J Comp Physiol B
DOI 10.1007/s00360-006-0122-7

ORIGINAL PAPER

Habitat requirements of the seabird tick, *Ixodes uriae* (Acari: Ixodidae), from the Antarctic Peninsula in relation to water balance characteristics of eggs, nonfed and engorged stages

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Received: 25 July 2006 / Revised: 25 August 2006 / Accepted: 27 September 2006

Abstract The seabird tick *Ixodes uriae* is exposed to extreme environmental conditions during the off-host phase of its life cycle on the Antarctic Peninsula. To investigate how this tick resists desiccation, water requirements of each developmental stage were determined. Features of *I. uriae* water balance include a high percentage body water content, low dehydration tolerance limit, and a high water loss rate, which are characteristics that classify this tick as hydrophilic. Like other ticks, *I. uriae* relies on water vapor uptake as an unfed larva and enhanced water retention in the adult, while nymphs are intermediate and exploit both strategies. Stages that do not absorb water vapor, eggs, fed larvae and fed nymphs, rely on water conservation. Other noteworthy features include heat sensitivity that promotes water loss in eggs and unfed larvae, an inability to drink free water from droplets, and behavioral regulation of water loss by formation of clusters. We conclude that *I. uriae* is adapted for life in a moisture-rich environment, and this requirement is met by clustering in moist, hydrating, microhabitats under rocks and debris that contain moisture levels that are higher than the tick's critical equilibrium activity.

Keywords Water balance · Dehydration · Tick · *Ixodes* · Antarctica

Communicated by I.D. Hume.

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Introduction

Only a single tick species, *Ixodes uriae*, lives in Antarctica. On that continent, the tick feeds predominantly on penguins, but it is distributed at high latitudes in both hemispheres, presumably spread by migratory seabirds (>48 avian species recorded) that serve as its preferred hosts (Wilson 1970). As a competent vector of *Borrelia* spirochetes to birds, *I. uriae* has epidemiological significance (Olsen et al. 1993, 1995). Physiologically, few experiments have probed *I. uriae*'s remarkable ability to survive in extreme cold and dry conditions. Those studies that have been conducted focus primarily on how this tick copes with extremely low temperature (Lee and Baust 1982, 1987). Interestingly, no physiological adaptations, i.e., cold tolerance or desiccation resistance (Lee and Baust 1987; Dautel and Knülle 1996), have been described

that would make Antarctica a suitable habitat exclusively for *I. uriae*, but not other ticks.

Under field conditions, *I. uriae* takes approximately 3 years to develop from larva to adult, annually spending more than 11 months off the host, residing in aggregations underneath rocks and debris that sometimes reach thousands of individuals (Eveleigh and Threlfall 1974). Each of these tick colonies consist of a mixture of ticks in all stages of development both before and after blood feeding, and are typically in close proximity to penguin rookeries. While clustered, these ticks remain relatively akinetic, huddling in direct contact with each other. They leave the aggregation in the protected, sheltered area only to feed, undoubtedly guided by bird host cues (kairomones; Sonenshine 1991). After each bloodmeal, the ticks immediately return to their colony under the rocks where they molt, and wait until the bird hosts return the following season or, in the case of the adult female ticks, mate (mating takes place off-host in this tick), lay eggs and die (Sonenshine 1991).

In this study, dehydration resistance of *I. uriae* was ascertained by determining standard water balance characteristics for each stage of the life cycle, from egg to adult. Dehydration tolerance was recorded as an indicator of the minimum amount of body water required for function. Water retention was assessed from water loss rate in relation to temperature. Clustering of this tick was evaluated as a method of water loss suppression. Free water drinking and water vapor absorption were examined as means to replenish losses and maintain their internal body water mass levels. Additionally, all developmental stages of the tick were compared to determine which periods in the life cycle were most vulnerable to water stress. In particular, we determined percentage body water content; dehydration tolerance limit; water loss rate; critical transition temperature (CTT), denoting the temperature where water loss increases abruptly; and critical equilibrium activity (CEA), the lowest amount of ambient moisture that is required for water vapor absorption to occur.

Materials and methods

Tick collection, storage and weighing

Ixodes uriae were collected on the Antarctic Peninsula (64°04′S, 64°03′W) from Humble Island near Palmer Station, Anvers Island in January 2006, and all experiments were conducted at Palmer Station. Male and female adults, nymphs and larvae were found grouped together under rocks, typically in sites near Adelie penguin (*Pygoscelis adeliae*) rookeries. Ticks were handled using soft-tipped forceps or an aspirator. In the experiments, eggs were used 7–10 days post-oviposition, larvae were used 10–14 days after ecdysis in the laboratory at 4°C, but the only age information we can provide for other stages is that they were acquired at the same time. Eggs were tested for viability by holding them in groups at 100% relative humidity (RH) at both 4 and 25°C for 1 month or until emergence. Experiments on engorged stages (larvae and nymphs) were conducted after they had ceased movement in preparation for molting, denoted by extension of the legs and failure by the tick to respond to stimuli (Kahl and Knülle 1988). Temperature (25 and 4 ± 1°C) and photoperiod (15:9 h light:dark) were controlled using environmental cabinets. Fed female adults were not analyzed because they die shortly after oviposition and adult males do not blood feed, thus larvae and nymphs were the only fed stages examined.

In the laboratory, ticks were held individually in 1 cc mesh-covered chambers placed on a perforated porcelain plate within a sealed glass desiccator (8,000 cc) that contained, at its base, a saturated salt solution to maintain RH. Each RH was controlled by saturated salt solutions containing an excess of solid salt; 33% RH ($MgCl_2$), 75% RH (NaCl), 85% RH (KCl) and 93% RH (KNO_3) or glycerol–distilled water mixtures of different concentrations (Johnson 1940; Winston and Bates 1960). Additionally, double-distilled water was used for 100% RH and $CaSO_4$ was used for 0% RH. Each RH was measured daily with a hygrometer (SD ± 0.5% RH; Thomas Scientific, Philadelphia, PA) and varied less than 1% over the course of the experiments.

An electrobalance (SD ± 0.2 μg precision and ±0.6 μg accuracy, CAHN, Ventron Co., Cerritos, CA) was used to weigh the ticks without use of anesthesia. Each specimen was taken from the desiccator, removed from its 1 cc enclosure with an aspirator, placed or allowed to crawl onto the weighing pan, weighed, picked up with an aspirator, placed back into the enclosure and then returned to the desiccator. This manipulation required approximately 1 min.

Measurement of water balance characteristics

Temperature used for basic observations was 25°C for comparison with previous studies of water balance (Hadley 1994) and 4°C as a temperature that is more representative of this tick's natural environment and for comparison with the cold hardiness literature (Lee and Baust 1982, 1987; Worland and Block 2003). For water balance purposes, activity units were used to

define water movement into and out of the tick; a_v is the activity of water as a vapor (a_v = %RH/100) and a_w is the activity of water as a liquid, which has been determined experimentally in terrestrial arthropods to be $0.99a_w$ based on mole fraction (Wharton 1985); thus, the activity of the tick's body water can be related directly to the water vapor in the surrounding atmosphere. Before experimentation, ticks were placed at $0.33a_v$ and monitored until loss of 4–6% body mass so that the ticks were physiologically standardized by removing any residual surface water and to minimize the effects of digestion, reproduction and defecation on mass changes; thus, mass measurements reflected only changes in the tick's body water levels (Arlian and Ekstrand 1975; Wharton 1985).

Water balance characteristics were determined based on Wharton (1985) with modifications by Benoit et al. (2005). In accordance with Wharton's (1985) general water balance equation (Eq. 1):

$$m = m_S - m_T, \quad (1)$$

m is the water mass (amount of body water that is available for exchange with water vapor) and is influenced by water gain due to sorption (m_S) and water loss by transpiration (m_T). When $m_S > m_T$ there is net water gain, when $m_T > m_S$ there is net water loss, and when $m_S = m_T$ the tick is in water balance (no change in water mass).

The water mass (m) was calculated by taking the difference between the initial mass (i) and dry mass (d). After the initial mass was determined, dry mass was found by freezing (–70°C, 12h) and placing them at $0.00a_v$ at 65°C until reaching a constant mass. The body water content was found using Eq. 2:

$$\text{percentage } m = 100(i - d)/i. \quad (2)$$

The maximum reduction of water mass a tick can tolerate before succumbing to dehydration-induced mortality was determined by exposure to $0.33a_v$, weighing them every 2 h. For nonfed stages this was based on when the tick failed to right itself and crawl ten body lengths, for eggs this was based on collapse of the chorion, lack of visible guanine accumulation internally and inability to hatch, and for fed stages it was the inability to emerge to the next developmental stage (Yoder and Spielman 1992; Yoder et al. 2006). To verify that individuals could not recover from dehydration, all specimens were placed at water saturation ($1.00a_v$) for 24 h and again evaluated for survival. At the completion of the experiment, dry masses (d) were obtained so that mass measurements could be converted to water mass (m). The critical mass (m_C), used to assess dehydration tolerance, was defined as the mass below which the ticks could not be rescued by placing them at $1.00a_v$. This value was used to calculate the percentage change in mass (dehydration tolerance) according to Eq. 3, with i serving as the initial mass

$$\text{percentage change in } m = 100(i - m_C)/i. \quad (3)$$

Water loss (m_T)

To prevent interference of adsorbed surface water from the atmosphere on mass changes, water loss rate (net transpiration rate = integumental plus respiratory water loss) was determined at $0.00a_v$ so that mass changes could be attributed solely to a reduction of the internal water pool; that is, under $0.00a_v$, $m = -m_T$ so water loss occurs with no gain because $m_S = 0$. In short, the ticks were weighed, held at $0.00a_v$, reweighed to find a change in mass six times and dried to a constant mass to obtain dry mass (d) so that mass changes reflected changes in body water (m), denoted m_t (water mass at any time t) as a proportion of initial water mass (m_0). These mass values were used to establish the rate of water loss ($-k_t$) by plotting the ln (m_t/m_0) against time according to Eq. 4 of Wharton (1985):

$$m_t = m_0 e^{-k_t}, \quad (4)$$

with the rate derived from the slope and expressed as %/h. Additionally, water loss rates were expressed as mg/h.

To determine the threshold of particularly rapid desiccation, water loss rate determinations ($0.00a_v$, following Eq. 4) were made for individual, isolated ticks and recorded at multiple temperatures. In some cases, a point when water loss begins to increase dramatically with temperature may occur and is denoted by the CTT. The CTT was determined using Arrhenius analysis based on the following equation:

$$\ln k = -E_a/R_{gas}T + \ln A, \quad (5)$$

where k is the water loss rate, E_a is the energy of activation, R_{gas} is the gas constant, T is absolute temperature and A is the frequency factor. Living, rather than killed, ticks were used because they give the same CTT value (Davis 1974; Yoder and Spielman 1992; Yoder and Tank 2006), while providing more physiologically relevant information.

To test for possible behavioral regulation on water conservation, group effects on water loss were similarly

assessed by placing individuals in groups of 5 or 10 (Yoder and Knapp 1999). Due to a shortage of field-collected adults, only nymphs, larvae and eggs were used in this section of the experiment. The mobile stages were held in groups within a 2 cc mesh-covered chamber, and eggs were held directly in the wells of a porcelain plate. Water loss rates were determined at $0.00a_v$ at 25°C, following Eq. 4, for an isolated individual within the group. After mass determination the individual was returned to the group. To discern individuals to be weighed within the group, a spot of paint (Pactra, Van Nuys, CA) was applied with a single bristle of a soft camel's hair brush to the dorsal idiosoma; paint had no effect on mass changes (data not shown).

In all cases, weighing intervals for determining water loss rates were 1 h except at higher temperatures and for smaller immature stages where losses were excessive and necessitated the use of shorter time intervals so that the water loss rate could be derived from six consecutive mass measurements with the tick displaying regular ambulatory activity and prior to reaching its critical mass (m_C).

Water gain (m_S)

The threshold for active uptake of atmospheric water, defined as the CEA (Wharton 1985), was examined by monitoring tick water mass at different water vapor activities for 10 days. Conforming to standard practice, 0.85, 0.93, 0.98 and $1.00a_v$ were the water vapor activities used as benchmark values. Additional water vapor activities generated with glycerol–water mixtures were used to narrow the range to obtain a more precise approximation of the CEA by lowering the water activity by $0.01a_v$ until water mass was not maintained, testing for the lowest water vapor activity where the tick maintained its water mass (m). Below the CEA, water loss occurs by simple diffusion because the activity of the surrounding air is less than the $0.99a_w$ activity of the tick's body water. Thus, in our study the CEA represents the lowest water vapor activity where the tick was able to maintain its water mass (m) for a period of 10 days.

To test for free water drinking, ticks were held at $0.33a_v$ and monitored until 10–12% body mass was lost. Experiments were conducted as described (Yoder and Spielman 1992; Kahl and Alidousti 1997). Ticks were then placed, 10 at a time, into 9 cm i.d. petri dishes with 10–15 droplets of deionized (DI) water stained with 0.1% Evans blue dye and observed (40×) for 15 min of every hour for 24 h to examine their reactions to the droplets. Sizes of droplets varied from 5 to 20 µl. After this exposure, the ticks were removed, washed with DI water to remove residual dye, and placed at $0.33a_v$ for 2 h. Ticks were dissected in 1.0% NaCl to liberate any blue coloration from their digestive tract as confirmation of drinking.

Sample size and statistics

Each experiment involved at least 24 ticks or eggs. Each tick was monitored individually. Data are presented as a mean ± SE and compared using an analysis of variance (ANOVA) with arcsin transformation in the case of percentages. A test for the equality of slopes of several regressions was used to compare characteristics derived from regression lines (Sokal and Rohlf 1995).

Results

Water content and loss at different stages

The water balance profiles for each life cycle stage of *I. uriae* are presented in Table 1. Among the nonfed stages, initial mass, dry mass, water mass and percentage body water content increased throughout development, with eggs having the lowest percentage body water content and adults having the highest (ANOVA, $P < 0.05$). Females had a smaller body size than males and smaller water content, but there was no significant difference in percentage body water content between the two sexes (ANOVA, $P > 0.05$). As anticipated, fed stages were characterized by having increased water mass compared to the preceding nonfed stage, a likely consequence of blood feeding. Ticks within each stage were consistently the same size, and in all cases, water mass correlated positively with dry mass: $R^2 > 0.93$ for nonfed females, >0.84 for nonfed males, >0.91 for nonfed nymphs, >0.87 for unfed larvae, >0.88 for fed larvae; >0.85 for fed nymphs, and >0.94 for eggs (ANOVA, $P < 0.001$). Corresponding water mass ratios (m/d) were similar among nonfed females (2.3), nonfed males (2.5), nonfed nymphs (2.3), fed larvae (2.3) and fed nymphs (2.4), but were lower for unfed larvae (1.9) and eggs (1.4), stages that have a lower percentage body water content as a consequence of having a greater dry mass. These observations imply that water balance characteristics are stage-specific.

Nonfed adult males and females had similar water loss rates, corresponding to a loss of 13–15% (0.71–0.86 mg) of their water content per day at $0.00a_v$ and 25°C, and 4% (0.21–0.25 mg) per day at $0.00a_v$ and 4°C (Table 1). These values are consistent with both adult

Table 1 Comparison of water balance characteristics of *Ixodes uriae* throughout development

Characteristics	Eggs	Larvae		Nymphs		Males	Females
		Unfed	Fed	Nonfed	Fed		
Water pool							
Initial mass (mg)	0.121 ± 0.010	0.093 ± 0.003	1.282 ± 0.092	0.888 ± 0.061	11.57 ± 0.41	8.64 ± 0.13	6.83 ± 0.09
Dry mass (mg)	0.051 ± 0.037	0.032 ± 0.005	0.386 ± 0.074	0.269 ± 0.031	3.48 ± 0.35	2.46 ± 0.16	2.04 ± 0.11
Water mass (mg)	0.070 ± 0.029	0.061 ± 0.006	0.894 ± 0.011	0.611 ± 0.021	8.39 ± 0.24	6.18 ± 0.09	4.79 ± 0.06
Water content (%)	57.85 ± 1.21	65.60 ± 0.64	69.73 ± 1.62	69.43 ± 0.91	72.51 ± 1.72	71.56 ± 0.54	70.13 ± 0.72
Water loss							
Loss tolerance (%)	33.4 ± 2.5	23.2 ± 1.02	26.4 ± 0.9	25.2 ± 0.8	28.5 ± 1.5	28.7 ± 0.9	27.1 ± 1.5
WLR (%/h)(Fig. 2)							
4°C	0.52 ± 0.06	1.04 ± 0.09	0.09 ± 0.01	0.43 ± 0.03	0.07 ± 0.02	0.17 ± 0.01	0.18 ± 0.03
25°C	2.01 ± 0.04	3.05 ± 0.03	0.41 ± 0.03	1.41 ± 0.05	0.21 ± 0.03	0.58 ± 0.02	0.62 ± 0.02
WLR (mg/h)							
4°C	$3.64 \pm 0.42 \times 10^{-4}$	$6.34 \pm 0.55 \times 10^{-4}$	$8.05 \pm 0.89 \times 10^{-4}$	$2.51 \pm 0.18 \times 10^{-3}$	$5.87 \pm 1.67 \times 10^{-3}$	$1.05 \pm 0.07 \times 10^{-2}$	$8.62 \pm 1.14 \times 10^{-3}$
25°C	$1.41 \pm 0.03 \times 10^{-3}$	$1.86 \pm 0.09 \times 10^{-3}$	$3.66 \pm 0.03 \times 10^{-4}$	$8.62 \pm 0.31 \times 10^{-3}$	$1.76 \pm 0.25 \times 10^{-2}$	$3.58 \pm 0.12 \times 10^{-2}$	$2.96 \pm 0.10 \times 10^{-2}$
CTT (°C)	–	30.1 ± 1.9	ND	32.5 ± 1.5	ND	37.8 ± 1.7	37.9 ± 0.9
Water gain							
Liquid water uptake	–	–	–	–	–	–	–
CEA (a_v) (Fig. 5)	≥0.99	0.84–0.86	≥0.99	0.86–0.88	≥0.99	0.89–0.90	0.89–0.92

Each value represents the mean ± SE of at least 24 individuals

Loss tolerance, percentage water loss at the critical mass; WLR, water loss rate at $0.00a_v$ and 25°C; CEA, critical equilibrium activity, where water vapor absorption begins; a_v, activity of water in the vapor phase = %RH/10; –, does not occur; ND, not determined

stages being able to lose approximately 28% of their water mass and surviving approximately 2 days at $0.00a_v$ at 25°C and 7 days at $0.00a_v$ at 4°C. Thus, survival estimates match dehydration tolerance given a corresponding rate of water loss.

Unfed larvae became irreversibly dehydrated after 8–10 h, and nonfed nymphs survived approximately 1 day at $0.00a_v$, survival times that are consistent with their respective water loss rates (Table 1). Eggs had a water loss rate of 2%/h (1.41×10^{-3} mg/h), amounting to a loss of almost 50% per day and failed to hatch after less than 1 day at $0.00a_v$. Consistently, at the ecologically relevant temperature of 4 C, water loss rates of all nonfed stages of *I. uriae* dropped by nearly one-third compared to the rates observed at 25 C (Table 1) which amounted to a 3–4-fold increase in survival time.

When analyzed as percentages of the initial water content, water loss also exhibits a size-rate relationship ($y = -0.38 \times -0.19$, $R^2 > 0.99$ at 25°C; $y = -0.42 \times -1.41$, $R^2 > 0.99$ at 4°C; Fig. 1), thus adults of this tick retain water more effectively than immature stages as a consequence of surface area to volume properties. Fed stages and eggs were not included in this size analysis because they differ so markedly from nonfed stages due to the fact that they are blood filled or contain inert heavy yolk in the case of eggs. Temperature produces an accelerated water loss rate, 25–30°C for unfed larvae and 30–35°C for nonfed nymphs, males and females as evidenced by curves denoting temperature effects on water loss rate (Fig. 2a). When examined by Arrhenius analysis (Fig. 2b–f), water loss rate increases correspondingly with increasing temperature and yields proportionate losses that are typical of standard Boltzmann temperature function (in all cases, $R^2 \geq 0.97$: ANOVA, $P < 0.001$). There is evidence of a CTT, corresponding to the temperature threshold of a particularly rapid water loss, at 30.1 ± 1.9°C for unfed larvae, 32.4 ± 1.5°C for nonfed nymphs, 37.8 ± 1.7°C for nonfed males, and 37.9 ± 0.9°C for nonfed females. Eggs did not have a CTT ($R^2 = 0.98$). Thus, larvae and eggs are more prone to temperature-induced desiccation stress than nymphs and adults.

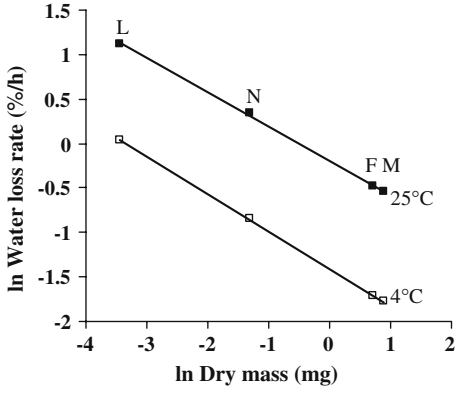

Fig. 1 Correlation between size of each developmental stage and water loss rate of *Ixodes uriae* at 4 (*open squares*) and 25°C (*closed squares*). Each point represents the water loss rates from at least 24 individuals. (L larva, N nymph, M male adult, F female adult)

The water loss rates for the fed stages were markedly lower in respect to their initial water content (0.41%/h for fed larvae and 0.21%/h for fed nymphs) when compared to unfed larvae (3%/h) and nonfed nymphs (1%/h) (Table 1; ANOVA, $P < 0.05$). The high water loss rates when shown as absolute values (mg/h) are typical of small, immature stages and unless you relate the water loss rates to the initial water content (%/h), no comparisons can be made between the water loss rates for each stage. By comparison, fed stages displayed lower water loss rates and were more resistant to desiccation, tolerating about 3% greater water loss (dehydration tolerance) and lost about 7× less water (water loss rate) than before it fed (ANOVA, $P < 0.05$; Table 1). Ranked statistically, taking into account water loss rate and dehydration tolerance and percentage water content (ANOVA, $P < 0.05$) with regard to ability to handle desiccation stress: fed stages > nonfed adults > nonfed nymphs > eggs > larvae, thus the larvae were the most sensitive to desiccation.

Group effect

Unfed larvae, nonfed nymphs and eggs in groups of 5 and 10 were more effective in preventing desiccation than isolated ticks (Table 2). For the unfed larvae and nonfed nymphs, the clusters formed were tightly packed and all individuals were in direct contact with each other, and upon re-introducing the marked tick to the cluster after mass determinations, this tick crawled back to the group. Eggs are laid as a mass. Each stage tested had a reduction of 10–15% in their water loss rates in clusters of only 5 individuals, and rates were further suppressed by nearly 65% for individuals in groups of 10, compared to the water loss rates of isolated individuals. Similar percentage reductions in the water loss rate also occurred when experiments were conducted at 4 and 30°C (data not shown), indicating that the clustering effect does not only occur as a result of high or low temperature stress. To diminish an effect of surface area to volume properties, eggs, unfed larvae and nonfed nymphs in groups had similar water mass

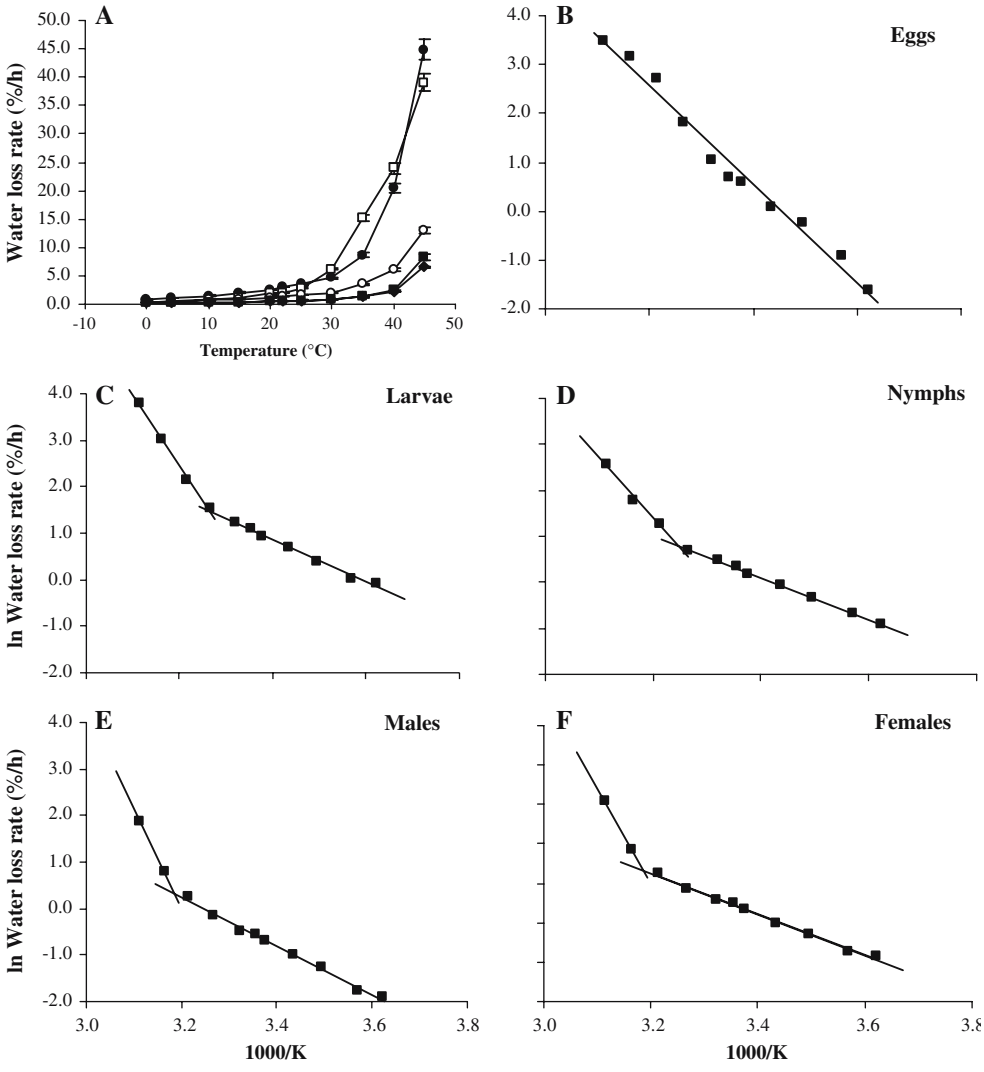

Fig. 2 Temperature effect on ability to retain water (water loss rate) by *Ixodes uriae* at different stages of development (**a** *closed circle* larvae, *open square* eggs, *open circle* nymphs, *closed square* females, *closed diamond* males). Same data plotted on an Arrhenius plot (**b**–**f**). Water loss rates were determined at 0.00 a_v from 24 individuals and are presented as mean ± SE in Fig. A

(Table 2) to those used in experiments on individual ticks (Table 1; ANOVA, $P > 0.05$), implying that the difference that we note in water conservation can be attributed to clustering.

Water replenishment

Liquid uptake did not occur for any developmental stage as evidenced by the absence of blue stain in the digestive tract (Table 1). Unfed larvae, nonfed nymphs and adults encountered the water by chance, thus they did not appear to be attracted nor repelled by the droplets. At no point during our observations did a tick lower and insert its hypostome into the droplet and appear to drink, though some did appear to rest near the edge of the droplets on occasion. Fed larvae and fed nymphs did not react to the droplets because they were used during apolysis and no longer have the ability to move. However, nonfed stages (larvae, nymphs and adults) could uptake water vapor, and the CEA increased with progressive development (Table 1), with larvae being able to absorb water vapor from drier air (0.84–0.86a_v) than adults that absorb water vapor closer to saturation (≥0.89a_v), the CEA of nymphs fell in between (0.86–0.88a_v). Eggs, fed larvae and fed nymphs could not utilize water vapor as evidenced by net water mass decreases at 0.98a_v (1 atmosphere lower than activity of the body water, 0.99a_w) of 0.20%/day by eggs, 0.08%/day by fed larvae and 0.04%/day by fed nymphs. We conclude that active water vapor uptake occurs in those stages that are not modified for water retention.

Discussion

We anticipated that *I. uriae* would display either a low water loss rate, low percentage body water content or

Table 2 Effects of clustering at 25°C on the water loss rate of eggs, unfed larvae and nonfed nymphs of the tick, *Ixodes uriae*

Group size	*N*	Water mass (mg)			Water loss rate (%/h)		
		Eggs	Larvae	Nymphs	Eggs	Larvae	Nymphs
1	30	0.070 ± 0.008[a]	0.061 ± 0.0011[a]	0.611 ± 0.021[a]	2.01 ± 0.024[a]	3.05 ± 0.025[a]	1.41 ± 0.05[a]
5	10	0.068 ± 0.013[a]	0.061 ± 0.0015[a]	0.607 ± 0.049[a]	1.69 ± 0.041[b]	2.72 ± 0.060[b]	1.21 ± 0.07[b]
10	10	0.073 ± 0.020[a]	0.060 ± 0.0016[a]	0.601 ± 0.090[a]	1.32 ± 0.061[c]	2.01 ± 0.020[c]	0.99 ± 0.05[c]

Each value represents the mean ± SE. Data followed by the same superscript are not statistically different (ANOVA, $P > 0.05$)

high tolerance for dehydration because these water conservation features typically coincide with tolerance to low temperature (Danks 2000). To the contrary, *I. uriae* is characterized by high water loss rates, classifying it as hydrophilic with regard to water balance, which implies that the emphasis for this tick is on water gain rather than water retention and desiccation-hardiness. To counter large body water losses, *I. uriae* seeks cool, moist reprieves under rocks, consistent with its hydrophilic nature, and this results in the formation of clusters. Importantly the moisture level under the rocks is greater than the CEA of the tick. In fact, what these data show are a remarkable similarity in water balance characteristics between *I. uriae* and other kinds of *Ixodes* ticks, suggesting that the occurrence of *I. uriae* on Antarctica is not directly related to a unique water balance characteristic, but instead is probably dictated by the presence of seabird colonies and attributes of cold tolerance. The propensity of *I. uriae* for moisture and absence of major water conservation features suggests that Antarctica is an ideal environment for *I. uriae* because it is cold, which enables the tick to control its rate of water loss. This is supplemented behaviorally by a water-conserving group effect as a result of aggregation formation. These ticks survive because they are cold tolerant (Lee and Baust 1987), but from a water balance perspective, the low temperature is critical for maintaining water balance.

On the islands near Palmer Station, the ground temperature remains relatively stable below 4°C throughout major portions of the year, especially under the rocks where *I. uriae* overwinters, indicating that cold exposure will not be debilitating to the populations (Lee and Baust 1987). During the summer, the temperature will surpass 20°C only on a few occasions, and the moisture content near the tick colonies is rather high, averaging $>0.96a_v$ under the rocks (field data 2006), allowing *I. uriae* to maintain its water balance after feeding and molting. These moisture conditions are greater than the CEA of the tick ($0.84–0.86a_v$ for larvae, $0.86–0.88a_v$ for nymphs and $0.89–0.92a_v$ for adults) and thus are sufficiently high to permit water vapor absorption and maintain water balance; that is, the microhabitat under the rocks exceeds the CEA of the tick. Thus, given the importance of water gain for *I. uriae* because it is hydrophilic, it seems reasonable to suggest that the moisture-rich microhabitat under the rocks is selected to satisfy an absolute moisture requirement, and this results in the formation of clusters.

Clustering has the side benefit of reducing water loss rates of individual members. The result is a nearly twofold decrease in the amount of water lost which makes a major contribution to the internal body water pool. Reduced rates of water loss occurred in tick clusters of five individuals, and further reduction occurred when group size was increased to ten (water loss rate dropped by nearly 30% when compared to the water loss rate observed for individuals). Whether the water loss rate continues to drop as group size increases, or reaches a threshold, is not known; neither is the mechanism. Conceivably, the cluster is a site of localized high water vapor activity generated by water loss of the members of the group producing a humidified boundary layer (Yoder et al. 1992) that perhaps can be used by nearby ticks. Behavioral regulation of water loss by group effects have been reported for other ticks (*Dermacentor variabilis*, *Amblyomma americanum*; Yoder and Knapp 1999), but only for larvae, the stage regarded as most sensitive to desiccation (Knülle 1966). Because nymphs and eggs of *I. uriae* have a group effect as well, the clustering is not solely a larval response to stress, rather a behavioral mechanism employed by multiple stages of this tick. In our recent field observations and those reported by Lee and Baust (1987), we typically observe clusters of hundreds of this tick indicating that water loss reduction by the formation of aggregations also occurs in nature and is not a laboratory artifact. The clustering effect of egg masses is especially important for *I. uriae* due to their high water loss rates, particularly if temperatures are high. Clustering offers the additional benefit of bringing males and females together for mating (plus pheromonal cues; Sonenshine 1991), such that these moisture-rich sites (preferred by fed females; Sonenshine 1991) under rocks are sites for oviposition, development and hatching.

Slowing down the water loss rate is the key survival element for *I. uriae*. Like many ticks, percentage body water content of *I. uriae* (70–75%) is relatively high (~70%; mean water content of most arthropods; Hadley 1994), thus to function properly they require a high body water content, only about 1/3 of which can be lost before reaching a lethal level of dehydration. Several aspects of *I. uriae* enable water loss suppression. (1) Stable cold temperature, averaging 4°C, has an appreciable impact on restricting water loss and keeping the water loss rates low. (2) Possession of a high CTT > 30°C for *I. uriae* largely safeguards this tick from experiencing any kind of abrupt, rapid lethal water loss. (3) Selection of moisture-rich sites at or above the CEA of *I. uriae* under the rocks, which has the advantage of exposing ticks to more hydrating atmospheres than dehydrating ones in addition to maintaining water balance. (4) Water loss is reduced behaviorally by group effects. Interestingly, eggs of *I. uriae* are nearly as prone to desiccation as the larvae, even though the egg has a lower water loss rate and a higher dehydration tolerance than larvae; the lack of a water uptake mechanism and the inability to move to more favorable conditions increases the likelihood of the eggs becoming irreversibly dehydrated. This suggests that both the eggs and larvae will dictate moisture requirements that may limit the expansion of *I. uriae*, particularly in relation to increasing temperature.

Our classification of *I. uriae* as hydrophilic is based mainly on its water loss rate in comparison to other species (Hadley 1994). Water loss rates match moisture requirements for life in a particular environment; species that are dry-adapted have low water loss rates and species that are wet-adapted have high water loss rates (Hadley 1994). Comparative water loss rates at $0.00a_v$, 25°C, conditions that offer the greatest potential for comparisons, of nonfed adult females are: 0.67%/h for *I. scapularis* (Yoder and Spielman 1992) and 0.28%/h for *A. americanum* (Sigal 1990), two species that are classified as having a hydrophilic distribution (Hair and Bowman 1986), 0.21%/h for *D. variabilis* (Yoder et al. 2004b), which has a distribution described as ubiquitous (Drummond 1998), 0.15%/h for *Rhipicephalus sanguineus* (Yoder et al. 2006), a xerophilic species that displays a preference for warm and dry climates (Heath 1979; Demma et al. 2005), and 0.08%/h for the extreme xerophilic, desert-adapted *Hyalomma dromedarii* (Hafez et al. 1970). This places *I. uriae* (0.54%/h) more toward the hydrophilic end of the spectrum. Indeed, a hydrophilic classification is common for species of *Ixodes* (Lees 1946; Kahl and Alidousti 1997; Kahl and Knülle 1988; Yoder and Spielman 1992), and this agrees with their distribution in moisture-rich environments that are typically near the coast (Drummond 1998).

As in other ticks, the capacity to uptake water vapor is specific to certain stages of the life cycle (Knülle and Rudolph 1982; Needham and Teel 1986). Larvae of *I. uriae* can use water vapor at a_v's as low as $0.84–0.86a_v$, which corresponds to CEA values for larvae of *A. americanum*, *A. cajennense*, *D. andersoni* and *D. variabilis* (Knülle 1966), *Haemaphysalis leporispalustris* (Camin and Drenner 1978) and *I. scapularis* (Yoder and Spielman 1992). The moisture requirement for adults, however, is greater, as demonstrated by CEA values that are closer to saturation: $0.89–0.92a_v$ in *I. uriae* and $0.92–0.96a_v$ for *I. canisuga*, *I. hexagonus* and *I. ricinus* (Lees 1946) and *A. americanum* (Sigal 1990). Nymphal CEA values are $0.86–0.88a_v$ for *I. uriae*, which are close to that ($0.87–0.89a_v$) for *I. scapularis* (Yoder and Spielman 1992) and *I. ricinus* (Kahl and Knülle 1988), and are intermediate between the CEA's of larvae and adults (Knülle and Rudolph 1982; Yoder and Benoit 2003). There are typically more hydrating atmospheres for larvae (CEA is lower) as a trade-off for its high water loss rates and more dehydrating atmospheres for an adult (CEA close to saturation) to prevent over hydration due to its lower water loss rate; nymphs are intermediate and represent the shift in priority from water gain as a larva to water retention as an adult (Yoder et al. 2006). Water vapor absorption does not occur in eggs (Teel 1984; Yoder et al. 2004a) as this stage is uniquely modified for water conservation by having low water content (due to presence of inert, heavy yolk) and high tolerance for dehydration (Heath 1979; Hinton 1981). Engorged stages of *I. uriae*, like engorged stages of *I. holocyclus* and *I. ricinus* (Heath 1981; Knülle and Rudolph 1982; Kahl and Knülle 1988) and *A. americanum* (Yoder et al. 1997), favor water retention rather than uptake and lose their ability to absorb water vapor from the air.

Ixodes uriae was incapable of drinking liquid water to maintain its off-host moisture levels, as also noted in two other *Ixodes* species, *I. scapularis* (Yoder and Spielman 1992) and *I. ricinus* (Lees 1946; Kahl and Alidousti 1997), as well as other ticks *A. cajennense* (Knülle 1966) and *D. nuttalli* (Kahl and Alidousti 1997). Ticks that can imbibe free water include *B. microplus*, *B. decoloratus*, *R. evertsi*, *R. appendiculatus*, *A. americanum* (summarized by Kahl and Alidousti 1997) and *R. sanguineus* (Yoder et al. 2006). In all of these cases, the ticks were observed to approach dye-stained droplets of water, insert their hypostome into the droplet and drink the colored liquid. Evidence of dye can be seen filling the gut diverticula and the color tracer is liberated upon dissection. Failure of *I. uriae* to

drink free water is thus a feature shared among many, but not all, tick species. Based on free water drinking, there does not appear to be any consistent ecological, phylogenetic or stage connection that would distinguish those species that can drink free water from those species that cannot. The fact that *I. uriae* does not drink directly from droplets of water is not unusual for *Ixodes*, but our preliminary mass change and behavioral data suggest that they may replenish high body water losses by absorbing water vapor from the humidity in the vicinity of the droplet, as observed in *I. ricinus* (Kahl and Alidousti 1997).

This study on *I. uriae* also addresses a crucial issue involving the rapid, water loss that occurs in response to rising temperature, a point known as the CTT. The CTT is sometimes, but inappropriately, used as an indicator of temperature tolerance to predict the potential of a species to spread into new geographic regions. The CTT for larvae of *I. uriae* is 30°C, a value that is within the 30–35°C range for larvae of *A. americanum*, *A. maculatum*, *D. variabilis*, *I. scapularis* and *R. sanguineus* (Yoder and Tank 2006). A higher CTT of 38°C is observed in adults of *I. uriae*, a value that is within the 35–40°C range for adults of *I. ricinus*, *I. canisuga*, *I. hexagonus* and *A. americanum* (Lees 1946). *I. uriae* is like *A. americanum* in that there is no CTT in the egg (Yoder et al. 2004a). Few reported CTT values are available for nymphs, but they tend to fall close to or within the range of larval values rather than the range for adults (Lees 1946; Yoder and Spielman 1992). There is no doubt that ixodid ticks experience an abrupt, rapid water loss as the temperature rises (Lees 1946; Yoder and Tank 2006). In most ticks, the CTT lies beyond the biological temperatures preferred by the species, suggesting that the CTT is of negligible importance (Needham and Teel 1986). Indeed, the CTT values of *I. uriae* are far higher than temperatures that this tick naturally experiences on Antarctica, except possibly while feeding. Interestingly, the CTT for *I. uriae* is within the ranges of the CTT's of larvae and adults of other tick species that represent an array of thermal habitats, suggesting that the CTT of ticks cannot be used for ecological interpretations.

Acknowledgments NSF grants OPP-0337656 and OPP-0413786 funded this research. We thank the Palmer Research Staff for their assistance.

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