Responses to temperature and relative humidity in the harvestman *Vonones ornatus* (Say) (Arachnida, Opiliones, Cosmetidae)

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Summary

Experiments were conducted to determine fecundity, and to assess the effects of relative humidity (RH) on lower (LLT_{50}) and upper (ULT_{50}) lethal temperatures and critical thermal minima (CTM_{min}) and maxima (CTM_{max}), and the effect of temperature on survival time in adults of the harvestman *Vonones ornatus* under controlled laboratory conditions. The number of eggs deposited per female ranged from 27–66 (mean 46.2 ± 6.1 SE). Females deposited their eggs within a 7-h period, between 2130 and 0430 h, in several small clusters. The number of clusters per female ranged from 1–4 (mean 2.1 ± 0.2 SE); number of eggs per cluster ranged from 7–32 (mean 15.6 ± 5.8). Females deposited eggs between 12 and 28 July. LLT_{50} and ULT_{50} values ranged from 1.73–2.25°C, and 37.04–39.45°C, respectively, with no significant differences between males and females. RH had a significant effect on lower and upper lethal temperatures for both sexes. Under mesic conditions (70% RH), adults exhibited an ability to withstand significantly lower and higher ambient temperatures. CTM_{min} and CTM_{max} temperatures for males and females ranged from 2.33–2.69°C, and 35.31–37.23°C, respectively. As with lethal temperatures, CTM_{min} and CTM_{max} values were significantly lower and higher, respectively, under mesic conditions, and there was no significant difference between the sexes at either condition of RH. Harvestmen maintained at 23 ± 0.2°C, 69–71% RH, and 12L:12D photoperiod, attained sexual maturity after 7 nymphal instars. Longevity for adults maintained at 30°C and 70° RH ranged from 78.8–83.4 days. Longevity was reduced significantly for adults tested at 10°C and 70% RH (10.3–13.1 days). Survival times were significantly reduced when animals were exposed to xeric conditions as compared with mesic conditions at both test temperatures.

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Introduction

There are many terrestrial arthropods for which little information is available on their natural history, ecology and behaviour (Punzo, 2000). This is especially true for arachnids known as harvestmen (Arachnida, Opiliones) (MacKay *et al.*, 1992). In fact, detailed field and laboratory studies exist for relatively few species of opilionids (Corey & Taylor, 1987; Elpino-Campos *et al.*, 2001; Hara & Gnaspini, 2003).

Many species of opilionids inhabit mesic habitats in forests, grasslands and caves, where they are commonly found under or within decaying logs, on the surface or within bark crevices on tree trunks, and under leaf litter (Rodriguez & Guerrero, 1976; Edgar, 1990).

The harvestman *Vonones ornatus* (Say) (Opiliones: Cosmetidae) occurs throughout central and southern Florida where it has been collected from several vegetative zones associated with pond pine, sand scrub, and flatwood communities (Corey & Taylor, 1987). As there are few detailed studies on the physiological ecology of opilionids (Edgar, 1971; Hadley, 1994; Punzo, 2000), this study was conducted to determine fecundity, and to assess the effects of relative humidity (RH) on lower and upper lethal temperatures and critical thermal minima and maxima, and the effect of temperature on survival time in adults of *V. ornatus* under controlled laboratory conditions.

Material and methods

Experimental animals and husbandry

All harvestmen used in this study, except those used to determine fecundity, consisted of third- and fourth-generation individuals which were captive-bred from adults originally collected in 2001–2002. These adults were collected in Lettuce Lake Park (LLP) in Tampa, Florida (Hillsborough County, USA). Voucher specimens have been deposited in the Invertebrate Collection at the University of Tampa. LLP consists of over 400 ha and is bordered on the west by the Hillsborough River, with adjoining southern hardwood forest and moist hammocks.

Harvestmen were housed in plastic cages ($40 \times 40 \times 30$ cm) and provided with water *ad libitum* by placing two water-soaked cotton balls in a glass dish at one end of the cage. Cages were housed in a climate-controlled room ($23 \pm 0.2°C$; 69–71% RH; 12L:12D photo-period regime). Each cage contained 10 adult males and 10 females, and cage floors were covered with a substrate consisting of a mixed layer of sphagnum moss and vermiculite. Cages were cleaned with soap and water on a weekly basis and provided with fresh sub-strate. A 20-cm long piece of semicircular-shaped synthetic bark strip (Bush Herpetological Supply, Neodosha, Kansas, USA) was placed at the end of the cage opposite the water dish, to provide a refuge for the harvestmen.

Although the natural diet of this species is not known, adults and subadults were fed *ad libitum* on a diet consisting of vestigial-winged fruitflies (*Drosophila melanogaster*), minced earthworms (*Lumbricus terres-tris*) and crickets (*Gryllus* spp.), and mashed sweet potato. Younger nymphal stages and hatchlings were fed a diet consisting of first-instar crickets (*Gryllus* spp.) and mealworm larvae (*Tenebrio molitor*), newly emerged spiderlings (*Pardosa* spp. and *Oxyopes salticus*), minced earthworms, and mashed banana. Food was always put in a circular food dish (2.0 cm deep; 5 cm in diameter) placed in the open area of the cages. Maintenance and breeding of *V. ornatus* in captivity have been successful under these rearing conditions (Punzo, unpubl. data).

Measurements of total body length and width were made to the nearest 0.1 mm using a Unitron Model 1100 dissecting microscope fitted with an ocular micrometer.

Determination of fecundity

All experiments described in this paper were con-ducted between 7 July 2004 and 17 August 2005. Fecundity was determined from females collected in their natural habitat and brought into the laboratory. Between 25 June and 5 July 2004, 264 adult females were collected in LLP, brought back to the laboratory, and housed individually in identical plastic cages ($17 \times 17 \times 15$ cm). Cages were provided with the same substrate as described above, and the females were maintained on the same diet and under identical climatic conditions as described previously for adults. A cube-shaped opaque chamber (7 cm per side), containing a small circular opening (1.5 cm in diameter), was placed in one corner of each cage. The opening of the chamber faced the centre of the cage. The floor of this chamber was provided with moistened blotting paper which pro-vided a substrate on which females could deposit their eggs. Previous pilot studies had shown that females of *V. ornatus* always deposited their eggs in these darkened, moist chambers rather than on the open floor of the cage (Punzo, pers. obs.). The blotting paper was checked daily for the presence of eggs. If no eggs were present, the previous paper was removed and replaced with fresh, moistened blotting paper. If eggs were present, they were removed and counted, and fresh blotting paper was placed in the opaque chamber. The number of eggs deposited was recorded for each female and used as an index of fecundity.

Determination of lower and upper lethal temperatures and critical thermal minima and maxima

Harvestmen used in all experiments on thermal toler-ances were 6–7 month old adults (from the day of emergence from eggs). Experiments for the determi-nation of lower (LLT_{50}) and upper (ULT_{50}) lethal temperatures were conducted following a modification of the protocol described by Punzo & Rosen (1984), using Percival Model 816 environmental chambers (Boone, Iowa, USA). The door of each chamber con-tained a one-way window which allowed observation of subjects within the chambers. To summarise, 3 replicates were run at RH values of 12% (xeric conditions) and 70% (mesic). Each replicate consisted of 10 males and 10 females subjected to each test temperature. Subjects were tested individually in test chambers consisting of 50-ml vials (Central Scientific, Chicago, Illinois, USA) whose openings were covered with cheese cloth to allow circulation of air. Food (0.5 g of minced earthworms soaked with water) was placed at the bottom of each vial. For LLT_{50} experiments, different subjects were exposed to low temperatures ranging from 1–5°C at 0.5°C intervals. Subjects were monitored at 1-h intervals and time of death was recorded. A subject was con-sidered dead if it was found on its side or dorsal surface and failed to respond to gentle prodding with a glass stirring rod. LLT_{50}s were determined using standard probit analysis (Finney, 1971). Identical procedures were used to determine ULT_{50}s, with the exception that subjects were exposed to high temperatures ranging from 30–55°C, at 12 and 70% RH.

The protocol described by Punzo & Jellies (1983) was used to determine critical thermal minima (CTM_{min}) and maxima (CTM_{max}). To summarise, CTM_{min} and CTM_{max} were defined as the low and high temperature, respectively, at which loss of the righting reflex occurred. For CTM_{min} determinations, environmental chambers were programmed to start at 10.0°C and decrease to $-2.0°C$ at 1°C/min. For CTM_{max} determinations, chambers were programmed to start at 30°C and increase to 60°C at 1°C/min. For both CTM_{min} and CTM_{max} experiments, different groups of subjects were tested under conditions of 12 and 70% RH. We conducted four replicates at each RH, each replicate consisting of 20 males and 20 females. In each replicate, subjects were placed individually in 50-ml condiment cups with a water-soaked cotton ball placed at the bottom of each cup to provide a saturated atmo-sphere. Subjects were monitored continuously until they collapsed (knock-down or torpor point).

Effect of temperature and RH on survival time (longevity)

Adult males and females were obtained from laboratory-bred cultures as described previously. In

these experiments, testing began on the day immediately following the animal's moult to the adult stage. In this species, adults can be readily sexed as males have markedly longer palpi than females, with a distinctive spur on the femur. Based on results obtained for lethal temperature determinations, the following stressful (but non-lethal) ambient test temperatures were chosen: 10°C and 30°C. Subjects were exposed to these temperatures under xeric (12% RH) and mesic (70% RH) conditions, in environmental chambers identical to those used in the experiments on upper and lower lethal temperatures. Animals were placed individually in 50-ml glass vials and were monitored daily to determine mean survival time in days as a function of temperature and RH. Subjects were considered dead when they were found lying motionless on the bottom of the vial and did not respond when prodded with a glass stirring rod after a period of 24 h.

Statistical analysis

All statistical tests followed procedures as discussed by Sokal & Rohlf (1995). A Bartlett's test for homogeneity of variances was performed on all data and indicated that parametric tests were appropriate because assumptions of normality were met.

Results

Deposition of eggs and fecundity

All females that deposited eggs ($n=60$) were of similar size (body length: mean 5.5 mm $\pm$ 0.05 SE, range 5.3–5.7 mm; body width: mean 4.2 mm $\pm$ 0.03 SE, range 4.0–4.4). At 23 $\pm$ 0.2°C, 65–70% RH, and 12L:12D photoperiod regime, the number of eggs deposited per female ranged from 27–66 (mean 46.2 $\pm$ 6.1 SE). All females deposited all of their eggs within a 7-h period, between 2130 and 0430 h (Eastern Standard Time, EST), in several small clusters on the blotting paper. The number of clusters per female ranged from 1–4 (mean 2.1 $\pm$ 0.2 SE); the number of eggs per cluster ranged from 7–32 (mean 15.6 $\pm$ 5.8). Females deposited eggs between 12 and 28 July.

Lethal temperatures and critical thermal minima and maxima

Results for LT_{50} determinations are shown in Table 1. LLT_{50} and ULT_{50} values ranged from 1.73–2.25°C, and 37.04–39.45°C, respectively. No significant differences were found between males and females ($p>0.60$). However, RH had a significant effect on lower and upper lethal temperatures for both sexes. Under mesic conditions (70% RH), adults exhibited an ability to withstand significantly lower and higher ambient temperatures ($p<0.05$).

CTM_{min} and CTM_{max} values for males and females are also shown in Table 1 and ranged from 2.33–2.69, and 35.31–37.23°C, respectively. As with lethal temperatures, CTM_{min} and CTM_{max} values were significantly lower and higher, respectively ($p<0.05$), under mesic conditions, and there was no significant difference between the sexes at either condition of RH ($p>0.50$).

Survival time for adults

These harvestmen, maintained at 23 $\pm$ 0.2°C, 69–71% RH, and 12L:12D photoperiod, attained sexual maturity (adulthood) after 7 nymphal instars. The effects of ambient test temperature and RH on survival time for adults are shown in Table 2. Mean longevity for adult harvestmen maintained at 30°C and 70% RH ranged from 78.8–83.4 days. Longevity was reduced significantly for adults tested at 10°C and 70% RH (10.3–13.1 days). Survival times for both sexes were significantly reduced when animals were exposed to xeric conditions as compared with mesic conditions at both test temperatures ($p<0.01$). There was no significant difference in mean survival times between males and females at ambient temperatures of 10°C or 30°C under xeric or mesic conditions ($p>0.50$).

Discussion

One important aspect affecting the survival of terrestrial arthropods is the extent of their ability to resist desiccation by limiting evaporative water loss (EWL) (Edney, 1977; Hadley, 1994; Punzo, 2000). These ani-

	Lethal temperatures (°C)			
	LLT_{50}		**ULT_{50}**	
%RH	**Males**	**Females**	**Males**	**Females**
12	2.21 $\pm$ 0.05a	2.25 $\pm$ 0.03a	37.04 $\pm$ 0.42a	37.22 $\pm$ 0.39a
70	1.73 $\pm$ 0.03b	1.79 $\pm$ 0.02b	39.12 $\pm$ 0.47b	39.45 $\pm$ 0.22b
	Critical thermal minima (CTM_{min})		**Critical thermal maxima (CTM_{max})**	
	Males	**Females**	**Males**	**Females**
12	2.68 $\pm$ 0.03a	2.69 $\pm$ 0.02a	35.31 $\pm$ 0.19a	35.47 $\pm$ 0.29a
70	2.33 $\pm$ 0.04b	2.36 $\pm$ 0.03b	37.15 $\pm$ 0.27b	37.23 $\pm$ 0.33b

Table 1: Lower (LLT_{50}) and upper (ULT_{50}) lethal temperatures, and critical thermal minima and maxima, for adult males and females of the harvestman *Vonones ornatus* under xeric (12% relative humidity, RH) and mesic (70% RH) conditions. Data are expressed as means $\pm$ SE. Values in columns followed by different letters are significantly different ($p<0.05$). Level of significance computed using a Student t-test.

Survival time (days)
Ambient test temperature

%RH	10°C		30°C	
	Males	Females	Males	Females
12	2.6 ± 0.3a	3.1 ± 0.2a	33.7 ± 6.2c	36.5 ± 5.8c
70	10.3 ± 2.4b	13.1 ± 3.3b	78.8 ± 8.6d	83.4 ± 4.8d

Table 2:   Effects of ambient temperature and relative humidity (RH) on survival time (in days) of adults of *Vonones ornatus*. Data expressed as means ± SE. Values in rows and columns with different letters and symbols are significantly different (*p*<0.01) based on Student t-tests.

mals lose water through their cuticle and/or respiratory surfaces. Because ambient temperature and relative humidity both affect rates of EWL, terrestrial arthropods must respond effectively to gradients of temperature and moisture within their microhabitats in order to survive. It is well known that the integument represents a main avenue for EWL in arthropods, and the degree of cuticular permeability determines, to a large extent, the rate at which EWL occurs (Punzo, 1991a). Terrestrial arthropods utilise various behavioural mechanisms of thermoregulation to escape potentially stressful conditions (Cloudsley-Thompson, 1991; Punzo, 1998). For example, many terrestrial arthropods avoid high ambient temperatures by nocturnal activity patterns. Others simply move away from an area when unfavourable conditions manifest themselves. Some respond to harsh conditions by burrowing beneath the ground surface or seek refuge under leaf litter, logs, or rocks. Because opilionids typically have lightly sclerotised cuticles that are not particularly effective as barriers to EWL (Edney, 1977; Edgar, 1990), they are especially susceptible to desiccation.

The results of the present study show that survivorship and longevity of *V. ornatus* adults are significantly reduced when they are exposed to xeric conditions at both lower and higher temperatures. This is in agreement with previous observations that opilionids are typically found in microhabitats where moisture levels are high (Phillipson, 1959; Juberthie, 1965; Edgar, 1971, 1990; Rodriguez & Guerrero, 1976; MacKay *et al.*, 1992; Gnaspini, 1995). In a previous study, Corey & Taylor (1987) showed that several species of harvestmen from central Florida, including *V. ornatus*, were strongly nocturnal and most frequently found in moist microhabitats: under moist leaf litter, within moist decaying logs, and on the surface of leaves containing numerous water droplets following summer rains. This is also in line with results of the present study showing that adults of *V. ornatus* exhibited a greater resistance to low and high temperature stress under mesic conditions, and is in general agreement with previous studies on other terrestrial arthropods, including spiders (Pulz, 1987; Punzo, 1991b), insects (May, 1985; Nation, 2002), centipedes (Cloudsley-Thompson, 1991), and millipedes (Crawford, 1981, 1991; Punzo, 2000).

In species of harvestmen that inhabit desert regions characterised by high seasonal temperatures and low relative humidity, egg deposition most frequently occurs during rainy seasons (Crawford, 1981; Cokendolpher, 1990). Hunt (1991) reported that harvestmen from arid regions in Australia are strongly nocturnal in their diel periodicity and frequently aggregate near ephemeral pools of water and permanent sink holes. Although some species of neotropical harvestmen have been reported to form intra- or interspecific aggregations consisting of several to many individuals (Machado *et al.*, 2000; Elpino-Campos *et al.*, 2001: Pereira *et al.*, 2004), no instances of aggregation were observed for *V. ornatus* in central Florida.

A relatively detailed study of a Nearctic harvestman was conducted by MacKay & Grimsley (1992) on *Trachyrhinus marmoratus* Banks, a species found in New Mexico (northern Chihuahan Desert) in a variety of habitats including tobosa-black grama grasslands and desert shrub consisting predominantly of creosote-lechuguilla plant associations. Rainfall is typically <25 cm/year, and occurs primarily in late spring, late summer, and early autumn. Air temperatures range from <0°C in winter to over 40°C in late summer. Population densities peaked by late May, and then declined rapidly during the hottest months. Most adults mated in early May, before spring rains, and deposited their eggs most frequently within 36–72 h after spring rains. With the appearance of higher ambient temperatures in late May, surface activity declined sharply. Thus, even this desert species deposits its eggs in cooler, moister seasons. Although detailed information on the phenology of *V. ornatus* from Florida is lacking, preliminary observations indicate that, although juveniles are most abundant in mid-spring and adults in August (Punzo, pers. obs.), because of the high rainfall levels in the state harvestmen can be found throughout most months of the year.

In view of the paucity of information available on the physiological ecology of harvestmen, future studies should focus on the combined effects of temperature and RH on survivorship of embryos, rate of embryonic development, postembryonic growth rates, age at sexual maturity, fecundity, temperature preferenda, rate of cuticular water loss, metabolic rate, and longevity. Furthermore, these studies should include individuals from different taxa that are found in a diversity of microhabitats.

Acknowledgements

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