In a total of twelve campaigns between 2017 and 2019, they spent countless nights looking for crushed carapaces on evident tire tracks on both beaches and dirt roads that lead to them. For each and every collision scene, they also recorded how urbanized the area was and its distance from the water. This meticulous method came about when Dr. Costa was collecting data for a colleague's road ecology project. Relying too much on what the eyes can see could produce errors, but he believes it to be "most feasible and real, especially in Brazil where we don't have too much money." Weighing in on this labor-intensive method, Dr. Sarah Boyle, an expert on animal behaviors and conservation biology at Rhodes College, believes that it would allow us to look at all aspects of the problem and more easily compare the case to other locations globally.

Dr. Costa used a null hypothesis for his study, which states that coastal traffic is completely irrelevant to Atlantic ghost crab mortality. "Scientific research," he said, "is only valid if we can refute the hypothesis. That way, my hypothesis could also be applied for management, which constantly seeks clues to put together actions."

And indeed, he was able to refute the hypothesis. The 47 crabs killed by vehicles on the beaches correlated positively to the number of tire tracks on sand. Most were killed in less urbanized beaches because they are more abundant here than at those with more human footprints. Atlantic ghost crabs are known to be natural eco-checkers: they retreat from places with human development and pollution to avoid dangers. Less urbanized beaches also have denser vegetation. As vehicles try to not trample vegetated areas, they end up in high intertidal zones, where the majority of ghost crabs live. An extreme instance is when 25 crabs, or 2.3% of their population, were killed in a single night during a high tourism season due to a traffic jam.

Tire tracks on the beach (credit: Kerry Trapnell)

Coastal road ecology and those who study it, like Dr. Costa, are entangled with more than just an ecological problem: "Sandy beaches are social and ecological systems and there are many conflicts. Normally, our suggestion is to perform isolation of beaches: for recreation and for conservation. But in the most urbanized areas, we can't do anything because of the tourism economy." Ghost crabs are becoming more vulnerable due to habitat loss, but since they have yet to be considered endangered, it's difficult to get management attention.

Nevertheless, his team is still walking the beach. Dr. Costa was inspired by a paper he reviewed that studied coastal road mortality of land crabs during spawning season in South Korea. He wanted to know if there are specific time periods during which more crabs are being crushed as they will be vital to management. So bi-weekly every month, they set out to check on these fleet-footed creatures and pay attention to many more details besides urbanization, such as moon phases, light pollution, temperature, and rainfall.

Turtles laying eggs along Brazil's northeast coast (credit: Paulista City Hall)

Dr. Costa believes this research can apply to other species as well, like marine turtles or opossums. The less mobile the species is, the more in danger they are because they can't run past the road fast enough. More importantly, he emphasizes the role of scientific outreach in the potential success of research like this: "In Brazil, we don't have funding because people don't know about what we are doing. I'm doing what I do because I know nature matters, but when we talk to people, we have to show them more direct impacts. For example, commercial fishing or charismatic animals are easier to communicate."

While Dr. Boyle agrees that charismatic animals attract more attention, she maintains that we must look at the bigger picture, that traffic on beaches worldwide is killing all sorts of species and destroying their habitats: "Even dog-walking is an imminent threat to ground-nesting birds! We need to encourage the public to think about the implications of even the most seemingly harmless actions." So, next time you're heading to the coast for that breezy and relaxing vibe, remember to look around and down to your feet!

The Future of Amphibian Conservation: Development of Novel Probiotics

Emma Root, Rhodes College

Amphibians around the world are facing large population declines and extinctions due to infection from Batrachochytrium dendrobatidis (Bd). When different amphibian species come into contact with Bd, clinical outcomes can range from little to no symptoms up to death and considerable population declines attributed to chytridiomycosis. Susceptibility to Bd can differ among amphibian species and populations, likely due to variation in defense mechanisms of the host, the virulence of Bd, and properties of the environment where the host and pathogen interact. Immunization and antifungal treatments are either ineffective or limited in scope when it comes to mitigating the global loss of biodiversity from Bd. The skin microbiome is a line of defense against disease for amphibians in addition to, or concurrently with, the innate and adaptive immune system. Host survival has been conclusively linked to presence and relative abundance of anti-Bd bacteria which inhibit Bd directly through production of inhibitory metabolites and indirectly through immunomodulation. In the light of Bd and the urgent need to develop effective mitigation strategies, the role of symbiotic bacterial communities in host protection against chytridiomycosis should be fully investigated in order to manufacture deployable antifungal probiotics to bolster amphibian conservation efforts.

Keywords: Amphibian, *Batrachochytrium dendrobatidis*, bioaugmentation, chytridiomycosis, probiotic

Introduction

Amphibians around the world are facing large population declines and extinctions due to chytridiomycosis, which makes research into management and prevention a high priority for amphibian conservation biologists. Chytridiomycosis is a highly infectious fungal disease caused by the pathogenic chytrid fungus, *Batrachochytrium dendrobatidis* (henceforth, *Bd*) (Longcore et al. 1999). Chytridiomycosis has been catastrophically devastating for amphibian biodiversity, affecting over 350 species, and is responsible for one of the most significant losses of biodiversity from disease in recorded history (Kilpatrick et al. 2010). One of the most crucial defenses against *Bd* and resilience to chytridiomycosis disease can be found in the vast prokaryotic populations found in amphibians' skin.

Prokaryotic symbionts that populate host organ systems are vital to amphibian host's immune system and ability to resist pathogens (Bletz et al. 2013); One such important microbiota in amphibians is the skin microbiome, its composition, and subsequent function. The amphibian cutaneous skin microbiome is shaped by different host factors and environmental factors such as temperature, water pH, forest type, and many others (Hughey et al. 2019; Loudon et al. 2020). Despite variation between individuals, species-specific communities persist and are consistent across habitats– thus, a recurring core microbe is likely to perform a significant function for the host, such as producing important metabolites that may function as antifungals or to activate the host immune system (Kruger 2020). Recent studies have revealed that some of these symbiotic bacterial species can defend their host against *Bd* by secreting antifungal metabolites, such as violacein (Becker et al. 2009). Thus, a comprehensive understanding of the community ecology of amphibian skins is crucial to also understanding the susceptibility of already vulnerable species to *Bd*.

Because *Bd*-inhibitory bacteria have been successfully isolated from wild-type amphibian microbiomes (Walke et al. 2015; Bell et al. 2018; Becker et al. 2009), understanding the potential for symbiotic microbes to mediate disease resistance may provide critical tools for amphibian conservation in controlling and mitigating this emerging disease. In a modern scientific context, bioaugmentation is defined as the improvement of a given environmental or biological concern by introducing specific microorganisms (Cycoń, et al. 2017). One such bioaugmentation strategy that warrants attention is the discovery and development of antifungal probiotics. This could be in the form of both deployable environmental probiotics and/or treating amphibians with probiotic bacteria directly, which has been both promising (Bletz et al. 2013) and unsuccessful (Becker et al. 2011) in different settings. At this point, research is ongoing to explore bioaugmentation strategies to harness the medicinal properties of symbiotic bacteria. In the light of *Bd* and the urgent need to develop effective mitigation strategies, the role of symbiotic bacterial communities in host protection against chytridiomycosis should be fully investigated in the hopes of manufacturing deployable antifungal probiotics to bolster amphibian conservation efforts.

Overview of *Batrachochytrium Dendrobatidis*

Batrachochytrium dendrobatidis is a pathogenic aquatic fungus that causes the high-morbidity skin infection, chytridiomycosis (Longcore et al. 1999). A member of the chytrid (Chytridiomycota) fungal phylum, *Bd* notably has a biphasic life cycle and starts out as flagellate-motile zoospores that seek out amphibian hosts in water sources by means of chemotaxis (Sewell et al. 2021). Once a zoospore senses a host environment, it will attach to the amphibian epidermis, encyst, and colonize the amphibian by developing and growing as its stationary life-stage, zoosporangia (Berger et al. 2005; Grossart et al. 2016; Sewell et al. 2021). The cycle then continues as the zoosporangia produces many more zoospores to be released back into the environment (Hibbett et al. 2007) or even to re-infect the original host animal (Bletz et al. 2013).

Unfortunately, *Bd* is not limited to a confined, native range; in fact, the fungus has been found in every continent except for Antarctica (Olson et al. 2013). To make matters worse, *Bd* does not appear to be host-specific and has been documented as capable of infecting over 600 species of amphibians around the globe (Xie et al. 2016). The situation is also far from stagnant, given the potential for climate change to worsen this emerging disease by altering habitat ranges of both amphibians and *Bd*. These environmental concerns may leave amphibians even more vulnerable by increasing pathogen virulence (Pounds et al. 2006), decreasing host fitness, and allowing *Bd* to further infiltrate even the most removed habitats that currently exist as infection coldspots (Mutnale et al. 2020). Looking through a conservation lens, it is safer to assume that any amphibian species that have managed to evade *Bd* exposure to-date may be vulnerable to *Bd* and chytridiomycosis, lest they prove to be the exception to the rule (Murray et al. 2011).

Even though *Bd* is not host-specific, there is significant variation in the virulence of *Bd* and the severity of chytridiomycosis across species and populations (Flechas et al. 2018; Bletz et al. 2013; Mutnale et al. 2020). The progression of events beginning with exposure to *Bd* culminating in full-blown chytridiomycosis infection is complicated and dependent upon many factors, including environmental and host-pathogen interactions. Most notably, morbidity and death rates of chytridiomycosis varies with pathogenic load, temperature, and amphibian host species (Berger et al. 2005; Sonn et al. 2017; Bletz et al. 2013). A study on the effects of temperature on *Bd* morbidity have determined that temperature affects a host amphibian's ability to cope with infection, specifically that pathogenicity is inversely related to environmental temperature (Sonn et al. 2017). The variability of species-specific susceptibility cannot be so easily condensed into a single trend, leading to a discussion of amphibian immune defenses.

Amphibian Skin Microbiome's Role in Immune System

The amphibian immune system at-large is comprised of innate and acquired immune defenses. Specific mechanisms of amphibian immunity include antimicrobial peptides, diversity in major histocompatibility complex genes, acquired immune response, and cutaneous microbial communities (Bataille et al. 2015; Kueneman et al. 2014). The skin microbiome, home to prokaryotic symbionts, is a key component of the amphibian innate immune system as it is the first line of defense against pathogens like *Bd* (Harris et al. 2006; Jiménez et al. 2019). The different aspects of a healthy, naturally occurring microbiota that contribute to pathogen-resistance include bacterial species diversity, microbial community composition, and key bacterial species that produce important metabolites (Jiménez et al. 2019; Bletz et al. 2013). Some skin bacteria can outcompete and/or kill *Bd* (henceforth anti-*Bd* bacteria) thus their presence is a major factor in the clinical outcome of the host (Flechas et al. 2019; Muletz-Wolz et al. 2017; Harris et al. 2009). Overall, an intact skin microbiota can minimize costs of infection for the host (Woodhams et al. 2012).

Influences on Structure and Function of Skin Microbiome

After establishing the host-benefits of a healthy skin microbiota, specifically composed of anti-*Bd* bacteria, attention must turn to understanding the factors that influence the structure and composition of amphibian cutaneous bacterial communities. In general, the major contributors to the skin microbiota are environmental microbes, parental microbes at time of hatching, and the host's own immune system (Loudon et al. 2020). Variation within a single species has been found to be influenced by environmental factors, such as forest type, which influences overall diversity of bacteria found on amphibian skin when populations of a single species are compared (Assis et al. 2020).

However, external environmental factors are not the only determinants of the amphibian skin microbiota composition. Prokaryotic communities taken from multiple amphibian species in the same habitat have been found to differ significantly and on the flip-side, species‐specific bacterial communities persist and are consistent in different locations (McFall‐Ngai et al. 2013; Loudon et al. 2020; Kruger 2020). Thus, host ecology affects the amphibian skin microbiota, which is comprised of consistent core species and environment-specific transient bacteria.

Cutaneous Microbiota Taxonomy

Narrowing focus to the prokaryotes themselves, the dominant bacterial taxa on amphibian skin included *Bacteroidetes*, *Gammaproteobacteria*, *Alphaproteobacteria*, *Firmicutes*, *Sphingobacteria* and *Actinobacteria* (Kueneman et al. 2014; Mutnale et al. 2020). Anti-*Bd* bacteria can protect their host through a variety of mechanisms. One of the primary methods is to produce secondary anti-fungal metabolites that inhibit *Bd* growth (Becker et al. 2009; Harris et al. 2009; Loudon et al. 2020). Some important anti-*Bd* species isolated from amphibian skin include *Janthinobacterium lividum* which produces "Violacein" (Harris et al. 2009) and *Serratia marcescens* which produces "Prodigiosin" (Woodhams et al. 2018), two metabolites that effectively disrupt the cellular membrane of *Bd* and prevent colonization (Cauz et al. 2019; Danevčič et al. 2016).

***Bd* Affects Composition of Skin Microbiota**

An inadvertent discovery made regarding the protective bacteria in amphibian cutaneous microbial population was how *Bd* infection can also modulate host skin microbiota. It is clear that *Bd* infection disturbs the microbiome of its host (Kueneman et al. 2014) but there appears to be variation between host species in their ability to return to a healthy, typical skin microbiome after a pathogenic/*Bd* disturbance (Weeks et al. 2020). Research has revealed that *Bd* is indeed a selective force on amphibian skin bacterial community structure and function (Walke et al. 2015) and the maintenance of ecological community function over time depends on community resistance and resilience, especially under pathogenic stress (Jani et al. 2020). Thus, an amphibian microbial community's stability depends on its ability to successfully recover to a pre-disturbance state, which in-turn may confer *Bd* disease resistance. In essence, resistance to *Bd* may be two-fold; first, the initial competition between skin microbes and *Bd*, then second, the microbiota's ability to recover to a pre-*Bd* state in order to stave off morbidity secondary to *Bd*.

Previous Conservation Efforts

To greater contextualize the dire need for novel *Bd* treatments, conservation efforts and other failed treatments attempted to-date should be briefly examined for efficacy and potential to work concurrently with bioaugmentation efforts. An early attempt at immunization with adjuvants in-combination with killed *Bd* culture was unable to stimulate a significant protective immune response (Stice & Briggs et al. 2010). Experimental antibiotic treatment revealed that safe concentrations of Hygromycin, Zeocin, and Blasticidin showed promising *Bd*-inhibition (Robinson et al. 2020). Currently, one of primary treatment options for *Bd*-infected amphibians is the administration of the antifungal drugs, including mandipropamid, steriplantN, and most notable: itraconazole (Woodhams et al. 2012). Itraconazole has been found to be successful in causing immediate reductions in *Bd* load but ultimately, pathogenic load and survival returned to pre-treatment levels and no long-term changes in population-*Bd* dynamics were observed (Knapp et al. 2022; Hudson et al. 2016). However, a fundamental limitation in the use of itraconazole is the potential for toxicity depending on the concentration of dosage (Woodhams et al. 2012). Thus, as a short-term measure, in-situ itraconazole treatment and specific antibiotic regiments are capable of increasing survival probability and could be used in accordance with other mitigation efforts.

Probiotic Supplementation of Single Anti-*Bd* Bacterial Species

To this point, there is considerable evidence that a bioaugmentation strategy may be an effective management tool to control *Bd* and the future of amphibian conservation may be in the discovery and development of probiotics for amphibians that can mitigate the threat of chytridiomycosis. The ideal candidate for a single-taxon probiotic would be a beneficial skin bacterium that can protect against the disease chytridiomycosis by producing secondary metabolites that inhibit the spread + colonization of *Bd*. Putting knowledge into action, the experimental addition of *J. lividum*, the previously mentioned antifungal bacterial producer of violacein, to *Bd*-susceptible amphibian species successfully increased violacein concentrations on the skin which was strongly associated with survival after experimental exposure to *Bd* (Harris et al. 2009; Becker et al.

2009; Keuneman et al. 2016). Thus, probiotic inoculation with a single anti-*Bd* bacterial species has been shown to reduce *Bd* infection intensity and increase survival probability. However, a single-taxon probiotic is likely not the ultimate solution to *Bd* because drastically altering the skin microbial community in favor of a single highly effective species (Harris et al. 2009) also reduces bacterial synergistic defenses which leads to interest in heterogenous probiotic communities (Bletz et al. 2013).

Probiotic Supplementation of Multi-Strain Community

Probiotic development strategies should also consider the addition of multi-strain antifungal bacterial community, given that pathogen resistance is an emergent property of microbial communities (Piovia-Scott et al. 2017) and greater microbial community diversity has been linked to increased pathogen inhibition (Jiménez et al. 2019). In a lab setting, multi-species communities inhibited *Bd* growth more than monocultures of individual species, further illustrating that species-species interactions in-part determine the effect of bacterial communities on *Bd* (Piovia-Scott et al. 2017). There are multiple possible mechanisms behind these emergent anti-*Bd* abilities. Bacterial species compete through the production of defensive compounds (Loudon et al. 2014) and the host obtains a defensive benefit as a by-product of microbial inter-species competition. Anti-*Bd* effects can be additive, if the anti-*Bd* species operate via different modes i.e., one microbe strain that directly secretes metabolites combined with another bacterial species that is capable of immunomodulation. Interestingly, monocultures of bacteria have demonstrated the potential to interact synergistically in which bacterial strains produced emergent metabolites when cultured together, with even greater pathogen inhibition (Loudon et al. 2014). Therefore, multi-taxon *Bd*-inhibitory probiotics may be more effective than single-taxon treatments because they would maximize the anti-*Bd* properties of heterogenous bacterial communities through species-species interactions.

Properties of an Effective Probiotic

The characteristics of an effective probiotic stem from the basic functional goal of probiotics, being to alter cutaneous microbial community structure and microbial interactions between symbionts and *Bd* to prevent a lethal disease outcome and overall spread of chytridiomycosis. At the fundamental level, the probiotic microbiota needs to be able to colonize and persist at sufficient levels in the target host's skin microbiome. One of the biggest hurdles found in *J. lividum* probiotic trials was that *J. lividum* appeared to colonize Panamanian golden frog skin microbiome temporarily, but it was not able to persist and thus, probiotic treatment did not prevent or delay mortality (Becker et al. 2011; Becker, Walke, Cikanek et al. 2015). These failed probiotic trials highlight a major issue in developing probiotics: incompatibility between bacterial probiotic strains and new amphibian host, where bacteria are unable to colonize and persist long-term in skin microbiota. This may be due to several factors including probiotics being inhibited by resident microbiota species or other host immune defense mechanisms (Chiu et al. 2017). If the probiotics can successfully colonize and persist, the conditions must also be correct for anti-*Bd* action, whether that's production of the metabolite, immunomodulation, etc. This leads to the next point; if probiotic bacteria can colonize one host animal, would probiotics be able to last in the community in some sort of self-disseminating system? For probiotic treatment to be a long-term solution, it should be able to last in the amphibian community via vertical, horizontal, and/or environmental transmission. The goal is that a complex community comprised of resident and probiotic microbiota that stabilizes resistance over time to achieve greater disease resistance (Weeks et al. 2020; Knapp et al. 2022).

Areas for Additional Research

Overall, more research is still to be done to achieve a fully integrated understanding of the variability and stability of the skin microbiome and its role in disease resistance. The next step in development and application of novel probiotic treatments is moving from ex-situ testing to in-situ trials. To do this safely, more research studies are needed to determine the biosafety of environmental broad bioaugmentation applications. This would include investigating the efficacy of vulnerable-habitat inoculation, with and without initially reducing naturally occurring microbial populations (Bletz et al. 2013). Future research of this nature should also be on the lookout for the effects of bioaugmentation on non-target species and overall unintended ecosystem effects. Also, after identifying success in probiotic mechanisms, studies should also aim to combine conservation tactics, such as combining antifungal treatment with probiotics, to maximize pathogen resistance.

Conclusion

To understand the disease dynamics associated with *Batrachochytrium dendrobatids* and chytridiomycosis, the interactions between the host amphibian, its resident cutaneous microbes and colonizing pathogens need to be characterized. *Batrachochytrium dendrobatidis* (*Bd*) is a pathogenic chytrid fungus that causes chytridiomycosis in amphibians (Longcore et al. 1999). Short term itraconazole anti-fungal treatment of *Bd*-infected individuals is effective in extended population survival during periods of high disease risk (Hudson et al. 2016). Different symbiont community structures result in distinct clinical *Bd* outcomes for the host (Kruger et al. 2020; Walke et al. 2015) and the characterizing microbial community composition differences among populations prior to infection can aid in predicting susceptibility in amphibian species (Becker et al. 2015). Anti-*Bd* bacteria achieve disease resistance directly, by production of anti-fungal metabolites, and indirectly by host immune modulation (Flechas et al. 2019; Muletz-Wolz et al. 2017; Harris et al. 2009). Low abundance of anti-*Bd* bacteria on frog's skin has been linked to high prevalence of *Bd* in the hotspots of infection (Varela et al. 2018) and vice versa, a high abundance of anti-*Bd* bacteria on frog's skin is associated with low prevalence of *Bd* infection in coldspots of infection (Mutnale et al. 2021).

The role of symbiotic bacterial communities in host protection against chytridiomycosis should be adapted to the development of an effective probiotic is to alter cutaneous microbial community structure to decrease morbidity associated with *Bd*. Beneficial effects of skin microbiota likely result from individual bacterial strain anti-*Bd* abilities as well interactions within the microbial community (Harris et al. 2009; Becker et al. 2009; Keuneman et al. 2016; Loudon et al. 2014;), thus probiotic therapy may be successful with both single-taxonomy and heterogenous consortia applications.

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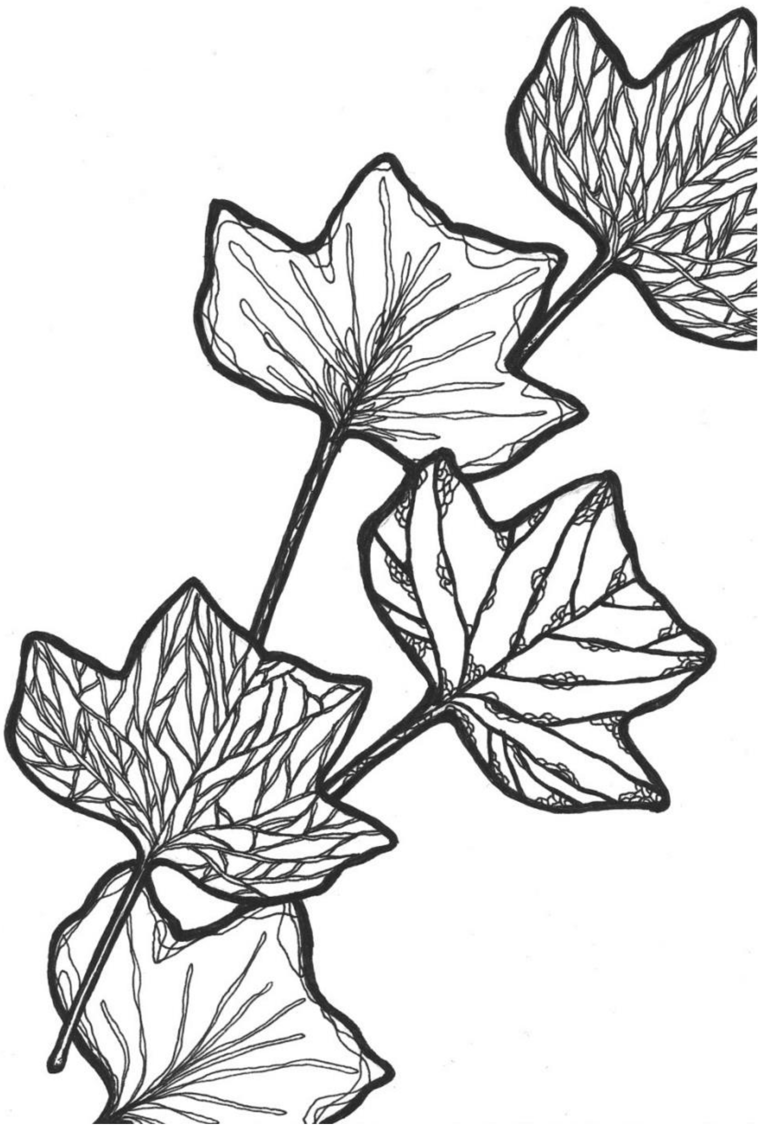

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