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Surveillance of *Ranavirus* in False Map Turtles (*Graptemys pseudogeographica*) along the Lower Missouri River, USA

Ranavirosis, the disease caused by pathogenic viruses of the genus *Ranavirus*, is a highly virulent systemic infection that can affect fish, amphibians, and reptiles (Daszak et al. 1999; Duffus et al. 2015). Ranaviruses are multi-host pathogens that have been reported to have the ability to transfer between amphibian and reptile species and are known to be the causative agent of mass-mortality events in many species, including turtles (Johnson et al. 2008; Brenes et al. 2014). Though *Ranavirus* has not been extensively screened for in turtles, *Ranavirus*-caused mass-mortality events have been reported in both terrestrial turtles, such as Eastern Box Turtles (*Terrapene carolina*; Allender et al. 2011; Kimble et al. 2017), and aquatic turtles, including Painted Turtles (*Chrysemys picta*; Goodman et al. 2013) and European Pond Turtles (*Emys orbicularis*; Blahak and Uhlenbrok 2010). Generally, *Ranavirus* infection in turtles is characterized externally by the presence of cutaneous abscesses, nasal or ocular discharge, oral plaque, or unenergetic behavior (Allender 2012). Internally, *Ranavirus* infection in turtles can cause systemic fibrin thrombi and hemorrhagic necrosis of kidneys, liver, heart, spleen, and the alimentary tract, ultimately leading to death (Johnson et al. 2007).

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Though ranaviruses have been detected across the United States, little is known about their geographic and host distribution in the Midwestern United States (Duffus et al. 2015). To our knowledge, no turtles in the region have been screened for ranaviruses, though recent efforts have detected ranaviruses in amphibians along the Missouri River in Nebraska (Davis and Kerby 2016) and in South Dakota (Davis 2018) as well as in sturgeon in the Missouri River at the Gavin's Point National Fish Hatchery in Yankton, South Dakota (Kurobe et al. 2011). Despite the presence of ranaviruses in the region, nothing is known about whether they occur locally in turtle species.

In South Dakota, conservation efforts are focused on maintaining populations of the state-threatened False Map Turtle (*Graptemys pseudogeographica*) in the Missouri River. The False Map Turtle was once considered the most abundant turtle in the Missouri River in South Dakota (Timken 1968); however, river modification due to the creation of large reservoirs and hydroelectric dams has resulted in the decline and extirpation of historical populations (DRD, unpubl. data). As a result of these declines, it is important to ensure the health of remaining populations of False Map Turtles. Further, given the detection of ranaviruses in amphibians from Nebraska and South Dakota and the ability for pathogen transmission to occur among vertebrate classes, there is concern over the potential transmission of ranaviruses to False Map Turtles. Here, we investigated the prevalence and infection load of ranaviruses in False Map Turtles from the lower Missouri River between South Dakota and Nebraska.

False Map Turtles were collected along the 59-mile stretch of the Missouri National Recreation River (MNRR) between Yankton and Elk Point, South Dakota, USA from 2015–2017 (Fig. 1). Turtles were primarily collected using partially submerged

TABLE 1. Sampling localities, sampling dates, tissue types (blood, muscle, skin), sample sizes, and *Ranavirus* (RV) prevalence of False Map Turtles (*Graptemys pseudogeographica*) sampled from the lower Missouri River between South Dakota and Nebraska, USA. Site numbers correspond to localities shown in Fig. 1.

Site	Locality	Latitude	Longitude	Date	Tissue Type	Sample Size	RV Prevalence
1	James River	42.87806°N	97.27972°W	17 July 2017	Blood	7	0/7 (0%)
2	James River, at confluence with Missouri River	42.86199°N	97.29495°W	3 May 2017	Muscle	1	0/1 (0%)
3	Missouri River, Myron Grove Game Production Area	42.77300°N	97.12580°W	31 May 2017	Blood	12	0/12 (0%)
4	Missouri River, Goat Island	42.76777°N	97.08527°W	25 July 2017	Blood	11	0/11 (0%)
5	Missouri River, Goat Island	42.76364°N	97.07773°W	19 July 2017	Blood	21	0/21 (0%)
6	Missouri River, above Clay County Park	42.76557°N	97.01894°W	29 August 2017	Blood	14	0/14 (0%)
7	Missouri River, above Clay County Park	42.76541°N	97.01795°W	10 September 2015	Muscle	1	0/1 (0%)
8	Missouri River, North Alabama Bend	42.76090°N	96.98494°W	10 September 2015	Skin	27	0/27 (0%)
9	Vermillion River, at confluence with Missouri River	42.73351°N	96.88969°W	28 June 2017	Blood	28	0/28 (0%)
10	Missouri River, Rosenbaum Water Access Area	42.56042°N	96.64425°W	4 June 2016	Muscle	1	0/1 (0%)
					TOTAL	123	0/123 (0%)

hoop traps baited with sardines, but individuals were also opportunistically collected by hand. Traps were left submerged near basking surfaces (e.g., fallen trees) for 24–48 h and captured individuals were weighed, measured, and given a unique identifying notch on their marginal scutes (following Ernst et al. 1974). Before turtles were released, we either: 1) collected a blood sample from the caudal vein using a sterile insulin syringe (EXELINT International Co., Redondo Beach, California, USA), or 2) collected a small skin tissue sample (ca. 5 mm^2) from the webbing on the hind foot. We also collected three individual False Map Turtles as voucher specimens during this study. We euthanized these individuals via an overdose of sodium pentobarbital injected through the caudal vein and collected a muscle tissue sample from the right hind limb. Tissue collecting equipment (e.g., scissors, forceps) was sterilized with a 10% bleach solution between individuals and sites to prevent cross contamination and gloves were worn throughout.

All tissue samples were stored in individual tubes containing 95% ethanol and kept at -20°C until processing. DNA was extracted from tissue samples using DNeasy Blood and Tissue Kits (Qiagen, Hilden, Germany) and following kit protocols. Extracted samples were then analyzed for *Ranavirus* infection via quantitative PCR (qPCR) following methods outlined in Forson and Storfer (2006). Numerous other studies have used this method successfully and have verified that this method provides reliable detection down to a single viral copy (Whitfield et al. 2012; Davis and Kerby 2016). Each qPCR plate included a negative control (water) and a 1/10 serial dilution series ($1e^2$–$1e^5$) of gBlocks (IDT, Coralville, Iowa, USA) containing a target sequence of DNA (major capsid protein) in order to create a standard curve to quantify infection loads. Each sample that was analyzed was run in triplicate and C_t (cycle threshold level) values were used to determine absence/presence of ranavirus: a sample was considered positive if at least two wells amplified with a $C_t < 45$. All analyses were run on an ABI 7300 Real-time PCR System using Real-time PCR System Sequence Detection Software v1.2.3 (Applied Biosystems, Foster City, California, USA).

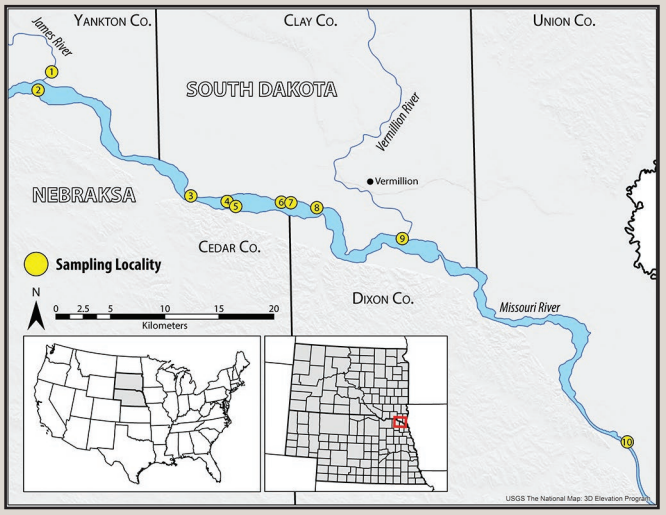

FIG. 1. Locations where False Map Turtles (*Graptemys pseudogeographica*) were sampled for *Ranavirus* along the lower Missouri River between South Dakota and Nebraska, USA. Sampling localities correspond to site numbers in Table 1.

A total of 123 False Map Turtle tissue samples were collected from 10 sites along the MNRR (Fig. 1; Table 1) including blood, muscle, and skin webbing. All samples were negative

for *Ranavirus* presence (Table 1). Lack of positive *Ranavirus* samples may have been influenced by a number of factors, including water temperature. Water temperatures are known to affect the persistence of *Ranavirus* in infected animals, but do not fully explain lack of *Ranavirus* detection. In 2017, the water temperature at sampled sites where 93 of the total 123 False Map Turtles sampled was 26.1 ± 0.3°C (mean ± 1 SE). Allender (2012) found that *Ranavirus* in semi-aquatic turtles has a higher and faster mortality at lower temperatures (22°C) when compared to higher temperatures (28°C). Therefore, given that *Ranavirus* has been detected in the region, elevated water temperatures may reduce the likelihood of infection in False Map Turtles, though further studies should investigate this possibility.

While the negative results are promising for the conservation of the False Map Turtle in the region, transmission of ranaviruses from infected hosts still may occur. With *Ranavirus* known from sites < 1.5 km away (Davis and Kerby 2016), the lack of infected False Map Turtles in this study may also suggest that ranavirosis progresses quickly, with high mortality of infected individuals. To test the vulnerability of False Map Turtles to the pathogen and ranavirosis, an experimental infection study may be a future direction to pursue. For this reason, additional research and continued surveillance for *Ranavirus* in the region, in both the False Map Turtle and other likely host species, is imperative to maintaining population health profiles and informing future conservation action for the surrounding aquatic and terrestrial ecosystems.

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