DISTRIBUTION AND INTERACTIONS OF TURTLE ACANTHOCEPHALANS IN TWO SPECIES OF FRESHWATER SNAILS

By:

RYAN WILLIAM KOCH

Bachelor of Science in Ecology, Evolution, and Natural Resources

Rutgers University

New Brunswick, New Jersey

2015

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
December, 2018

DISTRIBUTION AND INTERACTIONS OF

TURTLE ACANTHOCEPHALANS IN

TWO SPECIES OF FRESHWATER SNAILS

Thesis Approved:

Dr. Matthew Bolek

Thesis Advisor

Dr. Andrew Dzialowski

Dr. Monica Papeş

ACKNOWLEDGEMENTS

First, this thesis could not have been completed without the support of my friends and family. To all of my friends on the east coast, you have made the transition from New Jersey to Oklahoma smooth and easy. All of the visits during winter and summer break have been wonderful mini vacations after each completed semester of courses, teaching, and research. James, thank you for all of the intense conversations about life, liberty, and the pursuit of Karen. Will, Min Hwa, and Steve, our adventures are endless and are the highlight of my trips back home. Kelley, as Rutgers graduates, we share a special connection. We know a good towpath when we see one. Our obsessions with dinosaurs, plants, and the other eccentricities in life keep me going. I am extremely fortunate to have you in my life. Brian, you are like a brother to me; knowing you since preschool has brought us very close. I am glad to have you in my life, and I can't wait to see where life takes us next. Mom, your moral support and fascination of nature is the best thing I could ask for. Dad, all of our conversations about wildlife, research, and car problems have been extremely helpful and motivating. I have always looked up to you as a role model, and your many years as a refuge manager in the U.S. Fish and Wildlife Service has been my underlying inspiration towards science. Rachel and Travis; our family is everything to me. I cannot thank you enough for helping me get to where I am today.

Thank you to the Zoology Graduate Student Society (ZoGSS), which has brought me close to my community via outreach, science club, and networking. It has been my pleasure

Acknowledgements reflect the views of the author and are not endorsed by committee members or Oklahoma State University.

serving as ZoGSS president during the 2018-2019 academic year. Thank you especially to the Department of Integrative Biology for the wonderful support, including faculty, my committee, and laboratory facilities. A special thanks to the Bolek lab for your collaborative efforts in making my research possible, especially Ryan Shannon. You have opened my mind to the world of sequencing and all of the [tedious] troubleshooting it entails. I am a more competent researcher because of you. Thank you Scott Goeppner for sharing an interest in parasite and snail research with me; I greatly appreciate your help in designing the snail survival and cage experiments. Thank you to all of the undergraduates who have helped me with field and laboratory work, and thank you to the owners of Cow Pond for allowing me to conduct snail experiments over the past few years.

There are many funding sources I must thank, as they have supported me during my time as a master's student at OSU: the Bolek lab, the Reichard lab, the McBee lab, the American Society of Parasitologists, the Annual Midwest Conference of Parasitologists, the Graduate College, the Department of Integrative Biology, the Zoology Graduate Student Society, and the Graduate and Professional Student Governmental Association.

I would also like to thank all of my fellow colleagues at OSU. You have made my daily work environment the best a graduate student could ask for. In particular, thank you Angela Riley for being an amazing climbing partner, work partner, and friend. I love plant shopping with you, as well as cooking together. I'll always be there to catch you. Importantly, I must thank all of the coffee shops that have allowed me to produce this thesis. Specifically, thank you Balanced Coffee Co. for serving the best-tasting coffee in town, thank you Aspen Coffee Company for staying open the latest, thank you The Coffee House for providing the most comfortable workspace, and thank you Panera Bread for your warmth of a fire place.

Acknowledgements reflect the views of the author and are not endorsed by committee members or Oklahoma State University.

Last, but far from least, I thank my amazing advisor, Matt Bolek. Your extraordinary passion for parasites has no doubt rubbed off on me. I am so excited to have worked with this snail-acanthocephalan system, and I am even more excited to continue it during my PhD with you. We have worked well as collaborators over the past three years, and I know we will publish many exciting papers in the future. Every day I learn to appreciate parasitology and all it has to offer a little bit more. You have taught me so much of my training as a parasitologist, much of which I know I will use for the rest of my life. I am learning to be skeptical of science before I can accept it and to keep asking questions. After working in your lab for the past three years, I can see why all of your publications are quality work. I have not met any other parasitologist who thinks as critically as you. Overall, I am extremely grateful to have you as an advisor.

Acknowledgements reflect the views of the author and are not endorsed by committee members or Oklahoma State University.

Name: RYAN WILLIAM KOCH

Date of Degree: DECEMBER, 2018

Title of Study: DISTRIBUTION AND INTERACTIONS OF TURTLE ACANTHOCEPHALANS IN TWO SPECIES OF FRESHWATER SNAILS

Major Field: ZOOLOGY

Abstract: Acanthocephalans are an understudied group of parasites, with only 8% of their life cycles known. Nevertheless, acanthocephalans are a very complex group of organisms, parasitizing all classes of vertebrates from terrestrial, freshwater, and marine habitats. One interesting component in acanthocephalan life cycles is the addition of a paratenic host, which bridges an ecological gap between the intermediate and definitive hosts. Of all paratenic host records, approximately 99% are vertebrates, leaving only a few reports of acanthocephalans in invertebrates. Interestingly, freshwater snails have been reported as paratenic hosts for a species of turtle acanthocephalan, *Neoechinorhynchus emydis*. However, very little is known regarding these snail-acanthocephalan interactions and the importance of snails in acanthocephalan life cycles. The objectives of this thesis were to examine two common species of freshwater snails, *Helisoma trivolvis* and *Physa acuta*, for acanthocephalans in northcentral Oklahoma. Specifically, I conducted spatial and temporal snail surveys for acanthocephalans and used molecular techniques to identify acanthocephalans to species. Additionally, I investigated acanthocephalan pathology in snails with the use of lifespan experiments and histology. These data indicate that acanthocephalan prevalence and intensity in snails vary strongly in time and space, with *H. trivolvis* being the more commonly infected snail host. Based on complete ITS rDNA sequences, juvenile acanthocephalans in snails were 100% identical to adult *N. emydis* from turtles. Acanthocephalans did not significantly have an impact on snail survival, whereas trematodes and snail size did. Lastly, results from field infection experiments indicate that snails are not acquiring acanthocephalan infections during the winter, which may be a result of the absence of infected ostracod intermediate hosts or the crashing snail populations in the winter. This was the first comprehensive survey on the distribution of acanthocephalans in snail hosts and suggests that snails commonly use a turtle acanthocephalan in their life cycle. These observations strongly suggest that *N. emydis* may be more common in snail paratenic hosts than the current lack of reports suggests, and snails may play an important role in the transmission of *N. emydis* to turtle definitive hosts that reside in wetland habitats.

TABLE OF CONTENTS

LIST OF TABLES

Table Page

LIST OF FIGURES

CHAPTER I

OVERVIEW

Parasitism is one of the most common ecological relationships (Bush et al., 2001). Price (1980) estimated that more than 50% of all plant and animal species are parasitic at some point in their life cycle. Additionally, it has been estimated that almost 100% of all animals and plants serve as hosts for parasites (Bush et al., 2001). However, most ecological studies have been conducted on free-living animals with little attention placed on organisms such as parasites (Bush et al., 2001). As stated by Price (1980, 1984), "Small, highly specialized organisms are different in many aspects of their biology from larger, more generalized animals". The lack of attention towards parasites has recently been realized, and their importance is being portrayed with a growing body of literature (Combes, 2005; Poulin, 2007). However, most of these studies have concentrated on the negative effects of parasites on their hosts (Bush et al., 2001), and few studies have evaluated transmission strategies and host use by parasites in nature (Bolek and Janovy, 2007a, 2007b, 2008, Bolek et al., 2009, 2010).

In order for any parasite transmission events to occur, an infective stage of a parasite has to first encounter a host. This is known as an encounter filter (Euzet and Combes, 1980). For example, ecological conditions and their effects on species-specific life history characteristics of parasites and their hosts influence the survival of free-living stages of parasites in the external environment and the probability of host encounters. The more successful parasites are at encountering their hosts, the higher the probability of hosts becoming infected (Combes, 2005).

However, in order for a successful parasite infection to establish in a host, an additional compatibility filter must be overcome. In this case, host susceptibility to the parasite is controlled by a variety of host factors such as host genetics, immunity, and physiology, among others (Combes, 2005). As a result of these filters, the geographical overlap of hosts and their parasites, specific ecological conditions which allow host-parasite encounters, and host susceptibility factors are critical for successful parasite transmission (Combes, 2005).

To the parasite, the host represents a resource and a habitat where the parasite can grow and reproduce. Once produced, stages of the parasite are released from the host into the external environment where they undergo development and must find their way back into another host. Therefore, unlike most free-living organisms, one of the major problems for parasites is for individuals of a particular species to find the "appropriate" host to propagate the next generation and complete the life cycle. This is a statistical problem of colonization, where parasites face spatial and temporal difficulties of transfer from one host to another, which must be overcome by enormous reproductive outputs and/or by exploiting complex ecological associations between successive hosts (Tinsley, 1990).

Parasite life cycles have evolved by either adding or subtracting hosts based on trophic interactions of potential hosts. In complex trophically transmitted life cycles with two or more hosts, there are two hypotheses that support the addition of a host. One hypothesis suggests that the original host was preyed upon by other potential hosts higher up in the trophic food chain, and all other hosts have been added over time to the parasite life cycle (Smith-Trail, 1980; Parker et al., 2003; Poulin, 2007). Another hypothesis suggests the opposite. In this case, the original host was a top predator in the food web, and all other hosts with lower positions in the food web than the original host have been added secondarily to the parasite life cycle (Smith-Trail, 1980;

Gibson and Bray, 1994; Lafferty, 1999; Parker et al., 2003). Finally, hosts can also be lost if the life cycle no longer requires a particular host for completion (Parker et al., 2003). However, understanding how and why life cycles have evolved is difficult due to the lack of a fossil record for most parasites, complex host/parasite associations, and the lack of empirical data on host use for most parasite species in nature (Stigge and Bolek, 2015). For example, it is currently unclear if these processes occur gradually or require less time over evolutionary time (Stigge and Bolek, 2015). As a result of these difficulties, understanding how life cycles operate in nature and what hosts are used by those parasites can provide us with empirical data for future hypotheses testing on parasite life cycle evolution.

One group of parasites for which little is known about their life cycle diversity and transmission is the acanthocephalans (Kennedy, 2006). Known as thorny-headed worms, acanthocephalans are a phylum of parasitic worms that infect the intestinal track of vertebrate definitive hosts and the hemocoel of arthropod intermediate hosts. In a typical acanthocephalan life cycle (Nickol, 1985), adult males and females mate in the vertebrate definitive host, and mature eggs are released by female worms into the external environment within the definitive host's feces. In the external environment, eggs are ingested by a suitable arthropod intermediate host. Within the gut of the arthropod intermediate host, the acanthor hatches from the egg and penetrates into the hemocoel where it develops into the acanthella stage, and eventually into a cystacanth or juvenile stage, which is the infective stage to the definitive host. In some species of acanthocephalans, cystacanths can also infect a paratenic host, which are usually vertebrate hosts that are common prey item in the diet of the definitive host. Once in the definitive host, acanthocephalans develop into sexually mature adults, completing the life cycle (Kennedy, 2006).

Although approximately 1,150 species of acanthocephalans have been described from all classes of vertebrate hosts, life cycles are only known for 8% (97) of those species (Benesh et al., 2017). One reason for this lack of knowledge might be the complex nature of acanthocephalan life cycles. For example, adult acanthocephalans parasitize a diverse group of definitive hosts, comprising all classes of vertebrates, including hosts in terrestrial, aquatic, and marine environments (Kennedy, 2006). Second, and within some species of acanthocephalans, adult acanthocephalans can be transmitted from one definitive host to a second definitive host through post-cyclic transmission when an infected definitive host is ingested by an uninfected definitive host and the adult worms survive and re-establish in the intestinal track of the second definitive host (Nickol, 1985). As a result of post-cyclic transmission, sometimes acanthocephalans can wind up in inappropriate definitive hosts. For example, the acanthocephalan *Acanthocephalus tumescens* typically infects the common galaxias, *Galaxias maculatus*, a small fish as the definitive host, but can survive up to four weeks in rainbow trout, *Oncorhynchus mykiss*, a common predator of galaxias; however, not the natural definitive host for this acanthocephalan species (Rauque et al., 2002). Finally, as mentioned above, in numerous acanthocephalan life cycles, there is an additional paratenic host, which acts as a trophic "bridge" between the intermediate and definitive host but is not needed for life cycle development (Baer, 1951). In many cases the paratenic host facilitates and maintains transmission in many acanthocephalan life cycles. However, the diversity of paratenic hosts has likely been unexplored due to the numerous predator-prey interactions that have not been examined in nature (Nickol, 1985). As a result, even if the paratenic and definitive hosts are known, the intermediate host may still be unknown, creating several difficulties in solving acanthocephalan life cycles.

Despite reports of various groups of animals serving as paratenic hosts for acanthocephalans, our current understanding of paratenic hosts in life cycles of most helminths and including acanthocephalans is limited (Kennedy, 2006; Stigge and Bolek, 2015). Cystacanth stages of acanthocephalans that develop in intermediate hosts and/or encyst in the tissue of paratenic hosts have adult acanthocephalan features except for their size and sexual maturity (Schmidt, 1985). However, despite the morphological similarities between cystacanths and adult acanthocephalans, juvenile acanthocephalans cannot be identified to the species level based on morphology alone since the morphological characters used for acanthocephalan identification are usually based on sexually mature adults. For example, morphological characters which are important for species level identification of turtle acanthocephalans in the genus *Neoechinorhynchus* include the posterior end of sexually mature females and mature egg morphology (Barger and Nickol, 2004). Thus, without actually conducting experimental infections of definitive hosts with cystacanth stages of acanthocephalans, juvenile stages of acanthocephalans recovered from paratenic hosts cannot be identified to the species level based on morphology alone (Nickol, 1985).

More recently, this problem has been addressed with the use of DNA barcoding techniques. For example, Alcántar-Escalera et al. (2013), sequenced various nuclear and mitochondrial markers from juvenile acanthocephalans recovered from 15 species of freshwater fish paratenic hosts collected throughout Mexico. Based on these sequences, the authors were able to match these cystacanth stages with adults of *Polymorphus brevis* collected from three species of heron definitive hosts (*Botaurus lentiginosus*, *Egretta caerulea*, *Nycticorax nycticorax*) for which sequences were available. Clearly, completing such life cycles in the laboratory would be difficult and tedious due to the host involved and, in part, explain why very

little is known about acanthocephalan life cycles in general. However, in these situations the use of molecular barcoding techniques can provide new information on host use by acanthocephalans in nature. More importantly, over the past few decades more adult acanthocephalans have been sequenced using various nuclear and mitochondrial markers and are now available on GenBank, allowing for newly generated sequences to be matched against this ever-growing database (Alcántar-Escalera et al., 2013). Therefore, the use of molecular techniques has made it possible to discovering new acanthocephalan species, paratenic hosts, and/or life cycle association in nature.

Previous studies on acanthocephalan life cycles indicate that most animals reported as paratenic hosts are vertebrates, with few reports of invertebrates serving as paratenic hosts (Schmidt, 1985; Kennedy, 2006). However, there have been occasional reports of acanthocephalans in the genus *Neoechinorhynchus* from aquatic larval insect and freshwater snail paratenic hosts in Europe and North America, respectively (Whitlock, 1939; Hopp, 1946, 1954; Lincicome and Whitt, 1947; Lassiere, 1988). My preliminary surveys of ponds and wetlands in northcentral Oklahoma indicated that freshwater pulmonate snails were infected with juvenile acanthocephalans in the genus *Neoechinorhynchus*. The only previous reports of juvenile *Neoechinorhynchus* species in freshwater snail host are from four species of freshwater prosobranch from eastern North America snails including *Campeloma decisum*, *Campeloma rufum*, *Pleurocera semicarinata*, and *Pleurocera acuta* (Whitlock, 1939; Hopp, 1946, 1954; Lincicome and Whitt, 1947). Notably, these species of snails predominantly reside in lotic habitats (i.e., streams and rivers), and currently no information is available on the occurrence and infection parameters of acanthocephalans that infect freshwater snails in lentic habitats (i.e., ponds, lakes, and wetlands). Previous surveys and life cycle work by Hopp (1954) indicate that

juvenile acanthocephalans that infects freshwater snails in Indiana, U.S.A. belongs to *Neoechinorhynchus emydis*. The life cycle of *N. emydis* involves an ostracod intermediate host, a snail paratenic host, and an aquatic turtle definitive host (Hopp, 1954). However, all other reports of juvenile *Neoechinorhynchus* species recovered from freshwater snails did not provide any credible evidence for species identification. As a result, currently it is not clear if other species of *Neoechinorhynchus* infect freshwater snails throughout North America (Whitlock, 1939; Lincicome and Whitt, 1947).

In this thesis, I investigate various questions on the relationships of juvenile acanthocephalans in freshwater pulmonate snails. In Chapter II, I address the following questions: 1) what species of freshwater pulmonate snails, which reside in pond and wetland habitats, are infected with acanthocephalans?; (2) where are the juvenile acanthocephalans located within their snail hosts?; and (3) what species of acanthocephalans infect freshwater snails in northcentral Oklahoma? To evaluate if freshwater pulmonate snails were infected with juvenile acanthocephalans, I surveyed two species of common freshwaters snails (*Helisoma trivolvis* and *Physa acuta*) from 23 wetlands and streams in Payne and Logan counties located in northcentral Oklahoma. Juvenile worms recovered from snail hosts were evaluated for their morphological characteristics and location within each snail host. Additionally, to identify these juvenile acanthocephalans to species, I sequenced the complete Internal Transcribed Spacer region of nuclear rDNA (ITS) from juvenile worms and identified adult worms recovered from turtle definitive hosts from Oklahoma and compared those sequences to available acanthocephalan ITS sequences in GenBank. Finally, phylogenetic analyses were preformed to show relationships of juvenile acanthocephalans from snail hosts to other turtle and fish acanthocephalans for which ITS sequences were newly generated and/or available on GenBank.

In Chapter III, I evaluate the following questions: 1) how do acanthocephalan infections vary seasonally within *H. trivolvis* snail hosts and when do snails recruit acanthocephalans seasonally?; and 2) are there any consequences to the snail host of being infected with juvenile acanthocephalans? The temporal survey consisted of examining samples of *H. trivolvis* for acanthocephalan infections over a year at a single location known to contain infected snails. To investigate snail acanthocephalan recruitment, I conducted cage exposure experiments by placing laboratory-reared *H. trivolvis* snails in cages for periods of five weeks, at a pond with naturally occurring acanthocephalans in snail hosts. Snails were then sampled from cages for acanthocephalan infections. Because field populations of snails fluctuated in size seasonally and were commonly infected with other parasites, a snail survival laboratory study was conducted on field collected and naturally infected *H. trivolvis* to evaluate any consequences of acanthocephalan infections to these snails. Finally, I evaluated any snail-acanthocephalan associated pathology at tissue and cellular level in naturally infected *H. trivolvis* snails.

It is hoped that the results of my work will help elucidate some of the controversies regarding the role snails play in the life cycle of turtle acanthocephalans. Ostracods have been suggested to be the primary route of transmission of *N. emydis* to turtles. However, this has never been tested experimentally, nor have there been any comprehensive surveys of acanthocephalans of ostracods or snails. My study provides new data on the snail-host relationship with juvenile acanthocephalans, which hopefully will stimulate future studies on the life cycle and transmission of acanthocephalans that infect freshwater snails.

CHAPTER II

OCCURRENCE OF JUVENILE *NEOECHINORHYNCHUS EMYDIS* (PHYLUM: ACANTHOCEPHALA) IN FRESHWATER SNAILS FROM THE GREAT PLAINS REGION OF NORTH AMERICA

ABSTRACT:

Although little information exists on the role of invertebrate paratenic hosts in the life cycles of acanthocephalans, the North American turtle acanthocephalan, *Neoechinorhynchus emydis*, has been reported from freshwater snail paratenic hosts. However, few studies have examined freshwater snails for acanthocephalan infections. In this study, two species of freshwater snails, *Helisoma trivolvis* and *Physa acuta*, were examined for acanthocephalan infections from 23 wetlands and streams throughout northcentral Oklahoma. Additionally, the complete Internal Transcribed Spacer (ITS) region of nuclear rDNA was sequenced from multiple juvenile acanthocephalans from snail hosts and adult acanthocephalans from definitive hosts, red-eared slider turtles, *Trachemys scripta elegans*. Of the 23 locations sampled, 7 (30%) had at least one snail infected with juvenile acanthocephalans belonging to the genus *Neoechinorhynchus*. Most worms were encysted within a thin-walled cyst in the headfoot of their snail hosts. However, some worms were attached with their proboscis to the mantle collar of snail host, occurring between the snail's shell and visceral mass. The ITS sequences of juvenile acanthocephalans recovered from snail hosts had no variation in their ITS genetic

distance and were identical to ITS sequences generated from adult *N. emydis* from turtle hosts. In contrast, the ITS genetic distances of juvenile acanthocephalans from snails and adults of three other turtle *Neoechinorhynchus* species obtained in this study or from GenBank varied from 6.1% to 12.5%, and 33.3% for the most similar ITS sequences of *Neoechinorhynchus* species from fish definitive hosts. The inferred phylogenetic relationship supports the clustering of juvenile *Neoechinorhynchus* sp. from snails and *N. emydis* from a turtle as a monophyletic group. Finally, an additional juvenile *Neoechinorhynchus* sp. recovered from the introduced faucet snail, *Bithynia tentaculata* collected from west central Wisconsin is documented. The major contributions of this study include new locality records and the documentation of juvenile acanthocephalans from three previously undocumented but widely distributed snail families across North America. Additionally, this study provides baseline ITS rDNA sequence data to serve as a genetic barcode for species of *Neoechinorhynchus* from snail paratenic and turtle definitive hosts. Evaluation of dietary studies of the five species of freshwater turtles reported as definitive hosts for *N. emydis* indicate that aquatic snails are frequent and important components of these turtle diets, suggesting that snails play an important role in the transmission of these acanthocephalan species to turtle definitive hosts.

INTRODUCTION

Acanthocephalans are a phylum of parasitic worms that, as adults, inhabit the gastrointestinal tract of all classes of aquatic and terrestrial vertebrates (Schmidt, 1985). However, compared to other helminth groups, their transmission strategies and epizootiology have been poorly studied in nature (Nickol, 1985; Schmidt, 1985). The typical acanthocephalan life cycle includes a definitive vertebrate host, in which worms reach sexual maturity, reproduce

and release eggs into the external environment within the hosts' excrement. The eggs are subsequently ingested by an arthropod intermediate host, where the worm undergoes growth and development but does not reach sexual maturity (Crompton and Nickol, 1985). All acanthocephalan species for which life cycles are known involve a definitive and an intermediate host (Olsen, 1986; Schmidt, 1985). However, an additional paratenic host is commonly involved in the life cycle of acanthocephalans (Schmidt, 1985).

In the case of a paratenic host, the juvenile parasite does not undergo further developmental changes; thus, this type of host is not required for the development of infective stages of the parasites (Baer, 1951). Although not required for development, paratenic hosts have been associated with bridging a trophic gap between intermediate and definitive hosts (Olsen, 1986; Zelmer and Esch, 1998; Stigge and Bolek, 2015). As a result, paratenic hosts increase transmission probabilities of acanthocephalans between intermediate and definitive hosts, and in some cases are essential for completing life cycle transmission between successive hosts (Nickol, 1985).

Among studies that exist on the use of paratenic hosts by acanthocephalans, most report vertebrates such as fish, amphibians, reptiles, and mammals as paratenic hosts (see Schmidt, 1985; Kennedy, 2006). Nonetheless, there have been occasional reports of acanthocephalans in the genus *Neoechinorhynchus* using insects and snails as paratenic hosts, suggesting that invertebrate paratenic hosts may play important roles in acanthocephalan transmission and life cycles (Whitlock, 1939; Hopp, 1946, 1954; Lincicome and Whitt, 1947; Lassiere, 1988).

In North America, all reports of juvenile acanthocephalans from invertebrate paratenic hosts are from freshwater prosobranch snails which commonly reside in rivers and streams (Whitlock, 1939; Hopp, 1946, 1954; Lincicome and Whitt, 1947). In most of these cases, the

acanthocephalan was identified as *Neoechinorhynchus emydis* (Hopp, 1946, 1954; Lincicome and Whitt, 1947). Work on the life cycle of *N. emydis* by Hopp (1954) indicates that snails could not be infected with eggs of *N. emydis*; yet, ostracods could be easily infected, suggesting that snails act as true paratenic hosts and become infected when they ingest ostracod intermediate hosts. However, *N. emydis* cystacanths (juveniles) from snails were infective to turtles, but not cystacanths from ostracods (Hopp, 1954). This suggests that the snail may not be a paratenic host at all but a true intermediate host, and potentially the only example known to date of an acanthocephalan life cycle requiring two intermediate hosts. However, over the last 65 years, no other reports exist of acanthocephalan infections in freshwater snails. As a result, it is unclear as to the importance of snail hosts in the transmission of acanthocephalan species to their vertebrate definitive hosts. Therefore, this study had two goals: (1) to examine two species of commonly occurring freshwater snails from northcentral Oklahoma for acanthocephalan infections, and (2) to identify the species of juvenile acanthocephalans that infect these snail hosts.

MATERIALS AND METHODS

Snail survey

During August–December of 2016 and February–July of 2017, two species of freshwater snails, *Helisoma trivolvis* (Planorbidae) and *Physa acuta* (Physidae), were collected from 23 wetlands and streams in Payne and Logan Counties, OK, and examined for acanthocephalan infections (Fig 2.1). All sites were georeferenced, and an effort was made to collect 10-20 snails per site, which required multiple collection dates. However, only five snails were collected at one of the sites. All snails were collected by hand or by using a dip-net, and each snail species

was placed in 19-liter buckets filled with 8 L of pond water and brought back to the laboratory at Oklahoma State University.

Snail necropsies and worm morphology

In the laboratory, snails were identified to species using keys in Burch (1989) and descriptions by Gustafson et al. (2014). The shell diameter for *H. trivolvis* or shell length (from the aperture to the apex) for *P. acuta* was measured to the nearest 1.0 mm with a handheld caliper. Individual snails were then placed in a petri dish, gently crushed with a smaller petri dish, and the shell fragments were removed. Snails were then examined for any acanthocephalans attached to the body surface under an Olympus SZ-8145 stereomicroscope configured for incident illumination at 10 x to 45 x total magnification, the location of the worms was recorded, and each worm was gently removed with forceps. Next, based on snail size, the entire carcass of each snail was cut into approximately two to four equal pieces with a razor blade and each snail was then flattened between two glass slides. Once snail tissue was flattened, the entire snail carcass was scanned for juvenile acanthocephalans under an Olympus SZ-8145 stereomicroscope. When an acanthocephalan was observed in the snail tissue, the location was recorded, the top slide was removed, and the acanthocephalan was gently removed from the snail tissue with forceps. Any additional snail tissue was removed from the proboscis of each worm, the worm was placed on a clean glass slide, and a wet mount was prepared by adding a drop of water and a coverslip. Additionally, a few pieces of snail tissue with encysted worms were fixed in hot Bouin's fixative, embedded in paraffin, sectioned at 10 µm, affixed to slides, stained with hematoxylin and eosin, mounted in Canada balsam, and examined microscopically (see below).

All juvenile worms and sectioned worms in snail tissue were examined at 100 x to 400 x total magnification with an Olympus BX-51 upright research microscope configured for bright field (BF) and differential interference contrast (DIC) microscopy with plain fluorite objectives and a calibrated ocular micrometer and an Olympus CX-41 upright research microscope configured for bright field (BF) and dark field (DF) microscopy with plan achromat phase objective lenses. Finally, an additional acanthocephalan recovered from the faucet snail, *Bithynia tentaculata* (Bithyniidae) and collected on August 9, 2007 by Dr. Rebecca Cole (USGS) from Broken Gun SW Bay, Lake Onalaska, La Crosse Co., WI (43.904523, -91.249742), was examined.

Observations were made on several distinguishable features of juvenile worms, including the number of giant nuclei, the number of rows of spines and spines per row on the proboscis, and the shape of the posterior end. Additionally, multiple digital images were taken of a subsample of 30 juvenile worms for hook morphology and trunk length and width using an Olympus 5-megapixel digital camera for further identification following the terminology in Amin (2002) and Barger and Nickol (2004). Measurements were obtained from digital images of juvenile worms using ImageJ software (Schneider et al., 2012) and included the total length and greatest width of the trunk, and the length and base of hooks 1, 2, and 3 within a single row of the anterior, middle and posterior circle on the proboscis, according to Wayland (2010) and Alcántar-Escalera et al. (2013). All worms were then preserved in 100% ethanol for molecular work.

Statistical analyses

Prevalence, along with 95% confidence intervals and mean intensity, were calculated for total numbers of snails for each species collected from all sites and at each site according to Bush

et al. (1997). Mean shell size was calculated for each snail species among all sites and among infected and uninfected individuals. The chi-square test for independence was calculated to compare differences in prevalence among the two snail species. However, due to a low sample size of infected *P. acuta*, statistical tests were only performed on *H. trivolvis* snails. Because variances were heteroscedastic, a Student's t-test with unequal variance was calculated to evaluate any differences in mean shell diameter of infected and uninfected *H. trivolvis*. Lastly, Pearson's correlation was calculated to analyze the relationship between *H. trivolvis* shell diameter and acanthocephalan abundance and intensity. All values other than prevalence are reported as the mean ± 1 standard deviation (SD), followed by the range in parentheses.

Adult specimen collection for molecular characterization

Based on morphology alone, most juvenile acanthocephalans cannot be identified to species (Crompton and Nickol, 1985; Schmidt, 1985), and previous reports indicate that juvenile acanthocephalans reported from snails commonly use aquatic turtles as definitive hosts (Hopp, 1946, 1954; Lincicome and Whitt, 1947). As a result, adult acanthocephalans were obtained from two red-eared slider turtles (*Trachemys scripta elegans*) collected opportunistically as roadkill from Payne and McCurtain Counties, OK. These worms were used to compare the complete Internal Transcribed Spacer (ITS) region of nuclear rDNA from identified adult worms recovered from turtle definitive hosts to juvenile worms recovered from snail hosts. Briefly, turtles were necropsied by using a hand saw to detach the plastron from the carapace and then carefully removing the gastrointestinal tract. Using forceps, a total of 50 and 208 adult acanthocephalans were removed from the small intestines of each turtle, and all worms were left overnight in distilled water at 4 °C. The posterior end from a subsample of adult female worms was then cut using a razor blade, and a wet mount of eggs was prepared and examined with an

Olympus BX-51 upright research microscope. Multiple digital images were taken of mature eggs as previously described. Adult female worms with mature eggs from each of the two turtle hosts were identified to species based on mature egg morphology and characteristics of the posterior end according to Barger and Nickol (2004), and all worms were then preserved in 100% ethanol for molecular work.

DNA extraction, amplification, and sequencing

Ten juvenile and nine adult worms used for genetic DNA extractions were recovered from eight *H. trivolvis* snails, one *P. acuta* snail, one *B. tentaculata* snail, and two *T. scripta elegans* turtles, all collected from nine different locations at various times. Prior to DNA extraction, pieces of adult worms and entire juvenile specimens were lysed over night at 56 ℃ using Quiagen DNeasy Blood and Tissue Kit. With the exception of a few bases at the 5' and 3' ends, the entire ITS rDNA region (ITS 1 - 5.8S - ITS 2) was amplified by polymerase chain reaction (PCR) using a forward primer in the 18S region 5'-GTCGTAACAAGGTTTCCGT-3' and reverse primer in the 28S region 5'-TATGCTTAAATTCAGCGGGT-3' (Luton et al., 1992). Additionally, an effort was made to also amplify the mitochondrial cytochrome oxidase I gene (COI), but these attempts were unsuccessful.

PCR reactions were performed in a 25 µl total volume (10 µl DNA, 1.5 µl RNase-free water, 0.5 µl of each primer, and 12.5 µl HotStarTaq Plus Master mix) according to instructions accompanying the HotStarTaq Plus Master mix kit (Quiagen, Valencia, California). Reactions were run on an AB 2720 thermocycler (Applied Biosystems) under the following cycling conditions: denaturation at 94 °C for 5 min; 35 cycles of denaturation at 94 °C for 30 seconds; annealing at 50 °C for 1 min; extension at 72 °C for 2 min; and incubation at 72 °C for 7 min. PCR success was determined by running 4 µL of PCR product on a 1.5% agarose gel using 0.5x

TBE buffer on a horizontal electrophoresis system at constant voltage (100V) for 30 minutes. Bands were visualized using a UV light, and single, solid bands were deemed successful reactions. Unincorporated PCR primers and nucleotides were removed from PCR products using the Promega Wizard SV Gel and PCR Clean-up System. For sequencing PCR amplicons, forward and reverse internal primers were used with the Sanger sequencing chemistry on an Applied Biosystems 3730 capillary sequencer at the Oklahoma State University core facility.

Sequence analysis

All DNA sequences obtained in this study were compared with other sequences in GenBank using BLAST (Morgulis et al., 2008), which utilized program BLASTN 2.2.29 (Zhang et al., 2000). The 18 complete ITS rDNA gene sequences from nine snails and two turtles obtained in this study were then combined with eight other sequences retrieved from GenBank of *Neoechinorhynchus* species from turtles and a fish (Table 2.1) and aligned using the MUSCLE (Edgar, 2004) feature of MEGA 6.06 (Tamura et al., 2013). The alignment was inspected by eye, and ambiguous regions were removed from further analyses. The final alignment consisted of 774 base pairs (including gaps) and 26 taxa. Sequence divergences between life stages and species of *Neoechinorhynchus* were calculated using MEGA 6.06 and the Kimura 2-parameter model (Kimura, 1980; Kumar et al., 2004). A phylogeny was estimated using Maximum Likelihood (ML). The model testing feature of MEGA 6.06 (Tamura et al., 2013) yielded the HKY + G model (Hasegawa et al., 1985) as the best evolutionary model for the ML analysis. Maximum Likelihood trees were created in MEGA 6.06 (Tamura et al., 2013) using the HKY + G evolutionary model with 1000 bootstrap replications.

RESULTS

Spatial snail survey

A total of 310 *H. trivolvis* snails (5-76 per site) were collected from 20 sites; whereas a total of 225 *P. acuta* snails (1-30 per site) were collected from 17 sites (Table 2.2). Of the 23 sampling localities, 14 sites (61%) contained both snail species (Table 2.2). Overall prevalence of acanthocephalans in *H. trivolvis* snails was 20.3% (63/310; 95% CI 16-25) and mean intensity was 2.1 ± 1.26 (1-6), and overall prevalence of acanthocephalans in *P. acuta* snails was 1.8% (4/225; 95% CI 1-5) and mean intensity was 1 ± 0 (1). The chi-square test for independence indicated that significant differences existed in overall prevalence of acanthocephalan infections among *H. trivolvis* and *P. acuta* ($\chi^2 = 40.9281$, $P < 0.00001$).

Of the 23 sites sampled for snails, seven (30.4%) were positive for acanthocephalans (Fig. 2.1). For *H. trivolvis*, six of the 20 locations (30%) were positive for acanthocephalans, with prevalence and mean intensity ranging from 5-70% and 1-2.7 per site, respectively (Table 2.2). For *P. acuta*, two of the 17 locations (11.8%) contained infected snails, and prevalence ranged from 8-23% and mean intensity was 1 per site (Table 2.2).

Mean shell diameter for *H. trivolvis* was 11.0 mm ± 2.3 (range 5 – 19); whereas the mean shell length for *P. acuta* was 7.8 mm ± 2.0 (range 3 – 14.5). Infected and uninfected *H. trivolvis* snails had an average shell diameter of 11.4 ± 1.6 mm (range 8 – 17) and 10.9 ± 2.4 mm (range 5 – 19), respectively, but there was no significant difference in shell diameter between infected and uninfected *H. trivolvis* snails ($t = 1.97$; $P = 0.06$). However, there was a weak positive correlation between *H. trivolvis* shell diameter and acanthocephalan abundance ($r = 0.13$; $P < 0.02$), and snail shell diameter and acanthocephalan intensity ($r = 0.38$; $P < 0.01$).

Juvenile worm distribution within snails and worm morphology

In addition to the single worm obtained from the faucet snail, *B. tentaculata* collected in Wisconsin, a total of 137 worms were recovered from Oklahoma snails. These included 4 worms infecting *P. acuta* and 133 worms infecting *H. trivolvis*. Of the 133 worms recovered from *H. trivolvis*, 7 (5%) were attached with their proboscis to the mantle collar of *H. trivolvis*, with the worm's trunk located between the snail's shell and viseral mass (Fig. 2.2). Each of these worms was firmly attached with its proboscis to the mantle collar of their snail host, and each worm contained a significant amount of snail tissue on the proboscis when removed (Fig. 2.2). The remaining 130 (95%) worms from *P. acuta* and *H. trivolvis* were encysted in the headfoot region of snail hosts. Encysted worms within the snail's headfoot always occurred as single coiled worms surrounded by a dark pigment within thin-walled cysts (Fig. 2.2). Histological sections on three infected snails revealed that the coiled worm was surrounded by cellular debris and melanin-like pigment (Fig. 2.2).

Mean trunk length of 30 juvenile worms (*H. trivolvis*) was 1.15 ± 0.24 mm (0.7 – 1.8) and the greatest mean trunk width was 0.23 ± 0.05 mm (0.1 – 0.4). Living juvenile worm were partially translucent but white in color, and the aspinose trunk contained few subcuticular giant nuclei (Figs. 2.2 and 2.3). Some individuals (46%) contained a knob-like structure on the posterior end of the trunk (Fig. 2.3). The proboscis was short, globular, and slightly broader than long, and the proboscis receptacle contained a single muscular layer (Fig. 2.3). The proboscis hooks were arranged in three circular rows of six hooks: hooks of anterior circle, length 99.3 ± 9.0 µm (87-115) and base 27.0 ± 3.7 µm (18-34); hooks of middle circle, length 50.7 ± 7.5 µm (34-63) and base 7.4 ± 1.3 µm (4-10); and hooks of posterior circle, length 40.1 ± 5.7 µm (28-48) and base 5.7 ± 1.0 µm (4-7). The trunk length and greatest trunk width of two juvenile worms for *P. acuta* were 0.82-1.1 mm and 0.22-0.24 mm respectively. The terminal hook length was

91-97 µm and base 23-26 µm; middle hook length 33-36 µm and base 5-7 µm; and posterior hook length, 26-33 µm and base 3-4. Finally, the single juvenile worm examined from *B. tentaculata* had a trunk length of 1.4 mm and the greatest trunk width of 0.24 mm. The terminal hook length was 129 µm and base 35 µm; middle hook length 73 µm and base 10 µm; and posterior hook length, 35 µm and base 9. Based on these characteristics, all individuals recovered from snails were identified to the genus *Neoechinorhynchus*.

Adult worm identification from turtle hosts

All 258 adult worms recovered from two turtle hosts were identified to the genus *Neoechinorhynchus*. Worms were white in color and ranged from 11 to 27.5 mm in trunk length. All individuals had a short, globular, and slightly broader than longer proboscis with three circles of six hooks each. The trunk was aspinose and contained a few subcuticular giant nuclei. Seven adult female worms recovered from a single *T. scripta elegans* collected from Pane Co., OK, contained mature eggs. The posterior end of the trunk of these females was irregular but sometimes possessed a distinct knob. Mature eggs contained a prominent broad C-shaped equatorial vacuole, not entirely encircling the acanthor (Fig. 2.4A). Based on these characteristics, the worms were identified as *Neoechinorhynchus emydis*. Five adult female worms recovered from a single *T. scripta elegans* collected from McCurtain Co., OK, contained mature eggs. The posterior end of the trunk of these females contained a distinct cleft consisting of two prominent lateral lobes. Mature eggs were football shaped with radiating striations (Fig. 2.4B). Based on these characteristics, these worms were identified as *Neoechinorhynchus pseudemydis*.

Molecular characterization of juveniles and adults

Of the 19 worms for which an attempt was made to extract nuclear DNA, only the juvenile acanthocephalan from the faucet snail, *B. tentaculata* was not successful. The lengths of the ITS rDNA gene sequences from the remaining 18 worms generated in this study ranged from 722 to 777 bp (Table 2.1). For all 18 sequences, the first 57 BLAST results for each sequence had an 88 – 96% identity score for acanthocephalans in the genus *Neoechinorhynchus* from fish and turtle hosts. For all 18 sequences, the top BLAST search results included four hits for the turtle acanthocephalan *Neoechinorhynchus emyditoides* (query coverage: 99 – 100%; maximum score: 681 – 1107; maximum identity: 91 – 94%; mean *E*-value: 0.0 ± 0), and three hits for the turtle acanthocephalan *Neoechinorhynchus schmidti* (query coverage: 95 – 98%; maximum score: 685 – 790; maximum identity: 86 – 88%; mean *E*-value: 0). The remaining 50 hits included species of *Neoechinorhynchus* from fish hosts that had a sequence query coverage equal to or less than 33%.

The intraspecific genetic distances for the ITS rDNA barcoding region generated in this study for three samples of *N. pseudemydis* was 0.0 – 0.3 %, and the five samples of *N. emydis* was 0.0% (Table 2.3). Similarly, the intraspecific distance for ITS sequences from GenBank for four samples of *N. emyditoides* collected from *T. scripta* from two locations in Mexico was 0.0 – 0.3 %, and three samples of *N. schmidti* collected from *T. scripta* from two locations in Mexico was 0.0% (Table 2.3). In contrast, the interspecific distance between these four species of *Neoechinorhynchus* varied from 6.1-12.5% (Table 2.3).

The genetic distance of the complete ITS rDNA barcoding region for the nine juvenile *Neoechinorhynchus* sp. recovered from snail hosts was 0.0 % (Table 2.3). In addition, the ITS sequences from these nine juvenile *Neoechinorhynchus* sp. recovered from snail hosts were identical to the ITS sequences of five adult *N. emydis* individuals with a genetic distance of 0.0%

(Table 2.3). The inferred phylogenetic relationship supports the clustering of juvenile *Neoechinorhynchus* sp. from snails and *N. emydis* from a turtle as a monophyletic group (Fig. 2.5).

DISCUSSION

The major contribution of this study includes new locality records for juvenile acanthocephalans from freshwater snails in Oklahoma and Wisconsin, and the documentation of three new freshwater snail species as paratenic hosts for juvenile acanthocephalans from three previously undocumented snail families. In addition, this study generated new sequence data for juvenile and adult acanthocephalans from freshwater snail and turtle hosts. Despite the lack of previous studies on acanthocephalans in snails from other localities in North America and across the world, this was the first study to survey multiple snail species from multiple locations for acanthocephalan infections (Whitlock, 1939; Hopp, 1946, 1954; Lincicome and Whitt, 1947). Morphologically, all juvenile acanthocephalans recovered from *H. trivolvis*, *P. acuta* and *B. tentaculata* conformed to the genus *Neoechinorhynchus*. Comparisons of measurements of 33 juvenile *Neoechinorhynchus* sp. from snail hosts obtained during this study and similar data for 29 juvenile acanthocephalans identified as *N. emydis* from the prosobranch snail *Campeloma rufum* collected by Hopp (1954) from Indiana indicate that specimens from Oklahoma and Wisconsin overlap with juvenile *N. emydis* from snails from Indiana, in trunk length (0.7-1.8 mm vs. 1.2-1.9 mm), greatest trunk width (0.1-0.4 mm vs. 0.2); terminal hook length (87-129 µm vs. 83-88 µm); middle hook length (33-73 µm vs. 54 µm) and first hook length (26-68 µm vs. 47 µm).

Sequences of the complete ITS rDNA barcoding region of juvenile acanthocephalans recovered from one *P. acuta* and eight *H. trivolvis* snails, collected from six locations, indicated that there was no variation (0%) in the genetic distances among the nine juvenile acanthocephalans sequenced. Similarly, the intraspecific genetic distances for the available ITS rDNA sequences among four species of *Neoechinorhynchus* from turtle definitive hosts obtained in this study or from GenBank was also low, ranging from 0.0 to 0.3 % (Table 2.3). In contrast, the interspecific genetic distance between complete ITS rDNA sequences from juvenile acanthocephalans recovered from snails and the three other *Neoechinorhynchus* species (*N. emyditoides*, *N. pseudemydis* and *N. schmidti*) from turtle definitive hosts, and between the most similar sequence for a fish *Neoechinorhynchus* sp., differed by 6.9-8.6% and 33.3% (Table 2.3), respectively. Taken together, these data strongly suggest that juvenile acanthocephalans recovered from snail hosts from Payne Co., belong to the species *N. emydis*.

Of the four previous reports of juvenile acanthocephalans in freshwater snails, three studies identified the worms as *N. emydis* (Whitlock, 1939; Hopp, 1946, 1954; Lincicome and Whitt, 1947). However, only Hopp (1954) had credible evidence for his identification by infecting field-collected painted turtles, *Chrysemys picta marginata*, with juvenile worms from field-collected snails and recovering adult worms, which could be identified as *N. emydis*. Currently, three species of *Neoechinorhynchus*, for which museum vouchers are available, have been reported from freshwater turtles in Oklahoma, and include *N. chrysemydis*, *N. emyditoides*, and *N. pseudemydis* (Barger, 2004). However, Everhart (1957) examined red-eared sliders from six locations in the vicinity of Stillwater, Payne Co., Oklahoma and reported recovering 377 specimens of *N. emydis* from 11 of 23 turtles. Although Everhart (1957) did not deposit voucher specimens of *N. emydis* in a museum collection, the study clearly indicates that the eggs of

worms recovered from red-eared sliders from Payne Co., Oklahoma conformed to the description of *N. emydis* by Hopp (1954) and Cable and Hopp (1954). The single, road-killed red-eared slider collected from Payne Co., Oklahoma during this study was also infected with adult *N. emydis*. Taken together, the common occurrence of *N. emydis* in snail hosts in northcentral Oklahoma, and the current and previous reports of adult worms of this species in red-eared sliders from Payne Co., Oklahoma indicate that *N. emydis* is a common acanthocephalan of red-eared sliders in northcentral Oklahoma (Everhart, 1957).

Snail-acanthocephalan associations

As part of this work, there are now two more species of freshwater snails reported as hosts for acanthocephalans in the genus *Neoechinorhynchus*, making a total of seven snail host species from five families throughout North America (Whitlock, 1939; Hopp, 1946, 1954; Lincicome and Whitt, 1947). In this study, 30% (7/23) of the locations sampled for snails had at least one snail infected with an acanthocephalan. However, prevalence of *N. emydis* in snails at positive sites varied considerably, ranging from 5% – 70%. Among all snails examined, *H. trivolvis* was more commonly infected in terms of prevalence (20%) and mean intensity (2.1 ± 1.26), compared to *P. acuta* (2%; 1 ± 0.0). One possible explanation for these differences in infection parameters could be the differences in life histories of the two snail species. Both *H. trivolvis* and *P. acuta* are considered generalist feeders; however, *H. trivolvis* commonly feeds on detritus on the pond bottom, whereas most physid snails are considered to be grazers and commonly feed on carrion (Brown, 1982; Chase et al., 2001). As a result of these differences in foraging strategy and habitat preferences among these two species of snails, *H. trivolvis* may be increasing accidental ingestion of infected ostracods, which primarily reside in the bottom layer of the mud in wetlands (Thorp and Rogers, 2014). Additionally, differences in life span among

the two snail species examined in this study may affect the time allotted for snails to encounter infected ostracods. Both Boerger (1975) and Morris and Boag (1982) examined the life history of populations of *H. trivolvis* in the field in Canada, and they indicated that in nature *H. trivolvis* lives for one to two years but can live as long as five years. In contrast, most laboratory studies and field observations on populations of *P. acuta* indicate that these snails live for only three to four months (Gustafson et al., 2015; Gustafson et al., 2016). Therefore, a longer life span of *H. trivolvis* may increase this snail species' probability of ingesting infected ostracods, resulting in higher prevalence and intensities of *N. emydis* than in *P. acuta*. This hypothesis was supported by my study, as there was a significant but weak positive correlation between snail size and *N. emydis* abundance, indicating that larger and older snails had higher probability of being infected with acanthocephalans. Overall, these findings suggest that *H. trivolvis*, compared to *P. acuta*, is likely the more common host in this acanthocephalan life cycle.

One interesting observation during this study was the location of some *N. emydis* within *H. trivolvis*. Most of the worms (95%) were coiled within thin cysts of the headfoot of snails as previously reported (Whitlock, 1939; Hopp, 1946, 1954; Lincicome and Whitt, 1947). However, and surprisingly, 5% of the worms were attached with their proboscis to the snails' mantle collar, and the trunk of the worm was positioned between the snails' shell and visceral mass. This latter placement in a snail paratenic host is rather unusual, as cystacanths (juvenile worms) infecting intermediate and/or paratenic hosts are usually encysted within thin-walled cysts (Crompton and Nickol, 1985; Kennedy, 2006). In this situation, the worms that were attached with their proboscis to the mantle collar of snails were, in essence, acting as if they were in the intestine of turtle definitive hosts. However, none of these worms were sexually mature. The reason why some juvenile *N. emydis* were attached underneath the snails' shell is not clear, but assuming that

N. emydis is transmitted to snails when ostracods are ingested, some of these juveniles must migrate through the snail tissue until they hit the surface of the snail body where they attach with their proboscis. Once within the space of the shell and visceral mass, cues from snail mucus and other factors under the shell may be providing signals to the worms that they are in a definitive host. It is unclear if worms that are attached under the shell of snails are infective to turtle definitive hosts, but if they are, this would suggest that attached juveniles would undergo postcyclic transmission, when a definitive turtle host ingests a snail host which in essence is acting like a definitive host and the acanthocephalan attaches in the intestine of the turtle host.

The role of snails in the life cycle of *N. emydis*

Currently, *N. emydis* has been reported from five species of freshwater turtles in North America, including the common map turtle, *Graptemys geographica*, the false map turtle, *G. pseudogeographica*, the Texas map turtle, *G. versa*, the Blanding's turtle, *Emydoidea blandingii*, and the red-eared slider, *T. scripta elegans* (Hopp, 1954; Everhart, 1957; Barger, 2004). Studies on the diet content of various species of North American freshwater turtles reported as definitive hosts for *N. emydis* indicate that freshwater snails make up a significant portion of their diets (Rowe, 1992; Ernst et al., 1994; Lindeman, 2000; Herrel et al., 2002; Bouchard and Bjorndal, 2005; Pérez-Santigosa et al., 2011; Richards-Dimitrie et al., 2013; Wang et al., 2013). For example, the frequency of gastropod occurrence in the gut content (percent of individual turtles in which freshwater snails are found) can range from 45% in populations of Blanding's turtles to as high as 85% in populations of the common map turtle (Rowe, 1992; Richards-Dimitrie et al., 2013). More specifically, in a study on the diet content of introduced red-eared sliders in Spain, Pérez-Santigosa et al. (2011) indicated that the frequency of freshwater snails in the gut content of adult turtles was 58% and as high as 83% in juvenile turtles. Finally, of the six localities

snails have been sampled for *Neoechinorhynchus* infections, turtles have been reported as definitive hosts for *N. emydis* in the general area of four of those locations (Fig. 2.6). Unfortunately, no surveys exist for turtle parasites from Kentucky or turtle species that serve as definitive hosts for *N. emydis* from Michigan, the two other locations where snails have been reported to be infected with *Neoechinorhynchus* sp. (Whitlock, 1939; Lincicome and Whitt, 1947; Esch et al., 1979; Barger, 2004; Muzzall, 2005). Nevertheless, these anecdotal observations suggest that freshwater snails may play an important role in the life cycle of *N. emydis*.

However, Lindeman and Barger (2005) suggested that the primary route of transmission of *N. emydis* to North American freshwater turtles is most likely by incidental ingestion of ostracods via sessile aquatic organisms, such as aquatic plants, algae, bryozoans, and sponges. In their study, Lindeman and Barger (2005) examined female (N = 13) and male (N = 7) Texas map turtles for *N. emydis* and other helminth parasites and estimated their dietary intake by evaluating the stomach content, colon content, and feces of these turtles. The study found a significantly higher prevalence of *N. emydis* in female than male Texas map turtles. In addition, dietary analyses indicated that sponges, bryozoans, and filamentous algae were more common than snails in female turtles, whereas sponges, bryozoans, and filamentous algae were absent in male turtles. However, Lindeman and Barger (2005) also reported the presence of a digenetic trematode, *Telorchis corti*, with higher prevalence in female turtles (54%) than male turtles (14%). *Telorchis corti* requires snails as the second intermediate host in its life cycle which have to be ingested by turtle definitive hosts for the completion of its life cycle (Schell, 1962), indicating that female and male Texas map turtles in their study were consuming snails as part of their diet.

Although turtles may incidentally ingest large numbers of ostracods while feeding on aquatic plants and other sessile aquatic organisms, previous studies have reported very low prevalence (less than 1%) and intensities of *Neoechinorhynchus* species in ostracods (Ward, 1940; Hopp, 1954; Merrit and Pratt, 1964; Uglem and Larsen, 1969). Ostracod populations also experience drastic changes throughout the year, with environmental factors such as temperature, pH, and dissolved oxygen influencing population densities (Liberto et al., 2012; Thorp and Rogers, 2014). More importantly, long-lived snails such as *H. trivolvis* may extend the transmission window for *N. emydis* to turtle definitive hosts throughout the year. Clearly, controlled life cycle studies with complimentary field surveys of turtle definitive hosts will be critical to understand the roles of ostracod intermediate hosts and snail paratenic hosts in the transmission of *N. emydis* to turtle definitive hosts.

In conclusion, this study increases our knowledge on the distribution and snail-host use of *N. emydis* and provides new sequence data for multiple individuals for two acanthocephalan species that infect turtle definitive hosts. Importantly, all previous reports of *N. emydis* in snail paratenic hosts were from snails predominantly restricted to stream habitats in the eastern United States (Ward, 1940; Hopp, 1954; Merrit and Pratt, 1964; Uglem and Larsen, 1969; Burch, 1989). The current study is the first to document the occurrence of *N. emydis* in two species of freshwater snails that reside in ponds and wetlands across North America (Burch, 1989). These observations strongly suggest that *N. emydis* may be more common in snail paratenic hosts than the current lack of reports suggest, and snails may play an important role in the transmission of *N. emydis* to turtle definitive hosts that reside in wetland habitats.

It currently remains unclear when and how snails become infected with acanthocephalans in nature and what implication, if any, the infection has on the snail host. Future work should

aim to survey snail and ostracod populations on a seasonal basis in order to establish when recruitment of acanthocephalans in snails occurs. Ultimately, this will allow for experimental infections of ostracods, snails, and turtles under controlled conditions and the ability to evaluate what roles ostracod intermediate and snail paratenic hosts play in the establishment and reproductive success of *N. emydis* in turtle hosts. By doing so, this work will add to the understanding of acanthocephalan life cycle studies and provide novel data on the role of intermediate and paratenic hosts in parasite life cycle evolution.

Table 2.1 *Neoechinorhynchus* specimens used in this study, their hosts, geographical origin of specimens, sequence length, and GenBank accession numbers for vouchers of corresponding sequences of *Neoechinorhynchus*.

Species	Host species	Geographic origin	Accession no. (sequence length)	References
N. emydis (1)	*Trachemys scripta elegans*	Payne Co., Oklahoma	— (766)	This study
N. emydis (2)	*Trachemys scripta elegans*	Payne Co., Oklahoma	— (722)	This study
N. emydis (3)	*Trachemys scripta elegans*	Payne Co., Oklahoma	— (772)	This study
N. emydis (4)	*Trachemys scripta elegans*	Payne Co., Oklahoma	— (777)	This study
N. emydis (5)	*Trachemys scripta elegans*	Payne Co., Oklahoma	— (773)	This study
N. pseudemydis (1)	*Trachemys scripta elegans*	McCurtain Co., Oklahoma	— (738)	This study
N. pseudemydis (2)	*Trachemys scripta elegans*	McCurtain Co., Oklahoma	— (775)	This study
N. pseudemydis (3)	*Trachemys scripta elegans*	McCurtain Co., Oklahoma	— (772)	This study
N. pseudemydis (4)	*Trachemys scripta elegans*	McCurtain Co., Oklahoma	— (760)	This study
Juvenile (1)	*Helisoma trivolvis*	Site 17, Payne Co., Oklahoma	— (750)	This study
Juvenile (2)	*Helisoma trivolvis*	Site 1, Payne Co., Oklahoma	— (743)	This study
Juvenile (3)	*Physa acuta*	Site 11, Payne Co., Oklahoma	— (777)	This study
Juvenile (4)	*Helisoma trivolvis*	Site 21, Payne Co., Oklahoma	— (777)	This study
Juvenile (5)	*Helisoma trivolvis*	Site 5, Payne Co., Oklahoma	— (777)	This study
Juvenile (6)	*Helisoma trivolvis*	Site 7, Payne Co., Oklahoma	— (774)	This study
Juvenile (7)	*Helisoma trivolvis*	Site 17, Payne Co., Oklahoma	— (770)	This study
Juvenile (8)	*Helisoma trivolvis*	Site 1, Payne Co., Oklahoma	— (787)	This study
Juvenile (9)	*Helisoma trivolvis*	Site 1, Payne Co., Oklahoma	— (773)	This study
N. emyditoides (1)	*Trachemys scripta*	Lake Catemaco, Veracruz, Mexico	KY077108 (760)	Pinacho-Pinacho et al. (2017)
N. emyditoides (2)	*Trachemys scripta*	Lake Catemaco, Veracruz, Mexico	KY077109 (759)	Pinacho-Pinacho et al. (2017)

N. emyditoides (3)	*Trachemys scripta*	Papaloapan River, Tlacotalpan, Veracruz, Mexico	KC004174 (754)	Pinacho-Pinacho et al. (2014)
N. emyditoides (4)	*Trachemys scripta*	Papaloapan River, Tlacotalpan, Veracruz, Mexico	KC004175 (754)	Pinacho-Pinacho et al. (2014)
N. schmidti (1)	*Trachemys scripta*	Centla Swamp, Tabasco, Mexico	KC004172 (789)	Pinacho-Pinacho et al. (2014)
N. schmidti (2)	*Trachemys scripta*	Centla Swamp, Tabasco, Mexico	KC004173 (784)	Pinacho-Pinacho et al. (2014)
N. schmidti (3)	*Trachemys venusta*	Centla Swamp, Tabasco, Mexico	FJ388982 (856)	Martinez_Aquino et al. (2009)
Neoechinorhynchus sp.	NA	NA	KY077112 (784)	García-Varela et al. (2011)

Table 2.2 Site, location, prevalence, and mean intensity of acanthocephalans in *Helisoma trivolvis* and *Physa acuta* from 23 locations in Oklahoma.

Site	Latitude	Longitude	Total snails collected per site	*H. trivolvis* prevalence (no. infected/no. examined)	95% CI (%)	*H. trivolvis* mean intensity (range)	*P. acuta* prevalence (no. infected/no. examined)	95% CI (%)	*P. acuta* mean intensity (range)
1	36.138917	-97.048442	34	35 (7/21)	15 to 57	2.7 (1–6)	23 (3/13)	5 to 54	1 (1)
2	36.160067	-97.061293	10	0 (0/10)	0 to 31	–	–	–	–
3	36.100259	-97.079178	39	0 (0/22)	0 to 15	–	0 (0/17)	0 to 20	–
4	36.190928	-97.052852	5	20 (1/5)	1 to 72	1 (1)	–	–	–
5	36.146056	-97.077859	10	10 (1/10)	0 to 45	2 (2)	–	–	–
6	36.153034	-97.050865	10	0 (0/10)	0 to 31	–	–	–	–
7	36.152037	-97.050789	10	70 (7/10)	35 to 93	1.7 (1–3)	–	–	–
8	35.780295	-97.358463	21	0 (0/21)	0 to 16	–	–	–	–
9	36.105651	-97.123663	20	–	–	–	0 (0/20)	0 to 17	–
10	36.053917	-97.085672	24	0 (0/12)	0 to 27	–	0 (0/12)	0 to 27	–
11	36.071901	-97.108707	26	0 (0/13)	0 to 25	–	8 (1/13)	0 to 36	1 (1)
12	36.068345	-97.106652	15	0 (0/1)	0 to 98	–	0 (0/14)	0 to 23	–
13	36.118959	-97.026826	16	0 (0/11)	0 to 29	–	0 (0/5)	0 to 52	–
14	36.077897	-97.04958	11	0 (0/7)	0 to 41	–	0 (0/4)	0 to 60	–
15	35.999531	-97.053402	42	0 (0/12)	0 to 27	–	0 (0/30)	0 to 12	–
16	36.110386	-97.131479	20	0 (0/9)	0 to 34	–	0 (0/11)	0 to 29	–
17	36.038719	-97.265725	93	61 (46/76)	49 to 72	2.1 (1–5)	0 (0/17)	0 to 20	–
18	36.029391	-97.240452	16	0 (0/15)	0 to 22	–	0 (0/1)	0 to 98	–
19	36.143299	-97.283335	20	–	–	–	0 (0/20)	0 to 17	–
20	36.071996	-97.347077	10	–	–	–	0 (0/10)	0 to 31	–
21	36.06596	-97.353496	28	5 (1/20)	0 to 25	1 (1)	0 (0/8)	0 to 37	–
22	36.1158505	-96.8674904	17	0 (0/3)	0 to 71	–	0 (0/14)	0 to 23	–
23	35.8795665	-97.3958417	37	0 (0/21)	0 to 16	–	0 (0/16)	0 to 21	–
Total			534	20.4 (63/309)	16 to 25	2.1 (1-6)	1.8 (4/225)	1 to 5	1 (1)

Table 2.3 Pairwise genetic distance for ITS genotypes of *Neoechinorhynchus* species from turtle, fish, and snail hosts. Uncorrected p-distances are expressed as a decimal indicating percent difference between individuals.

NP = N. pseudemydis; *NE = N. emydis*; J = juveniles; *NS = N. schmidti*; *NEm = N. emyditoides*; *Nsp = Neoechinorhynchus* sp.

	*NP*1	*NP*2	*NP*3	*NP*4	*NE*1	*NE*2	*NE*3	*NE*4	*NE*5	J1	J2	J3	J4	J5	J6	J7	J8	J9	*NEm*1	*NEm*2	*NEm*3	*NEm*4	*NS*1	*NS*2	*NS*3	*Nsp*
*NP*1	–																									
*NP*2	0.003	–																								
*NP*3	0.000	0.003	–																							
*NP*4	0.000	0.003	0.000	–																						
*NE*1	0.069	0.071	0.069	0.069	–																					
*NE*2	0.069	0.071	0.069	0.069	0.000	–																				
*NE*3	0.069	0.071	0.069	0.069	0.000	0.000	–																			
*NE*4	0.069	0.071	0.069	0.069	0.000	0.000	0.000	–																		
*NE*5	0.069	0.071	0.069	0.069	0.000	0.000	0.000	0.000	–																	
J1	0.069	0.071	0.069	0.069	0.000	0.000	0.000	0.000	0.000	–																
J2	0.069	0.071	0.069	0.069	0.000	0.000	0.000	0.000	0.000	0.000	–															
J3	0.069	0.071	0.069	0.069	0.000	0.000	0.000	0.000	0.000	0.000	0.000	–														
J4	0.069	0.071	0.069	0.069	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	–													
J5	0.069	0.071	0.069	0.069	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	–												
J6	0.069	0.071	0.069	0.069	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	–											
J7	0.069	0.071	0.069	0.069	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	–										
J8	0.069	0.071	0.069	0.069	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	–									
J9	0.069	0.071	0.069	0.069	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	–								
*NEm*1	0.064	0.066	0.064	0.064	0.085	0.085	0.085	0.085	0.085	0.085	0.085	0.085	0.085	0.085	0.085	0.085	0.085	0.085	–							
*NEm*2	0.061	0.063	0.061	0.061	0.085	0.085	0.085	0.085	0.085	0.085	0.085	0.085	0.085	0.085	0.085	0.085	0.085	0.085	0.003	–						
*NEm*3	0.064	0.066	0.064	0.064	0.085	0.085	0.085	0.085	0.085	0.085	0.085	0.085	0.085	0.085	0.085	0.085	0.085	0.085	0.000	0.003	–					
*NEm*4	0.061	0.063	0.061	0.061	0.085	0.085	0.085	0.085	0.085	0.085	0.085	0.085	0.085	0.085	0.085	0.085	0.085	0.085	0.003	0.000	0.003	–				
*NS*1	0.109	0.111	0.109	0.109	0.086	0.086	0.086	0.086	0.086	0.086	0.086	0.086	0.086	0.086	0.086	0.086	0.086	0.086	0.125	0.121	0.125	0.121	–			
*NS*2	0.109	0.111	0.109	0.109	0.086	0.086	0.086	0.086	0.086	0.086	0.086	0.086	0.086	0.086	0.086	0.086	0.086	0.086	0.125	0.121	0.125	0.121	0.000	–		
*NS*3	0.109	0.112	0.109	0.109	0.086	0.086	0.086	0.086	0.086	0.086	0.086	0.086	0.086	0.086	0.086	0.086	0.086	0.086	0.125	0.121	0.125	0.121	0.000	0.000	–	
Nsp	0.354	0.351	0.354	0.354	0.333	0.330	0.333	0.333	0.333	0.333	0.333	0.333	0.333	0.333	0.333	0.333	0.333	0.333	0.329	0.334	0.331	0.334	0.333	0.333	0.333	–

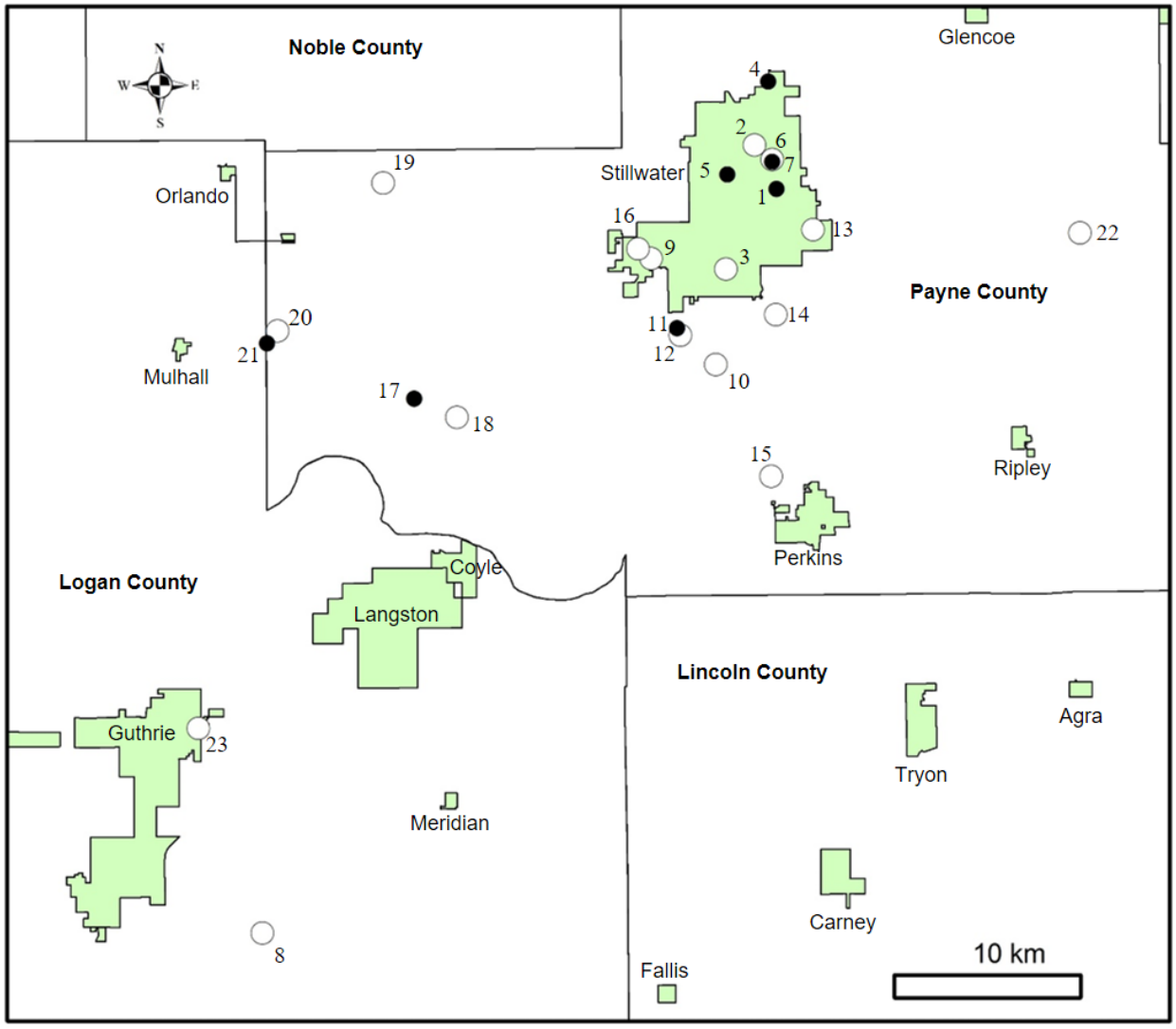

Figure 2.1 Positive (black circles) and negative (white circles) localities for acanthocephalans in snails from Oklahoma. Green polygons represent city limits, and numbers represent names of each site (see Table 2.2).

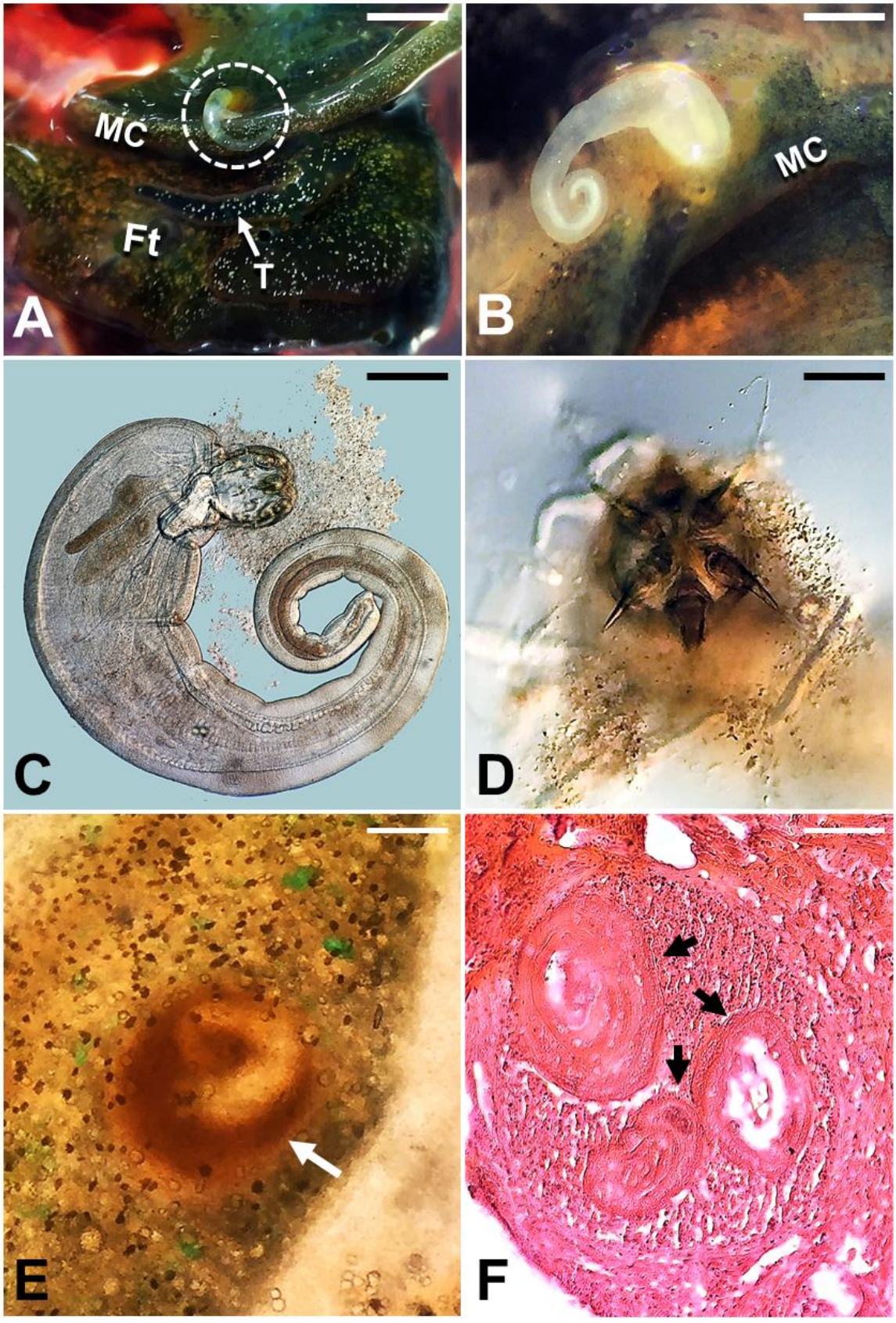
MC
Ft
T
A
MC
B
C
D
E
F

Figure 2.2 Bright field (BF) and differential interference contrast (DIC) micrographs of juvenile acanthocephalans attached or encysted in the snail *Helisoma trivolvis*, collected from Payne, Co., OK. **A**. Juvenile worm (white circle) attached to the mantle collar (MC) of *H. trivolvis* from which the shell has been removed. Note the snail foot (Ft) and tenticle (T) for worm attachment oriantation. Scale bar = 1 mm. **B**. Higher magnification of attached juvenile acanthocephalan on the mantle collar of *H. trivolvis*. Note the worm is attached to the mantle collar with its proboscis. Scale bar = 0.25 mm. **C**. and **D**. DIC micrographs of juvenile worms after being removed from the mantle collar of *H. trivolvis*. Note the presence of snail tissue on the proboscis. Scale bars = 0.1 and 0.05 mm, respectively. **E**. Light micrograph of a wetmount preperation of a juvenile worm encysted (white arrow) within the headfoot of *H. trivolvis*. Note the dark pigment surounding the coiled worm within the cyst. Scale bar = 0.05 mm. **F**. Histological section of a cyst within the headfoot of *H. trivolvis*, showing the position of a coiled worm (black arrows) surrounded by cellular debris and melanin-like pigment. Scale bar = 0.2 mm.

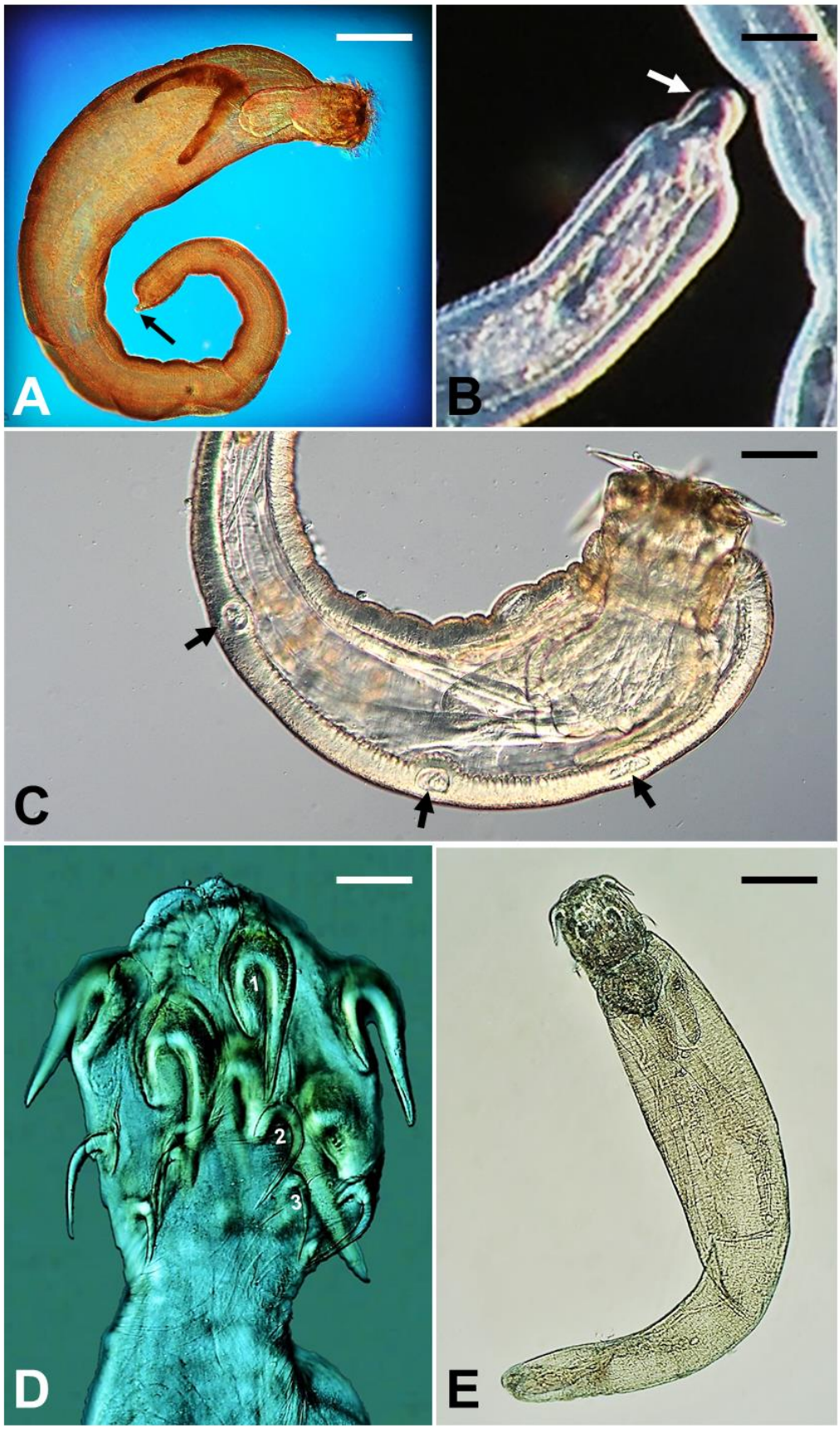
A
B
C
1
2
3
D
E

Figure 2.3 Bright field (BF), dark field (DF), and differential interference contrast (DIC) micrographs of juvenile *Neoechinorhynchus* sp. recovered from *Helisoma trivolvis* and *Physa acuta*, collected in Oklahoma, and *Bithynia tentaculata* collected in Wisconsin. **A**. and **B**. DIC and DF micrographs of a juvenile *Neoechinorhynchus* sp. removed from a cyst in the headfoot of *P. acuta*. Note the knob-like structure on the posterior end of the trunk (white arrow). Scale bars = 0.1 and 0.03 mm, respectively. **C**. DIC micrograph of a juvenile *Neoechinorhynchus* sp. removed from a cyst in the headfoot of *H. trivolvis*. Note the aspinose trunk contained subcuticular giant nuclei (black arrows). Scale bar = 0.09 mm. **D**. DIC micrograph of the everted proboscis of a juvenile *Neoechinorhynchus* sp. recovered from *H. trivolvis*. Note the three circles (numbered) containing six hooks each. Scale bar = 6 µm. **E**. BF micrograph of a juvenile *Neoechinorhynchus* sp. recovered from *B. tentaculata*. Scale bar = 0.2 mm.

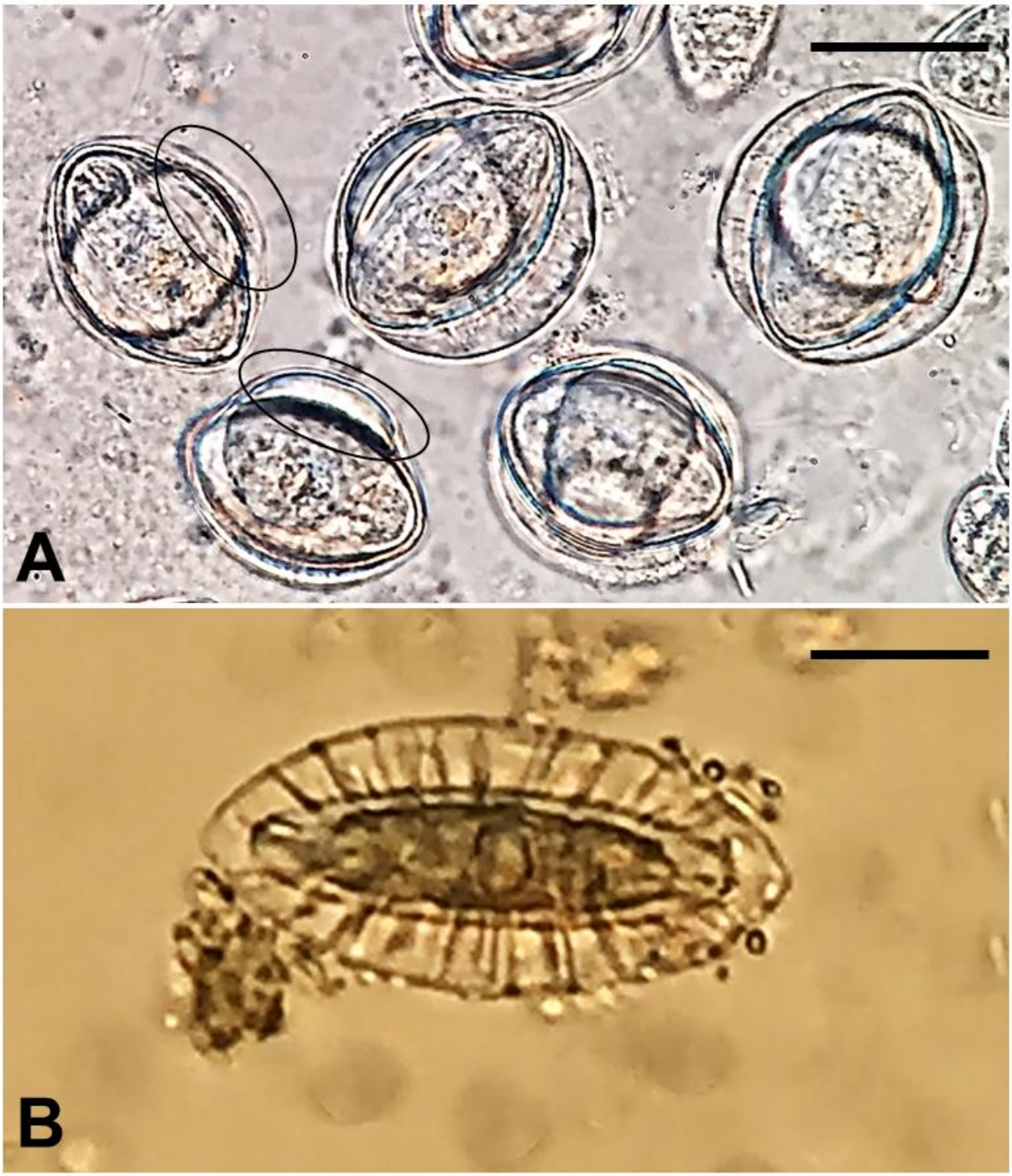
A
B

Figure 2.4 Bright field (BF) micrographs of mature eggs recovered from *Neoechinorhynchus* species collected from red-eared slider turtles, *Trachemys scripta elegans*, collected from Payne and McCurtain Counties, OK. **A**. Eggs of *Neoechinorhynchus emydis*. Note the C-shaped equatorial vacuole not entirely encircling the acanthor (black ellipses). Scale bar = 15 µm. **B**. Eggs of *Neoechinorhynchus pseudemydis*. Note the distinct football shape with radiating striations. Scale bar = 14 µm.

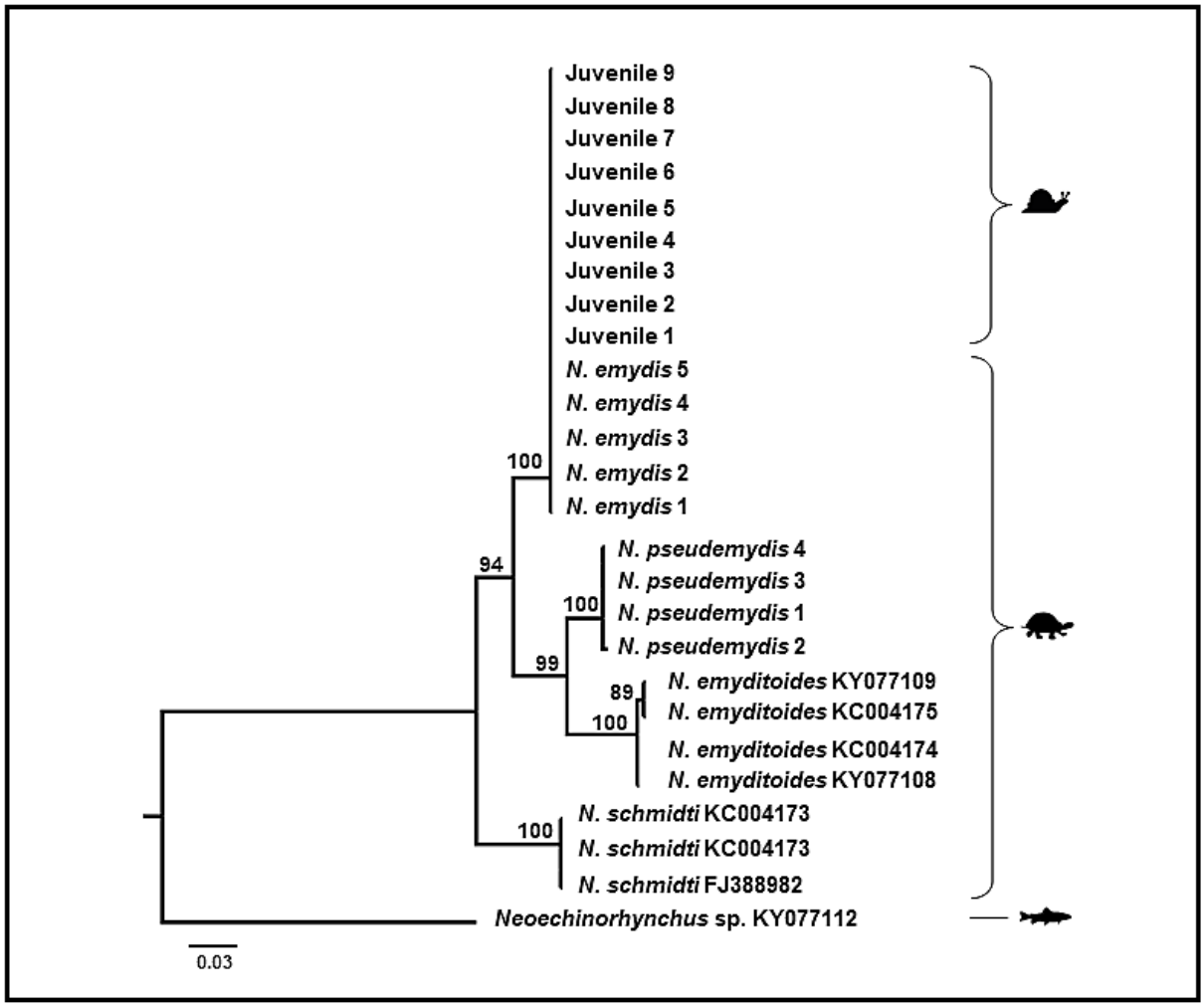

Figure 2.5 Relationships inferred by maximum likelihood (Log Likelihood: -2025.45). Tree is unrooted and drawn to scale indicating number of substitutions per site. Bootstrap values above 70 are shown.

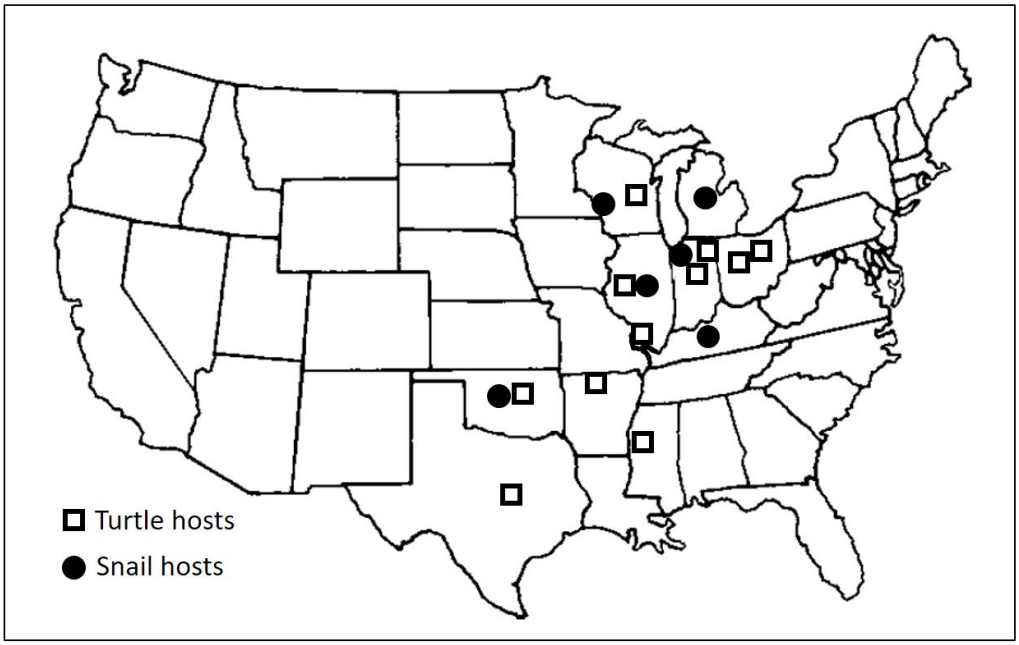

Figure 2.6 Geographic distribution of *Neoechinorhynchus emydis* from turtle definitive hosts (squares) and *Neoechinorhynchus* sp. reports from freshwater snails (black circles). Modified from Whitlock, 1939; Hopp, 1946, 1954; Lincicome and Whitt, 1947; Barger, 2004; and McAllister et al., 2014.

CHAPTER III

SEASONAL OCCURRENCE OF *NEOECHINORHYNCHUS EMYDIS* (PHYLUM: ACANTHOCEPHALA) IN THE FRESHWATER SNAIL, *HELISOMA TRIVOLVIS*, FROM THE GREAT PLAINS REGION OF NORTH AMERICA

ABSTRACT:

Neoechinorhynchus emydis infects freshwater turtle definitive and ostracods intermediate hosts, and is the only acanthocephalan species reported to infect freshwater snails as potential paratenic hosts. Currently, no information is available on the seasonal distribution of *N. emydis* in freshwater snail hosts. To address this, I examined the seasonal distribution of acanthocephalans and other parasite infections in the freshwater snail, *Helisoma trivolvis*, from a single location in northcentral Oklahoma. Overall prevalence and mean intensity of *N. emydis* in snails was 27% and 3.8 ± 5.3, respectively. Additionally, 33% of acanthocephalan-infected snails were co-infected with trematodes and/or nematodes. Finally, all acanthocephalans located within the headfoot region of snail hosts contained melanin-like pigment surrounding each worm, suggesting snails were mounting an immunological reaction to infections with *N. emydis*. Seasonally, prevalence of *N. emydis* in snails was 0% during the winter, increased to 50% during the summer, and declined to 17% in the fall; whereas mean abundance was more variable and ranged from 0 during the winter to as high as 1.9 ± 6 during the spring, but generally followed a similar pattern as prevalence. Snail shell diameter of snails was smallest during the spring, was

greatest during the fall, and decreased during the winter, suggesting that larger and/or older snails were dying during the winter. Because snails were commonly infected with trematodes and nematodes known to reduce the life span of snails, and snail size varied significantly over the collecting period, it was unclear whether the seasonal variation of acanthocephalan infections was a result of snail mortality due to snail age, parasite infections or a combination of these factors. To control for some of these factors, additional field and laboratory experiments were implemented. First, laboratory-reared *H. trivolvis* snails were exposed to naturally infected ostracods in field cages for five week intervals throughout the winter, spring, and summer, and snails were sampled on a weekly basis to examine short-term effects of infections during the fall. Second, a laboratory survival experiment was conducted by testing the life span of field collected snails of various sizes naturally infected with acanthocephalans and/or trematodes. Data from snail cage infections were consistent with the seasonal field survey, with *N. emydis* infections being highest in the summer (20%) and lowest (0%) in the winter, suggesting that snails were not ingesting infected ostracods during the winter. However, fewer snails survived in field cages during the winter than during the spring and summer, suggesting that snails may be succumbing due to the harsher winter conditions. The snail survival experiment indicated that larger snails, snails infected with trematodes, and snails co-infected with trematodes and acanthocephalans died at a faster rate than smaller uninfected snails or snails only infected with acanthocephalans. Taken together, these results suggest that the occurrence of acanthocephalans in snails throughout the year may be partially influenced by the abundance of infected ostracods that snails may be ingesting, co-infections with trematodes, and snail population fluctuations during the year. However, other abiotic factors, particularly harsh winter conditions on *N. emydis* infected snails, still need to be evaluated.

INTRODUCTION

Acanthocephalans require an arthropod intermediate and a vertebrate definitive host to complete the life cycle. Although not required, paratenic hosts may aid in completing acanthocephalan life cycles by increasing transmission from the intermediate host to the definitive host (Baer, 1951). Most reports of paratenic hosts for acanthocephalans are from vertebrate hosts, including mammals, fish, amphibians, and reptiles (Schmidt, 1985; Kennedy, 2006), with few reports in invertebrate hosts (Whitlock, 1939; Hopp, 1946, 1954; Lincicome and Whitt, 1947; Lassiere, 1988). Of those, freshwater snails have been reported as paratenic hosts for acanthocephalans in the genus *Neoechinorhynchus* (Whitlock, 1939; Hopp, 1946, 1954; Lincicome and Whitt, 1947). However, Hopp (1954) was the only one to accurately identify the species of acanthocephalan in snails as *Neoechinorhynchus emydis* by feeding naturally infected snails to turtles, and later using eggs from mature adult acanthocephalans for species identification.

Results from my previous work on the spatial distribution of acanthocephalans in snails from northcentral Oklahoma (chapter II) indicate that two species of freshwater snails are commonly infected with juvenile acanthocephalans. Sequences of the complete ITS rDNA barcoding region of juvenile acanthocephalans from snail hosts and adult acanthocephalans from turtle hosts collected from various ponds and streams in northcentral Oklahoma revealed that juvenile acanthocephalans infecting freshwater snails belong to the species *N. emydis* (Chapter II). In northcentral Oklahoma, the occurrence of juvenile acanthocephalans in snails was fairly common, with 30% (7/23) of sampled localities being positive for acanthocephalans. Between the two species of snails sampled, *Helisoma trivolvis* was found to be more commonly infected

with *N. emydis* (20.3% and 2.1 ± 1.26 worms per snail, respectively) than was *Physa acuta* (1.8% and 1 ± 0 worms per snail, respectively). Furthermore, limited histological analysis of infected snails indicated melanin-like pigment surrounding encysted worms, suggesting a possible immunological reaction as a result of acanthocephalan infections.

Despite these findings, no study has yet examined the seasonal distribution of acanthocephalan infections in freshwater snails. In the life cycle of *N. emydis*, snails are thought to become infected by ingesting infected ostracods, and turtles subsequently become infected by consuming infected ostracods and/or snails (Hopp, 1954). Thus, understanding the seasonal variation of acanthocephalans in snails will provide insight as to when acanthocephalans are being recruited from ostracod hosts to snail hosts. Additionally, acanthocephalan recruitment to turtles may also depend on when snail infections are highest. For example, Esch et al. (1979) found that four species of adult *Neoechinorhynchus* in red-eared slider turtles (*Trachemys scripta scripta*) from South Carolina persisted throughout the year at a high prevalence. However, based on their monthly size class data, there were two distinct recruitment periods (May and July) of acanthocephalans, which utilize ostracods as intermediate hosts to infect turtle hosts (Esch et al., 1979). However, and because freshwater snails have a much longer life span than ostracods (Thorp and Rogers, 2014), it is unclear how the addition of a snail paratenic host to the life cycle of *N. emydis* would affect the seasonal transmission of *N. emydis* to turtle definitive hosts.

Additionally, it is unclear whether there are any consequences to snail hosts while infected with juvenile acanthocephalans. For example, the trematode *Schistosoma mansoni* has been shown to decrease snail-host (*Biomphilaria glabrata*) survival (Lee and Cheng, 1971) and induces an immunological reaction within snail tissue (Souza and Andrade, 2006). Similar responses may also occur during establishment when juvenile acanthocephalans encyst in snail

tissue. Therefore, the goals of the present study were: (1) to determine the seasonal recruitment of acanthocephalans in *Helisoma trivolvis* snails, (2) to evaluate any snail-acanthocephalan associated pathology at the tissue and cellular level in naturally infected *H. trivolvis* snails, and (3) to evaluate the recruitment and survival of naturally infected snails with *N. emydis* under controlled laboratory/field conditions.

MATERIALS AND METHODS

Seasonal snail field survey and snail immunological reactions to acanthocephalan infections

To evaluate snail recruitment of juvenile *N. emydis* infections through time, *Helisoma trivolvis* snails were collected over a 12-month period (July 2016-June 2017) from a permanent man-made decorative pond at the Chapel Ridge of Stillwater Apartments (Apartment Pond), located in Stillwater, Payne Co., Oklahoma (site 1; 36.138917, -97.048442). Apartment Pond is 47 m long by 30 m wide and approximately 1 m deep with no emergent vegetation surrounding the pond. Apartment Pond was chosen for a temporal study due to previous surveys in the area indicating a relatively high acanthocephalan infection in *H. trivolvis* at this location (Chapter II). An attempt was made to collect approximately 20 *H. trivolvis* snails monthly over a period of 12 months. However, during certain months, snail abundance was very low, and no snails or fewer than 20 snails were obtained during some months (Table 3.1). All snails were collected by hand or by using a dip-net by walking around the periphery of Apartment Pond. All snails collected during each trip were placed in 50 ml capped plastic centrifuge tubes with 20-30 ml of pond water and brought back to the laboratory at Oklahoma State University.

In the laboratory, snails were identified to species using keys in Burch (1989) and processed for acanthocephalan infections within 12 hours of being collected. Briefly, each snail's shell diameter was measured to the nearest 1.0 mm with a handheld caliper. The shells were then removed from each snail by placing an individual snail in a petri dish and gently crushing it with a smaller petri dish. Next, the entire carcass of each snail was cut into approximately four equal pieces with a razor blade and flattened between two glass microscope slides, and each snail carcass was scanned for juvenile acanthocephalans under an Olympus SZ-8145 stereomicroscope configured for incident illumination at 10 x to 45 x total magnification. When an acanthocephalan was observed in the snail tissue, the location was recorded, the top slide was removed, and the acanthocephalan was gently removed from the snail tissue with forceps. Any additional snail tissue was removed from the proboscis of each worm, the worm was placed on a glass microscope slide, and a wet mount was prepared by adding a drop of water and a coverslip. In addition, the presence of other parasites (trematodes and nematodes) was recorded for each snail.

All acanthocephalans, were examined at 100 x to 400 x total magnification with an Olympus BX-51 upright research microscope configured for bright field (BF) and differential interference contrast (DIC) microscopy with plain fluorite objectives. Finally, multiple digital images were taken of a subsample of worms using an Olympus 5-megapixel digital camera for further identification following the terminology in Amin (2002) and Barger and Nickol (2004). Finally, nine infected snails containing acanthocephalan cystacanths within thin cysts of the headfoot were sectioned to evaluate any snail-acanthocephalan cellular interactions. Briefly, pieces of snail tissue with encysted worms in thin cysts were fixed in hot Bouin's fixative, embedded in paraffin, sectioned at 10 µm, affixed to slides, stained with hematoxylin and eosin,

and mounted in Canada balsam. The greatest length and width of the thin cysts were then measured and the cellular contents of each cyst was recorded. All histological sections were evaluated for the presence of cellular debris and melanin-like pigment.

Prevalence, along with 95% confidence intervals, mean intensity, and/or mean abundance for acanthocephalan infections were calculated for total numbers of snails collected on each date according to Bush et al. (1997), and mean shell diameter was calculated for snails collected on each date. However, because fewer than 20 snails were collected on certain months, and for statistical analyses, snail samples were grouped into four seasons including summer (June 1-August 31), fall (September 1-November 30), winter (December 1-February 28), and spring (March 1-May 31) and evaluated for any differences in prevalence and/or mean abundance of acanthocephalan infections.

Student's t-test was calculated to evaluate any differences in mean shell diameter for all infected and uninfected *H. trivolvis* collected throughout the year. Additionally, Pearson's correlation was calculated to analyze the relationship between *H. trivolvis* shell diameter and acanthocephalan abundance and intensity for all snails collected throughout the year. The Kruskal–Wallis and the Dunn's multiple comparison tests were used to compare differences in prevalence and mean abundance of acanthocephalan infections in snails sampled during each season. Finally, a one-way analysis of variance (ANOVA) followed by the Scheffé post hoc test was performed on shell diameter of snails to test for any differences in means between seasons. All statistical analyses were implemented in STATISTICA v10 (StatSoft, Inc., 2011).

Because the snail population at Apartment Pond was commonly infected with other parasites (trematodes and nematodes), which are known to reduce the life span and ultimately kill snails (Crews and Esch, 1987; Schmidt and Fried, 1997; Klockars, et al., 2007; Zimmermann

et al., 2011), and snail shell diameter varied significantly over the collecting period, it was not clear if the observed seasonal variation in snail infections with juvenile acanthocephalans was a result of snail mortality due to snail age, acanthocephalan infections, other parasite infections, their interactions, or other factors. Therefore, to control for these factors, field cage exposure experiments of laboratory reared snails and naturally infected snail survival experiments were run in the laboratory (see below).

Experimental field infections

Because snail abundance drastically declined during the spring of 2017 at Apartment Pond, a wild population of naturally infected *H. trivolvis* snails, with juvenile acanthocephalans and other parasites, was collected from a second location (Cow Pond) in Stillwater, Payne Co., OK (site 17; 36.038719, -97.265725). Previous surveys indicated that *H. trivolvis* snails had similar levels of acanthocephalan infections in Cow Pond as in Apartment Pond (Chapter II). Cow Pond is a privately owned pond, and is 46 m long by 37 m wide, approximately 2 m deep, surrounded by emergent vegetation, and contains approximately 20% canopy cover. Cattle and/or cow feces were commonly observed in or around the pond, hence the name. To evaluate when snails become infected with juvenile acanthocephalans during the year, an experiment was implemented in the field, by exposing laboratory-reared snails to naturally-infected ostracod hosts during summer, spring, and winter seasons of 2017-2018. Laboratory-reared snails were obtained by collecting a wild population of *H. trivolvis* snails from Cow Pond. Individual snails were housed in 500 ml plastic deli cups filled with 400 ml dechlorinated water and maintained under a 12:12 light-dark photoperiod at room temperature (21° C) throughout the entire experiment. On a weekly basis, water was replaced, and snails were fed TetraVeggie algae wafers (30% crude protein, 7.5% crude fat, 4% crude fiber). Once snails deposited egg masses,

eggs were transferred into new deli cups with 400 ml dechlorinated water until hatched. Groups of 10 to 12 hatched snail individuals were then transferred and housed in 24 3 L glass bowls of dechlorinated and water and aerated. Snails were maintained and fed in bowls as previously described, and these snails and their offspring were used for field cage experiments.

To minimize snails escaping from field cages, snails were removed from bowls and each snail's shell was measured with a hand caliper to ensure that all snails placed in field cages had a shell diameter of at least 9 mm in length which prevented them from escaping through the wire mesh of cages (see below). Additionally, to differentiate laboratory reared snails from any wild snails that could enter field cages through the mesh, each laboratory-reared snail that had a shell diameter of 9 mm or greater was removed from the water, dried, and two coats of green N.Y.C. New York Color® nail polish was applied to the surface of its shell as a marking technique (Henry and Jarne, 2007). All marked snails were left out of water to dry for 10 minute periods after each coat of paint was applied and then placed in a 19 L bucket filled with 9 L of dechlorinated water (Fig. 3.1). Snails were then transported to Cow Pond within an hour of being marked.

Cages used to house snails in the field were constructed from modified vinyl-dipped H_2O XPRESS™ 16-1/2" minnow traps. Each minnow trap was modified by cutting a 14 x 14 cm opening on the side, and a 15 x 15 cm piece of steel screen was attached with 18-gauge aluminum wire, acting as a modified door to access snails. Individual modified minnow traps were then attached vertically with 16 gauge galvanized steel wire approximately halfway up on a 1.8 m steel fence post. During the winter season, 22 snails were placed in each of 15 cages. However, because fewer laboratory-reared snails were available during the spring and summer, a total of 13-26 snails were placed in each of four cages during the spring and summer. Cages

were positioned 1-2 m apart along the periphery of the pond by hammering the steel fence post into the pond bottom, and allowing at least 15 cm of each cage to be above the water line to allow snails to surface to breathe (Fig. 3.1). All remaining living and dead snails were removed from cages after a period of 6 weeks and examined for acanthocephalans and other parasites as previously reported. The Chi-Square test of independence was calculated to compare differences in snail cage survival during the seasons. Because no snails became infected during the winter, the Fisher-Freeman-Halton Exact Test for 2 x 3 contingency table was used to evaluate any differences in prevalence of *N. emydis* infections in snails among all seasons (Freeman and Halton, 1951; Kroonenberg and Verbeek, 2018); whereas the Kruskal–Wallis and the Dunn's multiple comparison tests were used to compare differences in mean abundance of acanthocephalan infections in snails sampled during each season.

Finally, to evaluate any immunological reaction of snails to juvenile *N. emydis* infections, 21 laboratory-reared snails were placed in each of 10 cages at the periphery of Cow Pond during the fall season as previously described, and approximately 20 snails were sampled for *N. emydis* infections from the 10 cages weekly over a 5-week period. Snail tissue was examined under an Olympus SZ-8145 stereomicroscope configured for incident illumination at 10 x to 45 x total magnification, and the number of worms in each snail and the degree of melanin-like pigment in each cyst was recorded during each sampling week.

Snail survival laboratory study

Because snail survival in the seasonal cage study at Cow Pond was relatively low, an additional snail survival study was conducted to evaluate the life span of naturally infected snails from Cow Pond in the laboratory. Seventy-two snails were collected in March 2017 from Cow Pond with a dip-net and placed in a 19 L bucket filled with pond water and brought back to

Oklahoma State University. Snails were allowed to acclimate to room temperature in the bucket, and the shell diameter was measured to the nearest 1 mm using a hand caliper for each snail. Individual snails were maintained in deli cups and fed as previously described. Twice a week, snails were observed, and any mortalities were recorded. When no movement was observed, snails were stimulated by gently touching the foot with a pair of forceps. A snail unresponsive to stimulation was considered dead, and the date of death was recorded. Dead snails were examined for acanthocephalans, trematode and other parasite infections by flattening tissues between two glass slides and scanning the flattened tissue under an Olympus SZ-8145 stereomicroscope, as previously described.

To evaluate the effects of acanthocephalan infections, trematode infections, snail size, and their interactions on snail survival, a Poisson generalized linear model was implemented in STATISTICA v10 (StatSoft, Inc., 2011) with days of snail survival as the response variable.

RESULTS

Seasonal snail field survey and snail immunological reactions to acanthocephalan infections

A total of 186 *H. trivolvis* snails (3–25 per month) were collected from Apartment pond (Table 3.1). Mean shell diameter for *H. trivolvis* was 13.3 mm ± 3.1 (range 6 – 20). Overall prevalence and mean intensity of juvenile *N. emydis* in *H. trivolvis* snails was 27% (51/186; 95% CI 21-34) and 3.8 ± 5.3 (1-31), respectively. Infected and uninfected *H. trivolvis* snails had an average shell diameter of 14.7 ± 3.1 mm (range 8 – 20) and 12.8 ± 2.9 mm (range 6 – 19), respectively, and a significant difference existed in the shell diameter of infected and uninfected *H. trivolvis* snails ($t = 3.96$; $P < 0.001$). There was a positive correlation between *H. trivolvis*

shell diameter and acanthocephalan abundance ($r = 0.29$; $P < 0.001$), and snail shell diameter and acanthocephalan intensity ($r = 0.32$; $P < 0.05$). Additionally, 21% (39/186; 95% CI 15-27) of snails were infected with trematodes, 3% (6/186; 95% CI 0.7-5.8) of snails were infected with nematodes, and 33% (17/51; 95% CI 21- 48) of acanthocephalan infected snails were co-infected with trematodes and/or nematode. In total, 42% (78/186; 95% CI 35-49) of snails were infected with at least one type of parasite.

A total of 192 *N. emydis* were recovered throughout the yearly survey from snails at Apartment Pond. Of the 192 worms recovered from *H. trivolvis*, 4 (2%) were attached with their proboscis to the mantle collar of *H. trivolvis,* and the worm's trunk was located in the space between the snail's shell and viseral mass, whereas the remaining worms 188 (98%) were located in thin cysts within the headfoot region of each snail, and a dark melanin-like pigment surrounded each worm within the cyst.

Once removed from the thin-walled cyst within the snail's headfoot, or from the mantle collar of a snail, each juvenile worm vigorously everted and retracted its proboscis within the proboscis receptacle (Fig. 3.2). The proboscis was short, globular, and slightly broader than long with three circles of six hooks each (Fig. 3.2). Histological sections of the 9 infected and sectioned snails revealed that each coiled worm was surrounded by cellular debris and melanin-like pigment (Fig. 3.3). However, the amount of melanin-like pigment was variable among individual cysts. Cysts within the head foot of snails were 473 $\pm$ 78 µm in greatest length and 326 $\pm$ 75 µm in greatest width.

Seasonally, prevalence and mean abundance was 17% (10/59; 95% CI 7.4-26.5) and 0.6 $\pm$ 1.9 (0-12) during the fall, dropped to 0% (0/36; 95% CI 0-9.7) in the winter and increased to 50% (30/60; 95% CI 36.8-63) and 1.6 $\pm$ 2.9 (0-16) during the summer (Fig. 3.4). The Kruskal-

Wallis test revealed significant differences in seasonal prevalence and mean abundance of *N. emydis* in *H. trivolvis* (prevalence, $H = 33.05$, $P < 0.001$; mean abundance, $H = 33.46$, $P < 0.001$). The Dunn's multiple comparison tests indicated that prevalence of *N. emydis* during the summer was significantly higher than prevalence of *N. emydis* during the fall and winter seasons ($P < 0.001$; Fig. 3.4). Additionally, the Dunn's multiple comparison tests indicated that mean abundance of *N. emydis* during the summer season was significantly higher than mean abundance of *N. emydis* during the fall and winter seasons ($P < 0.001$; Fig. 3.4).

Seasonally, the mean shell diameter of snails was 14.4 $\pm$ 2.7 mm (6-19) during the fall, 12.0 $\pm$ 2.8 mm (8-18) during the winter, 10.5 $\pm$ 2.5 mm (8-20) during the spring, and 14.3 $\pm$ 2.6 mm (9-19) during the summer. The one-way ANOVA revealed significant differences in snail shell mean diameter during the seasons ($F = 20.47$, $P < 0.001$). The Scheffé post hoc test revealed significant differences in mean snail shell diameter for fall and winter ($P = 0.004$), fall and spring ($P < 0.0001$), spring and summer ($P < 0.0001$), and summer and winter ($P = 0.0009$).

Experimental field infections

Significant differences existed in the number of snails recovered from cages over the three seasons ($\chi^2 = 37.3904$, $P < 0.00001$). Snail recovery was lowest during the winter 29% (90/308; 95% CI 24-34), highest during the spring 65.5% (38/58; 95% CI 53-78), and 52% (54/103; 95% CI 43-62) during the summer. Prevalence and mean abundance of *N. emydis* from snails recovered from cages was lowest during the winter 0% (0/90), increased during the spring to 5% (2/38; 95% CI 0-12) and 0.05 $\pm$ 0.2 (0-1), and was highest during the summer 20% (11/54; 95% CI 9.6-31) and 0.26 $\pm$ 0.6 (0-4; Fig. 3.4). The Fisher-Freeman-Halton Exact Test indicated that significant differences existed in prevalence of *N. emydis* among different seasons (P_A =

0.00001); whereas there was no significant difference in the mean abundance of *N. emydis* from snails recovered from cages among seasons ($P > 0.05$; Fig. 3.4).

Overall survival of snails during weekly cage sampling in the fall was 50% (105/210; 95% CI 43-57). All dead snails that were recovered contained predatory leeches, which were in the process of consuming snails. Overall prevalence and mean abundance of *N. emydis* in all recovered snails during the fall was 15% (16/105; 95% CI 13-29) and 0.3 $\pm$ 0.5 (0-3), respectively. Snails began recruiting acanthocephalans within the second week of being placed out in cages. Week 2 had 4 infected snails with 5 worms, week 3 had 7 infected snails with 9 worms, week 4 had 3 infected snails with 5 worms, and week 5 had 2 infected snails with 2 worms. A total of 21 acanthocephalans infected snails over the 5-week sampling period, and the degree of melanin-like pigment varied depending on the week infected snails were recovered from field cages (Fig. 3.5). Of the acanthocephalans recovered from snails sampled on the second and third week, 40% (2/5) and 33% (3/9) contained melanin-like pigment within the cysts, respectively. In contrast, 60% (3/5) and 100% (2/2) of acanthocephalans recovered from snails sampled during week 4 and 5 contained melanin-like pigment surrounding the worms within the cyst, respectively (Fig. 3.5).

Snail survival laboratory study

Mean shell diameter for the 72 *H. trivolvis* snails collected from Cow Pond was 14.1 mm ± 1.6 (range 10 – 18). Overall, 71% (51/72; 95% CI 59-81) of snails were infected with acanthocephalans and/or trematodes. Prevalence and mean intensity for juvenile *N. emydis* in snails was 57% (41/72; 95% CI 45-69) and 2.0 ± 1.3 (1-6), respectively; whereas prevalence of trematode infections was 54% (39/72; 95% CI 42-66). Forty percent (29/72; 95% CI 29-53) of snails were infected with both acanthocephalans and trematodes.

In the laboratory, snails survived an average of 42 ± 35.5 days (range 3 – 128). The Poisson generalized model indicated trematode infections and snail shell diameter had consistent significant effects on snail survival. Acanthocephalan infections had significant effects when interacting with trematode infections or interacting with trematode and snail shell diameter (Table 3.2). In contrast, acanthocephalan infections or acanthocephalan infections by snail shell diameter interactions had no significant effect on snail survival (Table 3.2).

DISCUSSION

The present study is the first to document the seasonal variation of juvenile *N. emydis* infections in freshwater snails from Oklahoma. Additionally, my field cage exposure experiments with laboratory-reared snails provide insight into when snails acquire infections of *N. emydis* throughout the year. Finally, by conducting laboratory survival experiments on field collected snails, I was able to evaluate some of the factors influencing snail survival of field collected snails of various sizes and naturally infected with acanthocephalan and other parasites.

Morphologically, all juvenile acanthocephalans recovered from *H. trivolvis* during this study conformed to previous reports and descriptions of *N. emydis* infections in snails (Hopp, 1954; Chapter II). Overall prevalence and mean intensity of juvenile *N. emydis* in *H. trivolvis* snails from Apartment Pond was similar to previous reports of this acanthocephalan species infecting *H. trivolvis* in various ponds and streams throughout northcentral Oklahoma (Chapter II). As previously reported for *N. emydis* infections in *H. trivolvis*, there was a significant but weak positive correlation between snail size and *N. emydis* intensity and abundance, indicating that larger and/or older snails had higher probability of becoming infected with *N. emydis* (Chapter II). Finally, all acanthocephalans that were located within thin cysts of the headfoot of

field collected and infected snails from Apartment Pond contained various degrees of a dark melanin-like pigment surrounding each worm within the cyst. Once removed, all worms were viable and actively everted their proboscis.

Results from the seasonal field survey at Apartment Pond indicated that prevalence of acanthocephalan infections in snails was 0% during the winter, increased throughout the spring to 50% during the summer, and declined to 17% in the fall. Seasonally, mean abundance was more variable and ranged from 0 during the winter to as high as 1.9 worms per snail during the spring, but generally followed a similar pattern as prevalence, with some infected snails harboring as many as 31 worms. Additionally, field cage infections of laboratory-reared snails with *N. emydis* during the winter, spring, and summer from Cow Pond showed similar patterns of infections. None of the 106 recovered snails became infected during the winter; whereas both prevalence and mean abundance of *N. emydis* gradually increased during the spring to 20% in the summer. Taken together, these data suggest that snails may not be coming in contact with infected ostracods commonly during the winter.

There are several possible reasons for why snails are not acquiring infections of *N. emydis* during the winter. First, many aquatic turtle hosts overwinter and are generally less active during the colder months (Ultsch, 2006). As a result, turtles may not be releasing as many acanthocephalan eggs via feces, contributing to fewer ostracods becoming infected on the pond bottom. Second, even if acanthocephalan eggs are present throughout the year, ostracod densities may simply be very low during the winter (Liberto et al., 2012; Thorp and Rogers, 2014), preventing the life cycle to be completed. Third, snail hosts may not be feeding as much during the winter, and thus are not ingesting infected ostracods commonly. Previous work on four populations of overwintering *H. trivolvis* snails from New York State revealed significant

tissue degrowth in three of those populations, suggesting resources can be limited for some *H. trivolvis* populations during the winter, and snail feeding is reduced (Russell-Hunter et al., 1984). Although the lack of *N. emydis* infections in *H. trivolvis* snails sampled from field cages at Cow Pond during the winter strongly suggests transmission of *N. emydis* is reduced during the winter months, it does not explain why prevalence and mean abundance was reduced to 0 during the winter in field collected snails from Apartment Pond.

The reduction of acanthocephalan infections in snail hosts during the winter season in Apartment Pond suggests there is strong seasonal variation that may result from multiple factors. There are several reasons why snails infected with *N. emydis* may be disappearing during the winter season from Apartment Pond. Previous studies by Boerger (1975) and Morris and Boag (1982) on the longevity of Canadian populations of *H. trivolvis* indicate that in nature *H. trivolvis* live for one to two years but can live as long as five years. Data from my study indicated that mean snail shell diameter of field collected snails from Apartment Pond was smallest during the spring, increased throughout the summer, was greatest during the fall, and decreased during the winter, strongly suggesting that the population of *H. trivolvis* at Apartment Pond turned over during the winter season. Additionally, weekly samples of snails from cages during the fall season from Cow Pond indicated that over time, a higher proportion of *N. emydis* cysts contained melanin-like pigment surrounding each worm, suggesting that infections with *N. emydis* can stimulate an immune response from *H. trivolvis* snails. Because there was a weak but positive correlation between snail shell diameter and *N. emydis* intensity and abundance, larger infected snails which mounted an immune response to *N. emydis* infections may be dying more commonly than smaller uninfected snails during the winter. However, to make things more complicated, 42% of all snails collected from Apartment Pond were infected with trematodes,

nematodes, or acanthocephalans, and 33% of *N. emydis* infected snails were also co-infected with nematodes, trematodes or both that are known to reduce the life span and ultimately kill snails (Crews and Esch, 1987; Schmidt and Fried, 1997; Klockars et al., 2007; Zimmermann et al., 2011).

My survival studies on naturally infected *H. trivolvis* snails with trematodes and/or acanthocephalans from Cow Pond and maintained in the laboratory indicated that trematode infections and snail shell diameter had consistent significant effects on snail survival. Additionally, acanthocephalan infections had significant effects when interacting with trematode infections or interacting with trematode and snail shell diameter interactions. However, acanthocephalan infections or acanthocephalan infections by snail shell diameter had no significant effect on snail survival. These results strongly suggest that under laboratory conditions, larger snails and snails infected with trematodes or co-infected with trematodes and acanthocephalans had a reduced life span, yet acanthocephalans alone did not.

Importantly, my field cage experiments at Cow Pond with laboratory-reared snails indicated that snail survival was lowest during the winter and much higher during the spring and summer. Unfortunately, during this study, it was not feasible to collect data on the survival of snails in cages that were also infected with acanthocephalans under winter conditions, and it may be that the stress of reduced feeding by snails and the cold conditions during the winter months reduce the life span of *H. trivolvis* infected with acanthocephalans. This hypothesis is particularly plausible given the fact that *H. trivolvis* appears to mount an immunological reaction to infections with *N. emydis*. Previous field and laboratory studies by Lemly and Esch (1983, 1984a, b) demonstrated a seasonal negative effect exerted by the metacercariae of the strigeid trematode, *Uvulifer ambloplitis*, on juvenile bluegill (*Lepomis macrochirus*) second intermediate

hosts which mounted an immunological response to the parasite. In that system, when bluegills become infected with *U. ambloplitis*, they mounted an immune response to the metacercariae by encapsulating the worms with a black pigment around the metacercariae. In the laboratory, Lemly and Esch (1984a, b) demonstrated that this melanization reaction was correlated with the amount of stored fat available to the bluegill hosts. These combined controlled laboratory studies and field surveys indicated that juvenile bluegills, which melanized 50 or more metacercariae, used up all of their fat reserves. Finally, these field and laboratory studies indicated that juvenile bluegills with no fat reserves due to melanized *U. ambloplitis* infections did not survive the winter due to the lack of an available food source. Taken together, my observations indicate that in addition to snails not recruiting *N. emydis* during the winter months, infected snails may also have a reduced survival during the winter season.

The role snails and ostracods play in the transmission of *N. emydis* to turtle definitive hosts still remains unclear. Ostracods can live for up to 10 months (Mezquita et al., 2002), but snails such as *H. trivolvis* may live as long as two years in nature (Boerger, 1975; Morris and Boag, 1982). By infecting a longer-lived host, acanthocephalans may have an advantage in the transmission to turtle hosts. More time spent in snail hosts would potentially allow a parasite more time to grow to larger sizes, especially in larger hosts, which may ultimately increase fitness of the parasite as an adult, such as producing more eggs and establishing at higher frequencies (Steinauer and Nickol, 2003; Benesh and Hafer, 2012; Stigge and Bolek, 2015). Additionally, if snails have more time to acquire parasites and are increasing the time for turtles to become infected, snails may be acting as reservoir hosts for acanthocephalan infections throughout the year. However, if *N. emydis* infected snails are dying due to co-infections with other parasites, or due to stressful conditions during the winter, then the role of snails serving as

reservoir hosts is more complicated and may be regulated by other snail parasite infections, abiotic conditions, or a combination of these factors. In order to successfully determine the role of ostracods and snails in the transmission of *N. emydis* to turtle hosts, and to evaluate if infected snail hosts can survive during the winter months, it is essential to domesticate the *N. emydis* life cycle in the laboratory for experimental studies. The work here shows that *N. emydis* infections in snail hosts vary seasonally; however, the biotic and abiotic factors influencing this variation still need to be assessed. Finally, field studies should aim to quantify ostracod, snail, and turtle host densities while examining these hosts for acanthocephalan infections.

Table 3.1 Collection date, average snail size, prevalence, and mean intensity of acanthocephalans in *Helisoma trivolvis* from Apartment Pond.

Collection date (month/day/year)	Average size (mm) ± 1SD	Size range	Prevalence (no. infected/no. examined)	95% CI (%)	Mean Intensity (range)
7/1/2016	16.4 ± 1.6	13–19	90 (18/20)	54 to 89	3.6 (1–16)
8/1/2016	15.0 ± 2.0	12–18	45 (9/20)	23 to 69	3.3 (1–7)
9/1/2016	14.5 ± 1.9	12–19	33 (7/21)	15 to 59	2.7 (1–6)
10/1/2016	14.2 ± 3.7	6–19	15 (3/20)	3 to 38	4.7 (1–12)
11/1/2016	14.7 ± 2.4	10–18.5	0 (0/18)	0 to 19	–
12/1/2016	–	–	–	–	–
1/1/2017	12.1 ± 2.3	9–18	0 (0/21)	0 to 16	–
2/1/2017	11.8 ± 3.4	8–18	0 (0/15)	0 to 22	–
3/1/2017	13.7 ± 1.5	12–15	33 (1/3)	1 to 91	1 (1)
4/1/2017	11.7 ± 2.1	10–14	0 (0/3)	0 to 71	–
5/1/2017	10.0 ± 2.4	8–20	40 (10/25)	21 to 61	6 (1–31)
6/1/2017	11.7 ± 1.5	9–15	15 (3/20)	3 to 38	1.3 (1–2)
Total	13.3 ± 3.1	6–20	27 (51/186)	21 to 34	3.8 (1–31)

Table 3.2 A generalized linear/nonlinear Poisson link function model for life span of field collected *Helisoma trivolvis* of various sizes infected with acanthocephalans and/or trematodes.

Effect	df	Wald Statistic	*P*
Acanthocephalan infection	1	0.31	0.578
Trematode infection	1	27.99	0.000
Shell diameter	1	17.62	0.000
Acanthocephalan * Trematode infections	1	95.05	0.000
Acanthocephalan * Shell diameter	1	1.18	0.2778
Trematode * Shell diameter	1	25.80	0.000
Acanthocephalan * Trematode infections * Shell diameter	1	103.84	0.000

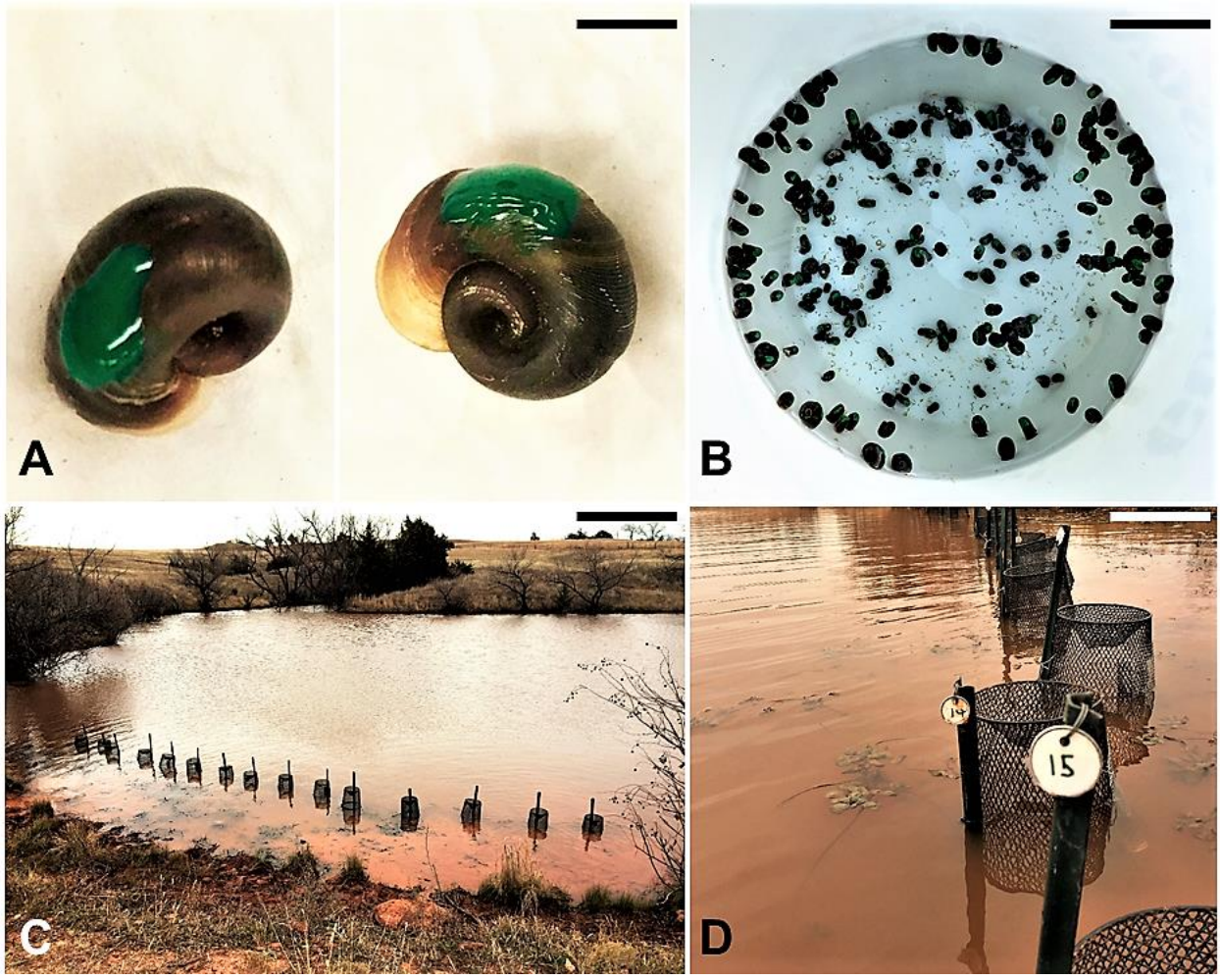

Figure 3.1 Snail marking and field exposure techniques. **A** and **B.** Example of laboratory-reared and marked snails used in field experiments. Scale bars = 4 mm and 6.5 cm, respectively. **C** and **D**. Example of cages with laboratory-reared snails placed out in Cow Pond during the winter season of 2017. Scale bars = 3 m and 26 cm, respectively.

Figure 3.2 DIC micrographs of juvenile *Neoechinorhynchus emydis* recovered from a field collected *Helisoma trivolvis* snail. **A-C.** A juvenile *N. emydis* in the process of everting and retracting its proboscis. Scale bars = 0.13 mm. **D.** An everted proboscis of *N. emydis* showing the arrangement and relative size of the three rows of hooks. Scale bar = 22 µm.

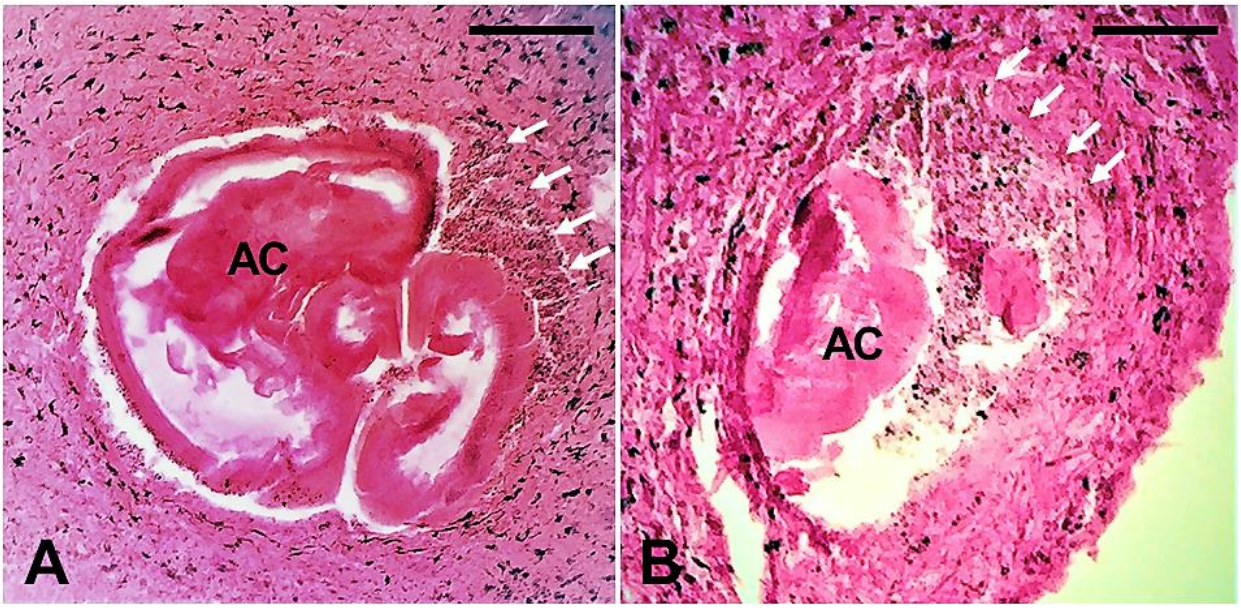

Figure 3.3 Histological sections of juvenile *Neoechinorhynchus emydis* cysts (AC) in the head-foot region of field collected *Helisoma trivolvis* snails. **A-B.** Showing the variation of cellular debris and melanin-like pigment (white arrows). Scale bars = 100 µm.

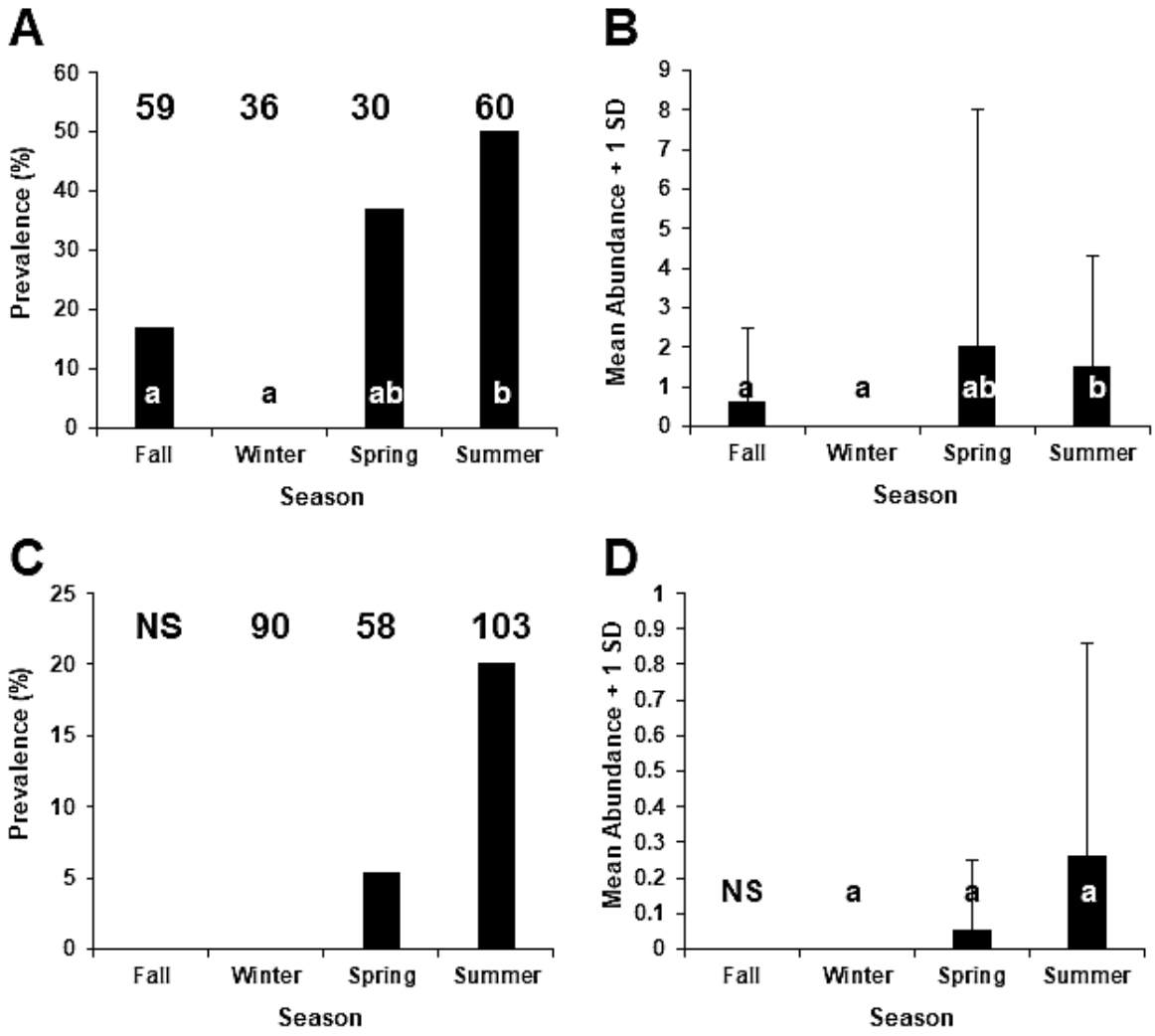

Figure 3.4 Seasonal recruitment of *Neoechinorhynchus emydis* in *Helisoma trivolvis* from northcentral Oklahoma. **A-B.** Seasonal prevalence and mean abundance of *N. emydis* from field collected *H. trivolvis* from Apartment Pond. **C-D.** Seasonal prevalence and mean abundance of *N. emydis* from laboratory-reared *H. trivolvis* snails maintained in cages at Cow Pond. Numbers indicate number of snails examined in each sampling period. Lower case letters represent significant differences among seasonal prevalence and mean abundances. NS = not sampled during the fall.

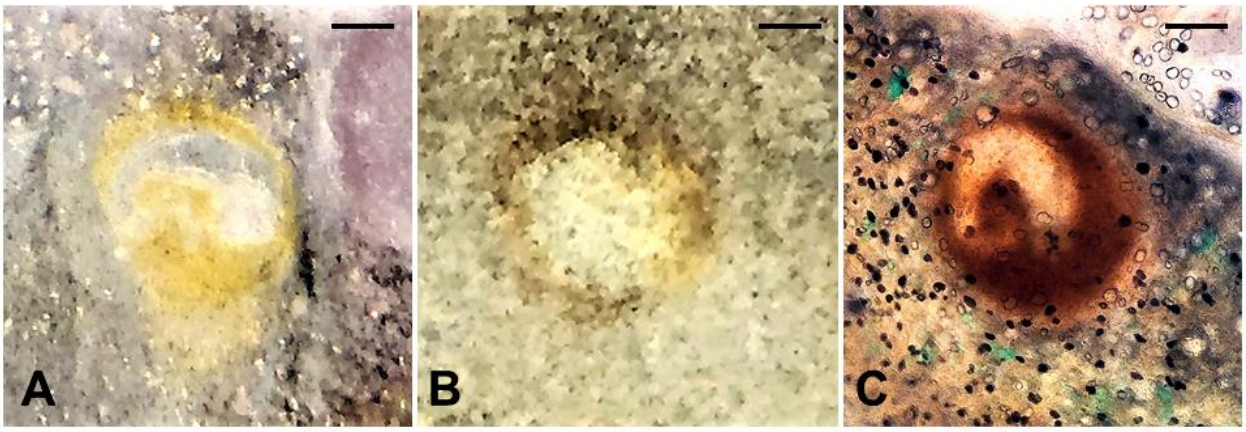

Figure 3.5 Degree of melanin-like pigment observed in *Neoechinorhynchus emydis* cysts within laboratory-reared *Helisoma trivolvis* snails maintained in cages over time at Cow Pond. **A.** *Neoechinorhynchus emydis* cysts from an infected snail maintained in a field cage for two weeks. Note the lack of melanin-like pigment. **B.** *Neoechinorhynchus emydis* cysts from an infected snail maintained in a field cage for three weeks. **C.** *Neoechinorhynchus emydis* cysts from an infected snail maintained in a field cage for five weeks. Note the high concentration of melanin-like pigment within the cyst. Scale bars = 1 mm.

CHAPTER IV

CONCLUSIONS

It is estimated that almost 100% of all plants and animal species serve as hosts for parasites, and over 50% of all plants and animal species are parasitic at some point during their life cycle (Bush et al., 2001). Despite the commonness of this relationship, few studies have addressed host-parasite interactions, the knowledge of their life cycles, and how they are transmitted among hosts in nature. One group of parasites in which this knowledge gap is more pronounced than others are the acanthocephalans. For example, among all acanthocephalan species, only about 8% of their life cycles are known (Benesh et al., 2017). One reason for this lack of knowledge may be due to the broad host spectrum acanthocephalans can infect. First, acanthocephalans are known to infect all classes of vertebrates from terrestrial, freshwater, and marine habitats (Kennedy, 2006). Second, acanthocephalans can undergo post-cyclic transmission in which an adult in a definitive host is ingested by another suitable definitive host and survives and reproduces (Nickol, 1985). Third, many acanthocephalan species use an additional vertebrate paratenic host in their life cycle to bridge the ecological gap from intermediate to definitive host (Baer, 1951). Taken together, the use of such a broad group of vertebrates in the transmission of acanthocephalans to their definitive hosts, and the variation in the ecological habitat of the definitive hosts infected with various species of acanthocephalans,

has made it difficult to identify common patterns of transmission in this group of parasites, making it difficult to solve novel life cycles of acanthocephalans.

Previous studies on paratenic hosts in acanthocephalan life cycles indicate that most are vertebrates, with few reports of invertebrates serving as paratenic hosts for acanthocephalans (Schmidt, 1985; Kennedy, 2006). In North America, all reports of invertebrate paratenic hosts of acanthocephalans include freshwater snails (Whitlock, 1939; Hopp, 1946, 1954; Lincicome and Whitt, 1947). Among these cases, the only species of acanthocephalan that has been identified is *Neoechinorhynchus emydis*, in which the life cycle has been suggested to involve an ostracod intermediate host, a snail paratenic host, and an aquatic turtle definitive host (Hopp, 1954). In his study, Hopp (1954) reported prevalence of *N. emydis* in snails and ostracods to be 87% and 0.8%, respectively. These reports suggest that snail hosts, compared to ostracod hosts, may play an important role in the life cycle, especially because snails are an important component of turtle diet (Rowe, 1992; Ernst et al., 1994; Lindeman, 2000; Herrel et al., 2002; Bouchard and Bjorndal, 2005; Pérez-Santigosa et al., 2011; Richards-Dimitrie et al., 2013; Wang et al., 2013). However, there have been no additional reports of acanthocephalan infections in freshwater snails in the last 65 years. As a result, the importance of snail hosts in the transmission of acanthocephalans to turtle hosts currently remains unclear. Thus, the objectives of this thesis were to 1) assess how common acanthocephalans infect snails by examining the spatial distribution throughout northcentral Oklahoma, 2) identify what species of acanthocephalans infect snails, and 3) determine the seasonal distribution of acanthocephalans in snail hosts.

CHAPTER II: OCCURRENCE OF JUVENILE *NEOECHINORHYNCHUS EMYDIS* (PHYLUM: ACANTHOCEPHALA) IN FRESHWATER SNAILS FROM THE GREAT PLAINS REGION OF NORTH AMERICA

In chapter II, I evaluated if two species of commonly distributed freshwater snails were infected with acanthocephalans by conducting a snail survey at wetlands and streams in Payne and Logan Counties in northcentral Oklahoma. Two of the most commonly occurring snail species, *Helisoma trivolvis* and *Physa acuta*, were sampled from 23 sites. Additionally, the complete ITS region of nuclear rDNA was sequenced from multiple juvenile acanthocephalans from snail hosts and adult acanthocephalans from turtle definitive hosts as controls. Of the 23 locations sampled, 7 (30%) were positive for juvenile acanthocephalans in snail hosts, with *H. trivolvis* being more commonly infected than *P. acuta*. The ITS sequences from juvenile acanthocephalans recovered from snail hosts had no variation in their ITS genetic distance and were identical to ITS sequences generated from adult *N. emydis* from turtle hosts. The inferred phylogenetic relationship supports the clustering of juveniles from snails and *N. emydis* from a turtle as a monophyletic group. The major contributions of this chapter include new locality records and the documentation of juvenile acanthocephalans from previously unreported freshwater snail hosts. Additionally, this study provides baseline ITS rDNA sequence data for acanthocephalans from snails and turtles, and strongly suggests that acanthocephalans of the species *N. emydis* use snail hosts as part of the life cycle.

CHAPTER III: SEASONAL OCCURANCE OF *NEOECHINORHYNCHUS EMYDIS* (PHYLUM: ACANTHOCEPHALA) IN THE FRESHWATER SNAIL, *HELISOMA TRIVOLVIS*, FROM THE GREAT PLAINS REGION OF NORTH AMERICA

The goals of chapter III were to examine the seasonality of *N. emydis* juveniles in *H. trivolvis* snails throughout the year and determine why any seasonal trends in acanthocephalans exist. I found that *N. emydis* infections were highest during the summer, decreased in the fall, and were lowest during the winter. However, because it was found that snail populations declined during the winter and snails were commonly co-infected with parasites known to reduce the life span and/or kill snails, snail cage experiments were conducted by exposing laboratory-reared *H. trivolvis* to naturally infected ostracods. Additionally, I tested the life span of wild-caught *H. trivolvis* snails of various sizes infected with acanthocephalans and/or trematodes in the laboratory. Results suggest that *N. emydis* infections in snails are lowest in the winter, most likely due to snails not consuming infected ostracods. However, fewer snails survived in field cages during the winter, suggesting that snails may die more often during the harsh winter conditions. In terms of laboratory studies on snail survival, acanthocephalans did not reduce the life span in snail, however, larger snails and snails infected with trematodes or trematodes and acanthocephalans had negative effects on snail survival. Thus, trematode infections, older snails, or the combination may also contribute to the seasonality of *N. emydis*.

Overall, this thesis provides the first comprehensive spatial and seasonal data for the occurrence of *N. emydis* in freshwater snails. Additionally, this is the first study to utilize molecular barcoding techniques to identify juvenile acanthocephalans in snail paratenic hosts. Future work should assess the role ostracods play in the life cycle of *N. emydis* and compare it to the data in this thesis.

REFERENCES

Alcántar-Escalera, F. J., M. García-Valera, E. Vázquez-Domínguez, and G. Pérez-Ponce De León. 2013. Using DNA barcoding to link cystacanths and adults of the acanthocephalan *Polymorphus brevis* in central Mexico. Molecular Ecology Resources **13**: 1116-1124.

Amin, O. M. 2002. Revision of *Neoechinorhynchus* Stiles & Hassall, 1905 (Acanthocephala: Neoechinorhynchidae) with keys to 88 species in two subgenera. Systematic Parasitology **53**: 1-18.

Baer, J. G. 1951. Ecology of animal parasites. University of Illinois Press, Urbana, Illinois, 224 p.

Barger, M. A. 2004. The *Neoechinorhynchus* of turtles: specimen base, distribution, and host use. Comparative Parasitology **71**: 118-129.

———, and B. B. Nickol. 2004. A key to the species of *Neoechinorhynchus* (Acanthocephala: Neoechinorhynchidae) from turtles. Comparative Parasitology **71**: 4-8.

Benesh, D. P., K. D. Lafferty, A. Kuris. 2017. A life cycle database for parasitic acanthocephalans, cestodes, and nematodes. Ecological Society of America **98**: 882.

Bolek, M. G., and J. Janovy Jr. 2007a. Evolutionary avenues for, and constraints on, the transmission of frog lung flukes (*Haematoloechus* spp.) in dragonfly second intermediate hosts. Journal of Parasitology **93**: 593-607.

———, and J. Janovy Jr. 2007b. Small frogs get their worms first: the role of nonodonate arthropods in the recruitment of *Haematoloechus coloradensis* and *Haematoloechus complexus* in newly metamorphosed northern leopard frogs, *Rana pipiens*, and woodhouse's toads, *Bufo woodhousii*. Journal of Parasitology **93**: 300-312.

———, and J. Janovy Jr. 2008. Alternative life cycle strategies of *Megalodiscus temperatus* in tadpoles and metamorphosed anurans. Parasite **15**: 396-401.

———, S. D. Snyder, and J. Janovy Jr. 2009. Redescription of the frog bladder fluke *Gorgoderina attenuata* from the northern leopard frog, *Rana pipiens*. Journal of Parasitology **95**: 665-668.

———, H. R. Tracy, and J. Janovy Jr. 2010. The role of damselflies (Odonata: Zygoptera) as paratenic hosts in the transmission of Halipegus eccentricus (Digenea: Hemiuridae) to anurans. Journal of Parasitology **96**: 724–735.

Boerger, H. 1975. A comparison of the life cycles, reproductive ecologies, and size–weight relationships of *Helisoma anceps*, *H. campanulatum*, and *H. trivolvis* (Gastropoda, Planorbidae). Canadian Journal of Zoology **53**: 1812-1824.

Bouchard, S. S., and K. A. Bjorndal. 2005. Ontogenetic diet shifts and digestive constraints in the omnivorous freshwater turtle *Trachemys scripta*. Physiological and Biochemical Zoology **79**: 150-158.

Brown, K. M. 1982. Resource overlap and competition in pond snails: an experimental analysis. Ecology **63**: 412-422.

Burch, J. B. 1989. North American freshwater snails. Malacological Publications, 80 p.

Bush, A. O., K. D. Lafferty, J. M. Lotz, and A. W. Shostak. 1997. Parasitology meets ecology on its own terms. Journal of Parasitology **83**: 575-583.

———, J. C. Fernández, G. W. Esch, and J. R. Seed. 2001. Parasitism: the diversity and ecology of animal parasites. Cambridge University Press, 510 p.

Cable, R. M., and Hopp, W. B. 1954. Acanthocephalan parasites of the genus *Neoechinorhynchus* in North American turtles with the descriptions of two new species. Journal of parasitology **40**: 674-680.

Chase, J. M., W. G. Wilson, and S. A. Richards. 2001. Foraging trade-offs and resource patchiness: theory and experiments with a freshwater snail community. Ecology Letters **4**: 304-312.

Combes, C. 2005. The art of being a parasite. The University of Chicago Press, Chicago.

Crews, A. E. and G. W. Esch. 1987. Histopathology of larval trematode infections in the freshwater pulmonate snail, *Helisoma anceps*. Journal of Invertebrate Pathology **49**: 76-82.

Crompton, D. W. T., and B. B. Nickol. 1985. Biology of the Acanthocephala. Cambridge University Press, Cambridge, U.K., 519 p.

Edgar, R. C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research **32**: 1792-1797.

Ernst, C., J. Lovich, and R. Barbour. 1994. Turtles of the United States and Canada. Smithsonian Institution Press, Washington, 578 p.

Esch, G. W., J. W. Gibbons, and J. E. Bourque. 1979. The distribution and abundance of enteric helminths in *Chrysemys s. scripta* from various habitats on the Savannah River plant in South Carolina. Journal of Parasitology **65**: 624-632.

Euzet, L., and C. Combes. 1980. Les problèmes de l'espèce chez les animaux parasites. Bulletin de la Société Zoologique Francaise **40:** 239–285.

Everhart, B. A. 1957. Notes on the helminths of *Pseudemys scripta elegans* (Wied, 1838) in areas of Texas and Oklahoma. Proceedings of the Oklahoma Academy of Sciences **38**: 38-43.

Freeman, G. H., and J. H. Halton. 1951. Note on an exact treatment of contingency, goodness of fit and other problems of significance. Biometrika **38**: 141-149.

Gibson, D. I., and R. A. Bray. 1994. The evolutionary expansion and host-parasite relationship of Digenea. International Journal for Parasitology **24**: 1213–1226.

Gustafson, K. D., B. J. Kensinger, M. G. Bolek, and B. Luttbeg. 2014. Distinct snail (*Physa*) morphotypes from different habitats converge in shell shape and size under common garden conditions. Evolutionary Ecology Research **16**: 77-89.

Gustafson, K. D., Belden, J. B., and Bolek, M. G. 2015. The effects of the herbicide atrazine on freshwater snails. Ecotoxicology **24**: 1183-1197.

Gustafson, K. D., Belden, J. B., and Bolek, M. G. 2016. Atrazine reduces the transmission of an amphibian trematode by altering snail and ostracod host-parasite interactions. Parasitology research **115**: 1583-1594.

Hasegawa, M., H. Kishino, and T. A. Yano. 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. Journal of Molecular Evolution **22**: 160-174.

Henry, P. Y., and P. Jarne. 2007. Marking hard-shelled gastropods: tag loss, impact on life-history traits, and perspectives in biology. Invertebrate Biology **126**: 138-153.

Herrel, A., J. C. O'Reilly, and A. M. Richmond. 2002. Evolution of bite performance in turtles. Journal of Evolutionary Biology **15**: 1083-1094.

Hopp, W. B. 1946. Notes on the life history of *Neoechinorhynchus emydis* (Leidy), an acanthocephalan parasite of turtles. Proceedings of the Indiana Academy of Science **55**: 183.

———. 1954. Studies on the morphology and life cycle of *Neoechinorhynchus emydis* (Leidy), an acanthocephalan parasite of the map turtle, *Graptemys geographica* (Le Sueur). Journal of Parasitology **40**: 284-299.

Kennedy, C. R. 2006. Ecology of the Acanthocephala. Cambridge University Press, Cambridge, U.K., 249 p.

Kimura, M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution **16**: 111-120.

Klockars, J., J. Huffman, and B. Fried. 2007. Survey of seasonal trematode infections in *Helisoma trivolvis* (Gastropoda) from lentic ecosystems in New Jersey, USA. Comparative Parasitology **74**: 75-80.

Kroonenberg, P. M. and A. Verbeek. 2018. The tale of Cochran's rule: my contingency table has so many expected values smaller than 5, what am I to do?. The American Statistician **72**: 175-183.

Kumar, S., K. Tamura, and M. Nei. 2004. MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief Bioinform **5**: 150-163.

Lafferty, K. D. 1999. The evolution of trophic transmission. Parasitology Today **15**: 111–115.

Lassiere, O. L. 1988. Host-parasite relationships between larval *Sialis lutaria* (Megaloptera) and *Neoechinorhynchus rutili* (Acanthocephala). Parasitology **97**: 331-338.

Lee, F. O., and T. C. Cheng. 1971. *Schistosoma mansoni* infection in *Biomphalaria glabrata*: alterations in heart rate and thermal tolerance in the host. Journal of Invertebrate Pathology **18**: 412-418.

Lemly, A. D., and G. W. Esch. 1983. Differential survival of metacercariae of *Uvulifer ambloplitis* (Hughes, 1927) in juvenile centrarchids. Journal of Parasitology **69**: 746-749.

———, and ———. 1984a. Population biology of the trematode *Uvulifer ambloplitis* (Hughes, 1927) in juvenile bluegill sunfish, *Lepomis macrochirus*, and largemouth bass, *Micropterus salmoides*. Journal of Parasitology **70**: 466-474.

———, and ———. 1984b. Effects of the trematode *Uvulifer ambloplitis* on juvenile bluegill sunfish, *Lepomis macrochirus*: ecological implications. Journal of Parasitology **70**: 475-492.

Liberto, R., F. Mesquita-Joanes, and I. César. 2012. Dynamics of pleustonic ostracod populations in small ponds on the Island of Martín García (Río de la Plata, Argentina). Hydrobiologia **688**: 47-61.

Lincicome, D. R., and A. Whitt. 1947. Occurrence of *Neoechinorhynchus emydis* (Acanthocephala) in snails. Transactions of the Kentucky Academy of Science **12**: 19.

Lindeman, P. V. 2000. Evolution of the relative width of the head and alveolar surfaces in map turtles (Testudines: Emydidae: *Graptemys*). Biological Journal of the Linnean Society **69**: 549-576.

———, and M. A. Barger. 2005. Acanthocephalan (*Neoechinorhynchus emydis*) Infections in Texas Map Turtles (*Graptemys versa*). The Southwestern Naturalist **50**: 12-16.

Luton, K., D. Walker, and D. Blair. 1992. Comparisons of ribosomal internal transcribed spacers from two congeneric species of flukes (Platyhelminthes: Trematoda: Digenea). Molecular and Biochemical Parasitology **56**: 323-328.

Merrit, S. V., and I. Pratt. 1964. The life history of *Neoechinorhynchus rutili* and its development in the intermediate host (Acanthocephala: Neoechinorhynchidae). Journal of Parasitology **50**: 394-400.

Mezquita, F., M. D. Boronat, and M. R. Miracle. 2002. The life history of *Cyclocypris ovum* (Ostracoda) in a permanent karstic lake. Archiv für Hydrobiologie **155**: 687-704.

Morgulis, A., G. Coulouris, Y. Raytselis, T. L. Madden, R. Agarwala, and A. A. Schäffer. 2008. Database indexing for production MegaBLAST searches. Bioinformatics **24**: 1757-1764.

Morris, J. R., and D. A. Boag. 1982. On the dispersion, population structure, and life history of a basommatophoran snail, *Helisoma trivolvis*, in central Alberta. Canadian Journal of Zoology **60**: 2931-2940.

Muzzall, P. M. 2005. Parasites of Amphibians and Reptiles from Michigan: A Review of the Literature, 1916-2003. Michigan Department of Natural Resources, Fisheries Division, 33 p.

Nickol, B. B. 1985. Epizootiology. *In* Biology of the Acanthocephala, D. W. T. Crompton and B. B. Nickol (eds.). Cambridge University Press, Cambridge, U.K., 307-346.

Olsen, O. W. 1986. Animal Parasites: Their life cycles and ecology. Dover Publications, Mineola, New York, 578 p.

Parker, G. A., J. C. Chubb, M. A. Ball, and G. N. Roberts. 2003. The evolution of complex life cycles in helminth parasites. Nature **425**: 480-484.

Pérez-Santigosa, N., M. Florencio, J. Hidalgo-Vila, and C. Díaz-Paniagua. 2011. Does the exotic invader turtle, *Trachemys scripta elegans*, compete for food with coexisting native turtles? Amphibia-Reptilia **32**: 167-175.

Poulin, R. 2007. Evolutionary Ecology of Parasites. 2nd ed. Princeton University Press, Princeton, New Jersey.

Price, P. W. 1980. Evolutionary Biology of Parasites. Princeton University Press, Princeton, New Jersey, pp. 237.

———. 1984. Communities of specialist: vacant niches in ecological and evolutionary time. In Ecological Communities; conceptual issues and the evidence, D. R. Strong, D. Simberloff, L. G. Abel and A. B. Thistel (eds). Princeton University press, Princeton, N. J. pp. 510-523.

Rauque, C. A., L. G. Semenas, and G. P. Viozzi. 2002. Post-cyclic transmission in *Acanthocephalus tumescens* (Acanthocephala: Echinorhynchidae). Folia Parasitologica **49**: 127-130.

Richards-Dimitrie, T., S. E. Gresens, S. A. Smith, and R. A. Seigel. 2013. Diet of Northern Map Turtles (*Graptemys geographica*): sexual differences and potential impacts of an altered river system. Copeia **2013**: 477-484.

Rowe, J. W. 1992. Dietary habits of the Blanding's turtle (*Emydoidea blandingi*) in northeastern Illinois. Journal of Herpetology **26**: 111-114.

Russell-Hunter, W. D., R. A. Browne, and D. W. Aldridge. 1984. Overwinter tissue degrowth in natural populations of freshwater pulmonate snails (*Helisoma trivolvis* and *Lymnaea palustris*). Ecology **65**: 223-229.

Schell, S. C. 1962. The life history of *Telorchis bonnerensis* Waitz (Trematoda: Reniferidae), a parasite of the long-toed salamander, *Ambystoma macrodactylum* Baird. Transactions of the American Microscopical Society **81**: 137-146.

Schmidt, G. D. 1985. Development and life cycles. *In* Biology of the Acanthocephala, D. W. T. Crompton and B. B. Nickol (eds.). Cambridge University Press, Cambridge, U.K., 273-306.

Schmidt, K. A., and B. Fried. 1997. Prevalence of larval trematodes in *Helisoma trivolvis* (Gastropoda) from a farm pond in Northampton County, Pennsylvania with special emphasis on *Echinostoma trivolvis* (Trematoda) cercariae. Journal of the Helminthological Society of Washington **64**: 157-159.

Schneider, C. A., W. S. Rasband, and K. W. Eliceiri. 2012. NIH Image to ImageJ: 25 years of image analysis. Nature Methods **9**: 671-675.

Smith-Trail, D. R. 1980. Behavioral interactions between parasites and hosts: Host suicide and evolution of complex life cycles. American Naturalist **116**: 77–91.

Souza, S. D. S., and Z. A. Andrade. 2006. On the origin of the *Biomphalaria glabrata* hemocytes. Memórias do Instituto Oswaldo Cruz **101**: 213-218.

Steinauer, M. L., and B. B. Nickol. 2003. Effect of cystacanth body size on adult success. Journal of Parasitology **89**: 251-254.

Stigge, H. A., and M. G. Bolek. 2015. The alteration of life history traits and increased success of *Halipegus eccentricus* through the use of a paratenic host: A comparative study. Journal of Parasitology **101**: 658-665.

Tamura, K., G. Stecher, D. Peterson, A. Filipski, and S. Kumar. 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution **30**: 2725-2729.

Thorp, J. H., and D. C. Rogers. 2014. Thorp and Covich's freshwater invertebrates: ecology and general biology. 4th ed. Elsevier Science & Technology, 1149 p.

Tinsley, R. C. 1990. Opportunism in parasite life cycles. In Parasitism and Host Behaviour, C.J. Barnard and J. M. Behnke eds. Burgess Science Press London. pp. 158-192.

Uglem, G. L., and O. R. Larson. 1969. The life history and larval development of *Neoechinorhynchus saginatus* Van Cleave and Bangham, 1949 (Acanthocephala: Neoechinorhynchidae). Journal of Parasitology **55**: 1212-1217.

Ultsch, G. R. 2006. The ecology of overwintering among turtles: where turtles overwinter and its consequences. Biological Reviews **81**: 339-367.

Wang, J., H. T. Shi, S. J. Hu, K. Ma, and C. Li. 2013. Interspecific differences in diet between introduced red-eared sliders and native turtles in China. Asian Herpetological Research **4**: 190-196.

Wayland, M. T. 2010. Proboscis profiler: A tool for detecting acanthocephalan morphotypes. Systematic Parasitology **76**: 159-167.

Whitlock, S. C. 1939. Snails as intermediate hosts of Acanthocephala. Journal of Parasitology **25**: 443.

Zelmer, D. A., and G. W. Esch. 1998. Bridging the gap: The odonate naiad as a paratenic host for *Halipegus occidualis* (Trematoda: Hemiuridae). Journal of Parasitology **84**: 94-96.

Zhang, Z., S. Schwartz, L. Wagner, and W. Miller. 2000. A greedy algorithm for aligning DNA sequences. Journal of Computational Biology 7: 203-214.

Zimmermann, M. R., K. E. Luth, and G. W. Esch. 2011. Complex interactions among a nematode parasite (*Daubaylia potomaca*), a commensalistic annelid (*Chaetogaster limnaei limnaei*), and trematode parasites in a snail host (*Helisoma anceps*). Journal of Parasitology **97**: 788-791.

VITA

Ryan William Koch

Candidate for the Degree of

Master of Science

Thesis: DISTRIBUTION AND INTERACTIONS OF TURTLE ACANTHOCEPHALANS IN TWO SPECIES OF FRESHWATER SNAILS

Major Field: Zoology

Biographical:

Education:
Completed the requirements for the Master of Science in Zoology at Oklahoma State University, Stillwater, Oklahoma in December, 2018.

Completed the requirements for the Bachelor of Science in Ecology, Evolution, and Natural Resources at Rutgers University, New Brunswick, New Jersey in 2015

Experience:
10 podium and 3 poster presentations at scientific meetings
6 semesters of teaching laboratories for 3 courses, including: Introductory Biology, General Ecology, and Biology of Fishes
Mentored 3 undergraduates, all who have presented a poster at the Karen L. Smith Undergraduate Research Symposium

Professional Memberships:
American Society of Parasitologists
Rocky Mountain Conference of Parasitologists
Southwestern Association of Parasitologists
Annual Midwestern Conference of Parasitologists
Society of Integrative and Comparative Biology