Temporal and Spatial Variation of the Intestinal Infracommunity of *Alligator mississippiensis*

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Examining the parasite infrastructure and community of a population of hosts in various locations over time can reveal cryptic information on environmental changes, as well as provide insight on the host biology, diet, and behavior. Various studies have illustrated how size, sex, location and dietary ontogenetic shifts influence parasite infrapopulations and component communities. However, abiotic factors such as temperature, precipitation, salinity, and heavy metals are also known to significantly govern host parasitism. The synthesis of these abiotic components partly contributes to the temporal fluctuations of infection and the spatial distribution of parasite species by determining the rate of succession of parasite life cycles. This affects the assemblage of parasite species richness localized within a host population, as well as demographically across a host range.

Heavy metals are common components of aquatic systems and represent the chemical composition of the local aqueous biotope. The increases in agricultural run-off, industry, as well as natural disasters, are contributing to toxic metal concentrations in water systems. In recent years, there has been an increase interest in biomonitoring as a technique to obtain higher accuracy of current environmental metal concentrations as a result of the bioaccumulation of chemical elements in organismal tissues. As a result of their interaction with both the environment and multiple trophic levels, and their ability to absorb and accumulate pollutants in their tissue more readily than their hosts, parasites are proving to be exceptional effect and accumulation bioindicators of metal pollution.

There are various documentations of parasitism in the American Alligator, but at present there have been no studies exploring the effects of environmental and anthropogenic disturbances on this host-parasite system. The goals of this study were: 1) explore variation of alligator intestinal parasite patterns between sample locations; 2) examine the significance of alligator size in parasite infracommunity structure; 3) verify parasite differences between alligator sex; 4) examine how abiotic and biotic factors effect parasite species richness, prevalence and intensity over a 3-year investigation; and, 5) identifying correlations of environmental and anthropogenic variables influence on parasitism between host size, sex, location or year.

Additionally, this study examines the quality of using parasites from an aquatic, long-lived, apex predator, *Alligator mississippiensis*, from Louisiana and Florida as bioindicators of environmental heavy metal pollution. Three parasitic groups from three different biological host sites will be examined for heavy metals in parallel to alligator levels to assess discrepancy between host and parasites, in addition to analyzing similarities and differences of metal uptake between parasitic groups of the different infection sites. Parasite prevalence, intensity, and abundance of individual hosts will also be examined and correlated to heavy metal concentration. Considering alligators are top-level predators in their localities, this information can be used to measure metal contamination in the area, as well as low-level pollutant discharge, providing information on the effects of environmental stress on host parasitism and biodiversity.

MATERIAL AND METHODS

Study Area

Alligators were collected with the assistance of the Louisiana Department of Wildlife and Fisheries (LDWF), nuisance hunters and licensed processing sheds for 3 annual alligator harvests (2009-2011). The alligator population of Louisiana is roughly 2 million, and about 300,000 farm and wild alligators are culled/harvested each year. The Alligator Management Program separates Louisiana into two zones during the 1-month harvest season. The East Zone (LAE) harvest begins in late August, and the West Zone (LAW) begins a week later in early September.

Host Collection
A total of 107 alligators were collected in 2009 (n= 17), 2010 (n= 45), and 2011 (n= 45) with legal trapping methods such as hook or line, bone and arrow or with a legal firearm. Location, sex, and size (juvenile: 30-90 cm; sub-adult 90-180 cm; adult ≥180 cm) were recorded. Intestinal tracts were removed from mesentery and either dissected upon removal or put into labeled jars fixed in 10% formalin and transferred to UCLA. Upon arrival, intestines were transferred to 70% ethanol.

Parasite Collection
Dissections were initiated at the beginning of the small intestine and continued down towards the rectum. A small portion of the intestine was cut, and its length and circumference was measured. Each piece was then examined under a stereomicroscope for parasites. Helminthes found were separated into labeled vials of 70% ethanol per host until identification, stained, and then identified.

Heavy Metal analysis
Pieces of livers (n= 71) or body fat (n= 8) were collected from alligators during the 2010-2012 annual Louisiana (LA) alligator harvest, and (n= 27) were collected from Florida (FL) during the 2011 and 2012 alligator harvests. Geographic origin, size and sex were recorded from all specimens. Parasites were recovered from lungs (LA, n= 19; FL, n= 16), stomach (LA, n= 24; FL, 26), and intestines (LA, n= 23; FL, n= 4), and put into labeled vials that were identified to the site of infection, host, and year. Liver or body fat samples were put into a cooler at -80°C, and parasite samples were either stored in a -80°C cooler or in vials of 95% ethanol, then transported to McNeese University or University of California, Los Angeles (UCLA) for heavy metal analysis.

Al, As, Cd, Cr, Cu, Fe, Mg, Ni, Pb, Pt, Se, and Zn are being analyzed in sample vials with an inductively coupled plasma atomic emission spectroscopy (ICP-OES) at McNeese State University and University of Mississippi.

RESULTS

Species Composition
A total of 10,421 trematodes and nematodes were collected from 104 infected alligator specimens. Overall parasite prevalence was 96%. Helminths were found in four microhabitats of the intestine, which included the duodenum, jejunum, ileum, and rectum. However, the majority of helminthes parasitized the duodenum and jejunum.

Variation in parasitism between alligator sex
There was a significant difference in total parasite abundance (W= 1022, p= 0.01107; Bootstrap t-test: p=0.0385) and total trematode abundance (W= 1015, p= 0.009757) between

the sexes. No difference between total nematode abundance, and nematode and trematode species richness was found. Prevalence between males and females was significantly different (Fisher's Exact: p= 0.024). Overall female helminth prevalence was 90% and mean intensity was 61.702 ± 65.988. Overall male helminth prevalence was 100%, and mean intensity was 136.745 ± 212.190.

Parasitic difference among alligator size categories

No significant difference was found between total helminth abundance, and sub-categories nematode and trematode abundance among alligator size. Additionally there was no significant difference in total nematode and trematode species richness. Infected proportion of categories did not differ significantly (Mood's median, p= 0.0895). Juveniles were 100% parasitized, with an average intensity of 4.4 ± 518.7. Examination of 38 sub-adults, 35 were parasitized (92%) with an average intensity of 68 ± 75.5. Helminth prevalence among adults was 97%, with an average intensity of 104 ± 145.6. Prevalence among alligator size was significantly different (Fisher's exact: p= 0.0485).

Geographic parasite variation

There was no significant difference of total helminth abundance, including nematode and trematode abundance, between LAE and LAW or habitat type. No significant difference was found among helminth community between exact GPS site locations, and longitude and latitude. Nematode and trematode species richness did not differ significantly. Examination of 53 alligators from LAE, 52 were infected (98%) with an average helminth intensity of 86.654 ± 96.870. Helminth prevalence in LAW was 93% with an average helminth intensity of 118.3 ± 215.251. Helminth prevalence between zones was not significantly different (Bootstrap 2-sample t-test, p= 0.3345).

Annual variation in parasitism

A significant difference was found between total abundance between harvests (Kruskal-Wallis chi-squared= 6.5051, df= 2, p= 0.03868), total trematode abundance (Kruskal-Wallis chi-squared= 6.6302, df= 2, p= 0.03633), and total nematode abundance (Kruskal-Wallis chi-squared= 13.6468, df= 2, p<0.01). Nematode and trematode species richness did not differ significantly. Analyses of sub-categories impact on helminth parasitism illustrate that variation in male parasitism differed significantly among harvest years. A significant difference was found among males between annual harvests in total helminth abundance (Kruskal-Wallis chi-squared= 8.6748, df= 2, p= 0.01307), trematode abundance (Kruskal-Wallis chi-squared= 8.0418, df= 2, p= 0.01794), and nematode abundance (Kruskal-Wallis chi-squared= 10.2714, df= 2, p= 0.005883). Nematode species richness also significantly differed amongst males between harvests years (Kruskal-Wallis chi-squared= 11.2443, df= 2, p= 0.03617). Harvests 2009 and 2010 both had 100% prevalence, yet differed in intensity (2009: 112.9 ± 102.1; 2010: 152.2 ± 230.9). Helminth prevalence in Harvest 3 was 91%, with an average intensity of 152 ± 230.9. No significant difference was found among harvests prevalence, yet proportion of infection among harvests differed significantly (Mood's median, p= 0.041).

Parasite Aggregation

Total parasite and trematode assemblage was found to be aggregated and heterogenous among sub-categories sex, size and temperature (Total parasite overdispersion coefficient: 1.6964; Trematode oversdispersion coefficient: 1.5051). The best fitting model based on lowest AIC for both total parasite intensity and trematode intensity propose that sex is the

only significant predictor of parasite aggregation, males having the highest parasite aggregation.

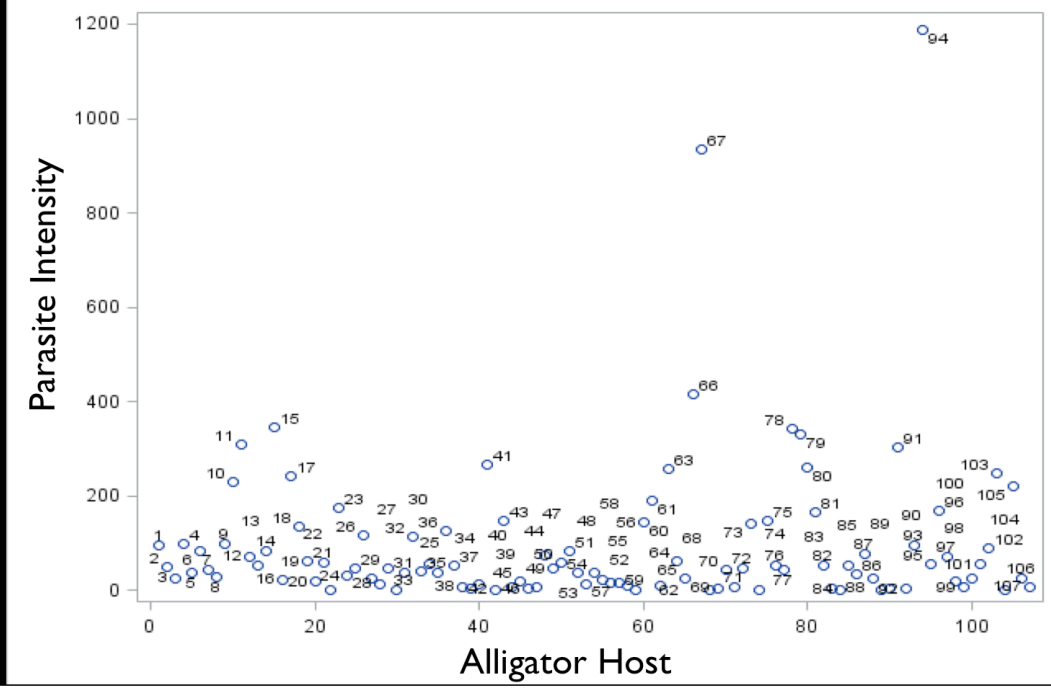
Heavy Metal Analysis
Analysis is being conducted at the moment.

DISCUSSION

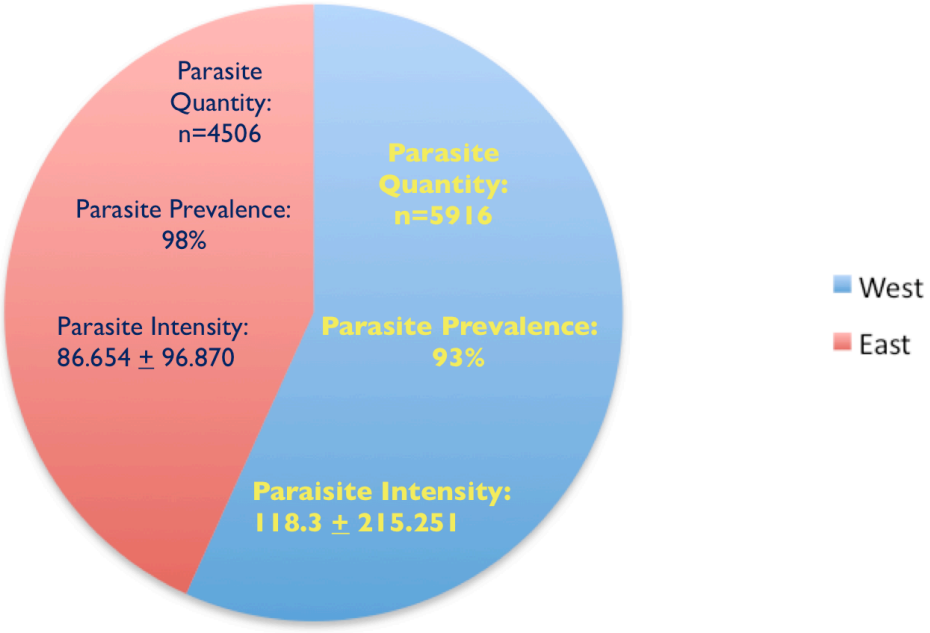
The present study examined utilizing parasites and alligators as heavy metal bioindicators, as well as analyzed the intestinal helminth community structure of alligator populations in Louisiana. Heavy metal analysis are still being conducted a present. The other part of the study has illustrated that alligator sex and harvest year significantly contribute to the parasite infra- and component community assemblage of the American Alligator. Parasite prevalence among the 107 alligators examined was high (96%). Unlike previous reptile-helminth studies, alligator parasites are dominantly host specialists, utilizing alligators as the final host.

Statistical analyses are still being processed and examined. In summary, males were overall more heavily parasitized than females, large sub-adults had higher parasitic intensity than adults and juveniles, and there was no significant different between LAE and LAW. Prevalence of infection was high for LAE and LAW indicating that alligators throughout the zones are frequently infected by helminths. Although statistical analyses did not prove to be significant among zones or particular geographic locations, LAE parasite prevalence was higher than LAW, yet parasite intensity was greater in LAW. Quantitative difference of prevalence and intensity among geographic zones may be explained by anthropogenic modification of environment and abiotic factors. Mining and natural resources are equally distributed throughout southern Louisiana, yet agriculture is significantly predominant in LAW than LAE, and mining and urbanization is predominant in LAE than LAW. These factors can greatly affect parasitism in hosts. It is possible environmental factors, such as hurricanes and drought, affected the annual variation in parasitism, yet this is still be analyzed.

Parasite Intensity

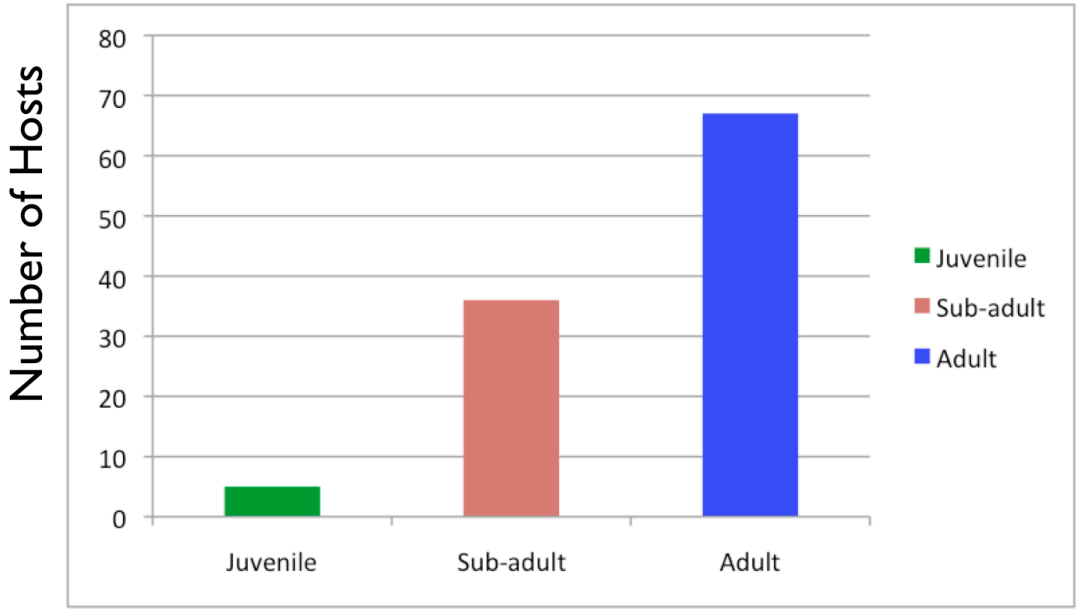


Geographic Parasite Variation



Parasite Variation in Alligator Size

Prevalence was significantly different: Fisher's exact, p=0.0485



Juvenile	Sub-adult	Adult
100% Prevalence Intensity: 4.4 ± 518.7	92% Prevalence Intensity: 68 ± 75.5	97% Prevalence Intensity: 104 ± 145.6