Gastrointestinal Helminth Prevalence in Lesser Scaup (*Aythya affinis*) Wintering on Lake Pontchartrain, Louisiana, USA

by

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Undergraduate honors thesis under the direction of

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Submitted to the LSU Roger Hadfield Ogden Honors College in partial fulfillment of the Upper Division Honors Program

April 2018

Louisiana State University
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Baton Rouge, Louisiana

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ABSTRACT

The continental population of Lesser Scaup (*Aythya affinis*; hereafter, “scaup”) has been in decline since the 1980s, and today exists at 51% of the original goal of 6.3 million set by the North American Waterfowl Management Plan. Endogenous nutrient stores accumulated by female scaup during the winter can influence initiation date, clutch size, and egg viability, and these factors are believed to drive scaup populations. Gastrointestinal helminths accumulated on wintering grounds could negatively affect nutrient absorption, leading to a decline in body reserves and consequently lowering reproductive success. We identified and enumerated helminth parasites in the lower gastrointestinal systems of 33 scaup collected from Lake Pontchartrain during the winter of 2016. We found a high prevalence of helminths, but at relatively low intensity. 75% of scaup were infected with a total of 465 helminths (mean 14.09 ± 4.07 SE). These helminth counts from scaup wintering on Lake Pontchartrain were lower than those observed in scaup in the upper Midwest and elsewhere in Louisiana. Helminth abundance significantly varied by age and sex, but these relationships were driven by one outlying juvenile female with high helminth counts. Importantly, we found no significant relationship between helminth abundance and body condition. In conclusion, while most scaup were infected with parasites, infections occurred at a relatively low intensity. This suggests that Lake Pontchartrain may be relatively free of gastrointestinal parasites and their intermediate hosts.

INTRODUCTION

The Prairie Pothole Region (PPR) is an important breeding ground for North American waterfowl (tribes Anatini and Aythyini), and recruitment varies with wetland conditions in the PPR. For example, most duck species declined 1986–1993 during drought conditions, but recovered between 1994 and 1997 (Smith 1995, Afton and Anderson 2001). However, the continental population of some species, such as the Lesser Scaup (*Aythya affinis*), have remained low. Since the late 1980s, the continental population of Lesser Scaup (hereafter, “scaup”) has remained below the continental population goal of 6.3 million established by the North American Waterfowl Management Plan (NAWMP) (Afton and Anderson 2001, Caithamer and Dubovsky 1997). The 2012 NAWMP estimated the continental scaup population at 4.1 million ducks, 2.2 million fewer than the continental management goal (USFWS 2012). Range-wide Midwinter Waterfowl Survey counts of scaup have indicated an average population decline at a rate of 24,260 annually (Afton and Anderson 2001). Additionally, harvest data between 1961 and 1996 revealed that the sex ratio and age structure of the continental population shifted such that male scaup outnumbered females, and adults outnumbered immature scaup (Afton and Anderson 2001). These patterns suggest poor recruitment of scaup to the breeding population and decreased survivorship of female scaup, two trends that perpetuate the decline of scaup across their continental range.

Louisiana provides important habitat for scaup in the Mississippi Flyway. Between 1961 and 1996, Louisiana overwintered 91% of all scaup recorded during midwinter counts of the Mississippi Flyway, which itself composes 40% of the continental scaup population (Afton and Anderson 2001). Louisiana hunters also harvest the most scaup (mean 70,918 ± 10,565 SE) of any U.S. state (Afton and Anderson 2001). One particularly important wintering area for scaup is Lake Pontchartrain, a dynamic estuarine system in southeast Louisiana (Stroud 2018). On Lake Pontchartrain, the most important food items for scaup are *Rangia cuneata* clams and *Mytilopsis leucophaeta* mussels, which constitute 42.54% and 25.78% of ingested food items, respectively (Stroud 2018). *Probythinella protera* snails account for an additional 17.10% and *Texadina sphinctostoma* snails account for 9.18% of items consumed (Stroud 2018). Both bivalves and aquatic snails serve as intermediate hosts for a number of enteric helminth parasites of waterfowl (Lapage 1961, McDonald 1969, and Wobeser 1981).

The geographic range of a given host-helminth system is bounded by the longitudinal and latitudinal extents of the duck host species' migratory flyway (Brown 1984, Fedynich and Pence 1993, Wallace and Pence 1986). Previous studies have noted a latitudinal gradient in parasite infestation in dabbling ducks, with the highest rate and intensity of helminth infections found on the breeding grounds where high densities of waterfowl forage upon seasonally abundant intermediate host species (Buschner 1965). Moreover, northward migrating ducks bring with them the helminths acquired from their species-specific food habits and geographically diverse wintering sites, thereby increasing the diversity of helminth fauna in breeding ground ponds. In a study of seasonal helminth fauna dynamics in Northern Pintail (*Anas acuta)*, Gadwall (*Mareca strepera)*, and Northern Shoveler (*Spatula clypeata),* Buschner (1965) found that adult ducks acquired 10 novel helminth species on the breeding grounds, in addition to the 17 species they brought with them from migration. Conversely, the lowest rate and intensity of helminth infections of dabbling ducks are observed in the wintering grounds of migratory waterfowl, where helminth infections may decrease to half the intensity of infections maintained on breeding grounds (Buschner 1965, Wallace and Pence 1986). Some proposed mechanisms for the decline in intensity of helminth infections are declines of intermediate host abundance, and changes in the food habits of migrating and wintering dabbling ducks as preference for these invertebrate forage items declines seasonally (Buscher 1965). The diets of dabbling ducks vary throughout the year, with dabblers predominantly consuming grains in the winter, and transitioning to more protein and calcium rich invertebrates in the spring (Baldassarre 2014). Therefore, it is perhaps unsurprising that dabbling ducks are infected with lower gastrointestinal parasite loads during the winter, as their consumption of invertebrate intermediate hosts during this time is minimal.

In contrast, diving duck diet composition remains more constant across seasons and throughout their continental range (Baldassarre 2014). Diving ducks, such as scaup, maintain a diet of primarily invertebrates, foraging on seasonally available gastropods and bivalves in the winter, and increasing consumption of amphipods in the spring (Baldassarre 2014). Because diving ducks forage on invertebrates (potential intermediate hosts of helminths) throughout their range and across the annual cycle, it seems possible that the helminth infections of diving ducks may be more severe, and may fluctuate less than in dabbling ducks. Nevertheless, helminth communities likely shift to some degree as the type of invertebrate consumed by scaup shifts across the species' continental range.

High intensities of helminth infection, especially infection with trematodes, have been associated with pathology in waterfowl. Trematodes have been indicated as the causative factor of hemorrhagic ulcerative enteritis in Anseriforms, but disease severity is a function of parasite prevalence and intensity (Ballweber 2004, McDonald 1969). Nevertheless, infection of waterfowl by gastrointestinal helminth parasites has the potential to impede the acquisition of nutrients (England et al. 2017, Wobeser 1981, Vest et al. 2006). Nutrients acquired by females on wintering grounds, especially those converted to lipid reserves, are a major determinant of breeding propensity, nest success, and juvenile recruitment (Alisauskas and Ankney 1992, Anteau and Afton 2004, Norris 2005). We examined the prevalence and intensity of gastrointestinal helminth parasites in scaup wintering on Lake Pontchartrain as a possible factor contributing to poorer scaup body condition, which could have cross-seasonal effects on breeding demographics that potentially contribute to the continental decline in scaup populations.

MATERIALS AND METHODS

Study Site

Lake Pontchartrain, a 1,630 km^2 brackish estuary in southeast Louisiana, is characterized by a well-mixed water column (mean depth 3.7 m), slow flushing time, and a saline gradient becoming fresher from east to west (mean salinity 107 ppt) (Saucier 1963, Sikora and Kjerfve 1985). The natural hydrology of Lake Pontchartrain has been altered by the construction of three commercial waterways that have changed the flow and salinity regimes of the system. The most dramatic modification of Lake Pontchartrain's natural hydrology was finalized in 1963 with the completion of the 120 km long, 157 m wide Mississippi River-Gulf Outlet (MR-GO) which connected New Orleans and Lake Pontchartrain with the Gulf of Mexico (Sikora and Kjerfve 1985). Lake Pontchartrain's connectivity to the Gulf of Mexico increases the influence of natural disturbance events, especially hurricanes and El Niño cycles, on the physiochemistry and aquatic biota (Sikora and Kjerfve 1985).

Field Procedures

A total of 60 scaup (42 adults, 18 juveniles; 53 males, 7 females), were collected by LSU School of Renewable Natural Resources MS student Clay Stroud between December 2016 and January 2017. Scaup were collected under federal collection permit MB 74481B-0 and state collection permit LNHP-15-074. Collection protocols were approved by the Louisiana State University Institutional Animal Care and Use Committee (permit A2015-20). All scaup were collected by shotgun from fast moving boats on Lake Pontchartrain, with collection sites incidentally concentrated on the northern region of the lake where scaup were found in larger flocks. Scaup were injected with 10% buffered formalin (CH_2O) immediately following collection to minimize post-mortem tissue degradation. The upper digestive tract (esophagus and proventriculus) were dissected by Clay Stroud to identify scaup diet items for another study. The lower digestive tract (gizzard, ceca, and intestines), was stored separately in 70% ethanol at room temperature.

I dissected the gizzard, ceca, and intestines of 33 scaup (20 adults, 13 juveniles) over a 10-month period from April 2017 to February 2018. I processed all female scaup (n=7), and randomly selected 26 males for processing. The lower gastrointestinal tract was dissected following protocols adapted from Sepulva and Kinsella (2013). The time expended between sample collection and sample preservation and processing was sufficient to allow posterior migration of helminths. Thus, I did not describe the linear distributions of helminth classes due to the possibility of post-mortem shifts in parasite ranges. I first removed the gizzard and intestines from the 70% ethanol solution and placed them on a 106 micrometer sieve. I used tap water to rise the external surface of the gizzard and intestines to remove remnant ethanol and blood coagulant resulting from collection. I gently detached the mesentery binding the intestines such that the intestines were able to lay straight across the dissecting board. I then put the intestines into deionized water to prevent desiccation, and to rehydrate the tissues. I bisected the gizzard along the longitudinal plane and scraped the digesta into a 50 mL beaker. I then flushed the lumenal surface of the gizzard with deionized water to remove remnant digesta. Once flushed, I examined the luminal surface of the gizzard under a dissecting microscope for imbedded parasites. I then suspended beaker of gizzard contents into approximately 20 mL of deionized water by manual agitation. I allowed the gizzard content-deionized water slurry to rest while I prepared the intestinal tissue samples. If I observed stratification of the solution after this time, then I pipetted the clear supernatant off of the surface of the solution.

I bisected the intestines longitudinally, and removed the ceca to a separate gridded petri dish. I then scraped the intestinal contents into a 50mL beaker using a razor blade. I suspended the intestinal contents into solution with deionized water in the same way as the gizzard contents. I then cut the evacuated intestines into segments roughly 3-inches in length. I examined the surface of these intestinal segments under a dissecting microscope for imbedded parasites, especially acanthocephalens. Next, I used a pipet to draw the gut content solution into a gridded petri dish. I first removed conspicuously large parasites with tweezers or a pipette and identified to one of four taxonomic classes of helminth parasites – Nematoda (roundworms), Cestoda (tapeworms), Trematoda (flukes), and Acanthocephala (thorny-headed worms). I examined the solution remaining in the petri dish for helminths, using grid squares to optimize search efficiency. I recorded helminth parasites as they were identified, and recorded and transferred them to screw-top glass sample vials labelled by helminth class and individual scaup. Sample vials were filled with 70% ethanol at a rate of no less than 5 parts ethanol per volume to 1-part parasite volume.

I recorded helminth prevalence by taxonomic class in Excel and completed statistical analyses in Program R. I used generalized linear regression with a Gaussian error structure to evaluate the effects of parasite abundance on scaup body condition (mass corrected for structural size). Clay Stroud estimated body condition as the residual of body mass regressed against the first principle component of structural size (taken from multiple morphometric measurements)

(Stroud 2018). I used generalized linear regression with a Poisson error structure to test whether parasite abundance varied with age or sex of scaup, and whether parasite abundance varied with the number of food items consumed. I chose an alpha value of 0.05 to designate significance of statistical relationships. Unless otherwise noted, data are presented as mean ± standard error.

RESULTS

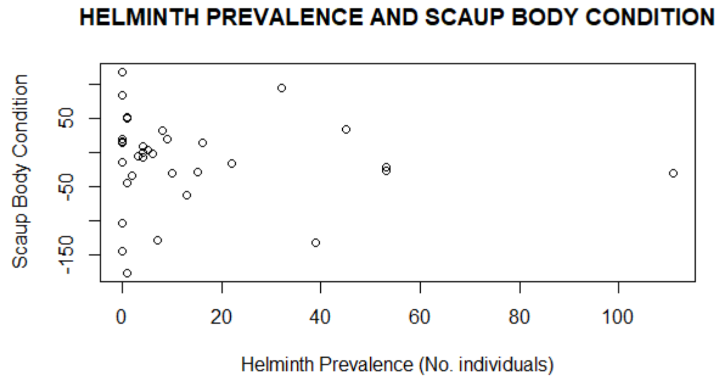
33 scaup were infected with three classes of gastrointestinal helminth: Nematoda, Cestoda, and Trematoda. 75% of scaup were infected with a total of 465 parasites, with a mean abundance of 14.09 helminths per scaup. No parasites were observed in the gizzards or ceca of dissected scaup. Helminths of class Trematoda (12.21 ± 3.89) were the most abundant parasites, followed by Cestoda (1.67 ± 1.19), and Nematoda (0.21 ± 0.09). No acanthocephalens were observed (Table 1). No lesions, evidence of hemorrhaging unrelated to collection, or other gross indicators of pathology were observed in the gastrointestinal tissues, even in those individuals with higher relative helminth intensities.

Figure 1. Minimum, mean, maximum, and relative frequency of helminths of classes Trematoda, Cestoda, Nematoda, and Acanthocepha found in the lower digestive tracts of scaup (n=33).

Helminth Class	Min	Mean ($\bar{x}$ ± SE)	Max	Relative Frequency
Trematoda	0	12.21 ± 3.89	106	0.867
Cestoda	0	1.67 ± 1.19	2	0.118
Nematoda	0	0.21 ± 0.09	39	0.015
Acanthocephala	0	0	0	0

Body condition was unrelated to the total abundance of parasites. No statistically significant relationship was observed between body condition and total helminth abundance (β = -0.15 ± 0.51, p = 0.77) (Fig. 2).

Figure 2. Relationship between total helminth prevalence (number of individuals of classes Trematoda, Nematoda, and Cestoda summed) and scaup body condition (scaup weight corrected for size) (p=0.77).



No significant relationship was observed between body condition and prevalence of trematodes (β = 0.094 ± 0.536, p=0.861), cestodes (β = -2.788±1.683, p=0.108), or nematodes (β = 0.023 ± 21.984, p=0.770 (Fig. 3–5). Statistically significant relationships between scaup sex and helminth prevalence (β = 1.038 ± 0.097, p<0.01), and scaup age and helminth prevalence (β = 0.677 ± 0.097, p<0.01), were observed, but biological effect sizes were small, and the relationship was largely driven by a single juvenile female infected with a large number of parasites. When the juvenile female outlier was excluded from data analysis, the correlation between helminth prevalence and scaup sex (β = 0.338 ± 0.1526, p= 0.026), and helminth prevalence and scaup age (β = 0.657 ± 0.106, p<0.01), remained statistically significant, but the strength of correlation, particularly the correlation between helminth prevalence and scaup sex, decreased.

Figure 3. Relationship between total trematode prevalence and scaup body condition (scaup weight corrected for size) (p=0.861).

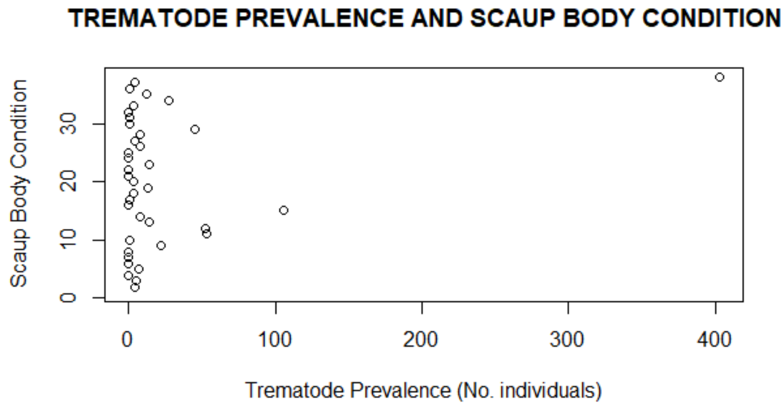


Figure 4. Relationship between total nematode prevalence and scaup body condition (scaup weight corrected for size (p=0.108).

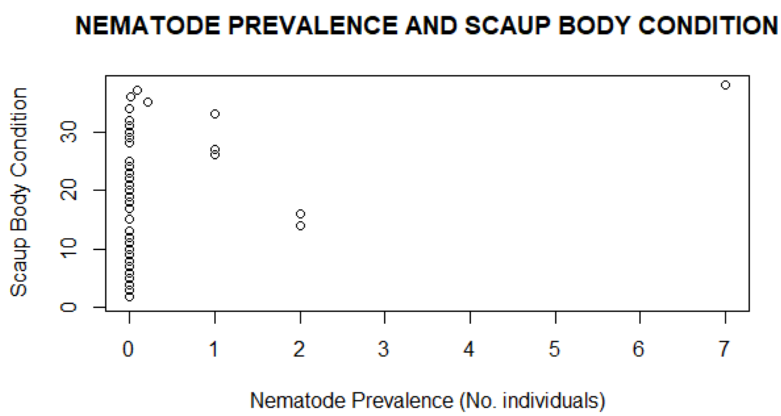
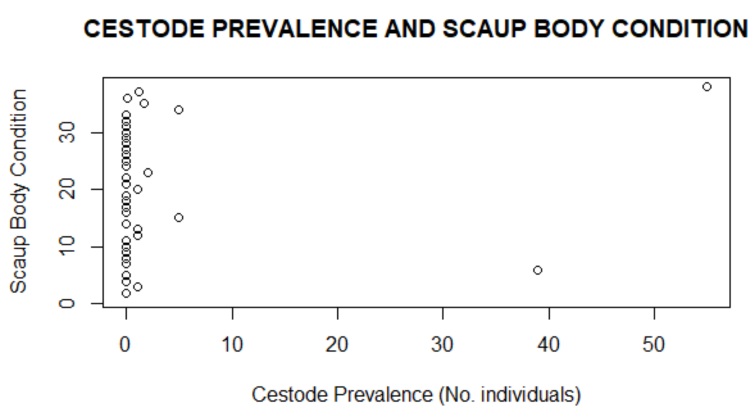
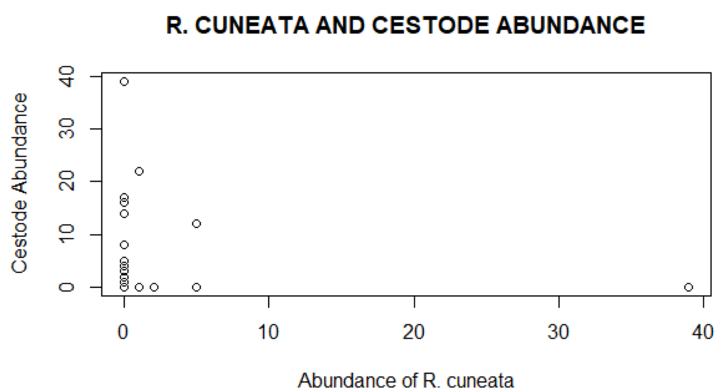


Figure 5. Relationship between total cestode prevalence and scaup body condition (scaup weight corrected for size)(p=0.999).



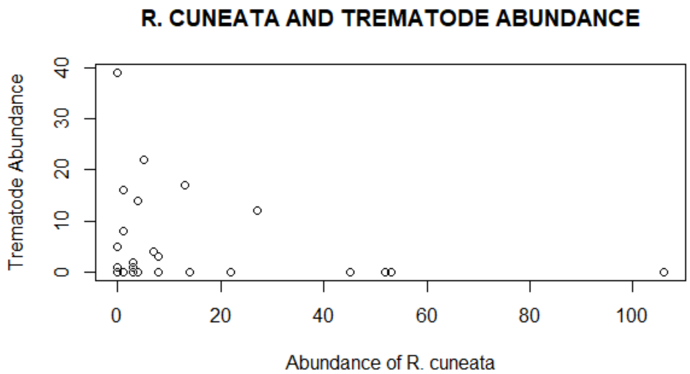
Rangia cuneata, the most common benthic invertebrate consumed by scaup, was unrelated to nematode ($\beta = -0.112 \pm 0.114$, p=0.325) prevalence, but was significantly related to cestode prevalence ($\beta = -0.086 \pm 0.033$, $p<0.01$) (Fig. 6) and trematode prevalence ($\beta = -0.063 \pm 0.010$, $p<0.01$) (Fig. 7), though again, biological effect sizes were trivial, and statistical significance was driven by outliers in the data (Fig 6–7). The significant relationship between *R. cuneata* consumption and cestode and Trematode prevalence disappeared ($p > 0.05$) when outliers were excluded from statistical analysis. *Mytopsilis leucophaeta*, the second most consumed forage item, was unrelated to trematode ($\beta = 0.010 \pm 0.005$, p=0.066) or Nematode ($\beta = -0.128 \pm 0.136$,

Figure 6. Relationship between consumption of *Rangia cuneata* and cestode abundance in scaup wintering on Lake Pontchartrain ($p<0.01$).



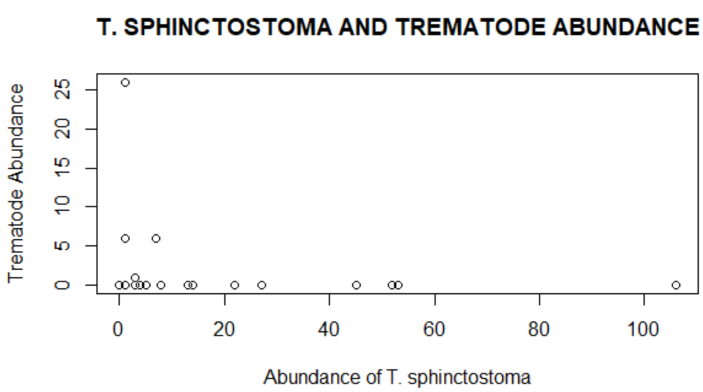
p=0.346) prevalence, but showed a statistically significant, but biologically insignificant, correlation with cestode prevalence ($\beta = -0.061 \pm 0.027$, p=0.026). Again, excluding outliers from the analysis eliminated the statistically significant correlation (p > 0.05) between consumption of *M. leucophaeta* by scaup and prevalence of cestodes.

Figure 7. Relationship between consumption of *Rangia cuneatea* and trematode abundance in scaup wintering on Lake Pontchartrain (p<0.01).



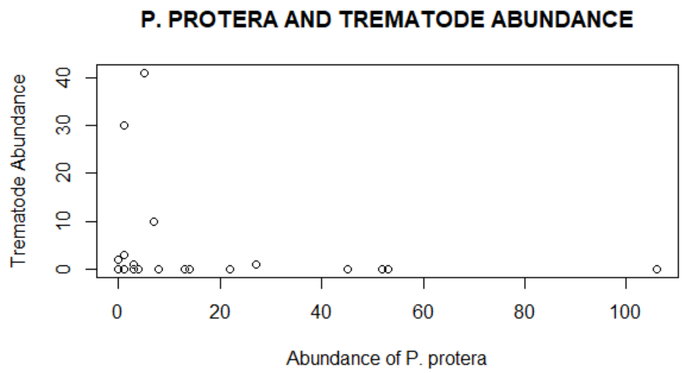
Texadina sphinctostoma was also commonly consumed by scaup, and was not related to nematode (β = -0.210 ± 0.429, p=0.625) or cestode (β = -15.411 ± 978.190, p=0.987) abundance, but displayed a statistically significant relationship with trematode abundance (β = -0.150 ± 0.045, p<0.01) (Fig. 8), but the model for *T. sphinctostoma* and cestode prevalence contained only 8 data points, making the output of the model is difficult to interpret. *Probythinella protera*

Figure 8. Relationship between consumption of *Texadina sphinctostoma* and trematode abundance in scaup wintering on Lake Pontchartrain (p=4.0e^{-4}).



was unrelated to nematode (β = -0.321 ± 0.621, p=0.605) or cestode (β = -0.047 ± 0.033, p=0.151) prevalence, but displayed a statistically significant relationship with trematode prevalence (β = -0.047 ± 0.012, p<0.01) (Fig. 9).

Figure 9. Relationship between consumption of *Probythinella protera* and trematode abundance in scaup wintering on Lake Pontchartrain (p<0.01).



DISCUSSION

I observed low prevalence of gastrointestinal nematodes, trematodes, and cestodes in the 33 scaup I dissected, and I observed no acanthocephalens. The low prevalence of gastrointestinal helminth parasites observed in scaup wintering on Lake Pontchartrain is consistent with the findings of previous studies examining the structure of helminth communities across the geographic range of migratory waterfowl hosts. Helminth prevalence has been found to decrease with distance from breeding grounds (Brown 1984, Buscher 1965, Fedynich and Pence 1993, Garvon et al. 2011, Gregory 1990, Poulin 2011). Our results are consistent with this trend when compared to other studies considering helminth prevalence in scaup in more northerly areas of the Mississippi Flyway. For example, Vest at al. (2017) surveyed helminths in female scaup at four spring migration stopover sites, and observed much higher abundance and richness of helminths. At a site in the central Mississippi River Valley, Vest et al. (2017) observed a mean abundance of 4367.4 Trematodes per scaup host, a value dramatically higher than the 12.21

trematodes per scaup found in our study at the southern terminus of the Mississippi Flyway. The higher parasite prevalence and intensity during spring migration observed by Vest et al. (2017) could suggest that helminth prevalence is sensitive to seasonal intermediate host abundance, and that intermediate host abundance, and subsequent abundance of helminth parasites in the environment varies by habitat type or time of year. Additionally, the distribution of rafts of wintering scaup across Lake Pontchartrain is clumped, not uniform, and it may be that low densities of definitive hosts (scaup) across the expansive 1,630 km^2 surface area of Lake Pontchartrain are an additional factor limiting helminth abundance, and thus is contributing to the low helminth abundance observed.

Despite the low abundance of parasites we observed in scaup wintering on Lake Pontchartrain, even low intensities of parasite infection have been shown to impact levels of blood metabolites, especially glucose, suggesting that host fitness is reduced even when parasite intensity is low and indications of gross pathology are absent (England et al. 2016). That said, the pathogenicity of low intensity nematode and cestode infections is generally low (Wobeser 1981). Furthermore, the lack of lesions due to parasite attachment in the intestinal lining suggest that helminth prevalence was not a source of gross pathology in the scaup I sampled.

The pairing status of wintering waterfowl can affect the intensity of helminth infection borne, especially in males (Grey et al. 1989). Unpaired males inhabit less favorable wintering habitat and behave more gregariously, two behaviors that could influence foraging behavior and infection by helminths (Gray et al. 1989). Though we observed a statistically significant relationship between scaup sex and total helminth abundance, this relationship was primarily driven by a single outlying data point. However, if the increase in sex ratio of male to female scaup described by Afton and Anderson (2001) continues on its current trajectory, and more males remain unpaired each year, the potential for increased helminth infection intensity in male scaup could increase to biologically significant levels.

We observed a statistically significant relationship between total helminth prevalence and scaup age, but that too was driven primarily by an outlier in the data. Nevertheless, previous studies on the effects of host age on helminth communities in waterfowl suggest that incomplete immune development predisposes juvenile waterfowl to more intense infections by parasites (Buscher 1965, Fedynich and Pence 1993, Wallace and Pence 1986). Thus it seems plausible that there is a potential difference in parasite prevalence and intensity between juvenile and adult scaup, but small differences I observed are likely due to the low overall abundance of helminths in Lake Pontchartrain. It is possible that the trend of juvenile scaup bearing greater parasite loads would become more pronounced if helminth abundance of the system were to increase.

Infection of scaup by gastrointestinal helminths is dependent on the consumption of infected intermediate hosts by birds (Fedynich and Pence 1993, Wobeser 1981). Positive covariation observed between common forage items of scaup and individual helminth classes could offer insight into the intermediate host dynamics of helminths in Lake Pontchartrain. However, our data showed a significant negative correlation value relating some forage items and helminth Class abundance. That was a trend driven by the diet items of a juvenile female outlier with relatively high parasite abundance. The negative correlation between cestode abundance and consumption of the potential intermediate host *M. leucophaeata* is statistically significant, but likely has low biological significance. Trematodes were the most prevalent and abundant helminth Class observed, and displayed statistically significant negative relationships with the presence of *T. sphinctostoma* and *P. protera* in the upper digestive tracts of sampled scaup. Trematodes commonly infect snails as their intermediate host (Wobeser 1981), so it is perhaps surprising that consumption of snails was associated with decreased Trematode abundance in the scaup sampled. This negative correlation between consumption of *T. sphinctostoma* and *P. protera* snails is likely driven by outliers in our sample group, and it is probable that the trend is a statistical artifact of scaup that had recently fed or had consumed relatively high volumes of food items, but were infected with few helminths. Inspecting the tissue of invertebrate forage items for encysted immature helminths could help bypass the confounding influence of outliers within the sample and help elucidate the role of Lake Pontchartrain benthos as intermediate hosts.

CONCLUSION

We observed low prevalence and intensity of gastrointestinal nematodes, trematodes, and cestodes in scaup wintering on Lake Pontchartrain during the winter of 2016. The low abundance of helminths is consistent with the general structure of helminth communities across the geographic range of migratory waterfowl hosts, in which helminth prevalence and intensity peaks in the northern breeding grounds and declines as waterfowl density and forage abundance decreases on stopover sites and wintering grounds. The dynamic nature of the Lake Pontchartrain system produces annual fluctuations in benthic invertebrate communities (intermediate hosts) and the wintering scaup (definitive hosts) that forage on these invertebrates. It seems possible that helminth parasite prevalence tracks the abundance of benthic invertebrates and wintering scaup on Lake Pontchartrain, and that seasonal helminth abundance in the system is governed by the same disturbance regimes that determine the size and structure of benthic invertebrate communities. Therefore, the abundance of helminths in Lake Pontchartrain, and subsequently the gastrointestinal parasite load borne by wintering scaup, may vary significantly from year to year.

Parasites do not seem to be a major detriment to nutrient store acquisition in ducks wintering on Pontchartrain. However, parasitism potentially increases during spring migration and upon arrival on the breeding grounds, and so remains a management concern in other geographies. More intensive helminth sampling efforts across a diversity of habitat types, and across and among migration corridors would provide a more detailed characterization of parasite-host dynamics across the continental range of scaup. Field teams conducting ongoing waterfowl habitat surveys throughout the continental range of scaup could easily collect water or soil samples for eDNA analysis while continuing to record traditional measures of habitat quality. eDNA analysis has potential as a monitoring tool to detect changes in helminth abundance and community composition in response to weather events, change of season, and changes in water chemistry (Bass et al. 2015). Understanding helminth response to environmental variation offers an opportunity to both characterize range-wide host-helminth dynamics of scaup and provides a tool to predict disease risk and success of nutrient reserve acquisition in scaup at various points in their annual migration cycle. eDNA analysis provides a more time and cost-effective tool for ecosystem-level sampling of parasites than do helminth specimen collection based sampling methods (Bass et al. 2015), and thus provide a promising avenue for range wide surveys of abundance in helminths of scaup.

APPENDIX

Photo A.1. Mediolateral view of a gastrointestinal parasite of Class Trematoda. Note diagnostic ventral sucker.

Photo A.2. Gastrointestinal parasite of Class Trematoda. The ventral sucker is visible at the cut end of the lower body fragment.

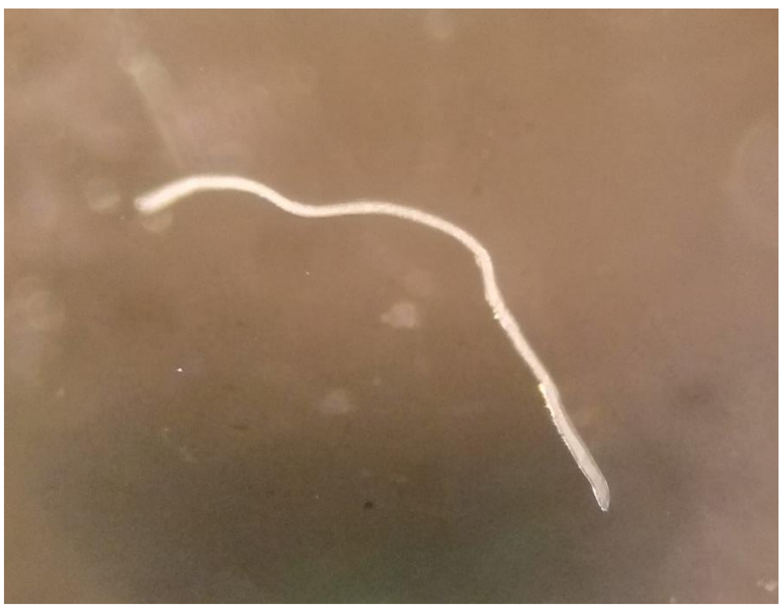


Photo A.3. Gastrointestinal parasite of Class Nematoda. Elongated cylindrical form and unsegmented body are diagnostic characters.

ACKNOWLEDGEMENTS

First and foremost, thanks are due to Dr. Kevin Ringelman, without whose generous guidance, patience, and encouragement this project would never have made it off the ground. I am forever grateful for your guidance in the classroom, in the lab, and in the duck blind.

Thanks also to Dr. Kelly Weinersmith, who kindly volunteered technical training and assistance with development of methodology and parasite identification throughout the duration of the project.

Plentiful thanks are due to Clay Stroud for allowing me access to his diet study data and scaup samples. Your generosity allowed me the opportunity to complete this project, which would have been otherwise unattainable on an undergraduate research budget.

Thanks to the humans of Collier Co. for standing by me even when I became a treacherous "duck gut traitor". I can't thank you enough for the 3 years of support and laughter that you've given me.

Thank you, Emily Hartdegen, for your sense of humor, steely stomach, and bewildering willingness to cut up duck guts. I couldn't have made it through this project without you.

Finally, I would like to thank my committee members Dr. Kevin Ringelman, Dr. Bret Collier, and Dr. Ginger Brininstool for their patience and for taking time out of their busy schedules to hear my defense and suggest revisions.

This project was made possible through funding from the Louisiana Department of Wildlife and Fisheries the LSU College of Agriculture Millard Perkins Undergraduate Research Grant, and Ducks Unlimited Canada.

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