

Moist habitats are essential for adults of the Antarctic midge, *Belgica antarctica* (Diptera: Chironomidae), to avoid dehydration

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Abstract. Desiccation resistance of adult males and females of the midge, *Belgica antarctica* (Diptera: Chironomidae) was evaluated to determine how this short-lived stage maintains water balance in the dry Antarctic environment. Both sexes had slightly lower water content ($\approx 60\%$) and a higher dehydration tolerance ($>30\%$ water loss) than most other insects. Water loss rates were high and increased rapidly at temperatures above 15°C , indicating that the adult midges are more hygroic than many other polar terrestrial arthropods. Water gain was accomplished by free water uptake with minimal or no contribution from absorption of water vapor or metabolic water production. Parameters related to water balance did not differ among populations from different islands. Overall, the high water requirements of the adult midge appear to be a significant challenge and presumably dictate that the adult midges must emerge during the brief period when free water is readily available and seek protected microhabitats that facilitate water retention.

INTRODUCTION

The midge, *Belgica antarctica*, is the southernmost insect and has been touted as the largest permanent, free-living terrestrial animal in Antarctica. It is locally abundant on the west coast of the Antarctic Peninsula and the South Shetland Islands where clusters of larvae can number in the thousands (Sugg et al., 1983). Only two other dipterans, also midges, exist in sub-Antarctic regions: *Parochlus steinenii* is limited to Tierra del Fuego in South America along with several islands north of Antarctica and *Eretmoptera murphyi* has recently been introduced accidentally to a northern region of the Antarctic Peninsula (Convey & Block, 1996). The life cycle of *B. antarctica* has been thoroughly detailed and is highlighted by a two-year period of development (Usher & Edwards, 1984), similar to that observed for both *P. steinenii* and *E. murphyi* (Convey & Block, 1996). Wingless adults emerge during the summer after a brief pupal period and mate within aggregations. The adults, which live less than two weeks, are considerably less tolerant of high temperatures than larvae (Peckham, 1979; Rinehart et al., 2006), thus suggesting that adult temperature tolerance, which is linked to the insect's water requirements, is an important parameter affecting survival in this challenging environment (Baust & Lee, 1987).

For an insect to function effectively water loss must be countered by water gain to maintain adequate internal water pools (Hadley, 1994). Water loss occurs by three major paths: cuticular loss, respiration and defecation. The small size of *B. antarctica*, with the accompanying high surface to volume ratio, exacerbates cuticular water flux. Uptake of water by vapor absorption and ingestion of liquid water directly or in the food are the two most common mechanisms to replenish water stores (Edney,

1977; Hadley, 1994). Less commonly water may be obtained from the metabolism of lipids (Hadley, 1985). Generally, arthropods with low rates of water loss maximize water retention as an adaptation for living in xeric environments, while rapid loss usually indicates a hydrophilic arthropod (Edney, 1977; Hadley, 1994; Benoit et al., 2005). For Antarctic terrestrial arthropods, maintaining water balance is a significant environmental driver due to long absences of free water and the prevalence of desiccating humidities (Baust & Lee, 1987). In these experiments we examined how adults of *B. antarctica* cope with the desiccating Antarctic environment. We did so by documenting water loss rates, minimum water content required for survival, clustering as a water-conserving mechanism, and by assessing potential routes for water uptake. Additionally, populations from different islands were compared to determine if this midge retains a general species- or island-specific water balance profile.

METHODS

Insects and experimental conditions

Belgica antarctica adults were field-collected in January 2006 on Torgersen, Cormorant and Norsal Point Islands near Palmer Station (Anvers Island, Antarctica; $64^{\circ}46'S$, $64^{\circ}04'W$). Exact ages of the adults were not known, but considering their short life span and highly synchronized emergence (Sugg et al., 1983; Usher & Edwards, 1984), they were likely to be similar in age. Once collected, the adults were held at 98% RH, 4°C and 20L : 4D for 1 d to eliminate the effects of digestion, secretion and defecation on mass changes (Wharton, 1985).

Midges were weighed individually using an electrobalance (CAHN 25, Ventron Co., Cerritos, CA; precision $0.2\text{ }\mu\text{g}$ SD and accuracy of $6\text{ }\mu\text{g}$ at 1 mg). Relative humidities (RH) were generated in glass desiccators with saturated salt solutions (98% RH with K_2SO_4 , 93% with KNO_3 , and 75% with NaCl ; Winston & Bates, 1960), double-distilled water (100% RH) or dry calcium

TABLE 1. Comparison of the water balance characteristics of male (M) and female (F) adult midges, *Belgica antarctica*, from three islands near Palmer Station, Anvers Island, Antarctica at 4°C. Blue coloration in the gut of the midges is denoted by + for free water drinking. CEH is the critical equilibrium humidity. Each point represents the mean \pm SE of 45 midges.

Characteristics	Islands near Palmer Station, Antarctica					
	Torgerson		Cormorant		Norsal Point	
	M	F	M	F	M	F
Water content						
Initial mass (mg)	0.31 \pm 0.01	0.69 \pm 0.01	0.31 \pm 0.01	0.60 \pm 0.01	0.31 \pm 0.02	0.68 \pm 0.02
Dry mass (mg)	0.11 \pm 0.01	0.27 \pm 0.01	0.11 \pm 0.02	0.23 \pm 0.01	0.11 \pm 0.01	0.26 \pm 0.01
Water mass (mg)	0.20 \pm 0.01	0.42 \pm 0.20	0.20 \pm 0.02	0.37 \pm 0.02	0.20 \pm 0.02	0.42 \pm 0.01
Body water (%)	64.5 \pm 1.1	60.9 \pm 1.0	64.5 \pm 1.2	61.7 \pm 1.3	64.5 \pm 0.9	61.7 \pm 0.8
Water loss						
Water loss rate (%/h)						
Individual	7.09 \pm 0.09	3.42 \pm 0.10	7.10 \pm 0.09	3.64 \pm 0.08	7.04 \pm 0.07	3.40 \pm 0.08
Group of 5	6.90 \pm 0.07	3.35 \pm 0.07	7.00 \pm 0.08	3.52 \pm 0.06	7.02 \pm 0.08	3.30 \pm 0.09
Group of 10	6.70 \pm 0.06	3.21 \pm 0.06	6.90 \pm 0.07	3.29 \pm 0.08	6.81 \pm 0.06	3.20 \pm 0.10
Group of 20	6.13 \pm 0.07	2.90 \pm 0.05	6.12 \pm 0.21	3.05 \pm 0.09	6.23 \pm 0.08	3.00 \pm 0.11
Dehydration tolerance (%)	34.4 \pm 1.0	32.4 \pm 1.1	34.1 \pm 0.9	32.2 \pm 0.9	33.9 \pm 1.0	32.1 \pm 1.2
Survivorship at 0%RH (h)	5.4 \pm 1.0	9.7 \pm 0.8	5.2 \pm 0.6	10.2 \pm 1.1	5.0 \pm 1.3	9.5 \pm 1.5
Water gain						
Free water uptake	+	+	+	+	+	+
CEH (RH)	100	100	100	100	100	100

sulfate (0% RH). In all cases observations were conducted at 4°C for ecological relevance or 25°C for comparison to other water balance studies (Wharton, 1985; Hadley, 1994) unless otherwise noted. During testing, the midges were housed in individual mesh-covered cages and were removed from the experimental conditions only for weighing, a procedure accomplished in less than 1 min. To measure individuals within a group, a spot of paint (Pactra, Van Nuys, CA) was placed on the abdomen of the midge; this had no effect on water loss or uptake and did not appear to be detrimental to the midge in any manner.

Water balance profile

The difference between the initial mass (i) and the dry mass (d; exposure to 0% RH, 65°C until constant mass) was calculated to find the water mass (m) that was also expressed as percent water content (m/i; Wharton, 1985). Hourly measurements at 0% RH were used to determine the water loss rate (cuticular plus respiratory water loss) by fitting the measurements to Wharton's (1985) model for exponential water loss. Water vapor absorption and drinking of liquid water were evaluated as mechanisms to replenish internal water pools (Hadley, 1994; Benoit et al., 2005). Water gain from the atmosphere was tested by monitoring (every 8 h) mass changes of individuals at different relative humidities. Maintenance of water mass at a subsaturated relative humidity is evidence of water vapor absorption (Wharton, 1985; Hadley, 1994). Imbibing of liquid water was examined by storing groups of 5 and 10 midges with droplets of Evans blue stained water (10%) and observing direct contact with the fluid. After 24 h, the midges were dissected to verify the presence of blue dye in the gut. In all experiments, both males and females were tested and characteristics were compared by analysis of variance (ANOVA) with arcsin transformation in the cases of percentages or by testing for equality of slopes (Sokal & Rohlf, 1981). Tukey test was used for pairwise post-hoc comparisons. Each experiment was replicated 3 times with 15 individuals.

Humidity attraction assay

Humidity preferences were assessed according to protocol established in Hayward et al. (2003) with modifications. To allow midges the choice between two different relative humidities,

a two-choice assay system was constructed. In each experiment, a single midge was inserted into a Y-tube with materials to generate each RH 5 cm from the junction. Groups were never tested due to possible chemical attraction between individuals. After 30 min, 1 h and 2 h, the location of the midge was recorded. This was replicated 5 times with 10 individuals. Conditions tested were 100 vs. 93% RH, 93 vs. 75% RH, 75 vs. 33% RH and 100 vs. 33% RH. Chi-square tests were used to determine if the distribution of the midges varied between relative humidities.

RESULTS

Water pool

Percentage water contents of adult midges from each collecting site were not statistically different (Table 1). Based on comparisons of dry mass, females were 2–3 \times larger and contained relatively less water than males (ANOVA/Tukey, $p < 0.05$). No differences in the water pool were noted for individuals tested at different temperatures (ANOVA; $p > 0.05$; data not shown). The only noticeable variation between island populations was the size of female midges from Cormorant Island (ANOVA/Tukey, $p < 0.05$); these females were consistently 5–10% smaller than individuals from the other two islands (Table 1). In all cases, the water mass and dry mass of individuals positively correlated ($r^2 = 0.96$), indicating that water flux was standardized to size in this study.

Water loss

Female and male midges lost water at a rate of 3–4 and 7–8%/h, respectively, when housed individually (Fig. 1, Table 1). This rate agrees with the ability of females to tolerate approximately 32% loss of water over a 10 h period and the male's ability to prevent desiccation-induced mortality until reaching a 34% loss of water after 5 h. No difference was noted in dry mass determined

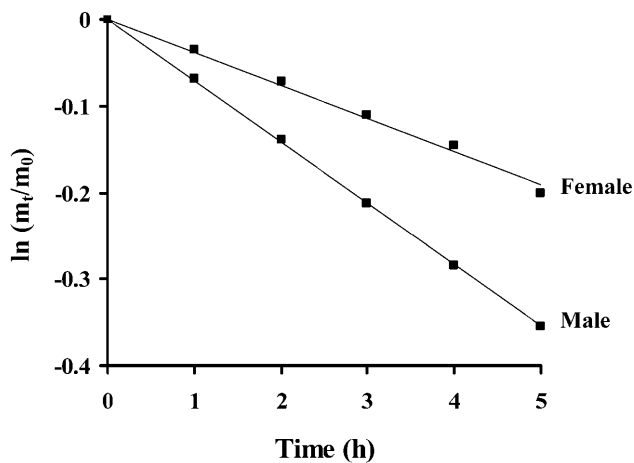


Fig. 1. Net water loss by males and females of *Belgica antarctica* at 0% RH (cuticular plus respiratory water loss) and 4°C. The rate of water loss is the slope of the regression through the plot. m_t represents the mass at any time t and m_0 is the initial mass. Data represents the mean for measurements from 45 individuals.

before and after desiccation, indicating that water was not likely created as a by-product of metabolism (Wharton, 1985; Benoit et al., 2005). The effect of temperature on water loss rates was determined by weighing the midges at different temperatures, and these data were also used to identify when water loss from the midges began to increase rapidly with temperature. For both males and females, this point was reached between 15 and 20°C (Fig. 2). Grouping had no effect on dehydration tolerance, water content or dry mass of male and female midges (data not shown) and had only a slight effect, <15%, on water loss, with the highest suppression occurring in groups of twenty (Table 1).

Water gain

Males and females lost water at all relative humidities except at water saturation (100% RH) when tested at both

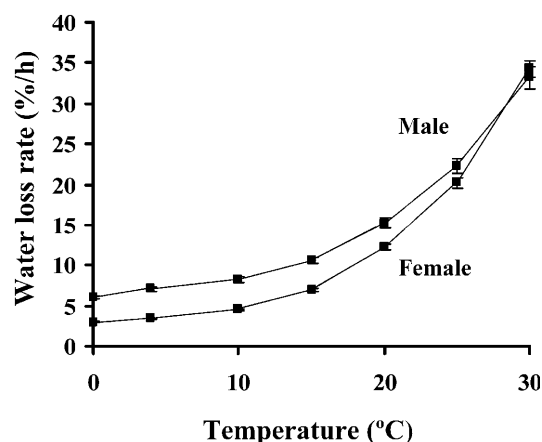


Fig. 2. Water loss rates of males and females of *Belgica antarctica* as a function of temperature. Each point represents the rate acquired according to Fig. 1 at the corresponding temperature.

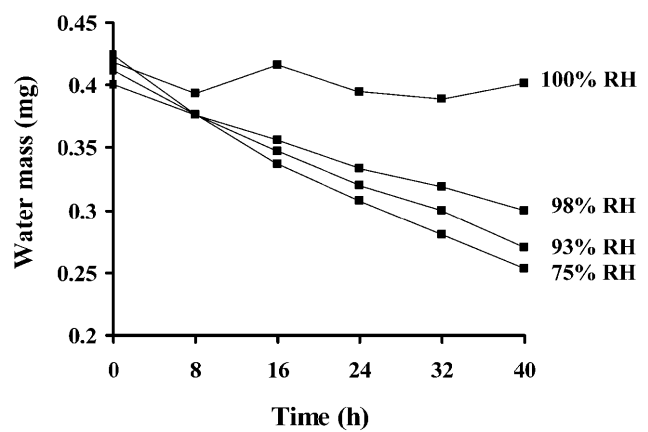


Fig. 3. Water mass changes of adult females of *Belgica antarctica* during exposure to different relative humidities (% RH). Maintenance of water mass occurs only at saturation (100% RH). The plot for males is nearly identical, but shifted downward as a result of their lower water mass (data not shown). Each point is the mean for 45 individuals.

4 (Fig. 3) and 25°C (data not shown), indicating their inability to maintain water content in subsaturated air. Additionally, this was verified by predessicating groups of both sexes at 85% RH for 15 h before exposure to 98%

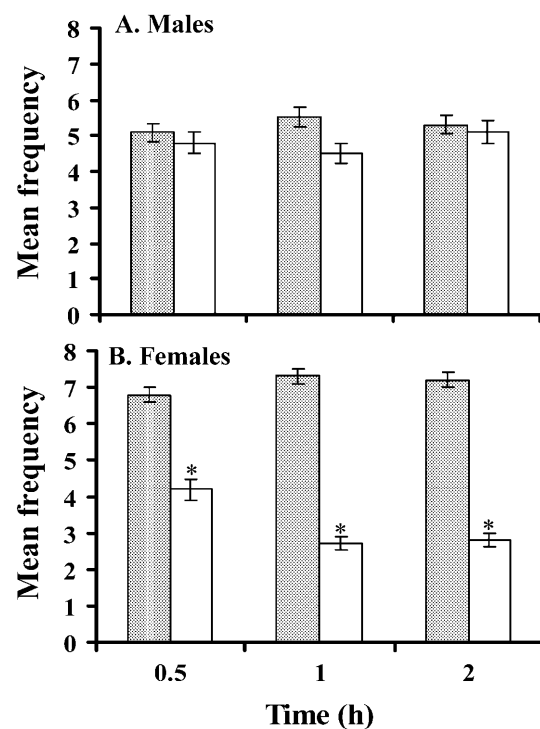


Fig. 4. Distribution of *Belgica antarctica* males (A) and females (B) during a choice assay between 100% RH (light gray) and 33% RH (white) at 4°C, recorded at different time intervals after the beginning of the assay. Measurements at 25°C yielded identical results and comparison between subsaturated relative humidities yielded no differences (data not shown). Each bar represents the mean \pm S.E. of 10 individuals replicated 5 times. * indicates significant differences within each couplet, χ^2 test, $p < 0.05$.

RH. As before, no increase in mass occurred, confirming that this midge cannot use water vapor as a source for water replenishment. Liquid water intake, confirmed by the presence of blue coloration in the digestive tract, was not observed in either males or females collected from the field and offered blue water (98% RH for 24 h at 4°C), but predesiccation (75% RH for 24 h at 4°C) in the laboratory caused both sexes to uptake liquid water after encountering the water by random movement.

Humidity attraction

During the test for water drinking, the midges showed no deliberate movement toward the water droplets, suggesting that there is no attraction to water vapor. For males this was also the result noted in the humidity preference experiments, regardless of temperature (Fig 4a; $\chi^2 = 5.4$, $P > 0.05$, d.f. = 3). Females, on the other hand, were slightly attracted to saturated conditions (Fig 4b; $\chi^2 = 18.4$, $P < 0.05$, d.f. = 3) but showed no differences when other subsaturated relative humidities were tested (data not shown; $\chi^2 = 6.0$, $P > 0.05$, d.f. = 3). This suggests the possibility that females seek areas of high relative humidity, possibly as a site for oviposition.

DISCUSSION

Body water content of most adult insects is approximately 70% (Hadley, 1994), but the values observed in both males (65%) and females (60%) of *B. antarctica* were lower by comparison (Table 1). Low levels of body water are usually associated with storage of large amounts of fats or a heavily water-proofed cuticle, but for the midges both are unlikely. With its short lifespan, excess lipid reserves, beyond what is needed by the females for egg maturation (Convey, 1992), are unnecessary and the midge's high rate of water loss suggests a thin cuticle with modest water proofing. Additionally, the dehydration tolerance of the adult midge (>30%) was slightly higher than the 20–30% loss tolerated by most terrestrial insects (Hadley, 1994), but was not nearly as high as the dehydration tolerance of larval chironomids such as *Chironomus* sp. that can tolerate the loss of 60–80% of their water stores (Suemoto et al., 2004) and *Polypedilum vanderplanki*, an African species that tolerates nearly complete desiccation (Kikawada et al., 2005). Few experiments have been conducted on adult dipteran species, but comparisons with *Drosophila* sp. from diverse habitats (Gibbs, 2002) show content (65–75%) and dehydration tolerance (25–35%) that are similar to our observations on *B. antarctica*. Even though *B. antarctica* contains less body water than other terrestrial arthropods, it may have the same relative amount of water available for exchange with the environment as a result of its high tolerance of dehydration, as noted for several other species that have low water content (Arlian & Eckstrand, 1975; Hadley, 1994).

Habitat suitability is related to moisture requirements and is best assessed using water loss rates (Wharton, 1985; Hadley, 1994). In general, arthropods that reside in dry environments have suppressed water loss rates, while those living in areas where water is easily accessible have

high rates of water loss (Hadley, 1994). The high loss rates for both males and females of *B. antarctica* (Fig. 1) suggest no special adaptations such as a thick cuticle, excess cuticular lipids or a lower metabolic rate to reduce water loss, and indicate that moist microhabitats are critical for this stage. In relation to other terrestrial polar arthropods, the relatively high water loss rate categorizes the adult midge with other hygric taxa such as collembolans and other larval midges, in contrast to mesic-adapted species such as the Antarctic oribatid mite, *Alaskozetes antarcticus* and the sub-Antarctic beetle, *Hydromedion sparsutum* (Worland & Block, 2003). The point where water loss began to increase exponentially with temperature was slightly higher than this point in other hygric polar arthropods but was significantly lower than is observed in most mesic arthropods (Worland & Block, 2003). This higher temperature for increasing water loss in relation to other polar arthropods, is presumably an adaptation for adult emergence during the summer and the midge's activity on the surface of the warm rocky substrate. Since this midge mates in aggregations (Sugg et al., 1983) it seemed possible that aggregations might facilitate water conservation as it does in some other species (Yoder et al., 1992), but suppression of water loss was minimal in clusters (Table 1). By comparison to previous studies of loosely-clustering species (Benoit et al., 2005), the small difference observed is not likely sufficient to prevent dehydration, especially when other environment factors such as air flow are considered, thus we suggest that the clustering effect we observed may be a laboratory artifact that has little ecological relevance. Based on our results, the adult stage of this midge is not well-adapted for a xeric habitat, thus adult emergence must occur when free water is available. Larvae reside in areas with high moisture content, thus the wingless adult are usually close to a water source.

Drinking liquid water was the primary mechanism used by the adult midges to maintain their internal water pools. No water vapor absorption occurred; neither males nor females could maintain water levels at any humidity below saturation (Fig. 3). Most arthropods cannot absorb water vapor, but rely on drinking as their primary water source (Hadley, 1994). Metabolic water production was not documented in this study but may occur at low levels that contribute only minimally (less than 0.5% of the water mass) to the water pool (Arlian, 1979).

With slight differences in chromosome variability (Atchley & Hilburn, 1979), morphology (Atchley & Davis, 1979) and concentrations of cryoprotectants (Baust & Lee, 1983) in populations of *B. antarctica* from different islands, the possibility that the water requirements differed from island to island seemed plausible. This was not the case; little variability was noted in the water balance profiles of midges from different islands (Table 1). Wharton (1985) originally hypothesized that water balance requirements of arthropods are species-specific, and if differences occur between two localities he suggested that different species or subspecies may be involved. Recent observations with other arthropods sup-

port this idea (Yoder & Benoit, 2003). Based on Wharton's hypothesis, the similarities we observed with *B. antarctica* collected from three different islands suggest that all midges are populations of the same species. The females collected on Cormorant Island were smaller than the others, but the nearly identical water content found in individuals from the different islands suggests that the difference is the result of less favorable conditions prevailing on Cormorant Island during larval development.

The temperature and dehydration vulnerability of midge adults suggest that slight increases in summer temperatures would have negative effects on adult survival. Summer temperatures on the surface of the substrate may rise to $>20^{\circ}\text{C}$, and water loss rates of adult midges at this temperature are extremely high, causing death unless areas with high moisture levels and free water are located. Interestingly, long-term climate changes may be detrimental, particularly increases in temperature, unless *B. antarctica* can adapt to reduce heat-accelerated water loss (Convey et al., 2002). We suspect that a major adaptation used by the adults to prevent desiccation is a behavioral response in which the midge seeks a microhabitat with high levels of moisture and access to free water. At the locality we examined near Palmer Station, peak adult emergence occurred in early to mid-January, a time when the environment is moist and free water is readily available. Though only the females have the ability to discriminate based on relative humidity, it is likely that temperature and other environmental factors contribute to habitat selection. Males are positively phototrophic, but females lack this response (Peckham, 1971; Convey & Block, 1996), suggesting that females are more likely to be found in darker microhabitats that are likely to be more humid and more suitable for oviposition. Thus, the adult phase of the life cycle exploits the optimal water environment of the austral summer, and females will tend to move to a humid environment.

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