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Jennifer T. Aronoff February 20, 2012

# "Effects of Stress and Environmental Factors on Patterns of Infection with Gastrointestinal Commensals and Parasites in the Critically Endangered Black Rhinoceros (*Diceros bicornis bicornis*) in Addo Elephant National Park, South Africa"

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a thesis submitted to the Faculty of Emory College of Arts and Sciences
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## Abstract

"Effects of Stress and Environmental Factors on Patterns of Infection with Gastrointestinal Commensals and Parasites in the Critically Endangered Black Rhinoceros (*Diceros bicornis bicornis*) in Addo Elephant National Park, South Africa"

by Jennifer T. Aronoff

This work examined gastrointestinal microbes in a population of black rhinoceros (*Diceros bicornis bicornis*) in Addo Elephant National Park, South Africa. The study was conducted to establish a health-monitoring program for this species by creating a profile of gastrointestinal parasites and commensals as a baseline indicator of rhinoceros vitality and health. Some 234 fecal samples were collected following direct and remote observations of defecation from January 2008 to June 2011 in two areas of Addo Park, Main Camp and Nyathi. The two collection sites vary with respect to environmental factors of elephant density, climate, predator density, habitat size, and tourism. Each sample was examined using fecal flotation and fecal sedimentation processes to identify and count microbes. Glucocorticoid analyses were done to evaluate levels of stress. Nyathi rhinos had significantly higher prevalence of most (14/16) of the commensal parasite species and individual Nyathi rhinos were concurrently infected with significantly more species of commensals compared to Main Camp rhinos. *Rhinozeta rhinozeta*, *Triplumaria corrugata*, *Monoposthiurn vulgaris*, *Gilchristata artemis* and Unidentified ciliates all showed significant trends for higher prevalence among rhinos in the Nyathi section. Co-infection occurred significantly more than expected between *Oesophagostomum* sp. and *Necator* sp. as well as *Strongyloides* sp. and *Necator* sp. No relationships were apparent among stress level, total precipitation and parasite/commensal richness. These results demonstrate that

commensal communities of herbivores can be sensitive to environmental variability even when parasites and host stress levels are relatively constant.

# "Effects of Stress and Environmental Factors on Patterns of Infection with Gastrointestinal Commensals and Parasites in the Critically Endangered Black Rhinoceros (*Diceros bicornis bicornis*) in Addo Elephant National Park, South Africa"

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## Introduction

Black rhino (*Diceros bicornis bicornis*) populations in Africa have declined more than 95% in the past century due to intensive poaching. Today, only about 3,600 black rhinos remain in the wild (compared to approximately 70,000 in the late 1960s), making them critically endangered (IUCN 2011). Humans represent the single greatest threat to the black rhino population. Rapidly increasing human population growth, combined with rising demands for land and resources pose a grave threat to the future of black rhinoceros populations throughout Africa.

Due to their large body size and long life spans many ecological and anthropogenic factors can influence the health of black rhino individuals. Compounded pressures, such as human population growth, commercial logging, droughts, and the capture and captivity of rhinos can lead to stress-associated immune suppression (Penzhorn et al. 1994). Immune suppression can increase the presence of opportunistic parasites. Habitat destruction not only concentrates animals into smaller spaces, it can create new food and social stressors that have not previously existed (Gillespie and Chapman 2006; Gillespie et al. 2008). These changes to the environment can lead to decreases in food, water, and viable habitats. Devastation of adequate vegetation hinders ciliate species from flourishing. The lack of these ciliate species, which aid in digestion and are proven to be beneficial to rhinos, can diminish the health of individuals. Anthropogenic habitat change also forces humans and wildlife into closer and more frequent contact therefore increasing the risk of disease transmission. Poaching of black rhinos (for the prized horn for traditional medicine or ornamentation) make them susceptible to transmission of pathogens due to their small population size.

Black rhinos select habitat based on a number of factors which extend beyond the quality of available browse. Those factors include distance to water, presence of roads, and fences that might surround potential habitat, and competition with other species (Morgan et al., 2009 and Santymire, 2010). Low precipitation combined with competition between other browsers can be detrimental to black rhinos health and reproductive success. Interspecific competition can lead to a lack of sufficient quantity or quality of vegetation (Foster, 1965) therefore increasing stress and potentially elevating parasite populations. Variation in availability and quality of vegetation (magnified by seasonal changes) can also impact the community structure of ciliates (Obanda, 2007). Different type of vegetation influences the intensity and species of ciliates found in individual rhinos. Ciliates such as e*ntodiniomorphs* and *holotrichs* are efficient converters of soluble sugar, starch and cellulose. Food sources that are high in key nutrients enrich the population of these ciliates (Abe and Iriki, 1978). Competition for food, water and shelter can affect gastrointestinal parasite infection rates and hormonal activity, which in turn negatively influence reproductive success along with the health of individuals.

Parasite infection can impact host survival and reproduction directly through pathologic effects and indirectly by reducing host condition (Chandra and Newberne, 1977; Boyce, 1990; Dobson and Hudson, 1992; Hudson et al., 1992; Coop and Holmes, 1996; Murray et al., 1998). Severe infection can lead to blood loss, tissue damage, spontaneous abortion, congenital malformations, and death (Chandra and Newberne, 1977; Despommier et al., 1995). However, less severe infections are more common and may impair nutrition, travel, feeding, predator escape, and competition for resources or mates, or they may increase energy expenditure (Dobson and Hudson, 1992; Hudson et al., 1992; Coop and Holmes, 1996; Packer et al., 2003).

Animal body condition and reproductive status can be compromised when parasites inflict substantial energetic costs (Hudson, 1986; Toque, 1993). However, parasites do not necessarily induce negative effects if hosts have adequate energy reserves or nutrient supplies concurrent with infection (Munger and Karasov, 1989; Gulland, 1992; Milton, 1996), suggesting that the outcome of host–parasite associations may be contingent on host status, as well as on the severity of infection.

Glucocorticoid levels (cortisol is one type of glucocorticoid) serve as an index of host stress. Prolonged elevated levels typically reduce survival and reproduction of captive animals and humans (Wasser et al., 1997; Creel, 2001; Creel et al., 2002; Bercovitch and Ziegler, 2002). Although data on fitness effects of elevated glucocorticoid levels in the wild are limited, the expectation is that fitness will decrease as population level stressors become more severe or more prolonged (Boonstra and Singleton, 1993; Creel et al., 2002).

Habitat destruction is not only the result of anthropogenic changes, but also results from overpopulation of species. For example, elephants do not feed on *Acacia* trees at heights below one meter, but they kill four percent of all seedlings (the primary food source for rhinos) (Birkett 2002). This destruction of food resources can harm less mobile herbivores that depend on such vegetation near permanent water supplies (Franz et al. 2010). Thus overcrowding and damage to vegetation reduces the potential for an increase in the rhino's population. When associated with drought, such habitat degradation can pose many challenges for the conservation of black rhinos.

Increased habitat fragmentation, whether due to environmental or anthropogenic factors may potentially lead to increased home ranges for individual rhinos. As habitats become increasingly fragmented, rhinos have to travel even further to find viable habitats with sufficient water, food, and cover (absent of human disturbances). Finding enough food necessitates

increased travel and associated increases in range overlap, increasing disease transmission through feces, aggressive behavior, and immigration and/or emigration of other species into the group. The cost associated with this prolonged stress may be responsible for decreased immune function (Linklater, et al. 2010). Anthropogenic changes, such as logging, can affect host ecology and modify infection prevalence and risk (Gillespie and Chapman 2006; Reid 2007). Increased habitat fragmentation and edges promote increased contact among humans and wildlife; however, edges may also create higher daytime temperatures from increased solar radiation, making the air and soil less moist and therefore reducing the risk of parasite infection (Kapos et al. 1997).

In sum, many environmental and anthropogenic pressures make rhinos vulnerable to infection (presence) of parasites and lack of infection of ciliates. Prevalence of ciliates and parasites along with stress levels can provide an index of population health that can be directly related to habitat quality and usage. Differences among demographic and climactic factors, such as elephant density, climate, predator density, habitat size, and tourism, between the Main Camp and Nyathi sections make Addo Elephant National Park (AENP) the ideal location for this study. The ultimate goal of this study was to non-invasively examine patterns of parasitism and mutualism in two black rhino populations in Addo Elephant National Park, South Africa relative to stress, reproductive hormone physiology, and ecological factors in order to create an integrated assessment of rhino health in the Addo ecosystem.

## Materials and Methods

### *Study Site*

From January 2008 to June 2011, black rhinoceros fecal samples were collected in AENP, South Africa between 33°22‘S-33°41‘S and 25°45’E-25°83’E (Figure 1). Camera traps

and direct observations ensured that each fecal sample corresponded to a specific rhino. Sampling occurred in two sections of the park, Main Camp and Nyathi, which vary with respect to several important factors including elephant density and sex ratio, climate, predator density, size, and frequency of tourism. Main Camp provides less rhino habitat (i.e. 11,500 hectares versus 14,000 hectares in the Nyathi section), substantially higher rates of tourism, a higher density of elephants, *Loxodonta africana* (3.58 elephants/km$^2$ in Main Camp and 0.71 elephants/km$^2$ in Nyathi), and predators such as lion (*Panthera leo*) and spotted hyena (*Crocuta crocuta*) not present in the Nyathi section, as well as reduced vegetation compared to the Nyathi section of the park (Table 1).

Table 1. Comparison of ecological factors between Main Camp and Nyathi Sections of Addo Elephant National Park, South Africa

| **Factors** | **Main Camp** | **Nyathi** |
|---|---|---|
| Rhinoceros Sex Ratio | Female-biased (9 females, 8 males, 1 unsexed) | Male-biased (11 females, 13 males, 2 unsexed) |
| Rhinoceros Density | ~0.15 rhinos/ km$^2$ | ~0.18 rhinos/ km$^2$ |
| Elephant Population<br>Elephant Density | High (~300)<br>3.58 elephants/km$^2$ | Moderate (~100)<br>0.71 elephants/km$^2$ |
| Rhinoceros Predators | Present | Absent |
| Vegetation | Limited | Abundant |
| Tourism | High | Low |
| Size of Study Area | 11,500 ha | 14,000 ha |


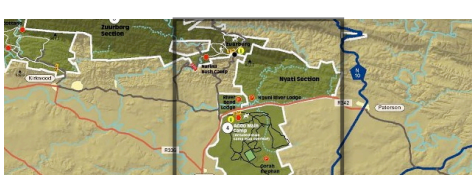

Figure 1. Location of Study Sites in Addo Elephant National Park, South Africa.

*Measurements/Statistical Analysis*

Rachel Santymire, Thando Mendela, Jordana Meyer, and SANparks assistants gathered the 234 samples from a total of 34 individual black rhinos (13 from the Main Camp section and 21 from the Nyathi section of the park). All samples were shipped to Atlanta in January 2011 and screened for gastrointestinal helminth eggs and larvae following standardized fecal flotation and fecal sedimentation methodologies developed for herbivorous hosts (Gillespie 2006).

Samples were first screened through fecal flotation processes. Test tubes were filled 1/3 to 1/2 full of the fecal sample then filled the rest of the way with distilled water. The tubes were then centrifuged at 1800rpm for 10 minutes and the resulting supernatant was discarded. Fecal material was re-suspended in a sodium nitrate solution and the tubes were centrifuged again for another 10 minutes at 1800 rpm with a cover slip on top of the tubes. After the tubes were centrifuged, the cover slip was removed and placed on a slide to be scanned under a compound microscope. Parasites were identified on the basis of egg, larvae, or cyst coloration, shape, contents, and size. Each individual parasite species per sample was counted (to determine the total number of that particular species found in the sample) and representatives measured at 40x to the nearest 0.1 $\mu$m with an ocular micrometer. If needed, one drop of Lugol's iodine solution was added to aid in species identification. Unknown and representative parasite species were photographed.

After fecal flotation analysis was finished, the samples were examined through fecal sedimentation processes. The remaining pellet from the fecal flotation process was suspended in a diluted soapy water solution then filtered through cheesecloth held over the lip of the beaker into a 50-ml centrifuge tube. The filtered suspension was allowed to settle until sediment was apparent. The resulting supernatant was removed by pipette and the remaining pellet was rinsed with the soapy water solution and filtered back through the same cheesecloth into the 50-ml centrifuge tube. Once the suspension settled, a couple drops of the sediment were placed onto a slide and covered with two cover slips. Samples were then read under a compound microscope and analyzed using the same method described above for fecal flotation processes.

In addition, Merifluor *Cryptosporidium*/*Giardia* Direct Immunofluorescent Detection Kits (Meridian Bioscience Inc, Cincinnati, Ohio) were used to detect *Crystoporidium* oocysts

and *Giardia* cysts based on monoclonal antibodies [Johnston et al., 2003]. Spiking negative fecal samples with known numbers of *Giardia* cysts and *Cryptosporidium* sp. oocysts validated this method.

The samples were examined using fecal hormone methodologies described in Santymire and Armstrong, 2009. Before analysis of the samples could occur, collected fecal matter was stored at -20 degrees C until processing when it was then thawed and thoroughly mixed. About 0.5 grams of fecal matter was weighed out and mixed with 5.0ml of 90% ethanol distilled water. Samples were agitated on a mixer for 30 minutes (setting of 60) and centrifuged for 20 minutes at 1500 rpm. The resulting supernatant was discarded and the remaining pellet was reconstituted in 1ml of methanol solution, vortexed briefly, sonicated for 20 minutes and diluted in a dilution buffer (0.2 M $NaH_2PO_4$, 0.2 M NaHPO, NaCl). Samples were then analyzed for glucocorticoid levels.

Prevalence, magnitude of multiple infections, and richness (number of unique parasite/ciliate species per host fecal sample) were determined to look at patterns of parasitism among the individual rhinos in relation to seasonality, inter-site differences, and stress levels. Prevalence, defined as the proportion of a population infected with a particular parasite or ciliate species at a given time, was calculated to determine the percentage of individual rhinoceros infected with particular protozoa. If a sample was positive for a specific parasite species at any time over the period of three years, than that individual rhino was also considered positive for that given parasite. Patterns of prevalence were examined annually as well. The Fisher Exact test was used to determine differences in parasite prevalence between individuals habituated in either the Main Camp or the Nyathi section of Addo National Park. A two-sample independent T-test was used to determine differences in the magnitude of multiple infections (average

number of parasite and ciliate species detected per individual rhino) between the two sections of AENP.

Seasonal comparisons were also made among the samples. Using the Fisher Exact test and Generalized Linear Model (GLM), individual rhinoceros positive for a pathogen were totaled to determine if climate (e.g. total precipitation (mm per month)) had a strong influence on the profile of gastrointestinal pathogens. If parasites were present in individual rhinos, GLM analysis was used to determine if the resulting ciliate richness was significant. Patterns of coinfection were analyzed using the Mantel-Haenzel Chi Square test. MS Excel 14.0, and Open Epi 2.3.1 were all used for the statistical analysis.

## Results

*Prevalence Pattern Results*

Patterns of prevalence among pathogenic parasite species were similar between the two sections of the park. With the exception of larval nematodes, no significant differences were found in parasite prevalence between the sites (Table 2).

Table 2. Comparison of infection prevalence in black rhinoceroses (*Diceros bicornis bicornis*) in Main Camp and Nyathi Sections of Addo Elephant National Park, South Africa (n=samples analyzed). $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

| Protozoa | Main Camp Section (% Positive) n=13 | Nyathi Section (% Positive) n=21 | Total Park (% Positive) n=34 | Significance |
|---|---|---|---|---|
| **Parasite Species** | | | | |
| Necator sp. | 46.15 | 42.86 | 44.12 | - |
| Trichostrongylus sp. | 92.30 | 90.48 | 91.18 | - |
| Strongyloides sp. | 53.84 | 28.57 | 38.24 | - |
| Oesophagostomum sp. | 53.84 | 52.38 | 52.94 | - |
| Larval nematodes | 7.69 | 47.62 | 32.35 | * |

| **Ciliate Species** | | | | |
|---|---|---|---|---|
| *Rhinozeta rhinozeta* | 38.46 | 90.48 | 70.59 | ** |
| *Rhinozeta triciliata* | 61.53 | 90.48 | 79.41 | - |
| *Rhinozeta addonenesis* | 30.76 | 66.67 | 52.94 | - |
| *Rhinozeta cristata* | 53.84 | 71.43 | 64.71 | - |
| *Rhinozeta caecalis* | 0 | 4.76 | 2.94 | - |
| *Rhinozeta unilaminatus* | 0 | 14.29 | 8.82 | - |
| *Blepharosphera ceratotherii* | 38.46 | 61.90 | 52.94 | - |
| *Blepharosphera intestinalis* | 23.07 | 47.62 | 38.24 | - |
| *Blepharoconus dicerotos* | 15.38 | 14.29 | 14.71 | - |
| *Charonina tenuis* | 7.69 | 14.29 | 11.76 | - |
| *Charonina odontophora* | 0 | 4.76 | 2.94 | - |
| *Charonina tortuosa* | 7.69 | 0 | 2.94 | - |
| *Triplumaria corrugata* | 15.38 | 66.67 | 47.06 | ** |
| *Monoposthiurn vulgaris* | 61.53 | 100 | 85.29 | ** |
| *Gilchristata artemis* | 15.38 | 85.71 | 58.82 | *** |
| Unknown Ciliate | 7.69 | 47.62 | 32.35 | * |

When prevalence was compared by year (Table 3), (2008, 2009, and 2010), significant differences in parasite prevalence between the two sections of AENP were found. In both 2008 and 2010, *Strongyloides* sp. had a higher prevalence in Main Camp ($p$=0.035 for 2008 and $p$=0.056 for 2010).

**Table 3.** Yearly comparison of infection prevalence in black rhinoceroses (*Diceros bicornis bicornis*) in Main Camp and Nyathi sections of Addo Elephant National Park, South Africa from 2008, 2009, and 2010 (n=samples analyzed). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

| Protozoa | % Pos Main Camp 2008 (n=3) | % Pos Nyathi 2008 (n=5) | Fisher Exact Test (2-Tail) p values for 2008 | % Pos Main Camp 2009 (n=5) | % Pos Nyathi 2009 (n=17) | Fisher Exact Test (2-Tail) p values for 2009 | % Pos Main Camp 2010 (n=7) | % Pos Nyathi 2010 (n=14) | Fisher Exact Test (2-Tail) p values for 2010 |
|---|---|---|---|---|---|---|---|---|---|
| **Parasite Species** | | | | | | | | | |
| *Necator* sp. | 66.67 | 0 | - | 40 | 29.41 | - | 57.14 | 28.57 | - |
| *Trichostrongylus* sp. | 100 | 80 | - | 100 | 94.12 | - | 85.71 | 85.71 | - |
| *Strongyloides* sp. | 66.67 | 0 | * | 0 | 25.53 | - | 71.43 | 21.43 | * |
| *Oesophagostomum* sp. | 66.67 | 20 | - | 60 | 41.18 | - | 28.57 | 42.86 | - |
| Larval nematodes | 0 | 40 | - | 0 | 52.94 | * | 14.29 | 28.57 | - |
| **Ciliate Species** | | | | | | | | | |
| *Rhinozeta rhinozeta* | 33.33 | 40 | - | 40 | 76.47 | - | 28.57 | 78.57 | * |
| *Rhinozeta triciliata* | 100 | 60 | - | 40 | 82.35 | - | 42.86 | 85.71 | - |
| *Rhinozeta addonenesis* | 66.67 | 60 | - | 20 | 64.71 | - | 14.29 | 64.29 | - |
| *Rhinozeta cristata* | 33.33 | 80 | - | 20 | 35.29 | - | 57.14 | 57.14 | - |
| *Rhinozeta caecalis* | 0 | 20 | - | 0 | 0 | - | 0 | 0 | - |
| *Rhinozeta unilaminatus* | 0 | 40 | - | 0 | 11.76 | - | 0 | 0 | - |
| *Blepharosphera ceratotherii* | 33.33 | 60 | - | 40 | 64.71 | - | 28.57 | 35.71 | - |
| *Blepharosphera intestinalis* | 0 | 60 | - | 0 | 35.29 | - | 28.57 | 42.86 | - |
| *Blepharoconus dicerotos* | 33.33 | 20 | - | 20 | 5.88 | - | 0 | 14.29 | - |
| *Charonina tenuis* | 0 | 0 | - | 0 | 0 | - | 0 | 21.43 | - |
| *Charonina odontophora* | 0 | 0 | - | 0 | 5.88 | - | 0 | 0 | - |
| *Charonina tortuosa* | 0 | 0 | - | 20 | 0 | - | 0 | 0 | - |
| *Triplumaria corrugata* | 33.33 | 40 | - | 0 | 64.71 | * | 14.28 | 50 | - |
| *Monoposthiurn vulgaris* | 66.67 | 80 | - | 80 | 94.12 | - | 28.57 | 100 | *** |
| *Gilchristata artemis* | 0 | 40 | - | 20 | 76.47 | * | 14.28 | 71.43 | ** |
| Unknown ciliate | 33.33 | 20 | - | 0 | 41.18 | - | 0 | 14.28 | - |

Prevalence of ciliates was significantly higher in Nyanthi compared to Main Camp rhinos. Over the three years sampled, there were statistically significant differences in *Rhinozeta rhinozeta*, *Triplumaria corrugata*, *Monoposthiurn vulgaris*, and *Gilchristata artemis* ($p$=0.002;

*p*=0.005; *p*=0.005; *p*=<0.001 respectively). *Rhinozeta addonenesis* and *Rhinozeta triciliata* were more prevalent in Nyathi samples than samples from Main Camp (Table 2).

Rhinoceroses in both Main Camp and Nyathi sections of AENP did not have all 16 ciliate species present. Three ciliate species were not found among rhinoceroses in Main Camp section (*Rhinozeta caecalis*, *Rhinozeta unilaminatus*, and *Charonina odontophora*). *Charonina tortuosa* was not found among rhinoceroses in the Nyathi section. *Cryptosporidium* and *Giardia* were not found in any of the rhinos examined.

*Magnitude of Infection Results*

No difference in the mean number of parasite species infecting individuals was apparent between Main Camp and Nyathi (Table 4). Individual rhinos in Main Camp were infected with an average of 2.53 parasite species per individual compared to 2.61 parasite species per individual rhino in the Naythi section of AENP (*p*=0.868). There was, however, a significant difference in the mean number of ciliate species infecting individuals between the two sections. While individuals in Main Camp only had about 3.76 ciliate species per rhinoceros, individuals in Nyathi has on average 7.80 ciliate species (*p*=0.0001).

Table 4. Number of parasite and ciliate species infecting individual black rhinoceroses (*Diceros bicornis bicornis*) in Main Camp and Nyathi sections of Addo Elephant National Park, South Africa.

| Section of AENP | Mean Number of Parasite Species Per Individual Rhinoceros | Mean Number of Ciliate Species Per Individual Rhinoceros |
|---|---|---|
| **Main Camp** | 2.538 | 3.769 |
| **Nyathi** | 2.619 | 7.809 |

*Patterns of Co-Infection Results*

Patterns of co-infection among pathogenic parasite species were found in rhinoceroses in AENP. Among the four pathogenic parasite species, two interactions were found to have a significant co-existance relationship (Table 5). Co-infection occurred significantly more than expected between *Oesophagostomum* sp. and *Necator* sp. as well as *Strongyloides* sp. and *Necator* sp. ($p$=0.009 and $p$=0.04 respectively).

*Stress Concentration and Parasite Burden Results*

Overall, the number of parasite species infecting a host was not related to fecal glucocorticoid metabolite levels ($r^2$=0.0014) and stress levels were almost equivalent between Main Camp and Naythi rhinos (Figure 1). Average glucocorticoid metabolite concentration in Nyathi was 154.88 ng/g feces compared to 155.39 ng/g feces in Main Camp ($p$=0.98).

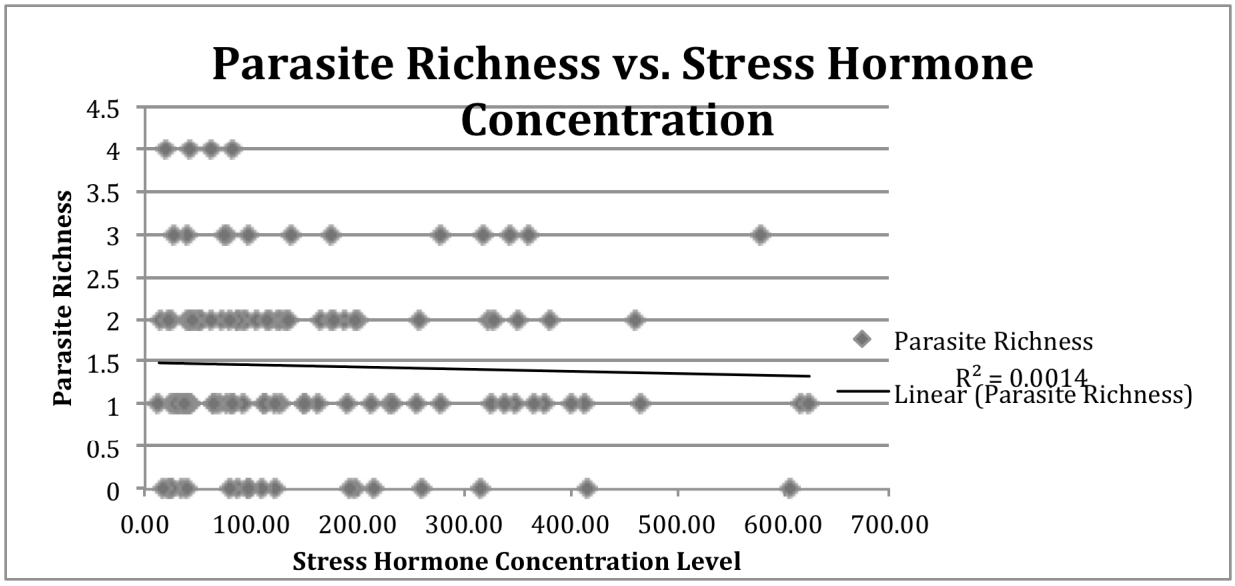


Figure 2. Number of parasite species recorded in individual rhinos in Main Camp and Nyathi sections of Addo Elephant National Park, South Africa compared with individual stress hormone concentration levels.

*Precipitation and Parasite/Ciliate Richness Results*

No correlations were found among total monthly precipitation and resulting parasite and ciliate richness ($r^2$=0.0073 and $r^2$=0.0016 respectively). Both relationships among precipitation and resulting parasite and ciliate richness were negatively correlated (Figure 2 and Figure 3 respectively).

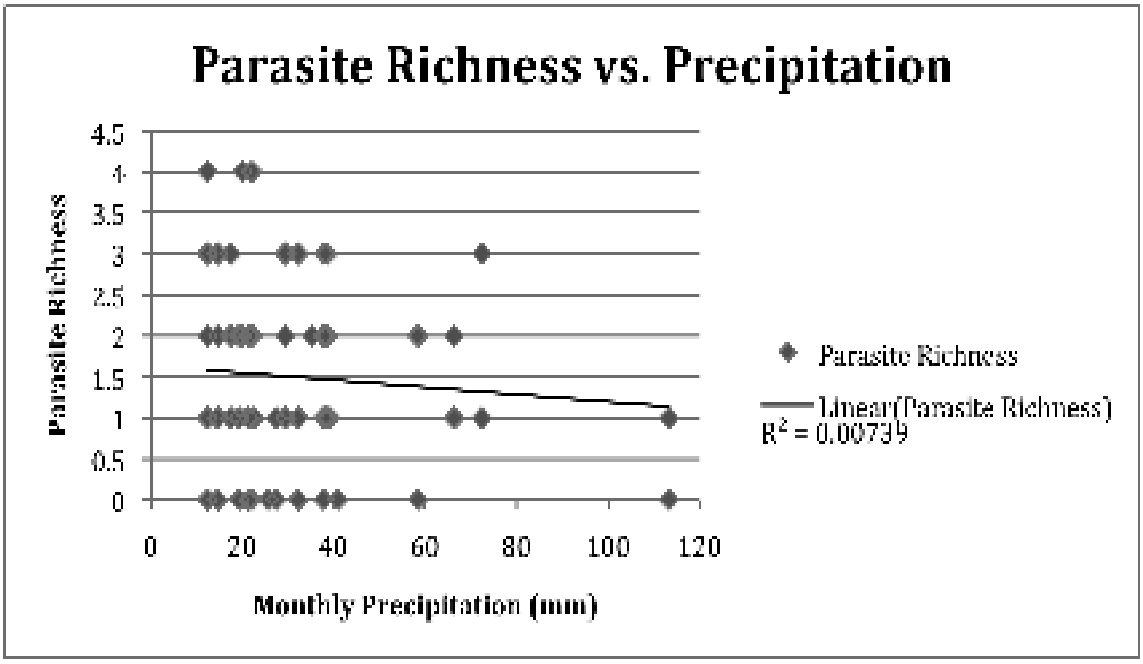


Figure 3. Number of parasite species recorded in individual rhinos in Main Camp and Nyathi sections of Addo Elephant National Park, South Africa in response to monthly rainfall.

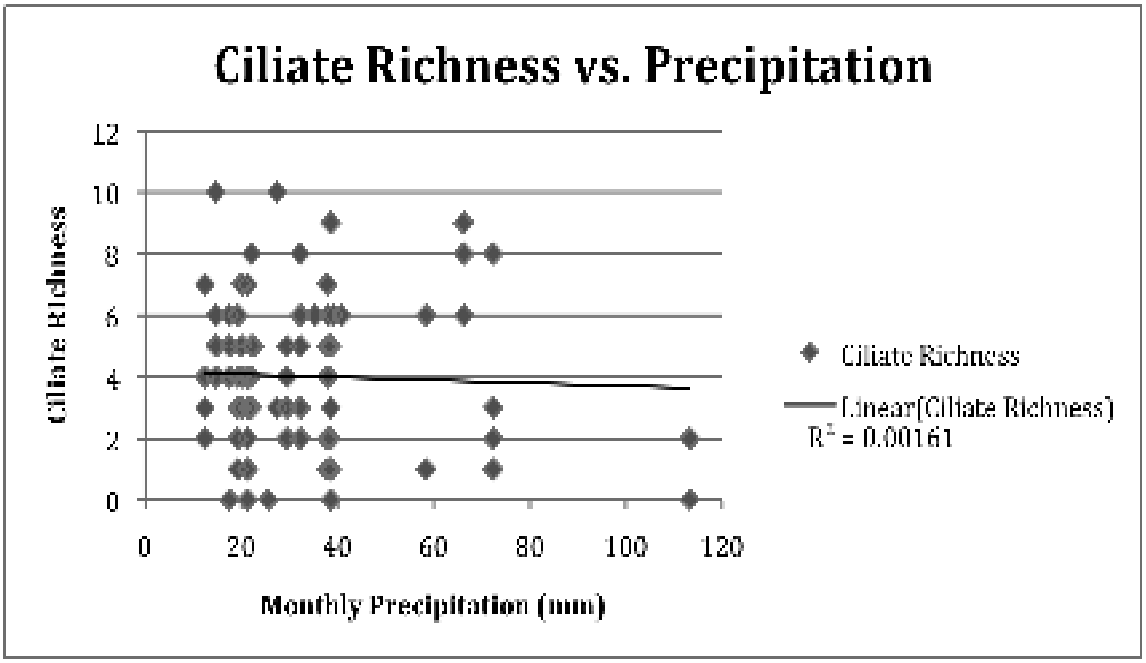


Figure 4. Number of ciliate species recorded in individual rhinos in Main Camp and Nyathi sections of Addo Elephant National Park, South Africa in response to monthly rainfall.

**Discussion**

The results of this study showed patterns of infection that provided an index of black rhinoceros population health directly related to habitat quality and usage. A lower prevalence and mean number of ciliate species per individual in Main Camp suggests greater environmental and anthropogenic disturbances in that region of Addo Elephant National. The black rhino population in the Nyathi section has a considerably higher prevalence of ciliate species. This pattern was significant for four ciliate species: *Rhinozeta rhinozeta*, *Triplumaria corrugata*, *Monoposthiurn vulgaris*, and *Gilchristata artemis*. When multiple samples per individual were accounted for, similar trends were seen among rhinoceros between the two sections of AENP. Although no variation existed among parasite prevalence, a lack of significant differences could be a result from different degrees of habitat fragmentation and disturbance among competitors, along with insufficient efforts to control contact between humans and wildlife in Main Camp. The results demonstrate that no negative differences exist impacting rhino health in AENP as parasites, which tend to be pathogenic, were seen at lower levels. A difference in the positive impacts of ciliates seen between the two sections in AENP (specifically *Monoposthium* sp. and *Rhinozeta* sp. which are found to aid digestion and serve as a proxy for overall nutritional health of the community (Ramsay)) suggests that the rhinos in the Nyathi section, with a higher prevalence of ciliate species, are experiencing a greater overall level of health.

In addition, 16 different ciliate species were found among black rhinos in both sections of Addo Elephant National Park; however, only 15 of those species were found in the rhinoceros population habituated in Nyathi and only 12 ciliate species were found among black rhinos habituated in Main Camp. The three ciliate species that did not occur in Main Camp rhinos were *Rhinozeta caecalis, Rhinozeta unilaminatus,* and *Charonina odontophora*. Because different ciliates serve various functions, differences in vegetation will greatly influence gastrointestinal

profiles of ciliate species in individual rhinos. The lack of ciliate species in Main Camp could be a result of differences in vegetation due to ecological variations such as seasonal impacts or habitat destruction. A more diverse ciliate community in Nyathi rhinos might indicate a greater range of forage options in Nyathi compared to only a limited amount of vegetation in Main Camp. This agrees with the finding that total number of rumen protozoa depends on the availability and condition of available vegetation (Demeyer, 1981).

Some parasites such as *Strongyloides* sp. and *Trichostrongylus* sp. have been shown to cause mild to high intestinal infections (Tiuria et al. 2006). Many nonpathogenic ciliates were also found among the rhinos in AENP such as the beneficial ciliate species mentioned above, *Monoposthium* sp. and *Rhinozeta* sp. A lower prevalence of symbiotic protozoa for Main Camp compared to Nyathi rhinos might suggest that Main Camp rhinos are experiencing an overall lower population health. This notion that the Main Camp population is perhaps at risk is further supported by the lower number of symbionts per rhino compared to Nyathi, since these symbionts aid in digestion and serve as a proxy for population health.

Patterns of co-infection were found among the parasite species infecting rhinoceros in AENP. Both significant relationships between *Oesophogostramum* sp. and *Necator* sp. as well as *Strongyloides* sp. and *Necator* sp. were found to compete among the rhinoceros. This pattern of coinfection may potentially lead to higher levels of mortality among the rhino population.

No relationships were found while looking at stress hormone concentration levels among the rhinoceros population in AENP in relation to anthropogenic and ecological factors. Numerous ecological factors such as diet, reproductive status, body condition, and natural daily rhythms can drastically influence hormone concentrations (Washburn and Millspaugh). Whether individually and/or synergistically, these have the potential to significantly influence fecal

glucocorticoid levels that might be partially driving the gastrointestinal profiles. However, a relationship among precipitation and parasite/ciliate richness was not observed. It is important to note that no climate data was available for AENP so climate data for Port Elizabeth, South Africa was used. This could imply that there was not much variation in vegetation cover across different seasons (Turner et al. 2011). The lack of increased parasite richness with elevated stress levels in the rhinoceros population might demonstrate successful acclimation to environments previously altered by anthropogenic or climactic factors as well as a variety of other factors mediating susceptibility to parasites (Zommers et al.).

This vulnerability to infectious disease will be better understood by integrating our results with corresponding data on reproductive hormones, diet, and demographics. Future work on this project by our team will examine this interplay in relation to patterns of human contact and various natural and anthropogenic stressors to these rhino populations. Looking at the homogeneity of the gastrointestinal pathogen communities among the rhinoceros between the two sections can also aid in determining host/environment/parasite interactions and relationships. Combining this knowledge with patterns relative to reproductive hormone physiology and diet will help to create an amalgamate assessment of the overall health of the rhino population, and determine the best management practices to create a self-sustaining population. The health assessment performed in this study shows no significant findings in negative influences on rhino health in AENP. Our results highlight the importance of anthropogenic disturbances on patterns of mutualism.

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**Appendix I.** Gastrointestinal nematoda found among black rhinoceroses (*Diceros bicornis bicornis*) habituated in Main Camp and Nyathi sections of Addo Elephant National Park, South Africa.

2a. *Necator sp*.

2b. *Oesophogotomum sp*.

2c. *Trichostrongylus sp*.

2d. *Strongyloides sp*.

2e-f. *Larval Nematodes*

**Appendix II.** Gastrointestinal entodiniomorphida found among black rhinoceroses (*Diceros bicornis bicornis*) habituated in Main Camp and Nyathi sections of Addo Elephant National Park, South Africa.

3a. *Didesmis synciliata*

3b. *Blepharosphaera intestinalis*

3c. *Blepharoconus dicerotos*

3d. *Blepharosphaera ceratotherii*

3e. *Charonina odontophora*

3f. *Charonina tenius*

3g. *Blepharoconus cervicalis*

3h. *Gilchristata artemis*

3i. *Triplumaria corrugata*

3j. *Monoposthiurn vulgaris*

3k. *Rhinozeta rhinozeta*

3l. *Rhinozeta triciliata*

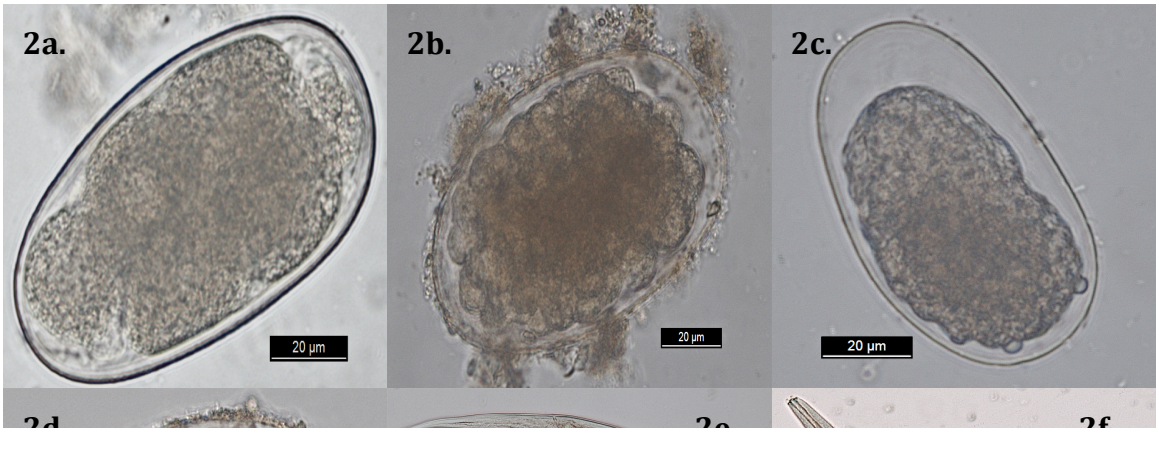
3m. *Rhinozeta addonenesis*

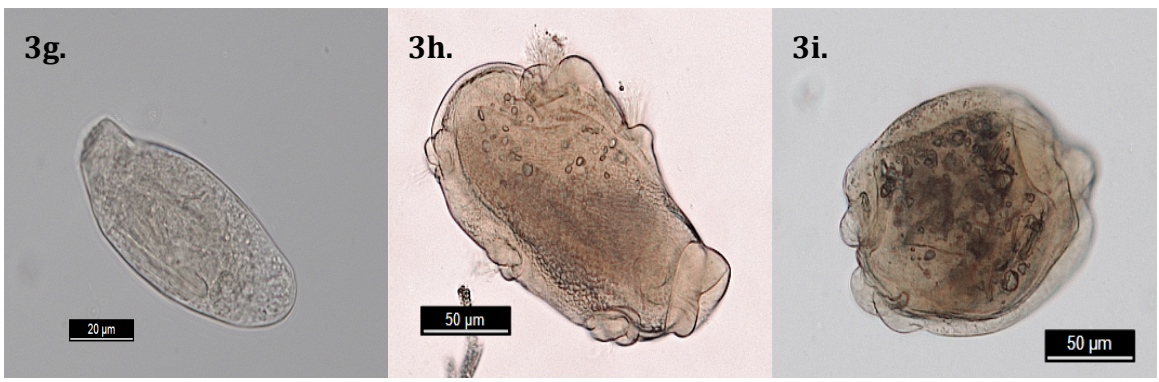
3n. *Rhinozeta cristata*

3o. *Rhinozeta caecalis*

3p. *Rhinozeta unilaminatus*

3q. *Triplumaria corrugata*

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