



Original article

Assessing the underwater survival of two tick species, *Amblyomma americanum* and *Amblyomma maculatum*Lindsey A. Bidder, Kristine M. Asmussen, Sean E. Campbell, Katerina A. Goffigan, Holly D. Gaff^{*}

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ABSTRACT

The hard (ixodid) ticks *Amblyomma americanum* and *Amblyomma maculatum* are found throughout the southeastern United States. To study the effects of water inundation, which is an increasingly common phenomenon in many coastal areas, unfed adult *A. americanum* and *A. maculatum* ticks were tested for survival by submergence in three water conditions: freshwater, brackish water, and saltwater. The results demonstrated a significant difference in survival between the two species in all three water conditions, with *A. maculatum* ticks surviving a shorter time underwater than *A. americanum* ticks. There is also a significant difference in *A. americanum* survival among the different water conditions, with the highest mortality in saltwater and the lowest in freshwater. *Amblyomma americanum* ticks survived the longest in freshwater (70 d), followed by brackish water (64 d), and the shortest survival was in saltwater (46 d), while the longest any *A. maculatum* tick survived was 24 d in freshwater. These findings demonstrate that any short-term flooding events, e.g., less than a week, would not likely eliminate these species of ticks in the flooded area.

1. Introduction

Climate change is believed to be one of many factors that have led to changes in the geographic range of ticks, their hosts, and the transmission of tick-borne disease agents (Childs and Paddock, 2003; Ogden et al., 2008; Gilbert et al., 2014). One part of climate change is an increase in heavy rainfall events over the last three to five decades, increasing flooding events across the United States including areas in the southeastern region (Walsh et al., 2014). Little research has been done to explore the consequences to tick populations from flooding events.

Amblyomma maculatum (the Gulf Coast tick) is native to Central and South America and commonly found along the United States Gulf Coast (Sumner et al., 2007; Teel et al., 2010). Recently, the *A. maculatum* tick moved into southeastern Virginia with established populations reported starting in 2010 (Fornadel et al., 2011; Wright et al., 2011). *Amblyomma maculatum* is the primary vector of *Rickettsia parkeri*, which is pathogenic to humans and can cause a spotted fever group rickettsiosis (Wright et al., 2011; Nadolny et al., 2014; Fryxell et al., 2015). Wright et al. (2011) reported that 43% of *A. maculatum* collected in the Hampton Roads area of Virginia in 2010 tested positive for *R. parkeri*, suggesting that persons in southeastern Virginia are at risk for infection with this pathogen.

Amblyomma americanum (the lone star tick) is the primary human-

biting tick in the southeastern United States (Stromdahl and Hickling, 2012; Nadolny et al., 2014), and it is the dominant tick species in southeastern Virginia (Nadolny et al., 2014). All three active life stages of *A. americanum* have been reported to bite humans (Childs and Paddock, 2003). *Amblyomma americanum* is a known vector of *Ehrlichia chaffeensis* and *E. ewingii*, causative agents of ehrlichiosis in humans and animals (Childs and Paddock, 2003; Stromdahl and Hickling, 2012), and is also a secondary vector of *R. parkeri* (Goddard, 2003; Wright et al., 2015).

Ticks have complex life histories that are directly related to their habitat and hosts. Studies that focus on how habitat influences the distribution of tick populations are vital to understanding the range of tick species and the associated risks to humans. Over the last century, the average temperature in southeastern Virginia has increased by 1 °C and precipitation has increased by 91 mm (NOAA, 2017). The Hampton Roads area of Virginia is in a tidal flood zone, and in the last few decades, there has been a significant increase of nuisance flooding occurrences, i.e., events with road closures but without significant loss of life or property, along the Atlantic Coast (Sweet et al., 2014). These recurrent floods result in submerged environments inundated with saltwater or brackish water for two or three days (Behr et al., 2016).

Unfed nymphal *A. americanum* ticks have been reported surviving up to 19 wk submerged in 20 °C freshwater in the laboratory and adults

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have been reported surviving up to 3 wk (Koch, 1986). No previous studies have examined the impact of water submersion on *Amblyomma maculatum*. In Europe, Honzáková (1971) reported the ability of *Ixodes ricinus* (the castor bean tick), *Dermacentor reticulatus* (the ornate cow tick), *Dermacentor marginatus* (the ornate sheep tick), and *Haemaphysalis punctata* (the red sheep tick) to survive for days underwater for a variety of fed and unfed life stages. In terms of the mechanism by which ticks survive submersion, Fielden et al. (2011) confirmed the use of plastron respiration, i.e., a small bubble of air between the spiracular plate and the water that allows for diffusion of dissolved oxygen, for underwater survival in *Dermacentor variabilis* (the American dog tick). Other studies have looked at the survival and egg-laying for engorged females that are submerged, and while *Amblyomma auricularium* (the reptilian tick) showed no differences after submersion of up to 96 h (Sá-Hungaro et al., 2014), *Rhipicephalus sanguineus sensu lato* (the brown dog tick) showed a significant decline in egg production and survival over the same time period (Giannelli et al., 2012).

Understanding how tick species survive in their habitat, while off-host and exposed to tidal flooding, could be key in determining tick dispersal, survival, and activity in tidal flood zones. Variations in morphology and physiology of tick species could aid or hinder a tick's ability to survive in these areas. The focus of this study was to explore the relationship between tidal flooding, which exposes ticks to a variety of salinity levels, and survival for unfed adult *A. americanum* and *A. maculatum* ticks. While the area vulnerable to tidal flooding is a small percentage of the range of these two species, the results of this study will also inform non-coastal areas subject to flooding events.

2. Materials and methods

2.1. Survival experiments

Survival studies were completed in a laboratory setting with three water conditions: fresh water (2 parts salt per thousand parts water (ppt)), brackish water (10 ppt), and saltwater (20 ppt). Approximately 20 liters of water representative of the examined water condition were collected from three different locations with fresh water from Back Bay, City of Virginia Beach, brackish water from Hoffer Creek, City of Portsmouth, and saltwater from the Atlantic Ocean, City of Norfolk, and placed in three 38-liter aquaria (Fig. 1). Pathogen-free male and female unfed *A. americanum* and *A. maculatum* ticks were purchased from the Oklahoma State University Tick Rearing Facility (Stillwater, OK, USA).



Fig. 1. Experimental aquaria used; without tick housing vials (top) and with tick housing vials (bottom): saltwater (left), brackish water (middle), freshwater (right).

Table 1

Initial number of *A. americanum* and *A. maculatum* ticks placed in housing vials and submerged underwater in the three water conditions (freshwater, brackish water, saltwater) in the initial 28 d study.

Water Condition	Species	Unfed Male Ticks	Unfed Female Ticks	Totals
Freshwater	<i>A. americanum</i>	42	42	84
	<i>A. maculatum</i>	42	42	84
Brackish water	<i>A. americanum</i>	42	42	84
	<i>A. maculatum</i>	42	42	84
Saltwater	<i>A. americanum</i>	42	42	84
	<i>A. maculatum</i>	42	42	84
Totals		252	252	504

Ticks were placed into plastic vials containing small stones for weight, and mesh cloth was placed over the mouths of the vials (Table 1). The vials were secured with plastic lids containing a 2 cm hole, and the vials were submerged in the aquaria.

The ambient room temperature and humidity was recorded daily with a digital thermometer/hygrometer, averaging to 21 °C and 51% RH over the course of the studies. Water temperature was also recorded daily for each aquarium and stayed consistent across all aquaria at 19 °C. Lighting was set on a timer to maintain a photo period of 12 light hours to 12 dark hours. Salinity measurements were recorded at the initial set-up with a refractometer.

For the initial experiment, six adult ticks (3 male, 3 female) were placed into each vial, for a total of 84 vials (14 per species per water condition). The vials were completely submerged underwater, and all air bubbles were cleared from each vial to ensure that no air was trapped in any vial. After 48 h, one vial per species from each aquarium was removed, and the ticks were checked for survival; this process was repeated after each subsequent 48 h period. Immediately upon opening a vial, ticks were removed and rinsed in fresh water (to remove any salts), carefully dried with a paper towel, then documented as immediately alive if moving or non-ambulatory. Warm breath and physical touch were used in attempts to stimulate non-ambulatory ticks for 30 min post water removal (Scifres et al., 1988; Needham et al., 1996) (Fig. 2). If movement was observed, ticks were recorded as alive. While the majority of live ticks responded immediately, if the ticks remained inactive, they were placed in an inescapable “corral” for a 24 h period. The corral consisted of an enclosed container with walls lined with double-sided adhesive tape to prevent escape. Ticks were placed dorsally on paper towels in the corral, and a circle was drawn around each tick (Fig. 2). Each tick circle was labeled with the tick's water condition, species, and sex. After 24 h, if the tick was able to right itself and move completely outside its designated circle in the corral, it was recorded as alive. Otherwise, the tick was considered nonviable.

Surviving *A. americanum* ticks from the final time period of the initial underwater experiment were used to conduct an additional study. There were no surviving *A. maculatum* so that species was not included in this second part of the study. In this follow-on study, one vial for each water condition was used to further observe underwater survival in *A. americanum* ticks. The surviving adult *A. americanum* ticks were placed into a vial and returned to their original water type: freshwater (3 male, 3 female); brackish (2 male, 3 female); and saltwater (1 male, 2 female) (Table 2). Once a week for a total of two additional months, the vial from each aquarium was removed, and the ticks were checked for survival. The same aforementioned protocols for viability were conducted. Any surviving ticks were re-submerged in the same aquarium as before. Time submerged was continued from the original study; thus, these ticks were recorded as already submerged for 28 d at the start of the study, concluding at 70 d.

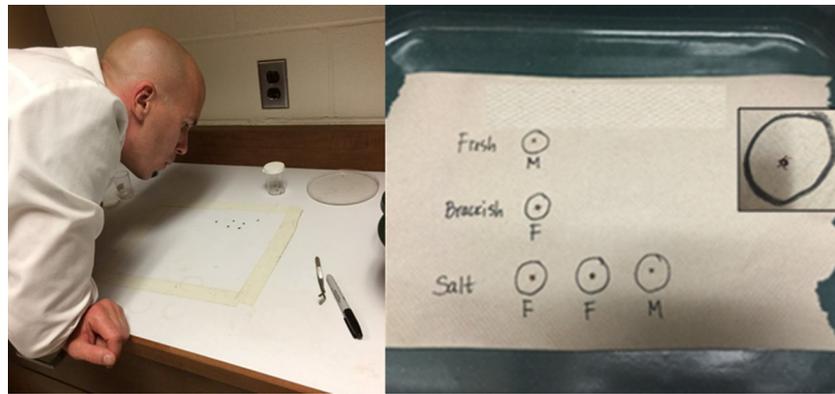


Fig. 2. Processes of checking for tick survival; warm breath was blown on ticks to stimulate movement (left). Tick corral used to observe tick survival over a 24 h period (right); inset shows enlargement of a female *A. americanum* in a circle.

Table 2

Initial number of *A. americanum* ticks in the 28 d to 70 d secondary study that were re-submerged underwater in the three water conditions (freshwater, brackish water, saltwater) post the conclusion of the initial study.

Water Condition	Unfed Male Ticks	Unfed Female Ticks	Totals
Freshwater	3	3	6
Brackish water	2	3	5
Saltwater	1	2	3
Totals	6	8	14

2.2. Statistical analysis

A log rank (Mantel-Cox) Kaplan-Meier survival analysis was run using the IBM® SPSS® version 22 program (IBM Corporation 1989, 2013) for each of the underwater survival studies. The analysis was run to determine if there were differences in the survival distribution for the different water conditions, species, and sex. A pairwise survival analysis was run to compare survival distributions for each species per water condition.

3. Results

3.1. Initial underwater experiment

In the initial underwater experiment, 84 vials (14 per species per water condition) containing 504 total *A. americanum* and *A. maculatum* ticks (Table 1) were submerged on Day 0, and vials were removed every other day for 28 d. Of the 252 total *A. americanum* ticks submerged across all water conditions, the majority of the nonviable ticks were in the saltwater aquarium with 21 nonviable ticks, followed by brackish water with 13 nonviable ticks, with the least observed in the freshwater aquarium with 3 nonviable ticks (Table 3). Of the 252 total *A. maculatum* ticks submerged across all water conditions, the majority of the nonviable ticks were in the saltwater aquarium with 61 nonviable ticks, followed by brackish water aquarium 59 nonviable ticks, with the least observed in the freshwater aquarium with 50 nonviable ticks (Table 4).

A log rank (Mantel-Cox) Kaplan-Meier survival analysis indicates there is a significant difference ($\chi^2 = 100.493$, $df = 1$, $p < 0.0005$) in total number of nonviable ticks between species across all water conditions over the 28-day study period (Fig. 3). When both species are combined, there is no significant difference ($p > 0.05$) in total number of nonviable ticks among water conditions, nor is there a significant difference ($p > 0.05$) in the totals by sex. A log rank (Mantel-Cox) Kaplan-Meier pairwise survival analysis indicates there is a significant difference ($p < 0.05$) in total number of nonviable *A. americanum* among the three water conditions. There is a significantly higher number of nonviable *A. americanum* ticks submerged in saltwater when

Table 3

Total number of *A. americanum* ticks declared nonviable over time during the initial study. On Day 0, six ticks were placed in each housing vial and submerged underwater in three water conditions (freshwater, brackish water, saltwater) for a total of 252 ticks. One vial was removed on Days 2, 4, ..., 28 to check for viability.

Day Removed	Nonviable <i>A. americanum</i> per Water Condition		
	Freshwater	Brackish water	Saltwater
Day 2	0	1	1
Day 4	0	0	2
Day 6	0	2	0
Day 8	0	0	1
Day 10	0	2	2
Day 12	0	0	2
Day 14	1	0	4
Day 16	0	1	1
Day 18	0	1	1
Day 20	1	4	1
Day 22	0	1	0
Day 24	1	0	2
Day 26	0	0	1
Day 28	0	1	3
Totals	3	13	21

Table 4

Total number of *A. maculatum* ticks declared nonviable over time during the initial study. On Day 0, six ticks were placed in each housing vial and submerged underwater in three water conditions (freshwater, brackish water, saltwater) for a total of 252 ticks. One vial was removed on Days 2, 4, ..., 28 to check for viability.

Day Removed	Nonviable <i>A. maculatum</i> per Water Condition		
	Freshwater	Brackish water	Saltwater
Day 2	0	1	0
Day 4	1	2	2
Day 6	2	2	2
Day 8	2	1	4
Day 10	1	5	5
Day 12	5	3	5
Day 14	5	3	2
Day 16	4	6	6
Day 18	5	6	6
Day 20	4	6	6
Day 22	4	6	5
Day 24	5	6	6
Day 26	6	6	6
Day 28	6	6	6
Totals	50	59	61

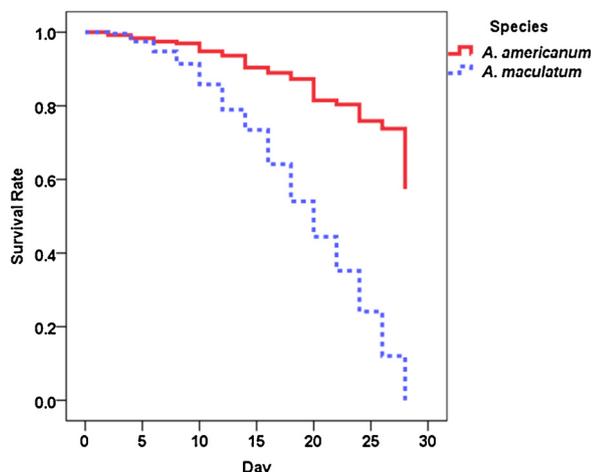


Fig. 3. Survival rate between *A. americanum* and *A. maculatum* for the initial study across all water conditions over the 28 d study period. There is a significant difference in survival between the species ($p < 0.05$).

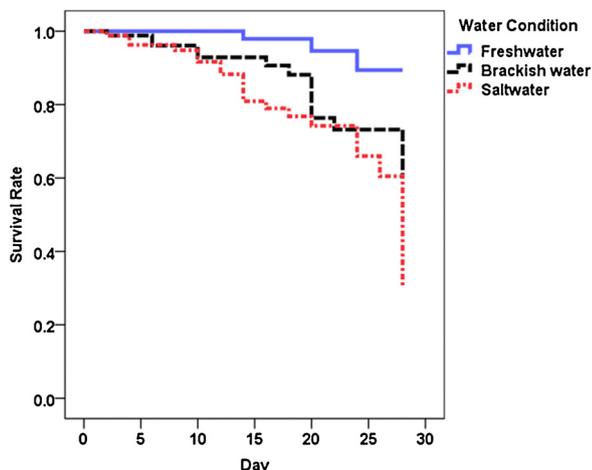


Fig. 4. Survival rate among water conditions for *A. americanum* ticks during the initial study over the 28 d study period. There is no significant difference between brackish water and saltwater survival; all other comparisons were significant ($p < 0.05$).

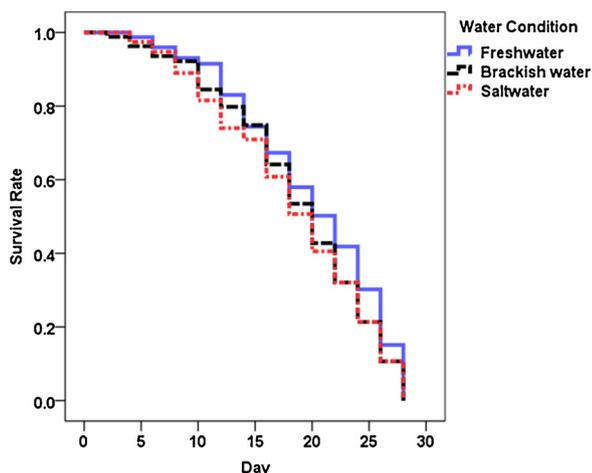


Fig. 5. Survival rate among water conditions for *A. maculatum* ticks during the initial study over the 28 d study period. There is no significant difference among water conditions ($p > 0.05$).

compared to freshwater ($\chi^2 = 14.093$, $p < 0.0005$) as well as for brackish water compared to freshwater ($\chi^2 = 6.398$, $p = 0.011$) (Fig. 4). There is no significant difference ($p > 0.05$) in survival for *A. maculatum* among the three water conditions (Fig. 5). There were no significant differences by sex for either species or water condition.

3.2. Secondary underwater experiment

In the follow-on study, 3 vials (1 per water condition) containing 14 total *A. americanum* (Table 2) were re-submerged on Day 28 and monitored until all ticks were declared nonviable. Number of nonviable ticks are reported for the total sampling event per vial over the study period. The purpose of this study was to observe the ability of *A. americanum* to survive long-term fluctuating inundation conditions; therefore, data are reported for observational applications but not statistically analyzed because of insufficient sample sizes. Additionally, the shift in methods of removing and re-submerging the same ticks allowed the ticks a chance to breathe, which quite likely affected survival.

All 3 *A. americanum* ticks in the saltwater vial were declared nonviable on Day 46. Of the 5 *A. americanum* ticks in the brackish water, one tick was declared nonviable on Day 40. The last tick in the brackish vial was declared nonviable on Day 64. Of the 6 *A. americanum* ticks in the freshwater vial, one tick was declared nonviable on Day 40, and the last tick was declared nonviable on Day 70 (Table 5, Fig. 6).

4. Discussion

This study was designed to explore the role that short-term coastal flooding events play in the mortality of tick populations in these environments. Similar to previous work on other tick species, we found variation in the length of time that these two species can survive with unfed *A. maculatum* surviving a much shorter period of time than *A. americanum*. Although *A. americanum* survive significantly ($p < 0.05$) longer underwater than *A. maculatum* (Fig. 3), some ticks from both species persisted in saltwater and brackish water over two weeks, with freshwater survival over three weeks. Additionally, some *A. americanum* survived in all three water conditions for more than a month, which is longer than was reported previously (Koch, 1986). Flood events in the Hampton Roads area of Virginia, similar to the entire Eastern seaboard, typically last only a few days, with waters quickly receding and uncovering affected areas. The results of this study would indicate this duration of tidal flooding would not increase tick mortality.

For both species, the first nonviable tick was recorded after just two days of water submersion. If short-term tidal flooding submerges an area, this study suggests a minor increase in tick mortality may occur, but the results also showed it is not likely to eliminate a population. All *A. maculatum* were declared nonviable after 16 d submerged for the

Table 5

Total number of *A. americanum* ticks declared nonviable in the secondary study over the 28 d to 70 d study period. On Day-28, ticks were re-submerged in their water condition (freshwater, brackish water, or saltwater). Once a week the vial from each aquarium was removed and the ticks were checked for viability.

Day Removed	Nonviable <i>A. americanum</i> per Water Condition			Totals
	Freshwater	Brackish water	Saltwater	
Day 32	0	0	0	0
Day 40	1	1	0	2
Day 46	1	1	3	5
Day 52	0	0	–	0
Day 58	0	2 ^a	–	2
Day 64	3 ^a	1	–	4
Day 70	1	NA	–	1
Totals	6	5	3	14

^a Mortality \geq LT50; Lethal Time where 50% of the ticks in the vial were nonviable.

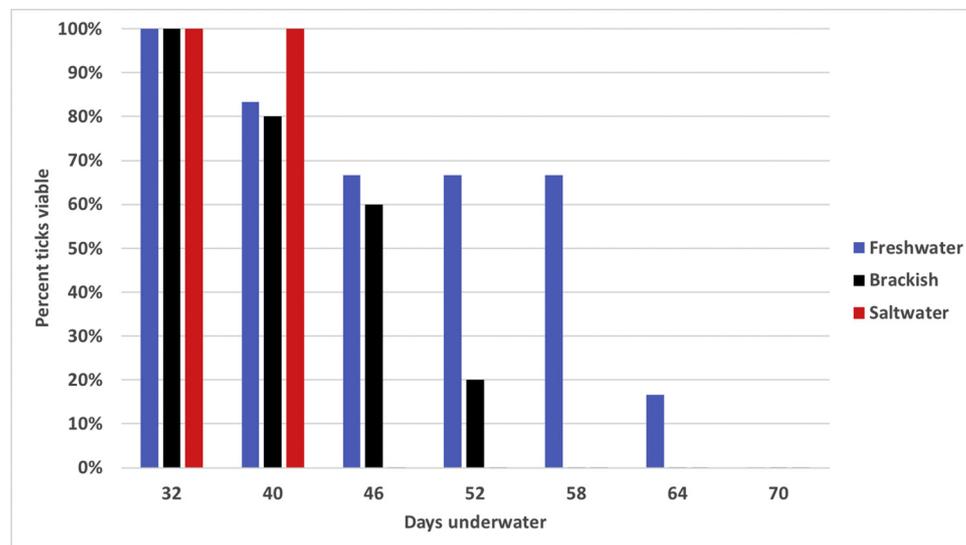


Fig. 6. Percent of viable ticks observed for *A. americanum* during the secondary study over the 28 d to 70 d study period. No viable *A. americanum* ticks were found in saltwater at Day-46; nonviable ticks were found at Day-70 in the freshwater vial.

brackish water and saltwater conditions, so this study suggests saltwater intrusions lasting longer than two weeks could eliminate an *A. maculatum* population at that location. Results also show *A. americanum* are much less susceptible to mortality from submersion in water than *A. maculatum*, and thus their population in the same area would not likely be eliminated.

Although ticks are resilient and can survive being submerged in water for an extended period of time, the higher salinity in tidal flooding may reduce population numbers for both species. With the *A. maculatum* established populations along mainly coastal habitats throughout the southeastern United States, it was thought that this species could withstand harsher environmental flood conditions where other tick species cannot survive. However, this study does not support that hypothesis.

This study did not assess the potential reduction in long-term tick survival due to the stressors associated with inundation, nor were energy analyses conducted. This study also did not analyze the dissolved oxygen in the aquaria, which could aid in understanding the mechanism of underwater survival. Finally, this study did not assess the potential negative impact on a tick's ability to take a blood meal and molt successfully following submersion in water. Overall, this study demonstrates that ticks can survive significant periods of time completely submerged in water. This finding confirms *A. americanum* and *A. maculatum* are not hindered from continuing to establish populations in habitats prone to flooding.

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