NATURAL SCIENCE

four pieces

THE EFFECT OF STRESS, TIME OF DAY, AND ADRENAL HORMONES ON BMAL1, PER2 AND C-FOS EXPRESSION IN RAT HIPPOCAMPUS AND AMYGDALA

Jeanelle France

The following is an excerpt from a longer piece. For full text, please visit www.honorsjournal.com

ABSTRACT

The term circadian rhythm refers to the behavioral and physiological changes an organism exhibits during an approximately 24-hour period. Plants, animals and fungi all maintain circadian rhythms, by part, with a molecular clock system. The molecular clock consists of a positive and negative feedback loop that alter gene transcription and translation. BMAL1 and CLOCK make up the positive transcription arm that stimulates the production of two classes of genes: period (Per 1-3) and cryptochrome (Cry 1-2). PER and Cry protein make up the negative feedback loop of the molecular clock and inhibit their own transcription in the nucleus.

The molecular clock system must be reset, or entrained, on a daily basis. The steroid hormone corticosterone (CORT) is thought to be the hormonal signal that entrains peripheral molecular clock systems. CORT is released when stressors activate the hypothalamic pituitary adrenal (HPA) axis. This study examines the role that stress, endogenous CORT, and time of day play in the expression of Per2, Bmal1 and c-Fos in rat hippocampus and amygdala. C-fos was chosen as a positive control to ensure that cells were generally responsive to stress. Acute restraint stress was used to model mild psychological stress in rats. Endogenous CORT was manipulated using sham and adrenalectomy (ADX) surgeries. Tissue samples were taken at ZT4 and ZT16 and analyzed via insitu hybridization to measure relative mRNA levels.

A robust stress effect was seen with c-Fos expression in all brain regions examined except for the superior portion of the dentate gyrus. Per2 shows a significant time of day effect in CA1, CA3 and BLA. CEA showed an interesting time of day by endogenous CORT status interaction. These data show that Per2 and Bmal1 are not rapidly induced by stress like the clock gene Per1. Future studies should analyze data at supplementary time points and alter the type of stressor utilized to see if those variables effects Per2 and Bmal1 expression.

[…]

CURRENT STUDY

Genes of Interest

Per 2: Circadian rhythms in hippocampus and amygdala are closely coupled with the sympathetic nervous system and HPA axis. Analyzing how Per2 expression is altered in stressful contexts is one of the first stepping-stones in discovering how stress, mood, memory and circadian rhythms all interact with one another. Examining Per2 in a stressful context may help shine light on possible cellular mechanisms that mediate various forms of circadian dysregulation. If there is a linkage between stress and dysregulation of Per2, future studies can focus on how to regulate this

component of the molecular clock in order to see if it induces therapeutic effects.

There have been no studies that examined the effects of stress on Per2 expression. Studies have shown that Per1 can be rapidly induced under stressful conditions. For this reason, it is predicted that Per2 expression will be stress and endogenous CORT sensitive in each region of interest.

Bmal1: Bmal1 was chosen as a gene of interest because it is involved in the positive transcription loop in the molecular clock. Expression patterns are typically antiphasic of Per2, due to the fact that these two clock genes participate in complementary transcriptional/translational activities (Figure 3)[12]. We predict that Bmal1 expression will display patterns of oscillation that are in opposite phase of Per2.

C-Fos: C-Fos was chosen as a positive control because it can be utilized as a biomarker for cellular activity[29,30]. Detectable levels of c-Fos would indicate that the cell was generally reactive to stress[29]. C-Fos also displays a circadian pattern of activation, with levels being highest during the animal's wake cycle. For this reason, c-Fos expression in the hippocampus and amygdala should display higher levels at ZT16 than ZT4. Stress will also prompt a rapid induction of c-Fos.

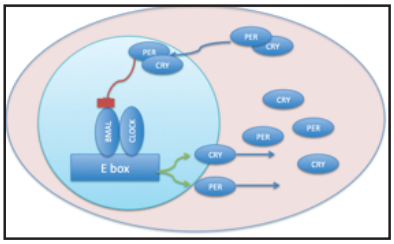


Figure 3: Simplified model of the mammalian molecular clock system. To initiate PER and CRY transcription, BMAL and CLOCK dimerize in the nucleus. This complex interacts with the E box to prompt transcription of cry1-2 and per1-3. When levels of CRY and PER reach their peak they will begin to dimerize. Phosphorylation tags the dimer for translocation into the nucleus where it inhibits the BMAL/CLOCK complex for initiation transcription of more PER & CRY proteins

Time of Day & Adrenal Hormones

Tissue samples were taken at ZT4 and ZT16. These time points are 4 hours past the start of the light or dark phase respectively. The four hours were used as an acclimation period. The status of adrenal hormones was altered surgically. Animals either received an adrenalectomy (ADX) or sham surgery. Removal of the adrenal gland also removes endogenous CORT. This manipulation allows us to examine if the stress effects observed are CORT mediated.

Regions of Interest (ROIs)

The ventral tegmental area (VTA) is a brain region that is important for learning and memory as well as mood regulation[31]. VTA sends direct dopaminergic projections to hippocampus and amygdala. Rodents that possess a Per2 mutation displayed greater depressive symptoms in the forced swim task. These animals also presented with elevated levels of dopamine in VTA[32]. Studies have also shown that acute stress can lead to a decrease in long-term potentiation, a phenomenon vital for learning and memory functions, in VTA[33]. Because both hippocampus and amygdala receive direct innervation from VTA, they are brain regions that are heavily implicated in the generation of depressive mood in humans who have been exposed to acute stress[34] and in animals that have received acute or prolonged stress[35].

Few studies have analyzed levels of Per2 and Bmal1 expression under stressful conditions in hippocampus and amygdala. This study is an attempt to contribute more data on mood and memory structures and their molecular clocks' reactivity to stress. In hippocampus we expect to see stress induced c-Fos expression in all regions[32]. No studies have examined the role stress plays in Per2 expression. If Per2 has a similar physiology as Per1, it should have stress-induced expression in both the hippocampus and amygdala.

METHODS

Subjects

The subjects were commercially obtained male Sprague Dawley rats aged 3 months (Harlan Laboratories; Harlan,

Indianapolis, IN). Rats were divided evenly into four different light/dark rooms as described in supplementary figure 1. Food and water were available ad libitum. After arrival at the facility, rats were given 2 weeks to acclimatize to their new environment. The acclimation period is vital for ensuring that any displayed stress effects are due to the experimental treatment rather than a reaction to a novel environment. Animals were ethically treated and their use in this experiment was approved by the University of Colorado's Institutional Animal Care and Use Committee.

[…]

Surgery

After the 2-week acclimation period, rats were given either adrenalectomies (ADX) or control (SHAM) surgeries. Both treatment groups received bilateral peritoneum incisions in order to expose the adrenal glands. In the ADX group, subjects' adrenal glands were located and removed using intestinal tissue forceps. The SHAM group followed the same procedure without removal of the adrenal glands. Both treatment groups were given one week to recover from surgery before test day. Trunk blood was then analyzed to ensure the absence of CORT in ADX animals.

The purpose of the surgery was to examine the role endogenous CORT plays in the expression of the aforementioned clock genes. In ADX subjects, no endogenous CORT is present while in the SHAM subjects, CORT is still being produced in a predictable manner.

Restraint Stress

In order to assess the role of stress in clock gene expression, half of the rats experienced restraint stress one week after surgery. 25 rats were kept in plastic restraint tubes acutely that allow for minimal movement for 30 min (diameter: 6.3 cm, length: 16cm). This type of stress has been shown to increase CORT in adrenal intact rats, which is a biomarker for stress effects[11].

Tissue Preparation

Immediately after restraint stress, decapitation took place at either ZT4 or ZT16. ZT16 subjects were sacrificed underneath a red light to reduce light induced clock gene expression[36,37]. Control rats were kept in their home cage until decapitation, whereas stressed rats were placed in restraint tubes 30 min before sacrifice. Brains were extracted and flash frozen in isopentane that was cooled with dry ice to between -20°C and -30 °C. Frozen brains were then wrapped in aluminum foil and kept in a -80°C freezer.

12µM coronal sections were obtained using a cryostat (Lecia CM 1850) at the level of the hippocampus/amygdala (Figure 7). Tissue sections were thaw mounted onto Colorfrost Plus microscope slides (Plus Slides, VMR) and stored at -70°C until subsequent use.

Insitu Hybridization & Densitometry

A two day insitu hybridization was conduced as previously described[36,37]. First, riboprobes for Per2, Bmal1, and c-Fos were generated in house following an established protocol[38].

[…]

To analyze the photo product of the insitu hybridization, the mean optical density of each slide was measured using ImageJ64(NIH). ImageJ allows for freehand definition of regions of interests (ROIs). For this analysis, the superior and inferior portions of the dentate gyrus of the hippocampus as well as the media, basolateral and central amygdala were examined bilaterally. Each subject had 8 different brain slices from the previously prepared coronal slices. Results from each slide were then averaged to find the mean optical density for each subject. The Paxinos and Watson Brain Atlas (1998) was used as a reference to locate ROIs.

[…]

RESULTS

The results from the ANOVA analysis of each gene within each ROI are summarized in Table 1. The significant main effects and interactions found for each gene are described below.

Per2

[…]

There was no significant effect of acute stress on Per2 mRNA expression in any ROI (Figure 8, Figure 9, Table 1). There were, however, time of day differences in CA1, CA3 and BLA, with greater levels of Per2 mRNA at ZT16 rather than ZT4. Interestingly, there was a significant adrenal status by time of day interaction in CEA. In contrast to the other brain regions, in CEA, Per2 mRNA levels of sham rats were lower at ZT16 than ZT4, but this time of day pattern was reversed in ADX rats.

Bmal1

[…]

There was no stress effect observed in any ROI. Time of day differences persisted in CA1, CA3 and BLA with ZT4 displaying more Bmal1 expression when compared to ZT16. This pattern is anti-phasic to patterns seen in Per2, which is to be expected given their roles in the molecular clock system. A significant adrenal effect was detected in SupraDG (Fig 10 and 11, Table 1) with SHAM animals having higher expression than those that received ADX.

C-Fos

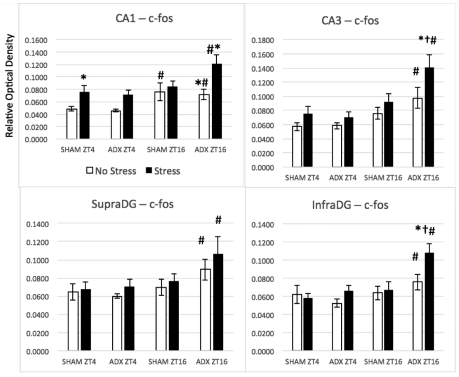


Figure 12: relative optical density reflecting c-fos expression in the following hippocampal nuclei: CA1, CA3, superior portion of the dentate gyrus and the inferior portion of the dentate gyrus. significant stress effect for subjects that share the same ZT and adrenal status. #significant ZT effect within groups that share the same stress and ZT conditions. ┼ adrenal status comparison within same stress and ZT conditions. Fisher's LSD test was used to establish significance in all cohorts (p<0.05)*

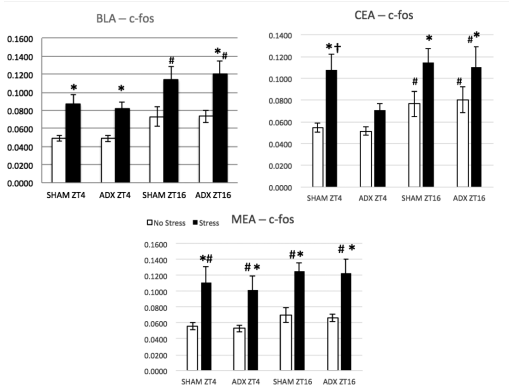


Figure 13: relative optical density reflecting c-fos expression in basolateral amygdala, central amygdala and medial amygdala. Post hoc analysis revealed significant stress effect for subjects that share the same ZT and adrenal status; #significant ZT effect within groups that share the same stress and ZT conditions. Fisher's LSD test was used to establish significance (p,0.05)*

In contrast to Bmal1 and Per2, there was a strong stress effect on c-Fos expression in all ROIs except for SupraDG. Stressful conditions consistently increased c-Fos levels. There was also a main effect of time for each ROI. In CA3, InfraDG and CEA there was an adrenal status effect (Figure 12, Figure 13, Table 1).

DISCUSSION

In contrast to previous studies examining Per121, there was no stress induction of Per2 or Bmal1 expression in any of the ROIs. However, two novel findings emerged from this study. First, a significant interaction of time of day and adrenal status was observed in Per2 induction in CEA. Second, an ADX effect was found for Bmal1 expression in SupraDG.

Per2

Per2 was expected to lose rhythmic expression in the amygdala following

ADX[35]; however, that trend was not directly observed. There was a main effect of time in CA1, CA3 and BLA. These rhythms are consisted with previously published research with adrenal intact animals[36].

Although insignificant, there was a general trend of ZT4 having higher expression than ZT16; however, this trend was reversed in sham animals in CEA. This result is consistent with other experiments that show Per2 mRNA and PER2 protein levels display opposite rhythms than BLA and hippocampus[35,36]. Interestingly, we found that this time of day difference had the opposite pattern in ADX rats, resulting in a significant time by adrenal status interaction.

Future studies should examine the role different types of stressors play in Per2 expression. Restraint stress activates the HPA axis, making it a good candidate to study mild stress. While mild stress did not alter Per2 expression, different stress models may yield dissimilar results. A good starting point would be analyzing prolonged and repeated stress because these conditions have been shown to modulate many processes that are under circadian control[39].

BMAL1

Stress had no effEct on BMAL1 expression in hippocampus or amygdala. The results show a significant time of day effect in CA1 and CA3 for Per2 with expression being higher at ZT16. Bmal1 displayed higher expression at ZT4 in both of these brain regions as well as BLA. The molecular clock model suggests that Per2 expression should be anti-phasic of Bmal1, which is consistent with these data[1]. This may be due to the time points selected. Additional time points could possibly see oscillatory pattern of Bmal1 expression.

Bmal1 also showed a significant ADX effect in SupraDG. This may have to do with the region's importance in memory consolidation. Bmal1 has the ability to alter MAPK functionality via circadian entrained processes[40]. CORT is the signal that is used by SCN to entrain the MAPK pathway. The effects of CORT can be modulated by Bmal1 via acetylation of GCRs (Figure 15)[16]. For these reasons, Bmal1 may be less responsive to the influence of stress induced CORT that is out of sync with its typical rhythms. This dampened response could help explain why some memory processes are disrupted with stress while others remain unaltered1[6,41].

c-Fos

c-Fos was the most reactive of the genes analyzed. It showed the greatest stress effects in amygdala and hippocampus (except for supraDG). Previous studies have established c-Fos as a marker for cellular activity. The robust stress response could be an indication that the restraint stress elicited physiological changes that are consistent with activation of the HPA axis.

There was also a trend for c-Fos expression to be higher at ZT16. This is consistent with other studies that show higher c-Fos expression during the active phase of animals. In all, these data show that c-Fos was a reliable positive control for this study. These findings are also consistent with behavioral studies. Rapid c-Fos induction has been seen in the hippocampus after swim stress and restraint stress in mice (Figure 15)[32].

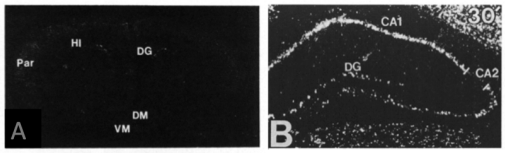


Figure 15: results from Cullinan et al. 1994. (A) Shows basal expression of c-fos in hippocampus(HI)(B)Shows immunolabeled cells that express c-fos mRNA 30 minutes after swimming stress. In our study, there was not a robust induction of c-fos in the hippocampus following restraint stress

Future studies should have animals that were restraint stressed and analyze their c-Fos mRNA levels minutes and hours after stress has stopped to see how long the stress effect is maintained.

CONCLUSION

The purpose of this study is to examine the role stress, endogenous CORT and time of day play in the expression of Per2, BMAL1 and c-Fos in hippocampus and

amygdala. These data will allow us to understand how stress influences the functionality of the molecular clock system in these extra SCN regions. Stress was measured using restraint stress and endogenous CORT was altered using adrenalectomy. Per2 and Bmal1 were chosen as the clock genes of interest because few studies have examined the role stress and adrenal status play on their expression in hippocampus and amygdala36,37. c-Fos was used as a positive control to confirm stress reactive brain regions. Insitu hybridization analyzed the relative amount of mRNA from each gene of interest.

Acute stress had no effect on clock gene expression; however, time of day effects were seen in CA1, CA3 and BLA for Per2 and Bmal1. These differences in expression likely display the circadian cycling of these mRNA transcripts. This is a heavy implication to make when only two time points are utilized, so future studies should examine the role of acute stress at additional time points.

Taken together, the data paves the way for new research. Further studies should be conducted on Per2 to see if its oscillatory pattern is fully maintained at different time points when exposed to stress. BMAL1 expression should be analyzed under different stress conditions to see if it can induce expression in some contexts rather than others, like Per1. C-Fos data can be used as a basis of comparison for future studies as well.

WORKS CITED

1. Buttgereit, F., Smolen, J. S.,Coogan, A. N. & Cajochen, C. Clocking in : chronobiology in rheumatoid arthritis. Nat. Publ. Gr. 1–8 (2015). doi:10.1038/nrrheum.2015.31

11. Kearns, R. R. & Spencer, R. L. Physiology & Behavior An unexpected increase in restraint duration alters the expression of stress response habituation. *Physiol. Behav.* 122, 193–200 (2013).

12. Duncan, M. J., Prochot, J. R., Cook, D. H., Smith, J. T. & Franklin, K. M. Influence of aging on Bmal1 and Per2 expression in extra-SCN oscillators in hamster brain. *Brain Res.* 1491, 44–53 (2013).

16. Becker-krail, D. & Mcclung, C. Implications of circadian rhythm and stress in addiction vulnerability [version 1 ; referees : 2 approved]. *F100Research* 59, 1–10 (2016).

21. Al-safadi, S., Branchaud, M., Rutherford, S. & Amir, S. Glucocorticoids and Stress-Induced Changes in the Expression of PERIOD1 in the Rat Forebrain. 1–13 (2015). doi:10.1371/journal.pone.0130085

29. Sagar, S. ., Sharp, F. . & Curran, T. Expression of c-fos Protein in Brain : Metabolic Mapping at the Cellular Level. *Science* (80-.). 240, 1328–1331 (1988).

30. Jon M. Kornhauser, Kell E. Mayo, J. S. T. Light, immediate-early genes and circadian rhythms. *Behav. Genet.* 26, 221–240 (1996).

31. Gerstner, J. et al. Cycling Behavior and Memory Formation. *J. Neurosci.* 29, 12824–12830 (2009).

32. W.E. Cullinan, J.P. Herman, D.F. Battaglia, H. Akil, S. J. W. Pattern and time course of immediate early gene expression in rat brain following acute stress.

33. Polter, A. M. & Kauer, J. A. Stress and VTA synapses: Implications for addiction and depression. *Eur. J. Neurosci.* 39, 1179–1188 (2014).

34. Gobinath, A. R. et al. Influence of sex and stress exposure across the lifespan on endophenotypes of depression : focus on behavior , glucocorticoids , and hippocampus. 8, 1–18 (2015).

35. Lamont, E. W., Robinson, B., Stewart, J. & Amir, S. The central and basolateral nuclei of the amygdala exhibit opposite diurnal rhythms of expression of the clock protein Period2. 6–10 (2005).

36. Chun, L. E., Woodruff, E. R., Morton, S., Hinds, L. R. & Spencer, R. L. Variations in Phase and Amplitude of Rhythmic Clock Gene Expression across Prefrontal Cortex , Hippocampus , Amygdala , and Hypothalamic Paraventricular and Suprachiasmatic Nuclei of Male and Female Rats. 30, 417–436 (2015).

37. Woodruff, E. R. et al. Adrenal-dependent diurnal modulation of conditioned fear extinction learning. *Behav. Brain Res.* 286, 249–255 (2015).

39. Tahara, Y., Shiraishi, T., Kikuchi, Y., Haraguchi, A. & Kuriki, D. Entrainment of the mouse circadian clock by sub-acute physical and psychological stress. *Nat. Publ. Gr.* 1–11 (2015). doi:10.1038/srep11417

40. Eckel-mahan, K. L. Circadian oscillations within the hippocampus support memory formation and persistence. 5, 1–4 (2012).

41. Giles, G. E., Mahoney, C. R., Brunye, T. T. & Taylor, H. A. Stress Effects on Mood , HPA Axis , and Autonomic Response : Comparison of Three Psychosocial Stress Paradigms. 1–19 (2014). doi:10.1371/journal.pone.0113618

EFFECTS OF CORAL BLEACHING ON ABUNDANCE, FEEDING BEHAVIOR, AND HABITAT USE OF THREE SYMPATRIC BUTTERFLYFISH

Madison Sankovitz

The following is an excerpt from a longer piece. For full text, please visit www.honorsjournal.com

ABSTRACT

The Great Barrier Reef is an extremely complex ecosystem, home to more than 1,500 fish species and countless other vertebrates and invertebrates. Currently, a massive coral bleaching event is occurring (the fourth of these events since 1998), which is killing live coral all over the reef. Coral bleaching occurs because of global climate change and subsequent rising ocean temperature. The purpose of this study was to investigate the question of how coral bleaching affects three sympatric butterflyfish species, *Chaetodon auriga, Chaetodon ocellicaudus*, and *Chaetodon plebeius*, at Horseshoe Reef and Palfrey Island Reef, both located off the coast of Lizard Island, Australia. The primary hypothesis states that coral bleaching has a negative effect on fish abundance because it decreases the amount of live coral for fish to feed on and use as shelter. The second hypothesis states that omnivorous fish, or generalists, will be able to survive better in a bleached environment than coralivorous fish, or specialists. Fish abundance and behavior data were collected using a visual belt transect method and substrate composition data were collected using a line transect. Results did not clearly support either hypothesis. Focal species were overall more abundant at Palfrey Island Reef, which showed the most coral bleaching, but focal species spent more time feeding at Horseshoe Reef compared to Palfrey Island Reef. In addition, *C. auriga* (omnivorous species) was observed utilizing a wider variety of microhabitats than *C. ocellicaudus* and *C. plebeius* (coralivorous species), but *C. auriga* was not more abundant than the other focal species at Horseshoe Reef (the most bleached site) as predicted. Although no strong conclusions were drawn from the findings of this study, the data allow humans to better understand the behavior of omnivores and coralivores in habitats being overcome by coral bleaching.

INTRODUCTION

The Great Barrier Reef is one of the largest and most complex ecosystems on the earth, housing more than 1,500 fish species, fostering a complex biodiversity of vertebrates and invertebrates alike, and covering approximately the same area as Japan ("Great Barrier Reef Facts," n.d.). Like all other ecosystems, the reef is regularly affected by natural disturbances such as cyclones and Crown of Thorns Starfish outbreaks. In addition to these natural phenomena, global climate change has caused ocean temperatures to rise and cause four massive coral bleaching events since 1998, including one that is happening currently ("Coral Bleaching," n.d.). Lizard Island, off the coast of Queensland, is settled on an exemplary section of the Great

Barrier Reef, in which the biodiversity of the reef is upheld by the interconnected systems of flora and fauna working in complex give-and-take relationships. Many of the reefs surrounding the island also have been affected by the current coral bleaching event. A previous study on the effects of coral bleaching on coral habitats and associated fishes examined the breadth of species that are negatively affected by coral bleaching (Pratchett et al., 2012). The research revealed that fish species that directly feed on coral are most strongly affected by coral bleaching and die off in the greatest numbers, but even non-coralivorous fishes still depend on the coral structure for their habitat and thus their population decreases from coral bleaching. Reef ecosystems are so tightly interconnected that all fish species are affected by climate change, rising ocean temperature, and subsequent coral bleaching events (Pratchett et al., 2012).

There were two identified hypotheses surrounding the question of how coral bleaching affects fish abundance and behavior. Possible explanations revolve around coral as a habitat and major food source in a reef ecosystem. The primary hypothesis states that coral bleaching has a negative effect on fish abundance because it decreases the amount of live coral for fish to feed on and use as shelter. Three species of butterflyfish were examined in this study: *Chaetodon auriga*, *Chaetodon ocellicaudus*, and *Chaetodon plebeius*. It is predicted that all three species of butterflyfish will be more abundant in reef habitats that are less bleached than in reef habitats that are strongly bleached. In addition, it is predicted that fish found in highly bleached areas will be observed swimming more frequently than actively eating, and vice versa for less bleached sites. A previous study examining resource partitioning among butterflyfish species revealed that bite rate is a good indicator of feeding habit and swimming is a good indicator of foraging (Zekeria et al., 2002). It is also predicted that butterflyfish will need to spend more time foraging in bleached environments where food is scarcer. These species depend on coral as an important habitat structure, and they must move elsewhere or die if their habitat is destroyed.

The second hypothesis states that omnivorous fish, or generalists, will be able to survive better in a bleached environment than coralivorous fish, or specialists. It is predicted that *C. auriga*, an omnivorous fish, will be more abundant and spend more time eating than swimming in a bleached environment than *C. ocellicaudus*, and *C. plebeius*, which are coralivorous fish.

There is a total of 114 species of butterflyfish found throughout the globe. Throughout this study three closely related species of butterflyfish that reside throughout the reef ecosystem surrounding Lizard Island, Australia were observed. The *C. auriga*, *C. ocellicaudus*, and *C. plebeius* are sympatric species that dwell in similar habitats and rely on interrelated resources. Abundance and feeding, swimming, shelter use, and interaction behavior were examined on two reefs off the shore of Lizard Island: Horseshoe Reef and Palfrey Island Reef. Data was also collected on the cover and level of bleaching of coral on these two reefs. The aim of the study was to investigate the question of how coral bleaching affects sympatric fish species on the Great Barrier Reef. The findings of the study will aid in better understanding how the abundance and behavior of generalist and specialist reef fish species is affected by coral bleaching.

RESULTS

Abundance

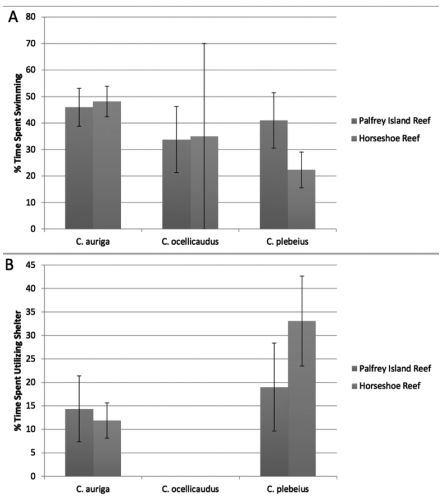
There were a total of 180 individual organisms counted throughout the abundance portion of this study: 62 *C. auriga*, 41 *C. ocellicaudus*, and 77 *C. plebeius*. Overall, Palfrey Island Reef had 4.4% more focus organisms (94 total) than Horseshoe Reef (86 total). Figure 1 shows a higher abundance of *C. auriga* at Palfrey Island Reef than at Horseshoe Reef. In addition,

Figure 1 displays a higher overall abundance of the three species on the reef flat compared to the reef crest at both sites.

[…]

Behavior

Observations were recorded for 32 *C. auriga*, 6 *C. plebeius*, and 23 *C. ocellicaudus*. Figure 2a displays the percentage of time each fish was observed swimming during the three- minute observation period. It shows that fish spent 6.7% greater time swimming at Palfrey Island Reef than at Horseshoe Reef, and *C. auriga* spent the most time swimming out of all the focal species. Figure 2b displays the percentage of time each fish was observed utilizing shelter during the observation period. It illustrates that fish spent 19.2% more time utilizing shelter at Horseshoe Reef than at Palfrey Island Reed, and *C. plebeius* utilized shelter the most out of all the focal species. Figure 2c shows the percentage of time each fish was observed feeding during the observation period. It illustrates that fish spent 6.2% more time feeding at Horseshoe Reef than at Palfrey Island Reef, and *C. ocellicaudus* spent the most time feeding out of all the focal species. Figure 2d displays the percentage of time each fish was observed interacting with other fish during the observation period. It shows that fish spent 27.3% more time interacting with other fish at Palfrey Island Reef than at Horseshoe Reef, and *C. plebeius* interacted the most out of all the focal species.



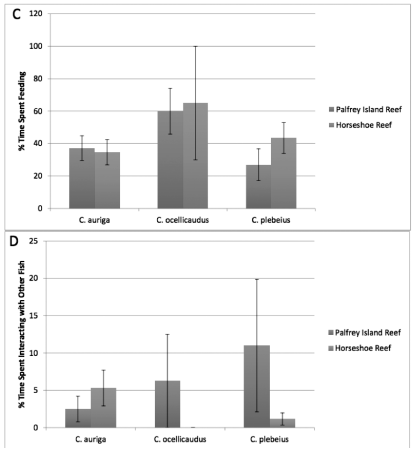


Figure 2. Percentage of time C. auriga, C. plebeius, and C. ocellicaudus were observed (a) swimming, (b) utilizing shelter, (c) feeding, and (d) interacting with other fish during the three-minute observation period at Horseshoe Reef and Palfrey Island Reef.

Tables 1 and 2 display the microhabitats each focal species was observed on or around during the three-minute observation period at Palfrey Island Reef and Horseshoe Reef, respectively. The two tables clearly show that hard coral was the utilized as habitat most often throughout the observation period at both reefs.

Table 1. Number of time C. auriga, C. plebeius, and C. ocellicaudus were observed utilizing microhabitats during the three-minute observation period at Palfrey Island Reef.

Palfrey Island Reef

	Hard Coral	Soft Coral	Dead Coral	Sand	Rubble
Blue Spot	10	0	0	0	0
Spot Tail	3	1	0	0	0
Thread-fin	14	0	2	0	0

Table 2. Number of time C. auriga, C. plebeius, and C. ocellicaudus were observed utilizing microhabitats during the three-minute observation period at Horshoe Reef.

Horshoe Reef

	Hard Coral	Soft Coral	Dead Coral	Sand	Rubble
Blue Spot	11	2	0	0	0
Spot Tail	1	1	0	0	0
Thread-fin	15	2	1	3	5

Substrate

Figure 3 displays the average percent cover of benthic coral and substrate observed along line transects at Horseshoe Reef and Palfrey Island Reef. Soft coral was most abundant at Horseshoe Reef, whereas rubble was the prominent ground-cover at Palfrey Island Reef. Although soft coral was dominant overall at Horseshoe Reef, there was a slightly greater amount of hard dead coral than soft coral on the reef crest at this site.

[…]

A total of 453 hard corals (228 at Horseshoe Reef and 225 at Palfrey Island Reef) and 632 soft corals (395 at Horseshoe Reef and 237 at Palfrey Island Reef) were observed along the line transects. 97.0% of hard coral (Figure 4a) and 97.7% of soft coral (Figure 4b) observed at Horseshoe Reef was bleached. 95.1% of hard coral and 98.7% of soft coral observed at Palfrey Reef was bleached. Overall, Horseshoe Reef had 0.4% more bleached coral (97.4% total bleached coral) than Palfrey Island Reef (97.0% total bleached coral).

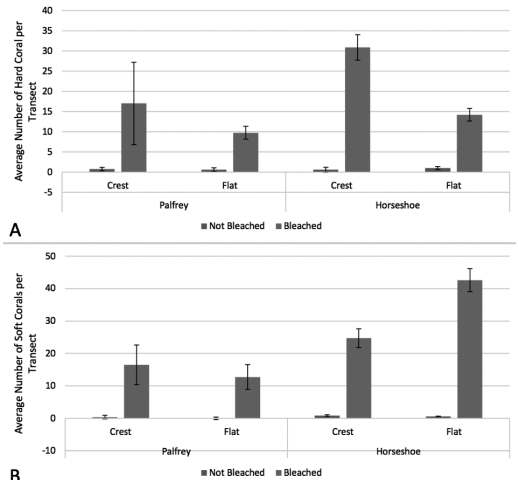


Figure 4. Average number of hard (a) and soft (b) corals observed per line transect on the reef crest and reef flat at Palfrey Island Reef and Horshoe Reef. Error bars show standard error for each category.

DISCUSSION

This study examines the effect of coral bleaching on the abundance and behavior of three species of butterflyfish. It was hypothesized that coral bleaching has a negative effect on fish abundance because it decreases the amount of live coral for fish to feed on and use as shelter. The results of this study only partially support this hypothesis, seeing as Palfrey Island Reef had 4.4% more focus organisms (94) than Horseshoe Reef (86) and Horseshoe Reef had 0.4% more bleached coral (97.4% total bleached coral) than Palfrey Island Reef (97.0% total bleached coral) overall. A possible explanation for this focuses on healthy coral as a food source and microhabitat. The three focal species feed on live coral and utilize it as a habitat structure, so the fish cannot survive in the absence of healthy coral. One study found that a loss of corals due to bleaching events negatively affects reef fish populations (Pratchett et al., 2012). There were more dead hard coral observed at Horseshoe Reef, which is a possible explanation of why fewer fish were observed at that site. Another study concluded that coral loss can immediately result in a loss of biodiversity in fishes that depend on coral as a food source (Pratchett et al., 2008). It was predicted that fish found in highly bleached areas will be observed swimming more frequently than actively eating, and vice versa for less bleached sites. Interestingly the results of this study do not agree with this prediction, seeing as fish spent 6.7% greater time swimming at Palfrey Island Reef than at Horseshoe Reef and 6.2% more time feeding at Horseshoe Reef than at Palfrey Island Reef.

The second hypothesis states that omnivorous fish, or generalists, will be able to survive better in a bleached environment than coralivorous fish, or specialists. The results of this study also only partially support this hypothesis. *C. auriga* is not the most abundant focal species at Horseshoe Reef, an outcome that was not predicted (Figure 1). Additionally, Figure 2c shows *C. auriga* spending more time feeding at Palfrey Island Reef than *C.*

ocellicaudus and *C. plebeius*, and Figure 2a shows *C. auriga* spending less time swimming (foraging) at Palfrey Island Reef than *C. ocellicaudus* and *C. plebeius*. These results to not support the second hypothesis. Contrastingly, Table 2 shows *C. auriga* occurring in every type of microhabitat, whereas *C. ocellicaudus* and *C. plebeius* only occur in live coral microhabitats, a finding that does support the hypothesis. *C. ocellicaudus* and *C. plebeius* eat a diet of solely coral, whereas *C. auriga* is omnivorous and only partially depends on live coral as a food source. The abundance and behavior data do not show that omnivores have an advantage over coralivores in bleached habitats, but the microhabitat data show omnivores dwelling in a wider range of coral and substrate than coralivores. These results do not lead to a clear conclusion about whether generalists or specialists are better suited to deal with coral bleaching events, like one study which found that generalists are more successful in a changing or disturbed environment than specialists (Wilson et al., 2008).

REFERENCES

Coral Bleaching. (n.d.) Retrieved March 28, 2016, from www.gbrmpa.gov.au/managing-the-reef/threats-to-the-reef/climate-change/what-does-this-mean-for-species/corals/what-is-coral-bleaching

Great Barrier Reef Facts. (n.d.). Retrieved March 28, 2016, from http://www.greatbarrierreef.org/about-the-reef/great-barrier-reef-facts/

Pratchett, M. S., Munday, P., Wilson, S. K., Graham, N. A., Cinner, J. E., Bellwood, D. R., … & McClanahan, T. R. (2008). Effects of climate-induced coral bleaching on coral-reef fishes. Ecological and economic consequences. *Oceanography and Marine Biology: Annual Review*, 46, 251-296.

Pratchett, M. S., Bay, L. K., Coker, D. J., Cole, A. J., & Lawton, R. J. (2012). Effects of coral bleaching on coral habitats and associated fishes. *Wildlife and climate change: towards robust conservation strategies for Australian fauna. ed by D. Lunney and P. Hutchings. Royal Zoological Society of NSW, Mosman, NSW*, 59-67.

Wilson, S. K., Burgess, S. C., Cheal, A. J., Emslie, M., Fisher, R., Miller, I., ... & Sweatman, H. (2008). Habitat utilization by coral reef fish: implications for specialists vs. generalists in a changing environment. *Journal of Animal Ecology*, 77(2), 220-228.

Zekeria, Z. A., Dawit, Y., Ghebremedhin, S., Naser, M., & Videler, J. J. (2002). Resource partitioning among four butterflyfish species in the Red Sea. *Marine and Freshwater Research*, 53(2), 163-168.

CAPITAL, LIVELIHOODS, AND RESILIENCE

A STUDY OF FARMING HOUSEHOLDS IN THE DRY ZONE OF SRI LANKA

Alejandra Pedraza

The following is an excerpt from a longer piece. For full text, please visit www.honorsjournal.com

EXECUTIVE SUMMARY

Anthropogenic climate change is transforming the natural environment, directly undermining the wellbeing of global populations and placing considerable stress on livelihood systems. In developing countries, the prominence of climate-sensitive sectors, primarily subsistence farming, promotes the conditional relationship between individual livelihoods and climate variability.

ADAPT – Sri Lanka carried out a survey (SEADS) encompassing responses from 25 different communities and 1148 households in the dry zone of Sri Lanka. The goal was to understand how farmers adjust their farming practices when they encounter challenges attributed to climate change. Utilizing data collected from SEADS, I analyzed and interpreted the relationship between the five types of capital, adaptive behavior, and sustainable livelihood outcomes in the context of farming households located within the dry zone of Sri Lanka. I associate adaptive behavior and sustainable livelihood outcomes as components of climate resilience. Climate resilience is the end goal for Sri Lankan farmers.

My statistical analysis concluded that the prevalence of adaptive behavior and sustainable livelihood outcomes is significantly dependent on a farmer having access to diversified capital. Based on my analysis, I identified financial and social capital as key players in promoting climate resilience.

Identifying valuable forms of capital is crucial in guiding both national and international efforts to build resilient systems in the face of climate change. Based on my research, I determined that financial capital receives the majority of attention. In turn, I argue that more attention needs to be devoted to providing the conditions favorable for the creation of social capital. Social capital creates the bonds of values, norms, and institutions that are a catalyst for development. Furthermore, development will facilitate the achievement of climate resilience.

Based on my analysis and research, I recommend Sri Lankan organizations to devote more resources towards Farmer Organizations and to invest in bringing people together. According to my analysis, a farmer that is part of a Farmer Organization is more likely to pursue adaptive behavior and in turn achieve sustainable livelihood outcomes. A greater flux of information and the creation of trust among farmers is a necessity to address the effects of climate change.

[…]

DISCUSSION

[…]

Research Question 1: Capital and Adaptation

The first goal of my statistical analysis was to shed light on the relationship between capital and adaptation. Specifically, I wanted to identify how having access to the different types of capital influenced adaptive behavior among farmers. Five dependent variables, discussed in the following subsections, were analyzed to represent a farmer's adaptability to climate change. This section breaks down my analysis from

Multivariate Regression Model 1.

Adaptive Efficacy

The first outcome variable I looked at was Adaptive Efficacy. Adaptive Efficacy refers to a farmer's perceived ability to overcome problems in cultivation. Overall, the data show that an increase in natural, financial/physical, and social capital increases a farmer's adaptive efficacy. On the other hand, human capital does not seem to have an influence.

The predictor variable used to represent natural capital is *Perceived Environmental Vulnerability*. Perceived Environmental Vulnerability refers to a farmer's perception of their yield's vulnerability to environmental threats. This variable was categorized as part of natural capital because a farmer's environmental vulnerability is closely tied to the availability of natural resources. For example, as actual rainfall conflicts with expected rainfall, a farmer may feel that his yield is threatened by a lack of water and will seek an alternative way to secure enough water for his cultivation. As a farmer becomes more aware of changing environmental conditions, his natural capital increases. Data from my analysis show that as a farmer feels more vulnerable, their adaptive efficacy increases. An explanation for this relationship can be that a farmer who feels especially vulnerable is more likely to seek help or information to decrease his or her vulnerability. By becoming more informed, and therefore more prepared, a farmer can foresee challenges and address them early on, thus building his adaptive efficacy.

Reliance on Machinery is indicative of financial/physical capital and refers to the diversity of equipment a farmer held. Potential examples include a tractor, sprayer, water pump, combine harvester, and water buffalo. In Sri Lanka, having access to such equipment is a symbol of economic wealth. Farmers that have access to a diverse range of machinery and infrastructure are better able to adapt to climate challenges. For example, a water pump can be especially helpful when there is little rainfall and water must be irrigated from a far distance. In this scenario, farmers with access to established irrigation infrastructure would be better able to adapt to drought than those who do not. As such, adaptive efficacy will be higher for farmers with irrigation, since they know they can address the challenges of a water shortage with their financial/physical capital.

Social capital is measured by the strength of social institutions and community dynamics that increase reciprocity, trust, and cooperation within a society. In this model, the predictor variable representing social capital is *Community Disaster Assistance*. This variable is indicative of farmers who when disaster strikes, expect assistance from community-level stakeholders. When farmers expect assistance from fellow community members during times of extreme vulnerability, insight is provided as to how strong social bonds are. With this variable, I assume that farmers who have received aid in the past from community stakeholders have positively benefited from such aid. An increase in a farmer's reliance on community stakeholders to offer support during disasters increases their adaptive efficacy because it assures farmers that they are not alone in dealing with challenges thrown their way. Aid provided at the community level is more appropriate because it better understands the needs of locals and is overall better suited to handle local challenges. Overall, it can be said, that social capital creates hope.

Human capital seemed to have no statistically significant relationship with Adaptive Efficacy. This may be because, regardless of education, farming background, age, and/or gender, a head-farmer could still find resources that allow them to feel less vulnerable and better prepared to address the challenges climate change creates.

[…]

Conclusions of Research Question 1

Based on Model 1, financial/physical and

social capital seem to be the largest contributors to promoting adaptive behavior among Sri Lankan farmers. Both financial and social capital create buffers that prevent a farmer from experiencing the shortcomings associated with trying something new or different from the established norm. Thus, a farmer who has large quantities of financial/physical and social capital will be better able to develop climate resilience and address the challenges associated with climate change.

Research Question 2: Capital and Sustainable Livelihood Outcomes

The second goal of my statistical analysis was to shed light on the relationship between capital and sustainable livelihood outcomes. Through my analysis, I wanted to understand how capital contributed to a farmer securing outcomes that promoted a sustainable livelihood. My analysis draws from Multivariate Regression Model 2, displayed in the Results section of this document. The four sustainable livelihood outcomes I studied are economic welfare, nutrition, health, and happiness.

Economic Welfare

An important outcome of a sustainable livelihood is economic welfare. To analyze economic welfare, I looked at two outcome variables: Maha Yields and Debt. Higher Maha yields supports economic welfare, but debt completely deteriorates it.

[…]

Overall, Maha yields were dependent on financial and social capital. R*eliance on Machinery, Hired Help, Wealth Index Score*, and *FO Participation* were all important determinates of the quality of a growing season for a farmer. Financial capital encompasses machinery and farming hands. Large dependence on farming equipment and a large pool of helpers allow a farmer to use resources efficiently and maximize output per hectare of cultivated land. Additionally, participating in farmer organizations provides farmers with information on what to do or not to do to maximize yields.

A second output variable looked at to provide insight into economic welfare was Debt. Debt was dependent on financial and natural capital. Farmers that relied heavily on machinery incurred the most debt. This may be due to the fact that farmers are willing to accrue debt to purchase machinery with the purpose or hope that such choice will increase overall yields, allowing them to pay back the debt. Additionally, natural capital was also a factor into how much debt a farmer had. Model 2 illustrates that a farmer who saw his cultivation fail because of insufficient water had debt. Since the farmer in this scenario lost a portion of his livelihood, he had no choice but to accrue debt in order to continue meeting his basic human needs.

[…]

Nutrition

Equally important to economic welfare is nutrition. In Sri Lanka, most farmers are subsistence farmers. This means that the entirety of their nutrition depends mainly on what they cultivate or what they can get in exchange for their cultivated goods. The outcome variable used to provide insight into nutrition was Dietary Diversity. This variable was created by calculating food groups produced per week, weighted by nutritional value. The food groups asked about are as follows: rice, grains, bread and starchy staples, tubers, vegetables, fruits, cooking oils/fats, dairy, and meat protein.

[…]

Overall, the relationship between economic welfare and nutrition is obvious. To ensure the proper nutrition, a farmer needs good economic welfare. As established earlier, economic welfare is dependent on financial and social capital. As such, I reach the same conclusion with nutrition. Financial and social capital are the most important types of capital to

ensure proper nutrition among households in the dry zone of Sri Lanka.

Health

Health is a major component of a sustainable livelihood. The outcome variable used to delve into this was *No Medical Attention*. Households were asked if anyone in the past three years had not received medical attention while being ill. 15% of the sample reported that this was indeed the case. Model 2 indicates that not receiving necessary medical attention was influenced by natural and financial capital.

[…]

Overall, health among Sri Lankan farming households was highly dependent on natural and financial capital.

Happiness

Along with health, happiness is an equally important outcome when insuring a sustainable livelihood outcome. Happiness may even be considered an extension of health since a person's happiness contributes largely to their mental health. Farmers in the study were asked to place themselves on a happiness ladder. Placing themselves at the bottom step (1) meant a farmer was unhappy with their life. Placing oneself at the top-most step (10) meant that the farmer was the happiest imaginable. The majority of famers placed themselves around the middle (5). Since a large portion of the sample was Buddhist, faith can be a reason for this explanation. Part of Buddhist doctrine is acknowledging that no one's life is or will be perfect.

Where a farmer stood on the ladder was dependent on financial, natural, and social capital. Financial capital was analyzed by looking at *Wealth Index Score* and *Paddy Land Holding*. Farmers who were financially stable and had large expanses of land tended to be on the higher step of the ladder. Natural capital was measured by looking at *Perceived Environmental Vulnerability*. This is an interesting relationship. The data show that farmers who feel more vulnerable tend to be happier. This too is counterintuitive. An explanation for this relationship can also be religion. A prime doctrine of Buddhist religion is that all life is suffering. Perhaps farmers that feel the most vulnerable are more in tune with their religion.

Finally, social capital also showed an influence on happiness. Social capital was studied by looking at *FO Satisfaction*. Farmers who were socially active, be that through farmer organizations, tended to be happy/live happier lives. This may be due to general human nature which craves emotional relationships.

Overall, happiness was dependent on financial, natural, and social capital. As such, it can be concluded that in order to ensure happiness a farmer must have access to a diversity of capital.

Conclusions for Research Question 2

Based on the significance of relationships outlined in Multivariate Regression Model 2, sustainable livelihood outcomes are largely dependent on financial/physical capital. Additionally, it seems that the livelihood outcomes of nutrition, health, and happiness are dependent on each other. I reach this conclusion based on each outcome variable sharing significance with the same input variables representing the different types of capital.

RECOMMENDATIONS

Financial/physical and social capital were the most significant determinants of adaptive behavior and sustainable livelihood outcomes among farming households in the dry zone of Sri Lanka. Financial capital is an observable conclusion. However, the role of social capital often goes unnoticed. Research shows that social capital gives rise to financial capital and economic development (Woolcock & Narayan, 2000). Additionally, it plays a prominent role in promoting sustainable livelihoods. A recent study found that the factors that best prescribe people's perceptions of livelihood recovery are formal networks in the community as well as leadership

and trust of community-based organizations (Minamoto, 2010). Most national and international aid groups focus on creating financial capital by trying to increase yields and savings, but the creation of social capital may require even fewer resources and be just as valuable.

Sri Lanka is still recovering from the impacts of its recent civil war, so social capital may not be at its peak simply because the legacy war leaves behind tends to be extensive. Also, those impacted the most by the war were people in the dry zone, my study group. As such, a different approach to social capital may be necessary. Research indicates that rather than focusing on engineering social capital, external agencies need to focus on better understanding the preconditions for social capital formation and how they can contribute to the creation of an enabling environment (Uphoff & Wijayaratna, 2000). Through my study, I reach a similar conclusion. Organizations should focus on creating conditions that contribute to the formation of social capital, rather than focusing on creating social capital itself.

In Sri Lanka, some ways to create conditions for social capital growth may be through the creation of weekly communal events and/or large celebrations for national holidays. Social events like these recommended do have costs associated with them, but it would be a worthwhile investment by aid organizations, governments, and community leaders hoping to increase social capital. Something that seems to work very well already is participation in Farmer Organizations. To increase participation, community leaders need to devise ways that further incentivize diverse participation in Farmer Organizations. Organizations should seek members from different demographics in order to create a greater sense of community engagement. The creation of social capital will create bonds of shared values, norms, and institutions that are a catalyst for development. Overall, the level of development a country experiences is the best indicator of climate resilience that will be achieved.

[…]

REFERENCES

Minamoto, Y. (2010). Social capital and livelihood recovery: post-tsunami Sri Lanka as a case. *Disaster Prevention and Management: An International Journal*, 19(5), 548-564.

Uphoff, N., & Wijayaratna, C. M. (2000). Demonstrated benefits from social capital: the productivity of farmer organizations in Gal Oya, Sri Lanka. *World development*, 28(11), 1875-1890.

Woolcock, M., & Narayan, D. (2000). Social capital: Implications for development theory, research, and policy. *The World Bank research observer*, 15(2), 225-249.

ENACTING ENVIRONMENTAL POLICY:

A BEAR OF A TASK

Torri Gladem

The following is an excerpt from a longer piece. For full text, please visit www.honorsjournal.com

ABSTRACT

In 2014, Colorado Parks & Wildlife enacted a multi-pronged strategy with the intention of mitigating threats to the mule deer population. This species has declined in recent decades across the state of Colorado. Various public meetings provided input that predation and degraded habitat were some of the most important factors to mitigate. In 2016, CPW attempted to do just that with the Piceance Basin predator control study. In this study, black bears are included as a predator species to be removed from the designated area to determine if mule deer populations respond positively. This case's issues, however, are that the prior public meetings were overwhelmingly held in rural places, and garnered little input from urban residents. Coupling this issue with CPW's second strategy goal of mitigating developmental impacts has left it on the defense. This is because many residents, as expressed in meetings as well as my study, felt that energy development destroyed valuable mule deer habitat. Since the agency's research has suggested otherwise, my goal is not to dispute this but rather, question the lack of holistic research in the heavily developed predator control area. Most residents like black bears, and the effects of energy development on this species has not been explored. My paper has provided a solution that mediates some of the most contentious issues. It involves a newly proposed fund for predator research. By conducting a survey, I determined that public receptivity to additional wildlife funding exists and there are options to pursue it.

[…]

RESULTS

My survey generated 96 responses total. Since I was primarily concerned with Coloradan stakeholders, however, I filtered out responses from non-residents. This left me with 75 responses to fully analyze. Overall, I received responses from 27 towns or cities, summarized in Figure 5 below.

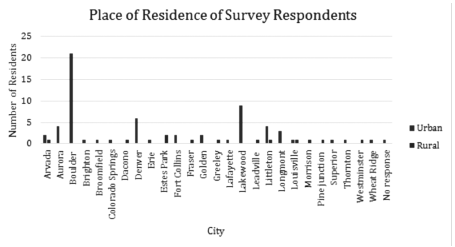


Figure 5. When participants' information was categorized by place of residence, it was clear that respondents to this survey typically resided in urban areas along the Front Range. Boulder, Denver, and Lakewood accounted for 48% of the responses. Amongst the remaining responses, a diverse group of 24 cities was represented in varying capacities.

Since 84%, or 63 out of 75, of my participants classified their place of residence as 'urban,' I chose to focus my analysis on the resulting answers from this overwhelming majority. This is not to say that the answers I received from 'rural' residents were not telling; they still helped me determine some of the general trends of my data. However,

they could not be used to estimate a true representation of the rural population of Colorado's opinions because my sample size was too small to create valuable confidence intervals. Along with this, a portion of my participants left answers blank as the survey progressed. To prevent this from skewing my results, I discarded all of the 'No responses' as I analyzed each question individually. This left me with slightly different sample totals for some questions, which are summarized below in Table 1, alongside other relevant results.

[…]

The general trend was that my survey results reflected my initial assumptions. As suspected, almost none of the participants had heard of the predator control plans previously. Of the nine respondents who did, five of them received their information through friends and family. The remaining four were split equally in regard to having heard of the plans through news and media coverage or the CPW agency themselves. My question about the public meetings as well as its follow-up had to be omitted due to a lack of responses. Almost no one had been aware of the public meetings; in fact, only one participant had previously heard of them.

The most important results from my survey were that most participants felt negatively or neutral about the predator control plans. In contrast, many respondents felt positively about black bears. A strong majority, 80%, was supportive of an optional two-tiered pricing system. Results were split almost equally amongst participants who did or did not buy a hunting/fishing license or season pass, but of those who did, the vast majority bought one every year. Of these 18 respondents, 8 would personally support building a predator research fund, 7 potentially would, and only 3 would not. This translated to a median estimate that 44% of the general population could be expected to support the proposal, with a 95% confidence interval of 22-69%. While 44% is a promising proportion, the large interval makes the true value harder to assume. 30 out of 51 respondents either agreed or strongly agreed that wildlife management has been overlooked in favor of energy development. Most of the remaining participants felt neutrally, and very few, 18%, disagreed.

In depth analyses of my results were limited due to low response rates to certain questions and answer choices. This issue was more pronounced when I tried to pair question responses, because it usually involved an analysis of fewer than 10 people. This would provide confidence intervals spanning nearly the entire range from 0-100. Such results lack relevant or useful information, and warrant further analysis of a large sample size.

DISCUSSION

My results reflect the perspectives of the public that was largely left out of the decision making process which led to the Piceance Basin predator cull. This group was urban residents. The majority of them did not know about the predator control plans prior to my study, nor did they know about the public meetings held on the issue. As it stands, CPW held public meetings inviting input in primarily rural areas that were located far from the communities on the Front Range. While they attempted to inform the public and invite participation, the locations they chose effectively cut those unwilling or unable to travel three hours out of the process.

The meetings resulted in concerns that predators were a primary cause of mule deer decline. Habitat degradation from energy development was another leading thought. This resulted in the implementation of the 2014 Colorado West Slope Mule Deer Strategy, where the Piceance Basin predator control study was announced. It was enacted without any prior research on potential causes of increased predation, which my survey respondents indicated prioritized energy development interests. Their attitude also reflected that a comfortable majority of the general population can be assumed to feel positively about predators, since 61% percent responded affirmatively. This resulted in a confidence interval estimating the true

sentiments of the general population to lie between 48-74%. Although the range of this interval is large, the interesting piece is where the ranges lies. One half to three quarters of the general population feeling positively about black bears implies a majority. This majority is crucial to implementing a new strategy for wildlife management that involves black bear conservation.

As expected, a large proportion of my respondents felt negatively or neutral about the predator control plans, while very few felt positively. This trend underpinned the gap that seems to span between CPW's goals and the general public's opinions. To assess the viability of my solution meant to mitigate this, I found that about 54% of people could be expected to buy a hunting/fishing license or season pass annually. In turn, the likelihood of these same individuals supporting my two-tiered fee system through their own purchases was about 58%, with an estimate that the true value for the population lies within the interval of 39-75%. While I would have liked to see a more obvious majority, these results show that there is receptivity to a predator research fund. There are also people willing to contribute to it. Further research with a larger sample size would be required to understand the true extent of this, but these results are hopeful.

CPW has many programs for different aspects of conservation and research, but none of them are specifically focused on predators. Since research is lacking on them in general, an idea supported by 61% of my respondents, a two-tiered pricing system is a potential option. It would give the public greater control over the wildlife that they already support heavily through their recreational activities. It would also give CPW a chance to show that they have looked at all the impacts from energy development, a sentiment that should be supported by a Coloradan public that has grown increasingly concerned with the environmental implications of such activities. If CPW pursued predator research before enacting drastic management experiments, they might determine new information that leads to predator success *along with* mule deer. If habitat degradation were found to be a limiting factor on predator species, attempts to address this may solve the biotic issues with mule deer decline. Such research is only possible with the proper funding, however. I cannot conclusively say that my solution is the best or even the most feasible, but it demonstrates a need for research as well as an option for garnering the resources necessary to conduct it. It is a talking point that could lead to improvements in the future.

CONCLUSIONS AND RECOMMENDATIONS

Public policy is a huge task to undertake, and not something CPW is required to make integral to their agency. Their previous efforts to include the public in meetings and decision-making processes about wildlife has shown that they are committed to making an attempt. To include a broader group of participants, they should hold future public meetings in a variety of locations, both urban and rural. It would be pertinent for the agency to also entertain new wildlife management strategies. My paper has shown that there is a dichotomy between the Piceance Basin predator control plan's objectives and the feelings of a large constituency. This same constituency expressed interest in creating a predator specific research fund through a two-tiered pass system. While more data is necessary to discern what the true support of the Coloradan population is, this result shows that there is, at the very least, receptivity to the idea. In future research, it would be interesting to determine what the opinions and perspectives of out of state residents are. While they do not make use of Colorado's resources to the same extent as residents, they are still valuable stakeholders. They contribute funds to non-resident licenses and passes and have a vested interest in our wildlife. If this group was also supportive of changes to the fee system to support more holistic research, funding may be viable. It would also be interesting to look at

other aspects of funding, because there may be available solutions unique from my proposal, but that accomplish similar goals.

My solution was an attempt to provide a more palatable answer to the vast number of people who feel the Piceance Basin predator control study was not planned or implemented properly. It works to reconcile impacts from energy development companies and the general attitudes of the urban constituency that was mostly removed from public meetings about the mule deer issue. While it is by no means perfect, it has the potential to provide CPW with a new wildlife fund, determined by the public. This public would be able to showcase their support through their own choices of funding predator research, which gives them a more tangible role in the fate of the wildlife that is considered a resource for all. In a similar sense, CPW would benefit from receiving the funds to present holistic, well-researched information before enacting management experiments. This solution would allow them to pursue true ecosystem health through adaptive management, in a way that is only becoming more necessary as developmental impacts are ongoing and increasing.

Although the mule deer strategy was implemented in 2014 and the Piceance Basin predator control study began this year and is set to run through 2019, this information provides an option for the future. The insights gained from observational predator research could be used to affect long-term positive change in the mule deer population, rather than short-term reactionary change. It could also mitigate current conflicts that make the strategy's goals less effective, and truly align CPW's goals with their studies and actions.