Hippopotamus movements structure the spatiotemporal dynamics of an active anthrax outbreak

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Abstract. Globally, anthrax outbreaks pose a serious threat to people, livestock, and wildlife. Furthermore, environmental change can exacerbate these outbreak dynamics by altering the host–pathogen relationship. However, little is known about how the quantitative spatial dynamics of host movement and environmental change may affect the spread of *Bacillus anthracis*, the causative agent of anthrax. Here, we use real-time observations and high-resolution tracking data from a population of common hippopotamus (*Hippopotamus amphibius*) in Tanzania to explore the relationship between river hydrology, *H. amphibius* movement, and the spatiotemporal dynamics of an active anthrax outbreak. We found that extreme river drying, a consequence of anthropogenic disturbances to our study river, indirectly facilitated the spread of *B. anthracis* by modulating *H. amphibius* movements. Our findings reveal that anthrax spread upstream in the Great Ruaha River (~3.5 km over a 9-day period), which followed the movement patterns of infected *H. amphibius*, who moved upstream as the river dried in search of remaining aquatic refugia. These upstream movements can result in large aggregations of *H. amphibius*. However, despite these aggregations, the density of *H. amphibius* in river pools did not influence the number of *B. anthracis*-induced mortalities. Moreover, infection by *B. anthracis* did not appear to influence *H. amphibius* movement behaviors, which suggests that infected individuals can vector *B. anthracis* over large distances right up until their death. Finally, we show that contact rates between *H. amphibius*- and *B. anthracis*-infected river pools are highly variable and the frequency and duration of contacts could potentially increase the probability of mortality. While difficult to obtain, the quantitative insights that we gathered during a real-time anthrax outbreak are critical to better understand, predict, and manage future outbreaks.

Key words: disease transmission; epidemiology; host–pathogen contact; hydrology; movement ecology; spatial.

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INTRODUCTION

Anthropogenic disturbances to ecosystems, such as habitat loss and climate change, are often attributed as the leading contemporary and future causal factors of wildlife declines. Critically, however, pathogens can interact with these disturbances, which could ultimately contribute to declines in wildlife abundance (Clark et al. 2011, Brearley et al. 2013). Thus, anthropogenic disturbances to ecosystems are often considered important drivers of emergent infectious diseases in wildlife (Plowright et al. 2008, Smith et al. 2009*a*). Human-modified landscapes can influence disease transmission rates and can dictate how a given species may respond to a disease by altering the host–pathogen relationship (Gallana et al. 2013).

Environmental predictability decreases as anthropogenic disturbances alter the distribution and abundance of key resources (Riotte-Lambert and Matthiopoulos 2020), which can have important consequences for animal movement and, ultimately, disease dynamics (Acevedo-Whitehouse and Duffus 2009, Harvell et al. 2009). In response to resource variability, nomadic movement behavior is expected (Mueller and Fagan 2008), with animal aggregations occurring around remaining resources (Wilmshurst et al. 1999). These responses can influence contact rates and the rate of disease transmission through density- and/or frequency-dependent effects (Smith et al. 2009*b*). Moreover, increased crowding and social interactions can increase physiological stress, which has been linked to increased susceptibility to infection (Martin 2009, VanderWaal and Ezenwa 2016). Thus, changes in environmental conditions not only influence the spatiotemporal distribution of wildlife, but they can also structure important processes in disease dynamics (Hampson et al. 2011, Peterson 2014, Escobar and Craft 2016).

Current climate forecasts predict increased variability in rainfall leading to prolonged droughts and diminished hydrological regimes across sub-Saharan Africa (Arnell and Gosling 2013, Nicholson 2017). Disease outbreaks are frequently linked with watershed hydrology, and research suggests a mechanistic link between river flow and disease outbreaks (Dorner et al. 2006, Collender et al. 2016). Furthermore,

hydrological models elucidate how different flow regimes may influence pathogen suspension in the water column and ultimately the extent to which pathogens may be distributed (Collender et al. 2016). For example, Driciru et al. (2018) found that an anthrax, *Bacillus anthracis*, outbreak spread longitudinally along a river flow gradient via water-borne mechanical propagation of infectious carcasses, and the physical characteristics of rivers influence the distribution of these carcasses (Subalusky et al. 2017). Because of the effects of global change on wildlife movement and the distribution of pathogens across watersheds, semi-aquatic species are likely to be highly vulnerable to anthropogenically driven climate and hydrological change (Lawler et al. 2010, Capon et al. 2013). Thus, we leveraged movements of the semi-aquatic common hippopotamus, *Hippopotamus amphibius*, during a disease outbreak in a hydrologically-disturbed environment to understand the relationship between global change, animal movement, and the spatiotemporal dynamics of a disease outbreak.

A global pathogen, the spore-forming bacterium, *B. anthracis*, which is the causative agent of anthrax, is a major cause of mortality in African wildlife and also poses a risk to human health (Turnbull et al. 1991, Hugh-Jones and Vos 2002). For *B. anthracis* transmission to occur, a host must die, whereby the carcass site creates an infectious reservoir within the landscape (Turner et al. 2014). Research on the pathways for *B. anthracis* transmission has primarily occurred in terrestrial environments. The classic pathway for *B. anthracis* infection in this context derives from grazing herbivores ingesting *B. anthracis* spores. Such infections can occur in a pulsed fashion because outbreaks and lethal doses of *B. anthracis* can be ingested at anthrax-infected carcass sites multiple years after initial carcass deposition (Turner et al. 2016). Turner et al. (2016) posit that water sources have a lower potential to transmit lethal doses of *B. anthracis* compared with terrestrial carcass sites due to lower concentrations of spores in water. Furthermore, transmission dynamics of *B. anthracis* in aquatic systems is poorly understood and whether lethal doses can occur in water sources is contested. However, aquatic environments aggregate susceptible wildlife, especially during the dry

season when water consumption increases, potentially resulting in wildlife consuming spore-contaminated water in lethal doses (Turner et al. 2016). In multi-species systems, there is a varying degree of susceptibility to *B. anthracis* infection. When anthrax outbreaks occur in both *H. amphibius* populations and other wildlife species, anthrax-related mortalities frequently occur first in *H. amphibius* populations and then in other wildlife species (Hugh-Jones and Vos 2002, Clegg et al. 2007, Wafula et al. 2008). Furthermore, anthrax outbreaks in *H. amphibius* populations can occur with no contemporaneous mortalities in other anthrax-susceptible wildlife species (Dudley et al. 2016), suggesting infectious reservoirs may not always be terrestrially based. Thus, the maintenance and transmission of lethal doses of *B. anthracis* in aquatic environments is plausible (Turnbull et al. 1991, Lindeque and Turnbull 1994, Driciru et al. 2018).

The majority of anthrax outbreaks in *H. amphibius* populations occur during the dry season (Turnbull et al. 1991, Clegg et al. 2007, Wafula et al. 2008, Dudley et al. 2016). Furthermore, *H. amphibius* populations frequently have higher anthrax-induced mortality compared with other species (Dudley et al. 2016). The following factors may jointly contribute to this observed phenomenon. First, because of the obligate dependency of *H. amphibius* on water, changes in water availability can play an important role in influencing their movement behaviors (Stears et al. 2019), which can also result in large aggregations forming in remaining aquatic refugia (Stommel et al. 2016a, Stears et al. 2018). Altered movements and increased aggregation sizes can influence exposure and transmission risk, ultimately influencing the spread of *B. anthracis*. Second, during prolonged periods of drought, *H. amphibius* can become nutritionally stressed as a result of loss of forage, which could heighten susceptibility, reducing the lethal dose required for infection and triggering disease outbreaks (WHO 2008, VanderWaal and Ezenwa 2016). Third, despite being an obligate herbivore, cannibalism and the consumption of carcasses of other species by *H. amphibius* is relatively common and has been observed in eastern and southern Africa (Dudley et al. 2016). The ingestion of spores via this pathway may increase anthrax mortality in *H. amphibius*. Thus, the seasonal nature of

anthrax in *H. amphibius* populations suggests that the effects of global change on river hydrology may exacerbate the severity of anthrax outbreaks because changes in river hydrology may alter *H. amphibius*–*B. anthracis* dynamics.

Here, we explored how altered *H. amphibius* movements, as a result of reduced water availability, influence the dynamics of an anthrax outbreak. Our study provides a novel opportunity to understand, in real time, how animal movements may structure disease outbreak dynamics. We linked carcass count transects with infected *H. amphibius* movements to assess the rate, extent, and directionality of an anthrax outbreak along the river course.

METHODS

Study site

The study was conducted in Ruaha National Park in central Tanzania (7°42′ S, 34°54′ E) during an anthrax outbreak between 17 August and 28 October 2017. Mean annual rainfall in this region is approximately 580 mm with most rainfall occurring during the wet season from November/December to May. The extensive dry season spans from June to November/December. Historically, the Great Ruaha River was a perennial river, maintaining dry season flow of 1–3 m^3/s (Mtahiko et al. 2006). However, since 1993, intensive water use from the Great Ruaha River for agricultural purposes has consistently reduced dry season river flow to zero (Mtahiko et al. 2006). As a result, approximately 60% of the river dries up and only isolated pools remain that are separated by large expanses of dry river bed (Coppolillo et al. 2004). We used river hydrology, which was monitored at the Msembe gauging station located (data were obtained from the Rufiji Basin Water Board, Iringa, Tanzania) within our focal sampling area, to relate the anthrax outbreak to river flow and seasonal drying.

Anthrax occurrence data and the spatiotemporal analysis of anthrax spread

Anthrax outbreaks have occurred sporadically throughout Ruaha National Park's history during both the wet and dry seasons. Key species that have been impacted by these outbreaks include the following: common hippopotamus, giraffe

(*Giraffa camelopardalis tippelskirchi*), and African elephant (*Loxodonta africana*). During the anthrax outbreak we studied in Ruaha National Park, *H. amphibius* was identified to be the species with the highest number of mortalities (see Results). This determination was confirmed by aerial wildlife census data that were concurrent with the outbreak. During these censuses, a single giraffe and single elephant carcass were found toward the end of the outbreak and therefore likely played little-to-no role in the spread of this particular outbreak. Three lines of evidence were used to confirm anthrax was the cause of death of *H. amphibius* during this outbreak (see Appendix S1 for more details).

At the watershed scale, *H. amphibius* population census and carcass location data were collected using aerial surveys, which covered the extent of the Great Ruaha River within Ruaha National Park (~120 km) and adjacent terrestrial habitats. From these data, we identified areas that contained carcasses as well as a number of surrounding pools to which the disease could spread. At the watershed scale, there were four main outbreak locations (see Fig. 1a); however, one area (hereafter referred to as our focal area, which covered ~6 km of river distance) had six isolated river pools that were close enough so that *H. amphibius* could move among them. Within this focal area, we conducted daily (when possible) walking transects (~6 km river distance) between 13 October and 28 October 2017 and recorded the date, location, and the number of *H. amphibius* carcasses as well as the number of live *H. amphibius*. These surveys allowed us to assess the spread of the outbreak at a finer scale as well as determine if the density of live *H. amphibius* in river pools influenced the total number of anthrax-induced mortalities that occurred after the first carcass appeared in each respective pool (see Appendix S1 for *H. amphibius* density calculations). We analyzed whether the total
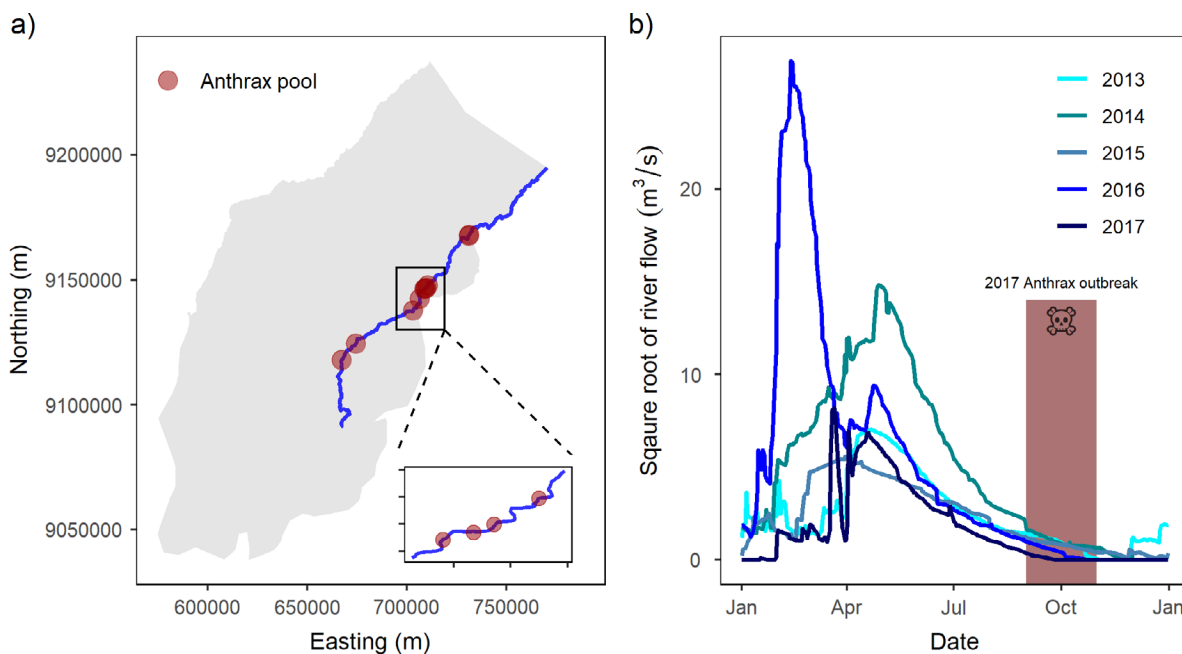

Fig. 1. Panel (a) shows Ruaha National Park, Tanzania, with the distribution of anthrax-related *Hippopotamus amphibius* mortality along the extent of Great Ruaha River. The inset plot depicts the focal area where we more closely monitored the anthrax outbreak by linking *H. amphibius* movements with the spread of anthrax. Panel (b) depicts seasonal river flow of the Great Ruaha River between 2013 and 2017. Note that in 2017, the Great Ruaha River dried earlier than all previous years. For graphical representation, we have square-root transformed river flow to more clearly show the differences in flow among years. The red bar and skull icon represent the anthrax outbreak that occurred in Ruaha National Park during the dry season of 2017.

number of anthrax mortalities was density dependent across *H. amphibius* pools using a generalized linear model with a gamma distribution. Using data from the census, we mapped the spatial distribution of carcasses to identify potentially infectious pools (hereafter referred to as anthrax pools). Because all carcasses only occurred within isolated river pools, we used the edge of the river pools as the extent of the infectious area. Finally, we used the location and date of new *H. amphibius* carcasses to assess the directional spread of anthrax-induced mortalities within the river course using the directional test statistic from the ClusterSeer2 software package (TerraSeer, Crystal Lake, Illinois, USA). The direction test uses individual case data to calculate the average direction in which cases advance during an outbreak (Jacquez 1996; see Appendix S1 for more details).

H. amphibius *movements*

We tracked 10 male *H. amphibius* in the Great Ruaha River using GPS-GSM UHF collars (Wireless Wildlife, Potchefstroom, South Africa). For more information on the collaring procedure, see Stears et al. 2019. All individuals were initially collared approximately one year before first detection of the 2017 anthrax outbreak. We used the aforementioned *H. amphibius* carcass distribution data to identify collared *H. amphibius* that had spatial and temporal overlap with river pools that contained confirmed *H. amphibius* anthrax carcasses. For the below analyses, we only used *H. amphibius* movement data collected during the anthrax outbreak, unless otherwise specified. Collars were programmed to acquire a location fix every 30 min between 1800 and 0600.

H. amphibius *movement and spatiotemporal overlap with anthrax pools*

To evaluate the relationship between *H. amphibius* movement and the spatial advance of the anthrax outbreak, we calculated modified net squared displacement (NSD) values from *H. amphibius* GPS fixes from November 2016 to November 2017. We modified the NSD values by taking into account that *H. amphibius* movements can either be away from the river (perpendicular), or alternatively, their movements could be focused parallel to the river. Thus, for each GPS fix, we simultaneously measured displacement along the

river and away from the river (see Appendix S1). This allowed us to overlay these modified NSD values with seasonal river flow and more specifically the anthrax outbreak. To link seasonal changes in river flow with overall *H. amphibius* ($n = 10$) movements both away from the river as well as along the river, we ran generalized linear mixed effect models (Gamma distribution and log-link function) using the *lme4* package in R (Bates et al. 2015). For these models, we used the maximum distance traveled both away from the river and along the river during each seasonal flow regime and we included *H. amphibius* ID as a random factor within the models. More specifically, during the anthrax outbreak, we analyzed the extent of upstream movements of *H. amphibius* ($n = 4$) by comparing the maximum distance traveled upstream by *H. amphibius* in the dry season to upstream movements in the wet season using a generalized linear mixed effect models (Gamma distribution and log-link function). As above, we included *H. amphibius* ID as a random factor within the model.

While moving along the river, *H. amphibius* can travel large distances away from their home pool (i.e., pool where they were initially collared) and therefore do not immediately return to these pools. This requires these individuals to seek out new transient river pools for daytime resting. Thus, we determined the frequency and duration of time that individual *H. amphibius* interacted with pools with anthrax cases during the outbreak period (i.e., did *H. amphibius* reside in an anthrax pool during the day after their nightly movements) using the *recurse* package in R (Bracis et al. 2018). We were not able to obtain location fixes while *H. amphibius* were submerged under water. Thus, we included an additional 50-m zone around the extent of the river pool using the *gBuffer* function from the *rgeos* package in R (Bivand and Rundel 2018). This additional zone around anthrax pools allowed us to identify and include visits to anthrax pools where the last location fix occurred away from the edge of the water before the *H. amphibius* was submerged.

Anthrax effects on H. amphibius *movement behavior*

Anthrax infections are rapid and large herbivores succumb to infection within one and seven days (WHO 2008, Easterday et al. 2020). Thus, we

assessed the potential of anthrax infection to influence nightly *H. amphibius* movement behavior by comparing movement patterns pre-anthrax infection with post-anthrax infection, which we split into two temporal scales. For pre-anthrax infection, we used data from 5 d prior to an individual *H. amphibius* interacting with an anthrax pool, whereas for post-anthrax-infected movement, we split these data into 5 and 3 d prior to death. These two temporal splits for post-anthrax-infected movement allowed us to capture the temporal period in which mortality from anthrax is most likely to occur for large mammals (WHO 2008, Easterday et al. 2020). For each movement path within these two time periods, we used the *trajr* package in R (McLean and Skowron Volponi 2018) to calculate the following indices: (1) the average speed at which *H. amphibius* traveled for the duration of its movement path, (2) how variable the speed was throughout the movement path, (3) the tortuosity of the movement path, (4) the temporal duration of each movement path, and (5) the total length of the trajectory. Finally, we performed non-metric multidimensional scaling (NMDS) using Bray-Curtis dissimilarities on the five trajectory indices (i.e., average speed, variation in speed, sinuosity, duration, and length) using the *vegan* package in R (Oksanen et al. 2019). We used a permutational multivariate analysis of variance (PERMANOVA) to test for significant differences in the movement trajectory indices between pre-anthrax infection and anthrax-infected movement periods in the NMDS plot. We ran two separate PERMANOVAs where we first compared pre-anthrax infection movements with the five-day anthrax-infected movements and then compared pre-anthrax infection movements with the three-day anthrax-infected movements. Finally, we tested the multivariate dispersion with an analysis of multivariate homogeneity of group dispersion using the betadispr function in the *vegan* package. For the above analyses, data were used from individuals that interacted with anthrax-infected pools.

RESULTS

Anthrax, river hydrology, and H. amphibius mortalities

During 2017, the Great Ruaha River experienced lower seasonal river flow compared with recent years (2013–2016), which caused the river to dry approximately one month earlier than prior years (Fig. 1b). This drier year occurred after the previous year's extremely high river flow that occurred during the wet season (2016 peak river flow 10-fold higher than 2017). A total of 60 *H. amphibius* carcasses were located at the watershed scale, which represents approximately 12% of the total *H. amphibius* population in the Great Ruaha River (TANAPA 2017 wildlife census). All carcasses were found in river pools, with no carcasses occurring in adjacent terrestrial habitats. Within our intensively-monitored focal outbreak region in the river (~6 km), we observed 20 *H. amphibius* carcasses across four pools, which represents a third of the total observed anthrax-related mortalities. The spread of *B. anthracis* along this focal river stretch occurred after the river had completely stopped flowing for approximately one month. Our *H. amphibius* counts showed that the reduction in the number of live *H. amphibius* during the study period was only due to anthrax-related mortalities (i.e., no emigration). Moreover, we did not observe any avoidance of floating carcasses by live *H. amphibius* during our sampling period. However, we found no relationship between the density of live *H. amphibius* in a pool and the number of anthrax-related mortalities that occurred subsequently after the first carcass appeared ($\chi^2 = 6.815$, $P = 0.494$). Within our focal areas of the river, the outbreak ceased approximately two and a half months before the start of the rainy season or an increase in river flow, despite there still being ~90 *H. amphibius* showing no signs of infection residing in the various anthrax pools within the focal area.

H. amphibius *movements and the spatiotemporal spread of anthrax*

When we linked the almost year-long NSD values with river flow for all collared *H. amphibius* ($n = 10$), we found that river flow influenced *H. amphibius* movements both away from the river ($\chi^2 = 14.329$, $P = 0.002$) and distance traveled along the river ($\chi^2 = 13.124$, $P = 0.004$). We used these NSD values to identify large-scale movements outside of individual *H. amphibius* home ranges. Specifically, we found that during periods of no or low flow, half of the collared *H. amphibius* exhibited large-scale movements

along the river course. Using these upstream movements, we identified individual *H. amphibius* that interacted with anthrax pools. Out of the 10 *H. amphibius*, four individuals interacted with anthrax-infected pools, with three individuals succumbing to the infection. The fifth individual that exhibited large-scale upstream movements was outside of the anthrax infection zone. Evidence of mortality of the collared *H. amphibius* was defined as cessation of all transmission of GPS signals in the region of an anthrax pool and confirmed identification of the dead *H. amphibius* in the last known location of final GPS transmission.

During the dry season, the average maximum distance traveled along the river away from their home pool by the *H. amphibius* that interacted with pools containing anthrax was approximately 3 times farther than their upstream movements when the river was flowing (χ^2 = 24.642, P < 0.001; Appendix S1: Fig. S1). In the dry season when *H. amphibius* moved upstream in search of sufficient water, they did not immediately return to their home pools. By contrast, when the river was flowing, upstream movements by *H. amphibius* were related to foraging and individuals always returned to their home pools. During the anthrax outbreak, *H. amphibius* moved along the river in an upstream direction (i.e., toward stretches of river that had not yet dried), which ranged in distance from 2 to 7 km (mean ± SE: 4 ± 1 km; Fig. 2). By moving upstream, these *H. amphibius* converged and interacted with river pools with active anthrax cases (Fig. 2). Out of the four *H. amphibius* that interacted with pools containing anthrax cases, the single *H. amphibius* that did not succumb to anthrax had the fewest visits and spent almost the least amount of time in pools with active anthrax cases compared with the three individuals that died (Appendix S1: Table S1).

The upstream movement by *H. amphibius* and the interaction with pools containing anthrax cases allowed for potentially infected *H. amphibius* to move upstream and spread the pathogen to a new pool via carcass deposition. The directional test revealed that the anthrax outbreak followed a consistent direction (angular concentration of 0.98; P = 0.001) along a bearing of 236.4° (pointing southwest against the natural gradient of river flow; Fig. 3). Ultimately, anthrax spread upstream by ~3.5 km over a 9-day period

and was likely vectored by the upstream movements of *H. amphibius*.

The effect of anthrax infection on H. amphibius movement behavior

We did not observe any effect of anthrax infection on *H. amphibius* movement (i.e., the five trajectory indices: average speed, variation in speed, sinuosity, duration, and length; Appendix S1: Fig. S2). This pattern was consistent irrespective of whether we compared five days of normal *H. amphibius* movement (e.g., movement prior to the anthrax outbreak) with *H. amphibius* movements five days prior to anthrax-related death (PERMANOVA pseudo-$F_{1,29}$ = 0.380, P = 0.729), or compared five days of normal *H. amphibius* movement with *H. amphibius* movements three days prior to anthrax-related death (PERMANOVA pseudo-$F_{1,23}$ = 0.563, P = 0.577). Furthermore, the non-significant dispersion tests on model residuals suggest anthrax infection did not influence the variability of *H. amphibius* movement (5-day pseudo-$F_{1,28}$ = 0.937, P = 0.365; 3 d $F_{1,22}$ = 1.357, P = 0.282).

DISCUSSION

By using movement data collected from infected individuals during an active outbreak, we show that reduced water availability indirectly facilitated the spread of *B. anthracis* by modulating *H. amphibius* movements. Within the focal area of the Great Ruaha River, the social behavior and movement of *H. amphibius* influenced exposure risk and the spread of *B. anthracis* between multiple *H. amphibius* pools. We reveal that anthrax spread upstream in the Great Ruaha River, which was counter to the assumption that anthrax would spread downstream in accordance with hydrological models. This upstream spread followed the movement patterns of infected *H. amphibius*, who moved upstream in search of aquatic refugia.

Changing water availability is a key mechanism that drives the exploratory and migratory movement modes of *H. amphibius* (Stears et al. 2019). This landscape change can influence disease dynamics by altering contact rates between susceptible individuals and *B. anthracis* reservoirs (e.g., Ostfeld et al. 2005). Thus, by moving upstream in search of suitable water, and outside
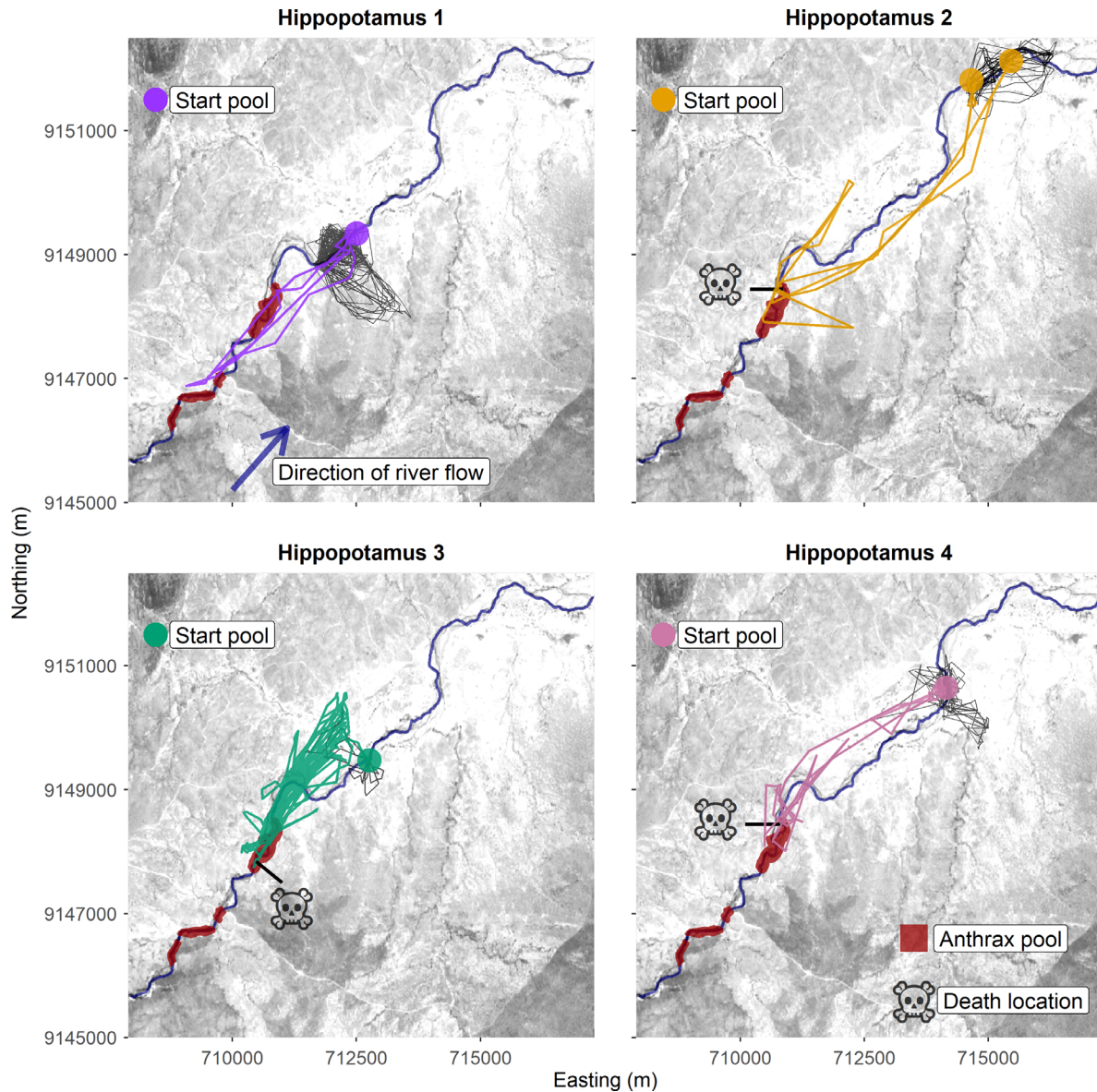
Fig. 2. *Hippopotamus amphibius* movements along the Great Ruaha River, Tanzania, during an anthrax outbreak. Typical movements within each *H. amphibius* home range are shown by the gray lines. The colored lines reflect the upstream movements of individuals away from their respective starting locations (i.e., the home pool where each *H. amphibius* was collared). During these upstream movements, individuals interacted with pools containing anthrax mortalities, which are highlighted in red. Three out of the four *H. amphibius* succumbed to anthrax infections and their death locations are denoted by the skull icon.

of their normal home range, *H. amphibius* increased their risk of exposure. Ultimately, with all *H. amphibius* carcass deposition occurring in river pools, and the dependency of *H. amphibius* on water, this resulted in individuals directly interacting with pools with active anthrax cases.

Within *H. amphibius* pools, transmission likely occurred via drinking pathogen-contaminated water and/or cannibalism (Dorward 2014, Dudley et al. 2016), although we did not observe cannibalistic behavior. Despite dilution effects expected in water (Turner et al. 2016), lethal
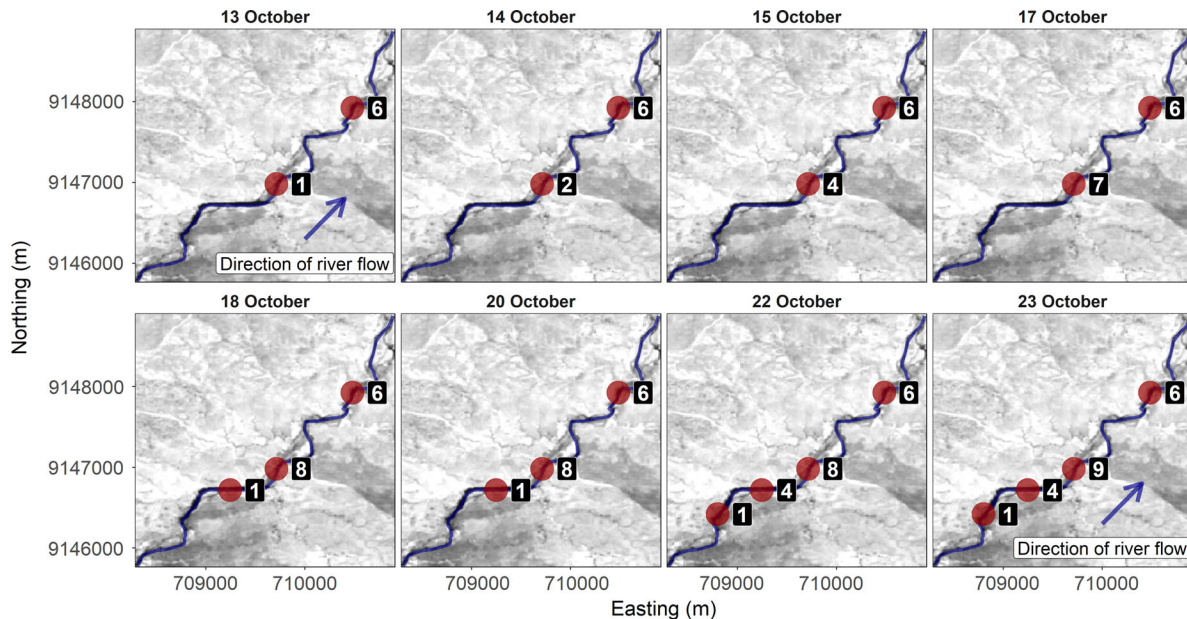
Fig. 3. The spatiotemporal spread of the 2017 anthrax outbreak along the focal area of the Great Ruaha River, Tanzania. Analyses revealed that the disease spread ~3.5 km along the river in a south-westerly direction against the direction of river flow (river direction shown in the first plot). The numbers denote the number of *Hippopotamus amphibius* carcasses in each pool. From 23 October to the end of the sampling period (28 October 2017), we did not observe an increase in *H. amphibius* carcasses. No anthrax-related mortalities occurred outside of the Great Ruaha River.

doses were maintained in pools most likely due to the prolonged presence of infected carcasses that can counter the effects of dilution (Turnbull et al. 1991, Wafula et al. 2008). Furthermore, the lack of individuals avoiding or leaving the pool after initial carcass deposition likely compounded the transmission risk. Finally, we found no differences in *H. amphibius* movement behaviors from when they were infected with *B. anthracis* compared with their behaviors prior to infection. Thus, with a single individual moving ~7 km in a night (Stears et al. 2019), an infected *H. amphibius* has the potential to vector *B. anthracis* up to 7 km even if the infected individual succumbs to infection in the fastest time possible (Zebra, *Equus quagga*, mortality is estimated to occur within 1–3 d of infection; Easterday et al. 2020). In less water-stressed environments, the high site fidelity of *H. amphibius* could limit the spatiotemporal spread of outbreaks, such as anthrax, especially if infectious reservoirs occur outside of an individual's home range (Dougherty et al. 2018).

Water availability can also influence the number of *H. amphibius* in the at-risk area (outbreak extent) because as water availability declines, there is often an increase (twofold–threefold) in the aggregation sizes of *H. amphibius* that congregate in the remaining river pools (Stommel et al. 2016*a*, Stears et al. 2018). Consequently, it has been posited that the spread of *B. anthracis* in *H. amphibius* populations is density dependent (Driciru et al. 2018). However, we found no relationship between the density of live *H. amphibius* in a pool and the number of anthrax-related mortalities that occurred subsequently after the first carcass appeared. This suggests that the transmission mechanism for *B. anthracis* is not density dependent for *H. amphibius* (see Driciru et al. 2018). It is plausible that we did not detect density dependency because of small sample sizes or that carcasses were potentially missed due to them being consumed and/or sinking prior to our counts. However, it is also possible that the number of carcasses in a river pool could influence the number of infected *H. amphibius*. We were unable to

assess whether individual *H. amphibius* were infected with *B. anthracis* because there was no change in movement behavior; we were only able to measure mortality. Thus, we do not completely rule out density dependency; however, our data suggest that contact rates between *H. amphibius* and *B. anthracis*-infected river pools are highly heterogeneous. The frequency and duration of contacts with pools containing anthrax cases increased the probability of mortality (Appendix S1: Table S1). Thus, we suggest that for *H. amphibius,* the spread of *B. anthracis* is likely due to the combined effects of density and frequency dependence.

Causal agents that disrupt river hydrology, such as climate variability and anthropogenic disturbances, are also likely to trigger anthrax outbreaks. Excessive drying has been attributed to mass anthrax mortalities in *H. amphibius* in other study systems (Turnbull et al. 1991, Wafula et al. 2008, Hampson et al. 2011). While most research on the persistence and transmission of *B. anthracis* has focused on terrestrial systems (Turner et al. 2016, Barandongo et al. 2018), we posit that this anthrax outbreak originated from within the aquatic system. In the year of the outbreak, the Great Ruaha River experienced unseasonably low river flow compared with previous years and completely stopped flowing approximately one month earlier than normal. Furthermore, the excessive drying during 2017 occurred after an abnormally-high peak river flow during the 2016 wet season. Flooding events can flush and disperse terrestrially-based concentrations of spores into aquatic systems (Hugh-Jones and Vos 2002, Collender et al. 2016) as well as scour the river course, potentially exposing spores from previous outbreaks. Mass carcass accumulation can occur within river systems (Subalusky et al. 2017), whereby carcasses from previous outbreaks form infectious reservoirs that are exposed during the drying of the river system (de Vos 1990). Combined, the excessive drying following a flooding event is the environmental conditions that likely triggered the 2017 anthrax outbreak in Ruaha National Park.

If the infectious reservoir was terrestrially based, mortalities would likely have also occurred in other susceptible grazing species (e.g., African buffalo, *Syncerus caffer*) that have a high degree of habitat overlap with *H. amphibius*

along the Great Ruaha River during the dry season (for buffalo space use, see Roug et al. 2020). However, aerial surveys did not locate buffalo carcasses or the carcasses of other wildlife species (apart from the single giraffe and elephant carcass mentioned earlier). Other wildlife species are unlikely to be infected with *B. anthracis* through drinking contaminated water at *H. amphibius* pools because, during the peak dry season, wildlife avoids *H. amphibius* pools due to *H. amphibius*-vectored nutrient inputs decreasing water quality and increasing bacterial loads (Stommel et al. 2016b, Stears et al. 2018). Instead, Stommel et al. (2016b) found that, in our study system, as bacterial loads in standing water increased, various species dug holes in the river bed to access clean water. Consequently, it is unlikely that there was a high degree of spatial overlap between anthrax pools and wildlife that used the river for drinking, thereby limiting the risk of exposure to infectious aquatic reservoirs.

It is generally assumed that the onset of the rainy season leads to the cessation of an outbreak due to the flushing of carcasses and dilution of spores. However, within our focal area, the outbreak ended well before the rainy season started (river flow only resumed in 2018) even though there were more than 90 susceptible *H. amphibius* remaining in the anthrax-contaminated river pools. Thus, this suggests a high degree of heterogeneity in transmission potential in *H. amphibius*, which can be influenced by the susceptibility of individuals to infection as well as the infectiousness of infected individuals (Tompkins et al. 2011). Furthermore, contact rates among individuals in a group can vary, with specific individuals having higher contact rates with carcasses and, thus, greater potential for infection and transmission of diseases (e.g., Clay et al. 2009). It is plausible that the death of the most at-risk individuals, which results in more resistant individuals remaining in river pools, could potentially lead to the outbreak ceasing before the onset of the wet season.

The effects of anthropogenic- or climate-related disturbances on zoonotic diseases have received considerable attention (Titcomb et al. 2017, Young et al. 2017). In order to predict the effects of these disturbances on future outbreaks, and ultimately our response, we not only need to identify the mechanistic drivers that influence

disease dynamics (Altizer et al. 2013), but we also need to track these dynamics in detail to understand how these epidemics play out in the field. Here, we show that water availability-induced *H. amphibius* movements structured the spatiotemporal spread of an anthrax outbreak. We can expect that anthropogenic disturbances that disrupt river hydrology, in conjunction with the effects of current models of climate variability on river flow (Mtahiko et al. 2006, Arnell and Gosling 2013, Haddeland et al. 2014), are likely to amplify *H. amphibius* movements and the spatial extent of future outbreaks. Across Africa, major watersheds that are home to the majority of *H. amphibius* populations are experiencing reduced flow as a result of anthropogenic disturbance (Stears et al. 2018). However, further research is required to understand how global stressors (i.e., anthropogenic disturbances) interact with local stressors (i.e., nutritional and competitive stress) to induce cumulative effects on infectious diseases.

We acknowledge that the number of tracked *H. amphibius* that interacted with anthrax pools in this study was fairly low. While tracking the dynamics of an anthrax outbreak in real-time is challenging, especially with relatively rare megaherbivores, these results provide important insights into the relationship between environmental change, animal movement, and disease dynamics broadly, and specifically elucidate how human disturbances to river hydrology may affect anthrax spread with consequences for wildlife and people. Our findings illustrate the critical need to obtain movement information for more individuals and taxa during real-time outbreaks and under a wider variety of environmental contexts.

Increased globalization and agricultural intensification in watersheds can increase human–livestock–wildlife contact, thereby creating an interface for transmission of zoonotic diseases (Mwakapeje et al. 2018, Walsh et al. 2019). Consequently, anthrax outbreaks in species such as *H. amphibius* are intimately linked with outbreaks in human populations because humans consume infected carcasses due to food insecurity (WHO 2008, Lehman et al. 2017). Thus, the fallout associated with anthropogenic disturbances to hydrological regimes extends beyond impacting wildlife to influencing human health. Ultimately,

because of the ecological role of *H. amphibius* in vectoring nutrients (see Subalusky et al. 2015, Stears and McCauley 2018, Stears et al. 2018), as well as their influence on the dynamics and maintenance of *B. anthracis* within ecosystems, which are both influenced by river hydrology, we consider *H. amphibius* to be the canary in the coalmine, indicating how anthropogenic- or climate-driven changes to river flow can influence overall watershed health.

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