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to promote fraternal relationships among those engaged in scientific work in Oklahoma;
to diffuse among the citizens of the State a knowledge of the various departments of science;
and to investigate and make known the material, educational, and other resources of the State.

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PROCEEDINGS OF THE OKLAHOMA ACADEMY OF SCIENCE

Volume 100

CONTENTS

The *Proceedings of the Oklahoma Academy of Science* is a publication with a long and storied history. For the last century, it served as a vehicle for disseminating research findings by Oklahoma Scientists in a wide range of scientific disciplines. To celebrate publishing the 100th issue of the *Proceedings*; we invited past and current presidents of the Oklahoma Academy of Science to reflect on the history and value of the Journal. Below are their contributions.

Dr. David Bass, University of Central Oklahoma
2000-2002

The *Proceedings of the Oklahoma Academy of Science* is an important scientific resource for many researchers for over a century, especially those of us interested in the natural history of Oklahoma. This journal serves as the written historical record of the flora and fauna of the state. Like many other biologists across the state, I have chosen to submit manuscripts to *POAS* describing Oklahoma's biological communities because it is the logical journal to publish these papers. I look forward with both curiosity and excitement to receiving each annual issue and reading about recent discoveries in Oklahoma.

Dr. Craig Clifford, Northeastern State University
2002-2004, 2012-2014

On this momentous occasion of the 100th volume of the *Proceedings*, I am honored to have this opportunity to add my congratulatory note. The *Proceedings of the Oklahoma Academy of Science* has long served as the vehicle by which scientific information of special significance to Oklahoma could find its way into print. The exceptional work of the many editors over the years has allowed new and established researchers to provide their findings to the widest audience possible while supporting the official publication of the Academy. My introduction to the *Proceedings* as a newly hired faculty member at Northeastern State University in Tahlequah revealed the work of dedicated fellow scientists in many fields in my new home state. The colleagues I have met and the activities I have participated in in a variety of venues provided by the Academy speak well of an institution that has served the state greatly over the many years since its inception. Congratulations to the *Proceedings* and its staff.

Dr. Sharon Young, Southern Nazarene University
2004-2006

To the former and present editors of the *Proceedings of the Oklahoma Academy of Science*: Thank you for your service! It is hard to imagine a job with more work and less glory. During officers' meetings, I heard the formal and informal reports from these servant volunteers. The editors who served with me were Kurtis Koll and Clark Ovrebo; and I remember Glenn Todd, Frank Leach and George Moore.

There was much more to the job than scientific and literary skills. One of the more odious tasks was cajoling authors, and reviewers to submit materials and revisions on time. During the earlier years there was no email, FAX or phone calls without "long distance" charges. My Friends, You did the skilled hard work and made The Academy proud. I hope that you find satisfaction in knowing that your grandchildren can research your names in the *Proceedings of the Oklahoma Academy of Science* and know that each of you were leading scientific professionals in the State of Oklahoma.

Dr. Kenneth R. Hobson, University of Oklahoma
2010-2012

A place for empirical science and the contribution of the *Proceedings of the Oklahoma Academy of Science*.

Great progress in science can come when we combine empirical experience with theoretical insight. Observations in biology, natural history, physics, chemistry, and all of science, inspire new questions and breakthroughs to new theory. The *Proceedings of the Oklahoma Academy of Science* is a place where the observations and data of empirical science can be published. Empirical science alone, can become lost in repetitive data collection without guiding direction. Theory alone, can miss or over-simplify important complexity and depart from reality. A healthy oscillation of empirical science to theory and a return, can provide fundamental advances, synthesis and broad understanding of general phenomena.

As a biologist, I appreciate the updates on range distributions, diversity records, taxonomic revisions and empirical science I find in the *POAS*. I browse through early editions online and discover lost bits of field biology that suggest new investigations. I find clever techniques, and inspiring ideas from decades ago.

POAS has a role in nurturing careers of new scientists. It serves as a place where scientists begin their careers, publish their work and establish a reputation in their field of study. The existence of *POAS* has encouraged communication and alerted potential colleagues to the arrival of new voices.

POAS has played a role in bringing scientists in Oklahoma and the region together, helping us to know our colleagues better. I enjoy looking back at early publications of senior scientists I've known, seeing how their thinking and science developed, the important new ideas, research, locations and collaborations that shaped their careers.

As we arrive at the publication of the one hundredth volume of the *POAS* we can be grateful for the role the Proceedings has played in supporting our science and our scientists.

Dr. Adam Ryburn, Oklahoma City University
2018-2020

Like many of my colleagues around the state, *POAS* and Oklahoma Academy of Science have had a profound impact on the development and success of my academic career. Of greater note, however, is the impact on the development of countless students that have benefited from being published in the *Proceedings*. From high school, to undergraduate, to graduate students, *POAS* has provided an opportunity for young scientists to experience the culmination of scientific investigation in the form of a scientific publication. Here's to the next 100 years of disseminating scientific knowledge.

Dr. Robert D. Mather, University of Central Oklahoma
2020-2022

The Current OAS President's Reflections on *POAS*

This is the centennial issue of the *Proceedings of the Oklahoma Academy of Science*! As we celebrate a century of the journal of record for the State of Oklahoma's scientists, I would like to share some of my experiences with OAS and *POAS*.

The Oklahoma Academy of Science has always been part of my life. As a child in the early 1980's, I attended Field Meetings with my father, Dr. Charles "Mike" Mather. It was fun to look for fossils and look at the stars with experts. It was also fun to watch scientists present their work to each other in a room with a screen, projector, and photographic slides that rotated when the scientist clicked a button. I will never forget the roar of laughter as the keynote speaker perfectly timed the rapid peek at the slide of naked scientists triumphantly circling the ceremonial South Pole at the South Pole Station. He said something like "This is a tradition among scientists who make it to the South Pole that we don't need to talk about." It may seem like a small moment, but it taught me how an intellectual giant can own a room of colleagues with quick wit and strategic irreverence. Of course, the best part of the Field Meetings was getting to spend time with my dad and learning so much!

After completing my masters degree, I wrote a manuscript on experimental psychology research that I thought would be a good introduction to the topic for the Academy. I submitted the manuscript to POAS in 2001. It was rejected with critical feedback from reviewers. However, the Editor took the time to soften the blow of the feedback and point out where the reviewers were wrong and I was right. It was a great lesson in writing to reviewers outside of my specialty and the Editor was a true teacher-scholar in his approach to mentoring a young scientist.

In 2006, I had a wonderful opportunity to return to my home state and give back to the scholarly communities that had trained me. My dad was still on faculty at USAO, and I thought it would be great to revive the Social Science Section, hang out at OAS meetings with my dad, and just let the Mather boys intellectually play together on the science playground. While sitting in the library at the University of Texas at Dallas (where I was teaching), I saw the archived *Texas Journal of Science*, which is published by the Texas Academy of Science. I immediately realized the lasting impact of the official journals of the state academies of science. I pitched the idea to write a Letter to the Editor in *POAS* that would be an emphatic statement to psychology faculty and students in Oklahoma about the need to participate in OAS. My dad went for it, Editor Clark Ovrebo went for it, my dad and I co-authored it, submitted it, and I had my first *POAS* publication accepted before I even crossed the Red River. I left copies of it in new faculty mailboxes for many years. It is one of my favorite articles, because it was the first one that I published with my dad (Mather & Mather, 2006). We subsequently published an experiment together that tested the persuasiveness of the OAS statement on science, religion and teaching evolution (Mather & Mather, 2009). Later my students and I published a small, interesting finding on emotion perception and eye tracking (Mather, Ray, McReynolds, & Jones, 2015).

POAS is important as an archive of our local science. I encourage readers to look at the online archives of the journal. You will be transported back in time to the era of your choice during the past century. You will read field notes from a new frontier as scientists explored, cataloged, and ultimately documented their discoveries. You can read about the problems on the minds of scientists in the decade after the 1929 stock market crash (Eaton, 1939), agricultural issues after the Dust Bowl (Coyner, 1939; Weese, 1939), and even early student health studies (Neill & Perkinson, 1930).

But there is no better sales pitch to convince you to read the old *POAS* articles than this intriguing line from the last article of the first issue of *POAS*. "A little black mongrel hen…hatched her brood and mothered them a few days when she turned cannibal, killing and eating her whole flock of chickens within a few hours" (Reiter, 1921). They did an autopsy and excused her for her crime, though being excused after an autopsy is not the ideal way to be vindicated as an accused criminal. The intent of the chicken did not factor in to the forensic behavioral explanation. Before your conspiratorial minds wander to other suspects such as coyotes, I will save you the trouble: The chicken did it. I believe it is fair to say that the chicken version of the M'Naghten rule was invoked in the offending hen's defense. There was no word on what became of the chicken carcass, but I have my suspicions. Go online and look up the article for the full report of the galluscidal event from a century ago.

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Performance of Early Juvenile Giant River Prawns (*Macrobrachium rosenbergii*) Fed Fish, Soybean, Shrimp and Four Insect Based Diets While Under Low Temperature Stress

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Abstract: Current guidelines for *Macrobrachium rosenbergii* advise introduction into outdoor ponds when water is > 16º C. However, little is known about possible interactions between diet quality and temperature tolerance. We examined use of seven rations for prawns raised at suboptimal temperatures. Juvenile prawns readily consumed all seven rations presented. Growth and survivorship were best on shrimp- and silkworm-based rations; whereas, the poorest growth was on fishmeal- and Black Soldier Fly larvae meal (BSFLM)-based rations. Soybean-, cricket-, mealworm-, and fishmeal-based diets had similar survivorship. Mortality occurred within 24 hr after each feeding of the BSFLM-based diet, possibly related to antimicrobial compounds produced by BSFL. Prawns raised at lower temperatures may require better quality feed compared to guidelines based on previous studies at ideal temperatures. Producers must determine whether it is better to stock prawns at higher rates or feed more expensive rations to manage survivorship of juvenile at lower temperatures.

Introduction

Ectotherms face continuous challenges imparted by interactions between the kinetics of their physiology and the quality of their diets. The rates at which stress response, pathogen defense, digestion, and growth ensue are directly tied to ambient temperature. This is a problem for outdoor production of the Giant River Prawn (*Macrobrachium rosenbergii*) in temperate regions. This is a tropical species of significant commercial importance that is native to rivers of the East Asian continent and surrounding Islands (New 1990; New 2002; New 2005). Temperatures of 26 – 31ºC (Sabdifer and Smith 1985) or 25 - 32ºC (Zimmermann 1998) are considered ideal for growth (New 1990; Satapornvanit 2006). The critical thermal minima for this species when acclimated to temperatures of 20 - 30ºC are 10 – 16.24ºC for postlarvae and 10.5 – 16.98ºC for juveniles (Herrera et al. 1998; Manush et al. 2004). Food consumption is significantly reduced when temperatures fall below 25ºC (Newman et al. 1982). Prawns start to manifest a physiologically compromised state with acute or slow changes in temperature below 15ºC and

are severely compromised with acute drop from 20ºC to 10ºC (Chung et al. 2012). Prawns raised for seven days under exposure to *Lactococcus garvieae* at 20ºC demonstrate low phagocytic activity, tolerance to ammonia and salinity (Cheng et al. 2003) and impeded immunological responses due to a sudden drop in temperature from their thermal ideal (28ºC) to 22ºC (Chang et al. 2015). Mortality is substantially elevated as temperatures fall below 12ºC (Sataporvanit 2006). Urea-N excretion is minimal at 17ºC and rises with temperature (Chen and Kou 1996). Growth nearly doubles for every 5ºC increase in temperature from 20 to 36ºC, without an appreciable change in conversion ratio (Faranfarmaian and Moore 1978). However, growth is more erratic at lower temperatures (Whangchai et al. 2007). Guidelines suggest cultivation temperatures should be above 16ºC (Herrera et al. 1998; New 2002). However, observations in research settings generally demonstrate higher survivorship than actually observed in commercial settings (Tidwell et al. 2005).

Diets resulting in higher survival and growth also tend to impart better responses to stressors (Racotta et al. 2003). Research with prawns has related temperature stress effects on neuroendocrine responses (Chang et al. 2015), oxygen consumption (Farmanfarmaian and Moore 1978; Chen and Kou 1996; Niu et al. 2003; Manush et al. 2004), nitrogen excretion (Chen and Kou 1996), pH and ion balance (Cheng et al. 2003), food consumption (Niu et al. 2003), growth (Farmanfarmaian and Moore 1978; Niu et al. 2003), and survivorship (Herrera et al. 1998; Chung et al. 2012; Manush et al. 2004). Additional nutritional studies with the *M. rosenbergii* are numerous; however, little work has approached the currently growing interest in more sustainable fish meal replacements such as insect meal.

Oklahoma has never had a prawn industry in the state despite apparent suitability of its climate and unique aquaculture infrastructure characteristics (McCallum and Tilahun [In Press]). Guidelines for earliest dates to stock ponds in Oklahoma are lacking. We set up a laboratory experiment comparing performance of juvenile freshwater prawns fed seven different diets comprising corn meal and selected protein feedstuffs. The study was conducted at temperatures comparable to those observed in surface waters in Oklahoma in late April through early May to help formulate guidelines for outdoor pond culture of prawns for Oklahoma producers and to test potential interactions between temperature, stress and diet. We hypothesized that prawns would grow slower at cooler temperatures and have lower survivorship than typically expected at higher temperatures, and that prawns may perform better on arthropod- and crustacean-based protein sources than on soybean or fishmeal diets. We predicted that growth and survivorship would be higher when fed arthropod- and crustacean-based protein sources than on fishmeal or soybean meal.

Materials and Methods

Prawns were obtained from Stickfin's Fish Farm (St. Augustine, Florida) and housed communally for one week after arrival in aerated 40-L aquaria containing moderately hard synthetic freshwater made with reagent grade chemicals (U.S. E.P.A. 2002). Following that week, 105 prawns were individually housed in 125 ml Pyrex Erlenmeyer flasks containing 100 ml of synthetic freshwater. An inverted Pyrex 25 ml flask was inserted into the neck of each larger flask to prevent contamination from airborne particulate. Each prawn was placed in a weigh dish and excess water removed using a transfer pipette and then weighed on an electronic analytical balance to the nearest 0.001 g. Next, each prawn was then placed in its flask where it resided for the duration of the study (28 days).

Water quality was monitored weekly and water was changed if it became cloudy or otherwise fouled. The pH (7.4 – 7.8), hardness (160 – 180 $CaCO_3$/L), and alkalinity (57 – 64 $CaCO_3$/L) never exceeded the typical range for moderately hard synthetic freshwater (U.S. E.P.A. 2002). Temperature was maintained at 21ºC throughout the study.

We obtained commercial fish meal and soybean meal from a local feed store (Stillwater Milling Company, Stillwater, Oklahoma). Dried silkworm pupae (*Bombyx mori*), mealworm larvae (*Tenbrio molitor*), "river" shrimp from Chubby Mealworms (Las Vegas, Nevada) and dried crickets (*Acheta domesticus*) came from Fluker Farms (Port Allen, Louisiana). Dried black soldier fly larvae (*Hermetia illucens*) came from Enviroflight, LLC (Yellow Springs, Ohio). Feedstuffs were ground and mixed with a household coffee grinder. Rations were balanced on a 35% protein basis (Table 1; New 2002; although even higher protein levels are effective [Millikin et al. 1980]) using bakery grade yellow corn meal (Quaker Oats Company, Chicago, Illinois). Prawns (n_{total} = 105 prawns, n = 15 prawns/treatment) were fed one of each ration (n = 7 treatments) *ad libitum* throughout the study, although the quantity varied according to bodyweight. Guidelines suggest prawns should be fed 10-20% of the total bodyweight (New 2002), but this is based on bulk weights, not individual measures. Because of this, it was impractical to feed such small quantities at the onset of the study. Each prawn received 15% of its last measured bodyweight. If food remained after 24 hr, we would reduce the quantity by 10% as fed on the next feeding. If food was consumed after 24 hr, we increased the feeding rate by 10% as fed, and added the additional quantity to the flask. Food was delivered by sprinkling the dried meal on the water surface.

If food settled on the bottom began to mold, we siphoned it off with a transfer pipette for disposal.

Daily observations of molting and mortality were recorded. At the end of the study, all remaining prawns were weighed. Growth data were tested for normality using an Anderson-Darling test. We compared the weight gain between treatments using a one-way ANOVA with a Tukey means comparison test. Survivorship and molting frequency was compared between groups using Chi Square. We used an alpha = 0.05 to assess significance.

Results

In all cases, prawns readily consumed food when presented and could be seen feeding on remaining food particles until the next feeding. None of the prawns consumed 100% of the food offered after 24 hrs, with obvious remnants of food typically remaining for days. Prawns did not perform equally well on all rations. Growth ($F_{6, 104} = 4.69$, $P < 0.001$) varied among treatments (Table 2, 3). Growth data were not normally distributed ($A^2 = 16.56$, $P < 0.001$) so we transformed these data using the normalize function in MiniTab 13.0. Prawns gained weight better on shrimp (-0.060, -1.708) and silkworm pupae (-0.040, -1.688) than on fishmeal. They also gained better on shrimp (-0.303, -1.9506) and silkworm pupae (-0.283, -1.931) than on black soldier fly larvae. No other significant differences in growth were observed.

There were marginal differences in survivorship ($\chi^2 = 10.81$, df = 6, P = 0.094) among treatments. Prawns survived equally well when fed fish meal, soybean meal, ground mealworms and ground crickets ($\chi^2 = 4.42$, df

Table 1. Nutritional breakdown on an as fed basis of the eight ingredients used to formulate diets for juvenile freshwater prawns (*Macrobrachium roosenbergii*).

	Fish meal	Soybean meal	Shrimp meal	Cricket meal	Black Soldier Fly meal	Silkworm pupae meal	Mealworm meal	Corn meal
DM %	90.12	91.26	92.40	93.0	95.7	94.0	94.8	88.0
Protein %	70	40	50	64	37	45	53.6	7.4
Fat %	14.82	18.48	13.13	19.0*	14.2*	29%	29.2	1.8
Gross energy MJ/KG DM	21.9	19.7	19.1	21.8*	23.8	25.8**	24.4**	13.8
Crude Fiber %	2.22%	7.10%	1.2%	8.5	7.0%	6%	18.5	7.4%

*Finke 2002

**http://feedipedia.org

Table 2. Performance of juvenile Giant Freshwater Prawns (*Macrobrachium rosenbergii*) on seven diets at the lower threshold of thermal tolerance.

Protein source	Starting BM (g) Mean (SE)	Ending BM* (g) Mean (SE)	Growth* (g) Mean (SE)	Molts (n)	Survivorship (%)
Fish meal	0.020 (0.002)	0.022 (0.002)	0.002 (0.001)	4	6.7
Soybean meal	0.020 (0.002)	0.028 (0.004)	0.008 (0.004)	4	33
Shrimp	0.025 (0.002)	0.039 (0.004)	0.015 (0.003)	8	86.7
Silkworm	0.018 (0.001)	0.033 (0.006)	0.016 (0.006)	10	66
Mealworm	0.030 (0.002)	0.035 (0.003)	0.014 (0.009)	6	40
Cricket	0.023 (0.002)	0.025 (0.003)	0.003 (0.001)	13	40
Black Soldier Fly	0.026 (0.002)	0.027 (0.002)	0.0003 (0.0003)	3	6.7
Overall	0.023 (0.001)	0.030 (0.001)	0.008 (0.002)	48	42

*Ending body mass (BM) and growth are the averages for those that survived to the end of the study; whereas, starting BM includes the entire starting population.

Table 3. ANOVA table for the response of growth to different foods.

Source	DF	SS	MS	F	P
Food type	6	15.778	2.630	4.69	< 0.001
Error	98	54.997	0.561		
Total	104	70.775			

= 4, P = 0.352). They performed just as well when fed ground shrimp or silkworm pupae (χ^2 = 0.196, df = 1, P = 0.158). Prawns had higher survivorship when fed shrimp meal or silkworm pupae meal than when fed black soldier fly or fish meal (χ^2 =10.84, df = 3, P = 0.013). There were no observed differences in molting frequency (χ^2 = 5.64, df = 6, P = 0.464) among treatments.

Within 24 hours of first feeding black soldier fly larvae to prawns, 27% died; whereas, the other feeds had 93 – 100% survivorship after 24-hr (Total 24-hr survivorship on other feeds = 97.8%). Mortality rose to 40% by the fifth day when fed the Black Soldier Fly larvae meal-based ration. After seven days, remaining food particles (mostly corn meal) were removed from all flasks, and new feed introduced. Then, 24-hr after being again fed the Black Soldier Fly larvae meal-based ration, another 47% of prawns died, with a total mortality of 93.3% in this treatment. The single remaining prawn survived until the end of the study, but its growth was negligible.

Discussion

Temperature and nutritional stress manifest in physiological trade-offs for ectotherms, and our results provide evidence that when raised at suboptimal temperatures freshwater prawns appear to become more sensitive to food quality compared to previous studies performed at ideal temperatures. Wild prawns feed on aquatic invertebrates, detritus and algae (Balaz and Ross 1976). Previous studies on nutrition of prawns were conducted within the thermal optima for this species and largely determine fishmeal and soybean meal to be adequate protein feeds (Hasanuzzaman et al. 2009; Gupta et al. 2007; Koshio et al. 1992) although reports as low as 11% survivorship on fishmeal are known (Kumlu 1999). Addition of shrimp oil to a balanced diet doubled the final biomass of prawns (Sandifer and Joseph 1976). Further, post-larvae fed entirely on *Artemia* nauplii perform well (Barros and Valenti 2003; New 2002). In general, prawns performed acceptably on proven protein sources (shrimp meal, soybean meal) but growth and survivorship on less suitable feeds (fishmeal, Black Soldier Fly larvae meal) was less impressive. Arginine, one of three key amino acids thought to be important to prawns (e.g., Methionine, Lysine [D'Ambramo and Sheen 1994]) is generally more abundant in soybean and shrimp meals than fish meal (Watts 1968); however, amino acid requirements have been difficult to elucidate (Reed and D'Abramo 1989).

Differences in survivorship and growth may be explained by changes in feeding behavior, more efficient digestion, and more effective stress responses when temperatures are warm compared to suboptimal. The time prawns spend

feeding is known to decline with temperature (Niu et al. 2003). Consequently, when at optimal temperatures, prawns may increase consumption sufficiently to overcome the minor inadequacies of amino acid composition. However, when housed at sub-optimal temperatures, feeding behavior is suppressed and they cannot fully accommodate for reduced limiting nutrient supply, leading to poorer performance. Further work is needed to elucidate amino acid needs of prawns (Mukhopadhyay et al. 2003; D'Ambramo and Sheen 1994) so that informed supplementation with imperfect feeds is possible.

Physiological processes in all ectotherms are tied to the ambient temperature (Wilmer et al. 2004; Manush et al. 2004; Manush et al. 2006), including digestion in prawns (Kumlu 1995; Newman et al. 1982). Each of these ingredients require different residency times and enzyme assemblages to digest and absorb, and each enzyme operates in an ideal temperature range. It is likely that digestive enzyme activity in prawns was suppressed by lower temperatures, leading to inefficient digestion of less perfect feeds. Further, as a kinetically controlled biochemical process, production of non-essential amino acids is altered at lower temperatures. Combined with feeding behavior, digestion and biochemical processes could explain why prawns fed fish, soybean and shrimp meal perform well at optimal temperatures; whereas, survival and growth is much reduced at cooler temperatures.

Unlike other ingredients in this study, freeze dried black soldier fly larvae appear unsuitable for feeding prawns, at least at suboptimal temperatures. Significant mortality occurred within 24 hours of feeding fly meal to the prawns. Black soldier flies are known to harbor compounds with antimicrobial properties (i.e., defensin-like peptide4, a 40 amino acid AMP; Elhag et al. 2017; Park et al. 2015) sufficiently powerful to suppress growth of *Escherichia coli*, *Salmonella* spp., antibiotic resistant *Staphylococcus aureus*, and gram positive *Pseudomonas aeruginosa* (Lalander et al. 2015; Liu et al. 2008). Their excretions are also known to inhibit growth in the larvae of other dipterids (Bradley and Sheppard 1984). This insect has been fed successfully to poultry (Cullere et al. 2016; Elwert et al. 2010; Dluokun 2000), livestock (Veldkamp and Bosch 2015; Veldkamp et al. 2012), dogs and cats (Bosch et al. 2014), and fish (Shakil Rana et al. 2015; Tran et al. 2015; Sealey et al. 2011; St-Hilaire et al. 2007).

Previous studies of black soldier fly larvae meal for decapod diets exist, though not for *M. roosenbergii*. Growth of juvenile white shrimp (*Litopenaeus vannamei*) was increasingly suppressed as black soldier fly larvae meal became a higher proportion of the diet (Cummins et al. 2017). Weight gain, final body weight, feed conversion ratio, and specific growth rate of shrimp became less acceptable as the dietary component of black soldier fly larvae meal increased. Survivorship of these shrimp was much higher than in our study (~91% vs. 6.7%). Other than the species involved, the primary differences in these two studies is that ours involved much younger/smaller animals (initial prawn BW = 0.023 g +/- 0.001 vs. initial shrimp BW = 1.24 +/- 0.01 g), diets with a much larger component of black soldier fly meal, smaller housing (100 ml vs. 110 L), individualized housing (vs. communal), and we maintained a lower temperature (21ºC vs 29.5ºC). These differences provide several avenues for the vastly different survivorship levels. The younger prawns may have less developed functional immune/stress response systems. Stress and immune responses are known to undergo ontogenetic changes as an organism grows (Manning and Turner 1976). The larger soldier fly component in the feed should deliver a larger dose of antimicrobial chemicals. The larger housing and resultant larger water volume used with white shrimp may be sufficient to dilute feed-borne compounds in the water column to non-toxic levels. The differences in temperature may also be a significant factor. Prawns were already under thermal and potentially nutritional stress. These two stressors are known to participate in a trade-off system that provides added risk to additional stressors (Padmanabha et al. 2011; Cotter et al. 2011; Karasov et al. 2007). Considering that

as an ectotherm, all physiological processes are bound to thermal optima (Wilmer et al. 2004), the stress response of prawns may be compromised by low temperature making them more susceptible to chemical stressors like those produced by soldier fly larvae. A confounding element to these results is that we did not provide perches for molting behavior. This may have provided across-the-board higher mortality (McCallum et al. 2018).

Black soldier fly has also been proposed for use in human diets (Wang and Shelomi 2017; Dossey and Morales-Ramos 2016; van Huis et al. 2015; Boland et al. 2013). The recent findings above suggest that incorporation of black soldier fly meal into the human food chain should be done with great consideration. The compounds black soldier fly larvae produce could lead to new strains of microbes afflicting humans and animals that are highly resistant to antibiotics. Further, there has been no testing to determine if these compounds have long-term health effects for the organisms ingesting them, or if these compounds could be transferred from food animals to humans. Until such studies are undertaken, it seems prudent to avoid using this protein source for humans or animals intended for human consumption. Other insects in this study do not appear to hold such risks. Thus far, we have found little evidence that the scientific community has considered the risks associated with antimicrobial compounds in black soldier flies. It has been more focused on heavy metal accumulation in the larvae, microbial decontamination prior to processing, and food allergies (Rouge and Barre 2017; Wang and Shelomi 2017).

Fish, shrimp, and soybean meals are among the most commonly used protein sources in animal feeds, including prawns (Hasanuzzaman et al. 2009; Koshio et al. 1992). Fish and shrimp meal continue to be sourced from wild fisheries and are increasingly expensive (Carter and Hauler 2000), thus discounting the sustainability of aquaculture operations (Love et al. 2014; Gatlin et al. 2007). Further, they can harbor contaminants from the wild environment (Costa 2007; Dorea 2006; Hardy 2002; Galindo-Reyes et al. 1999). Using soybean meal eliminates dependence on wild stocks for protein and reduces the contaminant problem, but brings an array of other environmental issues connected to crop farming such as pesticide use (van Meter et al. 2018; Mitsch et al. 2001; Pimentel et al. 1993). Soybean meal also contains phytoestrogens (Coward et al. 1993), which may adversely impact invertebrate reproductive potential, growth and development (Jefferson et al. 2005; Ryokkynen and Kukkonen 2006; McCallum et al. 2013). Hence, there are good reasons to desire alternatives to these two products, especially in aquaculture and aquaponics, the latter of which prides itself on being sustainable (Forchino et al. 2017; Konig et al. 2016; Goddek et al. 2015). If the freshwater prawn industry is positioned as a sustainable alternative to wild-caught shrimp, it can carve a larger place in the market and likely draw higher prices. Abandoning less-sustainable feed ingredients is an important step to reaching this goal (Sánchez-Muros et al. 2014). Although in its infancy, carefully selected and prepared insect meals may serve this purpose (Rumpold and Schlüter 2013).

The performance of prawns at temperatures similar to those in April – May in Oklahoma suggest that if producers intend to stock ponds that early, they will need to feed very high quality feeds or stock ponds at higher levels than typically recommended to ensure a harvestable product. Alternatively, producers could cover small ponds with greenhouse structures to extend the season (Pillai et al. 1999), restrict production to indoor recirculating or aquaponics facilities, or wait until later in the year when water temperatures are more suitable to stock ponds with juvenile prawns.

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Examination of the Current Oklahoma State Record Smallmouth Buffalo

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Abstract: Smallmouth Buffalo (*Ictiobus bubalus*) is found predominately in eastern Oklahoma and recognized as a non-game species by the Oklahoma Department of Wildlife Conservation. The designation of non-game allows the species to be recreationally caught or harvested using any legal method and without limits. We examined the current state record (restricted division: allowing recreational harvest with no restriction) Smallmouth Buffalo caught using rod and reel on May 3, 2019 from Broken Bow Reservoir. The lapilli otoliths were removed from this specimen to estimate age, back calculate length-at-age (growth rates), and back calculate spawning year. The final estimated age of this fish was 62 years old. The growth curve using back-calculated length-at-age suggested this Smallmouth Buffalo initially grew rapidly (80% of total length within first 17 years), but the growth increments constricted with increasing age. Based on the estimated age of this fish, the state record Smallmouth Buffalo was spawned in 1957, indicating this fish was spawned prior to impoundment of Broken Bow Reservoir. Further, the hatch year of this fish corresponds with flooding, after a prolonged drought, demonstrating the importance of river flow to successful spawning of this species. Although this study is limited to a single specimen, it improves our knowledge of a long-lived, understudied species in Oklahoma.

Introduction

Smallmouth Buffalo (*Ictiobus bubalus*) is a widely distributed, non-game species, once commercially harvested in Oklahoma waters. Smallmouth Buffalo is the most common of the 3 *Ictiobus* spp. in Oklahoma reservoirs and rivers (Miller and Robison 2004). This species is widely distributed throughout the Mississippi River basin from the Gulf of Mexico north to Ohio and west to the Dakotas (Miller and Robison 2004). Within Oklahoma, Smallmouth Buffalo are predominately found in the eastern part of the state (Miller and Robison 2004). *Ictiobus* spp. are commercially important in North America (Love et al. 2019) and were commercially harvested in Oklahoma from the 1930's to the mid 1980's (Houser 1957, ODWC unpublished data). Although records are limited, *Ictiobus* spp. comprised 44% (131,926 kg) of total annual commercial fish harvest from reservoirs between 1957 – 1969 (Elkin Jr. 1958, Jones 1961, Mensinger 1971). Currently, Smallmouth Buffalo are not commercially fished and are managed as a non-game species, which allows this species to be recreationally pursued via any legal methods with no regulated harvest (ODWC 2019).

Our current understanding of Smallmouth

Buffalo is limited to information obtained on smaller specimens through standard fish surveys. The Oklahoma Department of Wildlife Conservation (ODWC) currently has no management objectives for this species, but when these fish are encountered during standardized surveys (typically during fall gillnetting), they are enumerated, weighed and measured. However, the size of Smallmouth Buffalo sampled with the standard experimental gillnet is dictated by the mesh size (largest mesh size = 64 mm), so these samples are heavily biased to the capture of smaller Smallmouth Buffalo (mean TL = 428 mm, ranging 159 – 699 mm TL; ODWC unpublished data). This constricted size structure provides no insight into the growth potential or maximum size of Smallmouth Buffalo in these systems, which can be quite large (> 800 mm TL; Edwards and Twomey 1982, Miller and Robison 2004, Love et al. 2019). Regardless of sampling gear, encounters with large Smallmouth Buffalo are rare, but large fish are occasionally reported via angler reports when they believe they caught a state record. Collection and examination of these large, angler-caught specimens is a means to gain information on an otherwise understudied fish species.

On May 3, 2019, a new state record (1,015 mm TL, 30.1 kg) Smallmouth Buffalo was caught from Broken Bow Reservoir, Oklahoma. The fish exceeded the weight of the previous Smallmouth Buffalo record by 9.98 kg. Due to the anomalous size of this fish and the potential for individuals in this genus to reach very old ages (Bigmouth Buffalo *Ictiobus cyprinellus*, 112 years old; Lackmann et al. 2019), ODWC requested to obtain this fish for examination. Our objective was to examine the new state record Smallmouth Buffalo to estimate age, evaluate growth rate through back calculated length-at-age, and estimate hatch year (i.e., back calculated) to better understand environmental conditions that contributed to production of this fish. We realize this assessment is limited to a single individual but felt the opportunity to examine a large specimen would benefit future evaluations.

Methods

On 3 May 2019, ODWC southeast region fisheries staff weighed (using a large-capacity certified scale, Salter Brecknell Scale model 3255, Avery Weigh-Tronix, LLC, Fairmont, MN) and measured (TL and girth; mm) the potential new state record Smallmouth Buffalo. Meristic counts of lateral line scales, dorsal fin rays, anal rays, pectoral rays, pelvic rays, and gill rakers were taken to ensure species identification (Pflieger 1997, Miller and Robison 2004). Following inspection, this fish was verified as the new state record Smallmouth Buffalo.

Once certified, the fish was brought to the Oklahoma Fishery Research Laboratory (OFRL) in Norman, Oklahoma where the fish was dissected to remove the lapilli otoliths. Lapilli otoliths were cleaned of organic material and placed into an envelope to dry for a period > 24 hrs. After drying, lapilli otoliths were browned at 104°C on a hot plate to increase contrast between accretion and discontinuous zones (Secor et al.1992, Long and Snow 2016). After browning, otoliths were processed following methods described by Love et al. (2019), where otoliths were ground in a plane transverse to the nucleus using a rotary tool fixed with a grinding bit (#85422, Dremel, Racine WI). The rotary tool was held in a vice, and forceps coated in plastic tool dip (Plasti Dip International, Blaine MN) were used to securely hold the posterior portion of the otolith during the grinding process. Following grinding, otoliths were polished using wet 2000 grit wet/dry sandpaper.

To estimate age, the otolith was stood polished-side up in a dish containing modeling clay (aid in viewing), immersed in water (to reduce glare), and viewed with a variable-power stereomicroscope (capable of 130× magnification; aids in interpreting compressed annuli) using a fiber-optic filament (enables the reader to manipulate light angle to enhance annuli) attached to an external light source. Annuli, which appeared as dark rings on a light background, were counted to assign an age estimate. Otoliths were evaluated by two independent readers and if estimates were not

the same a concert reading was conducted to finalize an age estimate (Hoff et al. 1997). Once age estimates were finalized, the Dahl-Lea method was used to back-calculate length-at-ages to describe growth (mm; Quist et al 2012) and the final age estimate was subtracted from the capture year to estimate hatch year.

Results and Discussion

The new state record Smallmouth Buffalo was a female that measured 1,015 mm TL, had a girth of 974 mm, and weighed 30.31kg. The two independent readers estimated the age of this fish as 60 and 62 yrs old, respectively. The consensus age of this fish was 62 yrs old (Figure 1). This is the greatest longevity reported for Smallmouth Buffalo, although longevity information for this species is limited. However, Love et al. (2019) reported longevity of 39 yrs (majority of fish were 12 to 24 yrs old) for Smallmouth Buffalo from the middle Mississippi River. The oldest

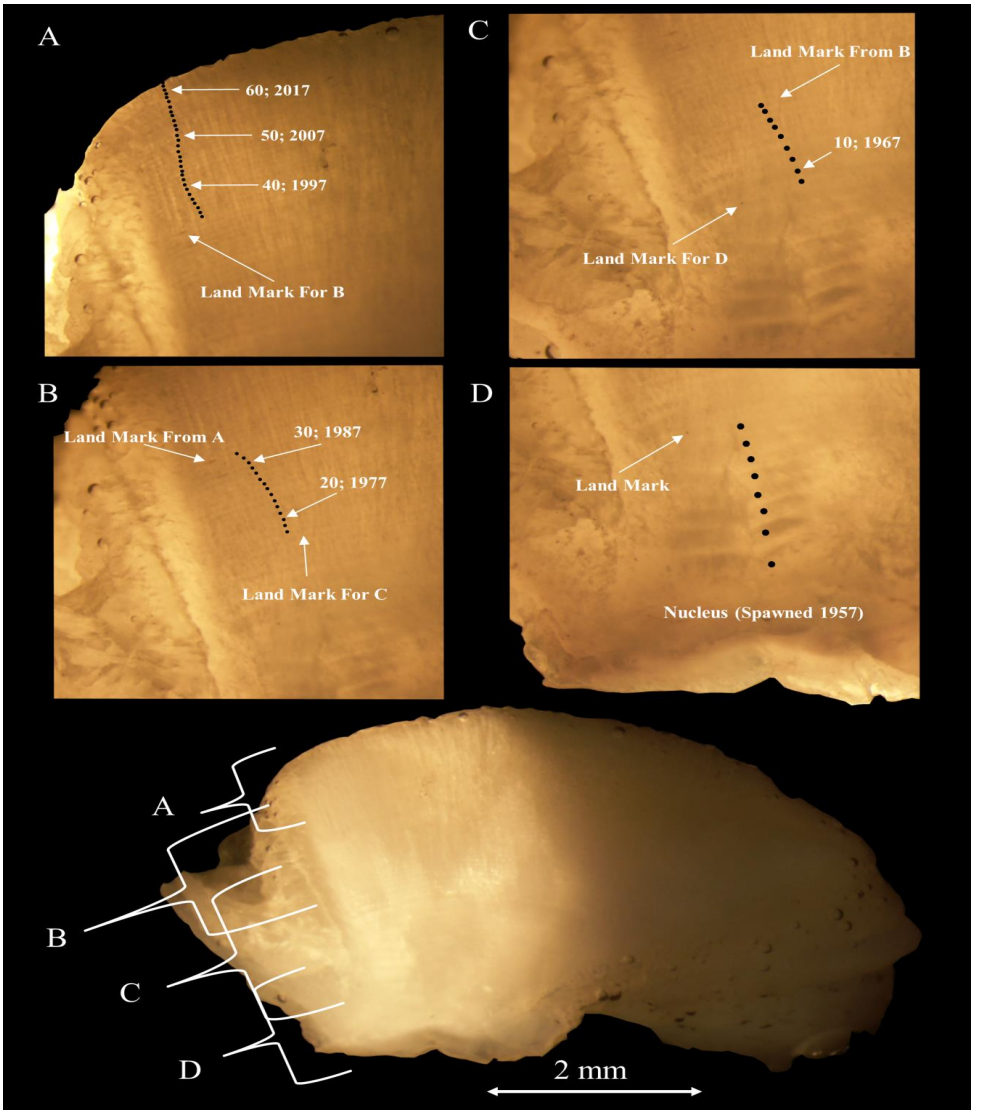

Figure 1. Photograph of a sectioned lapilli otolith from the current state record Smallmouth Buffalo (age 62) caught on 3 May 2019. ● = indicate annuli that reflect the age and year (age; year) on photographs A through D.

age reported for Smallmouth Buffalo using scale-derived age estimates was 18 yrs old (Martian et al. 1964, Jestor 1973). However, scale-derived ages are shown to underestimate the ages of catostomid species (Quist et al 2007, Muir et al. 2008, Grabowski et al. 2012). We cannot assess accuracy of this age estimate because no age validation studies using Smallmouth Buffalo otoliths have been conducted. However, the high precision between readers (within two years) for our specimen and validation of otoliths for other catostomid species (Paukert and Long 1999, Spurgeon et al. 2015), suggests this age estimate is reasonable.

Our evaluation is one of the few to present growth information for Smallmouth Buffalo. Using back-calculated length-at-age, we found that the state record Smallmouth Buffalo initially grew rapidly (attained 50% of TL in 6 years and 80% of TL by age 17), slowing with increasing age over the remainder of life (Figure 2). Our results are similar to Love et al. (2019) that found Smallmouth Buffalo growth to be rapid through the first 4 years of life (growing to 50% of their maximum TL). However, after age 10 (when they had achieved 71% of TL) Smallmouth Buffalo growth slowed drastically with increasing age (Love et al 2019).Similar growth patterns were observed with Blue Suckers (*Cycleptus elongates*) from both the Kiamichi and Red River in Oklahoma (Brewer and Dyer 2018) and three catostomid species from the Apalachicola River, Florida (Grabowski et al. 2012).

We were able to back-calculate the hatch year of this fish using the age estimate from annuli counts, which revealed that the state record Smallmouth Buffalo was spawned in 1957 (Figure 1). In that year, heavy precipitation and severe flooding impacted the region (Arkansas, Kansas, Missouri, Oklahoma, and Texas) during April through June (Kutschenreuter 1958). This flooding event ended the extended drought that began in 1952 (Kutschenreuter 1958, Nace

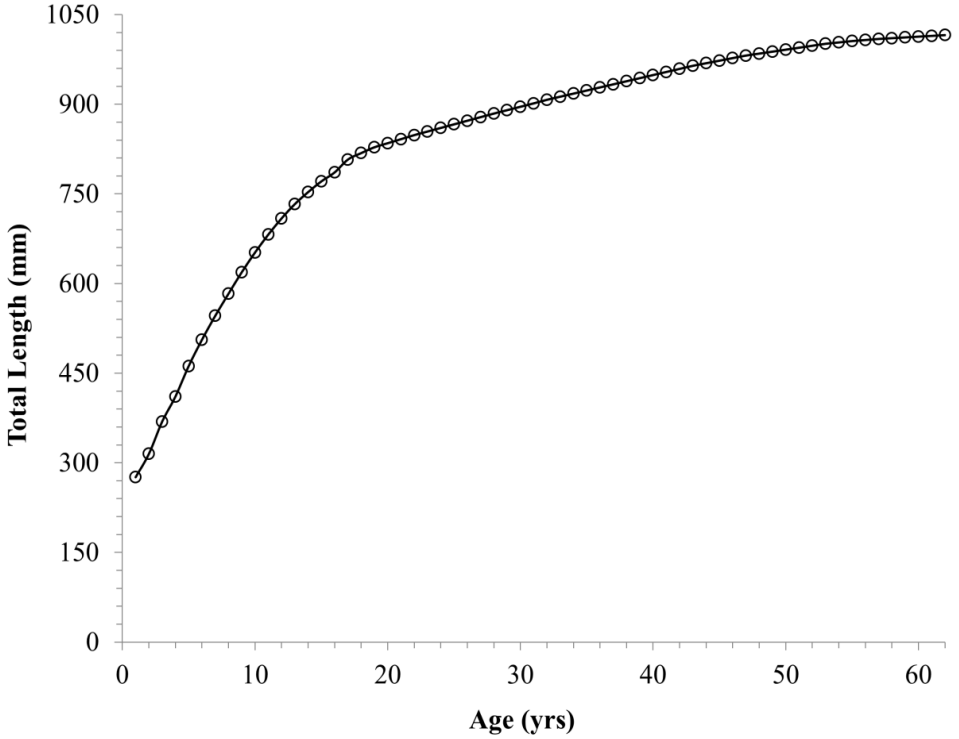

Figure 2. Back-calculated total length-at-age (mm) for the state record Smallmouth Buffalo captured in Oklahoma.

and Pluhowski 1965). In general, herbaceous terrestrial vegetation would have populated the river margins during extended drought conditions, which create spawning habitat and nursery areas for Smallmouth Buffalo when water levels rise following heavy rainfall (Etnier and Starnes 1993). It appears Smallmouth Buffalo were able to take advantage of the timing and magnitude of this flood event due to the spawning period for this species (April to early June; Becker 1983, Miller and Robison 2004). Catostomids, including *Ictiobus* spp., are cued to migrate up rivers and streams to spawn by inflow events where they spawn on inundated vegetation in the flood plain or in sloughs having shallow, vegetated shorelines (Martin et al. 1964, Hoyt and Flynn 1979, Becker 1983, Adams and Parsons 1998). It appears this extended flooding event (> 60 days) created conditions that resulted in successful spawning and recruitment of Smallmouth Buffalo to produce this state record fish 62 years ago.

Although this study is limited to a single fish, it provides considerably higher longevity estimates than previously described for Smallmouth Buffalo, and describes rarely-documented growth of this species. Additionally, large Smallmouth Buffalo are not encountered during typical fisheries assessments, so evaluating angler-caught specimens (even when it is a single fish) is important for understanding aspects of the life history of this species. An interesting finding from this evaluation was that this fish was spawned prior to impoundment of Broken Bow Reservoir. Therefore, this fish resided in Mountain Fork River, Oklahoma or migrated up stream prior to the completion of the Broken Bow Reservoir dam in 1968. This evaluation builds on our understanding of Smallmouth Buffalo biology and natural history, which is important for the conservation and management of this species.

Acknowledgments

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Common Carp Population Size and Characteristics in Lake Carl Etling, Oklahoma

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Abstract: Common Carp (*Cyprinus carpio*) is a highly invasive species and tolerates a diversity of habitats with a broad range of water-quality characteristics. Following extended drought conditions from 2005 - 2012, a noticeable increase in Common Carp densities was observed at Lake Carl Etling. Higher Common Carp numbers suggested other management techniques would be needed to control the population, which required knowledge of Common Carp population abundance. Therefore, our objective was to estimate the population size using a Schnabel estimator, body condition, and size structure of the carp population. We sampled the entire perimeter of the shoreline once monthly using boat electrofishing from May through November 2017. During the first sampling event in May, all Common Carp captured were measured (total length [TL], mm), weighed (g), given a hole punch mark through the left operculum, and released. In subsequent samples (June-November), each captured fish was examined for a hole punch mark on the left operculum, and if present was recorded as a recapture and released. However, if no mark was observed, the fish was measured, weighed, marked, and released. The mark recapture population estimate was constrained to Common Carp >200 mm because small fish (< 200 mm) were not fully recruited to the electrofishing gear. During the six month mark-recapture period, 2,848 Common Carp ranging 111 to 620 mm TL were collected. We marked and released 2,752 (≥ 200 mm TL) of the 2,848 fish captured. We recaptured 207 marked fish, resulting in a population estimate of 13,783 Common Carp (95% CI = 11,262-16,648). Common Carp density was estimated at 214 fish/ha^{-1} (95% CI = 181-267 fish/ha^{-1}) and biomass was 148 kg/ha^{-1} (95% CI = 125-184 kg/ha^{-1}). Most (93%) Common Carp in Lake Carl Etling were < quality size, but a small proportion exceeded preferred size. Condition of Common Carp in this population was below average (mean Wr = 90). Our results suggest the density of Common Carp in Lake Carl Etling is high and may be regulating size structure and condition of these fish. Further, based on our results fisheries managers can remove enough Common Carp from this system to improve water quality conditions and sport fish populations.

Introduction

Common Carp (*Cyprinus carpio*) is an invasive fish in North America that has negative consequences on invaded waters. Common Carp are native to Asia and Europe but have become widely distributed across the globe as a result of intentional stockings for aquaculture and recreational angling (Penne and Pierce 2008, Weber and Brown 2011, Weber et al. 2011, Carl et al. 2016). Their wide tolerance to temperature, salinity, and dissolved oxygen concentrations

allows them to survive a range of habitats, which makes them highly invasive (Bajer and Sorensen 2010). Once established, Common Carp populations can become extremely dense if not controlled through predation or by management biologists (Drenner et al 1997). As population densities of Common Carp exceed 100 – 250 kg/ha^{-1} in aquatic systems, negative effects on water clarity, aquatic macrophytes, macroinvertebrates, and native fish fauna occur as a result of their benthic feeding behavior, and nutrient release from this disturbance can cause excessive algal blooms (Koehn 2004, Weber et al. 2011, Carl et al. 2016).

Management biologists have attempted to control Common Carp populations but often success is difficult to evaluate due to lack of baseline abundance estimates. For example, commercial fisheries are commonly used to control Common Carp biomass but lack a formal stock assessment prior to removal (Colvin et al. 2012). Additionally, control techniques used by management biologists include water level manipulation, piscicide application, and removal efforts by agency personnel or commercial fishing (Weier and Starr 1950, Neess et al. 1957, Verrill and Berry 1995, Fritz 1987, Stuart et al. 2006), however these efforts have varying successes. To accurately assess the effects of a Common Carp removal effort and to implement effective management strategies, biologists require abundance estimates of Common Carp for removal efforts to have meaningful management targets. However, a management strategy to remove a specific percentage of the Common Carp population relies on an accurate population estimate.

Management of high-density Common Carp scenarios requires knowledge of baseline population characteristics, which is limited in Oklahoma. Although historical records suggest Common Carp are absent from Oklahoma's panhandle (Miller and Robison 2004), Lake Carl Etling (located in the furthest northwest corner of the Oklahoma panhandle) supports a robust population of Common Carp. The density of Common Carp in Lake Carl Etling is high enough to contribute to increased turbidity levels that follow annual spawning events in May (i.e., spawning activity increases suspended solids) that typically last through fall. The increased turbidity levels negatively impacted the foraging ability of stocked Tiger Muskellunge (*Esox masquinongy* × *E. Lucius*) and resulted in reduced survival and failure of the stocking program (Snow et al. 2017; Snow et al. 2018). This failed attempt to biologically control Common Carp numbers resulted in the need to evaluate the population size of Common Carp in Lake Carl Etling and consider other management techniques. Because the abundance of Common Carp is unknown and this information is important for management of this species, our study objective was to estimate the population size of Common Carp in Lake Carl Etling using mark-recapture. Further, because high fish abundances can result in deteriorated body condition and size structure, we described relative weight and proportional size distribution for Common Carp in Lake Carl Etling.

Methods

Study Area

Lake Carl Etling is a 64.3 ha impoundment located in northwest Oklahoma and is surrounded by the diverse Mesa de Maya/Black Mesa ecoregion (Snow et al. 2017). The reservoir was formed by impounding South Carrizo Creek, a tributary of the Cimarron River, in 1958. Lake Carl Etling is shallow (mean depth of 1 meter and maximum depth of 5.5 meters) and hypereutrophic. The shoreline, particularly in the upper one-third of the lake, is sparsely vegetated with submerged and emergent macrophytes. The Mesa de Maya ecoregion typically receives 42.4 cm annually (Woods et al. 2005; Kenton, Oklahoma, Mesonet station #52 for annual rainfall). Minimal precipitation in the watershed makes Lake Carl Etling prone to drought. During periods of drought, herbaceous and woody vegetation colonizes the shoreline and results in abundant woody habitat around the perimeter when the lake returns to normal pool conditions. The mean monthly secchi depth at Lake Carl Etling was 23.4 cm from October 2015 through May 2017 (Figure 1; Snow et al. 2017). Further, increased turbidity levels coinciding

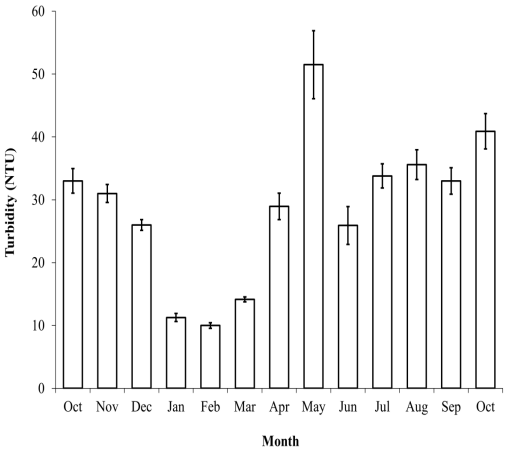

Figure 1. Turbidity measurements taken monthly during October 2015 - October 2016 from Lake Carl Etling, Oklahoma. Mean turbidity (NTU) measurements were taken using a turbidity tube (Myre and Shaw 2006). Error bars represent standard error of the mean.

with large numbers of Common Carp spawning was observed in 2015 and 2016, suggesting that Common Carp numbers were high enough to have detrimental effects on water quality. Lake Carl Etling experiences a wide range of water temperatures (1.7-33.4 ºC) during a typical year.

Sampling

Common Carp were sampled, using boat electrofishing (pulsed DC, high voltage, 7.5 GPP, Smith Root, Vancouver, Washington). The entire perimeter of the shoreline was sampled monthly from May through November 2017. All Common Carp captured were measured for TL (mm), weighed (g), administered a left operculum hole-punch mark, and released. On each following trip, any fish observed to have an operculum hole-punch mark were considered a recapture. If no mark was observed the fish was measured, weighed, and marked as directed above. All fish were processed, held in a live well, and released into an area of the lake that had already been sampled to avoid recapturing these fish and biasing the population estimate. We used mark-recapture to estimate population size and biomass of Common Carp in Lake Carl Etling. After capture Common Carp were marked by hole punching the left operculum of each fish using a 6.4 mm circular paper hole punch tool (Figure 2; Snow et al. 2020). This marking technique was shown to have 100% retention through 184 days (Snow et al. 2020).

Analysis

Common Carp population abundance was estimated using a Schnabel estimator (Seber 1982) with 95% confidence intervals based on a Poisson distribution (Krebs 1999). Mark-recapture sampling events occurred monthly (May to October 2017; n = 6 efforts). Based on the length distribution of Common Carp in Lake Carl Etling, it appears fish ≤ 200 mm were not fully recruited to the sampling gear, therefore fish of this size were not included in the population estimate. Although the primary assumption of a Schnabel estimator is a closed population (i.e., one with no immigration, emigration, recruitment, or mortality), it is robust to some departure from this assumption (Ricker 1975). We met the closure assumption over our study period (6 months) as there were no major rain events resulting in enough inflow or outflow from Lake Carl Etling to allow Common Carp immigration or emigration. It is possible that mortality and recruitment events could have occurred within the study period, however, we conducted the mark-recapture efforts over a short duration (6 months) to minimize the effects of these factors on estimated population

Figure 2. Photo illustrating a Common Carp with two distinct opercular hole punch scars, which was the marking technique used to estimate the population size of the Common Carp In Lake Carl Etling, Oklahoma.

size. Once population size was estimated for Common Carp, we estimated fish density (fish/ha^{-1}) and biomass (kg/ha^{-1}). Biomass by weight was calculated by applying the mean weight from the 2,848 Common Carp captured to the population estimate.

We used a variety of metrics to describe the size structure and condition of the carp population. A length-frequency histogram and proportional size distribution (PSD) of quality (410 mm, PSDq) and preferred (530 mm, PSDp; Anderson and Gutreuter 1983) sized fish were used to visualize and quantify Common Carp size structure. A weight to TL simple regression was used to describe the weight:length relationship of the population. Common Carp condition was evaluated by calculating relative weight (W_r) using the standard weight equation (W_s = -4.639 + 2.920 × log10 TL) presented by Anderson and Gutreuter (1983).

Results

We collected 2,848 Common Carp (ranging 111 to 620 mm TL; Figure 3) during our 6-month mark-recapture evaluation. Of the 2,848 Common Carp collected, 2,752 (individuals ≥ 200 mm TL) were marked and released to estimate population size. We recaptured 7% (207 of 2,752) of our marked fish in subsequent sampling efforts and marked 20% of the population, which produced a population estimate of 13,783 Common Carp (95% CI = 11,609 -17,161; Table 1). Common Carp density was estimated at 214 fish/ha^{-1} (95% CI = 181-267 fish/ha^{-1}) with an estimated biomass of 148 kg/ha^{-1} (95% CI = 125-184 kg/ha^{-1}).

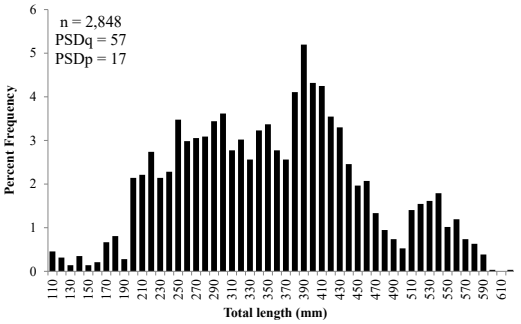

Figure 3. Length frequency distribution and proportional size distribution (PSD) of Common Carp collected from Lake Carl Etling, Oklahoma.

Most Common Carp in Lake Carl Etling were of stock and quality size (PSDq = 57), but a small proportion exceeded preferred size (PSDp = 17). The mean weight of Common Carp in Lake Carl Etling population was 692 g (range = 11- 2,965 g; Figure 4). The length-weight relationship of Common Carp was highly correlated (r^2 = 98; Figure 4). Common Carp from Lake Carl Etling were classified as below average body condition (mean W_r = 90), and condition was similar across length categories (stock = 91, quality = 89, and preferred = 90).

Discussion

This study provides a population estimate for Common Carp in a small Oklahoma impoundment based on mark-recapture methods. Our mark-recapture effort was confined to six months and larger Common Carp (≥ 200 mm TL) because of gear recruitment and the hole punch marks become less discernible for smaller fish after 7 months (Snow et al. 2020). Therefore, our density estimate of 214 fish/ha^{-1} is likely conservative given the exclusion of carp < 200 mm. This may explain why our estimate was lower than the median density estimates (15 to 569 fish/ha^{-1}) reported in the literature for small impoundments (4.7 to 159.2 ha; Bajer et al. 2009, Bajer and Sorensen 2010, Bajer et al. 2011, Bajer et al. 2012). Further, these population estimates are from impoundments in the northern United States, overall effects of climate and habitat could be driving differences.

Our biomass estimate (148 kg/ha^{-}1) for Common Carp in Lake Carl Etling was likely quite conservative but helps us identify a management target for removal. Our estimate is considerably lower than values reported in the literature, which can exceed 300 kg/ha^{-1} (Keohn 2004, Weber and Brown 2011, Bajar and Sorensen 2015). Negative impacts to aquatic systems have been observed when Common Carp reach a critical biomass of 198 kg/ha^{-1} (Vilizzi et al. 2015).Bajer et al. (2016) determined a Common Carp biomass of ~200

Table 1. Monthly mark-recapture information for Common Carp (≥200 mm total length) captured in Lake Carl Etling, Oklahoma from May through October 2017. Population estimates were calculated using the Schnabel method. The 95% confidence intervals (CIs) were produced using a Poisson distribution.

Month	Captured Monthly	Cumulative Marked	Cumulative Recaptured	Population Estimate	Lower 95% CIs	Upper 95% CIs
May	883	832	-	-	-	-
June	469	1,278	51	7,136	5,709	10,028
July	525	1,749	93	10,096	4,403	15,677
August	301	1,976	131	10,037	7,350	16,206
September	263	2,204	158	10,978	8,816	14,793
October	407	2,752	207	13,783	11,609	17,161

kg/ha^{-1} caused a 90% reduction in vegetation in Midwestern, US lakes. When Common Carp biomass exceeds 100 kg/ha^{-1}, negative impacts to aquatic systems can occur (Bajer et al. 2009). Therefore it seems maintaining a biomass < 100 kg/ha^{-1} is an appropriate management goal. Based on our estimates, we would need to remove 4,400 Common Carp from Lake Carl Etling to reduce the biomass below 100 kg/ha^{-1}. We captured 2,848 Common Carp using 7 sampling trips, so with a few days of additional effort, the remaining 1,552 Common Carp could have been captured to achieve a management goal of maintaining < 100 kg/ha^{-1} in Lake Carl Etling. A dedicated removal effort could be even more efficient if we identified and targeted areas where Common Carp congregate in the reservoir.

Common Carp aggregate during periods associated with overwintering and spawning, and these aggregations often occur at the same locations over consecutive years (Penne and Pierce 2008). Bajar et al. (2011) found Common Carp aggregations during winter in Midwestern lakes and showed that targeting these aggregations for removal could reduce 68% of a Common Carp population. We observed a Common Carp aggregation in the South Carrizo Creek confluence of Lake Carl Etling during winter over two consecutive years (2016-2018; Figure 5). Knowledge of this large, seasonal aggregation of Common Carp in Lake Carl Etling makes the goal to maintain abundance < 100 kg/ha^{-1} feasible, because these fish can easily be exploited for removal purposes.

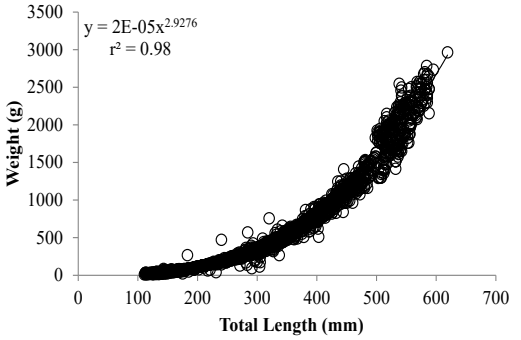

Figure 4. Length-weight relationship of Common Carp collected from Lake Carl Etling, Oklahoma.

Although our data are from one small impoundment in Oklahoma, this study provides important baseline data on abundance, body condition, and size structure of Common Carp. We do not have data from other Common Carp populations in Oklahoma for comparison, so it is unknown if the Lake Carl Etling Common Carp population is representative. Based on our results, the Lake Carl Etling population appears to be relatively high density and may be regulating its size structure (high abundance fish >200 mm TL) and body condition (below average W_r) of these fish. Common Carp are dense may improve stocking success or resident sportfish populations. Common Carp population assessments should be expanded to other Oklahoma small impoundments, as this information is lacking statewide and is critically important for managing sportfish populations. Our results suggest that it may be feasible for fisheries managers to remove enough Common

Figure 5. This photograph illustrates a Common Carp aggregation observed while electrofishing in the South Carrizo Creek confluence of Lake Carl Etling during the winters of 2016-2018.

Carp from this system to improve water quality conditions, based on results from previous evaluations.

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Length-Weight Relationships and Potential Biases for Alligator Gar (*Atractosteus spatula*) from Texoma Reservoir, Oklahoma

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Abstract: Alligator Gar (*Atractosteus* spatula) is the largest fish species found in Oklahoma, which makes obtaining field measurements (particularly weight) by fisheries managers or recreational anglers challenging. A method to overcome the logistical hurdles associated with handling and measuring large fish in the field would be beneficial to managers and anglers. Therefore, the objective of this study is to develop length-weight and length-length relationships for Alligator Gar collected from Texoma Reservoir, Oklahoma using total length (TL), standard length (SL), and girth (G) measurements to predict weights and length measurements of Alligator Gar. A total of 339 Alligator Gars averaging 1,666 mm TL (range = 590 to 2,357 mm TL) and weighing 38.2 kg (range = 0.77 to 105.3 kg) were used in this evaluation. No significant differences in weight of Alligator Gar were detected between sex or season (winter-spring and summer-fall), so all fish were pooled for the remaining analyses. All simple linear regression and multiple regression models produced significant relationships ($P \leq 0.05$), were highly correlated ($r^2 = 0.88$ to 0.99), and mean predicted error values were < 13% for all models. However, the simple linear regression model using TL and the multiple regression model using TL and G were the best predictive models to estimate weight in this evaluation. All weight and length estimates predicted with regression analysis were not statistically different than actual Alligator Gar weights and lengths. Visual inspection of weight bias plots suggests little bias between predicted and actual weights for Alligator Gar ≤ 60 kg, but became more variable for fish > 60 kg. However, the simple linear regression model using TL and the multiple regression model using TL and G produced less biased weight estimates for fish up to 80 kg, but appeared to underestimate weights of Alligator Gar ≥ 100 kg. All of the models evaluated in this study provide managers and anglers with a relatively accurate estimate of Alligator Gar weights from Texoma Reservoir and provides fisheries managers with weight-length information to which other Alligator Gar populations can be compared.

Introduction

Alligator Gar (*Atractosteus spatula*) has a limited distribution in Oklahoma due to anthropogenic influences such as dams, dredging, habitat change, unlimited recreational harvest and commercial fishing (Brinkman 2008, Inebnit 2009, Snow et al 2018). The Red River basin remains the stronghold for Alligator Gar in Oklahoma, although anglers occasionally report

fish caught from the Arkansas River (Oklahoma Department of Wildlife Conservation [ODWC] unpublished data). Because Alligator Gar have a limited distribution in Oklahoma, ODWC manages them as a species of greatest conservation need and regulates recreational harvest with a one fish daily bag limit (which must be reported to ODWC). Numerous angling methods are permitted to catch Alligator Gar, however bowfishing, rod and reel, and snagging are the most common (ODWC 2019). Alligator Gar anglers rarely have the means to weigh fish due to their large size, even though fish weight is often used as a measure of fishing success by trophy anglers (Meerbeek and Crane 2017). However, anglers often record total length or girth measurements to describe the size of the fish that they catch. Anglers and fisheries managers would benefit by having a method to estimate the weight of an Alligator Gar using the measurements commonly collected in the field.

Length-weight relationships provide fisheries managers with important basic biology information about fish populations, including fish condition, growth rates, morphological differences, and reproductive potential (Santos et al. 2012, Torres et al. 2012, Meerbeek and Crane 2017, Maurya et al. 2018). Further, the mathematical relationship between various fish length measurements and weight allows either measurement to be predicted simply by having the other (Sarkar et al. 2009, Nazir and Khan 2017). Development of length-weight equations for Alligator Gar would provide fisheries managers and anglers with a means to overcome physical and logistical issues associated with weighing large fish in the field, which reduces handling stress or unnecessary harvest just to obtain a weight. Therefore, the objective of this study is to develop predictive equations to estimate Alligator Gar weights using total length, standard length, and girth measurements. Additionally, bias was evaluated between weights predicted from length-weight relationships and actual Alligator Gar weights.

Methods

Alligator Gar were sampled in Texoma Reservoir using experimental gillnets (net dimensions described by Binion et al. 2015 and Schlechte et al. 2016). Nets were set in coves or on main-lake flats in depths ranging 2 - 9 m. Site selection was aided by the use of visual observation of Alligator Gar surfacing (aerial breathing) and side scan sonar. In deeper water or when fish were observed with side scan sonar near the lake bottom, weights (9.1 kg keg- style anchors) were attached to the lead line in the middle of the net to ensure the net maintained contact with the lake bottom. Nets were monitored every 15 to 30 min to ensure quick release of Alligator Gar and reduce mortalities. Besides gillnetting, several angling methods (jug fishing, rod and reel, and snagging) were used to collect Alligator Gar and harvested fish were donated by recreational anglers.

Upon capture, each Alligator Gar was measured using a fabric measuring tape for snout length (snout tip to anterior start of eye orbit), anal fin base length, girth (G; taken anterior of the pelvic fins), standard length (SL; snout tip to insertion of epichordal lobe of caudal fin) and total length (TL). These measurements were used to identify sex of each gar using methods outlined by McDonald et al. (2013). Due to their large size, individual Alligator Gar were placed into a fish sock (25.4 mm #60 green-twine mesh x 3.05 m long x 457.2 mm diameter), which were then hooked to a hanging scale (Intercomp CS200; Intercomp CO., Medina, Minnesota) attached to a winch (Badland 2500 ATV/Utility Winch; Badland Winches, Camarillo, California) and lifted to attain the weight (W; kg) of each fish (Figure 1).

To meet the assumptions of normality, length and weight data were log transformed prior to regression analyses. Differences in weight between seasons (winter/spring versus summer/fall) and sex of Alligator Gar were tested using ANCOVA. Simple linear regression models were used to describe the relationships between girth, length, and weight (TL:W, SL:W, G:W, TL:SL, TL:G, and SL:G) of Alligator Gar. Multiple regression analysis was used to determine the relationships between girth, length, and weight (TL/G:W and SL/G:W) for

Figure 1. Photographs depicting the winching apparatus used to weigh Alligator Gar and the fish sock (25.4 mm #60 green-twine mesh x 3.05 m long x 457.2 mm diameter) used to support Alligator Gar.

Alligator Gar. Differences between individual weights and lengths predicted from the simple linear and multiple regression equations were tested against actual fish weights and lengths using paired t-tests. Strength of each model was evaluated by comparing percent error [(Observed - Predicted)/Predicted*100] by averaging the percent predicted error across all observations for each model (Wood 2005, Scharf et al. 1998, Snow et al. 2017, Jeter et al. 2019). Additionally, bias plot were constructed to compare bias between actual and predicted girths, lengths, and weights of Alligator Gar. A bias plot was not constructed for TL:SL, as this relationship was highly correlated (r^2 = 0.99). All statistical analyses were conducted at a significance level of $P \leq 0.05$.

Results

A total of 339 Alligator Gar averaging 1,666 mm TL (range = 590 to 2,357 mm TL) and weighing 38.2 kg (range = 0.77 to 105.3 kg) were collected from Texoma Reservoir. The sex ratio of Alligator Gar was 52.5% male and 47.5% female. More Alligator Gar were collected during winter and spring (n=203) than during summer and fall (n=136). No significant difference in weight was detected between sex ($F_{1, 337}$ = 0.619, P = 0.57) or season ($F_{1, 337}$ = 0.116, P = 0.26), therefore all fish were pooled for the remaining analyses.

Simple linear regression models indicated significant relationships between all length and weight measurement combinations ($P \leq 0.01$;

Table 1. Simple linear and multiple regression equations for predicting length and weight measurements of Alligator Gar using total length (TL), standard length (SL), and girth (G) measurements, with associated r^2 and *P*-values.

Regression type	*n*	*P*-value	r^2	Regression equation
Simple Linear	338	≤ 0.01	0.91	logW = 3.074(logTL)-8.364
	331	≤ 0.01	0.88	logW = 2.839(logSL)-7.451
	227	≤ 0.01	0.91	logW = 2.501(logG)-5.536
	331	≤ 0.01	0.99	logSL = 1.011(logTL)-0.0878
	235	≤ 0.01	0.93	logG = 1.111(logTL)-0.7514
	234	≤ 0.01	0.93	logG = 1.095(logSL)-0.6431
Multiple	227	≤ 0.01	0.92	logW = 3.0806(logTL)-0.0037(logG)-8.3806
	227	≤ 0.01	0.90	logW = 2.8521(logSL)-0.0021(logG)-7.4931

Table 2. Percent error of length and weight measurements predicted from simple linear regression and multiple linear regression using total length (TL), standard length (SL), and girth (G) measurements taken from Alligator Gar, including outcomes of paired t-tests.

Regression type	Predicting variables	% error	t-statistic	df	*P*-value
Simple Linear	TL:W	8.1	0.596	337	0.28
	SL:W	12.4	1.54	330	0.06
	G:W	9.3	0.607	226	0.27
	TL:SL	1.1	0.108	330	0.46
	TL:G	-3.9	0.443	234	0.33
	SL:G	-8.8	0.436	233	0.33
Multiple	TL/G:W	2.86	-0.829	226	0.20
	SL/G:W	2.14	-0.191	226	0.42

Table 1), and these relationships were highly correlated (r^2 = 0.88 to 0.99). The simple linear regression model using TL was the best predictor of Alligator Gar weight ($P < 0.01$, $r^2 = 0.91$, mean % error = 8.1%; Table 2). Regardless of model, all predicted length and weight measurements were not significant different than actual values ($P > 0.05$; Table 2).

The multiple regression models using TL and G and SL and G to predict weight were significant ($P \leq 0.01$) and highly correlated (r^2 = 0.90-0.92; Table 1). The mean percent error was low for both multiple regression models (< 3%; Table 2). The weights predicted using the TL/G and SL/G models were not different than actual fish weights ($P > 0.05$; Table 2), suggesting both models predicted fish weights similarly.

Bias plots suggest predicted weights were relatively unbiased compared to actual fish weights for all models, particularly for Alligator Gar < 60 kg (Figure 2). As Alligator Gar weights exceeded 60 kg, increased variability was observed between predicted and actual weights for all linear regression and multiple regression models (Figure 2). The linear and multiple regression models using TL were the best predictors of fish weight compared to actual fish weights for large fish (80-100 kg). Visual inspection of bias plots suggests that predicted weights from all models underestimate the actual weights of the largest Alligator Gar (> 100 kg; Figure 2). Little bias was observed between girth measurements predicted using TL and SL and actual girth measurements for smaller fish, however the variability of predicted girth measurements increased with increasing Alligator Gar length (Figure 3).

Discussion

This study provides length-weight equations for Alligator Gar from Texoma Reservoir, Oklahoma. Both models (simple linear regression and multiple linear regression) that best predicted Alligator Gar weights used TL to predict weight, which had the highest correlation coefficients (r^2 = 0.91-0.92). Although all predictive models produced reliable estimates of Alligator Gar weight for smaller fish (≤ 60 kg), the weights predicted using simple linear (TL) and multiple regression (TL and G) models were less variable than weights predicted using other length measurements (SL or G), particularly for larger fish (≥ 80 kg). The simple linear regression model relating TL to weight was the best predictor of Alligator Gar weights for fish up to 100 kg. The variability in predicted weights of larger fish was likely due to low sample size of large fish, which comprised <1% of sample. Additionally, variability in predicted weights may have also been caused by intrinsic (gonadal development, age, sex and genetic makeup) and

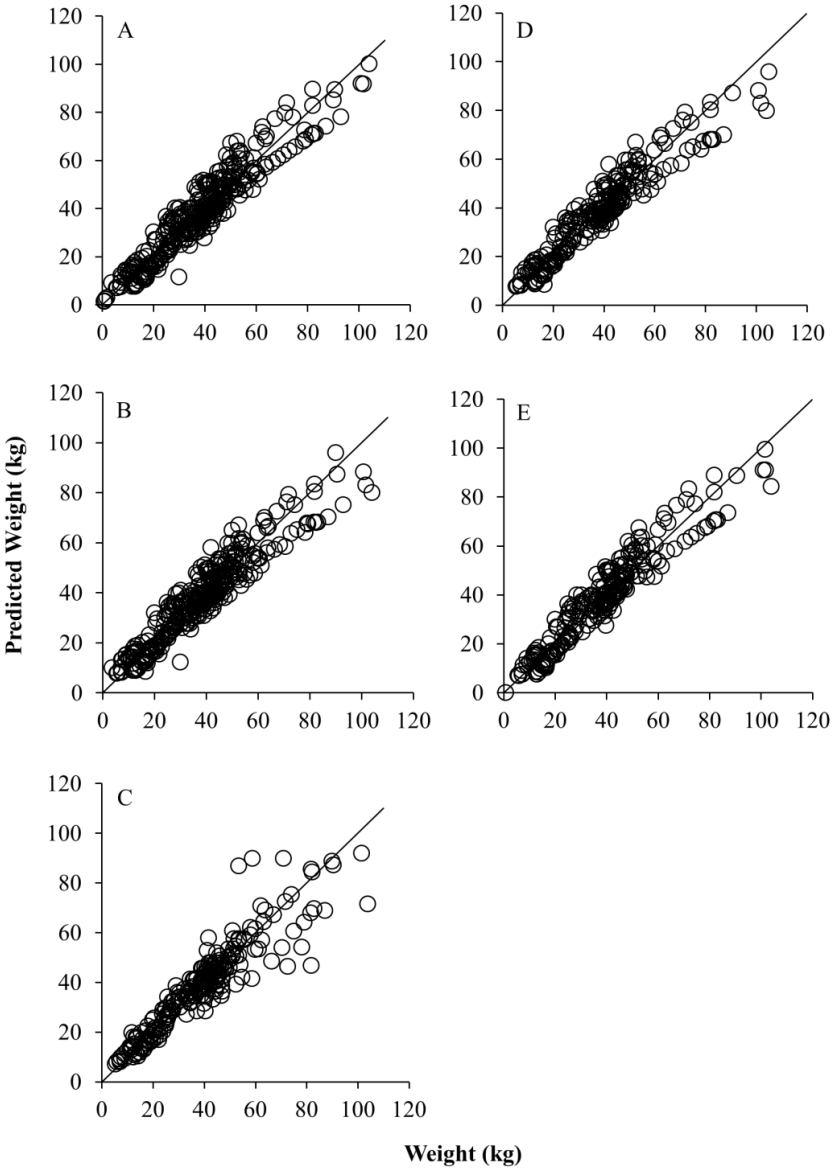

Figure 2. Bias plots comparing actual Alligator Gar weights to those predicted using regression anaysis: A) TL:W, B) SL:W, C) G:W, D) SL and G:W, and E)TL and G:W) of Alligator Gars. The diagonal line represents a 1:1 relationship between predicted and actual weights.

extrinsic factors (gut content, habitat, season; Neumann et al. 2012). Despite this variability, the best predictive models estimated the weight of the current Oklahoma state record Alligator Gar (not used in this study) to within 1.9 to 2.4% (TL:W = 118.05, TL and G:W = 117.49 kg) of the actual weight (115.27 kg).

Although Alligator Gars exhibit sexual dimorphism (McDonald et al. 2013), we found no significant differences in weights by sex or season. Similarly, García de León et al. (2001) found no difference in the weight-length relationships of Alligator Gar collected from Vincente Guerrero Reservoir, Mexico. Previous research produced similar results for other large-bodied fishes. Meerbeek and Crane (2017) found inclusion of sex and reproductive

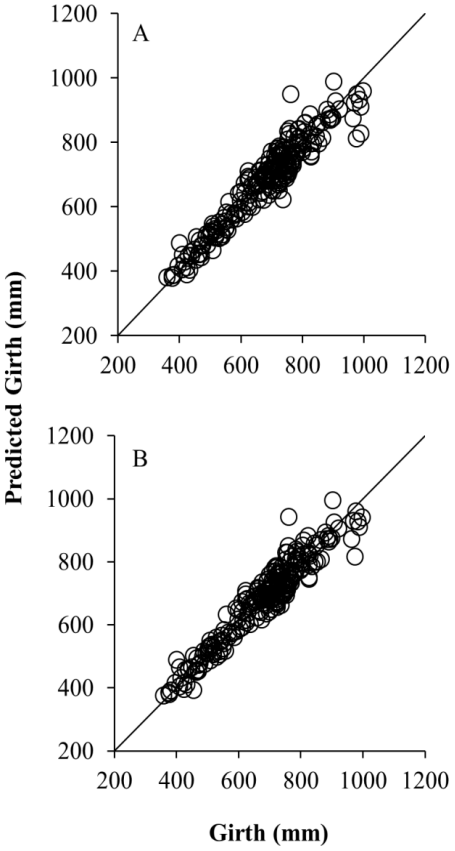

Figure 3. Bias plots comparing actual Alligator Gar girth measurements to those predicted using regression analysis: A) TL:G, B=SL:G) of Alligator Gars. The diagonal line represents a 1:1 relationship between predicted and actual girth measurements.

status did not significantly improve regression models for estimating weights of Muskellunge (*Esox masquinongy*). Similarly, Craig et al. (2005) found no differences in length-weight relationships between sexes of Lake Sturgeon (*Acipenser fulvescens*) from the St. Clair River, Michigan. However, they attributed this to a small sample size of old, large females in their study, which tend to not become larger than males until they have spawned several times (Bruch 1999). It is possible that our results are affected by a limited sample of old, large females, as Alligator Gars exhibit a periodic life history (Buckmeier et al. 2017), which may affect the ultimate size of female gar, similar to species of Acipenseridae (Bruch 1999). Past commercial harvest and unregulated harvest by anglers may have also reduced the number of old, large female Alligator Gar in Texoma Reservoir (Taylor et al. 2019).

The equations presented in this study to predict weight of Alligator Gar provide anglers and fisheries managers with an important tool that allows them to overcome physical and logistical issues associated with obtaining weights of large fishes. Obtaining weights of large-bodied fishes, like Alligator Gars, in the field requires specialized equipment (large capacity scales), and can increase handling time leading to additional physiological stress to a fish (Neumann et al 2012). For these reasons, numerous studies have provided length-weight relationships or predictive equations for large-bodied fishes including, Atlantic Tarpon (*Megalops atlanticus*; Ault and Luo 2013), Lake Sturgeon (Commanda 2018), Muskellunge (Meerbeek and Crane 2017), Pacific Goliath Grouper (*Epinephelus quinquefasciatus*; Boas et al. 2016), and White Sturgeon (*Acipenser transmontanus*; DeVore et al. 1995). Alligator Gar anglers are often not equipped to measure fish weights in the field, even though fishing success is measured by the number of fish caught, and the lengths and weights of those fish (Ault and Luo 2013, Meerbeek and Crane 2017). Our results promote Alligator Gar conservation by providing a method to assign weight to Alligator Gar, reducing the need to over-handle or harvest fish simply to obtain a weight.

The models presented in this study provide fisheries managers and anglers with a method to estimate measurements or weights of Alligator Gar that are typically captured in the field (G, SL, TL, and). However, readers should keep in mind that these models include only Alligator Gar from Texoma Reservoir and application of these equations to other Alligator Gar populations should be done with caution, due to population specific morphological differences. Further, use of these equations to predict measurements of Alligator Gar outside of the size distribution included in this study should be done cautiously. As future Alligator Gar conservation efforts increase in Oklahoma and across the range of the

species, models in this study provide managers with a method to compare body condition and growth potential across populations.

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Population Dynamics of a Stunted Blue Catfish Population in a Small Oklahoma Impoundment

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Abstract: Blue Catfish populations create popular recreational fisheries throughout the United States. Many of these populations were introduced due to their popularity as a sportfish. However, Blue Catfish introductions are not always successful, particularly in small reservoirs. In 2017, a Blue Catfish population was discovered in Meeker Reservoir, a small impoundment in central Oklahoma. Because Blue Catfish populations generally do not do well in small impoundments, an evaluation was implemented to describe population characteristics, recruitment dynamics, and estimate abundance of preferred-length (> 760 mm) Blue Catfish in Meeker Reservoir. Blue Catfish in this population have high longevity, slow growth, low annual mortality, and reach sexual maturity at small sizes. Recruitment of Blue Catfish was variable, although fish from 21 year classes were observed, of which three year classes were dominant (combine to make 58% of fish in the sample). Strong year classes were produced in years with higher mean annual temperatures (> 16.5°C). Overall, this population is overcrowded and stunted, but a small proportion of fish still reach preferred size. Slow growth of Blue Catfish in this population may be explained by some combination of competition, genetics, low reservoir productivity, and reproductive strategy. The small size structure of this population creates a challenging management scenario, because most fish are below the size that anglers are willing to harvest. Although this population may be anomalous, our results provide important information regarding Blue Catfish population characteristics and recruitment in a small impoundment.

Introduction

Blue Catfish (*Ictalurus furcatus*) are a very popular sportfish throughout their range in North America (Graham 1999, Arterburn et al 2002, Reitz and Travnichek 2004, Bodine et al 2013). In Oklahoma, Blue Catfish are native to the Arkansas and Red Rivers, but have been introduced throughout the state as a result of their popularity with anglers (Miller and Robison 2004, Kuklinski and Patterson 2011). However, Blue Catfish introductions are not always successful, particularly in smaller impoundments and in systems where environmental factors negatively affect recruitment (Bartram et al. 2011, Snow et al. 2017). Blue Catfish populations are more successful in larger, deeper rivers or impoundments with large river basins (Burr

and Warren 1986, Jenkins and Burkhead 1994, Graham 1999, Distler 2014). These systems likely provide a higher abundance of larger cavity spawning habitats, which is required to accommodate the overall larger body size of the male and female Blue Catfish for successful reproduction (Graham 1999, Wyatt et al 2006).

Although the factors resulting in successful reproduction and recruitment of Blue Catfish are not well known, it is suspected that Blue Catfish make spawning migrations triggered by flow events in large rivers and reservoirs to access spawning habitat (Bartram et al 2011, Snow et al 2017). In a study of 30 Blue Catfish populations in Texas, Bartram et al. (2011) determined that larger reservoir surface area, high productivity and longer growing season promoted establishment of robust Blue Catfish populations. Further, reservoirs with small surface area (mean = 335 ha; range = 166-961 ha) showed no evidence of natural reproduction, suggesting that Blue Catfish fisheries must be sustained through stocking in small impoundments.

In fall 2017, Blue Catfish (N = 114) were captured in fyke nets and hoop nets during a panfish (crappies *Pomoxis* spp. and sunfish *Lepomis* spp.) sampling assessment at Meeker Reservoir, a small impoundment in central Oklahoma (Porta et al. 2020). These fish ranged from 90 – 450 mm TL, and appeared to include multiple year classes. Blue catfish are not stocked into Meeker Reservoir by the Oklahoma Department of Wildlife Conservation, suggesting that this population is naturally reproducing, which is rare in small impoundments (Bartram et al. 2011). Further, Blue Catfish are not typically collected with the sampling methods used by Porta et al. (2020), so an assessment of this population with appropriate sampling techniques was warranted. Therefore, the objectives of this study are to 1) describe population characteristics (age and size structure, condition, growth, mortality, and age and size at maturity) of Blue Catfish in Meeker Reservoir using a multiple gear approach (low-frequency electrofishing, gillnets, and jug-lines), 2) determine environmental factors affecting year class strength of Blue Catfish, and 3) estimate the population size of preferred-length (≥760 mm) Blue Catfish in Meeker Reservoir.

Methods

Study Area

Meeker Reservoir is an 85.8 ha impoundment located 2.3 miles southwest of Meeker, Oklahoma in Lincoln County (35º 29′ 46.4″ N, 96º 56′ 10.2″ W; Figure 1). Meeker Reservoir was formed in 1970 by impounding Quapaw Creek. The primary purpose of the reservoir is for municipal water supply, flood control, and recreation. At full pool, Meeker Reservoir has 8 km of shoreline, a maximum depth of 7.4 m and a mean depth of 2.8 m (OWRB 2009). The river-reservoir interface of Quapaw Creek is shallow due to siltation, which has reduced the surface area of the lake by 21% (109.3 ha in 1970) since construction of the reservoir (OWRB 2009). The reservoir consists mostly of open water with areas of emergent aquatic vegetation, limited submerged or exposed standing timber, rock, coarse gravel, and clay or sand substrate. The reservoir is considered mesotrophic and is extremely turbid with a mean secchi depth of 10 cm (OWRB 2009). Salinity values range from 0.10 to 0.11 ppt. The water is neutral to slightly alkaline (7.33 - 8.37 pH).

Since impoundment in 1970, the Oklahoma Department of Wildlife Conservation (ODWC) has stocked Meeker Reservoir with Channel Catfish (*Ictalurus punctatus*), Flathead Catfish (*Pylodictis olivaris*), and Largemouth Bass (*Micropterus salmoides*). The first stocking occurred in 1972 and consisted of 6,000 fingerling Largemouth Bass, 600 fingerling Flathead Catfish, and 6,000 fingerling Channel Catfish. Since the initial stocking in 1972, only Channel Catfish have been stocked periodically (~20,000 fingerlings/year from 1981 – 1989; ~10,000 fingerlings/year from 2009 – 2013) into Meeker Reservoir. Based on ODWC historic stocking records and communication with representatives from the City of Meeker, there are no records of Blue Catfish stocking at Meeker Reservoir. It is possible that Blue Catfish were stocked via a contaminated stocking or an

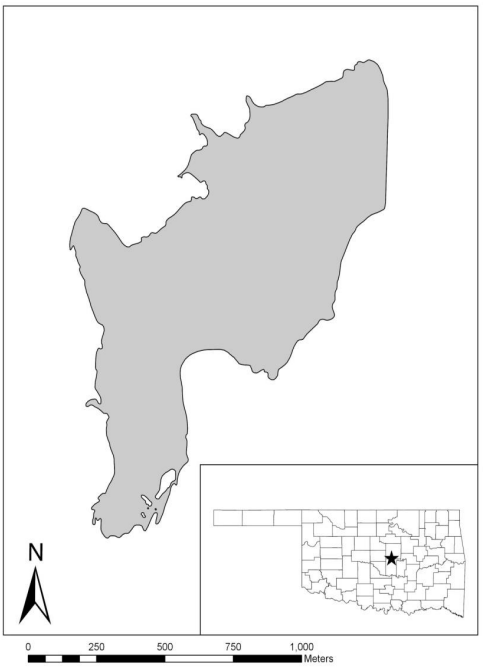

Figure 1. Map of Meeker Reservoir (35º 29′ 46.4″ N, 96º 56′ 10.2″ W) located in Lincoln County, Oklahoma.

angler introduction.

Study design

Blue Catfish were collected from Meeker Reservoir during June 2019, using low-frequency electrofishing (15 pulses/sec, pulsed DC, high voltage, Smith Root 7.5 GPP, set for optimal power; Miranda 2009, Bodine et al. 2011) using two chase boats to improve efficiency (ODWC Standardized Survey Protocol manual). Seven sites were chosen at random from the entirety of the reservoir and five-minutes of effort were applied to each site during daylight hours. At the end of each unit, all fish collected were measured for total length (TL; mm) and weighed to the nearest gram (g). Additionally, 20 Blue Catfish per 10-mm TL group were collected for age estimation and sex determination. Fish kept for age estimation and sex determination were placed on ice and processed at the Oklahoma Fishery Research Laboratory in Norman, Oklahoma. Fish were re-measured for total length (TL; mm), weighed (g), sex determined, and lapilli otoliths were removed for age estimation.

Sex determinations of fish kept for aging purposes, were assigned a maturity status (immature or mature) following methods of Davis and Posey (1958) and Perry and Carver (1972). Immature Blue Catfish were those showing no signs of gonadal development, the ovaries and testes are barely distinguishable or are readily distinguishable but not developed. However, mature female Blue Catfish had well developed ovaries that contained yellowish to creamy-yellow eggs or were spent. Mature males were those with enlarged testes that were white in color. These Blue Catfish were sampled and examined during the time of year when spawning typically occurs in Oklahoma (Miller and Robison 2004), which allowed for easy determination of fish maturity.

Lapilli otoliths were extracted from each fish (Long and Stewart 2010) and placed into an individually numbered envelope and allowed to dry for at least 24 h prior to processing (Secor et al. 1992, Snow et al. 2017). Once dried, otoliths were processed according to methods of Buckmeier et al. (2002) and Waters et al. (2020). After processing, otoliths were viewed using a stereo microscope (capable of 130x magnification) with a fiber optic filament attached to an external light source to illuminate annuli (Buckmeier et al. 2002, Waters et al. 2020). Each otolith was estimated in concert by two readers, however if the readers disagreed on the age of the fish, then that otolith was put aside and viewed again at a later date (Hoff et al. 1997). If an otolith was deemed unreadable, the second otolith was processed and age estimated, however if that otolith was also poor or disagreement persisted the fish was removed from the study. Each otolith was evaluated in random order with no reference of TL, weight or sex (Hoff et al. 1997).

Large Blue Catfish appeared to be underrepresented using only low frequency electrofishing, so we employed a multi-gear approach (large mesh gillnets and jug lines) to ensure all size classes of fish were collected and to estimate the population size of preferred-sized (≥760 mm) Blue Catfish. Blue Catfish were sampled from 2 March - 5 March 2020

using 4 gillnets (7.3 m tall with four 15.2 m long panels composed of 76.2, 101.6, 127, and 154.4 mm mesh, respectively) and 15 jug lines (one 8/0 circle hook/jug). Jug lines were baited with Bluegill (*Lepomis macrochirus*), Gizzard Shad (*Dorosoma cepedianum*), or White Crappie (*Pomoxis annularis*). All Blue Catfish were measured for TL(mm) and weighed (g). Blue Catfish ≥760 mm were tagged with a PIT tag (Biomark, Inc., Boise, Idaho), size 3 self-piercing tag (National Band & Tag Co., Newport, Kentucky), and a pelvic fin clip then released. PIT tags were inserted behind the left maxillary barbel and the self-piercing tag was crimped to the posterior side of the adipose fin. All Blue Catfish ≤759 mm were collected for age estimation purposes. Furthermore, on the last day of sampling all Blue Catfish captured were kept for age analysis to fill additional length groups.

Analysis

Size structure of the Meeker Reservoir Blue Catfish population was described with length-frequency histograms of all fish captured and proportional size distribution (PSD, stock ≥ 300mm, quality ≥510 mm, preferred ≥760; Anderson and Gutreuter 1983). A simple linear regression was used to describe the relationship between $\log_{10}$(weight):$\log_{10}$(length). The relationship of Blue Catfish length to weight was also used to evaluate fish condition by calculating relative weight (W_r) using the standard weight equation W_s = -6.067 + 3.400($\log_{10}$(TL)) (Muoneke and Pope 1999). A logistic regression model was used to determine the relationship between maturity at age for male and female Blue Catfish using binary variables (0 = immature, 1 = mature). Mean length at age was calculated for male and female Blue Catfish. These data were then log transformed data to linearize the relationship, and differences in growth between sexes were tested using analysis of covariance (ANCOVA). Because growth between sexes was similar ($F_{1, 322}$ = 0.485, P = 0.48) all fish were combined to estimate growth rates using a Richard's growth model (Richard 1959, Ogle et al. 2017). Total annual mortality of Blue Catfish was estimated using weighted catch-curve-regression where the slope of the relationship between numbers of fish caught (natural log transformed) at each age (instantaneous total mortality [Z]) was used to estimate total annual mortality ($A = 1 - e^{-Z}$; Ricker 1975). Blue Catfish < age-2 were not fully recruited to the sampling gears, so they were removed from catch-curve analysis.

Annual year-class strength of Blue Catfish was indexed using residuals of catch curves following methods of Maceina (1997). Because Blue Catfish recruitment is irregular (Kuklinski and Patterson 2011, Duck 2020) and to reduce bias caused by infrequent catch of older fish (Dunn et al. 2002), only residuals from the 2007-2017 year classes were used for analyses. Residuals were divided by corresponding $\log_{10}$ number of fish in each year class to calculate deviation between the observed and predicted numbers of fish (DEV), with year-classes having DEV values > 0.5 were considered strong (Catalano et al. 2009).

Residuals calculated from the catch-curve regression were used in a multiple regression equation (ln(catch) = b0 – b1(age) ± b2(exvar)) with the addition of an explanatory variable (exvar) to determine the effects of that variable on recruitment (Maceina and Bettoli 1998). Recruitment of reservoir fishes has been linked to variation in reservoir area, discharge, retention, volume, and productivity (Maceina 1997, Maceina and Bettoli 1998). However, those parameters were not available for Meeker Reservoir. Because environmental variables were limited, we chose to evaluate the effects of annual average rainfall (proxy for hydrological variables), May/June average air temperature (coincides with Blue Catfish spawning times in Oklahoma), and annual average air temperature (proxy for water temperature) on Blue Catfish recruitment. Rainfall affects the hydrology of reservoirs and lakes via runoff and river inflow (Patrick 2016). Suleiman and Ifabiyi (2015) reported a strong and positive correlation between rainfall and several reservoir variables (reservoir inflow, retention, and discharge). Additionally, water temperature is correlated with air temperature, and has been used as an explanatory variable in previous fish studies

(Chambers and Trippel 1997). Rainfall and temperature data were collected from the Shawnee, Oklahoma Mesonet Station #85 (35° 21' 53" N, 96° 56' 53" W) for the period of May 2007 to December 2017. This station is located 14.54 km south of Meeker Reservoir.

The population abundance of preferred-length (≥760 mm) Blue Catfish was estimated using a Schnabel estimator (Seber 1982) with 95% confidence intervals calculated using a Poisson distribution (Krebs 1999). Although the primary assumption of a Schnabel estimator is a closed population (assumes no immigration, emigration, recruitment, or mortality), it is robust to some departure from these assumptions (Ricker 1975). We used a short sampling duration (5 day mark-recapture period) to best ensure the assumptions of a closed system were met. During this time there were no major rain events that affected inflow or outflow from Meeker Reservoir that would allow Blue Catfish immigration or emigration. Additionally, we observed no anglers fishing for Blue Catfish and it is unlikely that other mortality or recruitment events occurred that substantially altered the number of individuals during the 5-day period. The density (fish/ha^{-1}) and biomass (kg/ha^{-1}) of Blue Catfish ≥760 mm was estimated using the population abundance estimate. All analyses were performed using XLSTST 2020 (Addinsoft Inc., New York City, NY). All significance tests were evaluated at $P \leq 0.05$.

Results

Population dynamics

A total of 455 Blue Catfish were collected using all sampling gears combined (Figure 2), of which 323 Blue Catfish were kept for age estimation and population assessment. Blue Catfish used for age analysis ranged from age-1 to age-29 and were 112 - 855 mm TL. More male (57%) than female (43%) Blue Catfish were represented in the sample. Both female and male Blue Catfish reached 100% maturity by age-8 (Figure 3). The earliest that a Blue Catfish reached maturity was age 2, and 50% of all Blue Catfish reached maturity by age 5. There was no significant difference in age at maturity (X^2 = 2.057, df = 1, P = 0.15) between sexes of Blue Catfish from Meeker Reservoir

This population was dominated by sub-stock (85%) and stock (11%) sized Blue Catfish (Table 1; Figure 2). As a result, PSD was low (PSDq = 20), but a small proportion of the population exceeded preferred size (PSDp = 6). Stock-length Blue Catfish averaged age-11, quality-length averaged age-19, and an average of 23.5 years was required to reach preferred-length (Table 1). The weight-length relationship of Blue Catfish was $\log_{10}(W) = 0.312(\log_{10}(TL)) + 1.75$, which was highly significant ($r^2 = 0.98$, $P < 0.01$; Figure 4). This weight-length relationship resulted in a mean W_r of 98 (Table 1), which is above average (near the 75th percentile). When

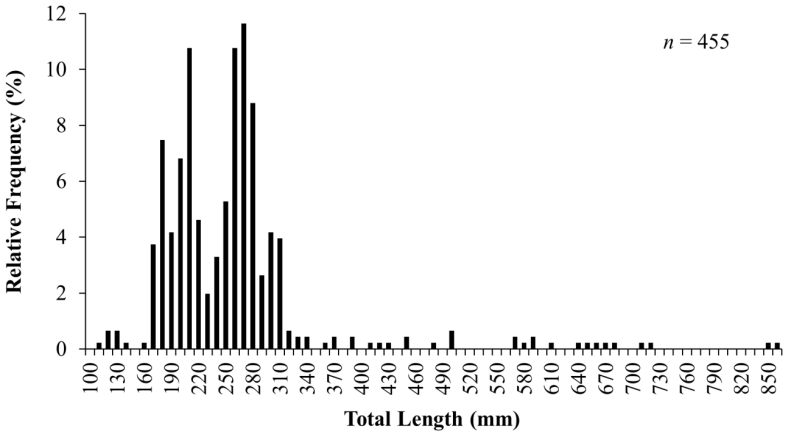

Figure 2. Length frequency histogram utilizing all gears to capture Blue Catfish collected from Meeker Reservoir, Oklahoma.

Table 1. Proportional size distribution (PSD; 95% confidence interval (CI)), mean age (range), and relative weight (Wr; 95% CI) of Blue Catfish by size class collected from Meeker Reservoir, Oklahoma.

Size Category	*n*	PSD Value (95% CI)	Mean Age (range)	W_r (95% CI)
Sub-stock	387	N/A	5 (1-12)	99 (98-100)
Stock (≥ 300 mm)	50	74 (61-87)	11 (7-17)	90 (88-93)
Quality (≥ 510 mm)	14	20 (8-32)	19 (16-29)	91 (85-98)
Preferred (≥ 760 mm)	4	6 (0-13)	23.5 (23-24)	95 (81-109)
Overall	455	N/A	6.5	98

evaluated by size classes, W_r ranged from 90 – 99. The Richard's growth model indicates that Blue Catfish approach L_∞ (L_∞ = 736 mm TL; predicted maximum total length) slowly (k = 2.1), with individuals in the population reaching approximately 50% of the L_∞ by age-11 and 75% of L_∞ by age-18 (Figure 5). The estimated annual survival rate was high (87%) and estimated total annual mortality was low (13%) for Blue Catfish in Meeker Reservoir (Figure 6).

Recruitment

Recruitment of Blue Catfish in Meeker Reservoir was sporadic and variable. Fish from 21 year-classes were represented in the age sample. Three strong year classes (DEV > 0.05; 2012, 2016, and 2017) comprised a large portion (58%) of the Blue Catfish population in Meeker Reservoir (Table 2). Blue Catfish recruitment patterns were not related to annual rainfall ($F_{2, 8}$ = 0.155, P = 0.86) or May/June air temperature ($F_{2, 8}$ = 0.503, P = 0.63; Table 2). However, Blue Catfish year class strength was positively correlated with annual air temperature ($F_{2, 8}$ = 9.431, $P \leq 0.01$, r^2 = 0.71, $P \leq 0.01$; Figure 7), suggesting that strong Blue Catfish year classes formed in warmer years (> 16.5°C) and weaker year classes formed in colder years (< 16.5°C). This relationship explained 24.3% (squared Pearson correlation coefficient) of the variation in abundance at age in the model after accounting for the influence of age.

Abundance of preferred size Blue Catfish

The estimated abundance, density, and biomass of preferred-length Blue Catfish in Meeker Reservoir were low. During the 5-day mark-recapture effort, we collected a total of 6 preferred-length Blue Catfish (ranging 762 to 855 mm TL), of which 3 (50%) were recaptured. This produced a population estimate of 9 preferred-length Blue Catfish (95% CI = 2.3 - 23.2) in Meeker Reservoir. Using the estimated

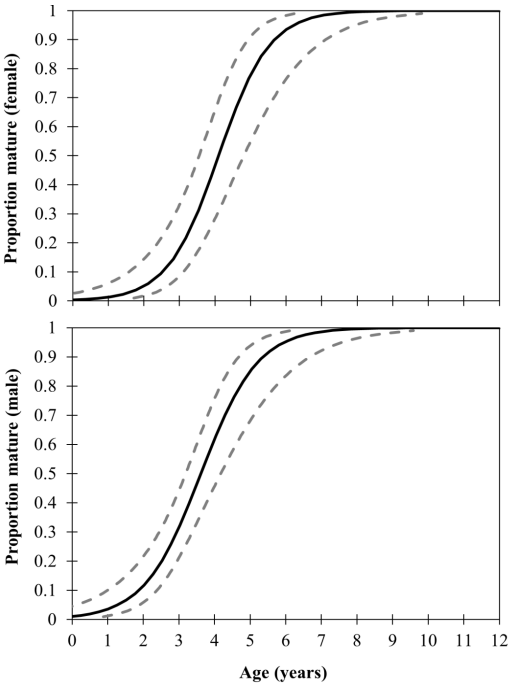

Figure 3. Results of logistic regression analysis displaying the proportion of mature female (top) and male (bottom) Blue Catfish by age. Only Blue Catfish age-0 to age-12 are presented graphically to allow clear visualization of these relationships. Grey dash lines represent 95% confidence intervals.

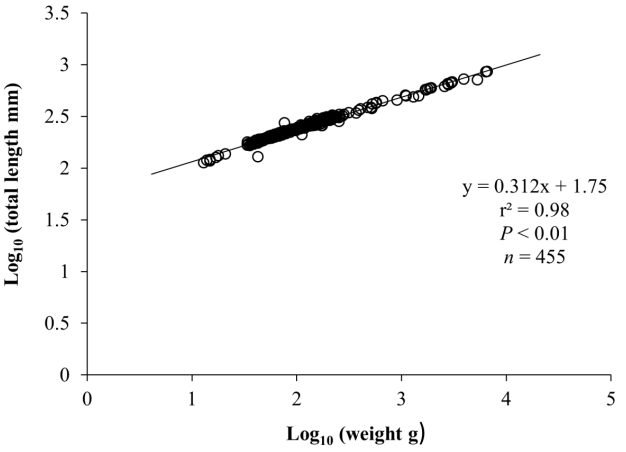

Figure 4. Weight-length relationship for 455 Blue Catfish collected from Meeker Reservoir, Oklahoma. The logarithmically-transformed weight-length equation is log10 (W) = 0.312 (log10 TL) + 1.75.

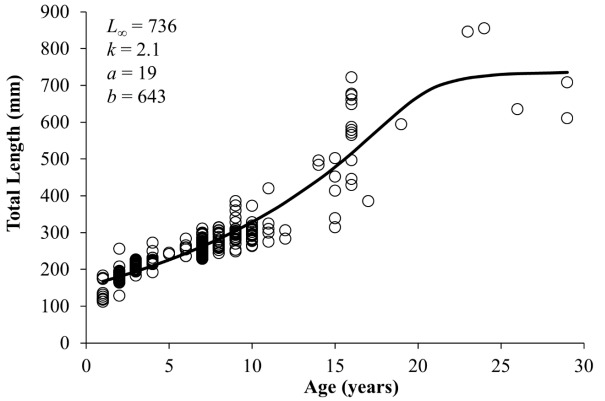

Figure 5. Richards growth curve calculated from 323 otolith age estimates for Blue Catfish collected from Meeker Reservoir, Oklahoma. L∞ = predicted maximum total length, k = growth constant, a =horizontal position of the inflection point, and b = vertical position of the inflection point.

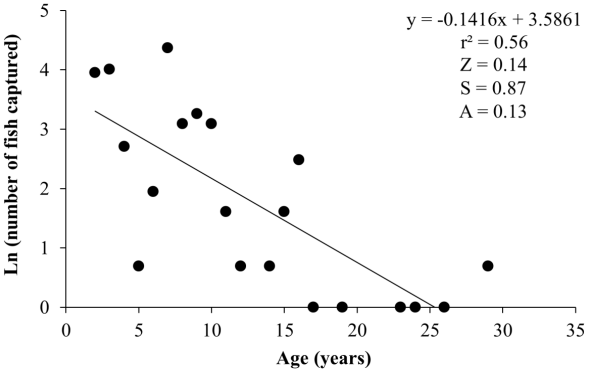

Figure 6. Weighted catch curve regression used to estimate total annual mortality (A) for Blue Catfish collected from Meeker Reservoir, Oklahoma. Z = instantaneous total mortality and S = total annual survival.

Table 2. Number (n) and percent (%) of total Blue Catfish collected by year-class from Meeker Reservoir, Oklahoma. Year-class strength (2007 – 2017) was estimated using catch-curve residuals divided by corresponding log predicted fish number to obtain deviation (DEV). Strong year-classes (DEV values >0.50) are identified in bold. Included are annual average rainfall (cm) and temperature (°C) data, which were used in multiple regression models. These data were collected from the Shawnee, Oklahoma Mesonet Station (35° 21' 53" N, 96° 56' 53" W) located 14.54 km south of Meeker Reservoir from May 2007 through 2017.

Year class	*n*	%	DEV	Average rainfall (cm)	Average May/June air temperature (°C)	Average annual air temperature (°C)
2017	**52**	**16.1**	**0.77**	**8.4**	**22.5**	**16.7**
2016	**55**	**17**	**1.88**	**9.4**	**22.6**	**16.7**
2015	15	4.64	-2.01	37.1	22.5	16.1
2014	2	0.62	-0.43	9	22.6	15
2013	7	2.17	-0.80	16.4	22.5	15
2012	**79**	**24.5**	**1.60**	**7**	**22.9**	**17.8**
2011	22	6.81	-1.31	4.9	23.1	17.2
2010	26	8.05	-0.85	16.7	23.2	16.1
2009	22	6.81	-0.57	5.9	23.2	15.6
2008	5	1.55	-0.33	14.4	23.2	15.6
2007	2	0.62	-0.35	27.3	23.0	15.6

abundance, preferred-length Blue Catfish density was estimated to be 0.11 fish/ha^{-1} (95% CI = 0.03 – 0.27 fish/ha^{-1}) with an estimated biomass of 0.08 kg/ha^{-1} (95% CI = 0.07- 0.08 kg/ha^{-1}).

Discussion

The establishment of a Blue Catfish population in a small impoundment like Meeker Reservoir is rare. In general, Blue Catfish do not do well in reservoirs with small surface area and low productivity. Bartram et al. (2011) found the most robust Blue Catfish populations in reservoirs with high productivity (Secchi depth < 65 cm) and large surface area (> 1,466 ha). Although water clarity of Meeker Reservoir is poor (mean secchi depth = 10 cm), it is the result of large amounts of suspended sediments and not productivity. Meeker Reservoir has low to moderate productivity, and has been classified as mesotrophic (OWRB 2009). The establishment of a Blue Catfish population in Meeker Reservoir appears anomalous, as conditions that typically result in robust Blue Catfish populations are not present. However, spawning habitat appears to be available and utilized, given year classes of Blue Catfish are produced in most years.

A combination of low reservoir productivity and high recruitment rates may explain the overabundant and slow growing Blue Catfish in Meeker Reservoir. In systems with low productivity, competition for forage resources may be high, resulting in reduced growth rates of fish (Andersen et al. 2017). Michaletz (2009) found that Channel Catfish growth was slow when fish density was high and lake productivity was low. Further, Nepal and Fabrizio (2020) suggested that juvenile Blue Catfish growth rates in the York River, Virginia may have been lower than those in other rivers evaluated in that study due to lower productivity in that system. Reproductive strategy may also explain the slow growth of Blue Catfish in Meeker Reservoir. Fishes that allocate energy towards

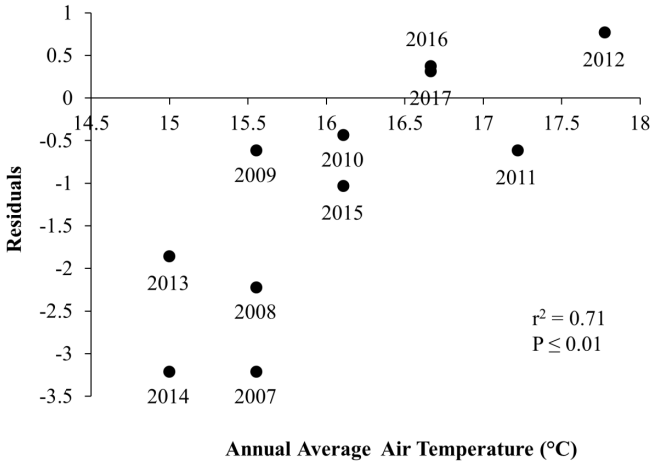

Figure 7. Relationship between catch-curve residuals annual air temperature (°C). Data points represent year classes.

reproduction rather than growth typically reach maturity at smaller sizes, which ultimately affects their overall growth potential (Enberg et al. 2012). Blue Catfish in Meeker Reservoir reached sexual maturity at smaller sizes than observed in other populations. We observed mature fish as small as 180 mm TL (age-2) and 50% of age-5 fish were mature (243 mm TL). Perry and Carver (1972) did not observe mature Blue Catfish until they exceeded 400mm TL in Louisiana. Hale and Timmons (1989) did not observe mature Blue Catfish until they were >445mm TL in Kentucky Lake, Kentucky. Graham (1999) reported mature Blue Catfish at lengths ranging 350-662 mm TL (age-4 to age-7). Overall, it appears a combination of factors are affecting growth of Blue Catfish in Meeker Reservoir resulting in growth rates that are much slower than other reservoir populations in Oklahoma (Boxrucker and Kuklinski 2006), and other areas of the United States (Graham 1999).

Growth of Blue Catfish in Meeker Reservoir was not consistent among age classes. Year-to-year changes in length between younger fish (< age-12) were smaller than year-to-year changes of older fish (> age-12). The observed differences in growth patterns between age classes required the use of a Richard's growth model instead of the more traditional von Bertalanffy growth model to describe growth of Blue Catfish from Meeker Reservoir. One possible explanation for the differences in growth between age groupings is the larger fish in this population may represent Blue Catfish that were originally stocked into Meeker Reservoir. Although it is unknown how and when Blue Catfish entered Meeker Reservoir, it is possible that these fish were transplanted from another system where Blue Catfish growth is faster, or that the initial small population size allowed ample growth for the first several years of this population's existence in Meeker Reservoir. Further, if fish were transplanted into Meeker Reservoir, it is possible that progeny produced from a small founder population are impacted by inbreeding depression that is negatively affecting growth, which has been observed for Channel Catfish (Bondari and Dunham 1987). Finally, size-specific differences in diet may explain the differences in growth between the age classes of Blue Catfish in Meeker Reservoir. Although our sample is limited (only fish captured for aging purposes were dissected to identify stomach contents), small Blue Catfish (ranging 112 – 496 mm) predominately consumed invertebrates (mayflies), while larger Blue Catfish (ranging 374 – 708) were able to consume fish (primarily small [< 150 mm TL] Blue Catfish), which has been described for other Blue Catfish populations (Edds et al. 2002). Larger fish have an advantage in that they can consume prey of a wider range of sizes, including fish, which would allow them to grow to larger sizes (Vanni

et al. 2009). Although the exact mechanism is unknown, divergence in growth among age classes is apparent in Meeker Reservoir.

In addition to slow growth, total annual mortality of Meeker Reservoir Blue Catfish was low (13%) compared to other populations. Boxrucker and Kuklinski (2006) observed a 26% average mortality rate across 9 Blue Catfish populations in Oklahoma. Similarly, Kuklinski and Patterson (2011) documented an average total annual mortality rate of 24% for Blue Catfish populations from 14 reservoirs in Oklahoma. Our mortality estimate for Meeker Reservoir fell at the lower end of the range (12-63%) reported by Graham (1999) for Blue Catfish populations across the U.S. However, the mortality estimates reported by Graham (1999) resulted from the use of multiple aging methods (i.e. otoliths and spines) that may affect the mortality estimates across populations' calculations (Boxrucker and Kuklinski 2006). Regardless, our mortality estimates for the Blue Catfish population in Meeker Reservoir are low compared to other Oklahoma reservoir populations that were described using similar aging methods (Boxrucker and Kuklinski 2006, Kuklinski and Patterson (2011).

The Meeker Reservoir Blue Catfish population was dominated (58%) by three year classes. Variable recruitment in Blue Catfish populations in Oklahoma is not uncommon (Kuklinski and Patterson 2011, Duck 2020). Similarly, Goeckler et al (2003) found 74% of the Blue Catfish population in a large reservoir in Kansas was comprised of two year classes. We found strong year classes of Blue Catfish were formed in Meeker Reservoir during years with higher average temperatures. Nepal and Fabrizio (2020) described increased growth of age-0 Blue Catfish in several tributaries to Chesapeake Bay during years when average temperatures were the warmest. Fish that attain larger sizes during their first year of life have a higher survival rate, which translates into increased year-class strength (Ludsin and DeVries 1997, Phelps et al. 2008). Improved growth of age-0 Blue Catfish in years with higher temperatures in Meeker Reservoir may explain the associations we found in this study. This is supported by findings of Bartram et al. (2011) that found growing season positively influenced Blue Catfish populations in Texas.

The Meeker Reservoir Blue Catfish population is dominated by a high abundance of slow growing fish. This creates a challenging management scenario because few fish exceed 406 mm TL, which is what catfish anglers consider to be eating size (Hunt and Hutt 2010). Therefore, it is unlikely that angler exploitation will be high enough to reduce competition and promote growth of this overcrowded, stunted population. An alternative option is for fisheries managers to manually remove small Blue Catfish from the Meeker Reservoir population in hopes that the reduction in biomass would result in reduced competition for forage resources and increased growth. To our knowledge there is limited information on targeted removals of catfish. Bonvechio et al. (2011) documented changes in the age structure, condition, size structure, and biomass of invasive Flathead Catfish in Satilla River, Georgia. Additionally, other species removal efforts have been successfully used to reduce biomass, for example Common Carp (*Cyprinus carpio*; Bajer et al. 2009). Although removal efforts are usually effective, they require a lot of labor intensive work.. Despite creating a challenging management scenario, our results provide important information regarding Blue Catfish population characteristics and recruitment in a small impoundment, which is a rare phenomenon.

Acknowledgments

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Conservation.

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Record of an Angler-Caught Blue Crab in Oklahoma

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The Oklahoma Department of Wildlife Conservation (ODWC) is often contacted by constituents or members of the general public that report wildlife observations or to have a species they caught identified. These reports often describe fish or wildlife species that are uncommon or are commonly occurring species that are misidentified. However, occasionally these claims are so bizarre they require further investigation. This was the case on July 7, 2020, when ODWC was contacted by a catfish angler who claimed to capture a large Blue Crab (*Callinectes sapidus*) in the tailwater below Overholser Reservoir, Oklahoma (Figure 1). The angler was asked if he could provide photographs for confirmation to identify the Blue Crab (Figure 2). Inspection of the photographs confirmed the angler caught a Blue Crab and he was asked to maintain the specimen for collection of scientific information. Upon taking possession of the Blue Crab, it was measured for carapace width and length (nearest mm), weight (g), and determined sex. Carapace width was measured as the distance between the tips of the posterior most lateral carapace spines and length was measured dorsally along the midline, between the frontal notch and the posterior margin of the carapace (Josileen 2011). The Blue Crab had a carapace width of 172 mm and length of 80 mm and weighed 170 g (Figure 3). Based on the shape of the apron and red coloration of the tips of the claws, this Blue Crab was identified as a mature female (Baldwin and Johnsen 2009).

Figure 1. Aerial photograph of the Overholser Reservoir tailwater detailing the Blue Crab capture location (x) and crab pot sampling sites (■).

Figure 2. Photographs taken by the angler and provided to ODWC for identification of the Blue Crab.

To determine if this was an isolated event, Blue Crabs were sampled in the tailwater below Overholser Reservoir using 6 crab pots (72 cm x 72 cm x 30.5 cm) baited with cut sunfish *Lepomis* sp. and Gizzard Shad (*Dorosoma cepedianum*) (Figure 1). However, no Blue Crabs were captured during this sampling effort. Further, ODWC has not received any additional reports of Blue Crabs being captured or observed in the tailwater of Overholser Reservoir or in surrounding tributaries.

It is impossible to know exactly how a Blue Crab entered this system, however we propose some possible explanations. First, this Blue Crab was captured from a waterway in Oklahoma City. It is possible that Blue Crabs were purchased at a live seafood market in the city, more were purchased than could be consumed, and the buyer decided to release the live Blue Crabs into a local waterway. Additionally, Blue Crabs could have been brought back during a vacation as a pet and released. Perhaps the more likely scenario, is that this Blue Crab may have been a bait bucket release resulting from an angler using Blue Crabs as fishing bait. Recent studies have documented the consumption of Blue Crabs by Blue Catfish (*Ictalurus furcatus*; Schmitt et al. 2019a, Schmitt et al. 2019b). Bait bucket introductions have resulted in establishment of many aquatic species in areas where they are not native (Moyle 1973, Ludwig1995, Killian et al. 2012, Drake and Mandrak 2014).

The capture of a Blue Crab from an Oklahoma water body is unique and unexpected observation, and therefore is the first report of this species in the state to our knowledge. Although Blue Crabs can reproduce and survive in water quality conditions similar to those in the river below Overholser Reservoir (salinity = 0.4 – 0.5‰; Eggleston et al. 2009, Roy et al. 2012), the risk of establishment is relatively low due to their complex early life history (Jivoff et al. 2007), life expectancy (3–4 years; Hewitt et al 2007) and intolerance to low water temperature (high mortality of mature females at water temperatures <5°C; Johnson 2015).

Figure 3. The photographs illustrate a dorsal (A) and ventral (B) view of a female Blue Crab caught in the Oklahoma River below Overholser Reservoir.

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Population Dynamics and Diets of Yellow Bass in New Spiro Reservoir, Oklahoma

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Abstract: Yellow Bass (*Morone mississippiensis*) are rare in Oklahoma and little information exists regarding the basic ecology in the state. However, a population exists at New Spiro Reservoir, which was studied in fall 2018 and spring 2019. Our objective was to describe population dynamics (age and size structure, condition, growth, mortality, and age at maturity) and diets (spring and fall) of Yellow Bass in New Spiro Reservoir, Oklahoma. This population was dominated by stock-sized (100 mm TL) fish that were primarily planktivorous in both seasons. Yellow Bass in New Spiro Reservoir is characterized by fast growth rates (L_∞ = 281 mm TL by age-2), short longevity (age-3), rapid maturity (100% mature by age-2), and high annual mortality rates. Relative weights (W_r) of Yellow Bass were average (mean W_r = 96), but W_r increased with fish size (substock = 90, stock = 92, quality = 98, preferred = 105, memorable = 114). This study improves our knowledge of the life history of Yellow Bass in Oklahoma and provides a basis from which future studies can be compared.

Introduction

Yellow Bass (*Morone mississippiensis*) are native to the Mississippi River system of North America (Miller and Robison 2004, Edds 2014), including Oklahoma. However, Yellow Bass have only been documented in a few Oklahoma reservoirs and rivers from three counties (Wagoner, Muskogee, and McCurtain) in the southeast portion of the state (Robison et al. 1974, Pigg and Hill 1974, Pigg et al. 1979, Pigg 1983, Pigg and Peterson 2000, Miller and Robison 2004). Due to their rarity, when Yellow Bass are captured during a survey in Oklahoma they are typically preserved and placed into museum collections (Pigg and Hill 1974, Pigg et al. 1979, Pigg 1983, Pigg and Peterson 2000). Recently, ODWC staff have documented Yellow Bass populations in two additional reservoirs (Robert S. Kerr Reservoir and Sally Jones Lake in Sequoyah County, OK) during standardized gillnet surveys since 2000 (ODWC, unpublished data), but follow-up studies were not conducted. In 2018, Yellow Bass were observed at a third reservoir, New Spiro in Le Flore county, during a spring electrofishing survey in 2018 (Porta 2019). Because little is known about Yellow Bass in Oklahoma, our objectives were to describe population characteristics (age and size structure, condition, growth, mortality, and age at maturity) and seasonal diets (spring and fall) for Yellow Bass from New Spiro Reservoir, Oklahoma.

Methods

Study Area

New Spiro Reservoir is a 100.8 ha impoundment of Holi-Tuska Creek formed in 1963 and is located 4.6 km south of Spiro, Oklahoma in Le Flore County (35º 11′ 35.7″ N, 94º 36′ 59.5″ W; Figure 1). This reservoir serves as a water supply for the city of Spiro and also provides flood control and recreational

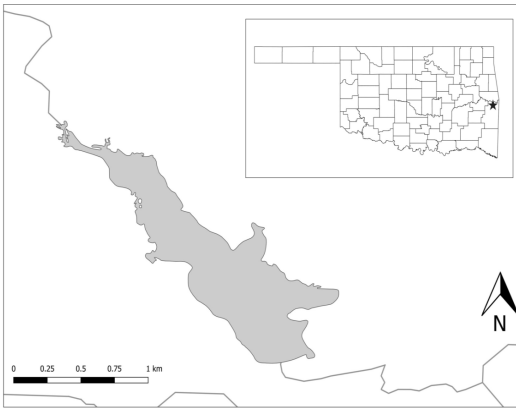

Figure 1. Map of New Spiro Reservoir (35º 11′ 35.7″ N, 94º 36′ 59.5″ W) located in Le Flore County, Oklahoma.

opportunities. At full pool, New Spiro Reservoir has 9.3 km of shoreline, a maximum depth of 6.7 m and a mean depth of 2.5 m (OWRB 2010). The reservoir consists of open water with areas of dense complex stands of submerged and emergent aquatic vegetation, with rock and/or coarse gravel points, and clay or sand substrate. The reservoir is considered hypereutrophic with a mean secchi depth of 47 cm (OWRB 2010).

Study Design

Yellow Bass (Figure 2) were collected from New Spiro Reservoir during October 2018, using experimental gillnets (61 m long x 1.8 m deep and constructed of eight 7.6 m panels [12.7, 15.9, 19.1, 25.4, 38.1, 50.8, 63.5, and 76.2 mm bar mesh]) at 6 randomly selected sites. Additionally, Yellow Bass were collected in April 2019 using boat electrofishing (pulsed DC, high voltage, Smith Root GPP), which was used to sample the entire shoreline. A multiple gear approach was implemented to ensure all age and size classes were represented in the sample (Kuklinski 2007, Porta and Snow 2017). All Yellow Bass were placed on ice immediately after capture, and processed at the Oklahoma Fishery Research Laboratory in Norman, Oklahoma. All fish were measured for total length (TL; mm), weighed (g), dissected to remove stomachs and determine sex, and sagittal otoliths were removed for age estimation. Although sex was determined for all fish collected, only Yellow Bass collected during spring 2019 (n = 165) were used to determine age at maturity as these fish were collected during the spawning season.

Figure 2. Photograph of a Yellow Bass (240 mm TL, 245 g) collected in April 2019 from New Spiro Reservoir, Oklahoma.

Following collection, otoliths were allowed to dry for >24 hrs before processing for age estimation. To estimate age, whole otoliths (concave side up) were submerged in water to reduce glare and viewed with a stereo-microscope (capable of 130x magnification) aided by an external light source to illuminate annuli. Annuli were counted to estimate age. Each otolith was estimated independently by two readers, however if the readers disagreed on the age, a concert read was performed by both readers to determine a final age estimate. If disagreement continued the otolith were broken in a transverse plane and polished using 2000-grit wet/dry sandpaper and re-estimated following the aforementioned process. If disagreement persisted, the sample was removed from the study. Each otolith was evaluated in random order with no reference of TL, weight or sex (Hoff et al. 1997).

To evaluate Yellow Bass diets, stomachs were thawed and prey items were removed, identified, enumerated, and weighed (g). Prey items were identified to order or family for invertebrates and species for fish using taxonomic keys (Oats et al. 1993, Miller and Robison 2004, Merrit et al. 2008, Traynor et al. 2010).

Analysis

Size structure of the Yellow Bass in New Spiro Reservoir was described with length-frequency histograms and by calculating proportional size distribution (PSD) using the

size categories described by Anderson and Gutreuter (1983; stock ≥ 100 mm, quality ≥180 mm, preferred ≥230 mm, memorable ≥280 mm). A simple linear regression was used to describe the relationship between $\log_{10}$ weight and $\log_{10}$ length. The relationship of Yellow Bass length to weight was also used to evaluate fish condition by calculating relative weight (W_r) using the standard weight equation (W_s = -5.142 + 3.133($\log_{10}$ TL)) presented by Bister et al. (2000).

Mean length at age was calculated for male and female Yellow Bass. These data were then log transformed to ensure a linear relationship, and differences in growth between sexes was tested using analysis of covariance (ANCOVA). Since growth between sexes was similar ($F_{2, 255}$ = 0.097, P = 0.09) all fish were combined to describe growth using a von Bertalanffy growth model. Total annual mortality of Yellow Bass was estimated using a weighted catch-curve-regression where the slope (instantaneous total mortality [Z]) of the relationship between numbers of fish caught ($\log_e$ transformed) at each age was used to estimate total annual mortality ($A = 1 - e^{-Z}$; Ricker 1975). Age-0 Yellow Bass were removed from catch-curve analysis because they were not fully recruited to the sampling gears. Yellow Bass diets were described using percent occurrence, percent composition by number, and percent weight by season (spring and fall; Bowen 1996).

Results

Population dynamics

A total of 257 Yellow Bass were collected for population assessment. Yellow Bass ranged from age-0 to age-3 and from 81 to 302 mm TL (Figure 3). Slightly more male (54%) than female (46%) Yellow Bass were represented in the sample. The majority (76%) of Yellow Bass reached maturity by age-1 and 100% of Yellow Bass were mature by age-2. The New Spiro population was dominated (74%) by stock-size fish, resulting in a relatively low PSD_q value of 19, but a small proportion of the population reached preferred and memorable sizes (Table 1). The weight-length relationship of Yellow Bass was $\log_{10}$ (W) = 0.2997 ($\log_{10}$ TL) + 1.68 and was highly explanatory (r^2 = 0.99, P < 0.01; Figure 4). Yellow Bass W_r averaged 94 but this value increased with fish size (substock = 90, stock = 92, quality = 98, preferred = 105,– memorable = 114). The von Bertalanffy growth model indicates that Yellow Bass approach L_∞ (281 mm TL) rapidly (k = 0.80), with individuals in the population reaching approximately 70% of the L_∞ by age-1 and 90% of L_∞ by age-2 (Figure 5). The estimated total annual mortality was high (83%) resulting in low longevity (3 years) (Figure 6).

Diet

A total of 257 (92 in the fall and 165 during spring) Yellow Bass were used for stomach content analysis. More Yellow Bass in the fall had empty stomachs (33 of 92; 36%) than those

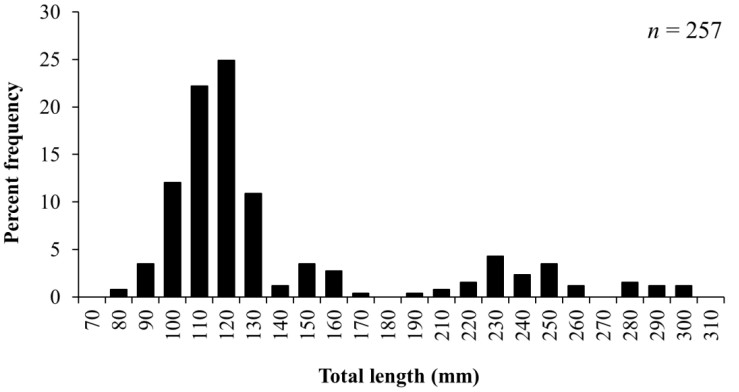

Figure 3. Length frequency histogram utilizing both fall and spring captured Yellow Bass collected from New Spiro Reservoir, Oklahoma.

Table 1. Proportional size distribution (PSD), mean age, and relative weight (Wr) by size category for Yellow Bass collected from New Spiro Reservoir, Oklahoma.

Size Category	*n*	PSD Value (95% CI)	Mean Age (range)	Mean W_r (95% CI)
Sub-stock	20	N/A	0 (0 - 1)	90 (84 - 97)
Stock (≥ 100 mm)	191	81 (73 - 88)	1 (0 - 1)	92 (90 - 93)
Quality (≥ 180 mm)	14	19 (12 - 10)	2 (1 - 3)	98 (94 - 102)
Preferred (≥ 230 mm)	25	14 (5 - 16)	2 (1 - 3)	105 (101 - 109)
Memorable (≥ 280 mm)	7	3 (0 - 6)	2 (2 - 3)	114 (109 - 119)
Overall	257	N/A	1 (0 – 3)	94 (92 – 96)

captured in spring (17 of 165; 10%). Yellow Bass consumed 19 different prey types during spring, but only 9 during fall (Table 2). Yellow Bass were primarily planktivorous regardless of season (86% in fall; 94% in spring).

During fall, zooplankton dominated the diets of Yellow Bass by percent occurrence (63%) and by number (86%), followed by invertebrates (O_i = 21% and N_i = 14%), and fish (O_i = 16% and N_i = 0.12%). However, fish contributed more by weight (64%) than invertebrates and zooplankton that contributed similarly (18%; Table 2). Individually, Copepods (61%) contributed the most to diets of Yellow Bass by percent occurrence followed by Diptera (19%), and shad spp. (13%). All other diet items contributed < 10% by occurrence (Table 2). By number, Copepods were found most often (84%), followed by Diptera (11%). All other prey items represented < 10% by number. Shad (*Dorosoma* spp.) contributed the most by weight (53%) to Yellow Bass diets, followed by Diptera and Copepods (17%), and unidentified fish (11%). The remaining prey items contributed < 10% by weight (Table 2).

Yellow Bass diets were dominated by zooplankton in the spring, contributing the most by occurrence (141%), number (94%), and weight (73%; Table 2). Cladocerans dominated the spring diets of Yellow Bass by percent occurrence (125%), followed by Copepods (14%), Diptera (4%), algae (3.6%), fish eggs (2.5%), and Ostracods (2.3%). All other diet items contributed < 5% by occurrence (Table 2). Cladocerans dominated diets of Yellow Bass by number (86%), followed by Copepods (7%) and fish eggs (2.2%). All other prey items represented < 3% by number. Cladocerans contributed the most by weight (66%) to Yellow Bass diets, followed by Diptera (12%), fish eggs (6.7%), Copepods (6%), and algae (3.3%). The remaining prey items contributed < 3% by

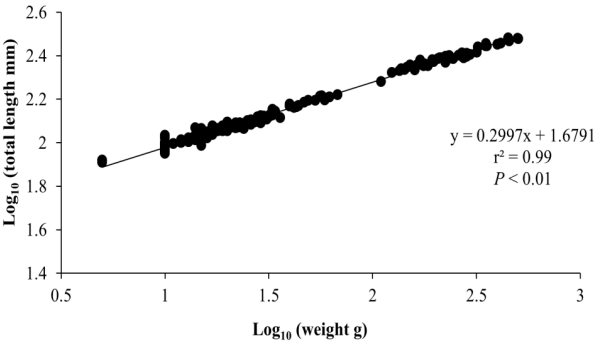

Figure 4. Weight-length relationship for 257 Yellow Bass captured from fall and spring sampling from New Spiro Reservoir, Oklahoma. The logarithmically-transformed weight-length equation is log10 (W) = 0.2997 (log10 TL) + 1.68.

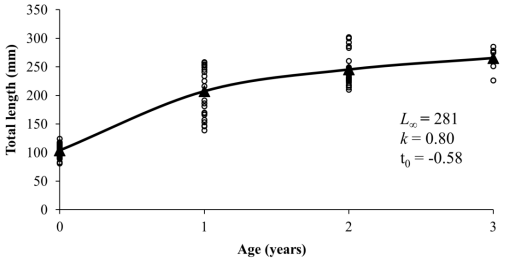

Figure 5. von Bertalanffy growth curve calculated using otolith age estimates for Yellow Bass captured in the fall and spring from New Spiro Reservoir, Oklahoma. L_∞ = predicted maximum total length, k = growth constant, and t_0 = theoretical time when total length = 0.

weight (Table 2).

Discussion

Past instances of Yellow Bass in Oklahoma were simply noted as present (e.g., Pigg and Hill 1974, Pigg et al. 1979, Pigg 1983, Pigg and Peterson 2000) but our evaluation is the first to describe population characteristics for this species in Oklahoma. We determined that Yellow Bass grew rapidly in New Spiro Reservoir, with most fish (90%) reaching L_∞ (281 mm) by age-2. Growth of Yellow Bass in New Spiro Reservoir was similar to populations from other parts of the species range (Lake Poygan, Wisconsin; Priegel 1975, Browning Oxbow, Kansas; Stein 2001, Barren River Lake, Kentucky; Zervas 2010, Little Grassy Lake, Illinois; Smith et al. 2011, Upper Baratartia Estuary, Louisiana; Fox et al. 2016) where maximum length approached 300 mm TL. However, growth of Yellow Bass in New Spiro Reservoir exceeded that of Yellow Bass in Crab Orchard Lake, Illinois (L_∞ = 209; Smith et al. 2011). Along with fast growth, Yellow Bass reached sexual maturity at young ages. We observed mature Yellow Bass starting at age-1 and, by age-2, 100% of Yellow Bass were mature. Stein (2001) reported similar maturity rates for Yellow Bass in Browning Oxbow, Kansas (87% by age-1). However, several studies did not observe mature Yellow Bass until they reached age-2 or older (Priegel 1975, Fox et al. 2016).

Catch-curve analysis revealed that total annual mortality was high (83%) for Yellow Bass in New Spiro Reservoir, which was nearly double that observed for other populations (31%, Browning Oxbow, Kansas; Stein 2001; 40%, Barren River Lake, Kentucky; Zervas 2010, 44%, Little Grassy Lake, Illinois; 31%, Crab Orchard Lake, Illinois; Smith et al. 2011). As a result, longevity of Yellow Bass in New Spiro Reservoir was low (age-3), which is considerably lower than previously documented for other populations (6-11 years; Priegel 1975, Stein 2001, Zervas 2010, Smith et al. 2011). Likely, water temperature in the southern climate of New Spiro plays a role in longevity. For example, although the critical thermal maximum is unknown for Yellow Bass, Neill and Magnuson (1974) determined water temperature preference for Yellow Bass was < 29°C in Monona Lake, Wisconsin. In another southern system, upper Barataria Estuary, Louisiana, longevity of Yellow Bass was also low (4 years; Fox et al. 2016). Suggesting that prolonged periods of temperatures exceeding their preference could explain the higher mortality rates and truncated age structure of Yellow Bass in New Spiro Reservoir

Diets of Yellow Bass collected from New Spiro Reservoir consisted of mainly zooplankton, although the breadth of prey

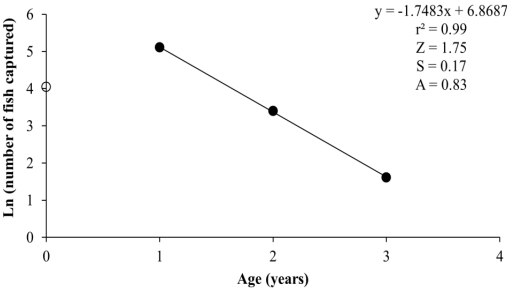

Figure 6. Catch-curve regression used to estimate total annual mortality (A) for Yellow Bass captured in the fall and spring from New Spiro Reservoir, Oklahoma. Z = instantaneous total mortality and S = total annual survival.

Table 2. Seasonal diets (spring and fall) of Yellow Bass from New Spiro Reservoir, Oklahoma described using percent occurrence (%O_i), percent composition by number (%N_i), and percent weight (%W_i).

	Fall			Spring		
Prey Items	%O_i	%N_i	%W_i	%O_i	%N_i	%W_i
Fish:						
Eggs				2.47	2.18	6.66
Gizzard Shad	13.16	0.08	52.79	0.02	0.005	0.02
Unidentified Fish	2.81	0.05	11.47	0.01	0.01	0.02
Total Fish	**15.97**	**0.12**	**64.26**	**2.49**	**2.19**	**6.69**
Invertebrate:						
Amphipod				0.05	0.01	0.01
Coleoptera				0.65	0.01	0.20
Diptera	18.56	10.81	17.08	4.07	2.10	11.85
Ephemeroptera				0.80	0.04	0.14
Grass Shrimp				0.36	0.11	0.42
Hemiptera				0.01	0.002	0.003
Odonta				0.14	0.06	0.20
Oligochaete	0.01	0.02	0.003	0.08	0.01	0.62
Tricoptera				0.33	0.05	0.11
Unidentified Invertebrate	2.76	3.01	0.65	0.66	0.03	0.35
Total Invertebrates	**21.33**	**13.84**	**17.73**	**7.15**	**2.43**	**13.91**
Zooplankton:						
Cladoceran	0.01	0.01	0.002	125.21	85.93	65.84
Copepod	60.79	84.10	17.45	13.96	6.98	6.15
Ostracod	1.43	1.62	0.46	2.25	0.66	0.57
Unidentified Zooplankton	0.47	0.30	0.10			
Total Zooplankton	**62.70**	**86.04**	**18.01**	**141.42**	**93.56**	**72.55**
Other:						
Algae				3.57	1.11	3.27
Detritus				0.40	0.14	0.07
Fishing Lure				0.32	0.002	0.09
Total Other				**4.29**	**1.25**	**3.43**

Shad spp. = Gizzard Shad and Threadfin Shad

items was higher in the spring. This is similar to other studies examining diets of Yellow Bass throughout their range. For example, Van Den Avyle et al. (1983) found that Yellow Bass fed largely on Cladocerans and Copepods, and Collier (1959) and Kraus (1963) found large numbers of Dipterans in diets. Zervas (2010) found Yellow Bass relied mostly on Chironomid larvae and pupae throughout spring, summer, and winter. In summer and in fall, Yellow Bass began feeding heavily on Copepods. For larger Yellow Bass in the spring and summer, diets consisted of young-of-year Gizzard Shad (*Dorosoma cepedianum*). Gizzard Shad were

not observed in the diets in fall, probably because of gape limitation from Gizzard Shad growth (Zervas 2010). Larger Yellow Bass are known to consume both zooplankton and small fish (Bulkley 1970), which is similar to our results. However, limited numbers of preferred size and larger Yellow Bass affected our ability to truly estimate their potential for piscivory.

It is unknown how long Yellow Bass have been in New Spiro Reservoir and could represent a robust population of a rare species in Oklahoma. However, it is equally plausible that this Yellow Bass population only recently became established in New Spiro Reservoir, such as during recent large flood events occurring in 2015. Regardless, this population should be routinely monitored to evaluate abundance trends through time and potential effects on the existing fish community. In some instances, Yellow Bass can negatively impact sport fish populations through egg predation (Driscoll and Miranda 1999) or competition for forage resources (Stein 2001) and our observations of fish eggs in the diets suggest this may be happening to some unknown degree. By completing the objectives of this study, we have established important baseline population dynamic and diet information against which future Yellow Bass populations in Oklahoma can be compared.

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A New Host and Geographic Record for *Paracapillaria sonsinoi* (Nematoda: Capillariidae) from Timber Rattlesnake, *Crotalus horridus* (Serpentes: Crotalidae) from Oklahoma

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Abstract: Compared to other members of the genus *Crotalus*, little is known about the helminth parasites of the timber rattlesnake, *Crotalus horridus*. Here, we report, for the first time, a species of capillariid nematode from *C. horridus* and from Oklahoma.

Introduction

The timber rattlesnake, *Crotalus horridus* L., 1758, is a heavily-bodied venomous snake that ranges from southcentral New Hampshire and the Lake Champlain region south to northern Florida and west to southeastern Minnesota and central Texas (Powell et al. 2016). In Oklahoma, *C. horridus* is found from the eastern tier of counties westward to the central part of the state (Sievert and Sievert 2011). This snake occurs in a wide variety of terrestrial habitat but typically occurs in mountainous regions but will also inhabit rocky woodland hardwood and pine hillsides, swampy wetlands, and river floodplains. Much is known about the natural history and ecology of this snake (Collins and Knight 1980). It is an ambush predator that feeds mostly on small mammals, birds, and snakes. The species is ranked S3 (vulnerable) by NatureServe (2020) in Oklahoma.

Surprisingly little is known about the helminth parasites of *C. horridus* (Ernst and Ernst 2006). A trematode and five species of nematodes have been previously reported from this host from the Catskill Mountains (New York), Louisiana, North Carolina, and Virginia (Fantham and Porter 1954; Solomon 1974; Bowman 1984; Ernst and Ernst 2006; Davis et al. 2016). More recently, McAllister et al. (2018) reported an unknown species of capillariid (egg) from *C. horridus* from Oklahoma. However, no adult helminths have been reported from *C. horridus* from Oklahoma. Here, we document a new host and geographic distributional record for a nematode from *C. horridus*.

Methods

Between June 2013 and July 2020, two adult and one juvenile *C. horridus* (snout vent length [SVL] = 750–1,030 mm) were collected in McCurtain County, Oklahoma, and examined for endoparasites. Snakes were euthanized with an intraperitoneal injection of sodium pentobarbital (Nembutal®). A midventral incision was made from the cloaca to oral cavity to expose the viscera and the gastrointestinal tract and associated organs (lungs, liver, gallbladder, gonads) were placed in individual Petri dishes containing 0.9% saline. Feces from the rectum was collected and placed in an individual vial containing 2.5% (w/v) potassium dichromate

($K_2Cr_2O_7$) and, after flotation in Sheather's sugar solution (sp. gr. 1.30), examined for coccidians by brightfield microscopy. Organ contents were examined at 20 to 30× under a stereomicroscope and parasites found were rinsed of mucus. Nematodes were fixed in near boiling water and preserved in 70% (v/v) ethanol. They were later cleared and identified in temporary mounts of lactophenol and then returned to the preservative.

Standard common and scientific names follow Crother et al. (2017). Voucher specimens of snakes were deposited in the EOSC collection, Idabel, Oklahoma. Voucher specimens of nematodes were deposited in the Harold W. Manter Laboratory of Parasitology (HWML), University of Nebraska, Lincoln, Nebraska.

Results

Eight very thin nematodes were found in the small intestine of a single *C. horridus*. No snakes were found to be passing coccidians. Information on the nematode species follows.

Nematoda: Trichuroidea: Capillariidae

Syn. *Trichosoma soninoi* Parona, 1897.

***Paracapillaria* (*Ophidiocapillaria*) *soninoi* (Parona, 1897) Moravec, 1986**

Type host: Green whip snake, *Hierophis viridiflavus* (Lacépède, 1789).

Location in type host: Intestine (Parona 1897).

Type locality: Pisa, Italy (Parona 1897).

Other hosts and localities: Viperine water snake, *Natrix maura* (L., 1758), southern France (Moravec 1986); diamondback watersnake, *Nerodia rhombifer* (Hallowell, 1852), Louisiana (Moravec 1986).

Location in other hosts: intestine and rectum; urinary bladder (Moravec 1986).

New host: Timber rattlesnake, *Crotalus horridus* L., 1758, collected on 2 July 2020, 750 mm SVL.

Specimens deposited: HWML 111651.

New locality: USA: Oklahoma: McCurtain County, Eastern Oklahoma State College campus, Idabel (33° 55' 17.1012'' N, 94° 46' 43.5612'' W).

Prevalence and intensity: 1/3 (33%); 8 female worms.

Site of infection: Intestine.

Remarks: Moravec (1986) reported that *Paracapillaria* included two subgenera, *Paracapillaria* and *Ophidiocapillaria*. Presently, three subgenera are now recognized: *Paracapillaria* Mendonça, 1963, *Ophidiocapillaria* Moravec, 1986, and *Crossicapillaria* Moravec, 2001 (Moravec 2001). Biserkov et al. (1994) disagreed with the revision by Moravec (1986) who suggested the synonymy of the species of *Paracapillaria* infecting snakes. The former concluded that the species *P. sonsinoi*, *P. mingazzinii* Rizzo, 1902, *P. colubra* Pence, 1970, *P. viperae* Biserkov, Georgiev and Genov, 1985, *P. ptyasi* Wang, 1982, *P. xochimilcensis* Caballero and Cercero, 1943, and *P. heterodontis* Harwood, 1932, be considered distinct species within the genus and we concur until molecular analysis can be conducted.

The average length of our specimens was 30 mm, eggs were near the vulva in a single row, but occasionally farther away in two rows, and eggs possessed a roughened surface. They fit the description of *P. sonsinoi* by Moravec (1986) very well.

Discussion

Other than the report of *P. sonsinoi* from the urinary bladder of *N. rhombifer* (Moravec, 1986), there are only two other species of *Paracapillaria* currently known from North American snakes. Harwood (1932) described

P. heterodontis from the rectum of eastern hog-nosed snake, *Heterodon platirhinos* Latreille, 1801 from Texas, and additional hosts include *N. rhombifer* and northern cottonmouth, *Agkistrodon piscivorus* (Lacépède, 1789) from Louisiana (Fontenot and Font 1996). Pence (1970) provided a description of *P. colubra* from the oviducts of southern black racer, *Coluber constrictor priapus* Dunn and Wood, 1939 from Louisiana. Several additional snake hosts have also been reported with *P. colubra*, including broad-banded watersnake, *Nerodia fasciata confluens* (Blanchard, 1923), plain-bellied watersnake, *Nerodia erythrogaster* (Forster, 1771), and northern watersnake, *Nerodia sipedon sipedon* (L., 1758) from North Carolina (Collins 1973). In addition, Davis et al. (2016) reported *P. colubra* from *A. piscivorus*, eastern copperhead, *Agkistrodon contortrix* (L., 1758), and *C. horridus* from North Carolina; however, they were not able to report the site of the infection from their salvaged hosts. We therefore document the first report of *P. sonsinoi* from a crotalid snake and of this nematode species from west of the Mississippi River in Oklahoma.

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A New Host and Geographic Record for *Subulura nevadense* (Nematoda: Subuluroidea: Subuluridae) from Rio Grande Ground Squirrel, *Ictidomys parvidens* (Rodentia: Sciuromorpha: Sciuridae), from West Texas

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Abstract: There is only one report of any helminth parasite from Rio Grande ground squirrels, *Ictidomys parvidens*. In April 2020, an adult *I. parvidens* was found dead on the road in Tom Green County, Texas, and examined for parasites. It was found to harbor 27 individual nematodes, *Subulura nevadense*. Here, we report *S. nevadense* for the first time from this host as well as provide a new geographic record for this nematode.

Introduction

The Rio Grande ground squirrel, *Ictidomys parvidens* (Mearns, 1896) is a small to medium-sized member of the genus that occurs in the United States in southern New Mexico and throughout much of southern and western Texas, north to almost the Red River just east of the Panhandle, and east as far as Erath and Travis counties (Schmidly and Bradley 1994; Helgen et al. 2009). It is a burrowing species inhabiting sandy and gravelly soils of the desert grasslands sometimes associated with cactus flats, mesquite, and shrub species at elevations between 200 and 3,000 m (Young and Jones 1982; Schmidly and Bradley 1994). This ground squirrel feeds on larval and adult insects, green plants, forbs, and grasses (Zimmerman 1999).

Although there is information on the natural history and ecology of this ground squirrel (as formerly included as a subspecies of Mexican ground squirrel, *I. mexicanus parvidens* (see Young and Jones 1982), there is only one report concerning its helminth parasites. Eads and Hightower (1952) reported the only endoparasite as an unknown species of "microfilaria" nematode taken from one of 14 (7%) individuals (as *Citellus mexicanus*) trapped in southwest Texas. Here, we document a new host and geographic record for a nematode from *I. parvidens*.

Methods

On 30 April 2020, a single *I. parvidens* was found dead on the road in San Angelo, Tom Green County, Texas, and immediately examined for parasites. This specimen appeared to be recently killed and showed no sign of putrefaction. The pelage was vigorously brushed over a white enamel tray in an attempt to find ectoparasites. A mid-ventral incision was made from the anus to throat to expose the viscera and the gastrointestinal tract and associated organs were placed in individual Petri dishes containing 0.9% saline. Feces from the rectum was collected and placed in an individual vial

containing 2.5% (w/v) potassium dichromate ($K_2Cr_2O_7$) and, after flotation in Sheather's sugar solution (sp. gr. 1.30), examined for coccidians and parasite ova by brightfield microscopy. Visceral contents were examined at 20 to 30× under a stereomicroscope and parasites found were rinsed of mucus. Nematodes were fixed in near boiling water and preserved in 70% (v/v) ethanol. They were later cleared and identified in temporary mounts of lacto-phenol and then returned to the preservative.

A host photovoucher was deposited in the EOSC collection, Idabel, Oklahoma. Voucher specimens of nematodes were deposited in the Harold W. Manter Laboratory of Parasitology (HWML), University of Nebraska, Lincoln, Nebraska.

Results

Twenty-seven nematodes were found in the small intestine and cecum of this ground squirrel. There were no ectoparasites present nor was this individual passing coccidian oocysts. Information on the nematode species follows.

Nematoda: Subuluroidea: Subuluridae

Subulura nevadense Babero, 1973 (Fig. 1)

Type host(s): White-tailed antelope squirrel, *Ammosphermophilus leucurus* (Merriam, 1889). Round-tailed ground squirrel, *Xerospermophilus tereticaudus* (Baird, 1858); Babero (1973) did not designate either species as type host.

Type specimen: USNM Helm. Coll. No. 70547.

Type localities: Clark, Lincoln, and Nye counties, Nevada; Babero (1973) did not designate any site within these counties as the type locality.

Prevalence and intensity: Not given (Babero 1973).

Site of infection: Cecum.

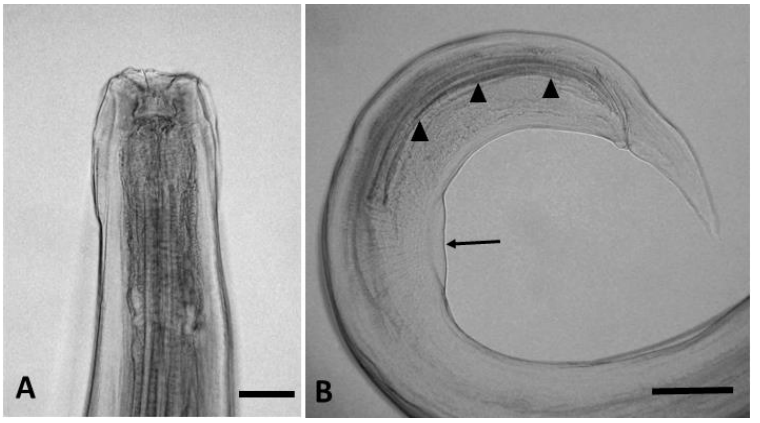
Other host and locality: Richardson's ground squirrel, *Urocitellus richardsonii* (Sabine, 1822), Beaverhead County, Montana (see Ubelaker et al. 2007).

New host: Rio Grande ground squirrel, *Ictidomys parvidens* (Mearns, 1896), adult lactating female.

Specimens deposited: HWML 112104.

Locality: USA: Texas: Tom Green County, San Angelo off Country Club Road (31° 22' 15.6252'' N, -100° 28' 22.1448'' W).

Prevalence and intensity: 1/1 (100%); 18 fourth-stage larvae, six females, and 3 males.



Figures 1A-B. *Subulura nevadense* from *Ictidomys parvidens*. (A) Female, anterior end showing buccal capsule; scale bar = 50 µm. (B) Posterior end of male showing spicules (arrowheads) and preanal sucker (arrow); scale bar = 200 µm.

Site of infection: Small intestine and cecum.

Remarks: Gravid females were 30 mm long and a single male was 20 mm long; spicule length ranged from 795 to 975 µm (mean 910) in three males. These measurements fit the description of *S. nevadense* given by Babero (1975).

Discussion

There are only two recognized species of *Subulura* from North American ground squirrels, *S. nevadense* and *S. novomexicanus* Ubelaker, Easter-Taylor, Marshall, and Duszynski, 2007 (Ubelaker et al. 2007). The latter was described from spotted ground squirrel, *Xerospermophilus spilosoma* Bennett, 1833, from Socorro County, New Mexico (Ubelaker et al. 2007, 2010). It differs from *S. nevadense* in being longer, having a larger egg size, and smaller spicules.

Acknowledgments

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A New Geographic Record and Reproduction of the Ouachita Mountain Crayfish, *Fallicambarus tenuis* (Decapoda: Cambaridae), from Southeastern Oklahoma

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Abstract: Little is known about the natural history and ecology of the Ouachita Mountain Crayfish, *Fallicambarus tenuis*. Here, we report a new geographic distribution record as well as some information on reproduction of this rare crayfish in Oklahoma.

Introduction

Crayfishes are important members of the invertebrate biodiversity of Oklahoma. Several new records and miscellaneous natural history information have been provided by the current authors or our research team over the last decade or more (Robison and McAllister 2006, 2008; Robison et al. 2009, 2018; McAllister et al. 2011a, b, 2016, 2019; McAllister and Robison 2014, 2016). We continue to provide new geographic records for crayfishes of the state, including here a new geographic record as well as novel reproductive data for a relatively uncommon species.

Crayfishes were collected by hand and aquatic dip net and preserved in 70% isopropyl alcohol. Voucher specimens were deposited in the Southern Arkansas University (SAU) Invertebrate Collection, Magnolia, Arkansas.

The collections are reported below in an annotated format as follows.

Decapoda: Cambaridae (cambarid crayfishes)

***Fallicambarus tenuis* (Hobbs, 1950) –** Ouachita Mountain Crayfish. Recently, the taxonomy of freshwater crayfishes was updated based on the last two decades of phylogenetic studies that have called into question family, subfamily, genus, and subgenus affiliations for various taxa (Crandall and De Grave 2017). The rare to uncommon Ouachita Mountain crayfish was moved taxonomically from the genus *Procambarus* into the genus *Fallicambarus*, thus becoming *Fallicambarus tenuis* (Ainscough et al. 2013; Robison et al. 2017).

Little is known about the biology of *F. tenuis*. Bergey et al. (2005) and Morehouse and Tobler (2013) reported this species is rare within its range. The species inhabits first and second order spring-fed streams and cool, clear perennial streams where it lives beneath rocks (Jones and Bergey 2007; Robison and McAllister 2008). Form I males are known from May and June; however, these authors reported no ovigerous females of the species. A single ovigerous female *F. tenuis* was captured on 24 March 2000 beneath rocks in a small first order tributary stream of the Kiamichi River, ca. 75 yards E of the Kiamichi River bridge at US 259, S of Big Cedar, Le Flore County (34° 38’ 13.848’’ N 94° 39’ 13.4892’’ W). This ovigerous female was carrying 34 eggs.

The entire range of *P. tenuis* includes the Arkansas, Ouachita, and Red River basins of eastern Oklahoma and western Arkansas (Hobbs 1989; Robison and McAllister 2008). In Oklahoma, *F. tenuis* has been previously reported from three counties, including Le Flore, Pittsburg, and Pushmataha (Robison and McAllister 2008) (Fig. 1). Morehouse and Tobler (2013) provided an ecological niche model for *F. tenuis* which indicated that suitable environmental conditions may be available in Atoka, Haskell, Latimer, and McCurtain counties. More recently, Dyer and Brewer (2018) reported that *P. tenuis* was a rare species that occurred exclusively in erosional channel units. *Procambarus tenuis* has been found burrowing adjacent to and within clear cool springs and streams, and also inhabits permanent flowing streams under rocks in exhibiting qualities of both a secondary and tertiary burrower (Hobbs 1989; Jones and Bergey 2007). We report a single form II male *F. tenuis* collected from McCurtain County, collected on 25 March 2000 beneath rocks in a ditch along St. Hwy. 4, ca. 2.9 km SW of Smithville (34° 27' 43.1136" N 94° 38' 7.7712" W). This finding documents the first report of this crayfish species from this county (Fig. 1), thus extending the Oklahoma range southward into northeastern McCurtain County.

Lastly, *P. tenuis* is currently listed by NatureServe (2020) as S1 (critically imperiled) in Oklahoma. It is unknown whether or not there are any major threats impacting *F. tenuis*. However, populations may perhaps be undergoing localized declines due to urbanization, alterations to the hydrological regime, and water pollution.

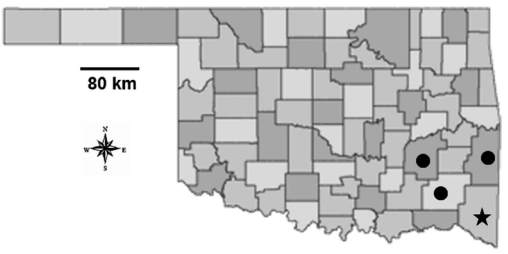

Figure 1. Distribution of *Fallicambarus tenuis* in Oklahoma. Dots = previous records; star = new record.

Acknowledgments

The Oklahoma Department of Wildlife Conservation issued a Scientific Collecting Permit (No. 1551546) to CTM.

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First Report of *Eimeria hydrophis* (Apicomplexa: Eimeriidae) from Plain-Bellied Watersnake, *Nerodia erythrogaster* (Serpentes: Colubridae: Natricinae), from Oklahoma

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Abstract: There are several reports of coccidian parasites (Apicomplexa) from plain-bellied watersnakes, *Nerodia erythrogaster* collected from various locales in Arkansas and Texas. However, there are no reports of coccidians from this host or any other watersnake in Oklahoma. Here, we document, for the first time, *Eimeria hydrophis* from a *N. erythrogaster* collected in Oklahoma, as well as provide the first photomicrographs of the coccidian.

Introduction

The plain-bellied watersnake, *Nerodia erythrogaster* (Forster, 1771) is a large, heavily-bodied reptile that ranges from the Delmarva Peninsula south to northern Florida and westward to Oklahoma, New Mexico, Texas, and adjacent México; there are isolated populations in the upper Midwest (Powell et al. 2016). In Oklahoma, this snake is found statewide (Sievert and Sievert 2011). It inhabits a wide variety of wetlands, but mostly larger, more permanent watersheds, river bottoms, floodplains, marshes, sloughs, swamps, and the edges of man-made ponds and lakes (Gibbons and Dorcas 2004). The taxon was formerly recognized as having four subspecies; however, using mitochondrial data, Makowsky et al. (2010) found little support for subspecific recognition and concluded this taxon represents a single, widespread species. We therefore follow Crother et al. (2017) in not recognizing any subspecies.

Several coccidian parasites have been previously reported from *N. erythrogaster* (Table 1), including *Eimeria attenuata* Wacha and Christiansen, 1974, *E. conanti* McAllister and Upton, 1989, *E. cyclopion* McAllister, Upton, and Trauth, 1990, an *E. helmisophis*-like coccidian, *E. hydrophis* Wacha and Christiansen, 1974, *E. natricis* Wacha and Christiansen, 1974, *E. sipedon* Wacha and Christiansen, 1975, and *E. tenuis* Upton and McAllister, 1988 (Duszynski and Upton 2009; McAllister et al. 2017). Here, we document a new geographic record for *E. hydrophis* from a *N. erythrogaster* collected from Oklahoma, and provide the first photomicrographs of the coccidian.

Methods

Between April 2012 and August 2020, four *N. erythrogaster* (660–745 mm snout-vent length [SVL]), two broad-banded watersnakes, *Nerodia fasciata confluens* (Blanchard, 1923) (178–223 mm SVL), and five northern diamond-backed watersnakes, *Nerodia rhombifer rhombifer* (Hallowell, 1852) (655–785 mm SVL) were collected from several sites in McCurtain County, and examined

for coccidians. They were killed with an intraperitoneal injection of sodium pentobarbital (Nembutal®). A mid-ventral incision was made and feces from the rectum was collected and placed in an individual vials containing 2.5% (w/v) potassium dichromate ($K_2Cr_2O_7$). Because the oocyst walls of some watersnake coccidians are known to wrinkle in sugar solutions used for flotation (Wacha and Christiansen 1974), an initial flotation was done in Sheather's sugar solution (specific gravity = 1.30), the coverslip was removed, and then rinsed with tap water. The sample was centrifuged (430×g for 10 min.) and wet mounts were examined for coccidia using an Olympus BX43 microscope with Nomarski DIC. Measurements were taken on 10 sporulated oocysts using a calibrated ocular micrometer and Lumenera Infinity Analyze software (Teledyne Lumenera, Ottawa, Ontario, Canada) and reported in micrometers (µm) with the means followed by the ranges in parentheses; photographs were taken using brightfield optics. Oocysts were 60 days old when measured and photographed. Descriptions of oocysts and sporocysts follow the standard guidelines of Wilber et al. (1998) including: oocyst length (L) and width (W), their ranges and ratios (L/W), micropyle (M), oocyst residuum (OR), polar granule(s) (PG), sporocyst length (L) and width (W), their ranges and ratio (L/W), sporocyst (SP), Stieda body (SB), substieda body (SSB), parastieda body (PSB), sporocyst residuum (SR), sporozoites (SZ) anterior (ARB) and posterior (PRB) refractile bodies, and nucleus (N).

Standard common and scientific names follow Crother et al. (2017). A host voucher was deposited in the EOSC collection, Idabel, Oklahoma. Photovouchers of coccidia were deposited in the Harold W. Manter Laboratory of Parasitology (HWML), University of Nebraska, Lincoln, Nebraska.

Results

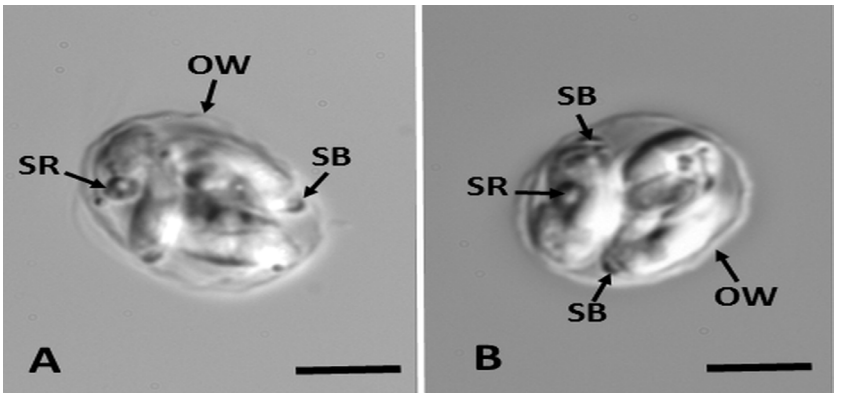
A single *N. erythrogaster* was found to be passing coccidia; none of the other 10 watersnakes were infected. Detailed information on the sample follows.

Apicomplexa: Eimeriidae

Eimeria hydrophis Wacha and Christiansen, 1974 (Fig. 1)

Description: Oocyst shape: subspheroidal to ellipsoidal; number of walls: 2; characteristics: smooth, walls wrinkle readily even in diluted Sheather's sugar solution; (L × W) (n = 10): 13.4 × 11.5 (11–16 × 10–13), L/W ratio: 1.2 (1.1–1.4); M, OR, PG: all absent. Sporocyst shape: ellipsoidal; (L × W) (n = 10): 9.6 × 4.8 (7–12 × 4–5), L/W 2.0 (1.6–2.4); flattened SB present; SSB, PSB: both absent; SR: present; composed of small, compact sphere of granules; SZ: Sausage-shaped (not measured); ellipsoidal ARB and PRB present, N in middle of SZ.

Type host: Northern watersnake, *Nerodia sipedon sipedon* (L., 1758).



Figures 1A-B. Sporulated oocysts of *Eimeria hydrophis*. Abbreviations: OW (oocyst wall); SB (Stieda body); SR (sporocyst residuum). Note wrinkled OW. Scale bars = 5 µm.

Table 1. Coccidians reported from plain bellied watersnake, *Nerodia erythrogaster*.*

Coccidian	Prevalence†	State	Reference(s)
Eimeria attenuata	2/20 (10%)	Arkansas	McAllister et al. (1995)
	2/23 (9%)	Texas	McAllister and Upton (1989)
E. conanti	2/20 (10%)	Arkansas	McAllister et al. (1995)
	2/23 (9%)	Texas	McAllister and Upton (1989)
E. cyclopion	1/1 (100%)	Arkansas	McAllister et al. (1990)
	2/9 (22%)	Arkansas	McAllister et al. (2017)
E. helmisophis-like‡	3/20 (15%)	Texas	McAllister and Upton (1989)
	5/20 (25%)	Arkansas	McAllister et al. (1995)
	1/2 (50%)	Texas	McAllister et al. (1995)
E. hydrophis	2/20 (10%)	Arkansas	McAllister et al. (1995)
	1/4 (25%)	**Oklahoma**	**This report**
	2/23 (9%)	Texas	McAllister and Upton (1989)
E. natricis	2/20 (10%)	Arkansas	McAllister et al. (1995)
E. sipedon	11/20 (55%)	Arkansas	McAllister et al. (1995)
	3/23 (13%)	Texas	McAllister and Upton (1989)
E. tenuis	2/22 (9%)	Arkansas	McAllister et al. (1995)

*Includes subspecies previously known for *N. erythrogaster*, including yellow-belly watersnake (*N. e. flavigaster*) and blotched watersnake (*N. e. transversa*).

†Number infected/number examined (%).

‡It is doubtful this coccidian is the same species found in worm snakes (*Carphophis* spp.) (see McAllister et al. 1995).

Type specimen: None deposited by Wacha and Christiansen (1974).

Type locality: USA: Iowa: Louisa County, 5 mi S Muscatine at Windy Hills Shooting Refuge.

Prevalence: 5/14 (36%).

Site of infection: Intestine.

Other hosts and localities: *N. erythrogaster*, Johnson County, Texas (McAllister and Upton 1989); midland watersnake, *Nerodia sipedon pleuralis* (Cope, 1892), Arkansas (McAllister et al. 1995); Brazo's River watersnake, *Nerodia harteri* (Trapido, 1941), Somervell County, Texas (McAllister and Upton 1989); *N. s. sipedon*, Bremer, Louisa, and Van Buren counties, Iowa, *N. r. rhombifer* (Hallowell, 1852), Muscatine County, Iowa (Wacha and Christiansen 1974, 1975).

Present host: *N. erythrogaster*.

New locality: USA: Oklahoma: McCurtain County, Hochatown (34° 09' 55.152" N, 94° 45' 35.8776" W).

Specimen deposited: HWML photovoucher 216631.

Prevalence: 1/11 (9%) overall; 1/4 (25%) *N. erythrogaster*.

Site of infection: Oocysts found in feces; bile contents did not contain oocysts. Wacha and Christiansen (1974) also recovered oocysts of *E. hydrophis* only in feces, and not bile, which indicated the site of endogenous development was the intestinal tract.

Remarks: Oocysts from the present sample were not significantly different than those described originally by Wacha and Christiansen (1974) from *N. s. sipedon* from Iowa. Our oocysts were, on average, slightly smaller in length (13.4 × 11.5 *vs.* 15.4 × 10.9 µm) but sporocysts were nearly identical in average sizes (9.6 × 4.8 *vs.* 10.3 × 4.9 µm) as well as all other morphological and mensural characteristics fitting *E. hydrophis*.

Discussion

There are several comprehensive surveys of various North American watersnakes for coccidian parasites from the surrounding states of Arkansas and Texas as well as Iowa (see summary in Duszynski and Upton 2009). Prevalence of infection in eight coccidians of *N. erythrogaster* is usually low depending on the eimerian species found and is 43 of 270 (16%) overall (Table 1).

In conclusion, a modest sample of three species have been examined to date for coccidians in Oklahoma, and information on a single infected host has been provided herein. As all four of the *Nerodia* spp. that occur in the state have been reported to harbor several coccidians from other states (Duszynski and Upton 2009), future surveys will surely provide new geographic distribution records as well as the possibility of discovering new species as additional Oklahoma watersnakes are examined.

Acknowledgments

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Two New Handsome Fungus Beetle (Coleoptera: Endomychidae: Lycoperdininae, Epopocinae) Records for Oklahoma

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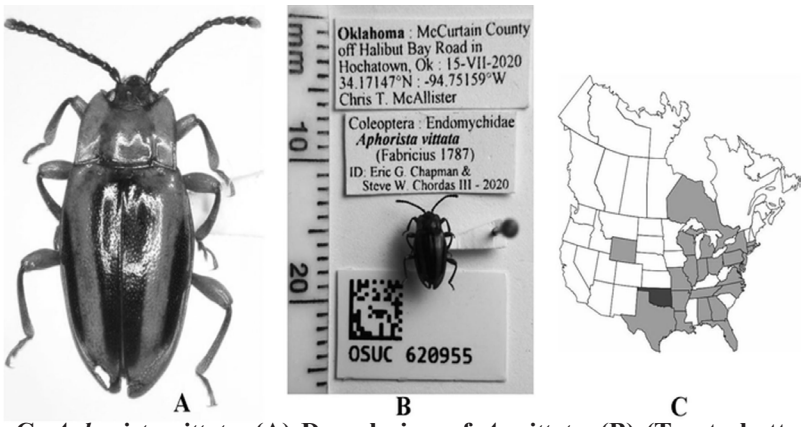
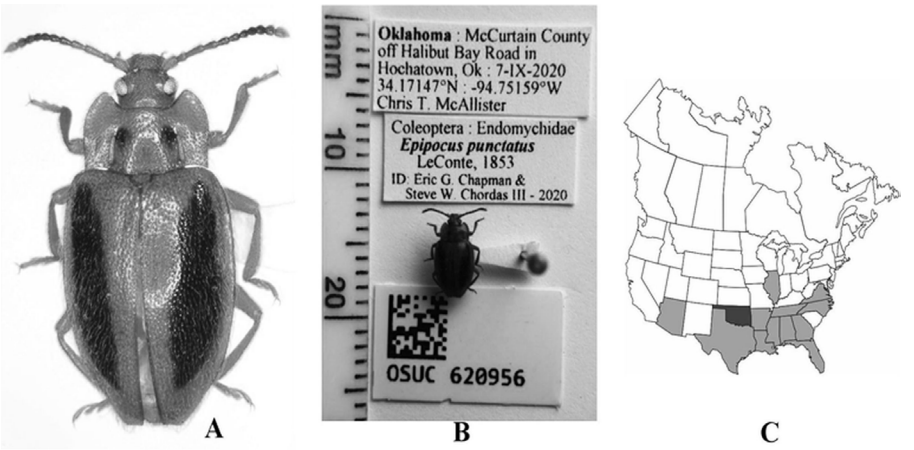
Insects belonging to the order Coleoptera include more described species (> 400,000) than any other group of organism on Earth. As such, they make up 40% of all insect species described to date, which is also about 25% of all animals (McHugh and Liebherr 2009).

The handsome fungus beetles of the family Endomychidae currently includes approximately 130 genera and 1,782 species and subspecies arranged among 12 subfamilies with the highest diversity in tropical and subtropical areas of Africa, Asia and the Americas (Shockley et al. 2009a, b). In terms of diversity in Oklahoma, there are 10 genera and 12 species in the family (http://entoweb.okstate.edu/museum/coleoptera/Endomychidae.htm). These beetles are relatively small to moderately sized, mostly reddish-brown in color and usually with contrasting markings on the pronotum and/or elytra. The subfamily Lycoperdininae constitutes the largest subfamily of Endomychidae, containing 38 genera and over 635 described species (Tomaszewska 2005).

During July and September 2020, two beetles were collected below a night light at a residence in Hochatown, McCurtain County. They were transferred to individual vials containing 70% (v/v) ethanol. Voucher specimens were deposited in the C. A. Triplehorn Collection at The Ohio State University, Columbus, Ohio. Dorsal habitus images of each species (Figs. 1A, 2A) were created via stacking digital



Figures 1A–C. *Aphorista vittata*. (A) Dorsal view of *A. vittata*. (B) (Top to bottom) location label, identification label, voucher specimen, unique museum number and code, scale bar in millimeters (mm) on left side of each image. (C) Distribution of *A. vittata* in North America. Light shade = prior literature records (Shockley et al. 2009); dark shade = new state record.



Figures 2A–C. *Epipocus punctatus*. (A) Dorsal view of *E. punctatus*. (B) (Top to bottom) location label, identification label, voucher specimen, unique museum number and code, scale in millimeters (mm) on left side of each image. (C) Distribution of *E. punctatus* in the United States. Light shade = prior literature records (Shockley et al. 2009); dark shade = new state record.

photographs (using CombineZP) of the curated voucher specimens captured with a Cannon EOS DLSR through an Olympus SZ60 dissecting microscope processed with Corel PaintShopPro 2021 (Corel Corporation 2020). Maps of literature records (Figs. 1C, 2C) were created with CorelDraw 2019 (Corel Corporation 2019), museum and data labels with voucher specimen and millimeter (mm) scale (Figs. 1B, 2B) were captured using a 10× close-up lens attachment on a Cannon EOS DLSR.

One beetle was identified as *Aphorista vittata* (Fabricius, 1787) (Fig. 1A) with the following collection data: ***Oklahoma***: McCurtain County, off Halibut Bay Road in Hochatown (34° 10' 17.0286"N, 94° 45' 5.7414"W); 15 VII 2020; C. T. McAllister (CTM), collector (unique museum specimen code: OSUC 620955) (Fig. 1B). Habitat of the area included various pines (*Pinus* spp.) and hardwoods (*Quercus* spp.) situated in the Southern Ouachita Mountain uplands.

Aphorista vittata is a species of handsome fungus beetle that occurs in North America and Southern Asia. It is a small to moderately-sized (5.5 to 8.0 mm), bright and attractively colored orange to brownish red mycetophagous beetle with elongate, tapering black stripes on the elytra down to the suture and a long black spot on each side; its front coxae are globular and distinctly separated (Tomaszewska 2005). Adults are attracted to light and often associated with wood rotting basidiomycete fungi of the family Boletaceae (Tomaszewska 2005; Ferreira 2016). This beetle has now been recorded from Ontario, Canada, and the following US states: Alabama, Arkansas, Connecticut, Delaware, Florida, Georgia, Illinois, Indiana, Louisiana, Massachusetts, Maryland, Michigan, Missouri, North Carolina, New Jersey, New York, Ohio, **Oklahoma (new state record)**, Pennsylvania, Rhode Island, Tennessee, Texas, Wisconsin, Wyoming, Virginia, and Washington, D.C. (Shockley et al. 2009a; Fig. 1C). In addition, Shepard (1983) reported three species of endomychids from the state but not *A. vittata*. Because this beetle has not been previously documented from Oklahoma, we report *A. vittata* here as a new geographic record for the state.

The other beetle was a handsome fungus beetle, *Epipocus punctatus* LeConte, 1853 (Fig. 2A) with the following collection data: ***Oklahoma***: McCurtain County, off Halibut Bay Road in Hochatown (34° 10' 17.0286"N, 94° 45' 5.7414"W); 6 IX 2020; CTM, collector (unique museum specimen code: OSUC 620956) (Fig. 2B). It belongs to the *tibialis* group and ranges in length from 4.8 to 7.3 mm, the ventral surface is entirely red, and possesses a pronotum with

narrow margins and two black spots and an M-shaped mark on its disc (Strohecker 1977). This beetle has been previously reported from Costa Rica, El Salvador, Guatemala, Honduras, México, Panama, and now 13 U.S. states, Alabama, Arkansas, Arizona, Florida, Georgia, Illinois, Louisiana, Mississippi, North Carolina, **Oklahoma (new state record)**, Tennessee, Texas, and Virginia (Fig. 2C). In México, specimens of *E. punctatus* have been collected from hard bracket poroid fungus (Polyporaceae) and banana debris (Arriaga-Varela et al. 2007).

Several species of handsome fungus beetles (including *A. vittata*) tend to orient to filter paper baited with cantharidin (2,6-dimethyl-4,10-dioxatricyclo-[5.2.1.0] decane-3,5-dione), an odorless, colorless, defensive compound produced by most meloid and some oedemerid beetles (Price and Young 2006). However, the role of cantharidin in the biology of endomychids is not known but one thought is it may mimic other terpenoid compounds in the environment, such as terpenoid fungal metabolites that may promote beetles in locating fungal hosts (Young 1984). Therefore, collection methods for endomycids should include cantharidin-baited pitfall traps. Using this sampling technique could increase the number of taxa of handsome fungus beetles reported from Oklahoma.

Acknowledgments

The Oklahoma Department of Wildlife Conservation issued a Scientific Collecting Permit to CTM. We thank Dr. Eric G. Chapman (University of Kentucky) for confirming the identity of these beetles.

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First Report of the Plant Bugs *Phytocoris depictus*, *Phytocoris fumatus* and *Phytocoris tricinctipes* (Hemiptera: Miridae) for Oklahoma

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Abstract: Hemipterans belong to the largest order of hemimetabolous insects. The number of species in the order is about 75,000, with a great diversity of forms, including the largest family, Miridae (plant bugs). It contains major insect pests and predatory groups that can be used as biological control agents. Here, we document three species of Miridae in Oklahoma, for the first time.

Introduction

Over the last decade, several new bug (Hemiptera) records have been reported for Oklahoma, including multiple species reported by the authors (see Chordas and McAllister 2012, 2016, 2018, 2019; Chordas et al. 2017; McAllister and Robison 2017). With over 200 species known for the United States north of México (Henry and Wheeler 1988), the genus *Phytocoris* Fallén, 1814 (Hemiptera: Miridae) is the most specious of the plant bugs. Taxonomic works including phytocorids are somewhat limited to regional or local treatments (none specific to Oklahoma) rendering the phytocorids, at times, extremely challenging to identify. As such, some specimens need to be evaluated using multiple references and the original description for genuine identification. Over the past few years we were able to identify three *Phytocoris* species not previously reported for the state. Here, we document three new geographic distributional records for these *Phytocoris* species for Oklahoma.

Methods

Miridae were collected with an insect aspirator under a porch light or from black light pan traps at a residence in Hochatown, McCurtain County (34° 10’ 17.0286”N, 94° 45’ 5.7414”W). Habitat of the area consisted of various hardwoods (*Quercus* spp.) and pines (*Pinus* spp.) in uplands bordering the Ouachita National Forest. Specimens were placed in individual vials containing 70% (v/v) ethanol. Blatchley (1926), Knight (1923, 1941, 1968), and Stonedahl (1988) were consulted for species identifications. Henry and Wheeler (1988), Knight (1941, 1968), Maw et al. (2000), Ratnasingham and Hebert (2007) and Stonedahl (1988) were used as distributional references. Further, verifiable records from BugGuide.net (2020) and iNaturalist (2020) were also incorporated. Voucher specimens (Figs. 1A–3A) were deposited in the C. A. Triplehorn Collection at The Ohio State University, Columbus, Ohio. Images of male genital claspers and associated terminal abdominal structures (Figs. 2D, 3D) and dorsal habitus of each species (Figs. 1A–3A) were created via stacking digital photographs (using CombineZP)

of the curated voucher specimens captured with a Cannon EOS DLSR through an Olympus SZ60 dissecting microscope processed with Corel PaintShopPro 2021 (Corel Corporation 2020). Maps of literature records (Figs. 1C–3C) were created with CorelDraw 2019 (Corel Corporation 2019), museum and data labels with voucher specimen and millimeter (mm) scale (Figs. 1B–3B) were captured using a 10× close-up lens attachment on a Cannon EOS DLSR.

New Records (Hemiptera: Miridae: Mirinae: *Phytocoris*)

Phytocoris depictus Knight, 1923 (Plate 1). A single male of this colorful species was collected on 8-IX-2020 (data label Plate 1B; unique museum code of voucher = OSUC 620954). Predominantly an eastern species in North America associated with oaks, *Quercus* spp. (Knight 1941). *Phytocoris depictus* is now known from Ontario and Québec, Canada, and the following 15 US states: *Connecticut* (via iNaturalist) Illinois, *Maryland* (via BugGuide), *Massachusetts* (via iNaturalist), Minnesota, *Mississippi* (Ratnasingham and Hebert [2007] BOLD SIHET515-13), Missouri, *New Jersey* (via BugGuide), New York, Ohio, **Oklahoma (new state record)**, *Pennsylvania* (via BugGuide), *Tennessee* (Ratnasingham and Hebert [2007] BOLD SIHET516-13), *Virginia* (via BugGuide), Wisconsin (Plate 1C) and Washington, DC.

Phytocoris fumatus Reuter, 1909 (Plate 2). A single male was taken on 11-V-2018 (data label Plate 2B; unique museum code of voucher = OSUC 620953). Left clasper and tubercle above clasper of male are shown in Plate 2D. With a spotty distribution in mid- and eastern North America (north of México), we did not anticipate finding this species in Oklahoma. *Phytocoris fumatus* is now known from Nova Scotia, Canada, and the following 12 US states: *Florida* (Ratnasingham and Hebert [2007] BOLD SIHET521-13), Georgia, Illinois, Maryland, Massachusetts, Missouri, New Jersey, New York, North Carolina, North Dakota, **Oklahoma (new state record)**, Pennsylvania (Plate 2C), and Washington, DC. We could not locate any ecological data for this species.

Phytocoris tricinctipes Knight, 1968 (Plate 3). As far as we could determine, this species has not been reported in any work since Stonedahl (1988). The current Oklahoma record is a significant eastern range extension of over 1,300 km (808 mi) for this uncommon species. A single male was collected on 8-V-2018 (data label Plate 3B; unique museum code of voucher = OSUC 620952). *Phytocoris tricincitipes* is now known from three US states: California, Nevada, **Oklahoma (new state record)** (Plate 3C). Species determination was arduous for us. However, the drawings in Stonedahl (1988) and the key and description were eventually confirmative for this western species. The left

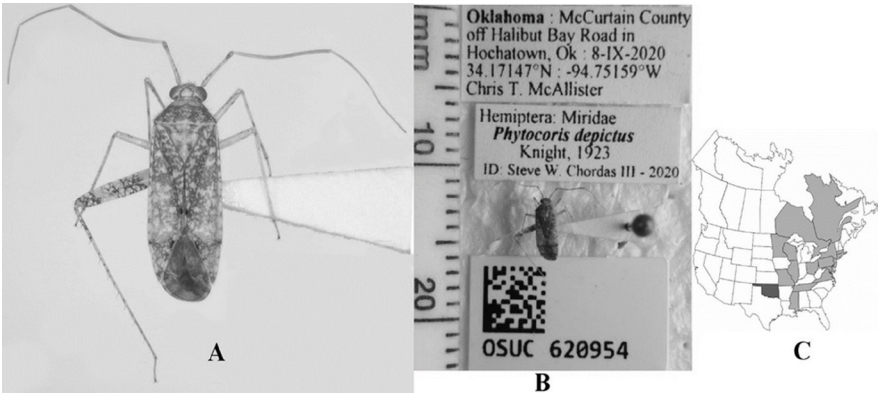

Plate 1 (Figures 1A–C). (A) Dorsal habitus of *Phytocoris depictus*; (B, top to bottom): location label, identification label, voucher specimen, unique museum number and code, scale in millimeters (mm) on left side of image; (C) distribution map (north of México), light shade = prior record, dark shade = new Oklahoma record.

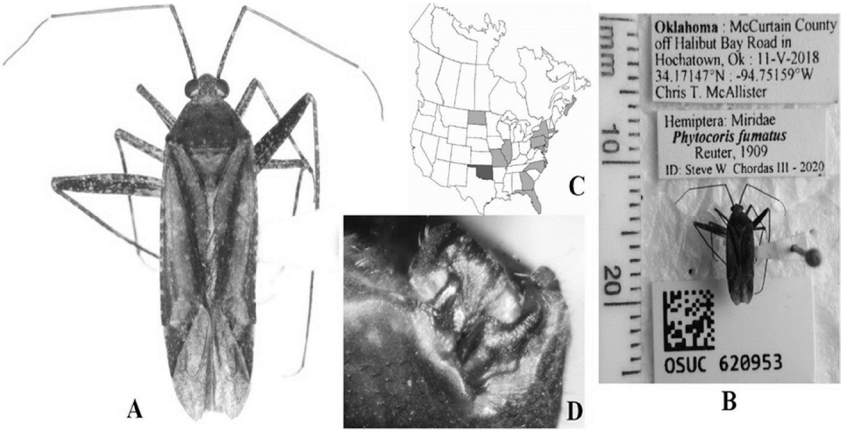

Plate 2 (Figures 2A–C). (A) Dorsal habitus of *Phytocoris fumatus*; (B, top to bottom): location label, identification label, voucher specimen, unique museum number and code, scale in millimeters (mm) on left side of image; (C) distribution map (north of México), light shade = prior literature record, dark shade = new Oklahoma record; (D) left male genital clasper, large tubercle above clasper and associated terminal abdominal structures.

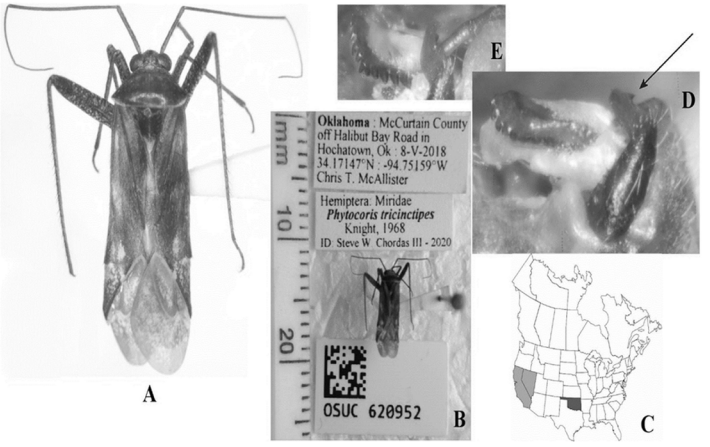

Plate 3 (Figures 3A–D). (A) Dorsal habitus of *Phytocoris tricinctipes*; (B, top to bottom): location label, identification label, voucher specimen, unique museum number and code, scale in millimeters (mm) on left side of image; (C) distribution map (north of México), light shade = prior literature record, dark shade = new Oklahoma record; (D & E) left male genital clasper, flagellum and associated terminal abdominal structures.

clasper shaft of the male has a clear posteriorly projecting protuberance, a morphological character illustrated by Stonedahl (1983, 1988), but not mentioned in the original description or in Knight's (1968) drawings (Plate 3D [arrow] and 3E). Most other characters and color combinations, with some variability, fit the descriptions in both Knight (1968) and Stonedahl (1988). Stonedahl (1988) reported *P. tricinctipes* to be a predaceous species that occurred in the Intermountain Sagebrush region of Nevada and eastern California (Inyo County) on pinyon pine (*P. monophylla* Torr. & Frem.); but was also attracted to lights (collection method of our specimen).

Additional collections of hemipterans, both aquatic and terrestrial, in the state should likely yield taxa yet unreported in the refereed literature for Oklahoma. Efforts to document

the bugs in Oklahoma is an ongoing endeavor.

Acknowledgments

The Oklahoma Department of Wildlife Conservation issued a Scientific Collecting Permit to CTM. We thank Dr. Thomas J. Henry (USNM, Smithsonian Institution, Washington, DC) for expertise and consultation of the phytocorids reported herein, and Janna Thompson (Ohio Dominican University, Columbus, OH) for assistance with images, graphics and maps in Plates 1–3.

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Additional Records of Hemoparasites (Apicomplexa) and Helminth Parasites (Trematoda, Cestoda, Nematoda, Acanthocephala) from Oklahoma Amphibians (Anura) and Reptiles (Testudines: Ophidia)

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Abstract: Over the last few years, we have learned more about hematozoan (blood) parasites and helminth parasites of Oklahoma herpetofauna than we have gained in many years past. To that end, we continue to provide information on various intraerythrocytic hematozoans and helminths of amphibians and reptiles of the state. We document several new host and distributional records for these parasites.

Introduction

During the last decade, our research consortium has made an attempt to help fill a void in our knowledge of hematozoans (McAllister 2015; McAllister et al. 2018a) and helminths of Oklahoma's herpetofauna (McAllister and Bursey 2012; McAllister et al. 2014a, b, 2015, 2016, 2018a, b, and references therein). Here, we complement some of that information by reporting new host and distributional records for select parasites of amphibians and reptiles from southeastern Oklahoma.

Methods

Between May 2017 and July 2020, single adult specimens of Fowler's toad, *Anaxyrus fowleri* (Hinckley), southern leopard frog, *Rana sphenocephala* (Cope), snapping turtle, *Chelydra serpentina* (L.), eastern river cooter, *Pseudemys concinna concinna* (LeConte), southern black racer, *Coluber constrictor priapus* Dunn and Wood, broad-banded watersnake, *Nerodia fasciata confluens* (Blanchard), western ratsnake, *Pantherophis obsoletus* (Say), Dekay's brownsnake, *Storeria dekayi* (Holbrook), and two each eastern hog-nosed snakes, *Heterodon platirhinos* (Latreille), and northern cottonmouths, *Agkistrodon piscivorus* (Lacépède) were collected by hoop net, hand or snake tong from Choctaw County (n = 1) and from several sites in McCurtain County (n = 11), and examined for hematozoan and helminth parasites. Specimens were placed in cloth collection bags, placed in a refrigerator, and necropsied within 24 hr. They were measured for straight-line carapace length (CL) or snout-vent length (SVL), killed by an intraperitoneal injection of sodium pentobarbital (Nembutal®) following accepted guidelines (SIH 2004), and examined for hematozoan and helminth parasites. A bone saw was used to remove the plastron from turtles to expose the heart and a mid-ventral incision from mouth to cloaca was made to expose the same in amphibians and other reptiles. Blood was obtained from all specimens by making a small incision in their heart and taking a sample using ammonium heparinized (75 mm long) capillary tubes. Thin films were smeared onto microscopic slides, air-dried, fixed for 1 min in absolute methanol, stained for 20–30 min with Wright-Giemsa stain, and rinsed in phosphate buffer (pH = 7.0). Slides were scanned at 100× or 400× and when infected cells were found, photographs were taken and length and width (L × W) measurements were made on gamonts of an intraerythrocytic parasite (n = 20) using a calibrated ocular micrometer under a 1,000× oil immersion lens and are reported in micrometers as means ±1SD followed by the ranges. For intravascular trematodes in turtles, we followed methods of Snyder and Clopton (2005). All visceral organs, particularly those of the GI tract from all specimens, were examined for helminths by removing and splitting organs lengthwise, placing separate organs in a Petri dish with 0.9% saline, and their contents scanned at 20–30× using a stereomicroscope. The liver and other suspected infected tissues from two anurans and two snakes were also biopsied and specimens processed for examination by light microscopy following Presnell and Schreibman (1997). Trematodes and cestodes were fixed in nearly boiling tap water without coverslip pressure, stained with acetocarmine, dehydrated in a graded ethanol series, cleared in methyl salicylate, and mounted in Canada balsam. Nematodes were fixed in hot tap water and studied as temporary mounts on a microscopic slide in a drop of glycerol. When acanthocephalans were found, they were rinsed of mucus and placed in dishes containing distilled water for 24 h in a refrigerator to evert their proboscides. Each specimen was placed on a glass slide, and a wet mount was prepared by adding a drop of tap water and a coverslip. All were examined at 100 to 400× with an Olympus BX-51 upright research microscope configured for Brightfield (BF) and Differential Interference-Contrast (DIC) microscopy. Digital images were taken of acanthocephalans using an Olympus 5-megapixel digital camera, and total length and greatest width of each worm were measured with ImageJ software (Schneider et al. 2012).

We follow the common and scientific names of North American herpetofauna of Crother (2017) except for adopting Yuan et al. (2016) in our usage of *Rana* rather than *Lithobates* for Oklahoma's ranid frogs. Host vouchers are deposited in the Arkansas State University Museum of Zoology (ASUMZ) Herpetological Collection, State University, Arkansas, or the Henderson State University Herpetological Collection (HSU), Arkadelphia, Arkansas. Actual vouchers or photovouchers of parasites are deposited in the Harold W. Manter Laboratory of Parasitology (HWML), University of Nebraska, Lincoln, Nebraska.

Results and Discussion

Fourteen taxa of endoparasites, including

three apicomplexans, four digeneans, two tapeworms, three nematodes, and two acanthocephalans were harbored by 14 hosts. An annotated list of the parasites found and the host data follows.

Apicomplexa: Adeleorina: Haemogregarinidae: Hepatozoidae

***Hepatozoon* sp. Miller, 1908** – Slender-elongate and slightly recurved gamonts of a *Hepatozoon* sp. (HWML 216370; Figs. 1A–B) was found in about 25% of the red blood cells (rbcs) of an adult *N. f. confluens* (675 mm SVL) collected on 10 June 2019 from an oxbow lake (Little River drainage), 3.2 km N of Idabel (33° 55' 56.93"N, 94° 43' 43.22"W). Gamonts were length × width, 18.5 × 7.1 (range 17–20 × 7–8) µm and caused a moderate hypertrophy to the host rbc by an increase in rbc length, but not width.

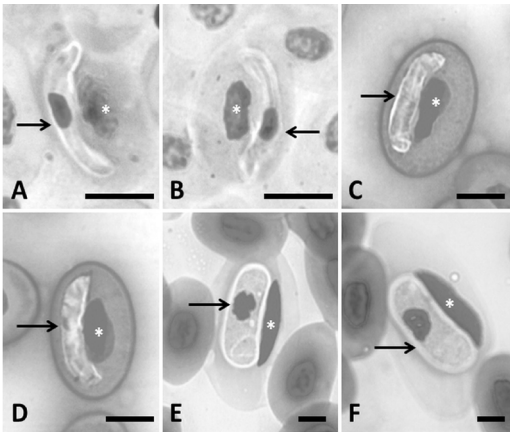
Lowichik and Yeager (1987) reported short and long "haemogregarine" gamonts in *N. f. confluens* from Louisiana, but unfortunately, did not provide photomicrographs. In addition, Telford et al. (2001) described *Hepatozoon pictiventris* in Florida watersnake, *Nerodia fasciata pictiventris* (Cope) from Florida. Gamonts (their figs. 25–26) were dissimilar to ours as they were elongate, but not slender and slightly recurved. This is the first time any haemogregarine has been reported from a *N. f. confluens* in Oklahoma along with the initial photomicrographs of the form observed in this host.

An adult *H. platirhinos* (650 mm SVL) collected on 15 October 2017 from Hochatown (34° 10' 17.0286"N, 94° 45' 05.7414"W) also harbored a few gamonts (Figs. 1C–D) of a *Hepatozoon* sp.; measurements were not taken and the other *H. platirhinos* was negative. Hilman and Strandtmann (1960) reported *H. serpentium* from three of four (75%) *H. platirhinos* from Texas. We report a *Hepatozoon* sp. from a *H. platirhinos* from Oklahoma for the first time.

A single adult *C. horridus* (1,030 mm SVL) collected on 10 May 2017 from the EOSC campus in Idabel (33° 55' 17.2632" N, 94° 46' 43.6548" W) were infected with gamonts (HWML 216371; Figs. 1E–F) of a *Hepatozoon* sp. McAllister (2015) previously reported *Hepatozoon* sp. from *C. horridus* collected from the same locale. However, a photomicrograph of the parasite was less than optimal and we here provide better photomicrographs from this host (Figs. 1E–F). Bean-shaped to elongate gamonts were prevalent that also resulted in the rbc nucleus becoming very elongate (Figs. 1E–F). Hematozoans have also been previously reported from *C. horridus* from New York (Fantham and Porter 1954) and *H. horridus* was described by (Telford et al. 2008) from timber rattlesnakes from Florida. The gamonts of *H. horridus* reported by the latter authors (their Figs. 5–6) do not resemble the current specimens.

Platyhelminthes: Digenea: Schistosomatoidea: Spirorchiidae

***Spirorchis scripta* Stunkard, 1923** – an adult *P. c. concinna* (320 mm CL) collected on 9 June 2019 from an oxbow lake (Little River drainage), 3.2 km N of Idabel (33° 55' 56.93"N, 94° 43' 43.22"W) had this digenean in its kidneys, liver and general body wash. *Spirorchis* spp. trematodes of turtles have a complex life cycle with turtles serving as



Figures 1A–F. Gamonts of *Hepatozoon* spp. from reptiles. (A–B) Gamonts from *Nerodia fasciata confluens*. (C–D) Gamonts from *Heterodon platirhinos*. (E–F) Gamonts from *Crotalus horridus*. Arrows indicate gamonts and asterisks (*) denote rbc nuclei; scale bars A–D = 10 µm; E–F = 5µm.

definitive hosts and snails as intermediate hosts. In the life cycle, for example, cercaria occurs in the planorbiid snails, the two-ridged ramshorn, *Helisoma anceps* (Holliman and Fisher 1968) and *Menetus dilatatus* (Goodchild and Martin 1969) and ancylid, *Ferrissia fragilis* (Turner and Corkum 1977). This blood fluke has previously been reported from *P. concinna* from Mississippi (Roberts et al. 2018) and Tennessee (Byrd 1939). It was originally described by Stunkard (1923) from Mississippi map turtle, *Graptemys pseudogeographica kohnii* (Baur) collected from Texas, and the red-eared slider, *Trachemys scripta elegans* (Wied-Neuwied) from North Carolina and has also been reported from the latter host from Oklahoma (Harwood 1931; Everhart 1975) as well as other turtles from Alabama, Georgia, Iowa, Nebraska, Ohio, Tennessee, Texas, Virginia, and Manitoba, Canada (Ernst and Ernst 1977; Timmers and Lewis, 1979; Roberts et al. 2016, 2019). However, this is the first time *S. scripta* has been reported from *P. c. concinna* in Oklahoma. Specimens are being retained for molecular analysis (VV Tkach, *pers. comm*.).

Plagiorchiida: Plagiorchiidae

***Styphlophora magna* Byrd and Denton, 1938** – Three *S. magna* was found in the gallbladder of an *A. piscivorus* (427 mm SVL) collected on 23 June 2019 from the same Hochatown site above. An additional *A. piscivorus* (510 mm SVL) collected from the same site on 5 September 2020 had a single *S. magna* in its gallbladder. Byrd and Denton (1938) described *S. magna* from the gallbladder of northern watersnake, *Nerodia sipedon sipedon* (L.) from Georgia and Mississippi. A single specimen from the gall bladder of Florida kingsnake, *Lampropeltis floridana* (Blanchard) from Florida was reported by Byrd et al. (1940). More recently, Fontenot and Font (2011) reported *S. magna* from Mississippi green watersnake *N. cyclopion* (Duméril, Bibron and Duméril), southern watersnake, *N. fasciata* (L.), northern diamond-backed watersnake, *N. rhombifer rhombifer* (Hallowell) and *A. piscivorus* from Louisiana. We document the second report of *S. magna* from *A. piscivorus* but more importantly, the first documentation of this digenean from west of the Mississippi River in Oklahoma. Specimens are being utilized in a molecular study (VV Tkach, *pers. comm.*).

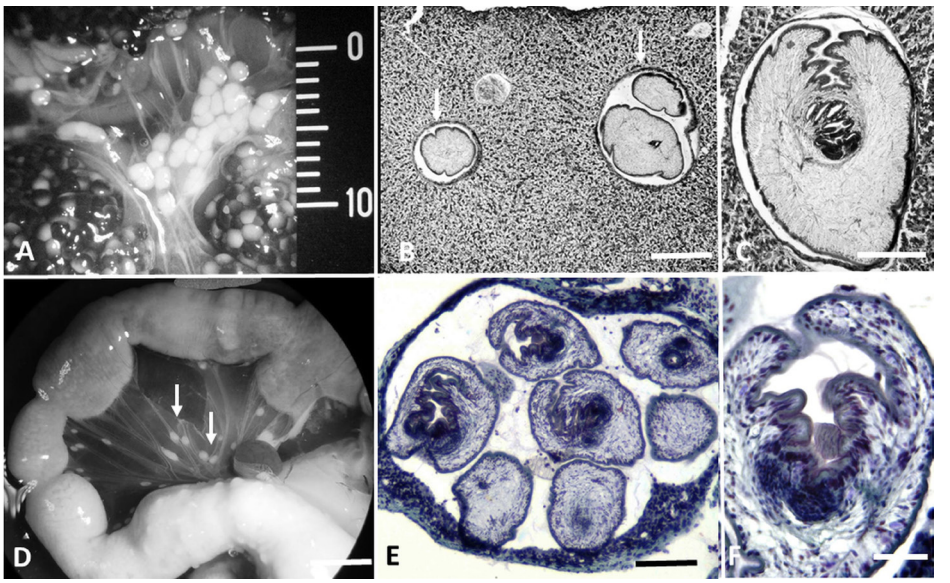
Ochetosomatidae

***Dasymetra conferta* Nicoll, 1911 –** Four *D. conferta* were found in the esophagus of an adult (1,020 mm SVL) *P. obsoletus* collected on 15 July 2020 from the same Hochatown site above. This trematode has been previously reported from Oklahoma in *N. r. rhombifer*, plain-bellied watersnake, *Nerodia erythrogaster* (Forster) and *H. platirhinos* (McAllister and Bursey, 2012; McAllister et al. 2016, 2018a). The parasite's range also includes hosts from Alabama, Missouri, and Texas (see Ernst and Ernst 2006). The western ratsnake is a new host record for *D. conferta*. Specimens are being utilized in a molecular study (TJ Fayton, *pers. comm.*).

***Renifer* sp. –** Six specimens of a *Renifer* sp. were found in the oral cavity of the same *N. f. confluens* above. Two species, *R. aniarum* (Leidy, 1891) and *R. magnum* = *magnus* (Byrd and Denton, 1938) have been reported from *N. f. confluens* (Ernst and Ernst 2006; Fontenot and Font 1996). The former taxon was reported from this host from Louisiana (Rabalais 1969; Brooks 1979; Fontenot and Font 1996) and from a *N. r. rhombifer* from Oklahoma (McAllister and Bursey 2012). However, this is the first time this host has been reported with a *Renifer* sp. from Oklahoma. The genus is badly in need of revision including molecular analyses, so we wait until a more definitive diagnosis can be made to identify our taxon here. These specimens have been retained for DNA studies (TJ Fayton, *pers. comm.*)

Cestoda: Cyclophyllidea: Mesocestoididae

***Mesocestoides* sp.** – two anuran and a snake species were found to possess tetrathyridia of *Mesocestoides* sp. One anuran host, an adult *R. sphenocephala* (80 mm SVL) collected on 25 August 2019 from Hochatown (34° 10' 17.0286"N, 94° 45' 05.7414"W) had tetrathyridia (HWML 216369) in its liver (Figs. 2A–C). *Mesocestoides* has previously been reported from *R. sphenocephala* from Arkansas by McAllister et al. (2014b). The other anuran

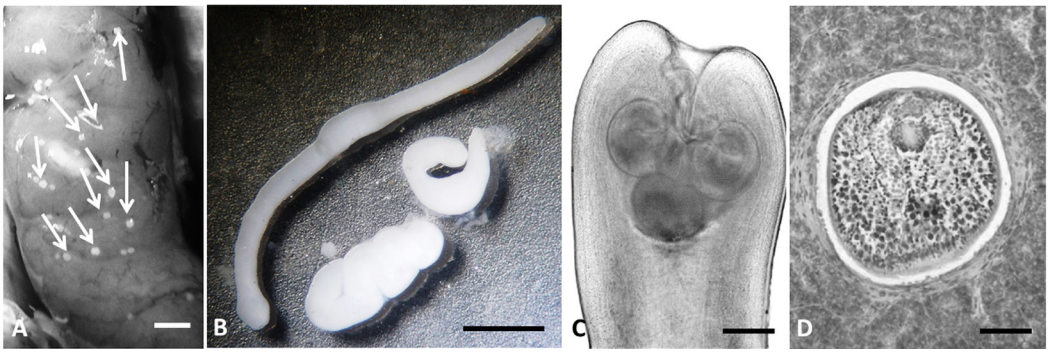


Figures 2A–F. *Mesocestoides* sp. tetrathyridia from anurans; note uniform size and shape with fully invaginated normal scoleces with suckers and normal organized body (C, F). (A) Multiple tetrathyridia in peritoneal cavity of *Rana sphenocephala*. (B) Individual tetrathyridium or dual tetrathyridia (arrows) in liver parenchyma of *R. sphenocephala*. (C) Single tetrathyridium from liver of *R. sphenocephala*. (D) Tetrathyridia (arrows) in omental tissue of *A. fowleri*. (E) Multiple tetrathyridia (n = 7) in single host-derived capsule of *A. fowleri*. (F) Single tetrathyridium from *A. fowleri*. Scale bars (A) each mark = 1 mm; (B) 1 mm; (C) 250 µm; (D) 5 mm; (E) 250 µm; (F) 250 µm.

host, an adult *A. fowleri* (60 mm SVL) collected from the same locality on 20 September 2016 was also found to possess tetrathyridia of *Mesocestoides* sp. (HWML 216368) in its liver (Figs. 2D–F) and mesenteries. This cestode was reported previously from *A. fowleri* in Arkansas and Michigan (McAllister et al. 2014b; Muzzall and Andrus 2014). It is being reported here for the first time in these particular host species from Oklahoma. These specimens are being processed further for molecular analysis of the genus *Mesocestoides* in amphibians (VV Tkach, *pers. comm.*). The tetrathyridia from both anuran hosts were uniform in size and shape, probably indicating similar age or time from initial infection (see McAllister et al. 2018b).

The same adult *H. platirhinos* collected from the same Hochatown locality above in October 2017 harbored *Mesocestoides* sp. tetrathyridia (Figs. 3A–C). We report *Mesocestoides* sp. tetrathyridia from this host in Oklahoma for the first time. The tetrathyridia from this host were of two distinct size and shape categories, with one group being small and rounded, and the other being larger and elongated, possibly indicating different ages or two distinct infection incidents (see McAllister et al. 2018b).

Given the frequent fact that some isolates of *Mesocestoides* tetrathyridia from various parts of the world are known to proliferate asexually, it is important to note that none of our specimens in this study showed any sign of asexual activity or abnormal development. On the contrary, all specimens exhibited highly organized musculature, parenchyma, and excretory systems, with no sign of supernumerary scoleces as described for *Mesocestoides vogae* Etges 1991, or distended excretory ducts and hyperplastic tegumentary invaginations associated with malignant transformation in aberrant proliferative *Mesocestoides lineatus* (Goeze, 1782) and other species (Conn et al. 2010, 2011; Conn 2016). Thus, our findings are consistent with all other field collections of *Mesocestoides* we have reported over many years from diverse hosts in North America



Figures 3A–D. *Mesocestoides* sp. tetrathyridia and *Spirometra* sp. from *Heterodon platirhinos*; note variations in size and shape of *Mesocestoides* sp. (A) Multiple tetrathyridia in situ (arrows) in liver. (B) Three individual tetrathyridia from peritoneal cavity or teased from liver capsules. (C) Anterior end of tetrathyridium; note fully invaginated normal scolex with four suckers and normal organized body. (D) *Spirometra* sp. (encapsulated plerocercoid) in liver tissue. Scale bars (A) 1 mm; (B) 2mm; (C) 100 µm; (D) 250 µm.

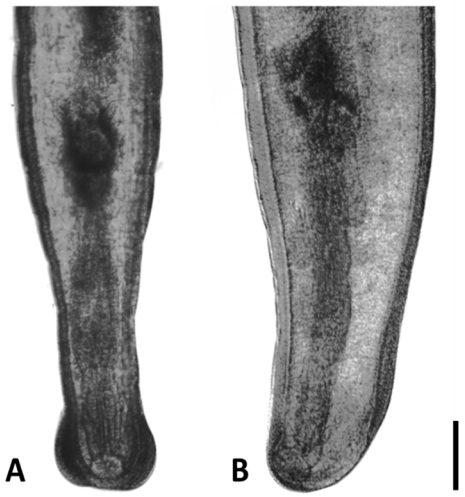
(McAllister et al. 1989, 1991a, b, c, 1992, 1995, 2004, 2005, 2017, 2018b). The only exception in three decades of our studies on the genus was a single moribund western coachwhip, *Coluber flagellum testaceus* Say, *in* James from Texas that harbored unidentified aberrant acephalic metacestodes co-occurring with normal non-proliferating *Mesocestoides* tetrathyridia (Conn and McAllister 1990); the aberrant forms in that unusual case were possibly not *Mesocestoides*, but, unfortunately, were unidentifiable due to lack of scoleces.

This enigmatic cestode, for whom no complete life cycle is known, has been previously reported from other Oklahoma herpetofauna, including Sequoyah slimy salamander, *Plethodon sequoyah* Highton, Hurter's spadefoot, *Scaphiopus hurterii* Strecker, plains spadefoot, *Spea bombifrons* (Cope), American bullfrog, *Rana catesbeianus* (Shaw), little brown skink, *Scincella lateralis* (Say, *in* James), and eastern gartersnake, *Thamnophis sirtalis sirtalis* (L.) (see McAllister and Bursey 2004; McAllister et al. 2005, 2017, 2018a, b). It is of interest to note that although the complete life cycle remains an enigma, it appears that from the numerous specimens (both frogs and snakes) collected from the identical Hochatown site that harbor tetrathyridia of this cestode, some unknown member or members that serve in the initial part of the life cycle occurs at this site. Further research is certainly warranted to help investigate this query.

Diphyllobothriidea: Diphyllobothriidae

***Spirometra* sp.** – Another adult *H. platirhinos* (650 mm SVL) collected in April 2020 from the same Hochatown locality also harbored this cestode in its liver (Fig. 3D) and body cavity.

The life cycle requires three hosts, members of the genus reproduces in wild and feral canines and felines and other mammals, but can also cause subcutaneous, visceral, ocular, and cerebral pathologies and zoonosis (sparganosis, a foodborne and waterborne disease) in humans, the majority of cases reported in China and Korea (Hwang et al. 2020). In some species within this genus, the vertebrate second intermediate host (and paratenic host) may be a frog, snake, bird, or mammal, and the first intermediate host is a copepod (Kuchta et al. 2015, 2020). At least four species are currently recognized (Kuchta and Scholz 2017); however, our metacestodes cannot be identified to species without molecular analyses. The forms in the Americas (both North and South America) are split into two groups that are currently recognized as the *S. decipiens* complex (Kuchta et al. 2020). Nevertheless, we document a new host and the first report of the genus in snakes from Oklahoma. Specimens from *H. platirhinos* are being processed further for molecular analysis of the genus *Spirometra* in reptiles (VV Tkach, *pers. comm*.).



Figures 4A–B. *Neoechinorhynchus* spp. from *Pseudemys concinna concinna*. (A) Posterior ends of non-gravid female *Neoechinorhynchus pseudemydis* and (B) *Neoechinorhynchus emyditoides*. Scale bar = 100 µm.

Ascaridida: Cosmocercidae

***Cosmocercoides dukae* (Holl, 1928) Travassos, 1931** – Twenty-four female and seven male specimens (HWML 111603) were taken from the lower intestine of a *S. dekayi* (125 mm SVL) collected on 15 May 2020 from Sweethome, N of Broken Bow (34° 03' 22.1796''N, 94° 46' 27.8832'' W). *Cosmocercoides variabilis* (Harwood, 1930) Travassos, 1931 was reported previously from *S. dekayi* from Oklahoma by McAllister et al. (2015). However, the current specimens are clearly *C. dukae* as they possess 12 pairs of plectanes, not the 16 or more possessed by *C. variabilis*. The former was reported by Harwood (1932) from *S. dekayi* from Texas. The life cycle involves terrestrial gastropods as intermediate hosts and amphibians and reptiles as definitive hosts (Anderson 2000). This is the first report of *C. dukae* in a *S. dekayi* from Oklahoma.

Nematoda: Strongylidea: Diaphanocephaloidea: Diaphanocephalidae

Kalicephalus inermis coronellae (Ortlepp, 1923) Lichtenfels, 1980 – A single *C. c. priapus* collected on 15 May 2019 from Broken Bow (34° 01' 7.4316'' N, 94° 45' 25.3512'' W) had one female *K. i. coronellae* (HWML 112120) in its esophagus. Ernst and Ernst (2006) previously listed *C. constrictor* as a host of this nematode but its subspecific identity was not provided; 11 subspecies of *C. constrictor* have been recognized (see Crother 2017). McAllister et al. (2018a) reported this nematode from Oklahoma in prairie kingsnake, *Lampropeltis calligaster* (Harlan). This hookworm has been reported from at least 25 snake (both colubrid and viperid) species from Colorado, Florida, Georgia, Louisiana, Massachusetts, New Mexico, North Carolina, and Texas, and Québec, Canada, and Guerrero, Michoacán, and Vera Cruz, México (Schad 1962; Baker 1987; Ernst and Ernst 2006).

Ascaridida: Anisakidae

***Contracaecum* sp. (larvae)** – two larval *Contracaecum* sp. (HWML 112116) were found deeply embedded in the intestinal mucosa of an adult (260 mm CL) *C. serpentina* collected on 1 October 2019 from a private lake in Ft. Towson, Choctaw County (34° 08' 32.5752'' N, 95° 20' 38.4756'' W). Crustaceans (primarily copepods and amphipods) serve as first intermediate hosts, freshwater and marine fishes are second intermediate/paratenic hosts, and fish-eating birds and mammals are definitive hosts (Anderson 2000). This is the first time the larvae have been reported from *C. serpentina*. *Contracaecum* larvae similar to our specimens have been reported from Florida softshell, *Apalone ferox* (Schneider) in Florida (Foster et al. 1998).

Acanthocephala: Eoacanthocephala: Neoechinorhynchida: Neoechinorhynchidae

***Neoechinorhynchus emyditoides* Fisher, 1960 –** the same *P. c. concinna* reported above harboring *S. scripta* was found to be infected with a non-gravid female of *N. emyditoides* (HWML 112102) in its lower intestine. The length and width (L × W) of *N. emyditoides* was 12.9 × 0.44 mm, respectively.

***Neoechinorhynchus pseudemydis* Cable and Hopp, 1954 –** a non-gravid female of *N. pseudemydis* (HWML 112103) was also found in the same host above. The L × W of *N.*

pseudemydis was 16.9 × 0.56 mm, respectively. Both acanthocephalans were identified based on posterior end morphology (Figs. 4A–B) (Barger and Nickol 2004).

Previously, *P. concinna* has been reported with *N. emydis* (Leidy, 1851) Van Cleave, 1916 from Illinois, *N. emyditoides* from Louisiana, and *N. chrysemydis* Cable and Hopp, 1954 from Alabama (as *P. c. hieroglyphica* × *concinna*), Louisiana, North Carolina, and an unknown locality (Van Cleave 1919; Johnson 1968; Acholonu 1969; Barger 2004). Additionally, McAllister et al. (2015) reported non-gravid acanthocephalans of either *N. pseudemydis* and/or *N. emyditoides* from *P. c. concinna* from Oklahoma, supporting the current findings. Here, we report a new host record for *N. pseudemydis* in *P. c. concinna*. *Neoechinorhynchus pseudemydis* has been reported from at least six species of turtles, including *C. serpentina*, painted turtle, *Chrysemys picta* (Schneider), red-eared slider, *T. s. elegans,* Blanding's turtle, *Emydoidea blandingii* (Holbrook), *Kinosternon* sp., and gopher tortoise, *Gopherus polyphemus* (Daudin) (Ernst and Ernst 1977; Barger 2004). These results strengthen the need to survey *P. c. concinna* for acanthocephalans, with future efforts dedicated to molecular identification.

In summary, we report some new host and geographic records for these parasites. There remains a need to continue surveying the diverse herpetofauna of the state, especially species found in the western part of Oklahoma that has been rarely examined. Additional new host and geographic distribution records are to be expected with extensive surveys, including the possibility of discovering novel taxa.

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The North American River Otter *Lontra canadensis* as a Late Pleistocene Fossil from the Canadian River, Oklahoma

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Abstract: A well-preserved left lower jaw of *Lontra canadensis* found in sands of the Canadian River in east-central Oklahoma provides the first fossil record of the species in Oklahoma. When subjected to pretreatment in advance of radiometric dating, the canine root extracted from the jaw did not preserve enough collagen to be datable. However, its preservation suggests a late Pleistocene or possibly early Holocene age.

Introduction

Otters of the genus *Lontra* are widely distributed in the western hemisphere, where they are represented by four recent species in North, Central, and South America (iucnredlist.org). The North American River Otter, *Lontra canadensis*, is widespread in North America. Prior to the European colonization of the continent, river otters probably occurred in streams across the southern Great Plains (Caire et al. 1989; Dalquest and Horner 1984; Schmidly 1983, 2002). Scant historical records indicate that otters were occasionally found in Oklahoma as far west as the confluence of Otter Creek (named for an otter killed there in 1852) and the North Fork of the Red River in Tillman County, as well as in Medicine Creek in the Wichita Mountains (Halloran and Glass 1964; Halloran 1975). Although the naming histories are otherwise unknown, at least eight other streams are named "Otter Creek," three in western Oklahoma and five in eastern Oklahoma (Table 1; Topographic Mapping Co., no date). Indeed, in the 19th century the Beaver River-North Canadian River in Indian Territory was known as the "Río Nutria" and is shown as such on at least one historic map (Creuzbaur 1848); "nutria" is Spanish for "otter."

During the period following colonization, many mammal species were adversely impacted by habitat destruction, commercial overexploitation, and other factors. These were extirpated from most of Oklahoma and much of the Great Plains by 1900, including river otters (Matthiessen 1959; Caire et al. 1989; Samson and Knopf 1996; Licht 1997; Shackelford and Whitaker 1997; White and Hoagland 1997; Raesly 2001; Barrett and Leslie 2010; Caire et al. 2016). In the late 20th century otters re-entered southeastern and central Oklahoma from rivers and wetlands farther to the southeast by natural re-immigration (Barrett and Leslie 2010). In addition, conservation measures during the same period included the re-introduction into southeastern Oklahoma of river otters from coastal Louisiana (Shackelford and Whitaker 1997; Barrett and Leslie 2010; Caire et al. 2016). By the beginning of the 21st century, these otters have re-entered parts of their ancestral range, including along the Canadian River at least as far as Norman, Cleveland/McClain counties, and the Cimarron River at least as far west as Kingfisher County (Barrett and Leslie 2010; Caire et al. 2016; pers. obs.) as well as along other rivers in eastern Oklahoma. In the 1990s, river otters were re-introduced to the Wichita Mountains Wildlife Refuge and are occasionally sited in tributaries and lakes within the refuge and in tributaries of the Red River near Altus, Oklahoma (K. S. Smith, pers. comm.).

Table 1. Occurrence of streams named "Otter Creek" in the state of Oklahoma, according to Topographic Mapping Co. (no date).

County or Counties and Part	Tributary of:	Course Relative to Landmarks and Nearby Towns
S Kiowa / NW Tillman Cos.	North Fork of the Red River	from Baker Peak through Mountain Park to N of Tipton
N Ellis / S Harper Cos.	Beaver River	near May
E Blaine / W Kingfisher Cos.	Kingfisher Creek	between Watonga and Kingfisher
SE Grandfield / N-C Logan Cos.	Skeleton Creek	E of Marshall
E Haskell Co.	Sans Bois Creek	S of Keota
E McCurtain Co.	Mountain Fork Little River*	SE of McCurtain Co. Wilderness Area
NW Osage Co.	Beaver Creek	W of Grainola
S Rogers Co.	Dog Creek near its confluence with Verdigris River	S of Claremore

* Here inundated by Broken Bow Reservoir.

Paleontologically, river otters are less well known in North America. A small Pliocene species, *Lontra weiri*, is known from Idaho (Prassack 2016). *Lontra canadensis* is known as a fossil in North America from the late Pliocene (late Blancan land mammal age) of Nebraska, through the early Pleistocene (Irvingtonian land mammal age) of California, Florida, Kansas, New Mexico, and Pennsylvania, to the Holocene (FAUNMAP online database). It has a relatively broad Holocene distribution with numerous records across North America (FAUNMAP). Its late Pleistocene record is substantial, with records from the Rancholabrean land mammal age and Wisconsinan glacial period in Alaska, Arizona, Arkansas, California, Florida, Georgia, Idaho, Illinois, Kansas, Missouri, Tennessee, Texas and (FAUNMAP). An earlier review of the literature (Smith and Cifelli 2000) revealed no record of fossil otters from Oklahoma. The purpose of this paper is to describe a fossil of *L. canadensis* from the Canadian River in eastern Oklahoma.

Methods

An otter fossil was formerly cataloged in the collection of Midwestern State University (Wichita Falls, Texas) as specimen 12945-8. The late Walter W. Dalquest donated this and other fossils from Oklahoma rivers to the Oklahoma Museum of Natural History (OMNH) in the early 2000s. The specimen is now re-cataloged as OMNH 77473. It was temporarily coated with ammonium chloride to circumvent the dark color, enhance surface morphology, and reduce glare for photography (Fig. 1). It was found between 1991 and 1993 along the Canadian River at Hoyt, Oklahoma, along the reach of the Canadian River now downstream of Eufaula Reservoir (OMNH locality V910). Other details of its discovery were not recorded, but it was one of many specimens found by amateur fossil collectors who were asked to search Oklahoma rivers after flooding events and donated their fossils to Midwestern State University. These fossils are often revealed by floods and periodic shifts in the river channels and sands, which removes them from their original depositional context in cross-bedded sands and places them in a new one, possibly repeated over time. This reach of the Canadian River is about 30 km upriver from its confluence with the Arkansas River at Sequoyah National Wildlife Refuge southwest of Vian, Oklahoma. This stretch of the Canadian River follows the boundary between

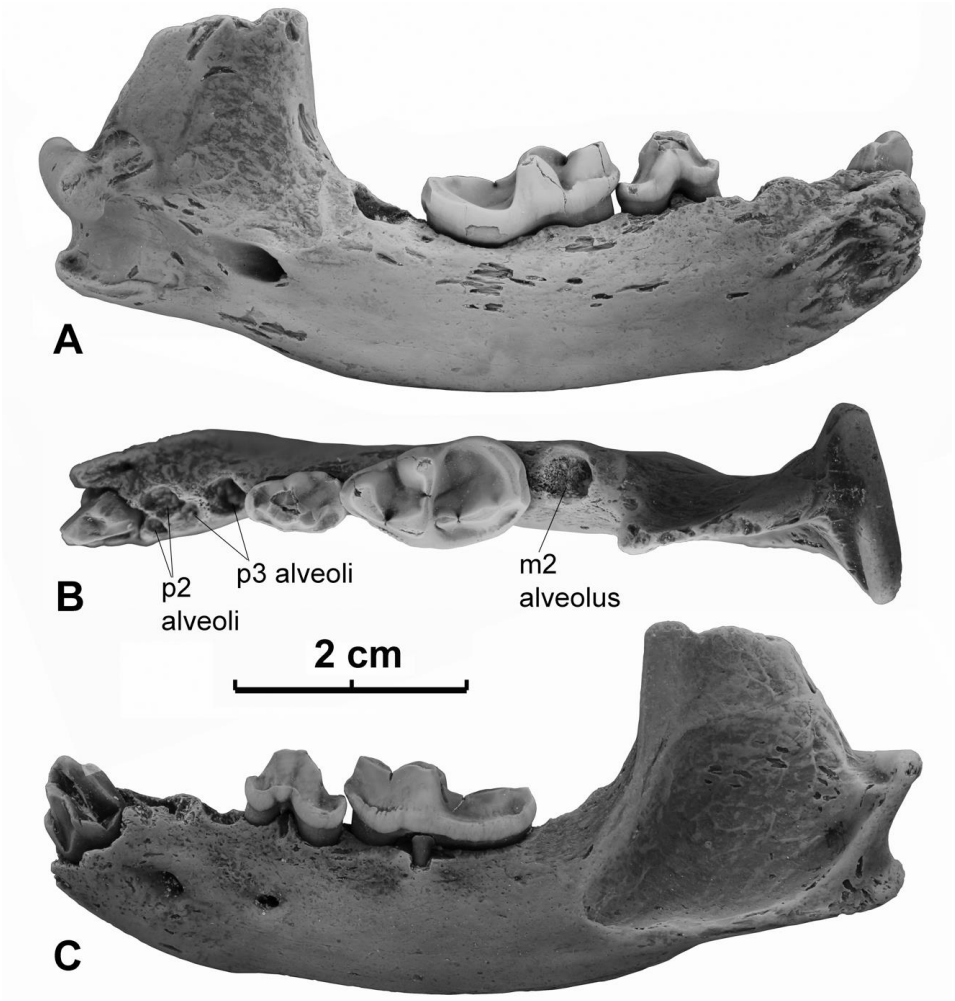

Figure 1. Fossil hemimandible with partial c1, p4, and m1 (OMNH 77473) of *Lontra canadensis* from the Canadian River near Hoyt, Oklahoma. A, medial view; B, occlusal view; C, lateral view.

Muskogee and Haskell counties. The otter jaw was identified by comparisons with six jaws of recent river otters from Oklahoma in collections of the OMNH.

A small portion of the dentary bone was carefully cut away so the lower canine could be extracted. The canine was sent to Beta Analytic, Miami, Florida, where it was subjected to pretreatment prior to radiometric dating. However, the sample did not preserve enough collagen to be datable (Beta Analytic sample Beta-574804, fide their email of 1 December 2020).

Results

OMNH 77473 is a nearly complete left dentary containing the base of the broken canine (extracted for radiometric dating) and the complete p4 and m1 (Fig. 1). The specimen is damaged anteriorly near the mandibular symphysis and missing the incisive alveoli but retains the empty alveoli for the p2, p3, and m2. The top of the coronoid process is broken off but the remaining portion shows a tall and straight vertical anterior edge. The condyloid process is transversely elongated, cylindrical, and situated slightly above the level of the

toothrow. The masseteric fossa is large. Two lateral mental foramina are present, one inferior to the p2 alveoli and one inferior to the p4. The dentary bone shows abrasion and rounding of broken edges typical of water and sediment-worn depositional settings, exposing some of the linear spaces between internal trabeculae as small linear holes near the eroded bone surface, especially on the lingual side of the horizontal ramus and both sides of the ascending ramus and angular process.

The p2 and p3 alveoli each indicate two obliquely oriented roots with the anterior of each pair smaller than the posterior, as in many mustelids. The p4 and m1 both show tooth morphology diagnostic of *Lontra canadensis*. The p4 has two main roots aligned with the long axis of the dentary, and with the posterior root larger than the anterior. The p4 has an extra labial rootlet (partly broken off) between its main roots. The p4 has an accessory cuspid directly lingual to the main cusp, whose tip is broken off. This swelling is better developed than on most modern specimens examined. The m1 has an extra rootlet situated laterally and between the main anterior and posterior roots as in typical *Lontra canadensis*. The lower carnassial (m1) has a broad crown with a well-developed trigonid and low and especially wide talonid. In the trigonid, the paraconid is located near the midline of the tooth and is as large as the protoconid. The metaconid is a little smaller and lower in height. The protoconid and paraconid form a high shearing carnassial blade with a deep, narrow, median carnassial notch. Another deep notch occupies the center of the crest between protoconid and metaconid. Cusps of the trigonid form a nearly equilateral triangle, with the protoconid-paraconid crest only slightly longer than the protoconid-metaconid crest. The talonid basin is wide, shallow, open lingually and occupied labially by an accessory cuspule on the posterior base of the protoconid (typical of lutrines; Baskin 1998) and mesiodistally elongated hypoconid, and bordered posteriorly by a curved crest; there is no distinct entoconid or hypoconulid. The accessory cuspule and hypoconid form an additional longitudinal shearing crest with a notch between these cusps. The talonid basin is closed off posteriorly by a curved ridge. The labial cingulum is very wide along the talonid, interrupted at the base of the protoconid, and well-developed and crenulated along the trigonid below the carnassial notch, protecting the gingiva below the shearing crests in life. Lingually, the cingulum is restricted to the anterior and lingual bases of the paraconid and the floor of the trigonid valley. The m1 has a large interdental wear facet on its distal surface resulting from contact with the m2. The preserved teeth show moderately heavy wear.

Measurements (in millimeters) of the specimen are: dentary length 68.5; dentary depth on medial side at anterior root of m1, 12.3; dentary depth on lateral side at anterior root of m1, 11.8; condyle width, 15.9; p4 anteroposterior length, 8.1; p4 transverse width, 5.1; m1 (carnassial) anteroposterior length, 14.9; m1 transverse width 8.8; length from anterior edge of c1 alveolus to posterior edge of m2 alveolus, 42.7; length from anterior edge of c1 alveolus to posterior margin of articular condyle, 67.6. Overall, the jaw length is relatively short, possibly indicating a female otter (recent otters show modest sexual dimorphism in which males are only 5% larger than females; Jackson 1961), but the p4 and m1 are more robust than in modern specimens of *L. canadensis*.

Discussion

The age of the otter specimen is uncertain because it did not retain sufficient collagen to enable radiometric dating, but other evidence suggests it is probably of late Pleistocene age. Vertebrate remains found in sand and gravel bars in Oklahoma rivers, including below Eufaula Dam, range in appearance from fresh bones often with adhering connective tissue to darkened and permineralized bones of extinct species such as *Mammuthus columbi* (Columbian mammoth), *Mammut americanum* (American mastodon), and *Bootherium bombifrons* (helmeted muskox) (W. W. Dalquest, in litt., and personal observation). Flooding events and releases from Eufaula Dam cause shifting river channels below the dam, exposing and transporting bones, removing them from their original depositional context and

their associated contemporaneous fauna. Thus, bones found on sand and gravel bars can be of varying ages ranging from late Pleistocene to late Holocene and do not represent an associated local fauna. Without these critical contextual data and without being able to date the otter fossils, there is no reliable means to determine the jaw's exact age. However, the modern and late Holocene bones found in the river are of low density, slightly elastic, pale in color, and little stained, and tend to fracture longitudinally. By contrast, the bones identifiable as those of extinct species are dense due to permineralization, dark brown or glossy blackish in color, inelastic, and tend to fracture transversely (W. W. Dalquest, in litt.; personal observation). Relative to these attributes, the otter jaw is dark brown with blackish tooth roots and light brown enamel, relatively dense for its size, and inelastic. Normally, the amount of collagen remaining within the bone (and especially within the denser roots of the teeth) decreases with age. A mastodont rib fragment from the Canadian River near Hoyt dated to the late Pleistocene at 11,560 ± 130 years before present (Beta-75145) by Dalquest (W. W. Dalquest, in litt.; this is an uncalibrated standard radiocarbon age). The lack of collagen in the otter canine root and the other features of its preservation suggest a relatively great age, probably late Pleistocene or possibly early Holocene.

Several characteristics of the jaw confirm its identity as a member of the mustelid subfamily Lutrinae (m1 with accessory cuspule behind protoconid and with distinct metaconid) and of the genus *Lontra* (p4 accessory cusp, m1 broad with high trigonid, broad shallow talonid basin, no entoconid) (Baskin 1998; Van Zyll de Jong 1972). Its size compares with and confirms the species as the Nearctic river otter, *Lontra canadensis*. Pleistocene fossils of river otters are rare in North America not only for the Nearctic river otter but also for the Neotropical river otter in Mexico (Arroyo-Cabrales et al. 2013). The Canadian River specimen documents the occurrence of the river otter in the late Pleistocene or early Holocene along the Canadian River about 30 km downstream from its historic confluence with the North Canadian River and 45 km upstream from its confluence with the Arkansas River. It constitutes the first fossil record of *L. canadensis* in Oklahoma. Together with the fossil records from other states, it helps to establish the Pleistocene distribution of the species in North America.

River otters occupy smaller rivers and streams, lakes, swamps, beaver ponds, and other wetlands with some shaded areas and log jams (Yeager 1938). They select clear streams and rivers with pools, log jams, and rapids with abundant fishes (Seton 1909; Melquist and Hornocker 1983). Otters are well known to feed mainly on medium-sized fish but also eat a variety of other small vertebrates such as frogs and salamanders (e.g., *Notophthalmus viridescens*, red-spotted newt) and invertebrates such as crayfish and gastropods (Hatcher 1984). Among the many late Pleistocene and Holocene fossils from the Canadian River bone assemblage are very few fish fossils, including only skeletal elements of large catfishes (Ictaluridae). The available fish fossils are fin spines of individual ictalurids much larger than the fishes typically eaten by river otters. However, the fish fauna of the late Pleistocene river certainly would have supported a high diversity of species and a fish fauna somewhat different from that of today.

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Review on Effects of Holding Time Exceedances on Ambient Water Quality

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Abstract: Globally, contamination of water bodies by microbial pathogens is a significant public health concern. Fecal indicator bacteria such as *Escherichia coli (E. coli)* typically identify the deterioration of water bodies. In order to determine water quality, monitoring *E. coli* concentrations is important. During collection, storage, and transportation of water samples, holding time can have a significant impact on the density of indicator pathogens. Although many studies have reported on the effects of holding time exceedances on water quality, there is a lack of comprehensive review of these studies. The objective of this work is to provide a complete review on the effects of holding time exceedances on water quality. The results of this study suggest that most *E. coli* samples can be analyzed beyond 8 hr and up to 48 hr after sample collection while still generating comparable data if the samples are stored below 10°C.

Introduction

Water used for domestic or recreational purposes has an important impact on human health. As many as 3.4 million people die from water-related diseases due to poor water quality (World Health Organization [WHO], 2014). Each day, 4,000 children die due to contaminated water according to the United Nations Children's Fund (2014). The presence of pathogens in the surface water is increasingly turning into a concern throughout the world. According to the Clean Water Act Sections §305(b) and §303(d), more streams and rivers remain impaired due to pathogens than any other pollutants (Figure 1). Pathogen impairment has negatively affected 480,000 km of rivers and 2 million hectares (ha) of lakes in the United States (US) (United States Environmental Protection Agency [USEPA], 2014).

Sources of Pathogens and Control Measures

Contamination of water bodies by pathogens may result from a point and non-point sources. Non-point sources include agricultural runoff, urban stormwater, and streams. Point sources consist of overflows from wastewater treatment plants, spills, or runoff from livestock housing or manure storage facilities. All these sources are linked to increase microbial loads to natural bodies of water (McLellan, 2004). Some microbial contaminants can be removed by water treatment coagulation and filtration processes.

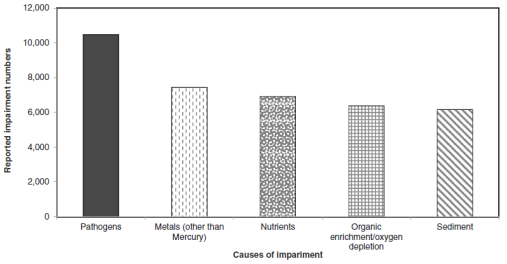

Figure 1: Causes of impairment in the U.S. (source: U.S. EPA, 2014)

The most important and cost-effective protection for water bodies is to prevent pathogen entry into source water. Point sources can often be controlled by treatment at the source. Control of non-point pollution requires a diminished release of pathogens to the atmosphere and run-off.

Indicator Organism

Indicator organisms are monitored to assess the level of microorganisms in water bodies. Water quality standards related to fecal contamination are measured in reference to *Escherichia coli* (*E. coli*) and *Enterococci* count. The presence of microorganisms such as *E. coli* in a water sample from an environmental source provides direct evidence of fecal contamination. The presence of microorganisms such as this is a great concern for human health.

Among the various microorganism, *E. coli* is a specific indicator of fecal pollution. According to Odonkor and Ampofo (2013), two key factors led to the use of *E. coli* as the preferred indicator for the detection of fecal contamination. First, some fecal coliforms could be of non-fecal origin. Second, the development of improved testing methods for *E. coli* makes testing more accurate. They are absent in uncontaminated water, survive at least as long as other waterborne microorganisms, and are thus considered by scientists as a good indicator organism (WHO, 2016).

The result of various studies demonstrates that *E.coli* is present in fecally contaminated water. The US Environmental Protection Agency (EPA) published a report recommending *E. coli* or *Enterococci* as the preferred fecal indicator bacteria (FIB) for freshwater (USEPA, 1986). This study is focused on *E. coli* in freshwater, where reports on its quantification are more prevalent than other water microorganisms.

Overview of Methods used to Enumerate *E. coli*

Over the years, differentiation of coliforms had come to a series of correlations that suggested indole production, gelatin liquefaction, sucrose fermentation, and Voges-Proskauer reaction were among the more important tests for determining fecal contamination. These developments culminated in the IMViC (Indole, Methyl red, Voges-Proskauer and Citrate) tests to differentiate fecal coliforms. One of the first generally accepted simpler methods for coliforms was called the Multiple-Tube Fermentation Test. The test method has evolved continually to become more specific. Some of the more significant developments were the so-called fecal coliform test, which selects for coliforms of fecal origin by using a higher incubation temperature (Odonkor and Ampofo, 2013).

Table 1. Approved CWA *E. coli* Test Methods

Number	Method Title
1103.1	*Escherichia coli (E. coli)* in Water by Membrane Filtration Using membrane-Thermotolerant *Escherichia coli* Agar (mTEC)
1603	*Escherichia coli (E. coli)* in Water by Membrane Filtration Using Modified membrane-Thermotolerant *Escherichia coli* Agar (Modified mTEC)
1604	Total Coliforms and *Escherichia coli* in Water by Membrane Filtration Using a Simultaneous Detection Technique (MI Medium)

(Source: USEPA, https://www.epa.gov/cwa-methods/approved-cwa-microbiological-test-methods)

Culture-based methods, multiple-tube fermentation (MTF) or membrane filtration (MF) followed by incubation on selective media are traditionally used for the enumeration of *E. coli* in waters. MTF and MF methods are now widely used for the routine analysis of microbiological water quality in Europe and North America(Prats et al., 2007). The US Environmental Protection Agency (USEPA) Method 1103.1 describes a membrane filter (MF) procedure for the detection and enumeration of *E. Coli* bacteria in ambient water (USEAP, 2010). EPA approved methods for *E. coli* Test in ambient water is listed in Table 1.

Eccles et al. (2004, examined the suitability of membrane filtration techniques and most probable number methods for isolating and enumerating *Escherichia coli*. The result showed that the tested methods gave comparable recoveries, and did not vary by greater than one order of magnitude (1 log). Likewise, Hamilton et al. (2006), examined enzyme-specific media and compared to levels determined with conventional culture media (m-FC and m-TEC) and concluded that levels observed with all tests were highly correlated, and significantly fewer *E. coli* were enumerated with m-TEC than with enzyme-specific media.

Water Quality and Holding Time

The Clean Water Act requires the US Environmental Protection Agency (USEPA) to update the environmental water quality status of our nation's waters on a regular basis. Water quality samples from various water bodies are collected and analyzed regularly by individual state agencies and their partners in order to accomplish this goal. Water samples collected for microbiological analysis should be examined as soon as possible. This is because changes could occur in the bacterial densities due to external factors such as storage temperature, time, and exposure to the atmosphere (United States Geological Survey [USGS], 2006). It is not possible to analyze the water sample immediately due to technical and procedural difficulties.

It is important to apply relevant standard operating procedures in the collection, handling, and preservation of water samples in order to ensure they are accurate representations of the water body being sampled (Ferguson, 1994). Holding time is critical during water quality sampling. The USEPA recommends analyzing samples immediately after the collection due to density differences over time. There is concern that the reliability of data is compromised because of holding time exceedances. It is important universally to determine proper holding time, which is defined as the length of time a sample can be stored after collection and prior to analysis without affecting the results.

Many investigations have shown that the increase in holding time results in a decrease in pathogen count (Aulenbach, 2010; Lonsane, Parhod, & Rao, 1967; Mcdaniels & Bordner, 1983). Pope et al. (2003) assessed the effects of holding time on *E. coli* in surface water samples that included 11 laboratories and 24 sites across the United States (US). Many previous studies measured *E. coli* concentrations between different holding times. Standridge and Lesar (1977) measured these differences between 4 hours (hr) and 24 hr. Pope et al. (2003) conducted similar studies between 0 to 48 hr. The Texas Commission on Environmental Quality's study looked at the differences between 6 and 30 hr and Harmel et al. (2016a) looked at times of ≤24 hr and >24 hr. They all concluded that increases in holding times resulted in decreases in pathogen counts.

Oklahoma Conservation Commission and Water Quality

In the state of Oklahoma, the Oklahoma Conservation Commission's (OCC) Water Quality Division is responsible for identifying waters impaired by non-point source pollution (NPS). NPS is pollution that comes from multiple diffuse sources such as pesticides, fertilizers, sediment, and animal waste run-off. OCC works to prioritize and implement projects to reduce pollutants and improve water quality. The bacteria analysis under OCC operating procedure is conducted only during the period from May 1 through September 30. Their analysis commonly includes *E.coli* and

Table 2. Required Containers, Preservation Techniques, and Holding Times Applicable to all Non-Potable Water Samples (includes wastewater, surface water, and groundwater)

	EPA Procedure[1]	OCC Procedure[2]
Bacterial Tests	*E. coli*	*E.coli*
Container	Plastic, Glass	Plastic, Glass
Preservation	Cool, <10 °C, 0.0008% $Na_2S_2O_3$	Ice, 4 °C
Maximum holding time	8 hours	6 hr/24 hr/48 hr[3]

(Sources: [1]USEPA, 1986; [2] OCC, 2014)
[3]standard violation 6 hr holding time is required; for OCC purposes, 24 hr is preferred but 48 hr is acceptable

Enterococcus. Table 2 lists OCC-recommended sampling and preservation procedures for bacteria samples.

Objective

The Oklahoma Conservation Commission is the state agency responsible for the state of Oklahoma's Non-Point Source Pollution Program. They are responsible for monitoring streams around the state for a wide variety of parameters, including pathogens. It would benefit the agency to better understand the effect of holding time on its pathogen counts.

There is currently inconsistency in the maximum acceptable holding times for water sampling of pathogens nationally. Although many studies reported on the effects of holding time exceedances on water quality, there is a lack of comprehensive review of these studies. The objective of this work is to provide an inclusive review on the effects of holding time exceedances on water quality.

Materials and Methods

A literature review was performed to collect and compile data pertaining to the effect of holding time exceedances on water quality. We searched handbooks, official guidelines, and scientific papers. They were qualitatively analyzed with respect to holding time and *E. coli* count in order to determine the relativity to this particular study. If the analyzed document discussed changes in bacteria count due to holding time, it was considered relevant.

Sources for the literature review included official websites of government agencies such as the USEPA, state agencies, and scientific peer review journals. Scientific papers were searched in databases such as Google Scholar and Web of Science using keywords: holding time, fecal indicator bacteria, and water quality. Literature sources were selected based on whether the results discussed the effects of holding time on pathogen counts, such as the number of *E. coli* or *Enterococci*. Nine reports, fourteen journal articles and one handbook were reviewed. Department of Environmental Quality or similar state agency websites of all 50 states and Washington DC were also reviewed. The literature source, holding time, sample storage hour, and effect of holding time on microbial density were tabulated for further discussion.

Results and Discussion

Holding Time and Bacteria Count

The USEPA-recommended water quality criteria for *E. coli* in freshwater systems is presented in Table 3 (USEPA, 2018). According to their recommendation, the maximum allowable value is 126 (MPN/100 ml) and it is expressed as the geometric mean value. The states in USEPA Region 6 all have a 126 maximum allowable value (MPN/100 ml) for freshwater that is used for primary contact recreation. For most of the other states, the standard required (a geometric mean during any consecutive 30-day period during the recreational season) is to be less than 126 CFU/100 mL (Table 4). Alaska, Illinois, North Carolina, and West Virginia apply a standard of 200 CFU/100 mL.

Collected water samples are preserved by placing the sample on ice and transporting

Table 3: Water quality criteria for *E.coli* in freshwater systems

Single Sample Maximum Allowable Values (MPN/100 ml) for *E. coli*		
	EPA Standard	Oklahoma standard
Geometric Mean Value	126[a]	126[a]
Designated Bathing Area	235	235[b]
Moderate Full Contact Recreation	298	-
Lightly used Full Body Contact Recreation	409	406[c]
Infrequent used Full Body Contact Recreation	576	-

(Source: USEPA, 2018)

[a] *E. coli shall not exceed a monthly geometric mean of 126/100 ml based upon a minimum of not less than five (5) samples collected over a period of not more than thirty (30) days*
[b] *in lakes and high use waterbodies*
[c] *all other primary body contact recreation beneficial use areas*

it to a refrigerator. It is best to minimize the time between the collection, storage, and analysis. As per the American Public Health Association (APHA) guidelines (1998) for non-potable water samples, the standard storage time between collection and processing is 6 hr, and the samples should be kept below 10ºC (Selvakumar, Borst, Boner, & Mallon, 2004). At present, the maximum acceptable holding time across the globe is inconsistent. The Clean Water Act Alternate Test Procedure, as described in Section 40 CFR Part 136 TABLE II, requires 8 hr as maximum holding time for all non-potable water samples (Table 2) (USEAP, 1986).

Comparison on maximum holding time varies by state (Table 4). The maximum allowable holding time found is 30 hr for Idaho and Iowa. Kansas, Montana, and Oklahoma allow up to 24 hr. In most of the other states, the water samples are required to be delivered to the lab within 6 to 8 hr of collection.

Previous studies (Table 5) have examined municipal and industrial effluent (Dutka & El-Shaarawi, 1980; Selvakumar et al., 2004; Standridge & Lesar, 1977), stormwater (Characklis et al., 2005; Selvakumar et al., 2004), water from lakes and rivers (Aulenbach, 2010; Dutka & El-Shaarawi, 1980; Pope et al., 2003; Standridge & Lesar, 1977), and water from municipal distribution systems (McDaniels & Bordner, 1983) for fecal indicator bacteria (FIB). According to these studies, survival rates for different FIB vary. Total coliform often decreases shortly after collection, whereas fecal coliform generally survives longer. It was noted that they could possibly live up to 62 hr (Aulenbach, 2010).

Lonsane et al. (1967) observed that the concentration decreased with an increase in storage time while the differences were not significant for marginally polluted water. Standridge and Lesar (1977) examined 28 water samples with initial coliform counts between 102/mL and 106/mL and found little change after storage at 2ºC to 4ºC for 24 hr. McDaniels and Bordner (1983) observed a significant decrease in coliform populations after 24 hr at temperatures 5°C and 22°C. The rate of decline was 2.5 magnitudes greater at 22°C than at 5°C. Average losses in 24 hr were 34% at 5°C and 87% at 22ºC. Some studies did not observe a significant decrease in *E. coli* density between 18 to 27r (Aulenbach, 2010; Selvakumar et al., 2004), while other studies suggested that up to 48 hr was acceptable (Pope et al., 2003).

In a study by the Texas Commission on Environmental Quality, decreases in *E. coli* concentrations were observed with an increase in holding time from 8 hr, 24 hr and 48 hr (Texas Commission on Environmental Quality [TCEQ], 2008). In a similar study where the number of *E. coli* was examined after a holding

Table 4: State by State comparison on holding time and maximum allowable value of *E.coli*

States	Holding time (hrs)	Geometric Mean*, Maximum Allowable Values (MPN/100 ml)**	Source
Alabama	8	126	www.adem.state.al.us
Alaska	6	200	www.dec.alaska.gov
Arizona	6	245 CFU[1]	www.legacy.azdeq.gov
Arkansas	-	126	-
California	6	126	www.waterboards.ca.gov
Colorado	8	126	www.colorado.gov www.colorado.gov/pacific/sites/default/files
Connecticut	8	126	www.portal.ct.gov
Delaware	6	100	www.dnrec.delaware.gov
Florida	6	126	www.floridadep.gov
Georgia	24[2]	126	www.epd.georgia.gov
Hawaii	-	-	-
Idaho	30	126	www.ci.moscow.id.us
Illinois	8	200	www.idph.state.il.us
Indiana	6	125	www.in.gov/idem
Iowa	30	126	www.iowadnr.gov
Kansas	24	160	www.kdheks.gov
Kentucky	8	130	www.water.ky.gov
Louisiana	6	-	www.deq.state.la.us
Maine	-	-	-
Maryland	6	126	www.health.maryland.gov
Massachusetts	8	126	www.mass.gov/doc/hudson-river-basin-water-quality-assessment-report-2002-appendices-0
Michigan	6	130	https://www.michigan.gov/documents/deq
Minnesota	6	126	www.pca.state.mn.us
Mississippi	6	126	www.mdeq.ms.gov
Missouri	6	126	dnr.mo.gov/env
Montana	24	126	waterquality.montana.edu
Nebraska	-	126	-
Nevada	6	126	www.ndep.nv.gov
New Hampshire	-	-	-
New Jersey	8	126	www.nj.gov /dep/srp/guidance
New Mexico	6	206	www.nmhealth.org
New York	8	200	www.wadsworth.org
North Carolina	6	200	www.files.nc.gov
North Dakota	-	126	
Ohio	6	126	www.epa.ohio.gov
Oklahoma	24	126	www.owrb.ok.gov
Oregon	6	126	www.deq.state.or.us
Pennsylvania	8	200	www.sfiles.dep.state.pa.us
Rhode Island	6	200	www.dem.ri.gov
South Carolina	-	200	-
South Dakota	6	126	www.denr.sd.gov
Tennessee	8	126	www. publications.tnsosfiles.com
Texas	8	126	www.tceq.texas.gov
Utah	8	126	www.deq.utah.gov
Vermont	8	126	www.dec.vermont.gov
Virginia	-	126	-
Washington	6	126	www.fortress.wa.gov
West Virginia	6	200	www.dep.wv.gov
Wisconsin	-	126	-
Wyoming	8	126	http://deq.wyoming.gov

** calculated using data from at least five different samples collected in separate 24-hr periods*

*** (Source: www.epa.gov)*

[1] Applicable Standard or Other Criteria

[2] All samples must be plated preferably as soon as possible, but no more than 24 hours after collection

Table 5. Summary of previous studies on effects of holding time on *E. coli* in water samples

Holding time (hours)	Temperature (°C)	Type of Bacteria	Conclusion/Remarks	Reference
24, 48, and 72	20 and 4	*E.coli*	– Concentration decreased with storage time	Lonsane et al., (1967)
4 and 24	4	*E.coli*	– Many samples can successfully be stored at 4°C for 24 hr	Standridge & Lesar, (1977)
2, 24, 30, and 48	1.5	*E.coli*	– More than 75% of the samples were microbiologically stable for at least 24 hr	Dukta & El-Shaarawi, (1980)
24, 30, and 48	22 and 5	*E.coli*	– Coliform populations declined significantly at both temperatures after 24 hrs. The rate of decline was 2.5 orders of magnitude greater at 22°C than at 5°C. Average losses in 24 hr were 34% at 5°C and 87% at 22 hr	McDaniels & Bordner, (1983)
9 and 18	20	*E.coli*	– Total coliform counts varied significantly when water samples were stored for either 9 or 18 hr – Results signify an inherent difference between samples collected manually and those collected automatically	Ferguson, (1994)
0, 8, 24, 30, and 48	10	*E.coli*	– If samples are held below 10°C and are not allowed to freeze, most surface water E. coli samples analyzed by commonly used methods beyond 8 hr – No significant difference in bacterial densities throughout the 48 hr	Pope et al., (2003)
24	4	*E.coli*	– The concentration of fecal coliform measured during the first 7 hr holding time was slightly greater than concentrations measured beyond 24 hr holding time	Selvakumar, et al., (2004)
6 and 24	<10°C	*E. coli*	– Samples can be analyzed 24 hr after sample collection and still generate data comparable to those generated at 6 hr after sample collection	USEPA, (2006)
Up to 62	-	*E. coli*	-Fecal and total coliform densities did not change significantly with holding times up to about 27 hr	Aulenbach, (2010)

time of 6 hr, decreases were observed by 20% (USEPA, 2006). A study (Karthikeyan's unpublished data, as cited in Harmel et al., 2016b) showed an increase in the concentration of *E. coli* in the first 2 hr by an average of 15%. It decreased from 3% to 17% for the next 3 to 48 hr. Karthikeyan et al. (unpublished data, as cited in Harmel et al., 2016b) observed higher uncertainty in counts when samples were stored at 25°C compared to 15°C. McCarthy et al. (2008) also examined the effects of temperature on pathogen count for samples stored for up to 24 hr, and the result showed that the number of hours a sample is stored in the field was not statistically significant.

A comprehensive study was performed by Pope et al. (2003) to examine the effects of holding time exceedances on pathogen counts. It included multiple laboratories and many sites across the US. It also included more than one monitoring method for *E. coli* (Table 6). There was no significant decrease in *E. coli* densities from samples that were analyzed with the Colilert method and stored at 4ºC and 10°C. Significant differences occurred when the samples had been held for at least 48 hr. *E. coli* densities for samples stored at 20°C and 35°C were significantly reduced within 8 to 48 hr.

Samples from the Southern Nevada Pumping Plant 1 showed a significant increase with time. The increase in *E. coli* density at this site was related to holding temperature and was attributed to samples not being maintained below 10°C after 12 hr (Pope et al., 2003). The study (Pope et al., 2003) demonstrates that 8 of 13 sites showed no significant difference in *E. coli* densities between time 0 and the 48-hr holding time, regardless of the evaluation

Table 6. Summary of test results for time 0 comparison (Source: Pope et al., 2003)

Laboratory	Site	Method	Coolant	Mean no. of *E. coli*/100 ml at time 0	No. of E. coli/100 ml (significant change in density) at indicated time (h) after sample collection[a]			
					8	24	30	48
Fairfax County Water	Potomac River	Colilert	Wet ice	73	NS	51 (D)	NS	NS
Fort Worth Water	Rolling Hills WTP[b]	Colilert	Utek	63	NS	NS	NS	NS
Indianapolis Water	Fall Creek	Colilert	Utek	337	NS	NS	NS	NS
	White River	Colilert	Utek	534	NS	NS	NS	NS
City of Phoenix	Squaw Peak WTP	Colilert	Wet ice	11	NS	NS	NS	NS
	Union Hills WTP	Colilert	Wet ice	69	NS	NS	NS	NS
Jefferson Parish	Mississippi River	mTEC	Wet ice	310	NS	NS	NS	NS
Southern Nevada	SNWS Pumping Plant	mTEC	Utek	17	30 (I)	32 (I)	34 (I)	44 (I)
Passaic Valley	Passaic & Ramapo Rivers	mFC/NA-MUG	Blue ice	193	NS	90 (D)	108 (D)	85 (D)
Portland Water Bureau	Station 2	mFC/NA-MUG	Blue ice	44	NS	55 (I)	NS	NS
Mohawk Valley	Hinckley Reservoir	mFC/NA-MUG	Utek	42	97 (I)	NS	NS	NS
Wisconsin State Laboratory of Hygiene	Willow Creek	mFC/NA-MUG	Wet ice	560	NS	NS	NS	NS
	Wingra Springs	mFC/NA-MUG	Wet ice	367	NS	NS	NS	NS

D, significant decrease in E. coli density compared to the time 0 results; I, significant increase in E. coli density compared to the time 0 results; NS, no significant difference in E. coli density compared to the time 0 results; WTP, water treatment plant

method and the coolant used. The results of the Pope et al. (2003) investigation suggested that *E. coli* samples can be analyzed beyond 8 hr and up to 48 hr after sample collection while still generating comparable *E. coli* data, provided that the samples are stored below 10°C.

To ensure that the most accurate data are generated, *E. coli* samples collected from surface waters should be analyzed immediately and within 6 to 8 hr when on-site facilities are available (Pope et al., 2003). Many of the studies (Aulenbach, 2010; Pope et al., 2003; Selvakumar et al., 2004) have reported that water samples can be analyzed beyond the 8 hr holding time and generate reliable data. However, an overall decrease in bacterial density with an extended holding time of > 8 hr was observed in some cases (Ferguson, 1994). The magnitude of the decrease is attributed to a decrease in nutrient concentrations and other parameters, including temperature, storage condition, and initial bacterial density (Volk & LeChevallier, 1999). As unpredictability exists within survival rates of *E. coli* and other pathogens, a comparison between the results of different studies is also challenging. The ability to compare water-quality of different sources largely depends on the uniformity in the sampling, preservation, storage, and transportation.

Collection/Storage Method and Bacteria Count

Sample preservation and storage protocols maybe even more critical for microbial samples due to their transient nature and susceptibility to environmental conditions. Typical preservation procedures involve placing the sample on ice after collection and transporting it to a refrigerator. The standard storage time between collection and processing is ≤ 8 hr, with the sample held below 10°C during this period (American Public Health Association [APHA], 1998). However, utilizing hold times longer than 8 hr for fecal indicator bacteria is supported by studies such as Pope et al. (2003) and Selvakumar

et al. (2004). Thus, numerous research studies have utilized 24 hr as a hold time threshold. Pope et al. (2003) reported that when samples were stored in coolers with wet ice or Utek ice packs, five of seven sites showed no significant difference between 0 and 24 hr of holding time, four sites showed no significant difference at 30 hr of holding time, and only two of seven sites showed no significant difference between 0 and 48 hr of holding time. Likewise, a study where samples were put on ice and brought back to the laboratory and refrigerated until processing indicated that bacteria densities do not change significantly with holding times up to about 27 hr for total coliform and possibly as long as 62 hr (Aulenbach, 2010). In another similar study (Harmel et al., 2016a), samples were stored in a cooler on the ice during transport to the laboratory and tested for short events (≤24 hr holding time) and long events (>24 hr). The results showed that there were no statistically significant *E. coli* concentration differences in samples stored for long runoff events (>24 hr). Storage uncertainty relates to the fact that collected samples can often be left in the field, without preservation or refrigeration, for a number of hours before analysis (McCarthy et al., 2008). According to, McCarthy et al. (2008) *Hours in Field* is not a significant factor for the *E. coli* level of stored samples up to 24 hr. However, when comparing holding times for samples stored in environmental conditions, McCarthy et al. (2008) reported an initial increase in *E. coli* concentrations (4 and 8 hr) but decreased after 24 hr.

Conclusion

It is not always possible to transport water samples to the testing facilities immediately, thus increasing holding time. Holding time and temperature can have a significant effect on the density of indicator species (*E. coli*). To ensure that the most accurate data are generated, *E. coli* samples collected from surface waters should always be analyzed as soon as possible. The results of this review suggest that pathogens (*E. coli*) present in water samples can be analyzed beyond 8 hr after sample collection while still generating comparable *E. coli* data. However, water samples need to be stored below 10°C.

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Abstracts of the
109th Oklahoma Academy of Science Technical Meeting
November 6, 2020
Held Virtually

FIRST-PRINCIPLES STUDY OF DEFECT FORMATION ENERGIES IN GRAPHENE AND CARBON NANOTUBES

Marasini, Bishal and Sanjiv Jha, East Central University

Presence of structural defects in graphene and carbon nanotubes (CNTs) can dramatically modify their properties. We apply first principles density functional computational method to study the formation energies of Stone-Wales defects and di-vacancies in carbon nanomaterials. Our calculations were performed using the Quantum ESPRESSO electronic structure package. Graphene sheets were modeled using 4x4, 5x5, and 6x6 hexagonal supercells containing 32, 50, and 72 carbon atoms, respectively. Armchair (10,10) CNT was modeled using a periodic supercell containing 160 carbon atoms. Stone-Wales defects in carbon nanomaterials were produced by the in-plane rotation of C-C bonds by 90° about their center, whereas the di-vacancy defects were created by removing two adjacent carbon atoms. Our calculated formation energies for studied supercells of graphene were in the range of 4.0 eV to 5.0 eV for Stone-Wales defects, and 6.0 eV to 7.0 eV for di-vacancies. In the case of the (10,10) CNT, the formation energy for Stone-Wales defects was calculated to be 4.39 eV. 1The computing for this project was performed at the OU Supercomputing Center for Education & Research (OSCER) at the University of Oklahoma (OU).

AN AUTOMATED QUALITY MEASURE OF SENTENTIAL PARAPHRASES

Duong, Thanh, Robert Owens, and Thanh Thieu, Oklahoma State University

Measuring quality/fluency of sentential paraphrasing is a challenging task that traditionally requires human annotation. By building learning-to-rank machine learning models, we present a novel method that works automatically and balances two conflicting criteria: semantic similarity and lexical diversity. Using machine translation features including edit distance, BLEU, ROUGE, and cosine similarity, we built models to measure quality of differentiation between a paraphrase and its reference sentence. Extrinsic evaluation on STS Benchmark and ParaBank Evaluation datasets resulted in a model ensemble with moderate to high accuracy. We applied our method on both small benchmarking and large-scale datasets as resources for the community.

TRANSPORT AND RECOVERY OF ALUMINUM OXIDE NANOPARTICLES THROUGH LIMESTONE AND DOLOMITE PACKED COLUMNS

Maples, Randall and Mitchell Wallis, East Central University

Metal oxide nanomaterials are used in applications such as materials coatings, sensors and even drug delivery. Because of this, there is the increased potential of these engineered nanoparticles being released into the environment as contaminants when devices and materials containing these are disposed of. The environmental toxicity of such materials has not been fully fleshed out due to the variety of engineered nanoparticles. It is important to assess the short and long-term fate of these engineered materials and their distribution in groundwater. This study looked at the synthesis, transport and recovery of water dispersible functionalized aluminum oxide nanoparticles through packed stone columns as models for the local groundwater environment.

COVALENT FUNCTIONALIZATION OF DEFECTIVE GRAPHENE AND CARBON NANOTUBES BY BENZYNE: A DENSITY FUNCTIONAL THEORY STUDY

Brown, Kellan and Sanjiv K. Jha, East Central University

We performed density functional theory calculations to study the covalent functionalization of pristine and defective graphene and carbon nanotubes (CNTs) with benzyne. Our calculations were carried out using the Quantum ESPRESSO electronic-structure package. Graphene sheets were modeled using 4x4, 5x5, and 6x6 hexagonal supercells containing 32, 50, and 72 carbon atoms, respectively. Armchair and Zigzag CNTs were modeled with periodic supercells containing 128 to 160 carbon atoms. Stone-Wales (SW) defects in carbon nanomaterials were created by rotating C-C bonds by 90° about their center, whereas the double vacancy (DV) defects were created by removing two adjacent carbon atoms from graphene and CNTs. The binding energies of benzyne functionalized graphene and CNTs were examined in cases of graphene and nanotubes containing no surface defects, containing Stone-Wales defects, and containing double vacancy defects. Our calculated results indicate that the presence of structural defects enhances the binding of benzyne to the surfaces of graphene and carbon nanotubes.

[1]Supported by OK-LSAMP and the McNair Scholars Program. [2]The computing for this project was performed at the OU Supercomputing Center for Education & Research (OSCER) at the University of Oklahoma.

COMPOUND OSW-1 INHIBITION OF SSRNA VIRUSES, INCLUDING CORONAVIRUS

Blewett, Earl L. and B.J. Reddig, Oklahoma State University Center for Health Sciences

Brett Roberts, and Anthony Burgett, University of Oklahoma Health Sciences Center.

The compound OSW-1, used at 1 - 30 nM concentrations, was shown to significantly inhibit the growth of many (+) ssRNA viruses. OSW-1 is a natural compound that extracted from the bulbs of the Giant Chincherinchee plant Ornithogalum saundersiae. The OSW-1 used in this study was synthesized in the laboratory of Dr. Burgett. The viruses tested were Enterovirus D68, Coxsackievirus A9, Coxsackievirus B2, Coxsackievirus B5, Coxsackievirus B6, Human Rhinovirus-1B and Feline Coronavirus. When cells were infected with virus then treated with OSW-1, viral growth was inhibited by as much as 4 logs. OSW-1 also significantly inhibited virus growth when the cells were prophylactically treated with the compound then infected with virus. We show that as a treatment, OSW-1 worked at much lower concentrations than other anti-enterovirus compounds such itraconazole. We also show that OSW-1 will work prophylactically to inhibit virus infection and itraconazole will not.

*GRAM-POSITIVE ANTIBACTERIAL SPECTRUM OF A NOVEL MELANIN-INSPIRED ANTIMICROBIAL.

Reed, Daniel, Toby Nelson, and Gabriel Cook, Oklahoma State University

Franklin R. Champlin, Oklahoma State University Center for Health Sciences.

The Melanin-inspired core represents a novel compound having the intrinsic ability to act as scaffolding for functional groups which may possess antibacterial properties. The purpose of this study was to investigate the antibacterial potential of Melanin-inspired antimicrobial EIPE-1 and EIPE-HCl which are hydrophobic and hydrophilic, respectively. A standardized disk agar diffusion bioassay was employed to determine the susceptibility and resistance levels of twelve gram-positive and thirteen gram-negative bacteria to the nonpolar and polar EIPE derivatives. Turbidimetric growth curves were generated from batch culture growth kinetic analysis to provide preliminary mechanistic information. Five strains of *Staphylococcus aureus*, plus *Bacillus* subtilis and *Staphylococcus* epidermidis were found to be susceptible to the hydrophobic derivative EIPE-1, while other gram-positive and all gram-negative organisms exhibited resistant phenotypes at the potencies tested. Batch cultural growth kinetics revealed EIPE-1 to cause immediate bacteriolysis of B. subtilis and S. epidermidis at a concentration of 0.2 µg/mL. The more polar EIPE-HCl derivative failed to inhibit growth of any of the organisms examined. These data support the conclusion that the hydrophobic EIPE derivative EIPE-1 possesses a gram-positive antibacterial spectrum and likely acts in a cytoplasmic membrane-directed manner. The susceptibility of two methicillin-resistant *S. aureus* strains suggests that its mechanism of action does not involve the penicillin-binding proteins of peptidoglycan biosynthesis targeted by mainstream β-lactam antibiotics. The uniform resistance of thirteen phylogenetically disparate gram-negative pathogens supports the notion that intrinsic outer membrane exclusion properties may play a role in the mechanism underlying their intrinsic phenotypic resistance. Further experiments will involve treating gram-negative organisms with an outer membrane permeabilizer in an attempt to increase the efficacy of EIPE-1 by chemical sensitization. Determining the mechanism of resistance to gram-negative organisms will be valuable for expanding the EIPE-1 spectrum and the development of other Melanin-inspired derivatives.

POSITIVE THINKING AND ANXIETY

Mitchell, LaDana and Alicia Limke-McLean, University of Central Oklahoma

This current research evaluated the effects of increased positive thoughts on anxiety. Anxiety experiences include negative feelings of excessive worry, stress, and discomfort. According to Badpar et al. (2017), thoughts can have an impact on human behavior along with mental and emotional states. The goal of this research is to test if increasing positive thoughts could help to alleviate some of the negative thoughts that accompany anxiety. College students (n=346) at the University of Central Oklahoma were recruited via the university email blast system to take part in an online experimental research study. Half of the participants underwent a positive thinking exercise video and the other half viewed a control video. The Positive Thinking scale (PTS) was used as a measurement for positive thinking (Diener et al., 2009). The Brief Symptom Inventory scale was used as a measurement for anxiety (Derogatis, 1975). A 2 (within: before and after) x 2 (between: positive and control) mixed factorial analysis of variance (ANOVA) was conducted on the data. The gain for positive thinking was marginally greater for individuals who watched the positive thinking video than for individuals who watched the control video. This marginally supports that increased positive thoughts can have a decreasing effect on anxiety.

DEVELOPING A PREDICTIVE MODEL FOR UNDERSTANDING THE CLIMATE CHANGE IN THE STATE OF OKLAHOMA

Akinwale, Emmanuel and Courtney William, Oklahoma State University.

Motivated by major challenges arising from the rapid change in the climate behavior around the world, the goal of this research project is to develop a predictive model for understanding the climate change in the state of Oklahoma using a common programming language called python. To this end, we study the last fifty years of historic climate data in the state of Oklahoma collected by the National Oceanic and Atmospheric Administration. To build a robust predictive model, we first need to clean the data to minimize the errors in them such as some missing dates then; we consider three main forecasting models, including the moving average scheme, the standard exponential smoothing, and Holt-Winters exponential smoothing. After implementing preliminary data cleaning and data preparation steps, we train each of the three models to tune their parameters. In the next step, we validate the best model within each category on the climate data of the recent few years. It was shown that the Holt-Winters model fits the Oklahoma climate data the best. This finding is further utilized to forecast the change of climate in Oklahoma in next few years. In addition to the developed predictive model, implementing a decomposition scheme, we discover that the average monthly temperature in Oklahoma has been consistently decreasing in the past few decades, particularly, in the 90s. This also proved that global warming was not the correct terminology to describe the change in the Oklahoma's climate. This research project is supported by Oklahoma Louis Stokes Alliance for Minority Participation (OK-LSAMP), conducted by the two undergraduate research scholars, Emmanuel Akinwale and Courtney Williams, and under the supervision of Dr. Farzad Yousefian a faculty member at the School of Industrial Engineering and Management in Oklahoma State University.

EFFECT OF TIME MANAGEMENT ON THE SLEEP HABITS OF AN UNDERGRADUATE STUDENT

Mitchell, LaDana and Vickie M. Jean, University of Central Oklahoma.

Approximately 28 to 64 percent of women were reported to have sleep disorders and time management training could significantly improve the sleep quality. (Wang & Wang, 2018). Effective time management can increase the quality of sleep one receives. Various time constraints can influence a student's sleep such as vigorous course loads along with managing part-time work. Adequate sleep increases academic performance. This study was designed to encourage time management strategies in a 21-year-old female undergraduate Psychology student at The University of Central Oklahoma. Participant collected baseline data for two weeks on hours of sleep to implement behavior modification regimen. This study used a single-subject research design. Measures used to collect data include a Microsoft word document for daily journaling of the time and variables; the Google sheets software was utilized to create the graphs for visual representation of this data. Following the baseline period, the participant collected data for an additional two-weeks on the time went to sleep following the implemented time-management behavior change. Participant also began to collect data on reinforcements given and amount of time to complete assignments. Time management-skills were implemented through the process of operant conditioning using positive reinforcement. Anticipated results are that the participant will sleep at least 8 hours a night after implementing the behavior change time-management strategies.

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OKLAHOMA ACADEMY OF SCIENCE
STATEMENT OF REVENUES COLLECTED AND EXPENSES PAID
FOR THE YEAR ENDED DECEMBER 31, 2019

REVENUES COLLECTED		
Membership Dues:	$2,940.09	$2,940.09
Investment Income:	$43.87	$ 43.87
Meetings:		
Registration - Fall Meeting	$3,713.01	
Registration - Technical Meeting	$8,600.38	$12,313.39
Donations:	$721.17	$721.17
Woody Plants:	$496.00	$496.00
POAS:	$4,190.52	$4,190.52
Transfer from OJAS	$1,172.48	$1,172.48
Other Income:	$3.50	$3.50
Total Revenue Collected		**$21,881.02**
EXPENSES PAID		
Stipends and other Compensation:		
Stipends	$6,141.24	
Social Security	$1,030.75	
Medicare	$241.05	$7,413.04
Professional Fees:		
Audit	$300.00	
Tax Preparation	$1,119.00	$1,419.00
Meeting Expenses:		
Fall Meeting	$3,825.00	
Technical Meeting	$3,128.97	$6,953.97
Dues:	$1238.17	$1238.17
POAS:	$3,528.58	$3,528.58
Woody Plants:	$487.13	$487.13
Other Expenditures:	$1,775.62	$1,775.62
Total Expenses Paid		**$22,815.51**
Revenues Collected Over Expenses Paid		**$-934.49**

OKLAHOMA ACADEMY OF SCIENCE
STATEMENT OF ASSETS, LIABILITIES AND FUND BALANCE
ARISING FROM CASH TRANSCATIONS
DECEMBER 31, 2019

ASSETS

Cash:		
Checking Account	$23,862.50	
Savings Account	$3,277.44	
Endowment Savings Account	$2,734.92	$29,874.86
Investments:		
Certificate of Deposit	$60,000.00	$60,000.00
Total Assets:		**$89,874.86**

LIABILITIES AND FUND BALANCE

Liabilities:	$0.00	
Fund balance:		
Beginning operation fund balance	$90,809.35	
Excess revenues collected over expenses	$-934.49	
Total Funds:		**$89,874.86**

OKLAHOMA ACADEMY OF SCIENCE

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All manuscripts must be refereed critically. The *POAS* Editors have an obligation to the membership of the Academy and to the scientific community to insure, as far as possible, that the *Proceedings* is scientifically accurate. Expert refereeing is a tested, effective method by which the scientific community maintains a standard of excellence. In addition, expert refereeing frequently helps the author(s) to present the results in a clear, concise form that exceeds minimal standards.

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A combination of these reasons is also possible grounds for declining to publish the MS. In most cases, the Editors rely on the judgment of the reviewers.

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All authors should approve all revisions (the corresponding author is responsible for insuring that all authors agree to the changes). A revised paper will retain its original date of receipt only if the revision is received by the Editor within two months after the date of the letter to the author(s).

B. Types of Manuscripts.

A manuscript may be a paper (report), review, note (communication), a technical comment, or a letter to the editor. All manuscripts should be submitted as a Microsoft Word document, 12-point Times New Roman font, double spaced, and include line numbers.

Paper (a report; traditional research paper). A Paper may be of any length that is required to describe and to explain adequately the experimental observations.

Review. The Editor will usually solicit review articles, but will consider unsolicited ones. The prospective writer(s) of reviews should consult the Editor; in general, the Editor needs a synopsis of the area proposed for review and an outline of the paper before deciding. Reviews are typically peer- reviewed.

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C. Manuscript Organization.

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For papers (reports), the subsections should typically include the following: Abstract, Introduction, Experimental Procedures (or Methods), Results, Discussion, Acknowledgments (if any), and References. In the case of notes or short papers, you may combine some headings, for example, "Results and Discussion":

I. The title should be short, clear, and informative; it should not exceed 150 characters and spaces (three lines in the journal), and include the name of the organism, compound, process, system, enzyme, etc., that is the major object of the study.

II. Provide a running title of fewer than 60 characters and spaces.

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VII. You may present the Results in tables or figures or both, but note that it is sometimes simpler and clearer to state the observations and the appropriate experimental values directly in the text. Present a given set of results *in only one form:* in a table, or figure, or the text.

VIII. The Discussion section should interpret the Results and how these observations fit with the results of others. Sometimes the combination of Results and Discussion can give a clearer, more compact presentation.

IX. Acknowledgments of financial support and other aid are to be included.

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Examples of References in CBE Style and Format

Journal Articles

Miller LF, Chance CJ. 1954. Fishing in the tail waters of TVS dams. Prog Fish-Cult 16:3-9.

Ortenburger AI, Hubbs CL. 1927. A report on the fishes of Oklahoma, with descriptions of new genera and species. Proc Okla Acad Sci 6:123-141.

Books

Book with Authors:

Miller RJ, Robison HW. 1980. The fishes of Oklahoma. Stillwater (OK): Oklahoma State University Press. 246 p.

Book with Editors:

Gilman AG, Rall TW, Nies AS, Taylor P, editors. 1990. The pharmacological basis of theraputics. 8th ed. New York: Pergamon. 1811 p.

Book with Organization as Author: International Union of Pure and Applied Chemistry, Physical Chemistry Division. 1993. Quantities, units, and symbols in physical chemistry. 3rd. Oxford (UK): Blackwell Science. 166 p.

Chapter in Book with Editors:

Hamilton K, Combs DL, Randolph JC. 1985. Sportfishing changes related to hydro- power generation and non-generation in the tailwater of Keystone Reservoir, Oklahoma. In: Olsen FW, White RG, Hamre RH, editors. Proceedings of the symposium on small hydropower and fisheries. Bethesda (MD): American Fisheries Society. p 145-152.

Theses: Knapp MM. 1985. Effects of exploitation on crappie in a new reservoir [MSc thesis]. Stillwater (OK): Oklahoma State University. 84 p. Available from: OSU Library.

Internet: Oklahoma Climatological Survey. 2003. Climate of Oklahoma [online]. Available from: http://climate.ocs.ou.edu. (Accessed August 15, 2005).

D. Review Process.

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