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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 XXXI marks the tenth 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.

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Image Credits

Lexi Perkins
This image is modeled off of one of the student articles featured in this edition of the journal on sleep depression

Editorial Staff

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The Role of Sleep Deprivation in Older Adults and the Development of Alzheimer's Disease

Alexis Smith

Sleep is an important part of human life, yet an increasing amount of the American population is getting less than the 7-9 hours of recommended sleep per night. Sleep deprivation can result in cognitive impairment that is comparable to excessive consumption of alcohol. Sleep deprivation also results in oxidative stress and an accumulation of β-amyloid proteins, both of which have well-established roles in the development of Alzheimer's disease. In addition, sleep deprivation reduces several different types of antioxidants. These negative effects are seen in both chronic and acute sleep deprivation, and the effects tend to worsen with age. Even the brains of older adults who did not suffer from Alzheimer's, though did experience poor sleep quality and less sleep duration, had the hallmarks of Alzheimer's disease. Even if sleep deprivation does not directly lead to the development of Alzheimer's, it still results in unnecessary stress in the brain.

Introduction

Sleep is an imperative part of a 24-hour day in the life of a human. According to the National Sleep Foundation, young adults and adults need a recommended 7-9 hours of sleep a night (National Sleep Foundation), though this is still debated among scientists today. However, despite this generally accepted recommendation among the public, Americans have decreased the amount of sleep they get per night by as much as 20% throughout the last century (Ferrara and de Gennaro, 2001 for review). Sleep deprivation is known to have effects on learning and memory, as well as cognitive functioning (Chee and Chuah, 2008). In fact, after 17 hours of sustained wakefulness, cognitive and motor performance is comparable to impairment seen with a blood alcohol level of 0.05% (Drawson et al., 1997). After 24 hours of sustained wakefulness, or what some college students might refer to as "all-nighters," impairment is similar to the blood alcohol level of 0.10% (Dawson et al., 1997), which is 0.02% over the legal driving limit in America and 0.05% over the limit in Australia (Rajaratnam and Arendt, 2001).

Sleep deprivation also causes oxidative stress, which is caused by a higher ratio of reactive oxygen species, a natural by-product of cellular activity, to antioxidant defenses within the brain (Melo et al., 2011; Silva et al., 2004; Vollert et al., 2011; Alzoubi et al., 2012). In addition, sleep deprivation can result in an accumulation of β-amyloid proteins (Xie et al., 2012). These proteins build up during wakefulness and sleep is necessary to clear them out of the interstitial fluid (Xie et al., 2012). Oxidative stress, one result of sleep deprivation, has a well-established role in the development of Alzheimer's disease, as does β-amyloid proteins buildup (Markesbery, 1997 for review). Therefore, because sleep deprivation leads to oxidative stress and β-amyloid protein accumulation, both of which are associated with Alzheimer's disease, it follows that sleep deprivation could play a role in the development of Alzheimer's disease.

Oxidative Stress as a Result of Sleep Deprivation Mediated by Age

Sleep deprivation leads to oxidative stress both during chronic and acute sleep deprivation. Rats that were sleep deprived for six weeks had an increase in oxidized glutathione (Alzoubi et al., 2012). Glutathione is an endogenous antioxidant, and thus an increase in oxidized glutathione, as opposed to reduced glutathione, indicates an increase in oxidative stress (Silva et al., 2004). Chronic sleep deprivation also resulted in a decrease in glutathione peroxidase, superoxide dismutase, and catalase, three endogenous enzymatic antioxidants in the hippocampus (Alzoubi et al., 2012). However, animals that were chronically sleep deprived and that also received Vitamin E, a known antioxidant, had levels of oxidized glutathione, glutathione peroxidase, superoxide dismutase, and catalase comparable to animals that were not sleep deprived (Ognjanovic et al., 2003; Alzoubi et al., 2012). Hence, the antioxidant Vitamin E stabilizes the changed levels of the parameters of oxidative stress from chronic sleep deprivation.

Similar effects are seen in acute sleep deprivation. In both rats and mice that were sleep deprived for 72 hours, glutathione levels were decreased, while the ratio of oxidized to reduced glutathione was increased (Silva et al., 2004; Kumar and Garg, 2008; Khadrawy et al., 2011). This change was not seen in control mice that experienced a stressful environment for that same period of time so these glutathione changes are not due to environmental stress (Silva et al., 2004; Kumar and Garg, 2008; Khadrawy et al., 2011). Lipid peroxidation, which is the cascade of the oxidation of fatty acids resulting in the generation of peroxyl radicals that can oxidize membrane proteins and

other nearby fatty acids was increased in the hippocampus after 72 hours of sleep deprivation (Halliwell and Chirico, 1993; Silva et al., 2004; Kumar and Garg, 2008; Khadrawy et al., 2011). However, hippocampal lipid peroxidation levels were lower in sleep-deprived animals treated with antioxidants, such as N-tert-butyl-a-phenylnitrone, a free radical spin-trapping compound, Vitamin E, and melatonin, (Carney et al., 1991; Silva et al., 2004). Sleep deprivation thus causes damage to lipids, which can have catastrophic effects on cells, but these effects are mediated by antioxidants. Another antioxidant measure that reduced oxidative stress from acute sleep deprivation is exercise (Vollert et al., 2011). Rats that exercised before the sleep deprivation did not experience the increase in any parameters of oxidative stress in the cortex, hippocampus, and amygdala that was seen in rats that were sleep deprived for just 24 hours but did not exercise (Vollert et al., 2011). Sleep deprivation anywhere from 24 hours and up to 6 weeks or longer leads to oxidative stress in the brain, but these effects are mediated by concomitant antioxidant treatment.

While chronic and acute sleep deprivation results in similar levels of oxidative stress markers, the effects can be dependent on age. Acute sleep deprivation in juvenile rats resulted in an increase in glyoxalase and glutathione reductase, an endogenous antioxidant enzyme, in the hippocampus (Vollert et al., 2011). However, enzymatic antioxidant levels in the hippocampus decreased in young adult rats that experienced chronic sleep deprivation (Alzoubi et al., 2012). This difference in antioxidant enzymatic activity could potentially be due to the length of the sleep deprivation, but it could also be due to the age at which the sleep deprivation occurred. Rats that were sleep deprived for 96 hours had a decrease in glutathione levels and superoxide dismutase activity in the hippocampus, but these decreases were greater in elderly rats compared to adults (Singh et al., 2008). In addition, older rats, even in the control group, had lower levels of glutathione and superoxide dismutase activity compared to the adults (Singh et al., 2008). However, further research needs to be conducted to more thoroughly understand the relationship between age and the oxidative stress effects of sleep deprivation.

Sleep Deprivation Leads to Toxic β-amyloid Protein Accumulation Characteristic of Alzheimer's Disease

Sleep deprivation can cause oxidative stress in the brain in other indirect ways, as well. In both mice and humans, β-amyloid proteins increased in the interstitial fluid during wakefulness throughout the day and decreased during sleep at night (Kang et al., 2009). During acute sleep deprivation, these β-amyloid proteins levels increased, while in chronic sleep deprivation in amyloid precursor protein transgenic mice, β-amyloid plaques formed (Kang et al., 2009). These plaques are also found in older adults who did not have Alzheimer's disease, and were associated with shorter sleep duration and lower sleep quality (Spira et al., 2013). Thus this hallmark of Alzheimer's disease is seen in individuals who also suffer from sleep deprivation. Oxidative stress can cause non-aggregated β-amyloid proteins to become aggregated (Dyrks et al., 1992). These aggregated β-amyloid proteins initiate lipid peroxidation and generate free radicals, and the concentration at which these radicals are formed is strongly correlated with the toxicity of the β-amyloid proteins (Butterfield et al., 1994; Harris et al., 1995; Hensley et al., 1994). Therefore, sleep deprivation causes a build-up of β-amyloid proteins and oxidative stress, and oxidative stress in turn causes an aggregation of β-amyloid proteins, which then leads to increased oxidative stress in the brain. Sleep deprivation is thus very toxic and could potentially play a role in the development of Alzheimer's disease.

This is especially true, since sleep deprivation in Alzheimer's disease is a common phenomenon (Craig et al., 2006). Patients with Alzheimer's disease have a more difficult time falling asleep, and they wake up more often during the night (see Wolkcove et al., 2007 for review). In patients with Alzheimer's disease, the rest-activity rhythm during sleep was very disruptive, and this correlated with the symptom severity (Witting et al., 1990; Bliwise et al., 1995). This correlation was stronger in patients who were taking sedating drugs, which would imply that those patients had severely disturbed sleep-wake rhythms before the medication was prescribed (Witting et al., 1990). However, age, gender, and the use of psychoactive medications were unrelated to sleep disruption (Bliwise et al., 1995). Thus, the factor that correlates with sleep disruption is severity of the dementia of Alzheimer's disease.

Conclusion

Sleep disruption is a very toxic, and potentially deadly, aspect of the busy 24-hour day that is now standard in our society (Ferrara and de Gennaro, 2001). With the wide use of artificial lights, video streaming services, longer workdays, social commitments, and hectic schedules in general, it is not surprise that Americans are sleep deprived. The effort that people put into their daily busy schedules should also be applied to sleeping at least the minimal recommended 7-9 hours each night because the result of sleep deprivation, particularly in older

age, could lead to the development of Alzheimer's disease. However, even if the end result were not the potential development of Alzheimer's, sleep deprivation still adds an unnecessary stress on the brain with the addition of oxidative stress and β-amyloid proteins, and all it would take to remedy this increase in stress would be to add a few extra hours of sleep every night (Alzoubi et al., 2012; Kang et al., 2009).

References

Alzoubi K, Khabour O, Rashid B, Damaj I, Salah H. 2012. The neuroprotective effect of vitamin E on chronic sleep deprivation-induced memory impairment: the role of oxidative stress. *Behav Brain Res*. 226:205-210.

Bilwise D, Hughes M, McMahon P, Kutner N. 1995. Observed sleep/wakefulness and severity of dementia in Alzheimer's disease special careunit. *J Gerontol A Biol Sci Med Sci*. 50(6): 303-306.

Butterfield D, Hensley K, Harris M, Mattson M, Carney J. 1994. B-amyloidpeptide free radical fragments initiate synaptosomal lipidperoxidation in asequence-specific fashion: implications to Alzheimer's disease. *BiochemBiophysical Res Comm.* 200(2):710-715.

Carney J, Starke-Reed P, Oliver C, Landum R, Cheng M, Wu J, Floyd R. Reversal of age-related increase in brain protein oxidation, decrease in enzyme activity, and loss in temporal and spatial memory by chronic administration of the spin-trapping compound N-tert-butyl-alpha-phenylnitrone. *PNAS.* 88(9):3633-3636.

Chee M and Chuah L. 2008. Functional neuroimaging insights into how sleep and sleep deprivation affect memory and cognition. *Current Opinion in Neurology.* 21:417-423.

Craig D, Hart D, Pasmore P. 2006. Genetically increased risk of sleep disruption in Alzheimer's disease. *Sleep.* 29(8):1003-1007.

Dawson D and Reid K. 1997. Fatigue, alcohol and performance impairment. *Nature.* 388(6639):235.

Dyrks T, Dyrks E, Harmann T, Masters C, Beyreuther K. 1992. Amyloidogenicity of βA4 and βA4-bearing amyloid protein precursor fragments by metal-catalyzed oxidation. *J Biol Chem.* 267(25):18210-18217.

Everson C, Laatsch C, Hogg N. 2004. Antioxidant defense responses to sleep loss and sleep recovery. Am J Physiol Regul Integr Comp Physiol. doi:10.1152/ajpregu.00565.2004

Ferrara M and de Gennaro L. 2001. How much sleep do we need? *Sleep Medicine Reviews*, 4(2):1-26.

Halliwell B and Chirico S. 1993. Lipid peroxidation: its mechanism, measurement, and significance. *Am J Clin Nutr.* 57:715S-725S.

Harris M, Hensley K, Butterfield D, Leedle R, Carney J. 1995. Direct evidence of oxidative injury produced by the Alzheimer's β-amyloid peptide (1 40) in the cultured hippocampal neuron. *Experimental Neurology*. 131(2).

Hensley K, Carney J, Mattson M, Aksenova M, Harris M, Wu J, Floyd R, Butterfield D. 1994. A model for β-amyloid aggregation and neurotoxicity based on free radical generation by the peptide: relevance to Alzheimer's disease. *Proc Natl Acad Sci.* 91:3270-3274.

Kang J, Lim M, Bateman R, Lee J, Smyth L, Cirrito J, Fujiki N, Nishino S, Holtzman S. 2009. Amybloid-β dynamics are regulated by orexin and the sleep-wake cycle. *Science*. 326:1005-1007.

Khadrawy Y, Nour N, Aboul Ezz H. 2011. Effect of oxidative stress induced by paradoxical sleep deprivation on the activities of Na+, K+-ATPase and acetylcholinesterase in the cortex and hippocampus of rat. *Translational Research*. 157(2):100-107.

Kumar A and Kalonia H. 2007. Protective effect of Withania somnifera Dunal on the behavioral and biochemical alterations in sleep-disturbed mice (Grid over water suspended method). *Indian J Exp Biol.* 45(6):524-528.

Kumar A and Garg R. 2008. A role of nitric oxide mechanism involved in the protective effects of venlafaxine in sleep deprivation. *Behav Brain Res*. 194(2):169-173.

Kumar A and Singh A. 2008. Possible nitric oxide modulation in protective effect of (*Curcuma longa, Zingiberaccae*) against sleep deprivation-induced behavior alterations and oxidative damage in mice. *Phytomedicine. 1*5(6):577-586.

Markesbery W. 1997. Oxidative stress hypothesis in Alzheimer's disease. *Free Radical Biology and Medicine*. 23(1):134-147.

Melo A, Monteiro L, Lima R, de Oliverira M, El-Bacha R. 2011. Oxidative stress in neurodegenerative diseases: mechanisms and therapeutic perspectives. *Oxidative*

Medicine and Cellular Longevity. 2011:1-14.

National Sleep Foundation. 2015. How much sleep do we really need? <https://sleepfoundation.org/how-sleep-works/how-much-sleep-do-we-really-need>

Ognjanovic B, Pavlovic S, Maletic D, Zikic R, Stajn A, Radojicic R, Saicic Z, Petrovic V. 2003. Protective influence of Vitamin E on antioxidant defense system in the blood of rats treated with cadmium. *Physiol Res*. 52:563-570.

Rajaratnam S and Arendt J. 2001. Health in a 24-h society. *The Lancet*, 358(9286):999-1005.

Silva R, Abilio V, Takatsu A, Kameda S, Grassl C, Chehin A, Calzavara M, Registro S, Anderson M, Machado R, Carvalho R, Riberiro R, Tufik S, Frussa-Filho R. 2004. Role of hippocampal oxidative stress in memory deficits induced by sleep deprivation in mice. *Neuropharmacology*. 46:895-903.

Singh A and Kumar A. 2008. Protective effects of alprazolam against sleep deprivation induced behavior alterations and oxidative damage in mice. *Neuro Res.* 60(4):372-379.

Singh R, Kiloung J, Sharma D. 2008. Effects of paradoxical sleep deprivation on oxidative stress parameters in brain regions of adult and old rats. *Biogerontology*. 9(3):153-162.

Spira A, Gamaldo A, An Y, Simonsick E, Bilgel M, Zhou Y, Wong D, Ferrucci L, Resnick S. 2013. Self-reported sleep and β-amyloid deposition in community-dwelling older adults. *JAMA Neurol.* 70(12):1537-1543.

Subbarao K, Richardson J, Ang L. 1990. Autopsy samples of Alzheimer's cortex show increased peroxidation in vitro. *J Neurochemistry*. 55(1):342-345.

Vollert C, Zagaar M, Hovatta I, Taneja M, Vu A, Dao A, Levine A, Alkadhi K, Salim S. 2011. *Behav Brain Res*. 224:233-240.

Witting W, Kwa I, Eikelenboom P, Mirmiran M, Swaab D. 1990. Alterations in the circadian rest-activity rhythm in aging and Alzheimer's disease. *Biol Psychi*. 27:653-572.

Wolkcove N, Elkholy O, Baltzan M, Palayew M. 2007. Sleep and aging: 1. Sleep disorders commonly found in older people. *CMAJ*. 176(9):1299-1304.

Xie L, Kang H., Xu Q, Chen M, Liao Y, Thiiyagarajan M, O'Donnell J, Christensen D, Nicholson C, Illif J, Takano T, Deane R, Nedergaard, M. 2013. *Science*. 342:373-377.

Designing a Genetic Screen for α1-Antitrypson Deficiency Associated Emphysema

Morgan Fuller and Lawrence T. Reiter, Ph.D., Department of Neurology, University of Tennessee

Drosophila melanogaster was used to design a genetic screen for the purpose of identifying suppressors and enhancers of ATZ pathology. The first step in this process was to find a screenable phenotype. The GAL4/UAS system was employed to over-express ATZ, the human Z mutation, and nec, the fly homolog, in the eye, wing, fat body, epidermis, dorsal vessel and neurons. A few lines produced a screenable rough eye phenotype in the eye. A climbing assay was used to measure the effects of ATZ on the muscles. This assay will be helpful in future projects to see if the functional A1AT protein can be rescued. The knockdown of various serpins was additionally tested because these serpins have been shown to be homologs of nec. In the future, we hope this research leads to the development of new drug therapies for effected individuals.

Introduction

The serpin or *ser*ine *p*roteinase *in*hibitor superfamily contains multiple proteins which function via a unique inhibition method that involves a conformational change (Huntington et al., 2000). This necessary conformational change unfortunately makes the serpins vulnerable to mutations that can result in a loss of function of the serpin protein (Carrell and Lomas, 2002). These mutations also result in the polymerization of the serpins, and the best characterized of these conditions remains α1-antitrypsin deficiency (Lomas et al., 1992; Lomas and Carrell, 2002).

α1-Antitrypsin (A1AT) deficiency affects 1 in 2800 individuals in North America with 96% of patients expressing the deleterious Z variant (Luisetti and Seersholm, 2004; Blanco et al., 2006). The Z mutation of A1AT (Glu^{342}→ Lys) results in liver cirrhosis and emphysema (Green et al., 2002). Under normal conditions, A1AT is produced in the hepatocytes and then released to circulate to the lungs (Green et al., 2002). A1AT ensures the lungs maintain elasticity by inhibiting neutrophil elastase and preventing degradation of elastin fibers (Brantly et al., 1988). Problems can arise when a homozygous Z variant (*ATZ*) results in a 10-15% decrease in functional A1AT (Brantly et al., 1988; Gooptu and Lomas, 2008). The accumulation of mutant α1-antitrypsin in the liver leads to cell death and inflammation that eventually manifests in cirrhosis of the liver (Gooptu and Lomas, 2008). In the lungs, the lack of A1AT causes a loss of organization and structure that exemplifies the pathophysiology of emphysema.

In this study, our goal was to identify suppressors or enhancers of *ATZ* pathology by performing an unbiased genetic screen in the common fruit fly *Drosophila melanogaster*. We specifically examined *nec* expression because it is a sequence homolog to A1AT in *D. melongaster* and because the nec protein is synthesized in the fly's fat body, which is analogous to A1AT production in humans (Green et al., 2002). We also identified modifiers of α1-antitrypsin to elucidate the gene network that leads to the development of α1-antitrypsin deficiency associated emphysema.

Methods

Fly Stocks

We used the GAL4/UAS system (Duffy, 2002) to overexpress *ATZ* in specific non-essential and easy to score structures in *D. melanogaster* in order to generate a phenotype for genetic screening (Table 1). We overexpressed *ATZ* and *nec* in the eye, wing, fat body, epidermis, dorsal vessel and neurons with the goal of identifying a line that produced a phenotype. We also knocked down *nec* expression using publicly available UAS-shRNAi lines and Transgenic RNAi Project (TRiP) lines (2016). We examined the effects of the knock down of various serpin genes that mimic the behavior of A1AT in the fly by crossing them to three GAL4 driver lines, *gmr*-gal4, *eye 3.5*-gal4, and *MS1096*-gal4. All Drosophila stocks were obtained from the Bloomington Drosophila Stock Center at Indiana University (2016).

Climbing Assay

Using a muscle driver, *mef*-GAL4, we looked for measurable muscle related phenotypes when *ATZ* is over-expressed. We used a climbing assay previously described in Hatfield *et al*. (2015) and quantified the effect of *ATZ* over-expression on climbing ability in Drosophila. We specifically collected the overexpression lines *mef*-GAL4;UAS-*ATZ* and w-;*mef*-GAL4 for these assays. Mouth pipetting was employed because anaesthetizing flies is known to have a negative effect on fly behavior. Each trial contained five flies that were collected three days post enclosure. To begin each trial, the tube was tapped to force the flies to bottom, and the time was

Stock Number		Genotype
UAS Lines		
6775		P{UAS-*f*::*nec*-dsRNA}1, w^{1118}
6774		w^{1118}, P{UAS-*f*::*nec*-dsRNA}6; P{UAS-*f*::*nec*-dsRNA}7
44228		w^{*}, b^{1}, nec^{20}, cn^{1}, bw^{1}/Cyo; P{UAS-nec^{9}}20
Donated by Dr. Kang		UAS-*ATZ*/TM6B
GAL4 Drivers		
	Where expressed?	
8221	Eye	y^{1}, w^{1118}, P{*eye 3.5*-GAL4.Exel}1
9146	Eye	w^{1118}, P{*GMR*-gal4, w-}^{2}/Cyo
27390	Muscles	y^{1}, w^{*}; P{w[+mc]=GAL4-*mef2*.R}3
2736	Dorsal Vessel	w^{*}; P{GawB}5108/Cyo
6982	Fat Body	w^{1118}; P{GawB}c564
2735	CNS	w^{*}; P{GawB}C1003
7026	Epidermis	$y^{1}w^{*}$; P{GawB}109-69/Cyo
7149	Epidermis	w^{*}; P{GawB}227
42736	Epidermis	P{GAL4}BG380; In(3L)D, D^{1}/TM3, sb^{1}
6990	Fat Body	w^{1118}; P{GawB}C8559
6984	Fat Body	P{Gaw}c754, w^{1118}
TRiP Lines		
	Serpin Knockdown	
31757	Srp42Dc	$y^{1}v^{1}$; P{TRiP.HM04068}attP2
31979	Srp42Da	$y^{1}v^{1}$; P{TRiP.JF03413}attP2/TM3, sb^{1}
41613	Srp42Da	$y^{1}v^{1}$; P{TRiP.GL01195}attP40
41722	Srp42Da	$y^{1}sc^{*}v^{1}$; P{TRiP.HMS02288}attP2
41972	Srp43Aa	$y^{1}sc^{*}v^{1}$; P{TRiP.HMS02370}attP40
42818	Srp43Aa	$y^{1}sc^{*}v^{1}$; P{TRiP.GL01353}attP40
41617	Srp42Dd	$y^{1}v^{1}$; P{TRiP.GL01199}attP40
43991	Srp42Dd	$y^{1}sc^{*}v^{1}$; P{TRiP.HMS02704}attP2
41687		$y^{1}sc^{*}v^{1}$; P{TRiP.HMS02251}attP2

Table 1. Fly Stocks Used in this Study

measured for how long it took the flies to climb four centimeters up the tube. We stopped the timer when the first fly crossed the four centimeter line marked on the side of the tube. We waited one minute between each trial to allow the flies time to recover. After all the trials, we calculated an average climbing time and conducted a Student's *t*-test to determine the statistical significance of our results.

Imaging

Pictures of fly eyes were taken with a Canon EOS Rebel T3 camera. Fly wings were dissected and mounted on slides with Cytoseal XYL. A Leica DM6000 upright microscope was used to capture images of the wings.

Results

ATZ Expressed in the Eye

The first experiment was to express wild type *ATZ* in the fly eye using the eye specific GAL4 driver *gmr*-GAL4. Over-expression of wild type human *ATZ* does not produce a rough eye phenotype (Figure 1). The rough eye phenotype develops when an over-expression of a specific gene in the eye leads to fused eye units called ommatidia. The rough eye phenotype appears as necrotic spots.

Expression in the Wing

As an alternative tissue phenotype, we generated a wing specific phenotype using the wing specific driver *MS1096*-GAL4. *MS1096*-gal4, a strong GAL4 driver across the entire wing, was used to drive *ATZ* expression (Figure 2). A possible phenotype appeared in offspring containing a knock down of *nec* and *f* using *MS1096*-gal4. The offspring with *MS1096*>6774 and offspring with *MS1096*>6775 appeared to have a thickening of bristles and a thicker cross vein. The offspring with *MS1096*>*ATZ* did not show any wing phenotypes. Note, that 6774 and 6775 lines contain a knockdown of both *nec* and *f.* A line with only a knockdown of *nec* was not publically

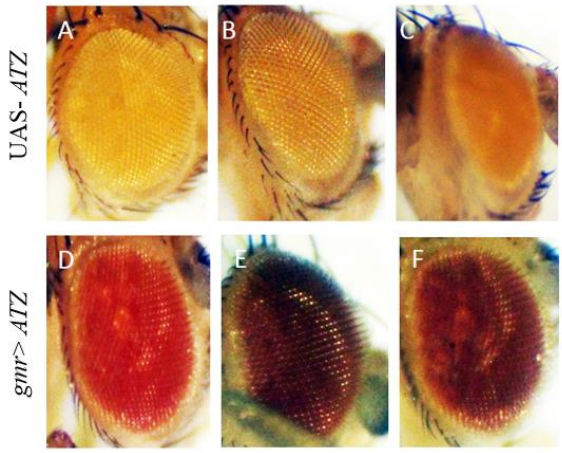

Figure 1. Over-expression of *ATZ* in the eye does not produce a rough eye phenotype. Three samples were taken from the experimental group, *gmr>ATZ* and three from the negative control UAS-*ATZ.* The red eye color signifies that these flies carry *gmr*. Because the eyes look normal and do not have any black, necrotic spots, a screenable phenotype was not found.

available at the time. Crosses with 6774 and 6775 were used to obtain preliminary data. In other words, we wanted to see if we could find a screenable phenotype first and if we did we would create a line of flies with only a knockdown of *nec.*

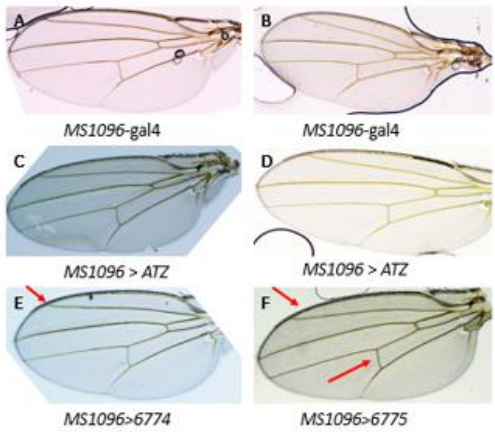

Figure 2. (A, B) Control W- flies did not produce a wing phenotype. (C, D) *MS1096>ATZ* flies also did not produce a screenable phenotype. (E) The arrow points to a possible thickened row of bristles in a 6774>*MS1096* sample. (F) 6775>*MS1096* flies may exhibit a thickening of the bristles and/or a thicker crossvein exhibited by the arrows.

Climbing Assay

In order to look for over-expression phenotypes related to the role of *ATZ* in muscle, a climbing assay was performed on flies expressing *ATZ* with the muscle driver *mef*-gal4 (Figure 3). A significant difference in climbing time was found with *mef*-gal4; UAS-*ATZ* flies (1.82 ± 0.09 seconds) (average climbing time ± standard error) showing a decreased ability to climb as compared to control *mef*-gal4;+ flies (1.13 ± 0.08 seconds) (*p* value ≤0.013).

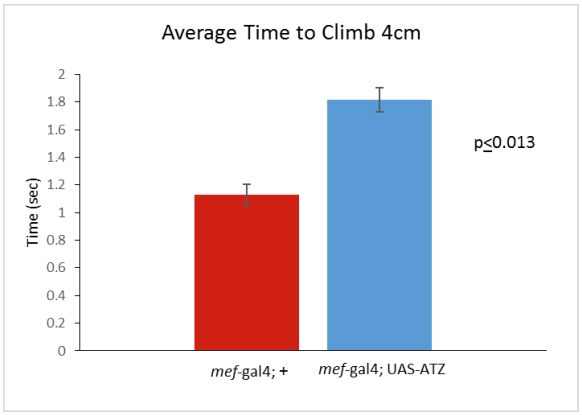

Figure 3. Virgin *mef*-gal4 flies were crossed with male UAS-*ATZ*/TM6B flies. Climbing assays were performed three days after enclosure. Four animals from each group were taken, and three trials were conducted on each fly. The error bars designate standard error and *mef*-gal4; UAS-*ATZ* flies showed a decrease in climbing speed when compared to control group ($p \leq 0.013$).

nec Knock Down in the Eye

Revisiting the *gmr*-gal4 driver, we crossed 6775 males and virgin *gmr*-gal4 flies in order to generate a screenable phenotype. In this case, we had to use fly stock 6775 which contained a knockdown of *nec* and a knockdown of *f* (Figure 4). An eye phenotype exhibiting necrotic spots was found only in female *gmr*> UAS-*f*::*nec*-dsRNA. No phenotypes were found in males because the UAS-*nec* insertion in stock 6775 is located on the X chromosome.

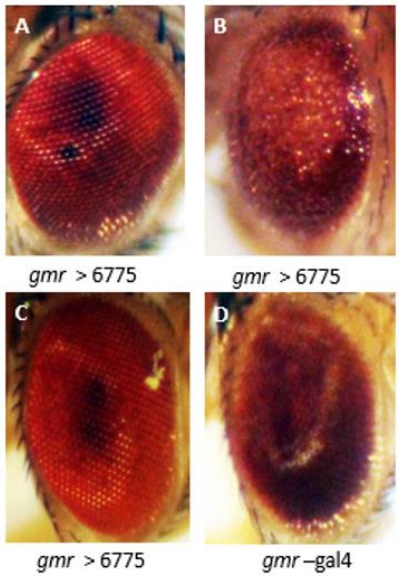

Figure 4. *ATZ* homolog, 6775, produces necrotic spots when over expressed in the eye (A). The rough eye phenotype varies in intensity and shows an example of an extreme rough eye phenotype (B). Not all *gmr*>6775 flies, however, had necrotic spots (C). The control fly (D) did not result in a screenable phenotype.

In order to independently confirm this phenotype, the 6774 stock was crossed to a different eye specific GAL4 driver, *eye 3.5*-GAL4 (stock 8221) (Figure 5). Note, that 6774 also contains a knockdown of *nec*. The offspring from this cross exhibited several necrotic spots beneath the ommatidia consistent with the results seen for *gmr*-gal4 (Figure 4).

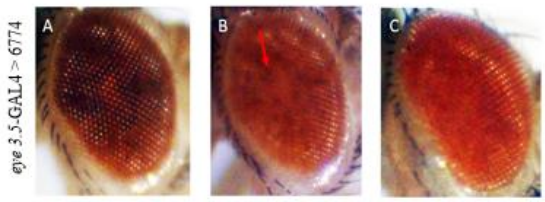

Figure 5. *ATZ* homolog, *nec*, was over-expressed in the eye using the *eye 3*.5-GAL4 driver and the offspring exhibited necrotic spots beneath the ommatidia (A, B, C). The arrow (B) points to one spot. In these samples, the rough eye phenotype had a more speckled appearance. There was no difference between genders.

To rule out the effects of the *f*-RNAi construct in the 6774 stock we crossed *gmr*-gal4 to UAS-RNAi-*f* (*f* TRiP) alone. The offspring from the *f* TRiP line and *gmr*-gal4 cross produced no abnormal eye phenotypes (Figure 6) and the same result was produced using the *eye 3.5*-GAL4 driver (Figure 7).

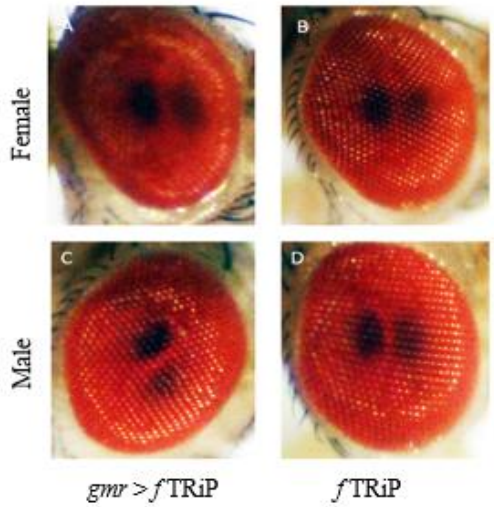

Figure 6. The offspring from the *f* TRiP and *gmr*-gal4 cross did not produce eye phenotypes. The experimental group (A, C), *gmr* > *f* TRiP, did not exhibit rough eyes which compares to the control group (B, D), *f* TRiP. There was no difference between genders. These results suggest that over-expression of *nec* is associated with the rough phenotype seen in Figure 4.

nec^{9} *Over-Expression in the Eye*

Returning to the *gmr*-gal4 driver, we crossed another *nec* line (44228 stock) to find a screenable phenotype. In the 44228 stock, *nec*^{9} is found in a P-element on the third chromosome, *nec*^{20} is also found on the second chromosome. This line contains the same mutation found in *ATZ* which made it a promising strain. The cross produced offspring with fused ommatidia, a screenable rough eye phenotype (Figure 8).

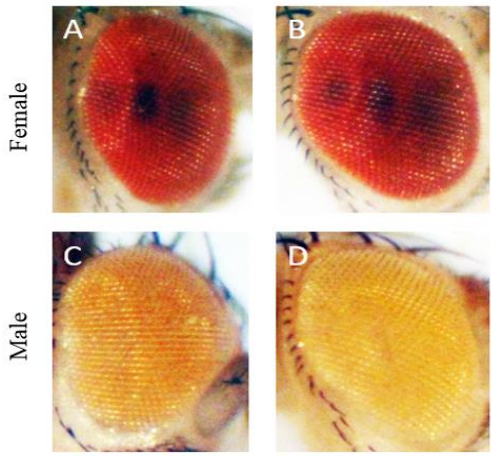

Figure 7. Two females (A, B) and two males (C, D) were selected from the experimental group, *3.5*-gal4 > *f* TRiP. No rough eye phenotype was present. There was no difference between genders. The lack of an abnormal phenotype suggests that over-expression of *nec* is associated with the rough eye phenotype seen in Figure 5.

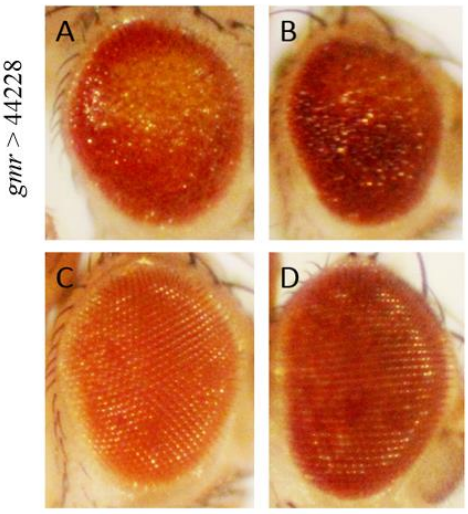

Figure 8. About 50% of offspring produced the expected rough eye phenotype (A, B). The remaining half of offspring did not have rough eyes, but the exact genotype cannot be determined as the same balancer was used in the GAL4 driver and UAS construct (C, D).

Discussion

The experiments described here were designed to generate a new Drosophila over-expression model of A1AT deficiency to use in a screen for genes and proteins that interact with mutant *ATZ* (Figure 1). For the wing and fat body drivers, we were unable to generate a phenotype for screening. However, we did generate a rough eye phenotype suitable for a

genetic screen by over-expressing the *ATZ Drosophila* homolog, *nec*, in the eye. There were a couple promising findings from a cross between UAS-*f*::*nec*-dsRNA; UAS-*f*::*nec*-dsRNA and *eye3.5*-GAL4 (Figure 5) and a second cross between UAS-*f*::*nec*-dsRNA and *gmr*-gal4 (Figure 4). However, we need to be cautious since lines 6774 and 6775 both contain a knockdown of both the *nec* and *f* genes. We predict that the knockdown of *nec* and not the knockdown of *f* alone results in the presence of necrotic spots in the eye since no phenotypes appeared after the cross between the *f* TRiP line and *gmr*-gal4 (Figure 6) and the cross of *f* TRiP to *eye 3.5*-gal4 (Figure 7). We will need to isolate UAS-*nec* alone for future studies using this approach.

We did find a strong phenotype for wild type *ATZ* over-expression using the muscle driver *mef*-Gal4 (Figure 3). However, this phenotype requires using a climbing assay for finding interacting genes, which is not practical on a large scale. This strong climbing assay defect could be used for validation of a gene interaction found using unbiased screening approaches described below.

Finally, we did find a rough eye phenotype suitable for a genetic screen using the *gmr*-gal4 driver to express the nec^{9} mutation which is analogous to human mutations in *ATZ* (Figure 8). Mutant *nec9* over-expression caused a robust phenotype, but the stock we used for these experiments (44228) also contains a nec^{20} mutation in the background. We are in the process of isolating the UAS-nec^{9} construct from the nec^{20} mutant background and will repeat the *gmr*-GAL4 crosses using this newly isolated UAS-nec^{9} transgene.

Once this UAS-nec^{9} line is created we will begin an unbiased screen for suppressors and enhancers of the *gmr*>nec^{9} rough eye phenotype. These new genetic interactions will help us to better understand the underlying genetic pathways resulting in A1AT in humans and can be further validated and tested in the *Drosophila* system for the production of new drug compounds for the treatment of A1AT deficiency.

Acknowledgements

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References

Blanco I, de Serres F, Fernandez-Bustillo E, Lara B, Miravitlles M. 2006. Estimated numbers and prevalence of PI*S and PI*Z alleles of α1-antitrypsin deficiency in European countries. *European Respiratory Journal*. 27(1):77-84.

Bloomington Drosophila Stock Center at Indiana University [Internet]. 1986. GAL4 Stocks. <http://flystocks.bio.indiana.edu/> Accessed 2016 March 16.

Brantly M, Courtney M, Crystal R. 1988. Repair of the secretion defect in the Z form of alpha-1 antitrypsin by addition of a second mutation. *Science*. 242:1700-1702.

Carrell R, Lomas D. 2002. $Alpha_1$-antitrypsin deficiency: a model for conformational diseases. *New England Journal of Medicine*. 346(1):45-53

Duffy J. 2002. GAL4 system in Drosophila: a fly geneticist's swiss army knife. *Genesis*. 34:1-15.

Green C, Brown G, Dafforn T, Reichhart J, Morley T, Lomas D, Gubb D. 2002. *Drosophila necrotic* mutations mirror disease-associated variants of human serpins. *Development and Disease.* 130:1473-1478.

Gooptu B, Lomas D. 2008. Polymers and inflammation: disease mechanisms of the serpinopathies. *The Journal of Experimental Medicine*. 205:1529-1534.

Hatfield I, Harvey I, Yates E, Redd J, Reiter L, Bridges D. 2015. The role of TORC1 in muscle development in Drosophila. *Scientific Reports*. 5:1-11.

Huntington J, Read R, Carrell R. 2000. Structure of a serpin-protease complex shows inhibition by deformation. *Nature*. 407:923-926.

Lomas D, Evans D, Finch J, Carrell R. 1992. The mechanism of Z α_1-antitryspin accumulation in the liver. *Nature*. 357:605-607.

Lomas D, Carrell R. 2002. Serpinopathies and the conformational dementias. *Nature Reviews Genetics*. 3:759-768.

Luisetti M, Seersholm N. 2004. α_1-Antitryspin deficiency 1: epidemiology of α_1-antitryspin deficiency. *Thorax*. 59(2):164-169.

The Transgenic RNAi Project at Harvard Medical School [Internet]. 2008. TRiP RNAi Stock Collection. <http://www.flyrnai.org/TRiP-HOME.html> Accessed 2016 March 16.

Plant Survival in Changing Climate Conditions Can be Predicted Through Niche Breadth and Presence of HSP101 and HSP70 in Germinating Seeds

Maddie Carwile

Climate change will cause worldwide temperature variations that are predicted to have significant impacts on plant survival, resulting in some species unable to survive due to an inability to adapt to a rapid change in climatic conditions. An indication of plant survival is germination, as seeds will not germinate unless the conditions are suitable. Plants that have a wider ecological niche are able to germinate at a wider range of temperatures, and are therefore more likely to survive fluctuating climatic conditions. Heat shock proteins are positively associated with increased seed germination during temperature stresses, with the proteins HSP101 and HSP70 particularly relevant. This review examines both niche breadth and heat shock proteins that are associated with plant survival in shifting climatic conditions, finding that a wide ecological niche and high levels of HSP101 and HSP70 are indicative of a plant's ability to survive climate change.

Introduction

Climate change is predicted to be the largest challenge to plant conservation in this century (Root et al., 2003). The effects of climate change are likely to be widespread and significant, correlating with a temperature-related shift present across taxa (Root et al., 2003). Many species of plants have already been affected by extreme temperature changes. For instance, Halloy and Mark (2003) found that 40-70 vascular species indigenous to New Zealand are at risk of extinction, and these extinction risks are likely to increase as temperatures continue to rise. A study conducted by Thuiller et al. (2005) found that more than half of studied species in Europe will become vulnerable or committed to extinction by 2080 if temperatures continue to increase at the predicted rate. In New Zealand, an additional 3°C rise in temperature is expected to cause a loss of 200-300 species of alpine vascular plants (Halloy and Mark, 2003).

Climate change threatens plants for a wide variety of reasons. Climate variations may cause changes in the geographic ranges of plants, which leads to an increased risk of local extinction (Thuiller et al., 2005). This reduction in geographic range implies that smaller stochastic events affect a larger proportion of the species' total population (Thuiller et al., 2005). In addition, climate change has already caused phenological events in plants to shift an average of 5.1 days per decade, which can cause a mismatch in time between activity of insect pollinators and when plants are flowering (Root et al., 2003; Bellard et al., 2012). These threats extend to the human sphere; climate warming may have significant effects on food security as many species of plants are unable to germinate and survive in extreme heat (Christensen and Christensen, 2007).

However, while climate change will likely cause extinction of some plants, differences in temperatures may have positive effects on other species of plants (Bellard et al., 2012). Increased levels of CO_2 may be beneficial to some species, and milder winters could increase the survival rate for threatened plants in colder regions (Bellard et al., 2012; Mondoni et al., 2012).

A plant's ability to germinate at a wide range of temperatures may be a strong indicator of plant adaptability in changing climatic conditions (Chochrane et al., 2010). The range of temperatures at which a seed is able to germinate is known as an ecological niche, and seeds with wide germination niches are more likely to survive climate change than seeds with narrow niches (Broennimann, 2006).

At the molecular level, accumulation of heat shock proteins (HSPs) 101 and 70 is correlated with greater germination at higher temperatures (Hong and Vierling, 2001; Quietsch et al., 2000; Burke and Chen, 2015). These proteins aid plant cells in responding to increased heat stress, and plants that are able to produce and accumulate these proteins may be more likely to survive increased temperatures caused by climate change than plants with lower levels of these proteins (Hong and Vierling, 2001; Quietsch et al., 2000; Burke and Chen, 2015).

Variance in Mean Time to Germinate and Optimal Germination Temperature

Seeds show an incredible amount of diversity in both timing of germination and optimal germination temperature. Mean time to germinate is based on both external temperature and a species-by-species basis, as well as on the climate in which that species evolved (Mondoni et al., 2012; Graae et al., 2008). Optimal germination temperature also depends on the season of germination for that species (Mott, 1972). Winter-germinating cotyledons in Western Australia showed optimal germination at temperatures between 15 and 20°C (Mott, 1972). Two species, *Helichrysum*

and *Helipterum*, had very little germination above 25 °C (Mott, 1972). However, *Aristida contorta*, a summer germinating plant, had optimal germination at 30°C, and its germination time and percentage of germination was reduced at lower temperatures (Mott, 1972). This diversity of reactions to climate change is also based on populations within a species. For example, Zhang et al. (2015) found that different populations of *Arabidopsis thaliana* have different responses to high temperature.

Some plants are more adapted to germinate at higher temperatures. In Mexican sunflowers (*Tithonia diversifolia*), temperatures of 50-55 degrees Celsius increased germination compared to colder temperatures (Wen, 2015). Moreover, Mexican sunflower seeds are able to germinate across a wide range of temperatures, and Wen (2015) hypothesizes that this higher tolerance in seeds to high temperature and limited availability of water is the main factor contributing to the species' success as an invasive plant. When heat treated for 30 minutes at 80°C, 20% of Mexican sunflower seeds were still able to germinate (Wen, 2015). However, a treatment at 85°C killed all Mexican sunflower seeds (Wen, 2015). Graae et al. (2008) found that a variety of dwarf shrub seeds in arctic, alpine, and boreal sites had increased percentages of germination at higher temperatures than at lower temperatures.

A study by Wilczek et al. (2014) examined relative fitness of local and migrated populations of *Arabidopsis thaliana*. They found that plants that had migrated from southern, historically warmer climates had higher relative fitness than non-migratory genotypes, and had increased levels and speeds of germination (Wilczek et al., 2014). In particular, when seeds were planted in the summer and fall and experienced warm temperatures for several months, natural selection favored plants that migrated from warmer climates (Wilczek et al., 2014). When plantings occurred in the cooler spring months, seeds that migrated from areas with an average April temperature of 11.4°C had a higher relative fitness compared to seeds from plants that migrated from warmer or cooler climates (Wilczek et al., 2014). The researchers also observed that there was an adaptation lag of several decades after plants migrated to a new climate before seed germination levels and speeds were fully restored (Wilczek et al., 2014). Due to this adaptation lag, plants may not be able to survive in the rapidly changing temperatures caused by climate change. Climate change will cause worldwide temperatures to increase at an unprecedented rate, with levels that have not occurred for the past 1,000 years (Smith et al., 2015).

Effects of Climate Change on Germination Rates and Percentages

Climate warming will likely shift the timing of seed germination in many plants (Mondoni et al., 2012; Milbau et al., 2009; Graae et al., 2008). In some species of plants, warmer weather increases germination while colder temperatures decrease germination (Milbau et al., 2009; Mondoni et al., 2012). Milbau et al. (2009) found that moderate summer warming accelerated germination in many species of shrubs and grasses, but did not affect germination percentages. Colder winter soil temperatures delayed germination in ten of their studied species and decreased percentage germination in four species: *E. angustifolium*, *V. myrtilllus V. uliginosum, and S. dioica* (Milbau et al., 2009). In four out of eight species of annuals and perennials, exposure to current or predicted summer temperatures led to 70% germination, while the other four species had less than 20% germination, demonstrating the wide variance in germination at warm temperatures (Ooi et al., 2009). A study by Mondoni et al. (2012) found that an increase in autumn temperature resulted in a significant increase in seed germination in six out of eight species of alpine plants. Germination stopped in all seeds that were transferred to winter temperatures of 0°C, and resumed when these seeds were transferred to simulated spring and summer conditions (Mondoni et al., 2012). While many studies have been conducted on increased air temperatures, soil temperature is likely to increase at double the rate of air temperature, and these predicted soil increases will likely affect seed dormancy and viability to a greater extent (Ooi et al., 2009).

Warming temperatures also have significant negative impacts on germination in some species of plants (Montesinos-Navarro, 2012; Akman, 2009). In a study by Montesinos-Navarro et al. (2012), *Arabidopsis thaliana* seeds that were exposed to a heat treatment at 30°C had a final germination that was 7% lower than seeds exposed to a treatment of 20°C. Exposure to warmer fall conditions reduced final germination by 33 ±3% compared to cooler spring conditions (Montesinos-Navarro, 2012). In addition to reducing the percent of germination, warming temperatures also slowed germination, with the study finding a slowed germination speed of 1.5 ± 0.6 days when seeds were exposed to 30°C compared to 20°C (Montesinos-Navarro, 2012). Akman (2009) found that increased temperatures decreased and delayed germination of maize, rice, and sorghum. In fact, maize germination was totally inhibited at 41°C (Akman 2009). However, germinating seeds are often able to recover from severe heat stress if they are later moved to cooler temperatures (Quietsch et al.,

2000). A study by Queitsch et al. (2000) exposed germinating *Arabidopsis* seeds to heat treatment and found that seeds that had developed for 30 hours had higher survival rates than germinating seeds that were heat shocked after 36 hours of development.

Cold temperatures are necessary for germination to occur in some species of plants (Graae et al., 2008). Arctic seeds may need more or longer periods of cold stratification than boreal seeds (Graae et al., 2008). For instance, seeds of *E. Nigrum* need several cold stratification periods in order to germinate (Graae et al., 2008). If climate warming is significant, *E. Nigrum* may be unable to germinate due to a lack of these cold periods.

Increased temperatures can also have indirect effects on seed germination in subarctic plants due to the melting of snow cover (Milbau et al., 2009; Graae et al., 2008). In experiments, colder winter temperatures often simulate an absence of snow cover that could be caused by climate warming (Mondoni et al., 2012). In some instances, thick snow cover may result in higher percentages of germination than thin snow cover because the snow creates an insulating layer for the seed (Milbau et al., 2009). The temperature under a normal snow cover in the arctic varies from between -17°C to -1°C (Graae et al., 2008). The snow cover clears following rapid snowmelt in May, and returns in September when daily mean temperatures decrease to around 0°C (Graae et al., 2008). In some locations, reduced snow cover is accompanied by summer warming, resulting in earlier germination and thus a longer first growing season, improving the chance of seedling survival in some species of plants (Milbau et al., 2009).

Climate change may also have effects on seed survival that cannot be easily predicted. A study by Graae et al. (2008) examined fungal attack on arctic species of plants and found that warmer winters may increase susceptibility to fungal disease by causing earlier germination. While warmer temperatures may increase germination overall, warming may also increase risk of extinction due to fungal attack (Graae et al., 2008).

Niche Breadth Helps Determine a Seed's Sensitivity to Climate Change

The term "niche breadth" refers to either a geographic range or a temperature range at which a seed can germinate and a plant can therefore survive (Chochrane et al., 2010; Broennimann et al., 2006). A plant's sensitivity to climate change partially depends on the niche breadth at which the seed can germinate (Chochrane et al., 2010). Niche breadths differ depending on the species and the climate in which they adapted evolutionarily to survive. Arana et al. (2015) found that the thermal characteristics of three species of *Nothofagus* ecological niche determined the seed's response to temperature. Seeds such as *D. drummondii*, *G. leakeanum*, *K. montana* and *V. foliosa* have wider niche breadths than *S. drummondii* or *A. echinocephala* (Cochrane et al., 2010). Species with a broad niche breadth will likely be less affected by future climate change, while seeds with a restricted niche breadth are the most vulnerable to climate change (Broennimann, 2006).

Chochrane et al. (2010) did not find data to support the hypothesis that germination niche breadth corresponded to breadth of geographic range as species with restricted or widespread geographic ranges did not have different levels of sensitivity to increased temperatures associated with climate change. However, Chochrane et al. (2010) found that species with narrow germination niches are more susceptible to environmental change. If temperatures increase slightly due to climate change, seeds of species with narrow niches will not germinate (Cochrane et al., 2010). Thuiller et al. (2005) found that plant species with a narrow niche breadth in terms of temperature were also expected to lose between 40 to 60% of their habitats. Marginal species in cold regions were predicted to be more sensitive to climate warming compared to marginal species in warm regions, which had been exposed to warmer temperatures and adapted previously (Thuiller et al., 2005).

HSP101 and HSP70 Levels Positively Correlated with Germination Thermotolerance Increase

Heat Shock Proteins 101 and 70 have been found to play a crucial role in thermotolerance during germination. HSP 101 renatures proteins aggregated by heat stress, while HSP 70 works as a chaperone and prevents aggregation while assisting in refolding of proteins denatured by heat shock (Nieto-Sotelo et al., 1999; Wang et al., 2003).

HSP101 has a high probability of adopting coiled-coil structures (Nieto-Sotelo et al., 1999). The function of these structures may be to form dimers or trimmers that could eventually assemble in the final hemohexameric particle (Nieto-Sotelo et al., 1999). A study by Hong and Vierling (2001) found that the timing of expression of HSP101 is likely during early germination. HSP101 was first linked to thermotolerance when it was found that HSP101 mRNA accumulates ten minutes following the onset of heat-shock treatment (Nieto-Sotelo et al., 1999). This accumulation of HSP101 mRNA could not be induced by any other stress treatments, such as cold treatment or increased osmotic pressure (Nieto-Sotelo et al., 1999).

Variation in HSP levels is positively associated with variation in thermotolerance (Zhang et al.,

2015). Queitsch et al. (2000) found that HSP101 is required for basal thermotolerance during germination. However, HSP 101 appears to be non-essential for normal seed development (Hong and Vierling, 2001; Quietsch et al., 2000). In the absence of severe heat stress, altered levels of HSP101 do not affect germination times or rates (Queitsch et al., 2000; Hong and Vierling, 2001). For instance, in a study conducted on cotton pollen at 23°C, there was no difference in germination for the transgenic and wild types, which had different levels of HSP101 (Burke and Chen, 2015). However, at temperatures of 39°C, both transgenic pollen and transgenic tobacco germinated at a significantly higher percentage than the null line (p-0.048 for transgenic pollen) (Burke and Chen 2015).

Higher levels of HSP101 are also linked to thermotolerance in *Arabidopsis.* When seeds were treated at 45 °C, higher HSP101 accumulation was associated with higher percent seedling survival, while plants with low HSP101 accumulation exhibited as low as 20% survival (Zhang et al., 2015). Burke and Chen (2015) conducted a study examining the results of AtHSP101 on heat tolerance of cotton pollen, finding that pollen in a control line had significantly reduced germination than pollen that was HSP101 transgenic.

HSP70 is another heat shock protein that is associated with increased thermotolerance during germination. A study conducted by Shufen et al. (2011) modified tomatoes with a codA gene, finding that transgenic seeds germinated faster and at a higher frequency than the wild type seeds. Glycinebetaine (GB) accumulated in transgenic plants but not in wild-type plants, and this accumulated GB enhanced the expression of heat-shock genes, thereby improving the tolerance of tomato seeds to high temperatures during germination (Shufen et al., 2011). Tomato plants with the cod-A gene had increased tolerance to high temperatures during germination, and there were higher levels of HSP70 produced by the transgenic seeds during heat shock (Shufen et al., 2011).

Summary

Responses to temperature changes are highly varied across plant species, populations, and location. This wide degree of variance means that climate change may increase or decrease germination based a plant's existing adaptations, evolutionary history, and ability to adapt rapidly to new conditions. However, there is an adaptation lag when seeds are exposed to new climatic changes, and due to the unprecedented rate at which climate change is occurring, many plants will not be able to adapt quickly enough to survive. The vast array of plant responses to climate change also means that it is impossible to know the exact results of climate change on seed germination in all species and populations.

In addition to the wide variety of responses to changing climatic conditions, climate change will also cause a variety of temperature changes in different locations. A majority of the reviewed studies measured plant responses to increased temperatures. However, climate change is shifting temperatures worldwide in a variety of ways, and will result in both increased and decreased temperatures depending on the location and season. Future studies should be conducted across a wider range of temperatures to gain a more accurate understanding of the effects of climate change on seed germination and plant survival.

In addition to climate change causing increased or decreased temperatures, there are also external factors that need to be considered in tandem. Temperature changes will affect snow cover, and may change the relationship between germinating seeds and other species, such as potentially infectious fungus or animals that consume the seeds. Although some of these factors have been considered and studied, others cannot be predicted, and climate change is likely to have many significant effects that will also increase and decrease seed germination rates, times, and percentages.

Plant survival in the face of climate change will have significant repercussions in terms of agriculture and food security. However, more studies have been conducted on model species, such as *Arabidopsis thaliana*, or on non-agricultural plants, rather than on plants typically consumed by humans. Due to the wide variety of factors involved and the relatively recent phenomenon of global climate change, impacts on food security remain to be fully understood. Future studies that examine the effects of increased temperatures on a wide variety of crop species will be especially relevant as climate warming increases.

The results of these studies will also raise questions concerning human response to the effects of climate change. Since some crop species will likely be unable to survive a rapidly changing climate, more people may become food insecure and rates of malnutrition are likely to increase. There are several possible solutions to increase crop yield in light of climate change. Once studies help demonstrate which species are most likely to survive, other, less adaptive plants can be genetically modified to be more resistant to climate change. However, these transgenic modifications are controversial due to the impact of genetically modified organisms on the environment and potential risks to human health. Another possible solution is to reduce the cultivation of plants that are unlikely to

survive climate change, and promote plants that are more likely to survive. However, this option poses problems of introducing new crops to other countries and cultures where they may not have been previously grown. Not only would these crops need to be viable across a wider range of environments, choice of food is also connected to culture, and people may be unwilling to switch to more adaptive plants. Overall, climate change and its potentially detrimental effects on seed germination will significantly impact food security and livelihoods throughout the world.

References

Akman Z. 2009. Comparison of High Temperature Tolerance in Maize, Rice and Sorghum Seeds by Plant Growth Regulators. *Journal of Animal and Veterinary Advances.* 8(2):358-361.

Arana M, Gonzalez-Polo M, Martinez-Meier A et al. 2015. Seed Dormancy Responses to Temperature Relate to Nothofagus Species Distribution and Determine Temporal Patterns of Germination across Altitudes in Patagonia. *New Phytologist.*

Bellard C, Bertelsmeier C, Leadley P, Thuiller W, Courchamp F. 2012. *Impacts of Climate Change on the Future of Biodiversity.* Ecology Letters. 15(4): 365-77.

Bita C, Geratas T. 2013. Plant Tolerance to High Temperature in a Changing Environment: Scientific Fundamentals and Production of Heat Stress-tolerant Crops. *Frontiers in Plant Science.* 4(273).

Broennimann O, Thuiller W, Hughes G, Midgley G, Alkemade J, Guisan A. 2006. Do Geographic Distribution, Niche Property and Life Form Explain Plants' Vulnerability to Global Change? *Global Change Biology.* 12(6): 1079-093.

Burke J, Chen J. 2015. Enhancement of Reproductive Heat Tolerance in Plants. *PLoS ONE.* 10(4).

Christensen J, Christensen O. 2007. A Summary of the PRUDENCE Model Projections of Changes in European Climate by the End of This Century. *Climatic Change.* 81(1):7-30.

Cochrane A, Daws M, Hay F. 2010. Seed-based Approach for Identifying Flora at Risk from Climate Warming. *Austral Ecology.* 36(8): 923-35.

Donohue K, Rubio De Casas R, Burghardt L, Kovach K, Willis C. 2010. Germination, Postgermination Adaptation, and Species Ecological Ranges. *Annual Review of Ecology, Evolution, and Systematics.* 41(1): 293-319.

Fernandez-Pascual E, Seal C, Pritchard H. 2015. Simulating the Germination Response to Diurnally Alternating Temperatures under Climate Change Scenarios: Comparative Studies on Carex Diandra Seeds. *Annals of Botany.* 115(2): 201-09.

Graae B, Alsos I, Ejrnaes R. 2008. The Impact of Temperature Regimes on Development, Dormancy Breaking and Germination of Dwarf Shrub Seeds from Arctic, Alpine and Boreal Sites. *Plant Ecol Plant Ecology.* 198(2): 275-84.

Halloy S, Mark A. 2003. Climate-Change Effects on Alpine Plant Biodiversity: A New Zealand Perspective on Quantifying the Threat. *Arctic, Antarctic, and Alpine Research.* 35(2):248-54.

Hong S, Vierling E. 2001. Hsp101 Is Necessary for Heat Tolerance but Dispensable for Development and Germination in the Absence of Stress. *The Plant Journal.* 27(1):25-35.

Johnston T, Chiotti Q. 2000. Climate Change and the Adaptability of Agriculture: A Review. *Journal of the Air & Waste Management Association.* 50(4): 563-69.

Jump A, Penuelas J. 2005. Running to Stand Still: Adaptation and the Response of Plants to Rapid Climate Change. *Ecology Letters.* 8(9): 1010-020.

Kronholm I, Picó F, Alonso-Blanco C, Goudet J, De Meaux J. 2012. Genetic Basis Of Adaptation In Arabidopsis Thaliana: Local Adaptation At The Seed Dormancy Qtl Dog1. *Evolution: International Journal of Organic Evolution.* 66(7): 2287-302.

Li S, Li F, Wang J et al. 2011. Glycinebetaine Enhances the Tolerance of Tomato Plants to High Temperature during Germination of Seeds and Growth of Seedlings. *Plant, Cell & Environment.* 34(11): 1931-943.

Milbau A, Graae B, Shevtsova A, Nijs I. 2009. Effects of a Warmer Climate on Seed Germination in the Subarctic. *Annals of Botany.* 104(2): 287-96.

Mondoni, A, Rossi G, Orsenigo S, Probert R. 2012. Climate Warming Could Shift the Timing of Seed Germination in Alpine Plants. *Annals of Botany.* 110(1): 155-64.

Montesinos-Navarro A, Picó F, Tonsor S. 2012. Clinal Variation In Seed Traits Influencing Life Cycle Timing In Arabidopsis Thaliana. *Evolution.* 66(11): 3417-431.

Mott J. 1972. Germination Studies on Some Annual Species from an Arid Region of Western Australia. *The Journal of Ecology.* 60(2): 293.

Nieto-Sotelo J, Kannan K, Martínez L, Segal C. 1999. Characterization of a Maize Heat-shock Protein 101 Gene, HSP101, Encoding a ClpB/Hsp100 Protein Homologue. *Gene.* 230(2): 187-95.

Ooi M, Auld T, Denham A. 2009. Climate Change and Bet-hedging: Interactions between Increased Soil Temperatures and Seed Bank Persistence. *Global Change Biology.* 15(10): 2375-386.

Queitsch C, Hong S-W, Vierling E, Linquist S. 2000. Heat Shock Protein 101 Plays Crucial Role in Thermotolerance in Arabidopsis. *Plant Cell.* 12(4): 479-92.

Root T, Price J, Hall K, Schneider S, Rosenzweig C, Pounds J. 2003. Fingerprints of Global Warming on Wild Animals and Plants. *Nature.* 421(6918): 57-60.

Smith S, Edmonds J, Hartin C, Mundra A, Calvin K. 2015. Near-term Acceleration in the Rate of Temperature Change. *Nature Climate Change.* 5(4): 333-36.

Thuiller W, Lavorel S, Araujo M, Sykes M, Prentice I. 2005. Climate Change Threats to Plant Diversity in Europe. *Proceedings of the National Academy of Sciences.* 102(23): 8245-250.

Thuiller W, Lavorel S, Araujo M. 2005. Niche Properties and Geographical Extent as Predictors of Species Sensitivity to Climate Change. *Global Ecology and Biogeography.* 14(4): 347-57.

Wang W, Vinocur B, Altman A. 2003. Plant Responses to Drought, Salinity and Extreme Temperatures: Towards Genetic Engineering for Stress Tolerance. *Planta.* 218(1): 1-14.

Wang W, Vinocur B, Shoseyov O, Altman A. 2004. Role of Plant Heat-shock Proteins and Molecular Chaperones in the Abiotic Stress Response. *Trends in Plant Science.* 9(5): 244-52.

Wen, Bin. 2015. Effects of High Temperature and Water Stress on Seed Germination of the Invasive Species Mexican Sunflower. *PLoS ONE.* 10(10):1-13.

Wilczek A, Cooper M, Korves T, Schmitt T. 2014. Lagging Adaptation to Warming Climate in Arabidopsis Thaliana. *Proceedings of the National Academy of Sciences.* 111(22): 906-913.

Zhang N, Belsterling B, Raszewski J, Tonsor S. 2015. Natural Populations of Arabidopsis Thaliana Differ in Seedling Responses to High Temperature Stress. *AoB Plants.* 7:1-13.

Technology Advances Valvular Replacement: A Tertiary Referral Center Experience -- Transcatheter Aortic Valve Replacement

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Transcatheter Aortic Valve Replacement (TAVR) is a less invasive alternative to surgical implantation of a bioprosthetic valve for native, calcified aortic stenosis in patients with multiple comorbidities considered not to be suitable candidates for surgery. The randomized Placement of Aortic Transcatheter Valves (PARTNER) study demonstrated a composite one year endpoint of death from any cause or repeat hospitalization of 42.5% with TAVR as compared with 71.6% with standard therapy. To assess clinical and demographic predictors of 30, 90, 180 and 365 day outcomes of TAVR patients treated through the highly structured Parkview Regional Medical Center Aortic Valve Clinic and receiving the Edwards Sapien device. A retrospective chart review of the first forty-one device implant patients between the dates of August 25, 2013 to June 30, 2015. The mean age of the TAVR patients was 82, with a mean STS (Society of Thoracic Surgery) score of 7.68. Implantation utilizing the transfemoral approach was favored (83.7%) over the direct aortic approach (16.3%). The 30-day mortality rate of 4.65%, was represented by two in-hospital deaths related to renal failure. There was a 12.2% overall 1-year mortality rate. There were no in-hospital, clinically significant, embolic strokes. No patients experienced acute myocardial infarction within the 1-year time frame. Post-operatively, no patients required aortic valve re-intervention with 11.6% requiring a pacemaker during the in-hospital timeframe. New onset atrial fibrillation developed in 4.76% of patients. The procedure was successful in the first 41 consecutive patients receiving a device based on post-procedural trans-aortic valve mean gradient by echocardiography. The in-hospital mortality rate of 4.78% and one year mortality of 12.2% compare favorably to the STS/ACC TVT Registry rates of 5.5% and 23.7% respectively. The PARTNER registry reports a lower mortality with the transfemoral approach and this approach was utilized in 83.7% of this study population. In-hospital deaths trended toward higher STS scores (average 11) as compared to those deaths outside of 30 days (average 4.7). Highly structured patient evaluations with attention to the STS pre-operative score will impact future patient selection and procedural success.

Introduction

Aortic stenosis (AS) is a pathophysiologic condition resulting in restricted mobility of the aortic valve leaflets during systole. Aortic valve disease accounts for 25,000 deaths annually; if left untreated, AS has an associated mortality rate of approximately 50% in the first two years after symptoms appear (Leon et al., 2010). Traditional surgical intervention of AS improve overall survival and lessen symptoms in surgically acceptable-risk patients as identified by a Society of Thoracic Surgeons Predicted Risk of Operative Mortality (STS PROM) score of less than 15.

Transcatheter aortic valve replacement (TAVR) is a less invasive alternative to surgical aortic valve replacement (SAVR) for high-risk patients. Included are those with severely diminished left ventricular function, advanced age, and comorbidities serving as technical limitations that increase risk of operative complications and mortality (Brecker et al., 2015; Smith et al., 2011). These surgically high risk patients are generally characterized by a STS PROM score greater than 15.

The TAVR procedure involves implanting a bioprosthetic valve through a catheter within the native, diseased aortic valve. Three approaches for access are available: transfemoral, direct aortic (requiring a mini-sternotomy), or transapical (requiring a small thoracotomy). The procedure's increasing popularity can be attributed to the randomized Placement of Aortic Transcatheter Valves (PARTNER) trial, which demonstrated improved survival rates and reduced symptoms in poor candidates for SAVR (Smith et al., 2011). Since then, various publications have arisen discussing the outcomes of TAVR post approval and in clinical practice compared to the randomized trials. Furthermore, there has been a need for studies to continue to research the long-terms outcomes of TAVR and the continuing success and efficacy for patients (Mack et al., 2013).

Due to technical improvements and increased operator experience, transcatheter aortic valve implantation success rate continues to grow. It has been noted that at 30 days post-TAVR, the reintervention rate is 0.5% (Holmes et al., 2015). Nevertheless, post procedural recovery in these high-risk patients is variable due to multiple comorbidities, and is frequently prolonged. The aim of this study is to evaluate clinical and demographic predictors of 30, 90, 180, and 365 day outcomes for patients receiving the TAVR procedure at the Parkview Heart Institute (PHI) in Fort Wayne, Indiana.

Methods

The initial patient population screened included 188 patients with aortic stenosis evaluated in the Aortic Valve Clinic within the Parkview Heart Institute in Fort Wayne, Indiana between August 2013 and July 2015. Inclusion criteria were patients with severe senile calcific aortic stenosis receiving an Edwards Sapien device. Those excluded from the study were those treated with traditional surgical intervention or medical management.

A two-year retrospective chart review was conducted on 41 patients meeting inclusion criteria, noting patient specific characteristics including demographics and baseline comorbidities: history of hypertension, chronic obstructive pulmonary disease (COPD), reduced ejection fraction (<40%), coronary artery disease (CAD), peripheral arterial disease (PAD), diabetes mellitus, chronic renal failure (CRF) (creatinine >1.5 mg/dL), atrial fibrillation (AFIB), previous pacemaker or implantable cardioverter defibrillator (ICD), congestive heart failure (CHF), New York Heart Association (NYHA) class, history of coronary artery bypass grafting (CABG), elevated systolic pulmonary artery pressure (>60 mmHg), and abnormal aortic valve gradient (>20 mmHg). Adverse events, including stroke, transient ischemic attacks (TIA), and cardiovascular related death, were noted at 30, 90, 180, and 365 day time intervals.

Collected data was then analyzed and compared to patient specific data from the STS/ACC TVT national registry. Comparative statistical analysis was completed, highlighting significant variance between data sets, utilizing the two-tailed Fisher's exact test, unpaired T tests, and Chi-square with Yates' correction tests.

Results

Procedural outcomes

The majority of patients, 83%, were accessed femorally, with 17.1% utilizing the direct aortic approach; there were no patients accessed apically (Table 1). No post procedural stroke was noted in the direct access cohort, of which 28.6% had prior CABG. One patient accessed directly had a valve-in-valve procedure. One procedure was aborted secondary to inadequate access and this patient was not included in the analysis. Prior to the procedure, 19.5% (8) patients had a pacemaker/ICD; 12.2% (5) of patients developed a conduction abnormality requiring a pacemaker/ICD following valve placement. Post procedurally, inotropic support was required for 4.9%. The in-hospital death rate was 4.9% (2) as a result of renal failure. Moderate to severe aortic regurgitation was noted in 11.8% (5) patients post procedurally with trace regurgitation noted in 17.1%. Hemoglobin levels dropped, on average, from 11.949 to 9.512 during the procedure; however, no patients experienced significant blood loss requiring transfusion.

Age, mean (SD)	82(7.14)
Sex, n (%)	
Male	25 (61)
Female	16 (39)
Race, (%)	
White	100%
Black	0%
Asian	0%
Other	0%
STS PROM, (%)	
Mean (SD)	7.7 (4.78)
Median (IQR)	6.3 (3.87-9.75)
<8	25 (61)
8-15	12 (29.3)
>15	4 (9.8)
History of Congestive Heart Failure, n (%)	
NYHA Class III/IV	89 (84.0)
Diastolic Dysfunction	22 (73.3)
Systolic Dysfunction	8 (26.7)
History of Hypertension, n (%)	40 (97.6)
History of Diabetes Mellitus, n (%)	20 (48.8)
History of CAD, n (%)	36 (87.8)
Previous CABG, n (%)	16 (39.0)
History of Stroke, n (%)	4 (9.8)
LVEF, %	
Mean (SD)	57.0 (11.84)
Median (IQR)	60 (55-65)
<30	1
30-45	7
>45	33
Atrial Fibrillation, n (%)	18 (43.9)
Serum Creatinine, mg/dL	
Mean (SD)	1.35 (1.06)
Median (IQR)	1.2 (0.9-1.45)
Chronic Renal Failure (>1.5)	10 (24.4)
COPD, n (%)	15 (36.6)
Permanent Pacemaker/ICD, n (%)	8 (19.5)
Pre-TAVR Mitral Insufficiency	
None/Trivial/Mild	11 (26.8)
Moderate	19 (46.3)
Severe	11 (26.8)
Access Site	
Transfemoral	34 (82.9)
Transapical	0 (0)
Direct Aortic	7 (17.1)

Table 1. Outlines clinical and demographic characteristics of the patient population (n=41) of which, 61% were white males, 39% were white females. The mean age was 82 ±7.14 with an average STS PROM score of 7.68±4.78. Comorbidities included: history of CAD in 87.8%, CHF in 84%, atrial fibrillation in 43.9%, CRF in 24.4%, history of PAD in 39.0%, moderate to severe mitral insufficiency in 73.1%, history of hypertension in 97.6%, COPD in 36.6%, and prior stroke in 9.8%. Abbreviations: SD, standard deviation; IQR, Interquartile range; CAD, Coronary Artery Disease; PAD, Peripheral Arterial Disease; LVEF, Left Ventricular Ejection Fraction; CVA, Cerebral Vascular Accident; AV, Aortic Valve; HGB, Hemoglobin.

30-day Outcomes

Post-TAVR, 24.4% (10) of patients had elevated levels of creatinine (>1.5mg/dL). The mean AV gradient decreased by 73.986% from pre and post procedural values of 46.03 mmHg to 10.95 mmHg.

Of the 46.3% (19) with AFIB pre-TAVR, 10.5% (2) did not present symptoms post procedurally; 9.1% (2) of the patients who did not have AFIB pre-TAVR developed it post procedurally. Severe aortic regurgitation was present in 2.4% (1) of patients at 30 days, with 24.4% (10) and 4.9% (2) experiencing less than mild and mild to moderate aortic regurgitation respectively.

Outcomes to 1-Year

There were a total of 5 deaths within a year of the procedure, with 2 deaths occurring within the 30-day time period. No deaths were a result of aortic valve complications; no patients required aortic re-interventions following their procedure. One patient (2.4%) experienced a hemorrhagic CVA within the year time frame. There were no embolic CVA's. Of the 9.8% (4) of patients with history of stroke, none experienced a CVA after their procedure.

Discussion

AV gradient

The AV gradient is an important metric in determining the severity of aortic stenosis in patients. Device implantation success is defined as a mean AV gradient <20 mmHg post-procedurally (Mack et al., 2013). In a national study, device implantation success was achieved in 92% of the 7710 cases (Mack et al., 2013). A 100% (41/41) device implantation success rate was noted for TAVR patients within the Parkview Heart Institute. This 8.0% difference in success rates was statistically significant (P=0.0461).

Two patients had low-flow, low-gradient aortic stenosis. Low-flow, low-gradient occurs when the degree of aortic stenosis is not made clear when analyzing the AV gradient due to the relatively low output syndrome commonly in the setting of cardiomyopathy. Two patients given a Dobutrex stress test experienced an 8 mmHg increased gradient from the average baseline gradient of 24 mmHg. The average pre-procedural LVEF of the two patients was 40.5%, significantly lower than the 58.0% average of the rest of the patients (P=0.0357). The average post-procedural LVEF for the two patients was 50.5; however, this value was insignificantly lower than the average post-procedural LVEF (61.957) of the other patients (P=0.2294). The average STS PROM for the low-flow, low-gradient patients was 3.15%, insignificantly lower than the total population average of 7.68% (P=0.1920). Other than the significantly different pre-procedural LVEF, the low-flow, low-gradient patients did not differ significantly in any other major category when compared to the rest of the patient population.

AFIB

In a national study, 40.8% (3148) of patients had pre-procedural AFIB[3], insignificantly differing from the 46.3% (19) patients who experienced AFIB pre-procedurally in the present study (P=0.6448). In the national study, new onset AFIB was found in 5.97% (460) patients. When compared to the 4.87% (2) of Parkview patients who experienced new onset AFIB, the difference is insignificant (P=1.0). Of the patients who were found to have AFIB postoperatively, all were anticoagulated with Warfarin. None of these patients evidenced a postoperative embolic event.

Stroke

By the 30-day mark after the procedure, 2.4% (1) of patients had experienced a hemorrhagic CVA while on Warfarin. Within a year following the procedure (30 days-1 year) 0% (0) of patients had experienced a CVA. When compared to the 2.02% (156) who experienced a postoperative stroke by the 1-year mark in the national study (Mack et al., 2013), there is an insignificant difference (P=1.0).

The percentage of Parkview patients who had experienced a CVA pre-procedurally was 9.8% (4). None of these patients, however, experienced a stroke postoperatively. Compared to the 13.02% (1004) of patients in the national registry had previously experienced a stroke, the difference is insignificant (P=0.6505).

Access Site

The retrograde transapical approach is associated with a 2-fold increased risk of mortality from heart failure when compared to mortality rates related to the antegrade transfemoral approach (Urena et al., 2015). Within the Parkview Heart Institute, no transapical procedures were completed in the first 41 consecutive patients, significantly less than the 29% accessed apically from national data (P<0.0001) (Mack et al., 2013) (Figure 1). This finding is supplemented by the lack of heart failure related deaths in the one year time frame in all patients studied. Parkview Heart Institute completed a significantly greater number of transfemoral procedures (83%) when compared to STS/ACC TVT registry data (64%) (P=0.0135) (Holmes et al., 2015) (Figure 1). The direct aortic approach was utilized in 17% (7) of patients, insignificantly greater than the nationally noted 7% (P=0.0788). Within the direct aortic cohort, 28.6% had history of CABG and none experienced adverse events within the one-year timeframe. This finding is consistent with the fact that CABG does not predict adverse outcomes in TAVR patients (Ducrocq et al., 2012). The in-hospital death rate of 4.9% (2) could not be correlated to access site; however, these deaths were

associated with a greater STS PROM score (average of 10.95) than those deaths beyond the 30 day timeframe (average STS PROM of 4.73) (Figure 2). Mortality rates associated with the transfemoral approach was 11.8%, highlighting an insignificant difference to the nationally noted 10.7% (P=0.6427); 14.3% direct aortic mortality also demonstrated an insignificant difference to the 15.3% listed nationally (P=1.000).

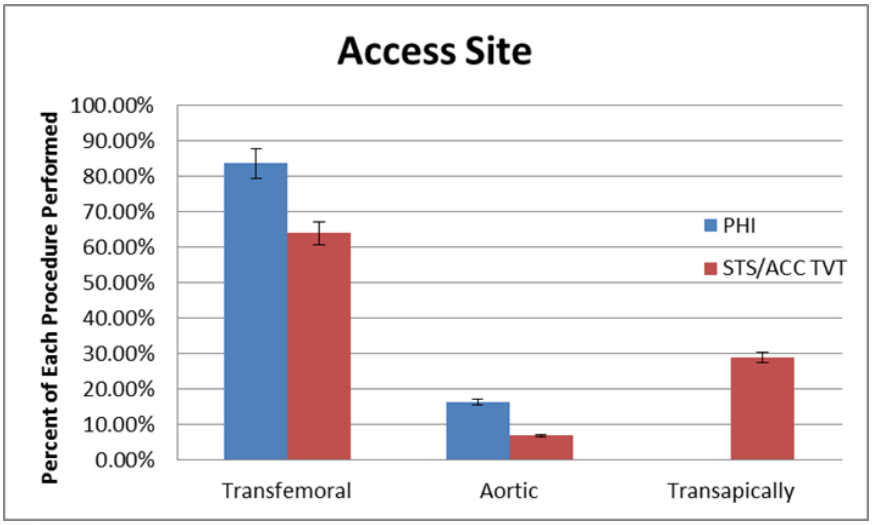

Figure 1. A comparison of access sites utilized to national STS/ACC TVT Registry data. The Parkview Heart Institute data performed a greater number of transfemoral and direct aortic procedures and no transapical procedures. All differences are statistically insignificant (P>0.050).

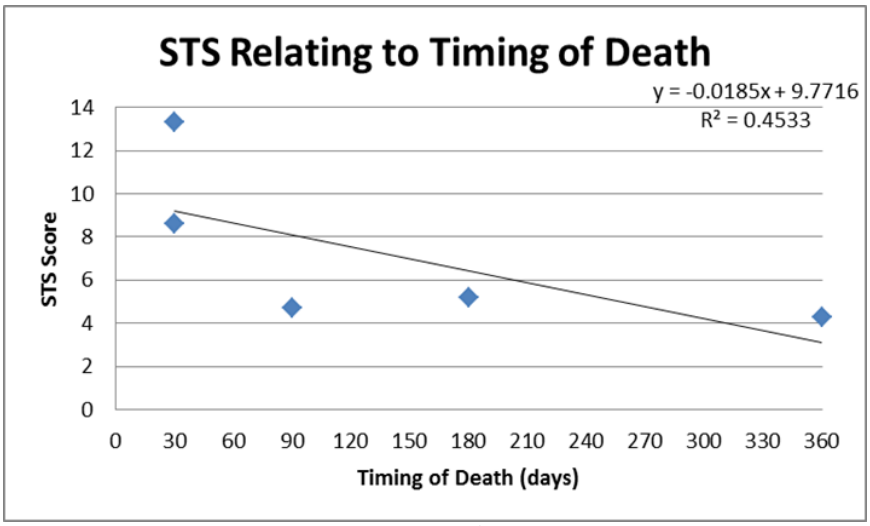

Figure 2. Delineates the relationship between STS PROM score and timing of all postoperative deaths noted. Those within the 30-day mark had an average STS PROM of 10.95 while those beyond the 30-day timeframe had an average STS PROM of 4.73.

Pacemaker/ICD

In the patient population, 19.5% had a preoperative permanent pacemaker or ICD, nearly equivalent with the registry's data of 19% with a preoperative device (Mack et al., 2013). Patients requiring a postoperative pacemaker or ICD without a preoperative device accounted for 12.2% (5) of the population, insignificantly differing to the 6.6% in the national registry (P=1.000). All patients that developed an arrhythmia postoperatively were accessed transfemorally. Of these patients that developed an arrhythmia, 100% were accessed femorally when compared to the registry noting 59.1% accessed femorally (P=0.0843). The consistency of the data from the first 41 consecutive patients with the national registry demonstrates excellent patient selection for TAVR recipients.

Mortality rate

The Parkview Heart Institute noted lower in-hospital, 30 day, and one-year mortality rates compared to national registry data (Figure 3). The in-

hospital death rate and 30-day mortality rate were noted at 4.65% (2) secondary to renal failure; this figure is insignificantly less than both the 5.5% in-hospital and 7.6% 30 day mortality rates listed in the national registry (Mack et al., 2013) (P=1.000). For the present study, the 12.2% (5) one year mortality rate was less than the 23.7% noted by the STS/ACC TVT registry (Holmes et al., 2015); none of the five deaths were related to stroke or cardiac causes, nationally noted to have an incidence rate of 3.1% and 5.8% respectively. Approximately two thirds, 60%, of all deaths in one year were related to renal failure while 40% were a result of natural causes. The noted lower mortality rate can be attributed to excellent patient selection, and application of well-organized care at Parkview Heart Institute.

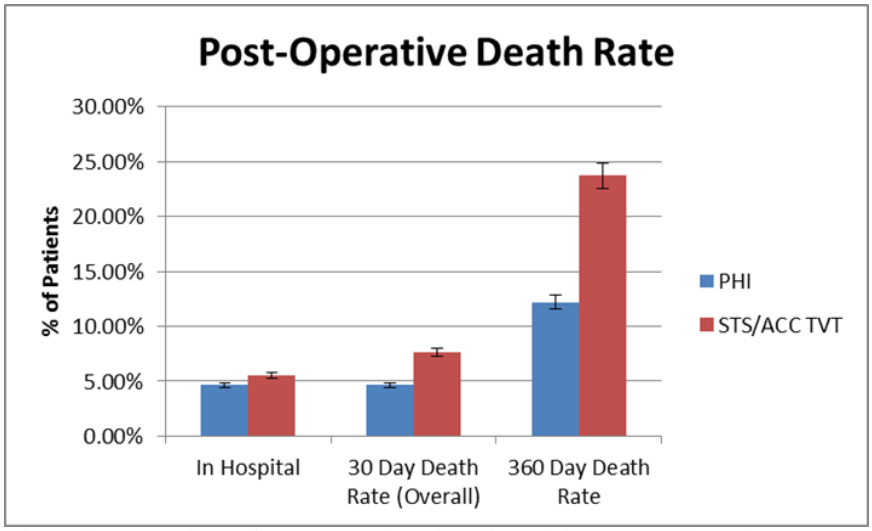

Figure 3. A comparison of mortality rates to the STS/ACC TVT Registry data. The Parkview Heart Institute's in-hospital, 30-day, and 360 day mortality rates were lower and those noted in the registry. All differences are statistically insignificant (P>0.050).

Serum Creatinine

It was noted that 17.1% (7) of the patient population (greater than or equal to) Stage III CKD with a pre-procedural serum creatinine value greater than 1.5 mg/dL. Of this cohort, 14.3% (1) patient died within the one-year time frame accounting for 20% of the total deaths. Of those who died within the one year time frame, the average pre and post-procedural serum creatinine was 1.3 and 1.8 respectively. This indicates that the benchmark of a pre-procedural creatinine of 1.5 was not an indicator of mortality while a post-procedural creatinine of greater that 1.5 was a strong indicator of mortality. No patients of the present study reached dialysis-dependent renal failure, which was less than the 1.9% noted by the national registry. One patient was on dialysis with a creatinine of 7.5 mg/dL before TAVR and had no adverse events up to the 90 day mark, no further information is available for the 180 and 360 day follow-up.

Conclusion

This population compared favorably with the demographics of the STS/ACC TVT registry. The procedure was successful in 100.0% of the first 41 consecutive patients receiving a device (based on post-procedural trans-aortic mean gradient <20 mmHg), with an in-hospital mortality rate of 4.9% and one year mortality of 12.2% comparing favorably to the STS/ACC TVT Registry rates of 5.5% and 23.7% respectively. In-hospital deaths trended toward higher STS PROM scores. With an average of 11.0 compared to those outside 30 days, which averaged 4.7; this should impact patient selection. Transfemoral approach was preferred in this population (83.7%) and is supported by registry outcomes data showing reduced morbidity with this route. We believe that a structured aortic valve clinic with dedicated personnel, protocol driven patient evaluations and follow-ups, and emphasis on physician education is critical to obtain excellent outcomes.

References

Brecker, S, Gaasch, W, Aldea, G. 2015. Transcatheter aortic valve replacement: Indications and periprocedural management. *UpToDate*. Retrieved from <http://www.uptodate.com/contents/transcatheter-aortic-valve-replacement-indications-and-periprocedural-management?topicKey=CARD/83110>.

Ducrocq G, Al-Attar N, Himbert D, Messika-Zeitoun D, Iung B, Descoutures F, Nataf P, Vahanian A. 2012. Early and Mid-term Outcomes in Patients Undergoing Transcatheter Aortic Valve Implantation after Previous Coronary Artery Bypass Grafting. *Eur J Cardiothorac Surg*. 9:499–504.

Goldbarg S, Elmariah S, Miller M, Fuster V. 2007. Insights into degenerative aortic valve disease. *J Am Coll Cardiol.* 50(13):1205-13.

Guyton R. 2012. The Placement of Aortic Transcatheter Valve (PARTNER) trial. *Circulation.125:3237*-3239. doi: 10.1161/CIRCULATIONAHA.112.093096.

Heart and Vascular Team. 2014. TAVR Procedure Safe for Elderly with Aortic Stenosis. *Cleveland Clinic.* Retrieved from <http://health.clevelandclinic.org/2014/10/tavr-procedure-safe-for-elderly-with-aortic-stenosis/>.

Holmes D, Brennan Jr. J, Rumsfeld J, et al. 2015. Clinical Outcomes at 1 Year Following Transcatheter Aortic Valve Replacement. *American medical Association JAMA.* 313(10):1019-1028. doi:10.1001/jama.2015.1474.

Leon M, Smith C, Mack M, et al. 2010. Transcatheter Aortic-Valve Implantation for Aortic Stenosis in Patients Who Cannot Undergo Surgery. *The New England Journal of Medicine.* 363(17), 1597-1607. doi: 10.1056/NEJMoa1008232.

Mack M, Brennan J, Brindis R, et al. 2013. Outcome Following Transcatheter Aortic Valve Replacement in the United States. *JAMA.* 310(19):2069-2077. doi:10.1001/jama.2013.282043.

Pibarot P, Dumesnil J. 2012. Low Flow, Low Gradient Aortic Stenosis in Normal and Depressed Left Ventricular Ejection Fraction. *J Am Coll Cardiol.*60:1845-1853.

Smith C, Leon M, Mack M, et al. 2011. Transcatheter versus Surgical Aortic-Valve Replacement in High-Risk Patients. *The New England Journal of Medicine.* 364(23), 2187-2198. doi:10.1056/NEJMoa1103510

Urena M, Webb J, Eltchaninoff H, et al. 2015. Late Cardiac Death in Patients Undergoing Transcatheter Aortic Valve Replacement: Incidence and Predictors of Advanced Heart Failure and Sudden Cardiac Death. *J Am Coll Cardiol.* 65(5):437-448.

Coral Reefs are the Hotspots for Fish Biodiversity

Alex Boss

Coral reefs make up less than 1% of habitable locations in the ocean for species of life, making them a rich hotspot for species diversity but also deserving of human conservation efforts. Scientists who have studied the evolutionary history of coral reefs pose two thoeries on how coral reef fish biodiversity came to populate reefs (1) biodiversity of coral reef fish formed immediately with the formation of coral reefs or (2) founder populations of non-coral reef fish flourished in coral reefs through coevolution alongside coral reefs allowing these species to adapt to coral reef environments over the course of multiple generations. One such study conducted by Dr. Price and colleagues reveals a new possible mechanism behind coral reefs promoting biodiversity in fish species. The study focuses mainly on the Labridae family of fish as the model organism and data reveals three possible mechanisms for coral reefs impacts on fish biodiversity (1) variety of potential prey (2) niche partitioning (3) coral reef fish differentiate faster than non-coral reef fish. Further research is required in this area to determine other possible reasons behind the rich biodiversity in coral reefs and what other species of reef fish are impacted. Results from this study and others should be used to further human conservation efforts to protect reef systems in order to protect biodiversity of its surrounding community.

Introduction

Recent analysis of coral reef studies has shown that there is a correlation between coral reefs and the morphological diversity in coral reef fish compared to non-coral reef fish. Dr. Samantha Price and her colleagues at the University of California, Davis researched this new breakthrough, as described in their recently published journal article (Price at al., 2011). On the reason for the interest in finding a correlation between coral reefs and fish biodiversity, Dr. Price stated, "People have already looked at speciation in coral reefs. Scientists have used phylogenies for comparison but nobody had gone further to look at the morphology and the ecology. These habitats within coral reefs promote diversification, which means if coral reefs disappear this could risk losing evolution in fish species in the future." To understand the correlation between these two intersecting components, comprehending the role of coral reef systems is necessary.

Coral reefs play an important role in the marine biome by serving as hotspots and protectors for diverse species of fish through the ecological niches they establish. Hotspots are defined as biogeographic regions with huge pools of significant biodiversity and can also play a significant role in species endemism, where a species is designated to a specific geographic location (Price et al., 2011). Unfortunately they are under threat from humans making them extremely important since coral reefs make up less than 1% of all habitable locations in the ocean (Price et al., 2011). For coral reef fish, species endemism is designated by the specific geographic locations of coral reefs. They are majority wide spread along the Indo-Pacific gradient, which consists of Papua New Guinea, Great Barrier Reef of Australia and the French Polynesia. Coral reefs are also endemic to the Red Sea and the Caribbean, which includes the Bahamas, Caribbean Sea and other locations in the Atlantic Ocean (Price et al., 2011; Holbrook et al., 2015).

However, despite the small overall space coral reefs inhabit, they are shown to hold a variety of diversity amongst different species of fish. Out of the top 11 biggest regions of coral reefs in the ocean 10 of the richest reefs hold up to 44 to 54% of marine species that are restricted by a certain range (Price et al., 2011). Further, because coral reefs promote such species richness amongst marine creatures, they also hold the greatest diversity amongst fish for the entire ocean, accounting for up to 1000 different species (Price et al., 2011). Coral reefs are also the center for speciation of a variety of fish and other marine creatures - by examining the evolution of coral reefs they are shown to export diversity from their habitats to other areas of the marine ecosystem. These coral reef habitats are rich with diversity in part thanks to the increase of prey and nutrients coral reefs gather from the surrounding ocean. Within a comparison of coral reef fish to non-coral reef fish, speciation has been shown to be higher and spread faster in coral reef fish groups compared to non-coral reef fish. This is exemplified in Dr. Price and colleagues' study on the Labridae fish family within selective coral reefs. Greater morphological diversity is shown in jaw sizes of species within this family from their results gathered (Price et al., 2011).

Figure 1. Four of the largest families of fish biodiversity. **(**Top left**)** The Pomacentridae family. **(**Top right**)** The Apogonidae family. **(**Bottom left**)** The Labridae family. **(**Bottom Right**)** the Chaetodontidae family.

The types of coral reef fish species with an increase in morphological diversity that have been analyzed are from many different groups. These include some species from the Labridae, Chaetodontidae, Pomacentridae and Apogonidae family of fish and rank as the highest within species rich families (Cowman and Bellwood, 2011). The Labridae family consists of the wrasses, which includes mostly brightly colored fish and ranges to around 500 species in different coral reef environments within the Indo-Pacific gradient. Most of these fish are smaller in size and include examples such as the Humphead Wrasse and the Yellowtail Coris Wrasse, which are found in the Great Barrier Reef and off the coast of Hawaii (Jonna, 2003). The Chaetodontidae family consists of Butterflyfishes, which are a type of tropical marine fish. Examples include the Copperband Butterflyfish found in the Indo-Pacific gradient and the Bluelashed Butterflyfish found from east Africa all the way Pitcairn islands north of Japan (Jonna, 2003; Animal-World). The Pomacentridae family of fish is a type of Perciform fish, which stands for "perch-like" and consist of around 335 species. Examples from the Pomacentridae family include the Damselfishes and Anemonefishes, which both can be found in the Indo-West Pacific and from the Philippines to Australia (Jonna, 2003). The Apogonidae family, also known as the Cardinalfishes, is nocturnal and lives in dark crevices within coral reefs. They consist of around 350 species with about 130 of these species living in Australian waters and include examples such as the Banggai Cardinalfish and the Large-toothed Cardinalfish (Bray and Gomon, 2012). Many more types of species rich families of fish exist, which will not be focused on. These four families out of many other species of fish display the greatest diversity and species richness (Price et al., 2011).

Coral Reef Evolution and Origins of Associated Fish Species

Understanding the correlation between coral reef systems and fish biodiversity is important, but in order to delve deeper into the mechanism behind this correlation, we first must know how coral reef systems were first established. Therefore, two

specific questions scientists have emerged. One, whether it is possible that the biodiversity of coral reef fish were formed immediately with the formation of coral reefs or two, did founder populations of non-coral reef fish species flourish in coral reefs through coevolution alongside coral reef formation, therefore allowing these species to adapt to the coral reef environment over multiple generations (Briggs, 2004). A meta-analysis of multiple research studies was done leading to different hypotheses about the origins of coral reefs and their associated fish species.

For understanding coral reef George D. Stanley describes the evolutionary history of coral reef formation (Stanley, 2006). Corals provide an example of photosymbiosis, a process in which symbionts, photosynthetic organisms, in this case live inside a host (coral) (Stanley, 2006). Often, there is a mutualistic relationship between the two; corals, with the nutrients derived from their photosynthetic suppliers, build bigger coral systems (Stanley, 2006). From here corals, algae and other calcifying organisms extract a specific compound, calcium carbonate, from the surrounding seawater to build massive skeletons we see as modern reefs today (Stanley, 2006). These huge coral reefs create massive hotspots of species richness and are much more rich compared to the rest of the ocean (Stanley, 2006).

Coral reefs then expand rapidly with help from a type of photosynthetic algae called zooxanthellae, which lives inside coral (Stanley, 2006). Zooxanthellae uptake the nitrogenous and carbon monoxide wastes from the coral and use their photosynthesis process to release carbon back into the coral making themselves the major source of nutrition and waste management for coral (Stanley, 2006). Because of this relationship that zooxanthellae have with coral, the area of coral growth is limited to shallow waters close to sunlight, where zooxanthellae can thrive, making coral structure over time to be modified to accommodate more sunlight (Stanley, 2006). The problem then becomes how to decipher how long coral reefs have been around since the symbionts in corals are not preserved. In order to measure the coevolution between the photosynthetic algae and corals, scientists use the photosymbiosis process. This is accomplished by using carbon and oxygen isotopes since coral reefs do not leave a fossil record behind like mammals. Using this method, scientists have been able to estimate when corals and photosynthetic algae coevolved together to form the modern day coral reef system (Stanley, 2006).

Living corals and non-reef building corals existed about 250 million years ago before the Permian mass extinction event wiped out almost all corals (Stanley, 2006). This cataclysmic extinction event nearly destroyed 96% of the earth's biodiversity due to climatic changes followed by a large event, possibly the vast eruption of volcanoes. 8-10 million years later scleractinians formed, which would later serve as the living corals to build modern day reefs (Stanley, 2006). During the Triassic period, which followed immediately after the Permian mass extinction, corals began to diversify given the fast turnover of biotic life and adaptive radiation (Stanley, 2006). This allowed corals to diversify into different forms filling other ecological niches. Scientists estimate living corals and photosynthetic algae coevolved together using the carbon and oxygen isotope methods with data analysis around the same period (Stanley, 2006). Additionally, other mass extinctions, such as the Triassic mass extinction, caused coral reef eclipses and more biotic turnover, which led to the modern day reef ecosystems by the end of the Neogene, 1.6 million years ago (Stanley, 2006; Cowman and Bellwood, 2011). During this time period, coral reefs were adapting, and stronger surviving symbionts, such as the zooxanthellae, were replacing less favorable ones (Stanley, 2006). Stanley and other researchers both attest the success and adaptability of the symbionts leading to modern day ones with our coral reef systems to be associated with the corals geographic expansion against different climatic changes (Stanley, 2006). Further research, though, is necessary for understanding the exact moment of coevolution between corals and photosynthetic algae and the other processes that led to photosymbiosis and our modern day coral reefs.

Coral reef evolutionary history is imperative, especially when understanding the origins of evolution of coral reef fish. The question then is whether coral reef fish co-evolved with coral reefs or whether non-coral reef species of fish started to inhabit coral reef systems later adapting to the new ecological niches offered (Cowman and Bellwood, 2011). When asked this question concerning the origins of coral reef fish, Price et al. (2011) stated, "For all species of fish its undecided if coral reef fish originated from a non-coral reef line." Price et al.'s research has primarily focused on the Labridae family of fish and within this family, "fossils found reveal that the environment these fish lived in was not specifically near a reef" (2011). Their research indicated that the Labrids, the ancestors of this family of fish, did not originate near coral reefs (Price et al., 2011). "Our data using models suggests Labrids evolved in different habitats and then arrived in the coral reef habitat," says Price et al. (2011), "and there were two periods, 66 million years ago and one other, where increased movements were shown from Labrids onto these coral reefs." The modern day coral reef was formed around the Neogene period, which

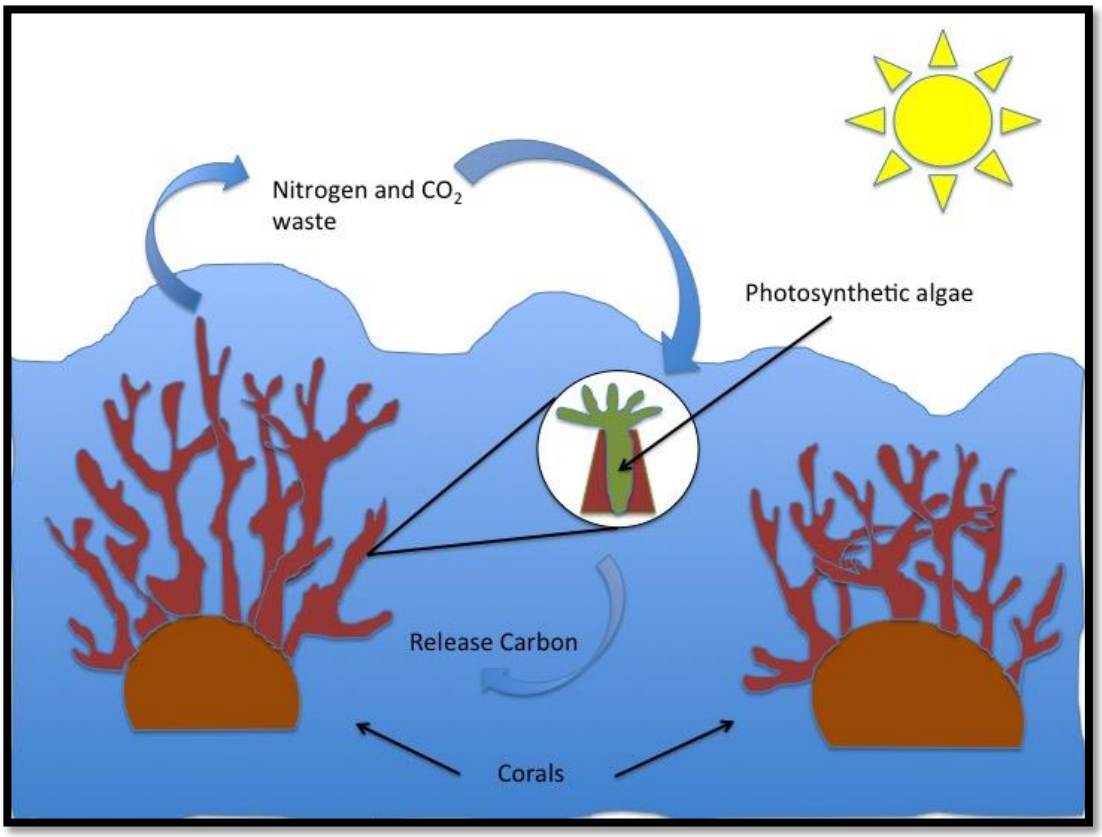

Figure 2. Diagram of photosymbiosis process demonstrated in living corals and photosynthetic algae. Corals and photosynthetic algae have a beneficial mutualistic relationship, where photosynthetic algae consumes the waste products from corals while also providing carbon ,a resource of energy, to them.

lasted from 23 to 1.6 million years ago (Stanley, 2006).

Although it has been challenging to prove the coevolution of corals and fish, Cowman and Bellwood present information which lays the foundation to find more evidence to prove these hypotheses of coevolution(Stanley, 2006). Cowman and Bellwood (2011) indicate that there is evidence showing strong correlations between the living corals, scleractinian, and the early ancestors of modern coral reef fish groups. The Permian mass extinction that wiped out nearly all-living corals played a significant role in the diversification of reef lineage fish (Cowman & Bellwood, 2011). Coral reefs expanded after this mass extinction event during the Triassic period right concurrently with these reef fish lineages (Cowman & Bellwood, 2011). Even more impressive is that around the time corals and symbionts were evolving to form our modern day coral reef systems during the Neogene period, Cowman and Bellwood (2011) were able to show similar expansion within their coral reef fish studied. They looked at three other families besides the Labrids: the Chaetodontidae, Pomacentridae, and Apogonidae (Cowman & Bellwood, 2011). For the Labridae, Chaetodontidae and Pomacentridae families of fish, all three showed increased radiation after the Permian mass extinction event (Cowman & Bellwood, 2011). During the Neogene time period these same four families of fish showed an increase in cladogenesis (Cowman & Bellwood, 2011).

This means that new formations of these fish families were forming and diverging from their original ancestral form. New coral reef habitats are known to have formed during this time, showing potential correlation between new reef fish lineages being established with formation of modern day coral reefs. Even though the origin coral reef fish formation has not been answered, the evidence available supports the hypothesis that modern coral reef fish were most likely established from a non-coral reef lineage but possibly coevolved with coral reef expansion.

Mechanism

Even if coral reefs did not show an increase in fish biodiversity, molecular evidence and data gathered from past research would show a correlation between coral reef expansion and growth of reef fish lineages. Thankfully, new research has been

conducted to support the statement that coral reefs promote fish biodiversity (Price et al., 2011). The model organism was the Labridae family of fish species analyzed from three big coral reef geographic locations, the Caribbean, Indo-Pacific gradient and the Red Sea (Price et al., 2011). The research revealed three significant factors contributing to morphological diversity in Labrids: variety of potential prey, niche partitioning, and the fact that coral reef fish differentiate faster than non-coral reef fish (Price et al., 2011; Messmer et al., 2011).

Establishment of coral reefs also brings along different ecological niches or positions organisms take within the community (Price et al., 2011). Different niches allows for a variety of potential prey to be available for fish species within the Labrid family. As the potential prey diversifies, so do the morphological aspects of the fish species in order to have an advantage over the prey (Price et al., 2011). Therefore, by looking at phylogenies, which are the evolutionary history of the developments and diversifications of a group of organisms, Price et al., noticed the vast diversification in jaw sizes for the fish species with the Labrid family (2011). Having a variety of potential prey led to the diversity and novelty of feeding methods demonstrated in the morphological diversity of the Labrids (Price et al., 2011). Coral reefs, as mentioned, contain diverse environments or niches for a variety of organisms (Price et al., 2011). Different ecological niches allow for diverse species of fish to breed and diverge into new lineages (Price et al., 2011). Another concept explaining further fish biodiversity is niche partitioning, which also allows for species co-existence (Price et al., 2011). Because coral reefs are very complex habitats and are home to a variety of species, niche partitioning can occur given the broad spectrum of prey for fish species to feed on (Messmer et al., 2011).

This is demonstrated with the groups, *Cheilinus undulatus* (Humphead Wrasse) and *Wetmorella nigropinnata* (Yellow Banded Possum Wrasse) (Price et al., 2011). These two groups prey on invertebrates such as mollusks, sea urchins and shrimp (Jonna, 2003). Because of the different diets each species preys upon, jaw sizes have modified to accommodate each species specific prey (Price et al., 2011). *C. undulatus* is bigger in size and has stronger jaw strength to capture prey with harder shells while *W. nigropinnata* is smaller in size and has less jaw strength to capture softer shelled prey (Price et al., 2011). These varying diets requires each species to look in specific niches for their desired prey, allowing for niche partitioning to occur. Because each species inhabits a different, they can co-exist peacefully instead of competing with each other over the same prey in the same niche (Price et al., 2011).

Variety of potential prey and niche partitioning contribute to coral reef fish differentiating faster than non-coral reef fish, which promotes diversification in fish biodiversity (Messmer et al., 2011). Because coral reefs are such complex habitats, each hosts a variety of niches and, therefore, diverse prey. Since niche partitioning is prevalent and is shown to support different fish assemblages along with habitat diversity, coral reef fishes' environment results in faster speciation compared to non-coral reef fish (Messmer et al., 2011). This was shown to be true in Price et al. (2011) research with the Labrid family. Species within the Labrid family contain coral reef fish and non-coral reef fish; therefore, this made the Labrid family a model organism for comparison between coral and non-coral reef fish speciation (Price et al., 2011). A phylogeny of the Labrid family shows that with coral reef species within this family greater morphological diversity was seen in jaw sizes compared to non-coral reef species (Price et al., 2011).

Conclusions

Despite the identification of these mechanisms, there are still other viable possibilities that explain why coral reefs promote extraordinary fish biodiversity. More research is necessary starting with smaller study groups, looking at different species of fish, and even branching out to other marine life within coral reefs. The fact that coral reefs promote fish biodiversity raises the concern of keeping coral reef environments from dying. Human actions and influences have caused much damage to the coral reefs left (World Wildlife Fund). Pollution, waste run off, and over fishing are just few careless actions humans make that contribute to coral reef degradation (World Wildlife Fund). If this continues, coral reefs will deteriorate faster leading to the loss of the novel and natural methods for evolution in fish species. Educating the general public is the most important way to raise awareness on the benefits of coral reefs to fish biodiversity (World Wildlife Fund), but in the meantime new research will continue to be conducted in hopes of offering new insights in the possible mechanisms of fish biodiversity within coral reefs.

References

Animal-World Pet and Animal Information [Internet]. 2015. Bennett's Butterflyfish. < http://animal-world.com/encyclo/marine/butter/BennettsButterflyfish.php> Accessed 2016 Mar 22.

Bray D, Gomon M. 2012. Fishes of Australia [Internet]. Cardinalfishes, Apogonidae. <http://www.fishesofaustralia.net.au/home/family/22#summary> Accessed 2016 March 22.

Briggs J. 2004. Older Species: a rejuvenation on coral reefs? *J. Biogeogr*. 31:525-530.

Cowman P, Bellwood D. 2011. Coral reefs as drivers of cladogenesis: expanding coral reefs, cryptic extinction events, and the development of biodiversity hotspots. *J Evol. Biol*. 24:2543–2562.

Holbrook S, Schmitt R, Messmer V, et al. 2015. Reef Fishes in Biodiversity Hotspots Are at Greatest Risk from Loss of Coral Species: e0124054. *PLoS One*. 10:1-12.

Jonna, R. 2003. Animal Diversity Web [Internet]. Labridae Wrasses. < http://animaldiversity.org/accounts/Labridae/> Accessed 2016 Mar 22.

Jonna, R. 2003. Animal Diversity Web [Internet]. Chaetodontidae Butterflyfishes. <http://animaldiversity.org/accounts/Chaetodontidae/> Accessed 2016 March 22.

Jonna, R. 2003. Animal Diversity Web [Internet]. Pomacentridae Damselfishes. <http://animaldiversity.org/accounts/Pomacentridae/> Accessed 2016 March 22.

Messmer V, Jones G, Munday P, Holbrook S, Schmitt R, Brooks A. 2011. Habitat Biodiversity as a Determinant of Fish Community Structure on Coral Reefs. *Ecology*. 92:2285–98.

Price S, Holzman R, Near T, Wainwright P. 2011. Coral reefs promote the evolution of morphological diversity and ecological novelty in labrid fishes. *Ecol Lett*. *14*:462–469.

Stanley G. 2006. Photosymbiosis and the Evolution of Modern Coral Reefs. *Science*. 312:857–858.

World Wildlife Fund [Internet]. 2015. Coral reefs: threats. <http://wwf.panda.org/about_our_earth/blue_planet/coasts/coral_reefs/coral_threats/> Accessed 2016 March 22.

Land Cover Modification Assessment for Protected Areas Containing Hippo Study Sites

Brooke Rose

Habitat loss represents a major threat to the common hippopotamus (Hippopotamus amphibious). Although hippo populations still persist throughout eastern and southeastern Africa, recent assessments show that their numbers are declining, largely due to human impact. Furthermore, the common hippo remains relatively understudied, particularly from a conservation perspective and the majority of hippo studies have been conducted in protected areas (PAs). In order to better understand habitat modification in the hippo's range as well as the role that PAs play in mitigating habitat modification, the current study compared land cover modification within PAs in which hippos have been studied and the surrounding 10 kilometer area. I found that there was no difference in land modification within the 30 PAs in which hippos have been studied and the 10 km areas surrounding each PA. Both areas were comprised of less than 3% modified land cover, suggesting that these PAs have successfully mitigated land modification within their borders and in the surrounding 10 km area. These findings present a positive outlook for the common hippo's future and demonstrate that hippo habitat remains relatively intact within the PAs in which hippos have been studied. However, there were three PAs and their surrounding buffer regions that showed strikingly high levels of modification: Masai Mara Game Reserve, Lake Naivasha, and Murchison Falls National Park. More research is needed to determine the current hippo populations in these areas and identify appropriate management solutions to ensure that more habitat is not lost within these PAs.

Introduction

Dispersed along river and lake systems, the common hippo (*Hippopotamus amphibius*) primarily occupies in large, yet discontinuous areas of eastern and southeastern Africa (Lewison, 2008). According to the IUCN, the common hippo is currently a Vulnerable species, a designation that indicates a species is "facing a high risk of extinction in the wild" (Lewison and Oliver, 2008). Previous research suggests that hippos can have a detrimental effect on native plants and crops, and this research has been used as justification for reducing wild hippo numbers (Lock, 1972). In past studies on common hippos, large-scale culling remained a valuable part of assessing wild hippo ecology, such as understanding their diet by examining the stomach content of deceased individuals (Field, 1970). Dam and weir construction have led to reduced riparian habitat and smaller hippo populations along the Limpopo River in South Africa (Jacobsen and Kleynhans, 1993). Although little research has been conducted on the effects of decreasing hippo numbers, a study that recommended regular hippo culling measures in Queen Elizabeth National Park acknowledged the importance of hippos in maintaining high biodiversity in the park's waterways (Bere, 1959).

Habitat loss due to human development in the form of agriculture, urbanization, and waterway diversion represents a significant threat to hippo populations across their range (Smuts & Whyte, 1981; Jacobsen & Kleynhans 1993). A recent predictive population model suggests that mild human disturbances and environmental changes could lead to a decrease of more than 50% in hippo populations throughout Africa (Lewison 2007). Therefore, understanding the extent of modified land cover throughout the hippo's current range remains essential to ensuring the stability of wild hippo populations. Habitat loss is among the primary drivers behind declines in hippo numbers and the majority of studies conducted on wild hippo occur within protected areas (PAs). Therefore, analyzing the role of national parks and reserves in mitigating habitat loss can better inform management decisions, i.e. setting aside riparian land for existing common hippo populations.

This study assesses land cover modification within PAs in which the common hippo has been studied. Humans have directly modified 33% of Earth's non-ice surface through agriculture and residency, a testament to our ability to alter natural ecosystems and modify global land cover (Ellis & Ramankutty, 2008). As a result, PAs provide habitat for entire ecosystems that would otherwise be threatened by human activity (Dudley, 2008). Regarded as necessary for biodiversity conservation (Dudley, 2008), PAs can limit land cover modification and, therefore, preserve wildlife and plants species. Comparing habitat and land cover differences between a PAs interior and surrounding, unprotected areas can offer insight into the PA's effectiveness (Mas, 2005; Bruner et al., 2001; Andam et al., 2008). By comparing land cover modification within PAs in which hippos have been studied to land cover modification in the unprotected area immediately adjacent to the PA, we can better understand the importance of each PA in preventing

land cover modification that could have detrimental effects on hippo populations. Moreover, high levels of modification in the area surrounding the PA could suggest that the PA represents the only nearby refuge for common hippos, indicating fragmentation in the hippo's habitat.

Methods

I conducted a literature search to locate previous studies conducted on common hippos in their native habitat using the search engines Google Scholar and ProQuest. I located 61 articles related to wild common hippo populations; however, I only recorded the study site locations for 55 of the research articles. The six excluded articles were either reviews of past studies or contained unclear delineations for the study sites assessed. The publication year for these studies ranged from 1959 to 2013. For each study, I recorded the study site location (e.g. Kruger National Park) along with the area's corresponding geographic coordinates. Three of the reviewed studies included river censuses in which a section of a river was monitored for hippo activity (Jacobsen and Kleynhans 1993; Viljoen 1980; Karstad and Hudson 1984). For these study sites, I recorded the censuses' start and end coordinates.

Geographic Information Systems (GIS) represents a popular and effective tool for generating unprotected buffer areas for comparison to PAs in terms of forest cover and land degradation rates (Andam et al., 2008; Liu et al., 2001). For my analysis, I used Esri ArcGIS 10.2, and displayed the coordinate data for the hippo study sites on a base map of the African continent with country delineations (Figure 1). In order to determine which hippo study sites were conducted within some type of protected area, I downloaded the World Database on Protected Areas shapefile (IUCN and UNEP-WCMC 2015). Through a Search-by-Location query, I determined which hippo studies were conducted within a protected area and which protected area contained a hippo study site (Figure 2). I then created 10 kilometer buffers around each protected area in which a hippo study was conducted. Past studies that examined land cover differences between protected area interior and exterior frequently used 10 kilometers to define the protected area exterior (Bruner et al., 2001; Joppa & Pfaff, 2010; Mas 2005; Francoso et al., 2015). According to Mas (2005), a 10-kilometer distance provides a reliable land cover modification assessment and allows for the analysis to be comparable to those used in previous studies.

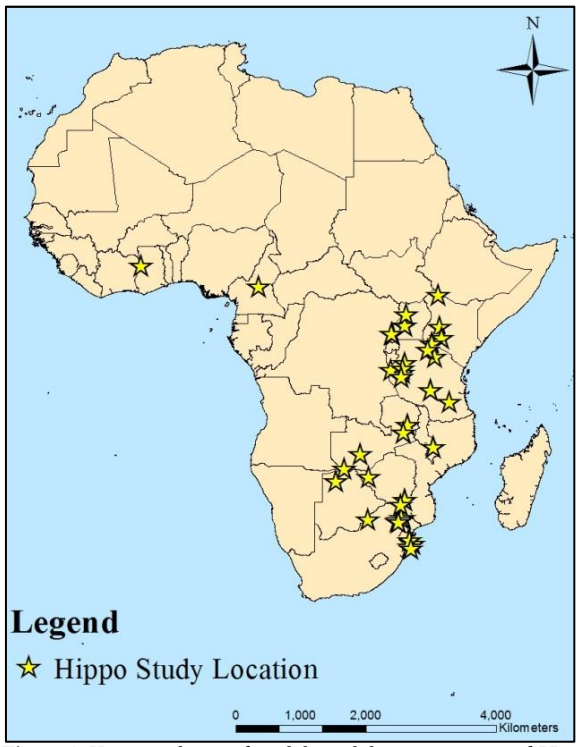

Figure 1. Hippo study sites found through literature review of 55 research articles of wild common hippos

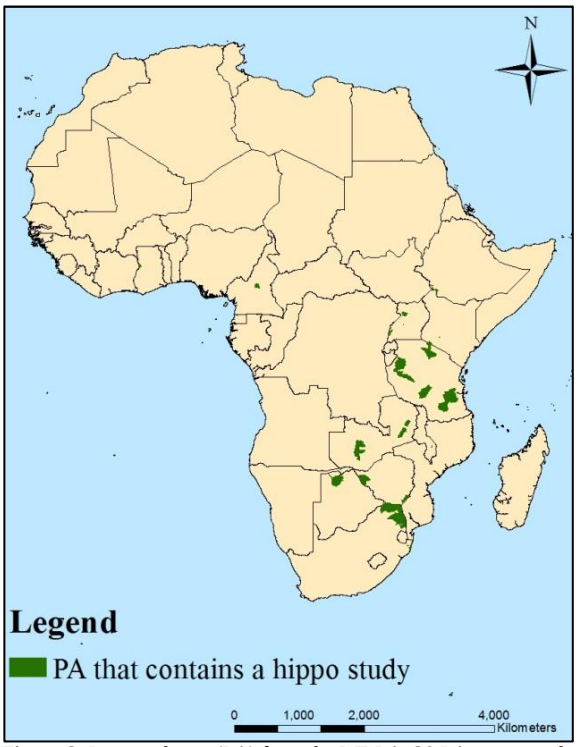

Figure 2. Protected area (PA) from the WDPA; 30 PAs contained at least one hippo study site

I used GlobCover 2009 (300 meter pixel resolution) data to analyze land cover modification differences between the interior of PAs in which hippo research had been conducted and 10 kilometers surrounding these PAs (Bontemps et al., 2011). For this analysis, modified land cover included the GlobCover 2009 categories irrigated croplands, rainfed croplands, mosaic cropland/vegetation, and artificial areas. Although previous studies have also included the land cover classification of mosaic vegetation/croplands as a modified land cover type (Boyle, 2014), I included it as a natural land cover modification for this study because it is comprised of >50% grassland, shrubland, and forest land cover (see Figures 3 and 4 for GlobCover 2009 maps). I clipped the GlobCover 2009 land cover data to the extent of each protected area in which a study or report was conducted on wild hippos (Figure 5) and calculated the percentage of modified and natural land cover in each PA. I used the same method for calculating the land cover within the total buffer areas (which also encompassed the PA) and subtracted the land cover percentages within the PAs to determine the land cover makeup in the 10 kilometer area outside of the PAs. Using Microsoft Excel, I conducted a paired t-test to compare percent modified land cover within each protected area to the 10 kilometer region surrounding each protected area.

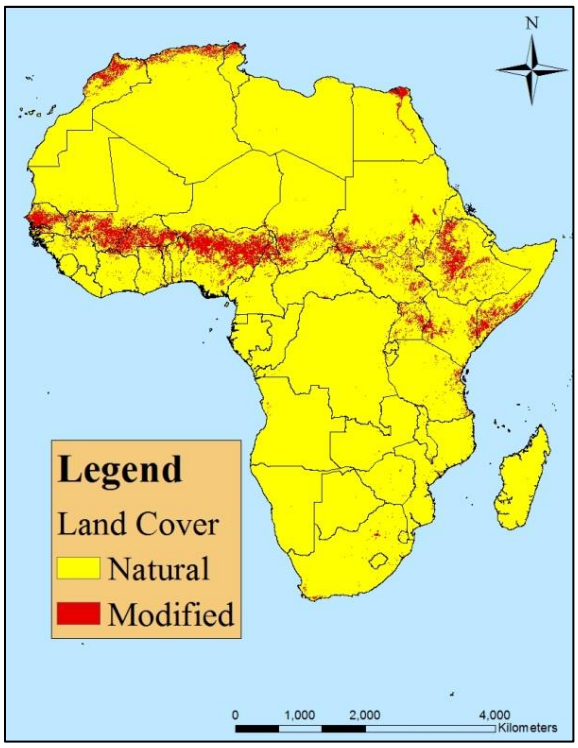

Figure 3. GlobCover 2009 land cover map of Africa

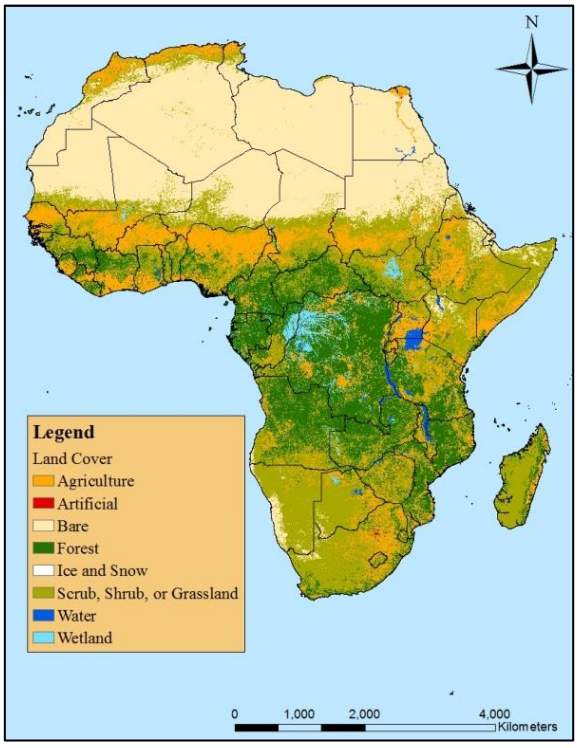

Figure 4. Natural and modified land cover; modified land cover includes artificial areas as well as agricultural land (with the exception of vegetation/cropland mosaic)

Results

Of the 55 reviewed hippo studies, I identified 37 separate hippo study sites throughout Africa. There was some site overlap between articles, i.e., twelve different hippo studies were conducted within Queen Elizabeth National Park in Uganda. In addition, some of the studies included multiple study sites; e.g. Okello et al., 2005 included ten study sites in an assessment of hippo mitochondrial DNA. In total, 30 PAs contained a past hippo study site (see Table 1 for list of PAs, their designations, and their sources). These PAs fell under 11 different protection designations: biosphere reserve, conservation area, controlled hunting area, forest reserve, game controlled area, game management area, game reserve, national park, nature reserve, Ramsar site, and World Heritage site.

There was no significant difference between the percent of modified land cover in PAs and the surrounding 10 kilometer buffer area ($t(29)=0.38$; $P=0.71$). On average, 2.91% (±1.17) of the protected areas were comprised of modified land cover and 2.72% (±1.04) of the surrounding 10 kilometer area was comprised of modified land cover (Figure 6). The percentage of modified land cover within PAs ranged from 0 to 29 and from 0 to 29 in the 10 km

buffer areas surrounding the PAs (Figure 7). Although the average land cover modification for the PAs and their surrounding buffer regions was less than 3%, the modified land cover percentages for Masai Mara Game Reserve, the Lake Naivasha Ramsar site, and Murchison Falls National Park ranged from 14-29% (Figure 8).

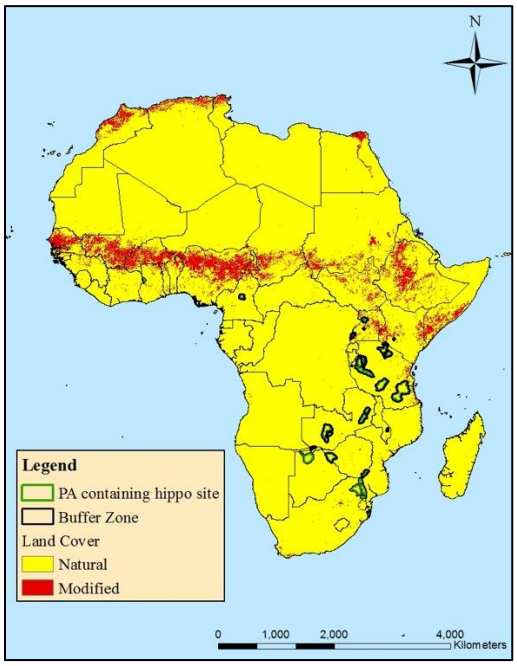

Figure 5. Location of PAs that contained hippo study sites and their surrounding 10 km buffer zones

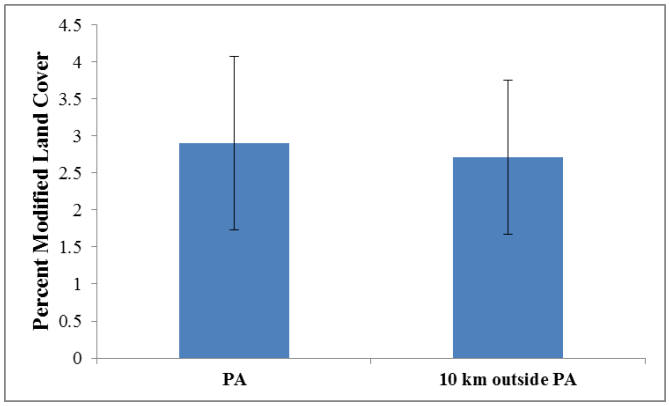

Figure 6. Mean and standard error for land cover modification percentage within PAs and surrounding 10 kilometer buffer zone.

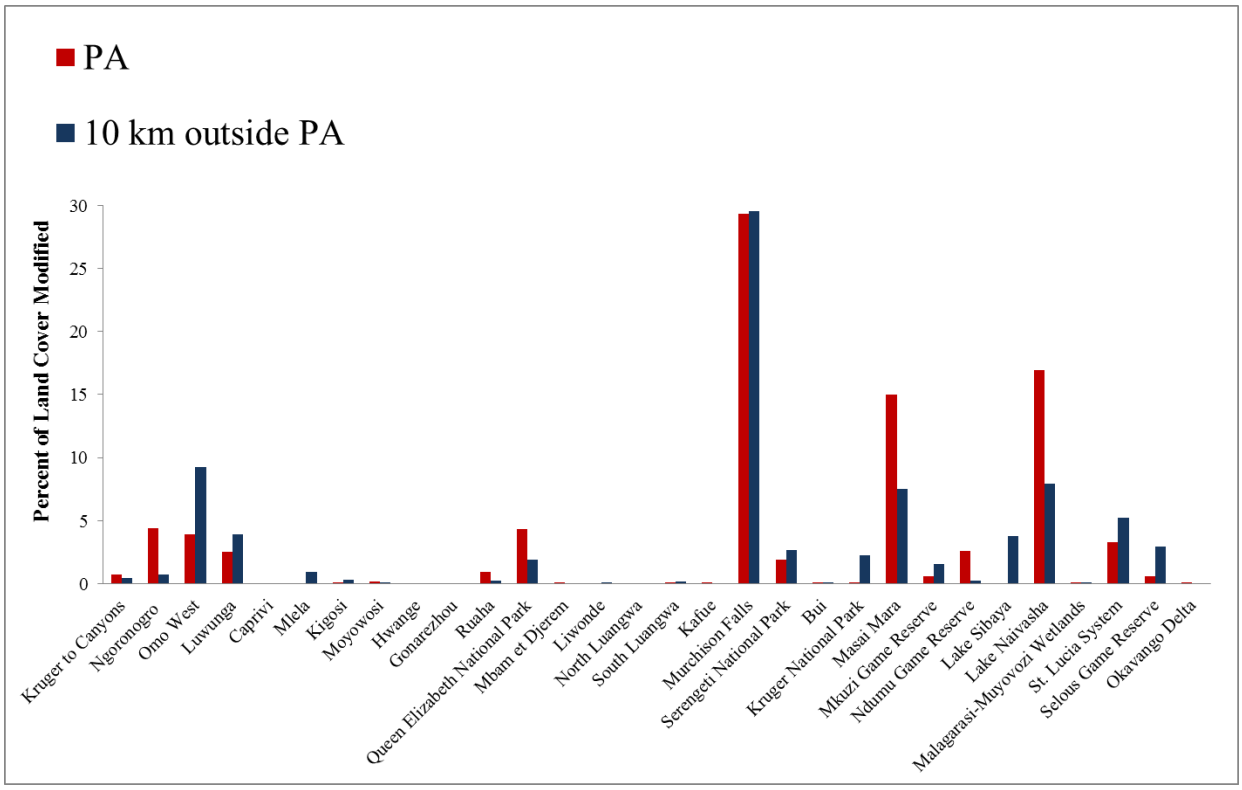

Figure 7: Comparison of % modified land cover within the PAs containing hippo studies and the surrounding 10 kilometer region

Name of Protected Area	Country	Designation	Source
Kruger to Canyons	Republic of South Africa	Bioshpere Reserve	Viljoen, 1980
Ngoronogro	Tanzania	Conservation Area	Deocampo, 2002
Omo West	Ethiopia	Controlled Hunting Area	Fuller, 1975
Luwunga	Uganda	Forest Reserve	Chansa et al., 2011; Sayer and Rakha, 1974; Tembo, 1987
Caprivi	Namibia	Forest Reserve	Griffin and Grobler, 1990
Mlela	Tanzania	Game Controlled Area	Okello et al., 2005
Kigosi	Tanzania	Game Reserve	Okello et al., 2005
Moyowosi		Game Reserve	Okello et al., 2005
Hwange	Zimbabwe	National Park	Dudley, 1996
Gonarezhou	Zimbabwe	National Park	Mackie, 1976; O'Connor & Campbell, 1986; Zisadza et al., 2010
Ruaha	Tanzania	National Park	Barklow, 2004; Kendall, 2011
Queen Elizabeth National Park	Uganda	National Park	Bere, 1959; Cowan et al., 1967; Eltringham, 1974; Field, 1970; Jewell, 1963; Laws, 1968; Lock, 1972; Okello et al., 2005; Petrides & Swank, 1965; Plowright et al., 2005; Thornton, 1971; Wright, 1987
Mbam et Djerem	Cameroon	National Park	Nchanji & Fosto, 2007
Liwonde	Malawi	National Park	Bhima, 1996; Harrison et al., 2007
North Luangwa	Zambia	National Park	Marshall & Sayer, 1976; Okello et al., 2005
South Luangwa	Zambia	National Park	Marshall & Sayer, 1976; Okello et al., 2005
Kafue	Zambia	National Park	Okello et al., 2005
Murchison Falls	Uganda	National Park	Okello et al., 2005
Serengeti National Park	Tanzania	National Park	Oliver & Laurie, 1974
Bui	Ghana	National Park	Bennett et al., 2000
Kruger National Park	Republic of South Africa	National Park	Dauth et al., 1964; Des Vos et al., 1980; Pienaar et al., 1966; Smuts & Whyte, 1981; Van Hoven, 1974; Van Hoven, 1977; Van Hoven, 1978; Viljoen, 1995
Masai Mara	Kenya	National Reserve	Kanga et al., 2011; Kanga et al., 2013; Karstad & Hudson, 1984; Karstad & Hudson, 1986; Okello et al., 2005;
Mkuzi Game Reserve	Republic of South Africa	Nature Reserve	Ellery et al., 2003
Ndumu Game Reserve	Republic of South Africa	Nature Reserve and Ramsar Site	Pooley, 1966; Schutte, 1973; Scotcher, 1973; Scotcher, 1978
Lake Sibaya	Kwazulu	Nature Reserve and Ramsar Site	Bruton, 1978
Lake Naivasha	Kenya	Ramsar Site	Grey & Harper, 2002; Okello et al., 2005
Malagarasi-Muyovozi Wetlands	Tanzania	Ramsar Site	Okello et al., 2005
St. Lucia System	Republic of South Africa	Ramsar Site	Henwood & Keep, 1989
Selous Game Reserve	Tanzania	World Heritage Site	Okello et al., 2005
Okavango Delta	Botswana	World Heritage Site	McCarthy et al., 1998

Table 1. List of PAs that contained a hippo study site and their sources

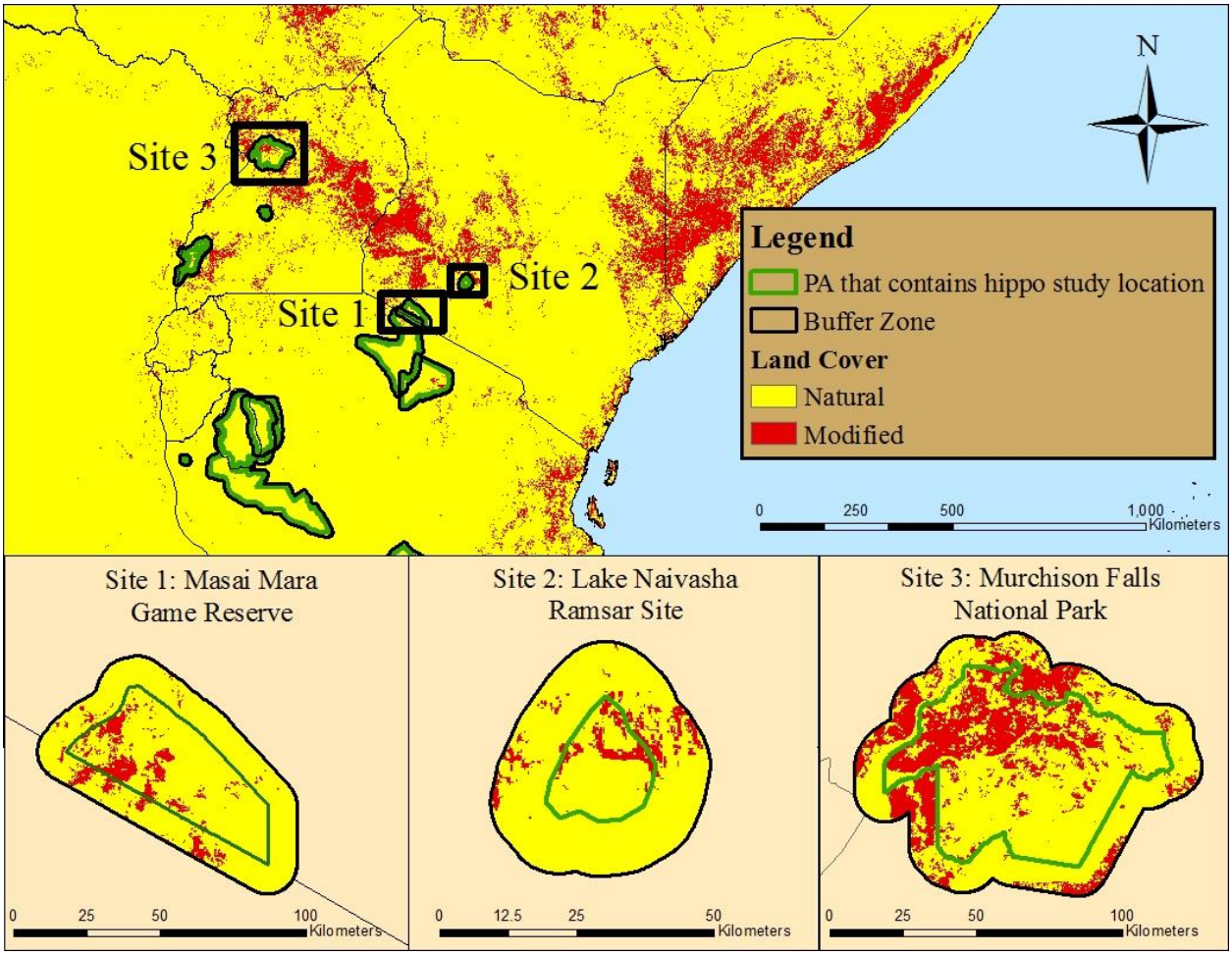

Figure 8: A closer look at the PAs that exhibited the highest percentages of land cover modification and their 10 kilometer buffer regions. Map above figure shows zoom extent.

Discussion

The results of the current study indicate that land cover modification did not differ between the PAs that hosted hippo study locations and the 10 km surrounding unprotected areas. Furthermore, with an average modified land cover of less than 3% for both the PA interiors and the surrounding 10 km area, land modification remains low within and immediately outside of the PAs examined in this study. Although the results of this study do not refute the claim that hippos are currently experiencing habitat loss and degradation throughout their range, it does show that natural land cover remains prevalent and intact within PAs in which wild hippos have been studied (Smuts & Whyte, 1981; Jacobsen & Kleynhans, 1993). Furthermore, for at least the 10 kilometers areas surrounding these PAs, the hippos are not limited by a lack of natural land cover to remain within the borders of national parks and other PAs.

However, as previously stated, three of the PAs exhibited noticeably higher percentages of land cover modification, both within their borders and the 10 km comparison region. Masai Mara Game Reserve and the Lake Naivasha Ramsar site are located in Kenya in regions that are dominated by agricultural production and showed high levels of land cover modification within their borders (>14% modified land cover). Masai Mara is located on the border of Kenya and Tanzania, with the Serengeti National Park located on the Tanzanian side. Comparisons between land policy and private land use in both countries suggest that large-scale agricultural production driven by land privatization has severely modified land cover in Kenya and led to subsequent declines in wildebeest populations (Homewood et al., 2001). In contrast, government control over agricultural land use in Tanzania has prevented similar degradation (Homewood et al., 2001). Recent research suggests that agricultural lands could restrict hippo ranges in the Mara River region if land cover change is not mitigated (Kanga, et al. 2011). Nevertheless, stricter land use regulation and

conservation incentives for farmers are necessary to ensure that the remaining natural land cover within Masai Mara and the surrounding area are protected from encroachment of industrialized agriculture (Homewooed et al., 2001).

In contrast to Masai Mara Game Reserve and Murchison Falls National Park, the Lake Naivasha Ramsar site represents an area that is considered ecologically important on an international level (Awange et al., 2013). However, as demonstrated by the current study, this PA contains a relatively large degree of land cover modification, particularly in the form of agriculture. The area surrounding the lake is known for its floriculture, a factor that has likely lead to declines in the lake's water levels since 2000 (Awange et al., 2013). The catchment area surrounding the lake has also shown significant declines, which have also been attributed to irrigation that supports the local flower industry. Although additional study is required to examine the impact of floriculture and the resulting water level declines on hippo populations, changes in the area's water patterns have likely affected hippo numbers in the area surrounding Lake Naivasha. As demonstrated by the negative impact that dams and weirs have on these large, semi-aquatic mammals (Jacobsen & Kleynhans, 1993), hippos are sensitive to changes in lake and wetland habitats. Because of the ecological importance of Lake Naivasha and the common hippo's specific sensitivity to declines in riparian habitats, water use management should be revisted to ensure that the lake's water levels do not continue to decline.

With the highest percent modified land cover of the 30 analyzed PAs, Murchison Falls National Park represents the largest, most visited national park in Uganda (Segan et al., 2012). Tourist traffic and the subsequent pollution have been acknowledged as substantial issues for wildlife within Murchison Falls (Segan et al., 2012). There is also evidence that small- and large-scale agriculture have increased within the landscape surrounding the park over the last decade; however, another assessment suggests that agriculture is not a prevalent land use within the park (Segan et al., 2012). Nevertheless, the current assessment found that almost 30% of Murchison Falls and the surrounding 10 kilometer region are dominated by agriculture and more research is needed to determine if this area represents industrialized or small-scale agricultural pursuits. Additionally, the current results suggest that this park could represent an area of concern for hippo populations in Uganda and conservation efforts should focus on protecting the remaining hippos of Murchison Falls.

Although this study found that land cover modification remains low in and around PAs that are known to contain hippo populations, several highly modified outliers were identified. Future conservation efforts to ensure that agricultural enterprises do not reduce habitat for hippos (and other wildlife) are crucial. Because these PAs are dispersed throughout several different countries with varying government systems, the management strategies for protecting hippo populations will differ from country to country. While this study only examined land cover modification within and around PAs that hosted previous hippo studies, understanding land cover modification in and around all PAs that represent hippo habitat remains crucial to gauging habitat loss for this iconic species. Future studies should examine other hippo population location data to assess land cover modification in all PAs that are known to contain hippos.

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References

Andam K , Ferraro P, Pfaff A, Sanchez-Azofeifa G, Robalino J. 2008. Measuring the effectiveness of protected area networks in reducing deforestation. *Proceedings of the National Academy of Sciences*. 105: 16089-16094.

Awange J, Forootan E, Kusche J, Kiema J, Omondi P, Heck B, Fleming K, Ohanya S, Goncalves R. 2013. Understanding the decline of water storage across the Ramsar-Lake Naivasha using satellite-based methods. *Advances in Water Resources.* 60:7-23.

Barklow W. 2004. Amphibius communication with sound in hippos *Hippopotamus amphibius*. *Animal Behaviour*. 68: 1125-1132.

Bennett D, Green N, Basuglo B. 2000. The abundance of *Hippopotamus amphibius* in the Black Volta River at Bui National Park, Ghana. *African Journal of Ecology*. 38: 372-373.

Bere R. 1959. Queen Elizabeth National Park: Uganda The Hippopotamus Problem and Experiment. *Oryx*. 5:116-124.

Bhima R. 1996. Census of hippopotamus (*Hippopotamus amphibius* (l)) in the Upper Shire River, Malawi. *African Journal of Ecology*. 34: 83-85.

Bontemps S, Defourny P, Van Bogaert E, Arino O, Kalogirou V, Perez J. 2011. GlobCover 2009: Product description and validation report. 52- 53.

Bruner A, Gullison R, Rice R, da Fonseca G. 2001. Effectiveness of Parks in Protecting Tropical Biodiversity. *Science*. 291: 125-128.

Bruton MN. 1978. Recent Mammal Records from Eastern Tongaland in Kwazulu, with Notes on Hippopotamus in Lake Sibaya. *The Lammergeyer*. 24: 19-27.

Chansa W, Milanzi J, Sichone P. 2011. Influence of river geomorphologic features on hippopotamus density distribution along the Luangwa River, Zambia. African Journal of *Ecology*. 49: 221-226.

Cowan D, Thurlbeck W, Laws R. 1967. Some diseases of the hippopotamus in Uganda. *Veterinary Pathology*. 4: 554-567.

Dauth J, Dreyer M, Raubenheimer E, Lemmer L, de Vos V. 1964. Blood lead concentrations in hippopotami (*Hippopotamus amphibius*) in the Kruger National Park. *South African Veterinary Association*. 59: 153-154.

de Vos V, de Klerk B. 1980. Tetanus in a free-living Hippopotamus *Hippopotamus amphibius capensis* from the Kruger National Park. *Koedoe*. 23: 171-175.

Deocampo D. 2002. Sedimentary Structures Generated by *Hippopotamus amphibius* in a Lake-margin Wetland, Ngorongoro Crater, Tanzania. *Palaios*. 17: 212-217.

Dudley J. 1996. Record of carnivory, scavenging and predation for *Hippopotamus amphibious* in Hwange National Park, Zimbabwe. *Mammalia*. 60: 486-488.

Dudley N. 2008. Guidelines for Applying Protected Area Management Categories. IUCN, Glant, Switzerland. (p. x+86).

Ellery W, Dahlberg A, Strydom R, Neal M, Jackson J. 2003. Diversion of water flow from a floodplain wetland stream: an analysis of geomorphological setting and hydrological and ecological consequences. *Journal of Environmental Management*. 68: 51-71.

Ellis E, Ramankutty N. 2008. Putting people in the map: anthropogenic biomes of the world. *Frontiers in Ecology and the Environment*. 6: 439-447.

Eltringham S. 1974. Changes in the Large Mammal Community of Mweya Peninsula, Rwenzori National Park, Uganda, Following Removal of Hippopotamus. *Journal of Applied Ecology*. 11: 855-865.

Field C. 1970. A Study of the Feeding Habits of the Hippopotamus (*Hippopotamus amphibious* Linn.) in the Queen Elizabeth National Park, Uganda, with some management implications. *Zoological Africana*. 5: 71-86.

Francoso R, Brandao R, Nogueira C, Salmona Y, Machado R, Colli G. 2015. Habitat loss and the effectiveness of protected areas in the Cerrado Biodiversity Hotspot. *Natureza and Conservacao: Brazillian Journal of Nature Conservation.* 13: 35-40.

Fuller G. 1975. *Hippopotamus amphibius* along the Omo River. *Walia*. 6:4-7.

Grey J, Harper D. 2002. Using stable isotope analyses to identify allochthonous inputs to Lake Naivasha mediated via the hippopotamus gut. *Isotopes in Environmental and Health Studies*. 38: 245-250.

Griffin M, Grobler H. 1990. Wetland-associated mammals of Namibia-a national review. *Madoqua*. 17: 233-237.

Harrison M, Kalindekafe M, Banda B. 2007. The ecology of the hippopotamus in Liwonde National Park, Malawi: implications for management. *African Journal of Ecology*. 46: 507-514.

Henwood R, Keep M. 1989. The capture and translocation of hippopotamus by means of chemical immobilization. *Lammergeyer* 40:30-38.

Homewood K, Lambin E, Coast E, Karluki A,
Kikula I, Kivelia J, Said M, Serneels S, Thompson M. 2001. Long-term changes in Serengeti-Mara wildebeest and land cover:Pastoralism, population, or policies? *Proceedings of the National Academy of Sciences*. 98: 12544-12549.

IUCN, UNEP-WCMC. 2015. The World Database on Protected Areas (WDPA). July 2015.Cambridge, UK: UNEP-WCMC. Available at: www.protectedplanet.net.

Jacobsen N, Kleynhans C. 1993. The importance of weirs as refugia for hippopotami and crocodiles in the Limpopo River, South Africa. *Water South Africa*. 19: 301-306.

Jewell P. 1963. Wild life research in East and Central Africa. *Oryx*. 7:77-87.

Joppa L, Pfaff A. 2010. Global protected area impacts. *Proceedings of the Royal Society B*.283: 1-6.

Kanga E, Ogutu J, Olff H, Santema P. 2011. Population trend and distribution of the Vulnerable common hippopotamus

Hippopotamus amphibius in the Mara Region of Kenya. *Oryx*. 45: 20-27.
Kanga E, Ogutu J, Piepho H, Olff H. 2013. Hippopotamus and livestock grazing: Influences on riparian vegetation and facilitation of other herbivores in the Mara Region of Kenya. *Landscape and Ecological Engineering*. 9:47-58.
Karstad E, Hudson R. 1984. Census of the Mara River hippopotamus (*Hippopotamus amphibius*), southwest Kenya, 1980-1982. *African Journal of Ecology*. 22: 143-147.
Karstad E, Hudson R. 1986. Social organization and communication of riverine hippopotami in southwestern Kenya. *Mammalia*. 50: 153-164.
Kendall C. 2011. The spatial and agricultural basis of crop raiding by the vulnerable common hippopotamus *Hippopotamus amphibius* around Ruaha National Park, Tanzania. *Oryx*. 45: 28-34.
Laws R. 1968. Dentition and ageing of the hippopotamus. *African Journal of Ecology*. 6:19-52.
Lewison R. 2007. Population responses to natural and human-mediated disturbances: assessing the vulnerability of the common hippopotamus (*Hippopotamus amphibius*).*African Journal of Ecology*. 45: 407-415.
Lewison R, Oliver W. (IUCN SSC Hippo Specialist Subgroup). 2008. *Hippopotamus amphibius*. The IUCN Red List of Threatened Species. T10103A3163790.
Liu J, Linderman M, Ouyang Z, An L, Yang J, Zhang H. 2001. Ecological Degradation in Protected Areas: The Case of Wolong Nature Reserve for Giant Pandas. *Science*. 292: 98-101.
Lock J. 1972. The effects of hippopotamus grazing on grasslands. *Journal of Ecology*. 60: 445-467.
Mackie C. 1976. Feeding habits of the hippopotamus on the Lundi River, Rhodesia. *Arnolidia Rhodesia*. 7:1-16.
Marshall P, Sayer J. 1976. Population ecology and response to cropping of a hippopotamus population in Eastern Zambia. *Journal of Applied Ecology*. 13: 391-403.
Mas J. 2005. Assessing protected area effectiveness using surrounding (buffer) areas environmentally similar to the target area. *Environmental Monitoring and Assessment*. 105: 69-80.
McCarthy T, Ellery W, Bloem A. 1998. Some observations of the geomorphological impact of hippopotamus (*Hippopotamus amphibius* L.*)* in the Okavango Delta, Botswana. *African Journal of Ecology*. 36: 44-56.
Nchanji A, Fosto R. 2007. Common hippopotamus (*Hippopotamus amphibius*): a survey on the River Djerem, Mbam-Djerem National Park, Cameroon. *Mammalia*. 70: 9-13.
O'Connor T, Campbell B. 1986. Hippopotamus habitat relationships on the Lundi River, Gonarezhou National Park, Zimbabwe. *African Journal of Ecology*. 24: 7-26.
Okello J, Nyakaana S, Masembe C, Siegismund H, Arctander P. 2005. Mitochondrial DNA variation of the common hippopotamus: evidence for a recent population expansion. *Heredity*. 95:206-215.
Olivier R, Laurie W. 1974. Habitat utilization by hippopotamus in the Mara River. *East African Wildlife Journal*. 12: 249-271.
Petrides G, Swank W. 1965. Population densities and the range-carrying capacity for large mammals in Queen Elizabeth National Park, Uganda. *Zoologica Africana*. 1: 209-225.
Pienaar U de V, Van Wyk P, Fairall N. 1966. An experimental cropping scheme of hippopotami in the Letaba river of the Kruger National Park. *Koedoe*. 9: 1-33.
Plowright W, Laws R, Rampton C. 1964. Serological evidence for the susceptibility of the hippopotamus (*Hippopotamus amphibius* Linnaeus) to natural infection with the rinderpest virus. *Journal of Hygiene*. 62: 329-336.
Pooley A. 1966. Bird/crocodile and bird/hippopotamus commensalism in Zululand. *The Ostrich*. 38: 11-12.
Sayer J, Rakha A. 1974. The age of puberty of the hippopotamus (*Hippopotamus amphibious* Linn.) in the Luangwa River in eastern Zambia. *East African Wildlife Journal*. 12: 227-232.
Schutte G. 1973. Hippopotamus behaviour after shooting an adult female. *Lammergeyer*. 19: 36.
Scotcher J. 1973. Observations on the breeding behaviour of hippopotamus. *Lammergeyer*. 19: 35-36.
Scotcher J. 1978. Hippopotamus Numbers and Movements in Ndumu Game Reserve. *The Lammergeyer*. 24: 5-12.
Segan D, Watson J, Nangendo G, Ayebare S, Plumptre A. 2012. Avoiding conflict and balancing trade-offs: Biodiversity Conservation in the context of Competing Land Uses. New York: Wildlife Conservation Society.

Smuts G, Whyte I. 1981. Relationships between reproduction and environment in the Hippopotamus *Hippopotamus amphibius* in the Kruger National Park. *Koedoe*. 24: 169-185.

Tembo A. 1987. Population status of the hippopotamus on the Luangwa River, Zambia. *African Journal of Ecology*. 25: 71-77.

Thornton D. 1971. The effect of complete removal of hippopotamus on grassland in the Queen Elizabeth National Park, Uganda. *African Journal of Ecology*. 9: 47-55.

Van Hoven W. 1974. Ciliate protozoa and aspects of the nutrition of the hippopotamus in the Kruger National Park. *South African Journal of Science*. 70: 107-109.

Van Hoven W. 1977. Stomach fermentation rate in the hippopotamus. *South African Journal of Science*. 73: 216.

Van Hoven W. 1978. Digestion physiology in the stomach complex and hindgut of the hippopotamus (*Hippopotamus amphibius*). *South African Journal of Wildlife Research*. 8: 59-64.

Van Niekerk J, Pienaar U de V. 1962. Adaptation of the immobilizing technique to the capture, marking and translocation of game animals in the Kruger National Park. *Koedoe*. 5: 137-143.

Viljoen P. 1980. Distribution and numbers of the hippopotamus in the Olifants and Blyde Rivers. *The South African Journal of Wildlife Research*. 10: 129-132.

Viljoen P. 1995. Changes in number and distribution of hippopotamus (*Hippopotamus amphibius*) in the Sabie River, Kruger National Park, during the 1992 drought. *Koedoe*. 38: 115-121.

Wright P. 1987. Thermoregulation in the hippopotamus on land. *South African Journal of Zoology*. 22: 237-242.

Zisadza P, Gandiwa E, van der Westhuizen H, van der Westhuizen E, Bodzo V. 2010. Abundance, distribution, and population trends of hippopotamus in Gonarezhou National Park, Zimbabwe. *South African Journal of Wildlife Research*. 40: 149-157.

An Analysis of the Role that Large-Scale Feedlots and Aquaculture Play in the Spread of Antibiotic Resistance

Morgan Fuller

The increase of antibiotic resistance over the last few decades has become a global concern both socially and scientifically (Smith et al., 2002). According to Smith et al. (2002), over prescribing and misusing antimicrobials in human medicine, and the increased widespread use of veterinary antibiotics for purposes other than their intended therapeutic use have contributed to the global increase in antibiotic resistance. In this review, I will specifically examine how the sub-therapeutic uses of antibiotics in animals contribute to the transmission of antibiotic resistance from bacteria to food animals to humans.

Introduction

A Public Health Issue

The human population continues to increase at an exponential rate which infers a greater risk that infectious diseases will evolve, emerge, or spread quickly through the population (Gilchrist et al., 2007). In order for this increasing human population to survive, there must be a sufficient amount of food to sustain them, such as a greater population of livestock (Gilchrist et al., 2007). However, a higher concentration of humans and/or animals in one geographical location enhances the risk of potential transmission of bacteria, including antibiotic resistant bacteria, between the groups (Gilchrist et al., 2007). To combat this potential transmission and to meet a high consumer demand for food, farmers use antibiotics to fight disease and improve animal productivity (Mathew et al., 1999). Because of this rise in large-scale animal feeding operations, there has also been an increase in supplements added to animal feed in order to promote animal growth (Zhao et al., 2010). Many times these supplements belong to a class of drugs generally referred to as antimicrobial growth promoters (AMGPs) (van den Bogaard et al., 2001). Some estimate that 16 million kilogram (kg) of antibiotics are used every year in the United States with 70% implored for non-therapeutic purposes (UCS, 2011). The Union of Concerned Scientists (2011) estimates that farmers administer 11.2 million kg of antibiotics to livestock every year in order to help the animals grow faster while medical doctors prescribe 1.4 million kg of antibiotics to humans each year. Because we have seen a significant increase in antibiotic resistant genes in the environment since 1940 (Knapp et al., 2010), many experts worry that we might be approaching a "post-antibiotic era" in which we will no longer have effective antibiotics to treat life-threatening infections (Gilchrist et al., 2007). With this review, I hope to first elucidate the problems associated with raising livestock and aquaculture. Secondly, this paper will propose possible solutions to combat the spread of antibiotic resistance.

The Mechanism

At the most basic level, resistance develops from selection of resistance populations over antibiotic susceptible populations (Figure 1). In other words, individuals in a bacteria population spontaneously develop mutations that allow for resistance to antibiotics (Khachatourians, 1998). After two generations, the susceptible bacteria die and only the antibiotic resistant bacteria survive to further colonize their host (Khachatourians, 1998). As a result, there is an increased risk for human infections that could become fatal or life-threatening, a mechanism which I will elaborate on later. For example, in food animals, farmers use prolonged exposure to low levels of antibiotics to treat infections in their animals and to promote growth. Unfortunately, this method does not kill all the bacteria present, which promotes the development of resistant bacteria by selection of the mutants (Gilchrist et al., 2007).

Bacteria can also develop resistance to one or more antibiotics through a process referred to as horizontal gene transfer. There are many types of horizontal gene transfer or ways in which bacteria can uptake resistance genes from its environment (Figure 2) (Davies, 1994). According to Smith et al. (2002), with this horizontal exchange of information and a large diversity of hosts, every external source of resistance genes attenuates the probability of a pathogen establishing drug resistance.

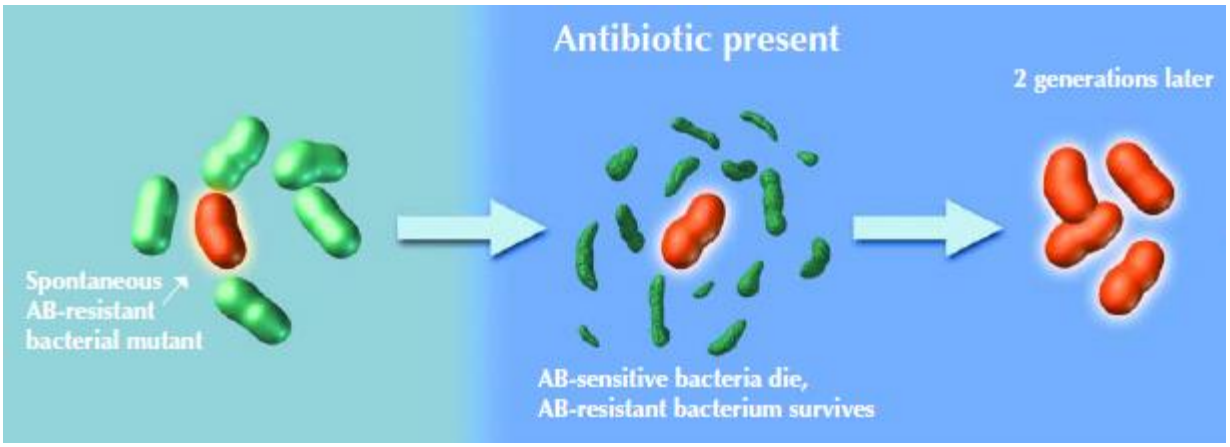


Figure 1. The selection for antibiotic resistant bacteria in bacterial populations (Khachatourians, 1998). The bacteria develop mutations that helps them survive antibiotic treatments. Then, the mutated bacteria colonize the immediate environment.

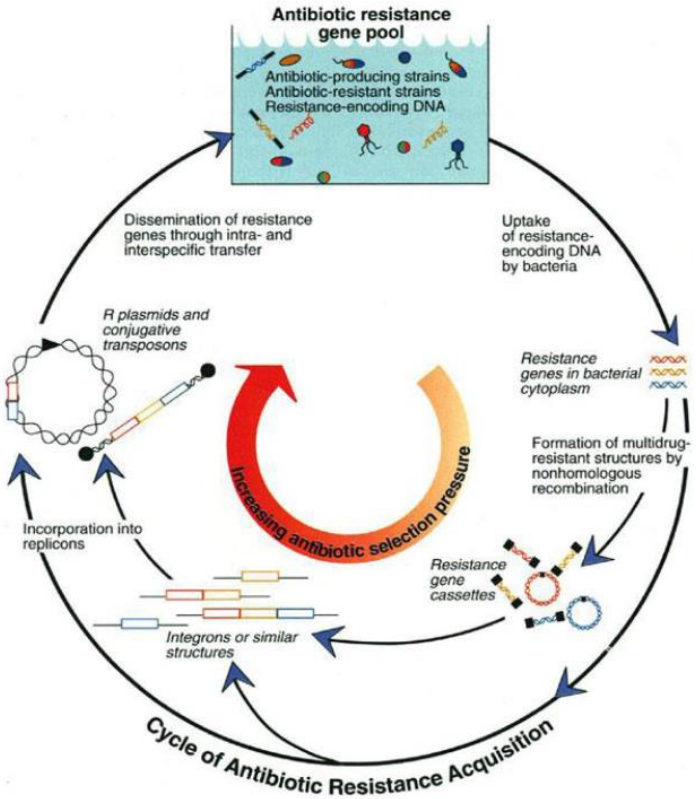


Figure 2: The cycle of antibiotic resistance acquisition (Davies, 1994). This illustration highlights the ability of bacteria to uptake genetic material from their environment, whether that is from another bacterium or from its immediate surroundings.

Physical factors, in addition, can distribute antibiotic resistant bacteria from soils to urban environments, increasing the probability that humans will come in contact with such bacteria (Heuer et al., 2011a). The transport of aerosol or particles containing antibiotic resistant bacteria can lead to hospital stays for humans as these bacteria can cause infections or create chaos in the benevolent, commensal populations already present within humans (Figure 3) (Heuer et al., 2011b). The resistant bacteria also have the ability to attach to crops, which leads to human exposure to these pathogenic bacteria upon consumption of infected plants (Heuer et al., 2011a). While I will not elaborate on the agriculture perspective in this paper,

I will analyze the role consumption of contaminated meat plays in spreading resistance.

We, as a global community, must develop techniques to stop the transmission of antibiotic resistance on farms. Factors, such as antibiotic use, overcrowding and poor sanitation, increase this transmission. I will present evidence that elucidates the details of the transmission (van den Bogaard et al., 2001).

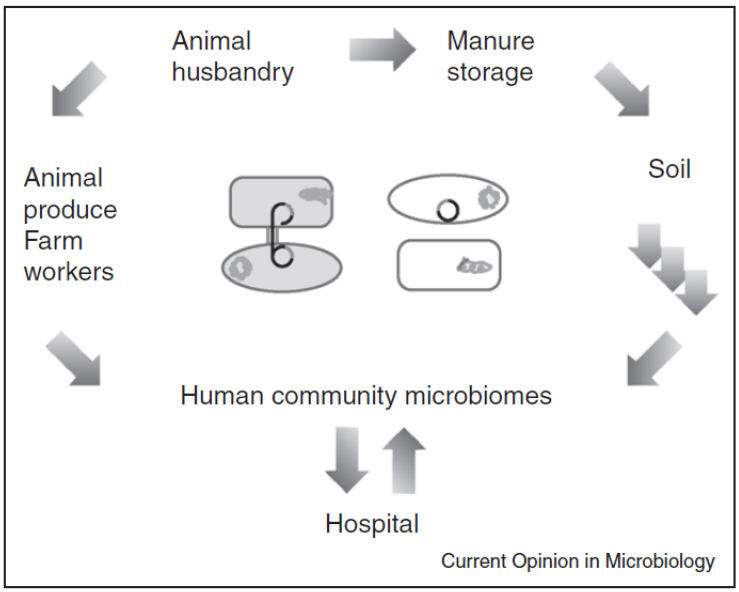


Figure 3: The possible transmission routes of antibiotic resistant genes from the environment to humans (Heuer et al., 2011a). Several arrows signify transmission of genetic elements via diverse environmental routes, such as water, air, birds, etc. Antibiotic resistance genes can travel from animals to humans on mobile genetic elements via multiple routes.

Measuring Antibiotic Resistance

Before examining how antibiotic resistance travels from bacterial populations to human food to humans, we must establish a method of measuring the amount of resistance in bacteria. One common and precise measurement involves determining the minimum inhibitory concentration (MIC), which can be used to depict resistance trends (Huang et al., 2015). The MIC value represents the minimum dosage of antibiotics required to effectively treat a specific bacterial infection (Huang et al., 2015). Determining the MIC normally involves agar plates with various antibiotic concentrations and growing bacteria colonies (van den Bogaard et al., 2001). Van den Bogaard et al. (2001) implored the use of Levine agar plates that select for specific bacteria populations, which is helpful when a study aims to examine resistance in one particular bacterial species.

The Treatment

One major concern with the antibiotic use in animals for non-therapeutic purposes is the fact that many of the antibiotics given to animals are also prescribed in humans (Michigan State University, 2015). Many people still argue for antimicrobial treatments in animals while many activist groups and concerned parents encourage consumers to buy products from local farms. In China, for instance, Zhao et al. (2010) examined three of the most frequent drugs used for both human and livestock purposes in order to establish a source of antibiotic resistance in eight rural provinces. Specifically, Zhao et al. (2010) studied the fluoroquinolone, sulfonamide and tetracycline drug classes which respectively account for 15%, 12% and 14% of the total amount of antimicrobials used in human and livestock populations in China. The use of similar drugs in animals and in humans could affect the treatment of human infections in the future. For example, Acar et al. (2000) attempted to answer this concern by suggesting that the rise of vancomycin-resistant enterococcal (VRE) infections in one European community arose from farmers using a glycopeptide antibiotic, avoparcin, as a growth promoter in their livestock. Avoparcin and vancomycin have similar chemical properties, which could explain why VRE rose in this area.

Antibiotics in the Environment

Antibiotics and antibiotic resistant genes (ARGs) enter the environment in various ways. According to Alcock et al. (1999), the majority of antibiotics are water-soluble meaning animals absorb them at a low rate in their gastrointestinal tract excreting 30-90% of

the parent compounds in their feces or urine. The excretion rate of antibiotic residues and ARGs, however, depend on the type of drug, the dose, and the age of the animal (Katz, 1980). The high presence of antibiotics in feces thus represents an environmental and human health concern as land application of manure is a common practice around the world (Zhao et al., 2010). Exposing bacteria in the soil to the antibiotics and ARGs present in the manure has the potential to create reservoirs of resistant traits in soil bacteria, which could ultimately affect human health upon consumption of food from these fields (Knapp et al., 2010). Terracumulation, or the amount of accumulated antibiotics and ARGs in the soil, will thus become a huge concern when the rate at which the antibiotics deposited on the soil surpasses the rate of degradation (Michigan State University, 2015). The accumulation of antibiotics in aquatic environments also concerns those involved with aquaculture because aquatic creatures, such as farm-raised salmon, are also susceptible to infectious diseases (Huang et al., 2015). On these farms, antibiotics are administered to the animals and there is a major risk of antibiotic resistance developing in bodies of water that has the ability to transfer to the humans drinking that water (Driscoll and Crank, 2015). Thus, soil types, irrigation patterns, fertilization techniques, age of animals, amount of antibiotic used, and sanitation techniques all play a part in the dissemination of antibiotic resistance (Knapp et al., 2010; Katz, 1980; Tao et al., 2014).

Discussion

As mentioned above the transmission of antibiotic resistance to humans can happen in various ways. In this section, I will provide evidence for this transmission and examine controversies in the debate.

Antibiotic Resistance Spread through Fecal Matter

According to Zhao et al. (2010), antibiotic residue excreted into animal manure enters the environment one of two ways: (1) using manure as fertilizer or (2) entering as sludge after manure collection and storage. These processes result in the accumulation of antibiotics in agriculture fields and adjacent areas to the farms (Roman et al., 1999) and add a dramatic amount of bacteria with ARGs to the soil (Heuer et al., 2011b). Zhao et al. (2010) found that multiple classes of antimicrobial compounds could be detected in manure samples, and the compounds present were dependent on type of animal, geographical locations and prescribing habits. Mathew et al. (1999) demonstrated that increased incidence of antibiotic resistance in nursery pigs was a result of pathogens colonizing young pigs at an increased rate and an increased use of antibiotics. The antibiotics used led to single and multidrug resistance patterns (Mathew et al., 1999). Of note, Zhao et al. (2010) tested fecal samples from swine, cows, and chickens while Mathew et al. (1999) tested only pigs. Both studies explain how the type of animal on the farm factors into the amount and type of antibiotic present in the manure. Heuer et al. (2011b) decided to examine manure spreading further by comparing fields without manure, with manure and with manure treated with antibiotics. Specifically, Heuer et al. (2011b) tested the impact of a sulfonamide drug, sulfadiazine (SDZ), on the presence of two ARGs, sul1 and sul2. They found that manure treated with SDZ results in an increase of ARGs (Heuer et al., 2011b). Knapp et al. (2010) provides further evidence of an increase in ARGs overtime with their examination of five-long term soil-series in The Netherlands from 1940 to 2008.

Furthermore, when the active ingredients penetrate to the upper soil layer, there is the potential that the antibiotic residues will runoff into groundwater or surface bodies of water from which humans draw their drinking water (Jongbloed and Lenis, 1998). Since humans receive constant exposure to bacteria in their environments due to the accumulation of ARGs in the soil, the world is experiencing an increase in antibiotic resistance, which threatens infectious disease therapies (Heuer et al., 2011b).

Handling Live Animals and Contaminated Meat

Those workers employed by large-scale animal operations are at risk of becoming colonized by resistant organisms because they come in contact with hundreds of animals that have been treated with antibiotics and that subsequently have developed resistant pathogens due to the mechanisms previously mentioned (Gilchrist et al., 2007). Armnad-Lefevre et al. (2005) conclude that resistant organisms frequently move from pigs to pig farmers. Levy et al. (1976a) tested farms with chickens raised with and without feed containing tetracycline, a human antibiotic, to track the spread of tetracycline-resistance. On farms without tetracycline supplements neither the farmers nor the animals tested positive for resistance in their intestinal flora (Levy et al., 1976a). After implementation of supplements, 31.3% of samples from farm members and 6.8% of samples from neighbors showed tetracycline-resistance (Levy et al., 1976a). This result suggests a transmission of antibiotic resistance from animals to humans. Van den Bogaard et al. (2001) elucidated a similar trend in turkeys and turkey farmers in the Netherlands by establishing a transmission route of ciprofloxacin-resistance. Controversy arises here on whether resistance can

actually travel from animals to humans. Many studies on this topic involved the use of serotyping, a method to subcategorize strains of Escherichia coli (van den Bogaard et al., 2001). This technique examines the o-antigen on the surface of the E. coli, which is the outermost part of the lipopolysaccharides that line the surface of this bacteria. These antigens can be used to track the origins of bacterial strains and provide evidence for or against the transmission of resistance from animals to humans (Linton et al., 1977; Shooter et al., 1974). For instance, Linton et al. (1977) found the same serotype in chickens and in humans suggesting a transmission route. In contrast, Shooter et al. (1974) complicates the issue by providing some evidence that animal and human strains of E. coli come from two separate resistance pools. This issue has several perspectives, but some can agree that as the number of resistant bacteria increase within a food animal population, the probability of transferring resistance to human populations will rise (van den Bogaard et al., 2001).

Wastewater Treatment Plants

Another way that ARGs and antibiotic residues leave farms is through wastewater treatment plants that are either on the farm property or nearby (Tao et al., 2014). Five ARGs were tested at four common sanitation steps on six farms in Taiwan (Figure 4) (Tao et al., 2014). According to Tao et al. (2014), ARGs were higher in anaerobic units, and lower in aerobic tanks and at the effluent. Although the anaerobic tank induces growth of resistant bacteria, it is still a necessary step in the sanitation process (Tao et al., 2014). These plants should increase the amount of time that the water is exposed to the sun and increase oxygen content to promote the degradation of organic compounds by aerobic bacteria (Tao et al., 2014). Overall, these plants effectively remove bacteria from the water, but there is still more work to do in order to combat more ARGs and increase the purification level of the water (Tao et al., 2014).

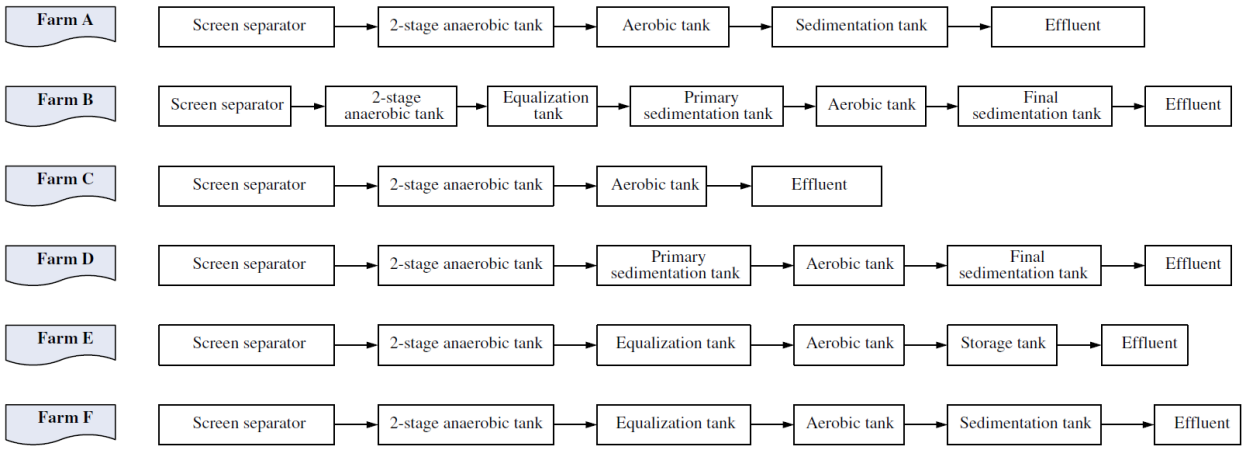


Figure 4: Flowchart illustrating the process that wastewater from six swine farms in Taiwan follows. The dirty water full of ARGs enters at the screen separator step and theoretically is clean at the effluent stage when it is released back into the environment (Tao *et al*., 2014).

Aquaculture

Specific antibiotic resistant determinants have been discovered in isolates from both aquaculture and clinical settings suggesting a connection between food animals and humans (Huang et al., 2015). For instance, McIntosh et al. (2008) detected ARGs, bla_{CYM-2}, sugE, and blc, in Aeromonas salmonicida ssp isolated from an Atlantic Canadian salmon farm that were identical to genetic elements widely distributed in clinical and food-borne Salmonella and other Enterobacteriaceae in Asia and the United States. Huang et al. (2015) tested 4767 isolates from an aquaculture ecosystem and found that 3771 or 79.1% exhibited resistance to more than one antibiotic. The majority of isolates from this ecosystem and the fish were found to be resistant to two or more antibiotics (Huang et al., 2015). Also, if the fish feed is not processed properly in the manufacturing step, a high risk of spreading antibiotic resistance down the food chain will result because the antibiotic resistant bacteria are allowed to persist in the environment (Huang et al., 2015). Multidrug-resistant bacteria, however, were found in fish farms that had no history of antibiotic use, which complicates this puzzle (Huang et al., 2015). Multiple factors, such as antibiotic resistant rich fish feed or environmental factors, thus intersect with the development of resistance besides the amount of antibiotics administered to animal populations (Huang et al., 2015).

Solutions

Now that we have outlined a few various routes of resistance transmission from animals to humans, we need to address possible solutions to this public health issue. At the most basic level, Gilchrist et al. (2007) recommends reducing animal crowding, improving hygiene, controlling temperature and ventilation, and reducing stress on the animals in order to help the animals better resist disease. If farmers can prevent disease spread in the first place, antibiotic use and development of resistance will not be a concern. As for the wastewater treatment plants, using solid reservoirs rather than earthen waste lagoons could prevent manure contamination (Gilchrist et al., 2007). The WHO (2001) implores veterinarians to use antibiotics prudently and to develop guidelines for antibiotic use for the populations they serve. For food animal producers, the WHO (2001) agrees with Gilchrist et al. (2007) that these farms need to improve hygiene and adds that they should reduce the amount of AMGPs they use while trying to improve animal husbandry. Seppala et al. (1997) provides support for the benefits of reducing antibiotic use. They found a 50% reduction in macrolide-resistant group A *streptococci* in Finland after farmers reduced the administration of macrolides to their animals (Seppala et al., 1997). This solution, however, is not as simple as it seems because Enne et al. (2001) found that a 6.2% increase in frequency of sulfonamide resistance followed a 98% decrease in sulfonamide prescriptions in the UK during the 1990s. Reversing antimicrobial resistance depends on several factors, such as rates of reacquisition of resistance and mutations that cause changes in microbial physiology to reduce the fitness costs of ARGs (Johnsen et al., 2009). Thus, in order to make a large impact on slowing down or preventing the spread of antibiotic resistance people must have a holistic approach. Most importantly, we must continue to monitor trends in the spread of antibiotic resistance, such as those trends exemplified by the Danish with their Danish Integrated Antimicrobial Resistance Monitoring and Research Program (DANMAP) (Michigan State University, 2015). Programs, such as DANMAP, will help us quantify and qualify the problem.

Summary

The spread of antibiotic resistance has been on the rise since the first prescription of the first antibiotic, penicillin, in the 1940s (CDC, 1999). In 1998, the Institute of Medicine estimated the annual cost of infections due to antibiotic resistant bacteria fell between $4 and $5 million (McGowan, 2001). From the evidence presented above, I argue that large-scale feedlots and aquaculture are contributing to the spread of antibiotic resistance worldwide. In summary, overcrowding, poor sanitation and antibiotic usage are the major factors that further spread antibiotic resistance in our food and our environments. Unless we can find new alternatives or develop new antimicrobials, we could move into an era in which we no longer have treatment options for clinical infections (Johnsen et al., 2009).

Future Work

Research is needed to determine alternatives to antibiotics, such as probiotics or promising vaccines (Gilchrist et al., 2007). Probiotics can add beneficial bacteria to the GI tract of food animals, which could potentially help them resist infections caused by pathogenic bacteria (Gilchrist et al., 2007). Farms and slaughterhouses also need to investigate ways to prevent the spread of resistance through the air, water and direct contact to workers in order to protect their employees and neighbors from unnecessary drug resistant infections (Gilchrist et al., 2007). The federal government could also play a role in regulating farms and slaughter houses. The general populace has the power to appeal to their legislators to pass further restrictions to control antibiotic usage in order to protect the environment and themselves. Society must be aware of what goes into their food and the potential effects on future generations and the environment.

References

Acar J. Casewell M, Freeman J, Friis C, Goossens H. 2000. Avoparcin and virginiamycin as animal growth promoters: a plea for science in decision-making. *Clin Microbiol Infect*. 6(9):477-482.

Alcock R, Sweetman A, Jones K. 1999. Assessment of organic contaminant fate in wastewater treatment plants I: selected compounds and physiochemical properties. *Chemosphere.* 38:2247-2262.

Armand –Lefevre L. Ruimy R, Andremont A. 2005. Clonal comparison of Staphlococcus aureus isolates from healthy pig farmers, human controls, and pigs. *Emerg Infect Dis.* 11:711-714.

CDC (Center for Disease Control and Prevention). 1999. Achievements in Public Health, 1900-1999*: Control of Infectious Diseases*. [Internet]. [cited 15 Nov 2015]. Available from http://www.cdc.gov/mmwr/preview/mmwrhtml/mm4829a1.htm

Davies J. 1994. Inactivation of antibiotics and the dissemination of resistance genes. *Science.* 264(5157):375-382.

Driscoll T, Crank C. 2015. Vancomycin-resistant enterococcal infections: epidemiology, clinical manifestations and optimal management. *Infection and Drug Resistance*. 8:217-230.

Enne V, Livermore D, Stephens P, Hall L. 2001. Persistance of sulphonamide resistance in *Escherichia coli* in the UK despite national prescribing restriction. *Lancet*. 357:1325-1328.

Gilchrist M, Greko C, Wallinga D. 2007. The potential role of concentrated animal feeding operations in infectious disease epidemics and antibiotic resistance. *Environmental Health Perspectives*. 115(2):313-316.

Heuer H, Heike S, Smalla K. 2011a. Antibiotic resistance gene spread due to manure application on agricultural fields. *Current Opinion in Microbiology.* 14:236-243.

Heuer H, Solehati Q, Zimmerling U, et al. 2011b. Accumulation of sulfonamide resistance genes in arable soils due to repeated application of manure containing sulfadiazine. *Applied and Environmental Microbiology.* 77(7):2527-2530.

Huang Y, Zhang L, Tiu L, Wang H. 2015. Characterization of antibiotic resistance in commensal bacteria from an aquaculture ecosystem. *Frontiers in Microbiology*. 6:1-7.

Johnsen P, Townsend J, Bohn T, Simonsen G, Sundsfjord A, Nielsen K. 2009. Factors affecting the reversal of antimicrobial-drug resistance. *Lancet.* 9:357-364.

Jongbloed A, Lenis N. 1998. Environmental concerns about animal manure. *J Anim Sci.* 76:2641-2648.

Katz S. 1980. The effects of human health. Subtherapeutic use of antimicrobials in animal feeds. Washington, DC: National Academy of Sciences.

Khachatourians G. 1998. Agricultural use of antibiotics and the evolution and transfer of antibiotic-resistant bacteria. *Canadian Medical Association Journal*. 159(9):1129-1136.

Knapp C, Dolfing J, Ehlert P, Graham D. 2010. Evidence of increasing antibiotic resistance gene abundances in archived soils since 1940. *Environ Sci Technol*. 44:580-587.

Levy S, Fitzgerald G, Macone A. 1976. Changes in intestinal flora of farm personnel after introduction of a tetracycline-supplemented feed on a farm. *N Engl J Med*. 295(11):583-588.

Linton A, Howe K, Hartley C, Clements H, Richmond M, Osborne A. 1977. Antibiotic resistance among *Escherichia coli* O-serotypes from the gut of carcasses of commercially slaughtered broiler chickens: a potential public health hazard. *Journal of Applied Bacteriology*. 42:365-378.

Mathew A, Saxton A, Upchurch W, Chattin S. 1999. Multiple antibiotic resistance patterns of *Escherichia coli* isolates from swine farms. *Applied and Environmental Microbiology*. 65(6):2770-2772.

Michigan State University. 2015. Veterinary Public Health Module [Internet]. [cited 15 Nov 2015]. Available from http://amrls.cvm.msu.edu/veterinary-public-health-module

McIntosh D, Cunningham M, JI B, et al. 2008. Transferable, multiple antibiotic and mercury resistance in Atlantic Canadian isolates of *Aeromonas salmonicida* subsp. Salmonicida is associated with carriage of an IncA/C plasmid similar to the Salmonella enterica plasmid pSN254. *J Antimicrob Chemother*. 61:1221-1228.

Roman H, Thomas T, Klaus H, Kari-Ludwig K. 1999. Occurrence of antibiotics in the aquatic environment. *Sci Total Envirn*. 225:109-118.

Seppala H, Klaukka T, Vuopio Varkila J, et al. 1997. The effect of changes in the consumption of macrolide antibiotics on erythromycin resistance in group A streptococci in Finland. *N Engl J Med*. 337:441-446.

Shooter R, Cooke E, O'Farrell S, Bettelheim K, Chandler M, Bushrod F. 1974. The isolation of *Escherichia coli* from a poultry packing station and an abattoir. *Journal of Hygiene, Cambridge.* 73:245-247.

Smith D, Harris A, Johnson J, Silbergeld E, Morris J. 2002. Animal antibiotic use has an early but important impact on the emergence of antibiotic resistance in human commensal bacteria. *Proc Natl Acad Sci*. 99:6434-6439.

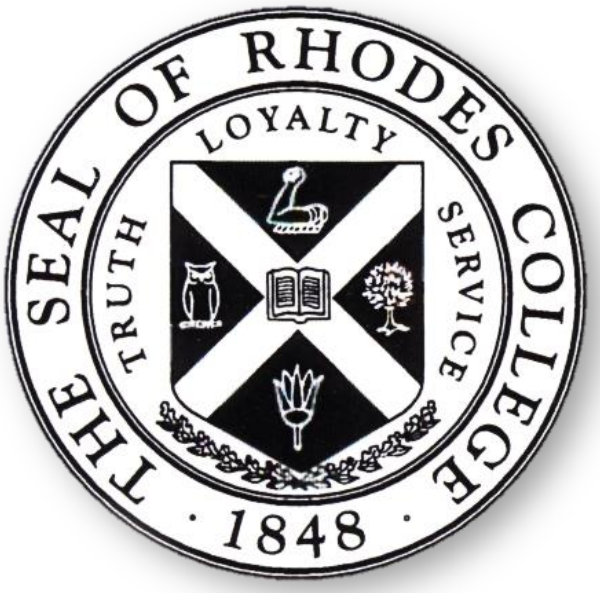
Tao C, Hsu B, Ji W, et al. 2014. Evaluation of five antibiotic resistance genes in wastewater treatment systems of swine farms by real-time PCR. *Science of Total Environment*. 496:116-121.

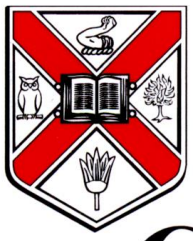
UCS (Union of Concerned Scientists). 2001. Hogging it: estimates of antimicrobial abuse in livestock [Internet]. [cited 15 Nov 2015]. Available from http://www.ucsusa.org/food_and_agriculture/our-failing-food-system/industrial-agriculture/hogging-it-estimates-of.html#.VklQyb90d9M

WHO (World Health Organization). 2001. Antibiotic resistance: synthesis of recommendations by expert policy groups [Internet]. [cited 15 Nov 15]. Available from http://www.who.int/csr/resources/publications/drugresist/WHO_CDS_CSR_DRS_2001_10/en/

Van den Bogaard A, London N, Driessen C, Stobberingh E. 2001. Antibiotic resistance of fecal *Escherichia coli* inn poultry, poultry farmers and poultry slaughterers. *Journal of Antimicrobial Chemotherapy*. 47:763-771.

Zhao L, Dong Y, Wang H. 2010. Residues of veterinary antibiotics in manures from feedlot livestock in eight provinces of China. *Science of the Total Environment*. 408:1069-1075.





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