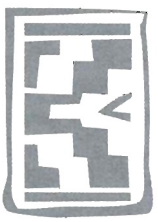

Studies on the critical water mass, rehydration capability and potential, acute chill tolerance and supercooling point of *Argas (Persicargas) walkerae* (Acari: Argasidae)

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ABSTRACT

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The critical water mass, defined as the water mass remaining in a dehydrated tick in the non-ambulatory state, differed only slightly between light and heavy mass groups of *Argas walkerae* and averaged 23.6 % and 23.2 %, respectively, in males and 28.4 % and 28.0 %, respectively, in females. All ticks survived dehydration to 50 %, 75 % or 100 % of their critical water mass, and 95 % of them rehydrated during their subsequent incubation at 95 % relative humidity (RH) and 28 °C for 14 days and regained their ambulatory status. Unfed adults were able to balance water loss frequently over a period of several months. When ticks were repeatedly dehydrated at 0 % RH for 14 days, females and males suffered 50 % mortality after 16 and 19 cycles of de- and rehydration, respectively, over a period of 278 days and 337 days, respectively. Water itself was not attractive to either dehydrated or non-dehydrated ticks and drinking was not observed. After submergence in water for 3 days, most of the dehydrated adult ticks gained mass. Judged by 50 % mortality, larvae tolerated short-term extreme chilling to –24 °C, nymphs I to –22 °C, nymphs II to –20 °C, females and males to –19 °C. None survived tissue freezing. At a chilling rate of 0.3 °C/min, mean supercooling points (SCP) ranged from –25.9 °C in eggs to –16.5 °C in unfed females. The SCP of all other stages was significantly higher than that of eggs. Mean SCPs of unfed adult ticks dehydrated to 50 % or 75 % of their critical water mass were significantly lower than that of fully hydrated ticks. The SCPs of ticks acclimated by several weeks exposure to 0 °C or to 38 °C were significantly lower than those of adult ticks kept constantly at 28 °C.

Keywords: *Argas walkerae*, cold-hardiness, critical water mass, rehydration

INTRODUCTION

Argas (Persicargas) walkerae is widespread in southern Africa, parasitizing domestic fowls. Wild hosts are unknown. The species occurs commonly in peasant-type fowl runs and adjacent trees and is the most important ectoparasite of fowls. It causes considerable economic losses, especially where it transmits *Aegyptianella pullorum* and *Borrelia anserina* (Gothe 1992a, b). In addition, larvae secrete a neurotoxin during feeding, frequently resulting in fatal paralysis (Gothe 1999).

The documented distribution of *A. walkerae* includes the Republic of South Africa, Lesotho, Namibia, Zimbabwe and Zambia only (Kaiser & Hoogstraal 1969; Eastwood 1971; Gothe & Schrecke 1972; Colbo 1973; Gothe & Verhalen 1975; Norval, Short & Chisholm 1985). Its occurrence throughout much of southern Africa indicates that it experiences various levels of environmental stress depending on the climate

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of its immediate habitat. It thus seems that this argasid species possesses a marked ecological plasticity and can tolerate extremes of both ambient temperature and relative humidity (RH) for long periods. This assumption is supported by laboratory investigations which revealed that larvae can still hatch when eggs were incubated in 28 °C and 95 % RH after exposure to −10 °C for 2 weeks (Pfeifer 1990). The mean survival periods of unfed larvae were always longest at a relative humidity above the critical equilibrium activity (CEA) reaching 33.4 days in 10 °C, 76.6 days in 20 °C and 28.1 days in 30 °C and shortest at 15 % RH amounting to 28.9 days in 10 °C, 24.1 days in 20 °C and 16.3 days in 30 °C.

Engorged larvae moulted at temperatures between 20 °C and 40 °C only, but also developed to nymphs I at 28 °C and 95 % RH after exposure to 10 °C for 7 months and to −10 °C for 10 days. The mean survival periods of unfed nymphs increased from stage I to stage III and were markedly longer than those of larvae. These periods became progressively longer the lower the saturation deficit fell and were always more than 200 days even at 15 % RH and 10–40 °C. Unfed nymphs I, II and III tolerated extremes of −10 °C and 15 % RH for mean periods of 23.9, 35.8 and 41.5 days, respectively.

Engorged nymphs I, II and III moulted to the subsequent stages at › 20 °C only, but they were still capable of completing their metamorphosis at 28 °C and 95 % RH after exposure to 10 °C for 10 months and to −10 °C for at least 5 days. Mean survival times for unfed adults were more than 270 days at 10 °C and 20 °C irrespective of whether the ticks were kept at 15 % RH or 100 % RH. At 15 % RH and −10 °C or 40 °C adult ticks survived approximately 2 months. Engorged females laid eggs at › 20 °C only, but they were able to oviposit even after exposure to −10 °C for 4 days. The lowest relative humidity at which water is taken up by adult ticks by active absorption from the atmosphere was determined to be 75.5 % RH (Pfeifer 1990).

However, it remained unsolved whether adult *A. walkerae* could survive and restore their water balance when either very large quantities or even almost all the total exchangeable water is lost. It also remained to be determined how frequently dehydrated adult ticks are capable of taking up water from the unsaturated air above the CEA or whether they drink liquid water. The crystallization temperature and lower lethal temperatures have also not yet been considered. The present studies were conducted to examine the critical water mass and the rehydration capability and potential of the ticks, as well as the supercooling point and the effect of short and rapid exposure to very low subzero temperatures on their survival in order to better understand the ecological and zoogeographic associations of *A. walkerae*.

MATERIALS AND METHODS

Ticks

The *A. walkerae* were the laboratory-reared offspring of ticks collected on a farm near Pretoria (Republic of South Africa) in 1986. Chickens were used as hosts for the larvae, nymphs and adults. All host animals were infested only once. Off-host ticks were kept at 28 °C and 95 % RH in the dark until the start of the experiments. Adult ticks were used 4–8 weeks after ecdysis, nymphs and larvae 2–4 weeks after metamorphosis and hatching, respectively.

Critical water mass and rehydration capability of unfed adult ticks dehydrated to 50, 75 and 100 % of their critical water mass

In this study the critical water mass is defined as the percentage of total body water determined in a hydrated tick that remains in this tick after its desiccation until it becomes unable to walk. For the estimation of the critical water mass, two mass groups each of 15 unfed females and 15 males, were used (Table 1). The mass of the ticks was determined to the nearest 0.01 mg on an electrobalance (Type BP 210 D, Satorius, Göttingen, Germany). The ticks were put singly into perforated Eppendorf vials of known mass and were then transferred to 0 % RH and 28 °C and were weighed at intervals of 2 days. At the same time the degree to which the ticks could move was classified. They were qualified as being not (a), definitely (b) or severely (c) affected when the ticks were ambulatory (a), moved stiffly with slightly bent legs, or thrashed their completely bent legs with no locomotion (b), or exhibited only barely recognizable movements of their legs (c) during exposure to light, CO_2 or mechanical stimuli. The severely affected ticks were dried for 24 h at 120 °C, their dry mass was determined and the critical water mass was calculated from the loss of mass.

Further experiments were conducted to determine whether dehydrated ticks are capable of rehydration. For this purpose, 10 females and 10 males each of two mass groups (Table 2) were dehydrated at 0 % RH and 28 °C until water loss reached 50, 75 or 100 % of the critical water mass. The dehydrated ticks were then transferred to 95 % RH at 28 °C and their mass and survival were recorded at intervals of 24 h for 14 days.

The relative humidities of 0 % and 95 % were generated using concentrated H_2SO_4 and water, respectively, in airtight chambers and were continuously measured using hairsynthetic hygrometers (Merck, Ismaning, Germany). The chambers were stored in an incubator (Memmert, Schwabach, Germany) maintained constantly at 28 °C, controlled by thermographs (Lambrecht, Göttingen, Germany). The statistical evaluation was performed by variance analysis (SAS system).

Rehydration potential of unfed adult ticks

To determine their rehydration potential, measured as the frequency with which repeatedly dehydrated ticks can take up water vapour from the unsaturated air above the CEA, unfed adult ticks were alternated between dehydration at 0 % RH and rehydration at 95 % RH and 28 °C. This change between 0 % RH and 95 % RH was repeated until the ticks had died or did not rehydrate again to at least 93.5 % of their mass after the previous rehydration.

In this experiment, 60 females and 60 males were used. The mean initial mass was 14.0 mg for females and 11.56 mg for males (Table 3). The ticks were weighed singly on the electrobalance and put in perforated Eppendorf vials of known mass in groups of three to six specimens with the same mass and sex. The vials were immediately reweighed and then transferred to 0 % RH and 28 °C for 14 days. Thereafter, dead ticks were removed, the vials were weighed and the mean mass per tick was calculated. The vials were then transferred to 95 % RH at 28 °C until the mass gained had reached at least 93.5 % of the previous rehydration mass. At intervals of 2 days the vials were weighed and the survival of ticks was checked. The ticks were then incubated in 0 % RH and 28 °C for 14 days again and the cycle was repeated as often as described above.

As a control, equal numbers of females and males of the same age and of similar mass (Table 4) were permanently incubated at 95 % RH and 28 °C. Their mean mass and survival were recorded at corresponding intervals. The abiotic conditions were generated and controlled and the statistical evaluation was performed as described above.

Uptake of liquid water by unfed adult ticks

To determine whether unfed adult ticks are capable of rehydrating by means of liquid water, several experiments were conducted. Twenty females and 20 males were used in each experiment. Single ticks were weighed on the electrobalance, transferred to perforated Eppendorf vials and dehydrated at 28 °C and 0 % RH for 28 days in airtight chambers and reweighed.

In Experiment 1, the dehydrated ticks were singly transferred to a 52 mm diameter petri-dish with a centrally drilled hole 2,5 mm in diameter in the bottom. The petri-dish was covered with gauze and placed in another petri-dish 57 mm in diameter, which was filled with tapwater so that the water formed a cone at the drilled hole and was accessible to the ticks. The water surface around the smaller petri-dish was covered with parafilm. Both petri-dishes were transferred to a chamber with a temperature of 28 °C and 55 % RH measured by a thermograph and hygrometer, respectively. The ticks were kept in the smaller petri-dish for 72 h. They were dried with pulp and weighed singly after 24 h, 48 h and 72 h.

To examine whether dehydrated ticks orientate themselves towards water or are attracted to water, two experiments were conducted. In Experiment 2, females and males were dehydrated as in Experiment 1 and then exposed in a test arena which consisted of a petri-dish 120 mm in diameter. In this petri-dish, four 5 µl water droplets were placed at a distance of 5 cm from the centre and at right angles to each other. Single ticks were released at the centre of the arena and breathed on for 3 s. During the following observation period of 15 min, the time until each started to move and its movement paths and distances were recorded. It was specially noted whether the ticks turned towards the water, had direct contact with it or inserted their mouthparts into it. Ticks which touched the water were reweighed immediately afterwards.

Since adult ticks react phototactically negatively (Gothe, Koch & Leuterer 1989; Beelitz & Gothe 1991), they were observed at a crepuscular-analogous irradiance of 2.5×10^{-6} W/cm^2 measured with a precision radiometer (Model IL 1700, International Light, Massachusetts, USA). Experiment 3 was done as a control of Experiment 2 and was performed in the same way except that non-dehydrated ticks were tested. In Experiment 4, dehydrated ticks were submerged in tapwater in glass vials and left there for 72 h. The mass of the ticks was determined as in Experiment 1.

In Experiment 5, dehydrated ticks were incubated at 28 °C and 95 % RH for 72 h to determine the extent of rehydration by uptake of water from the atmosphere. The mass loss after dehydration of the ticks used in Experiments 1, 2, 4 and 5 is given in Table 5. Relative humidities of 0 %, 55 % and 95 % were generated using concentrated H_2SO_4, Mg (NO_2) x 6 H_2O and water, respectively.

Acute chill tolerance of unfed larvae, nymphs and adult ticks

Acute chill tolerance was assessed by determining the lowest temperature at which any survival was observed. The procedure to monitor the lower lethal temperature corresponded to that described for the SCP determination. Nymphs and adult ticks were chilled singly and larvae in cohorts of 20 specimens in a close-meshed plastic cage at a rate of 0,3 °C/min to a low of −16 °C to −26 °C always using 20 specimens for each temperature point (Table 6). After the chill period, ticks were placed in a 95 % RH chamber at 28 °C for 1 week and survival was evaluated daily. Ticks that moved normally and survived the 1-week observation were categorized as survivors, whereas those that were dead or obviously impaired were considered dead.

Supercooling point (SCP) determinations of eggs, larvae, nymphs and adult ticks

The supercooling point was determined by attaching Ni/CrNi-thermocouples (testo, Lenzkirch, Germany) to the tick integument or egg surface by means of paraffin wax (MP 30 °C). This assembly was placed in a copper tube (8 cm in length, 4 cm in diameter) and fixed there using paraffin wax. The copper tube was then suspended in a refrigerated circulating bath (Julabo, Seelbach, Germany) containing a mixture of ethanediol and water (1:1). Cooling started at 20 °C and the specimens were then chilled at a rate of 2 °C/min, 1 °C/min, 0.5 °C/min or 0.3 °C/min. Adult ticks and nymphs were tested singly, and larvae and eggs in groups of five and ten specimens, respectively.

The thermocouples were connected to a precision temperature recorder (testo, Lenzkirch, Germany), which continuously registered the drop in temperature. The measuring data were stored in a computer data base and were analyzed by the computer software "Professional" V2.22 (testo, Lenzkirch, Germany). The lowest temperature recorded prior to the transient increase of temperature due to heat released as water crystallized within the tick was considered the SCP. The statistical evaluation was performed by variance analysis (SAS system).

Several experiments were conducted using adult ticks, nymphs and larvae and eggs from the first day of oviposition. The number of specimens per stage are summarized in Tables 7–10. In Experiment 1, unfed females and males were weighed singly to the nearest 0.01 mg on the electrobalance and were chilled at a rate of 0.3 °C/min. After reaching the SCP, the ticks were examined for survival and were then dried for 24 h at 120 °C. Their dry mass was determined and the water content was calculated for every tick based on its loss of mass. In addition, the SCP of engorged females, unfed nymphs and larvae as well as of eggs was determined (Table 7).

Experiment 2 corresponded to Experiment 1 and included unfed adult ticks only. However, the chilling

TABLE 1 Mean period (days) to reach definite and severe impairment of locomotor ability as well as mean mass loss (%) and critical water mass (%) of unfed males and females of light (l) and heavy (h) mass groups of *A. walkerae* kept at 0 % RH (± SD, n = 15 per group)

		Males		Females	
		l	h	l	h
Body mass (mg) before dehydration		10.14 ± 0.11	14.11 ± 0.13	12.24 ± 0.20	16.21 ± 0.15
Period (days) locomotor ability was impaired	Definitely	125.90 ±15.20	147.70 ±18.20	108.40 ±25.90	123.10 ±15.20
	Severely	127.60 ±14.70	149.60 ±18.60	111.20 ±25.40	125.70 ±15.40
Mass loss (%) at the time locomotor ability was impaired	Definitely	59.70 ± 2.40	60.40 ± 3.10	53.50 ± 1.80	53.90 ± 2.80
	Severely	61.50 ± 2.00	62.30 ± 3.30	56.20 ± 2.30	56.40 ± 2.30
Critical water mass (%)		23.60 ± 1.90	23.20 ± 3.40	28.40 ± 2.50	28.00 ± 2.40

TABLE 2 Mean mass loss (%) of unfed adult *A. walkerae* belonging to light (l) and heavy (h) mass groups dehydrated to 50 %, 75 % or 100 % of the critical water mass (CM) after incubation at 0 % RH as well as mass loss compensation (%) after subsequent incubation at 95 % RH and 28 °C for 14 days (± SD, n = 10 per group)

	Males		Females	
	l	h	l	h
Body mass before dehydration (mg)	10.37 ± 0.19	14.53 ± 0.15	12.36 ± 0.22	16.46± 0.23
Mass loss at 50 % of the CM (%)	30.70 ± 0.10	30.20 ± 0.20	28.20 ± 0.20	28.20± 0.10
Mass loss compensation (%)	54.10 ±10.60	42.20 ±11.60	51.80 ±13.60	67.90±10.60
Body mass before dehydration (mg)	10.35 ± 0.19	14.56 ± 0.15	12.33 ± 0.19	16.45± 0.19
Mass loss at 75 % of the CM (%)	46.00 ± 0.20	46.70 ± 0.20	42.20 ± 0.30	42.60± 0.10
Mass loss compensation (%)	56.10 ± 9.30	42.60 ± 9.20	54.80 ±11.80	49.80±11.60
Body mass before dehydration (mg)	10.44 ± 0.14	14.39 ± 0.24	12.31 ± 0.23	16.48± 0.24
Mass loss at 100 % of the CM (%)	61.10 ± 0.10	62.20 ± 0.20	56.10 ± 0.80	55.40± 0.60
Mass loss compensation (%)	32.70 ± 8.90	29.50 ± 6.10	29.70 ±11.20	37.50± 9.10

TABLE 3 Number of de- and rehydration cycles of unfed adult *A. walkerae* with regard to loss and gain of mass within and at the 50 % mortality period (± SD, n = 60 per group)

	Males	Females
Mean initial mass (mg) before first dehydration	11.56	14.0
Number of cycles within the 50 % mortality period	19.00	16.0
Maximum number of cycles	24.00	21.0
50 % mortality period (days)	337.00	278.0
Maximum survival time (days)	428.00	362.0
Mean mass loss after dehydration (%) within 50 % mortality period	8.50 ± 0.7	8.5 ± 0.7
Mean mass gain after rehydration (%) within 50 % mortality period	6.30 ± 2.3	6.3 ± 1.5
Mean mass loss of rehydrated ticks at 50 % mortality period (% of initial mass)	51.90	43.4

TABLE 4 Survival of unfed adult *A. walkerae* kept permanently at 95 % RH and 28 °C and mass loss at the 50 % mortality period of de- and rehydrated ticks (± SD, n = 60 per group)

	Males	Females
Mean initial body mass (mg)	11.50	13.97
50 % mortality period (days)	360.00	362.00
Maximum survival time (days)	444.00	442.00
Mean mass loss at 50 % mortality period of de-/rehydrated ticks (% of initial mass)	48.00	29.40

rate was increased to 0.5 °C/min, 1 °C/min or 2 °C/min (Table 8). In Experiment 3 (Table 9), unfed females and males were used. Single ticks were weighed on the electrobalance and then dehydrated at 0 % RH and 28 °C to 50 % and 75 % of their critical water mass. The SCP of the ticks was determined at a chilling rate of 0.3 °C/min. After reaching the SCP, the water content was calculated as described in Experiment 1. In further trials (Table 10), unfed females and males acclimated with 4 weeks exposure to 0 °C (Experiment 4) or with 6 weeks incubation at 38 °C (Experiment 5) were used. SCP and water content were determined as in Experiment 1.

TABLE 5 Mean mass loss (%) of unfed adult *A. walkerae* after dehydration at 0 % RH and 28 °C for 28 days (± SD, n = 20 per group)

Experiment	Males	Females
Experiment 1	11.7 ± 1.5	10.7 ± 1.2
Experiment 2	13.5 ± 2.2	16.0 ± 3.1
Experiment 4	12.0 ± 2.9	10.9 ± 1.6
Experiment 5	14.3 ± 2.4	12.4 ± 2.1

TABLE 6 Number of surviving unfed larvae, nymphs and adult ticks chilled at a rate of 0.3 °C/min to a low of −16 °C to −26 °C (n = 20 per temperature point)

Temp. (°C)	Larvae	Nymphs I	Nymphs II	♀♀	♂♂
−16	20	20	20	20	20
−17	20	18	20	18	18
−18	20	19	17	18	13
−19	17	19	14	14	10
−20	19	19	14	9	4
−21	19	12	6	5	1
−22	16	12	8	1	0
−23	11	3	1	0	0
−24	15	3	0	0	0
−25	2	1	0	0	0
−26	0	0	0	0	0

RESULTS

Critical water mass and rehydration capability of unfed adult ticks dehydrated to 50, 75 and 100 % of their critical water mass

Incubation at 0 % RH and 28 °C resulted in continuous mass loss in all ticks, but it always occurred very slowly. However, the ticks were only impaired in their locomotor ability 1–3 days before reaching the critical water mass, when they passed into the non-ambulatory state (Table 1). The time at which the critical water mass was reached varied considerably, but was significantly shorter (P = 0.0001) in females and in ticks of the light mass group than in males and in ticks of the heavy mass group. The mean period at which the water mass became critical for ticks of the light and heavy mass group was 111.2 days and 125.7 days, respectively, in females and 127.6 days and 149.6 days, respectively, in males.

The percentage mass loss at the point when the critical water mass was reached was significantly less (P = 0.0001) in females than in males. The mass loss of the light and heavy group averaged 56.2 % and 56.4 %, respectively, in females and 61.5 % and 62.3 %, respectively, in males (Table 1). The mass groups of females and males did not differ significantly. The critical water mass, calculated as a percentage of the total body water of fully hydrated ticks was significantly larger (P = 0.0001) in females than

TABLE 7 Mean SCPs (± SD) of various stages of *A. walkerae* at a cooling rate of 0.3 °C/min including mean body mass (mg), dry mass (mg) and absolute (mg) and relative (%) water content (Experiment 1)

Developmental stage	*n*	Body mass	Dry mass	Water content		SCP (°C)
				Absolute	Relative	
Eggs	30					–25.9 ± 1.3[a]
Unfed larvae	15					–22.3 ± 1.1[b]
Unfed nymphs I	15	1.63 ± 0.19	0.38 ± 0.05	1.24 ± 0.16	76.3 ± 2.3	–20.4 ± 2.0[c]
Unfed nymphs II	15	5.17 ± 0.41	1.39 ± 0.16	3.78 ± 0.36	73.1 ± 2.8	–17.9 ± 2.8[d]
Unfed ♂♂	15	15.14 ± 4.29	4.16 ± 1.21	10.98 ± 3.09	72.6 ± 1.2	–17.7 ± 2.2[d,e]
Unfed ♀♀	15	18.77 ± 5.65	5.31 ± 1.92	13.46 ± 3.80	72.2 ± 3.0	–16.5 ± 2.2[e]
Engorged ♀♀	15	60.46 ± 10.12	17.36 ± 2.61	43.20 ± 7.65	71.4 ± 1.3	–18.5 ± 1.0[d]

SCP Values with the same letter do not differ significantly

TABLE 8 Mean SCPs of unfed adult *A. walkerae* at a cooling rate of 0.3 °C/min, 0.5 °C/min, 1.0 °C/min and 2 °C/min including mean body mass (mg), dry mass (mg) and absolute (mg) and relative (%) water content (± SD, $n = 15$ per group) (Experiment 2)

Cooling rate (°C/min)	Sex	Body mass	Dry mass	Water content		SCP (°C)
				Absolute	Relative	
0.3	♂♂	15.14 ± 4.29	4.16 ± 1.21	10.98 ± 3.09	72.6 ± 1.2	–17.7 ± 2.2[a]
	♀♀	18.77 ± 5.65	5.31 ± 1.92	13.46 ± 3.80	72.2 ± 3.0	–16.5 ± 2.2[a]
0.5	♂♂	11.02 ± 2.92	2.80 ± 0.81	8.22 ± 2.13	74.7 ± 1.6	–19.7 ± 0.9[b]
	♀♀	14.82 ± 3.59	4.04 ± 1.05	10.78 ± 2.57	72.8 ± 1.4	–19.3 ± 1.6[a]
1.0	♂♂	13.92 ± 3.65	3.75 ± 0.98	10.17 ± 2.68	73.0 ± 1.4	–19.1 ± 1.6[b]
	♀♀	14.60 ± 3.92	3.97 ± 1.14	10.62 ± 2.81	72.8 ± 1.4	–19.5 ± 1.3[b]
2.0	♂♂	13.65 ± 4.30	3.59 ± 1.23	10.06 ± 3.09	73.9 ± 1.8	–20.2 ± 1.8[b]
	♀♀	12.48 ± 2.39	3.36 ± 0.68	9.12 ± 1.75	73.1 ± 1.6	–20.2 ± 1.9[a]

in males, amounting to about 28 % in females and 23 % in males (Table 1). The percentage of total body water lost in non-ambulatory ticks indicating the exchangeable water mass in ticks of the light and heavy mass group averaged 71.6 % and 72 %, respectively, in females and 76.4 % and 76.8 %, respectively, in males.

All ticks survived water losses corresponding to 50, 75 or 100 % of their critical water mass, 114 of the 120 ticks used in the experiments rehydrated during subsequent incubation at 95 % RH and 28 °C for 14 days and were able to walk again and six ticks died during rehydration. However, the extent of rehydration varied considerably, independently of the dehydration status (Table 2), but was significantly larger ($P = 0.0001$) in ticks dehydrated to 50 % or 75 % of their critical water mass than in ticks which had lost almost all their exchangeable water. Water losses were compensated significantly more ($P = 0.081$) in females than in males.

Rehydration potential of unfed adult ticks

Unfed females and males were capable of regaining water lost during dehydration at 0 % RH by frequent incubation at 95 % RH during a period of several months. However, replenishment of the water lost to at least 93.5 % of the initial mass did not occur after the first cycle of de- and rehydration, but after sub-

TABLE 9 Mean SCPs of unfed adult *A. walkerae*, fully hydrated or dehydrated to 50 % and 75 % of their critical water mass (CM), at a cooling rate of 0.3 °C/min including mean body mass (mg), mass after dehydration (mg) and absolute (mg) and relative (%) water content (± SD, n = 15 per group) (Experiment 3)

Hydration state	Sex	Body mass	Mass after dehydration	Dry mass	Water content		SCP (°C)
					Absolute	Relative	
Fully hydrated	♂♂	15.14 ± 4.29		4.16 ± 1.21	10.98 ± 3.09	72.6 ± 1.2	−17.7 ± 2.2[a]
	♀♀	18.77 ± 5.65		5.31 ± 1.92	13.46 ± 3.80	72.2 ± 3.0	−16.5 ± 2.2[a]
Dehydrated to 50 % of CM	♂♂	10.42 ± 0.23	7.24 ± 0.16	2.29 ± 0.13	4.95 ± 0.16	68.4 ± 1.7	−20.5 ± 2.2[b]
	♀♀	12.52 ± 0.23	8.98 ± 0.17	2.96 ± 0.26	6.01 ± 0.24	67.0 ± 2.7	−19.2 ± 1.8[b]
Dehydrated to 75 % of CM	♂♂	10.27 ± 0.18	5.54 ± 0.10	2.09 ± 0.19	3.45 ± 0.23	62.3 ± 3.6	−21.5 ± 1.4[b]
	♀♀	12.22 ± 0.14	7.07 ± 0.08	2.75 ± 0.28	4.32 ± 0.31	61.1 ± 4.1	−20.1 ± 1.4[b]

SCP values with the same letter do not differ significantly

TABLE 10 Mean SCPs of unfed adult *A. walkerae* constantly kept at 28 °C, acclimated with 4 wk exposure to 0 °C (Experiment 4) or with 6 wk incubation at 38 °C (Experiment 5) at a cooling rate of 0.3 °C/min including mean body mass (mg), dry mass (mg) and absolute (mg) and relative (%) water content (± SD, n = 15 per group)

Acclimation	Sex	Body mass	Dry mass	Water content		SCP (°C)
				Absolute	Relative	
Constantly kept in 28 °C	♂♂	15.14 ± 4.29	4.16 ± 1.21	10.98 ± 3.09	72.6 ± 1.2	−17.7 ± 2.2[a]
	♀♀	18.77 ± 5.65	5.31 ± 1.92	13.46 ± 3.80	72.2 ± 3.0	−16.5 ± 2.2[a]
Acclimated to 0 °C	♂♂	18.39 ± 2.93	5.05 ± 1.05	13.34 ± 2.13	72.6 ± 3.2	−19.5 ± 1.3[b]
	♀♀	17.85 ± 3.66	4.69 ± 1.16	13.16 ± 2.55	73.9 ± 1.6	−19.0 ± 1.3[b]
Acclimated to 38 °C	♂♂	11.23 ± 2.23	2.89 ± 0.67	8.33 ± 1.59	74.4 ± 1.7	−20.5 ± 1.7[b]
	♀♀	14.25 ± 3.01	3.60 ± 0.82	10.65 ± 2.26	74. 8 ± 1.7	−19.3 ± 1.2[b]

SCP values with the same letter do not differ significantly

sequent cycles. Fifty percent of the females had died after 16 cycles of de- and rehydration in 278 days and males after 19 such cycles in 337 days (Table 3). The mean mass loss after each dehydration period in both females and males was 8.5 % and was compensated after incubation in 95 % RH with a mean mass gain of 6.3 % of the dehydration mass. Maximum rehydration in females was obtained after 21 cycles in 362 days and in males after 24 cycles in 428 days. When 50 % of the ticks had died, the mass reduction compared with their initial mass averaged 43.4 % in females and 51.9 % in males. When 50 % of the repeatedly de- and rehydrated ticks had died, females permanently incubated at 95 % RH had lost an average of 29.4 % of their mass and males 48 % (Table 4).

Survival differed significantly between ticks permanently kept at 95 % RH and ticks de- and rehydrated in regular intervals. At constant incubation in 95 % RH, the 50 % mortality level was reached after 362 days in females and 360 days in males (Table 4). In contrast, half the repeatedly de- and rehydrated females died in a mean of only 278 days and males in 337 days (Table 3).

Uptake of liquid water by unfed adult ticks

Unfed adult ticks used in Experiments 1, 2, 4, 5 had lost mass after incubation at 0 % RH and 28 °C for 28 days (Table 5). Experiment 1, offering water in a test arena for 3 days to allow drinking, revealed that the body mass of dehydrated ticks increased in one

female only, which regained 8.1 % of her mass. All other ticks lost further mass. Drinking of water was never observed. In Experiment 2, during a 15 min period observing dehydrated ticks for orientation towards water or attraction to water in a test arena, only four females and two males very briefly (< 1 s) put their first pair of legs into the water, then immediately removed them and moved away quickly. They were not seen putting their mouthpart in the water. The body mass of the ticks remained unchanged. In Experiment 3, observing fully hydrated ticks in the test arena, nine females and six males very briefly (< 1 s) touched the water with the first pair of legs only, then they immediately changed direction. The body mass of all the ticks remained unchanged.

In Experiment 4, using dehydrated ticks, 15 males and 19 females survived submersion in water for 3 days. Except in one male, their body mass increased by a mean of 15 % in males and 32.7 % in females. In Experiment 5, nine males and 17 females of the dehydrated ticks incubated for 3 days at 95 % RH and 28 °C showed gains in mass that averaged 8.9 % in males and 30.4 % in females. A further mass loss during incubation in 95 % RH occurred in nine males and three females, which averaged 0.7 % and 0.2 %, respectively.

Acute chill tolerance of unfed larvae, nymphs and adult ticks

The number of surviving ticks decreased continuously the lower the temperature dropped below −16 °C. Males and females died faster than nymphs II which in turn died quicker than nymphs I and larvae (Table 6). Judging by the 50 % mortality level, short periods of extreme chilling to −24 °C was tolerated by larvae, −22 °C by nymphs I, −20 °C by nymphs II and −19 °C by females and males. The lower lethal temperature was similarly graduated (Table 6).

Supercooling point (SCP) of eggs, larvae, nymphs and adult ticks

None of the ticks survived tissue freezing. At a chilling rate of 0.3 °C/min (Experiment 1), mean SCPs ranged from −25.9 °C in eggs to −16.5 °C in unfed females. Compared with eggs, the SCP of the other stages was significantly higher. The next lowest SCP was determined in larvae, which differed significantly from nymphs and adults (Table 7). At chilling rates of 0.5 °C/min, 1 °C/min or 2 °C/min (Experiment 2), there were no significant differences in SCPs of unfed adult ticks, but, compared with the chilling rate of 0.3 °C/min, the SCPs were significantly lower (Table 8). Mean SCPs of unfed adult ticks dehydrated to 50 % or 75 % of their critical water mass and chilled at a rate of 0.3 °C/min (Experiment 3) did not differ significantly, but were significantly lower compared with fully hydrated ticks (Table 9). Chilling of unfed adult ticks at a rate of 0.3 °C/min revealed no significant differences in SCPs between ticks acclimated with several weeks exposure to 0 °C (Experiment 4) or to 38 °C (Experiment 5). Compared with ticks constantly kept at 28 °C, however, the SCPs were significantly lower (Table 10). Comparing the results of all the experiments, there was a weak but significant negative correlation between relative or absolute water content and SCP.

DISCUSSION

Judging by the results of the experiments on water balance the integument of unfed adult *A. walkerae* ticks is an efficient waterproofing barrier, which minimizes water loss and ensures their survival even in extreme environmental saturation deficits for long periods between bloodmeals. This conclusion is particularly justified when the long periods tolerated in 0 % RH at 28 °C until the water loss becomes critical are considered. Compared with other tick species, e.g. *Amblyomma cajennense* (Strey, Teel, Longnecker & Needham 1996), the period before the ticks reached their critical water mass was at least four times longer in *A. walkerae* and lasted distinctly more than 100 days. It is remarkable that the ticks survived despite loosing more than 70 % of their whole body water, and yet still retained their rehydration capability. Accordingly, adult ticks possess a large exchangeable water mass, while their transpiration rate, which reflects their integumental permeability to water, is low. The results on critical water mass clearly indicate that adult *A. walkerae* are provided with an ecological potency with regard to relative humidity, which is sufficient to counteract and overcome environmental moisture stress in their immediate habitats for long periods.

Earlier studies revealed (Pfeifer 1990) that both males and females incubated far below the CEA at 15 % RH survived a mean of > 274 days at 10 °C and 20 °C, > 221 days and 181 days, respectively, at 30 °C and even 71 days and 43 days, respectively, at 40 °C. The ecological potency, however, is particularly evident in the capability of adult ticks to rehydrate even after dehydration to their critical water mass. This is of great ecological and biological significance and demonstrates not only the ability of adult ticks to survive long dry periods while maintaining their rehydration capability, but also indicates that adult ticks that have lost almost all their exchangeable water can use an ambient humidity above the CEA briefly to obtain sufficient water even from the unsaturated air to regain their ambulatory status, which is necessary for host seeking. These studies suggest that *A. walkerae* is not only capable of occupying a variety of ecological zones but even of expanding into areas with long-term moisture deficit stress.

In addition, the ability of adult ticks to compensate water losses frequently and quickly in relative humidities above the CEA reaching a hydration level nearly corresponding to ticks permanently kept at 95 % RH is impressive. Mass loss experienced during dehydration at 0 % RH for 14 days was almost completely regained within only 2–3 days.

Furthermore, a high rehydration potential, defined as the frequency to balance water loss after dehydration, becomes particularly impressive in that 50 % of the ticks survived 19 and 16 cycles of de- and rehydration in males and females, respectively. The repeated change between de- and rehydration, however, influences survival because, compared with ticks constantly kept in 95 % RH, survival times of ticks repeatedly de- and rehydrated were significantly shorter, the 50 % mortality period of males and females only occurred in 337 days and 278 days, respectively. In contrast, the 50 % mortality period of ticks permanently incubated at 95 % RH was reached in males after 360 days and in females after 362 days. Repeatedly dehydrated ticks compensated their water loss very effectively because their body mass after rehydration almost corresponded to those ticks permanently kept at 95 % RH. However, body mass decreased continuously irrespective of whether the ticks were permanently incubated at 95 % RH or were repeatedly de- and rehydrated. Since the decline of dry mass in long fasting adult ticks is negligible (Pfeifer 1990), it is strongly suggestive that males and females cannot rebalance their water content to the original level. Thus unfed adult ticks permanently kept at 95 % RH as well as ticks experiencing numerous cycles of de- and rehydration are able to survive for months without a bloodmeal, but with a continuously decreasing water content.

Adult *A. walkerae* are able to compensate for water loss by using not only atmospheric water vapour but also liquid water. Like several ixodid species (Splisteser & Tyron 1986; Kahl & Alidousti 1997), however, active drinking from water droplets may be excluded, since dehydrated ticks were neither attracted to liquid water nor inserted their mouthparts into water droplets. Only one dehydrated female gained mass after incubation in a petri-dish in which water droplets were offered for 3 days. However, this tick probably did not use the liquid water to rehydrate, but the water vapour evaporating from the droplets. When contact with liquid water was forced, however, uptake of water occurred because, after submerging dehydrated ticks in water for 3 days, most of them gained mass. Compared with the uptake of atmospheric water vapour at saturation deficits above the CEA, adult ticks compensated mass loss even slightly more after submerging in liquid water. Thus, the possibility that liquid water, e.g. raindrops or dew droplets, offers a reliable moisture source in habitats of *A. walkerae* should not be ignored.

The experiments on cold-hardiness demonstrated that all unfed postembryonic stages of *A. walkerae* possess a marked tolerance to acute chill, since direct chilling injuries resulting in mortality were negligible at temperatures above –22 °C for larvae, –21 °C for nymphs I, –19 °C for males and –18 °C for nymphs II and females, when they were chilled at a rate of 0.3 °C/min. At these subzero temperatures, all stages suffered a chill coma but most of them regained ambulatory status after transfer to 28 °C at 95 % RH. The lower lethal temperatures observed were similarly ranked, being lowest in larvae and highest in females, and were close to or within the respective subzero temperature range causing crystallization. This implies that the supercooling points really only indicate the lowest temperature at which survival does not occur.

The acute chill tolerance determined by far exceeds the low temperature that *A. walkerae* may encounter in its habitats of southern Africa. However, it indicates that all postembryonic stages of this argasid species are capable of resisting at least short-term episodes of very severe frost. The cold-hardiness of all unfed postembryonic stages is supported by their long survival periods at a subzero temperature more representative of the climatic conditions in southern Africa as previously demonstrated (Pfeifer 1990). These studies revealed that –10 °C is tolerated (mean) for 19.4 days by larvae, for 41.5 days by nymphs I, for 49.6 days by nymphs II, for 54.4 days by nymphs III, for 73.6 days by males and for 76.5 days by females. Even the ability to embryonate with subsequent larval hatch, to moult and to oviposit is not impaired when eggs, engorged immature stages or engorged females are incubated in 28 °C at 95 % RH after previous exposure to –10 °C for a few days (Pfeifer 1990). Thus the various life cycle stages of *A. walkerae* are capable of resisting not only short episodes of severe frost, but also of surviving relatively long periods in very low subzero temperatures. Bridging of long cold periods within the distribution range of *A. walkerae*, however, is managed essentially by nymphs and adult ticks, but not by eggs or larvae as indicated by the values of the SCP.

None of the stages survived the supercooling point which indicates that *A. walkerae* is freeze intolerant as has been demonstrated for other tick species irrespective of their geographical origin (Burks, Stewart, Needham & Lee 1996; Dautel & Knülle 1996, 1997; Needham, Jaworski, Chen & Lee 1996). Almost all tick species investigated possess a high potential for supercooling and the SCP values of *A. walkerae* are fairly similar for comparable life stages to those of *Ixodes uriae* (Lee & Baust 1987), a species which has a circumpolar distribution in both hemispheres, parasitizes seabirds and experiences subzero temperatures during any month of the year. It is thus strongly suggestive that both argasid and ixodid

species have a similar, and always rather high supercooling capacity indicating common inherent physiological traits. The lower SCP of eggs and larvae compared to the postlarval stages of *A. walkerae* corresponds to that of other tick species such as *I. ricinus* and *Argas reflexus* (Dautel & Knülle 1997), *Amblyomma americanum* (Needham, Jaworski, Chen & Lee 1996) or *I. uriae* (Lee & Baust 1987) and can probably be explained in that the smaller tick stages supercool to lower temperatures than larger ones (Needham, Jaworski, Chen & Lee 1996). This is consistent with the lower SCPs of unfed adult *A. walkerae* ticks dehydrated to 50 % or 75 % of their critical water mass compared with fully hydrated ticks, because freezing is directly proportional to the volume of body water. The significantly lower SCPs in unfed adult ticks acclimated with several weeks exposure to 0 °C or to 38 °C compared with ticks constantly kept at 28 °C are difficult to explain and are in contrast to other species like *I. uriae* (Lee & Baust 1987), *A. americanum* (Needham, Jaworski, Chen & Lee 1996) and *I. ricinus* and *A. reflexus* (Dautel & Knülle 1997). Cold acclimation at 5 °C for 7 days of *A. americanum* had no influence on the SCP of various stages. None of the postembryonic stages of *A. reflexus* and *I. ricinus* reflected any seasonal fluctuation of the SCP in Central Europe and the SCPs of engorged immobile nymphs of *I. uriae* acclimated to various temperatures between −12 °C to 25 °C remained essentially constant. In conclusion, apparently the SCP has no predictive value for ticks in an ecological context, but only represents the lower temperature limit of survival.

REFERENCES

BEELITZ, P. & GOTHE, R. 1991. Investigations on the host seeking and finding of *Argas (Persicargas) walkerae* (Ixodoidea: Argasidae). *Parasitology Research*, 77:622–628.

BURKS, C.S., STEWART, JR., R.L., NEEDHAM, G.R. & LEE, JR., R.E. 1996. The role of direct chilling injury and inoculative freezing in cold tolerance of *Amblyomma americanum, Dermacentor variabilis* and *Ixodes scapularis*. *Physiological Entomology*, 21:44–50.

COLBO, M.H. 1973. Ticks of Zambian wild animals: a preliminary checklist. *The Puku*, 7:97–105.

DAUTEL, H. & KNÜLLE, W. 1996. The supercooling ability of ticks (Acari, Ixodoidea). *Journal of Comparative Physiology* B, 166: 517–524.

DAUTEL, H. & KNÜLLE, W. 1997. Cold hardiness, supercooling ability and causes of low-temperature mortality in the soft tick, *Argas reflexus*, and the hard tick, *Ixodes ricinus* (Acari: Ixodoidea) from Central Europe. *Journal of Insect Physiology* 43: 843–854.

EASTWOOD, E.B. 1971. A brief review of the genus *Argas* (Ixodoidea: Argasidea) in southern Africa. *Journal of the South African Veterinary and Medical Association*, 42:333–336.

GOTHE, R. 1992a. *Aegyptianella*: an appraisal of species, systematics, avian hosts, distribution, and developmental biology in vertebrates and vectors and epidemiology. *Advances in Disease Vector Research*, 9:67–100.

GOTHE, R. 1992b. Argasid ticks as vectors of pathogens. *First international conference on tick-borne pathogens at the host-vector interface: an agenda for research, proceedings and abstracts*, page 33–36. University of Minnesota College of Agriculture, Department of Entomology, and Minnesota Extension Service. Saint Paul, Minnesota, USA.

GOTHE, R. 1999. Tick toxicoses. *Hieronymus Munich*.

GOTHE, R., KOCH, B. & LEUTERER, G. 1989. Zur Sensitivität von *Argas (Persicargas) walkerae* auf optische Strahlung sowie zur Lokalisation und Organisation lichtperzipierender und durchlässiger Areale des Integumentes. *Journal of Veterinary Medicine Series B*, 36:303–313.

GOTHE, R. & SCHRECKE, W. 1972. Zur epizootiologischen Bedeutung von *Persicargas*-Zecken der Hühner in Transvaal. *Berliner und Münchener Tierärztliche Wochenschrift*, 85:9–11.

GOTHE, R. & VERHALEN, K.-H. 1975. Zur Paralyse-induzierenden Kapazität verschiedener *Persicargas*-Arten und -Populationen bei Hühnern. *Zentralblatt für Veterinärmedizin*, Reihe B, 22:98–112.

KAHL, O. & ALIDOUSTI, I. 1997. Bodies of liquid water as a source of water gain for *Ixodes ricinus* ticks (Acari: Ixodidae). *Experimental and Applied Acarology*, 21:731–746.

KAISER, M.N. & HOOGSTRAAL, H. 1969. The subgenus *Persicargas* (Ixodoidea, Argasidae, *Argas*). 7. *A.(P.) walkerae*, new species, a parasite of domestic fowl in southern Africa. *Annals of the Entomological Society of America*, 62:885–890.

LEE, JR., R.E. & BAUST, J.G. 1987. Cold-hardiness in the Antarctic tick, *Ixodes uriae*. *Physiological Zoology*, 60:499–506.

NEEDHAM, G.R., JAWORSKI, D.C., CHEN, C.-P. & LEE, JR, R.E. 1996. Cold-hardiness of a laboratory colony of Lone Star ticks (Acari: Ixodidae). *Journal of Medical Entomology*, 33:706–710.

NORVAL, R.A.I., SHORT, N.J. & CHISHOLM, M. 1985. The ticks of Zimbabwe. XIII. The distribution and ecology of *Argas walkerae*. *Zimbabwe Veterinary Journal*, 16:44–53.

PFEIFER, R. 1990. Zur Ökologie von *Argas (Persicargas) walkerae* Kaiser und Hoogstraal, 1969. *Inaugural-Dissertation der Tierärztlichen Fakultät der Ludwig-Maximilians-Universität München*.

SPLISTESER, H. & TYRON, U. 1986. Untersuchungen zu faunistischen Besonderheiten und zur Aktivität von *Dermacentor nuttalli* in der Mongolischen Volksrepublik. *Monatshefe für Veterinärmedizin*, 41:126–128.

STREY, O.F., TEEL, P.D., LONGNECKER, M.T. & NEEDHAM, G.R. 1996. Survival and water-balance characteristics of unfed adult *Amblyomma cajennense* (Acari: Ixodidae). *Journal of Medical Entomology*, 33:63–73.