Agriculture-Induced Aquatic Contamination and Disease Dynamics in Reptile Populations

April 2016

Shane M Hanlon, ***American Geophysical Union, Washington, DC 20001, USA***
e-mail: shane.michael.hanlon@gmail.com

William Peterson, ***Wapanocca National Wildlife Refuge, US Fish and Wildlife Service, Turrell, Arkansas USA***

James E Moore, ***Department of Biology, Christian Brothers University, Memphis, Tennessee 38104, USA***

In recent years, anthropogenic stressors have drastically altered both terrestrial and aquatic ecosystems. One of the greatest threats to organismal health are agricultural pesticides (Berny 2007). Such chemicals have the potential to affect a vast majority of flora and fauna that persists in areas of agriculture exposure. One area of intense agriculture is the Mississippi River Basin (MRB). With fields adjacent to the Mississippi River, some of the most fertile land in the United States exists along this corridor. However, with increased agricultural comes increased pesticide application (Fischer & Lindenmayer 2007). While policies are in place to prevent the direct application of such chemicals to non-target areas (i.e., non-croplands), incidental exposure through incidental direct exposure, or more commonly through runoff or spraydrift, is commonplace (Norris et al. 1983).

Wapannoca National Wildlife Refuge (WNWR), located in Turrell, Arkansas is as an ideal habitat to examine how contaminants alter ecosystem health in both fluvial and closed systems due to its close proximity to both agricultural systems and the MRB. The refuge contains a high diversity of habitats surrounded mainly by agricultural lands (~71%). In relation to contamination, these habitats can be grouped into two primary land-use types: areas with little/no point-source inflows (i.e., contaminant "free"), and areas with direct point-source runoff (i.e., direct input from agriculture). While these habitat types differ in their contamination input, they do share many of the same aquatic or semi-aquatic species. Perhaps the most common groups of vertebrates at WNWR are chelonids, or turtles.

A specific threat to turtle health are ranaviruses, a group of viruses with low host specificity; reptiles, amphibians, and even fish can be lethally or asymptomatically infected and can serve as reservoirs for other vulnerable species (Chinchar 2002; Schock et al. 2008). While ranaviruses are extremely lethal and have been attributed to mass herptile (reptiles and amphibians) die-offs nationwide (Gray et al. 2009), recent work has shown that ranaviruses may persist in populations without causing immediate die-offs, though the disease could "flare" at any moment to cause mass mortality. Ranavirus outbreaks and prevalence also vary seasonally; however, the underlying cause(s) of this variation is currently unknown. To date, surveys for the detection of ranaviruses have not been conducted in Arkansas; however, recently Hanlon et al. (2014) showed the amphibian chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) to be present in amphibian populations at WNWR. Ranaviruses have been detected previously in areas of *Bd* occurrence and the conditions necessary for both pathogens to persist are similar (Schloegel et al.

2010).

While much research has investigated the singular effects of ranaviruses on amphibian and/or reptile populations (Gray et al. 2009, Sparling et al. 2010), few studies have investigated how anthropogenic stressors may alter susceptibility to, or the resultant impact(s) of infection, from these pathogens. WNWR presents a unique opportunity to investigate how these biotic and abiotic stressors affect reptile populations. The effects of aquatic contaminants on ranavirus infections is a poorly investigated area. As turtles at WNWR persist in both the drainage ditches and ephemeral habitats, they are the perfect candidate organism to determine if and how pesticides alter ranavirus infections in natural systems. Moreover, WNWR contains habitats that are ideal for ranavirus persistence (e.g., aquatic ecosystems, wetlands [Petranka et al. 2007, Gahl and Calhoun 2010]).

Objectives

Our goal was to survey multiple locations within both habitats to determine:

1) The overall prevalence and species specificity of ranavirus in turtle populations
2) How ranavirus infection prevalence differs between habitats
3) The types and concentrations of pesticides in each habitats
4) How the presence of pesticides alter ranavirus infections in turtles and how such interactions vary seasonally.

Survey Area

We chose 3 reference sites (little agricultural input) and 3 agricultural sites as our study areas (Fig. 1). Reference sites receive no direct input from croplands while the agricultural sites receive direct input, primarily through runoff. While the reference sites will likely have small amounts of pesticides residues, the levels will be much less than the agricultural sites where pesticides are directly inputted into the systems. Moreover, because of increased agricultural land-use worldwide, “pristine” environments are extremely rare and the chosen study sites represent more realistic natural settings.

Methods

Turtles will be trapped using baited hoop nets (diameter 1m, mesh 2.5cm). Five traps/site were deployed for ~4 days per sampling period (4 sampling periods [Fig. 2], 20 trap nights/site/sampling period, 480 trap nights total) and the traps were checked daily. To ensure that no turtle was resampled, each captured turtle was marked with a unique identifier on the carapace using a Dremmel® tool. Upon capture, we determined the species, sex, mass, length, and checked for any clinical signs of ranavirus infection. Ranavirus can be detected through a tail clip (Gray et al. 2012). Each clip was placed in a snap-top tube, and upon returning to the lab, stored at 4°C until processed. Water samples was also collected from each site per sampling period. The samples were sent to Pacific Agricultural Laboratories and tested for a broad range of pesticide and fertilizer residues.

As pesticide accumulation and ranavirus loads vary seasonally, we sampled populations at four points throughout the year (roughly once every season). Previous work has shown that ranavirus loads peak in late spring/early summer (Gray et al. 2009). Using quantitative polymerase chain reaction (qPCR), ranavirus infection presence and intensity were determined. DNA was extracted from the clips using DNeasy Blood and Tissue Kit (Qiagen Inc., Valencia, CA, USA). From this extracted DNA, we used the qPCR methods of Picco et al. (2007) for virus detection.

Turtles were trapped using baited hoop nets (diameter 1m, mesh 2.5cm) at a single site in WNWR (Fig. 3). Five traps/site were deployed on 10 October 2015 and checked the following day. Upon capture, we determined the species, sex, maturity, mass, length, and checked for any clinical signs of ranavirus infection. Ranavirus can be detected through a tail clip (Gray et al. 2012). Each clip was placed in a snap-top tube (Fisherbrand®, Cat. # 02-681-272), and upon returning to the lab, stored at 4°C until processed.

Ranavirus confirmation

Using quantitative polymerase chain reaction (qPCR), ranavirus infection presence and intensity was determined. DNA was extracted from the clips using DNeasy Blood and Tissue Kit (Qiagen Inc., Valencia, CA, USA). From this extracted DNA, we used the qPCR methods of Picco et al. (2007) for virus detection.

Results

Contaminant levels

Results of pesticide residue analysis is presented in Table 1.

Trapping outcomes and ranavirus confirmation

During the year of contaminant sampling, we only succeeded in capturing turtles during the first sampling period from 17-19 August 2014. However, conditions prevented the confirmation of ranavirus in any captured individual (life history characteristics are presented in Table 2A). Sampling efforts continued until 11 October 2015 when we trapped 61 turtles (Table 2B). No ranavirus infections were detected in any of these turtles. Samples were tested via qPCR in three repeated tests, ensuring the accuracy of the test and the absence of ranavirus infections.

Discussion

Due to the inability to perform ranavirus tests in conjunction with contaminant testing, we were unable to test the effects on contamination on disease prevalence in turtle populations. In regards to contamination, we found residues from all major types (fungicide, insecticide, pesticide) and across numerous classes. Types of pesticides vary by sight and by sampling period. As predicted, a greater diversity of residues were present in May and August, shortly before and after the agricultural growing season, compared to the fall/winter months of November and February. While an abundance of residues are present in numerous parts of WNWR we cannot conclude that such residues have any effect on turtle abundance or diversity.

Counter to our predictions, ranavirus infections were not detected in any turtle that was captured on the refuge. We recognize that our predictions were based on the presence of a pathologically different disease (*Bd*). The lack of detection of ranavirus in the population measured allows us to conclude that the virus is not present in WNWR. However, such findings do not preclude an absence of ranavirus in the whole of Arkansas. Future surveys must be conducted to ensure the creation of an accurate mapping program.

References

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Fig. 1. Sampling areas within Wapanocca National Wildlife Refuge.

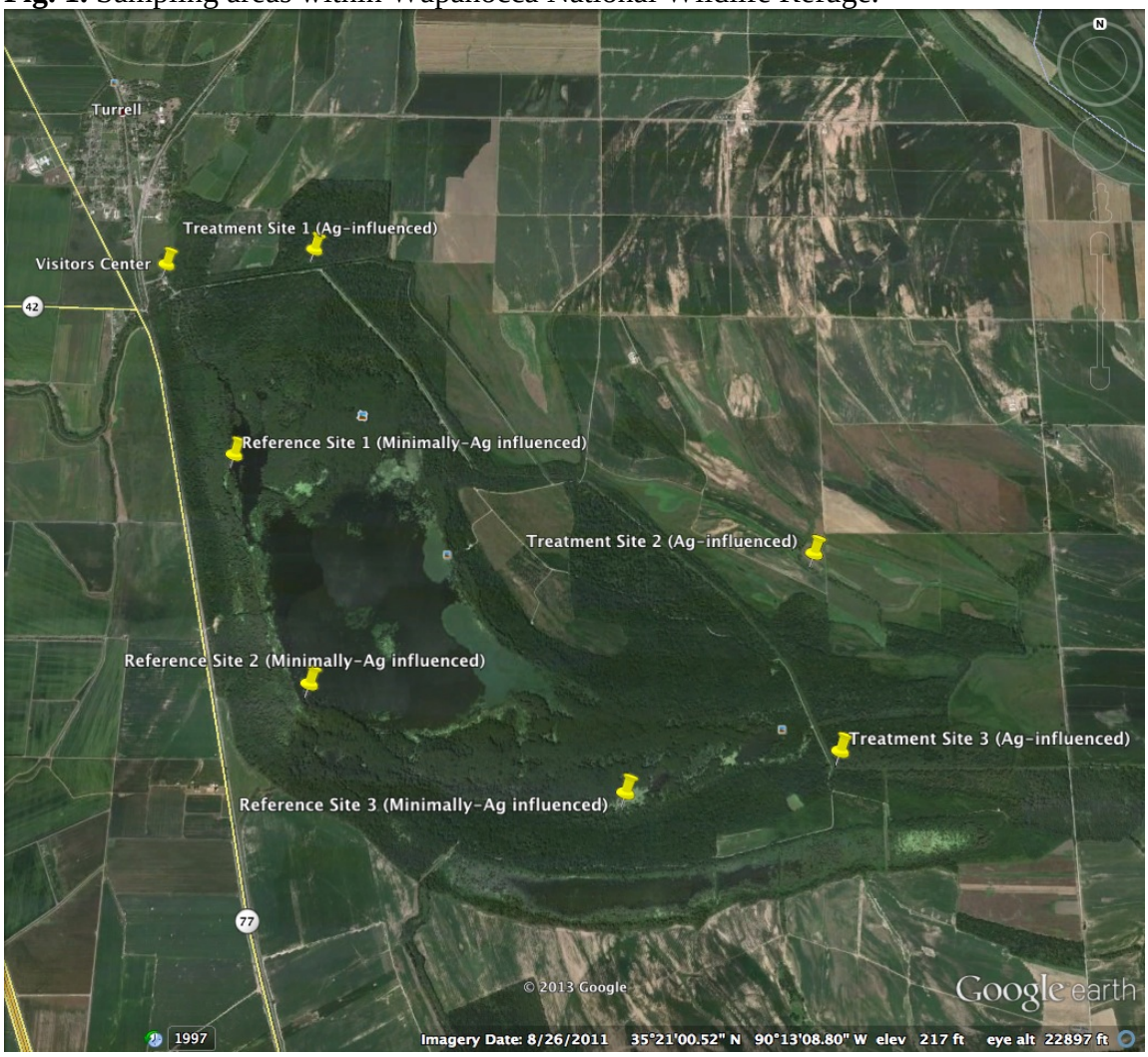

Fig. 2. Project timeline.

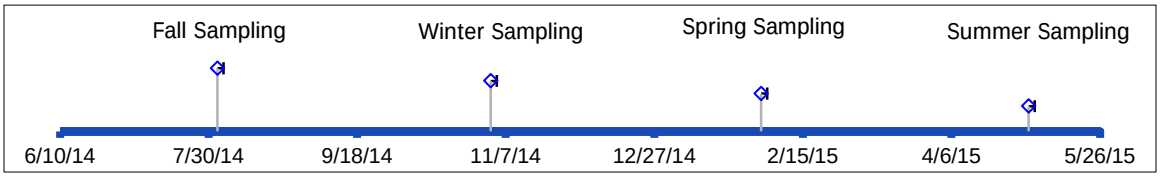

Fig. 3. Site of 10 October turtle sampling

Table 1. Contaminant residues collected in Wapanocca National Wildlife Refuge

August 2014				
Site	**Pesticide**	**Concentration (ug)**	**Pesticide Type**	**Pesticide Class**
BR	Metolachlor	5.20	Herbicide	Chloroacetanilide
BR	Propiconazole	0.46	Fungicide	Triazole
BR	Azoxystrobin	0.99	Fungicide	Strobilurin
BR	Simazine	0.70	Herbicide	Triazine
BR	DCPMU	0.80	Herbicide	Phenylurea
BR	Diuron	0.20	Herbicide	Phenylurea
D2	Metolachlor	1.80	Herbicide	Chloroacetanilide
D2	Propiconazole	0.54	Fungicide	Triazole
D2	Azoxystrobin	1.00	Fungicide	Organonitrogen
D2	Diuron	0.45	Herbicide	Phenylurea
D2	DCPMU	0.21	Herbicide	Phenylurea
November 2014				
D1	Methoxychlor	1.10	Herbicide	Chloroacetanilide
D1	Azoxystrobin	0.01	Fungicide	Organonitrogen
D2	Metolachlor	2.30	Herbicide	Chloroacetanilide
R2	Azoxystrobin	0.11	Fungicide	Organonitrogen
R2	Sulfentrazone	0.10	Herbicide	Triazolinone
February 2015				
BR	Boscalid	0.08	Fungicide	Anilide
D1	Metolachlor	0.58	Herbicide	Chloroacetanilide
D2	Metolachlor	0.88	Herbicide	Chloroacetanilide
PR	Carbaryl	0.10	Insecticide	Carbamate
May 2015				
BR	Atrazine	0.07	Herbicide	Triazine
D1	Atrazine	7.20	Herbicide	Triazine
D2	Metolachlor	17.00	Herbicide	Chloroacetanilide
D1	Flumioxazin	0.20	Herbicide	-
D1	Imidacloprid	0.11	Insecticide	Neonictinoid

D1	Propazine	0.09	Herbicide	Triazine
D1	Sulfentrazone	0.08	Herbicide	Triazolinone
D2	Atrazine	9.30	Herbicide	Triazine
D2	Metolachlor	18.00	Herbicide	Chloroacetanilide
D2	Imidacloprid	0.18	Insecticide	Neonictinoid
D2	Fluometuron	0.17	Herbicide	Urea
D2	Propazine	0.10	Herbicide	Triazine
D2	Methomyl	0.06	Insecticide	Carbamate
D2	Flumioxazin	0.06	Herbicide	-
PR	Metolachlor	0.31	Herbicide	Chloroacetanilide
PR	Atrazine	0.11	Herbicide	Triazine
CR	Atrazine	0.08	Herbicide	Triazine
DP	Atrazine	0.09	Herbicide	Triazine

Table 2. Life history characteristics of collected turtles in, A) August 2014, and b) October 2015.

A

17-Aug-2014

Species	**N**	**Sex**	**Mass (kg)**	**SE**	**Length (cm)**	**SE**
Apalone spinifera	1	F	1.5	NA	24.5	NA
	4	M	0.615	0.05	14.78	1.07

19-Aug-2014

Species	**N**	**Sex**	**Mass (kg)**	**SE**	**Length (cm)**	**SE**
Apalone spinifera		F	1.35	0.58	22.38	3.77
		M	0.48	0.11	13.99	2.42
Sternotherus minor		M	0.25	NA	10.5	NA

B

10-Oct-2015

Species	**N**	**Sex**	**Mass (kg)**	**SE**	**Length (cm)**	**SE**
Kinosternon subrubrum	1	F	1	NA	70	NA

Chysemys picta	5	M	0.9	0.08	124.2	12.21
Trachemys scripta	25	F	0.97	0.09	109.76	3.5
	20	M	0.31	0.05	13.94	3.12
Pseudemys coccina	8	M	0.9	0.09	116	2.2
Apalone spinifera	1	F	1.3	NA	160	NA
	1	M	0.4	NA	85	NA