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ABSTRACT ✓

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Ecophysiological Adaptations to Dry Thermal Environments Measured in Two Unrestrained Namibian Scorpions, *Parabuthus villosus* (Buthidae) and *Opisthophthalmus flavescens* (Scorpionidae)

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ABSTRACT

The daily changes in body temperature experienced by *Parabuthus villosus* (Buthidae), a scorpion found on the gravel plains around Gobabeb, Namibia, and by *Opisthophthalmus flavescens* (Scorpionidae), a dune-dwelling species from the same area, were measured under similar field conditions. Thermocouples implanted under the segments of the mesosoma measured maximum temperatures as high as 43°C in the shade. Air temperatures reached a maximum of 33°C during the daytime and a minimum of 12°C at night. Very low metabolic rates compared with those of other nonsedentary invertebrates were recorded in both species; oxygen consumption ranged from 8 $\mu\text{L g}^{-1} \text{h}^{-1}$ at 16°C to 115 $\mu\text{L g}^{-1} \text{h}^{-1}$ at 40°C. A pulsed Doppler system was used to measure heart rate in situ in free-moving scorpions. At night, heart rate declined to about 4 beats min^{-1} in resting undisturbed scorpions. During daylight excursions and while scorpions hunted for food, heart rates as high as 180 beats min^{-1} were observed. Heart rate was linearly correlated with temperature in *P. villosus*, with a slope of 2.37 ($Q_{10} = 2.18$), but in *O. flavescens* only a limited correlation was observed, with a slope of 1.18 ($Q_{10} = 1.69$). In *O. flavescens*, heart rate showed hysteresis as body temperature rose during daylight and then decreased during the late afternoon and evening; the reverse was observed in *P. villosus*. In both species, haemocyanin-oxygen affinity was independent of temperature, with a higher oxygen affinity and a larger pH sensitivity in *O.*

flavescens. The Q_{10} 's of oxygen consumption and heart rate are quite different in *O. flavescens* but not as different in *P. villosus*. Although changes in the cardiovascular system, such as stroke volume, may also play a role in meeting increased oxygen demand, the features of the haemocyanin oxygen transport system, such as the absence of temperature sensitivity and a marked pH sensitivity, can also influence the maintenance of \dot{V}_{O_2} under temperature stress. The differences in the normal thermal habitats of the two species may be used to explain the distinctions between the evolved physiological responses to temperature increase shown by the two species.

Introduction

Although the fossil record of scorpions is fragmentary, we have a tentative understanding of how this arthropod has evolved from an aquatic habitat to colonize some of the most extreme terrestrial environments (Sissom 1990). The present study investigates the ecophysiological adaptations or tolerance to temperature extremes in situ in representatives of two evolutionarily different scorpion families, *Parabuthus villosus*, a buthid, and *Opisthophthalmus flavescens*, a scorpionid.

Relatively little is known about the ecophysiology of the large scorpion fauna of southern Africa (Robertson et al. 1982), although Lamoral (1979) has provided an extensive review of the systematics and biology of the 56 species found within Namibia. *Parabuthus villosus* and *O. flavescens* are both burrowing scorpions, but the former is both pelophilous (soil or clay loving) and lithophilous (rock loving) (Newlands 1978), digging shallow scrapes under rocks in soils of variable hardness (Lamoral 1979). The latter is a true psammophilous species (Newlands 1978) and digs burrows 30–50 cm deep at the sides of small to large consolidated sand dunes (Lamoral 1979). *Parabuthus villosus* and *O. flavescens* exist in proximity to each other in Gobabeb but within distinctive habitats. The former is confined to the rocky gravel plains and the latter usually to more sandy substrates, although specimens have been found in the Kuiseb riverbed (Holm and Edney 1973).

As in most buthids, *P. villosus* has a powerful neurotoxin that it uses to paralyse or kill its prey after stinging (Newlands 1978). *Opisthophthalmus flavescens*, however, uses its large pedipalps to crush its prey and has a less powerful sting (Newlands 1978), similar in strength to that of a bee (W. J. van Aardt,

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personal observation). The two species also exhibit very different reproductive strategies; *P. villosus* is apoikogenic, producing large eggs with copious yolk, compared with the katoikogenic *O. flavescens*, which produces small eggs and no yolk (Polis and Sissom 1990). The gestation periods and life span are shorter in the buthids than in the scorpionids, and food availability and, indirectly, temperature control growth (Polis and Sissom 1990). Specimens of *P. villosus* from the Namib Desert have been known to go for a year without food in the laboratory (Newlands 1978).

With the exception of Robertson et al. (1982), most of the previous studies on the ecophysiology of desert scorpions have concentrated on the problems of water loss and osmoregulation in response to temperature changes in North American, Mediterranean, and North or West African species (Cloudsley-Thompson 1962a; Hadley 1970a, 1974, 1990, 1994; Riddle et al. 1976; Warburg et al. 1980; Warburg 1986). Metabolic rate changes with temperature have also been reported (Dresco-Derouet 1960, 1964; Hadley and Hill 1969; Riddle 1978, 1979; Paul et al. 1989). Recently, the circulatory system (Farley 1985, 1987, 1990) and the function of the book lungs in scorpions were investigated (Fincke and Paul 1989; Paul and Fincke 1989).

Ecophysiological studies examine the functioning of a particular species in relation to the environment. The few published field measurements of body temperature (T_b) and environmental (air) temperature (T_a) in scorpions are provided by Hadley (1970b), who made measurements on scorpions within their burrows. There are earlier reports of daily microclimatic environment changes in various deserts, including the Red Sea coastal plain (Cloudsley-Thompson 1962b), Arizona (Hadley 1970b), the pre-Sahara steppe (Heatwole and Muir 1979), and the Namib Desert (Holm and Edney 1973; Willmer 1982; Lancaster et al. 1984; Seely et al. 1988). All these desert environments are characterised by large daily changes in soil temperature and differences in their tautochrones.

In contrast, on an annual basis, summer and winter mean T_a differences in the Namib Desert are not extreme, averaging between 4.2°C on the coast and 6.2°C inland (Lancaster et al. 1984). The Namib is a typical coastal desert, with early morning fogs providing as much as 50% of the annual precipitation.

The aim of the present study was to investigate how T_b and T_a vary in the field at the same environmental site in two scorpion species with very different life histories. These investigations were for the first time combined with physiological measurements of heart rate made noninvasively in unrestrained scorpions under in situ conditions. Although each species normally colonizes a different habitat, there is some overlap. *Parabuthus villosus* can occupy consolidated sand areas (Lamorale 1979), and *O. flavescens* was found in proximity to the gravel plains in the Kuiseb riverbed. They were therefore both measured under the same conditions, that is, in scrapes under rocks, for comparative purposes, although it must be borne in

mind that responses may differ according to substrate. In further laboratory experiments, the effects of temperature on metabolism and oxygen transport were investigated by measuring oxygen consumption ($\dot{V}O_2$) and haemocyanin-oxygen affinity at different temperatures. The resulting data were then used to interpret the ecophysiological strategies of each species.

Material and Methods

Animal Capture and Maintenance

Specimens of *Parabuthus villosus* were collected on the gravel plains within a 3–4 km radius of the Desert Ecology Research Unit at Gobabeb, Namibia. This species is reported to be night active, but no specimens were found at night despite extensive searches with portable UV lights. This may, however, have been due to the bright moonlight at the time, since animals are best collected with UV lamps on dark nights when the moon is less than half full (Sissom et al. 1990). In this study, specimens were found only during daylight hours, by following their tracks back to their scrapes under rocks or by catching them whilst they were traversing open ground.

Opisthophthalmus flavescens were collected in the dry sandy bed of the Kuiseb River, which lies between an extensive dune system and the gravel plain described above. Specimens were found under the bark of fallen trees or under stones, which indicates that they are both psammophilous and epigeic. Again, these animals were obtained during dusk, since UV light searches at night along the riverbed were unsuccessful, probably for the same reason as above. After capture, both scorpion species were allowed to recover in individual glass aquaria (40 cm × 20 cm × 30 cm) with sand and stones under a natural light/dark cycle for 24 h without food before $\dot{V}O_2$ measurements were made. This was to ensure that any increase in metabolism due to feeding would not influence the measurements (Riddle 1978). Food in the form of sand crickets and water was then provided ad lib.

Animal Restraint

For implantation of thermocouples and piezoelectric Doppler crystals, specimens of each species were restrained by a rectangular bag made out of thick aluminium foil (van Aardt 1991). Forceps were used to grasp the scorpion by the tail and place it in the bag. The sides of the bag were folded together, and a wad of cloth was used to press the foil down to give a tight fit over the body of the scorpion. Scorpions are positively thigmotropic and are easily handled and restrained in this way after an initial struggle. An operating area was exposed by opening a small window in the aluminium foil bag above the dorsal surface of the animal. Animals were prepared with thermocouples and piezoelectric Doppler crystals at least 12 h in advance of experimentation.

Thermocouples

For measurement of T_b , thermocouples were implanted under the third or fourth segment of the mesosoma. The thermocouples were made out of copper-constantan wire 0.005 inches thick (TW36, Physitemp, Clifton, N.J.) and attached to a digital laboratory thermometer BAT 12 (Physitemp, Clifton, N.J.). Care was taken to place the ends of the thermocouples 2–3 mm under the overlapping cuticle without puncturing the haemocoel. The ends were then fixed in place with dental wax and epoxy resin.

The analogue recorder output of the BAT 12 was connected to a data acquisition system consisting of an A/D card (Metrabyte DASH-16, Taunton, Mass.) together with an AT computer using Asystant+ software (Asyst Software Technology, Rochester, N.Y.) to store and analyze the signals. For in situ recordings, the BAT 12 analogue output was connected by a 20-m coaxial cable to the input box of the data acquisition system. The thermocouples were calibrated in the field by measuring millivolt changes on a digital voltmeter and the computer display and comparing these with the temperature face display of the BAT 12 (T_a). In the laboratory, a water bath (Lauda Thermostat, MT, Königshofen, Germany) was used to generate known temperatures, and the measurements were made with the same instruments as used in the field. The following regression equations were calculated: results for the field, $mV = 10.05(T_a) - 68.79$ ($r = 0.99$); results in the laboratory, $mV = 10.09(\text{water temperature}) - 68.74$ ($r = 0.99$). This shows excellent correlation between the two measurement regimes.

Pulsed Doppler Measurements of Heart Rate

Small (1 mm diameter) piezoelectric transducer crystals (DBF-120A XS, Crystal Biotech, Holliston, Mass.) were implanted in each scorpion. The same restraining techniques as described above were used. Transducers were implanted dorsally under the tergites of either the third or fourth mesosomal segment. They were fixed in place with dental wax and histoacryl glue. Care was taken not to damage or penetrate the haemocoel. The Doppler transducers were connected to a directional pulsed Doppler flowmeter (545C-4, Bioengineering, University of Iowa, Iowa City), and accurate positioning was achieved by monitoring the signal strength over an audio coupler. Signals from the pulsed Doppler device were recorded acoustically and also on the data acquisition system (as described above).

In Situ Field Enclosure Experiments

An oval enclosure (approximately 1.5 m in diameter) was constructed to confine experimental animals on the gravel plain, within 20 m of the laboratory and 100 m from the point where

standard meteorological data were gathered. A number of large stones within the enclosure were arranged to form seminatural shelters above scrapes made in the soft gravel and sand substrate (depth, 5–10 cm). This simulated the natural conditions for *P. villosus* and, to some extent, for *O. flavescens*, although the latter species ideally prefers soft sand. On being released into the separate parts of the enclosure, both species immediately sought refuge under different stones and were only infrequently observed in the open and then only during darkness, which suggests that these species are mainly nocturnal. Recordings of T_b and heart rate were made in most cases between 0630 and 2300 hours, since all electrical power was supplied by electric generators that were in operation only during this time period. On one occasion, however, it was possible to make measurements over a 24-h period for both species to obtain temperature and heart rate data over a wider range.

Meteorological Data

Meteorological data were supplied by the staff of the Desert Ecology Research Unit from a first-order weather station at Gobabeb. Readings for T_a and sand temperature at depths of 5, 20, 30, 60, and 120 cm were made at 0800, 1400, and 2000 hours each day from February 18, 1992, until March 7, 1992. A permanently fixed thermometer system was used in either a standard Stephenson screen for T_a or implanted in the sand for sand temperature.

$\dot{V}O_2$ Measurements

The $\dot{V}O_2$ at STPD was determined with a Scholander respirometer specially constructed for measuring oxygen uptake in quiescent scorpions (van Aardt 1991). The scorpions were placed in the adjustable respirometer chambers (vol = 20–30 mL, van Aardt 1991), which were left open to the atmosphere. The scorpions were allowed to acclimate to handling and the temperature change for 1 h. Measurements were then taken at 30-min intervals (a total of three intervals) at both 35°C and 40°C and at 1-h intervals (a total of four intervals) at both 16°C and 25°C. $\dot{V}O_2$ was calculated as the mean of three consecutive readings. The relative humidity in the respirometer chamber was kept at or near 100% saturation for each temperature with the aid of pieces of Whatman no. 1 filter paper soaked with water (van Aardt 1991).

Haemolymph Sampling and Oxygen Equilibrium Curves

Haemolymph was sampled as described by van Aardt (1991) after the animals were kept for 2 h at 10°C to prevent the accumulation of anaerobic metabolites. The samples from nine *P. villosus* (mass = 2.03–12.92 g) and eight *O. flavescens* (mass = 2.19–8.47 g) were pooled within species and stored frozen in the laboratory at Gobabeb (–12°C) or in the laboratory in

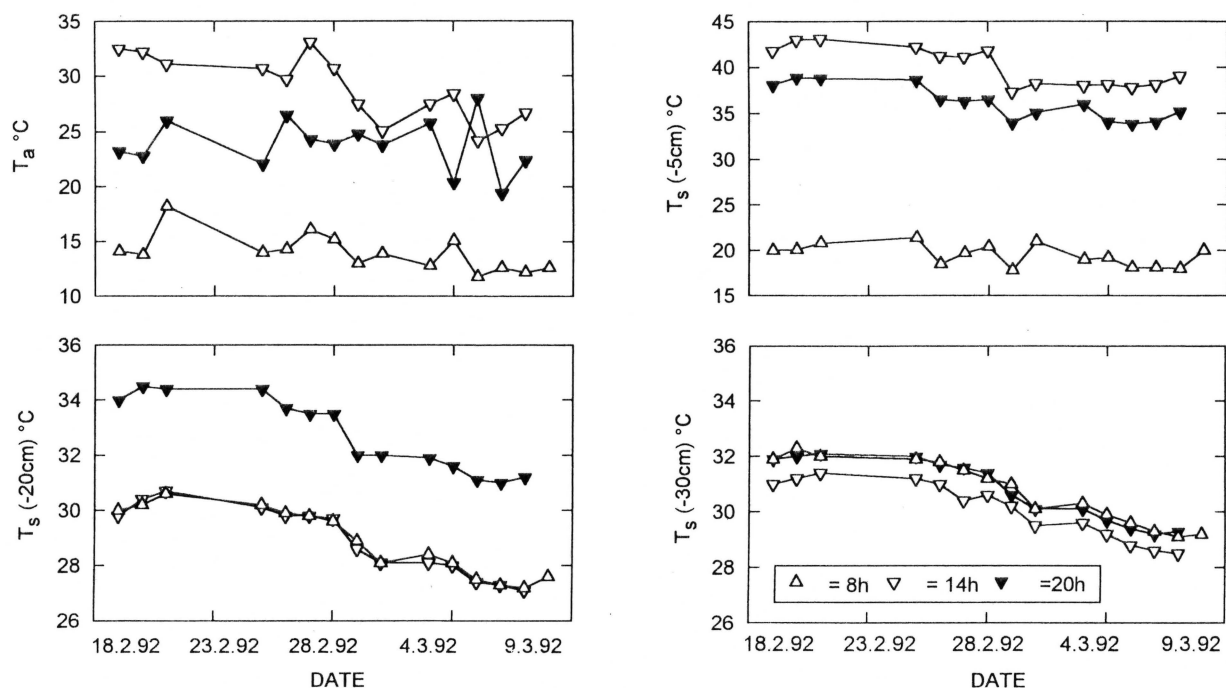


Figure 1. T_a and sand temperature (T_s) ($^{\circ}\text{C}$) measured at the meteorological weather station at the Desert Ecology Research Unit, Namibia, for the period from February 18, 1992, until March 3, 1992. Dry-bulb measurements were made daily at 0800, 1400, and 2000 hours in air and at depths of -5 , -20 , and -30 cm in sand.

Potchefstroom (-17°C) for 5 wk before measurement of oxygen equilibrium curves. No attempt was made to measure the in vivo pH because of the small size of individual samples. Aliquots (80 μL) of the pooled haemolymph samples were equilibrated for 30 min at 15°C , 25°C , 30°C , 35°C , and 40°C in a microtonometer system (BMS 2, Radiometer, Copenhagen) in the presence of 0%, 1%, 3%, and 5% CO_2 in air provided by a gas-mixing pump (M100/a-f, Wösthoff, Bochum). After equilibration, haemolymph pH was then measured with a micro-pH electrode (E5021, Radiometer, Copenhagen) that had previously been calibrated with precision buffers at the respective temperature.

The oxygen-binding properties of the haemocyanin in native haemolymph were measured by means of a thin layer optical cell at 340 nm by means of the step method (Dolman and Gill 1978) at 15° , 25° , 35° , and 40°C . A special software program (van Aardt and Naude 1990) was used to analyze the saturation and oxygen tension data as a Hill plot. The data between 25% and 75% saturation were then used to calculate oxygen affinity, expressed as P_{50} , and cooperativity, expressed as n_{50} . The haemolymph samples in the optical cell were exposed to the same CO_2 concentrations as used in the tonometry for pH measurements.

Results

Meteorological Data

Data for environmental parameters over the 3-wk study period are shown in Figure 1. T_a rose from 14°C in the morning to 33°C at 1400 hours and declined to 25°C in the evening. Sand temperature at -5 cm reached a maximum of 43°C at 1400 hours and cooled to 35° – 38°C by 2000 hours. Sand temperature values for -20 cm remained relatively constant (30°C) between 0800 and 1400 hours but rose after a delay to 34°C by 2000 hours. Sand temperature values at depths greater than -30 cm remained constant throughout the daily cycle at 31°C . The measurement at 1400 hours was, however, always 1°C lower than the measurements at 0800 and 2000 hours. No such difference was observed at -60 cm, although over the study period a steady decrease ($-0.1^{\circ}\text{C d}^{-1}$) was observed as winter approached.

Scorpion T_b 's

Figure 2A illustrates data for *Opisthophthalmus flavescens* T_b in unrestrained animals, mainly quiescent, lying in scrapes under stones in the shade. Initially T_b was similar to T_a . Between 0700 and 1400 hours, T_b rose almost linearly at a rate of $4.4^{\circ}\text{C h}^{-1}$, reaching a plateau between 1400 and 1800 hours at a level approximately equal to the sand temperature at -5 cm. T_b then declined exponentially, and by 2000 hours it was lower than sand temperature at -5 cm but usually above T_a . In unrestrained, quiescent *Parabuthus villosus* in scrapes and un-

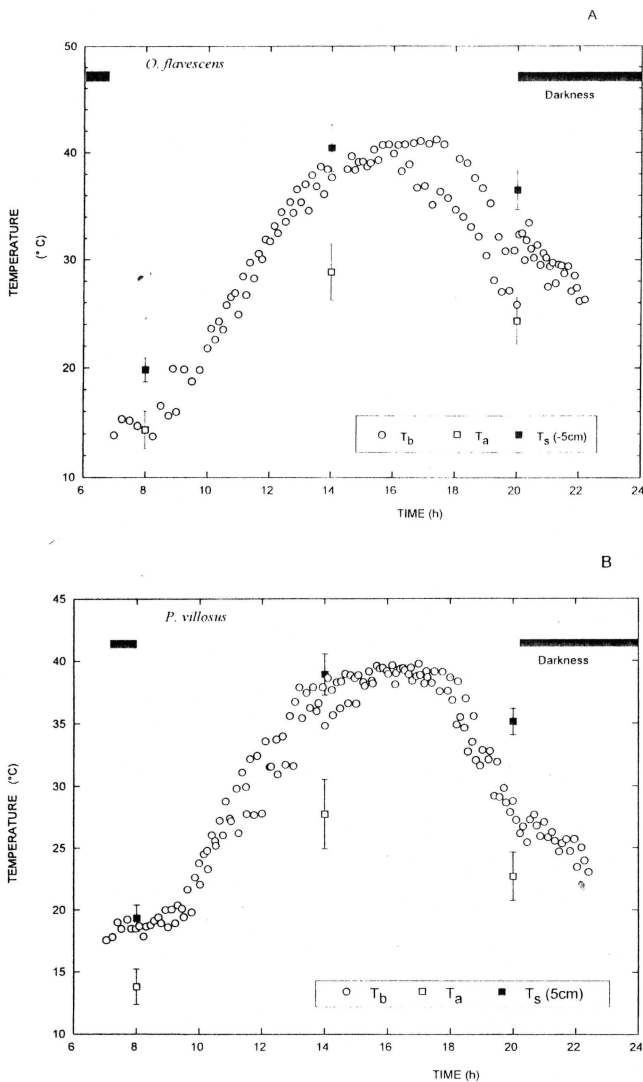


Figure 2. Daily changes in T_b ($^{\circ}\text{C}$) in *Opisthophthalmus flavescens* (A) ($n = 8$) and *Parabuthus villosus* (B) ($n = 10$) in a field enclosure. Mean values are shown for 5–6-min periods. Comparative mean values ± 1 SD are shown for T_a and sand temperature (T_s) at -5 cm.

der stones in the shade, T_b rose linearly during the morning at $3.9^{\circ}\text{C h}^{-1}$, reaching a plateau at 40°C between 1400 and 1800 hours before decreasing exponentially (Fig. 2B).

Figure 3 shows details of temperature measurements made over a 24-h period simultaneously for both species under separate stones in the same enclosure. As the sun rose and insolation occurred, T_b increased in both species. Although the two species were only 40 cm apart under separate stones, there was a 3°C temperature difference in T_b between the two. This may have been due to behavioural differences or to differences in microclimate under the stones, but neither species was observed to be active during this period. At approximately 1600 hours, T_b began to decline exponentially in both species. The

scorpions made a number of separate excursions out from under the rocks, as indicated by the arrows in Figure 3, and this led to a rapid decrease in T_b . T_b recovered to the previous higher value on return to the covering rocks. This nocturnal activity pattern corresponds with that reported for other scorpions (Warburg and Polis 1990). The lowest T_b 's were recorded at around 0600 hours, which coincided with the absence of solar radiation and the presence of high humidity associated with the morning mists. T_a 's in the present study represent values from the weather station (height = 1.5 m), and temperatures at ground level correspond approximately to sand temperature at -5 cm.

One criticism of the measurements in the field on *O. flavescens* in the present study is that this species is not often found on the gravel plain; rather, it inhabits burrows in the sand dunes (Lamoral 1979). A number of burrows from *O. flavescens* (identified by tracks and also excavation) were found during daylight searches near Gobabeb in the dune system. These were deeper than 20 cm, which indicates that they were at a level where daily temperature changes would be small. In a pilot study, an animal used in the temperature enclosure field study was taken to the dunes and allowed to enter a burrow system in the dunes. T_b remained relatively constant at around 30° – 32°C , whereas sand temperature at -5 cm rose to 37°C over a trial period of a few hours.

Oxygen Consumption

\dot{V}_{O_2} was measured for adult animals ($n = 5$) weighing between 2.69 and 8.47 g (5.26 ± 2.3 g [mean \pm SD]) for *O. flavescens* and from 2.03 to 12.92 g (6.03 ± 3.2 g [mean \pm SD]) for *P. villosus* ($n = 8$). \dot{V}_{O_2} rose linearly in both species with an increase in T_a (Fig. 4). Fitted regression lines for these rates gave the following linear equations: *O. flavescens*, \dot{V}_{O_2} ($\mu\text{L O}_2 \text{ g}^{-1} \text{ h}^{-1}$, STPD) = $4.77 (T_a) - 74.21$ ($r = 0.93$); *P. villosus*, \dot{V}_{O_2} ($\mu\text{L O}_2 \text{ g}^{-1} \text{ h}^{-1}$, STPD) = $3.99 (T_a) - 48.63$ ($r = 0.98$). A Q_{10} value of 3.06 was calculated from the overall log \dot{V}_{O_2} versus temperature relationship (Cossins and Bowler 1987) for *O. flavescens*, and a Q_{10} value of 2.50 was calculated for *P. villosus*. At temperatures between 16° and 25°C , the Q_{10} values were much higher, at 6.0 and 5.4 for *O. flavescens* and *P. villosus*, respectively. A greater variability in \dot{V}_{O_2} was observed above 30°C in both species. *Opisthophthalmus flavescens* died when exposed to temperatures above 40°C for longer than 3 h, although *P. villosus*, being an extremely hardy species (Newlands 1978), did not.

Heart Rate

Scorpion heart rates were elevated after implantation of piezoelectric Doppler crystals but soon returned to lower baseline levels characteristic of resting undisturbed animals. Figure 5A shows the typical response of a specimen of *O. flavescens* placed

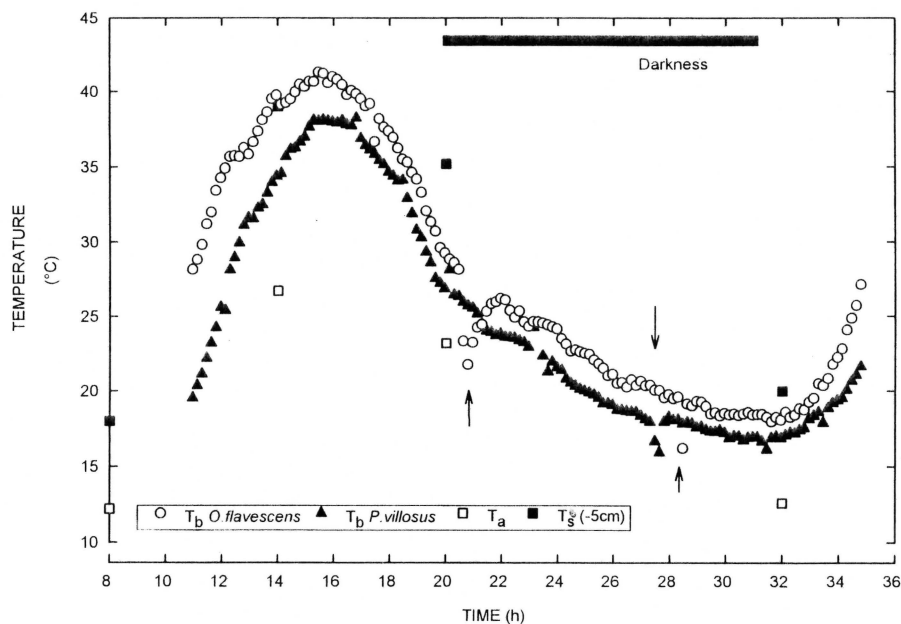


Figure 3. Changes in T_b ($^{\circ}\text{C}$) plotted against elapsed time in an individual *Opisthophthalmus flavescens* and an individual *Parabuthus villosus* during a 24-h in situ experiment. The daily values for T_a and sand temperature (T_s) are also shown. Arrows indicate periods when one of the scorpions emerged from its scrape and then retreated.

in the experimental enclosure where T_b and heart rate were recorded simultaneously. From consecutive measurements (15-min intervals), it is evident that heart rate remained relatively constant at low levels as T_b rose from 12° to 32°C during the morning. Above this temperature, heart rate began to increase and continued to rise, even though T_b reached a maximum at 1400 hours and then decreased. At approximately 1600 hours, heart rate reached a maximum and then began to decline. This general hysteresis pattern was observed for all specimens of *O. flavescens*.

The typical response of *P. villosus* is shown in Figure 5B. Heart rate rose rapidly on warming and continued to increase until approximately 1700 hours. Thereafter, heart rate declined more rapidly than the corresponding decrease in T_b . Exactly the opposite observations were made in *O. flavescens*. The maximum heart rate observed in *O. flavescens* was $152 \text{ beats min}^{-1}$ after handling, with a minimum value of 5 beats min^{-1} measured early in the morning. The corresponding values for *P. villosus* were $178 \text{ beats min}^{-1}$ after handling and 4 beats min^{-1} at 0600 hours.

For the combined heart rate and T_b data for six specimens of each species (shown in Fig. 6A for *O. flavescens* and Fig. 6B for *P. villosus*), the following linear regression lines were fitted to the data for heart rate and T_b : *O. flavescens*, heart rate = $1.18(T_b) - 5.59$, $r = 0.50$; *P. villosus*, heart rate = $2.37(T_b) - 28.87$, $r = 0.72$. Heart rate rose with increasing T_b at a faster

rate in *P. villosus* than in *O. flavescens*, with calculated Q_{10} values from the log heart rate versus T_b relationship of 2.18 and 1.69, respectively. Because of technical difficulties of operating in the field, only heart rate data could be analyzed, and not absolute flow values. Data points represent the heart rate for 1-min intervals measured every 15 min. Continuous recording and storage was not attempted, as this was technically beyond the scope of the available field equipment.

Oxygen Equilibrium Curves

Figure 7 shows that both oxygen affinity and the Bohr coefficients ($\Delta \log P_{50}/\Delta \text{pH}$) were independent of temperature in both species. Therefore, a combined regression line was fitted to the data. At pH 7.5, oxygen affinity, expressed as the half-saturation value P_{50} , was higher in *O. flavescens* (10.7 Torr, where Torr = 133.32 Pa) than in *P. villosus* (20.2 Torr). The pH sensitivity of the oxygen affinity of the haemocyanin of *O. flavescens* was larger, with a Bohr coefficient of -1.06 compared with -0.59 in *P. villosus*. In both species, the cooperativity of the haemocyanin, expressed as n_{50} , was independent of temperature, but in *O. flavescens*, n_{50} appeared to be pH-dependent, increasing with increasing PCO_2 values. The mean value for n_{50} in *O. flavescens* was 3.6 ± 0.79 , and for *P. villosus*, 3.48 ± 0.49 .

Discussion

Under the experimental conditions used in the present study, there was less opportunity to use behavioural means to avoid thermal stress, but both species survived exposure to high temperatures. Some behavioural mechanisms, however, have been observed to prevent cooling. In softer substrates where *Opis-*

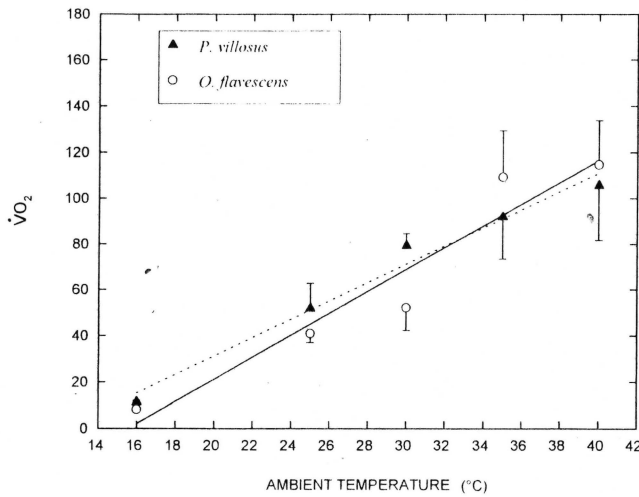


Figure 4. Weight-specific $\dot{V}O_2$ in $\mu\text{L O}_2 \text{ g}^{-1} \text{ h}^{-1}$ plotted as a function of T_a for *Opisthophthalmus flavescens* ($n = 5$) and *Parabuthus villosus* ($n = 8$). See text for equations of regression lines. Values are means ± 1 SD.

and Edney (1973) found little change in temperature after a depth of 20 cm was reached.

These in situ changes in meteorological temperature data in Gobabeb indicate that species such as *Parabuthus villosus*, dwelling within the top 5 cm of the substrate, may be exposed to daily temperature changes of 23°C. Those species that adopt a burrowing mode of life below 20 cm, such as *O. flavescens*, may escape these large changes. They may, however, then be exposed chronically to relatively high temperatures (30°–32°C) with a maximum around 2000 hours. Although precipitation and water balance were not considered in this study, Gobabeb has effectively been without rain for periods of up to 82 mo. From 1962 to 1981, the yearly rainfall averaged 27.5 mm and fog precipitation averaged 30.8 mm (Lancaster et al. 1984).

T_b Measurements

The rapid warming of both species under similar conditions and the similarity between T_b and sand temperature after heat-

Opisthophthalmus flavescens is able to construct burrows, exposure to large temperature variations may be limited. These two evolutionarily different species have adopted distinctive eco-physiological strategies in terms of the circulatory system and the contrasting properties of the haemocyanin to survive thermal stress.

Meteorological Data

Environmental parameters are particularly important in controlling the distribution of animals, and they also give an indication of the necessary physiological adaptations required to live in a particular habitat (Willmer 1982). Cloudsley-Thompson (1962b) conjectured that temperature extremes, more than the mean temperature, influence animal distribution.

In the present study, maximum T_a values (33°C) were 10°C lower than maximum sand temperature values (43°C). This is a common feature and has been reported elsewhere (Cloudsley-Thompson 1962b; Hadley 1970b; Hawke and Farley 1973; Heatwole and Muir 1979). Maximum temperatures were reached around 1400 hours in Gobabeb, and similar findings were made by Hadley (1970b) in Arizona deserts and Heatwole and Muir (1979) for the Tunisian steppe.

The overlying sand affected both the temporal changes in temperature and also their magnitude (Fig. 1). At -20 cm, a delay, probably due the insulating effect of the overlying sand (Hadley 1970b; Lancaster et al. 1984), caused maximum temperatures to be reached only at 2000 hours. The lower temperatures observed at 1400 hours compared with those at 0800 hours or 2000 hours at 30 cm depth may be due to evaporative cooling in the daytime. At depths greater than 30 cm, no change in temperature due to insulation was observed. Holm

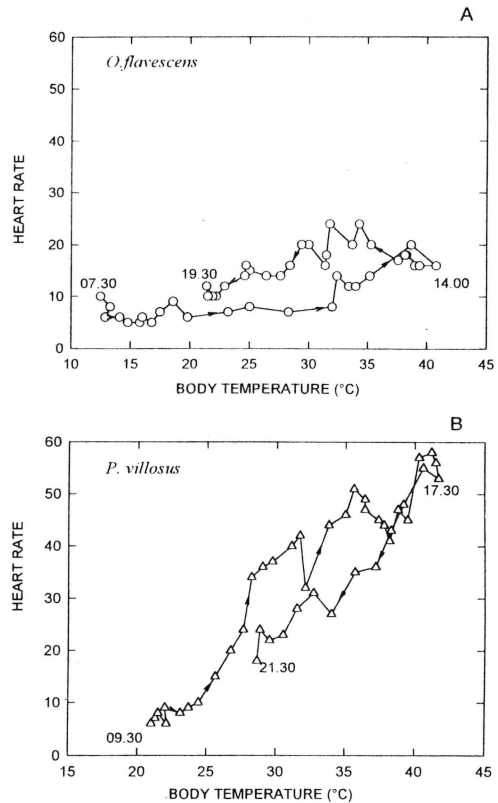


Figure 5. Changes in heart rate (beats min^{-1}) with T_b for a typical specimen of *Opisthophthalmus flavescens* (A) and *Parabuthus villosus* (B) in a field enclosure on March 4, 1992, and February 29, 1992, respectively. Lines connect consecutive measurement points made at approximately 15-min intervals, and the arrows indicate warming or cooling phases. The numbers within the figures indicate the time of day at which the measurement was made.

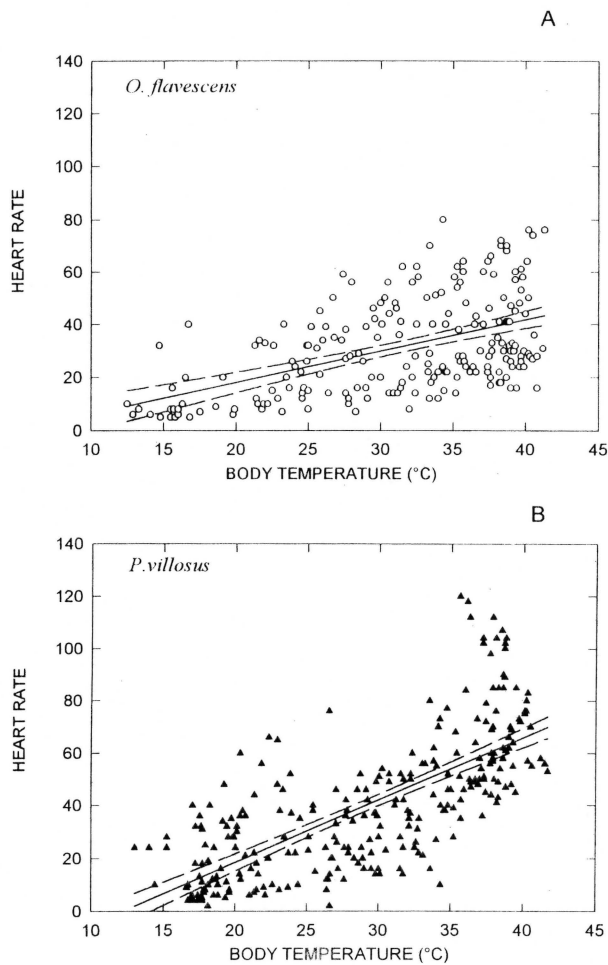


Figure 6. Dependence of heart rate (beats min⁻¹) on T_b (°C) in six specimens each of *Opisthophthalmus flavescens* (A) and *Parabuthus villosus* (B) in a field enclosure. The solid line is the regression line, and the dotted lines indicate the 95% confidence limits.

ing indicate that there was little opportunity in the present study to behaviourally regulate T_b at high temperatures (Fig. 3). However, T_b was normally slightly lower than sand temperature (Figs. 2, 3), which indicates perhaps that the shade under the rocks does help the species to tolerate extremes. The long exponential decline in T_b with time may indicate the passive change in T_b as the substrate cools.

In both species, the maximum temperature reached 42°–43°C. This compares with a lethal temperature of 47°C for *Leiurus quinquestriatus* (Cloudsley-Thompson 1962a) and a temperature of 44°C in *Hadrurus arizonensis* within a burrow system (Hadley 1970b). A temperature of 40°C for 3 h caused death in *O. flavescens* in the confined respirometer experiments but not in the more tolerant *P. villosus*. The former probably digs burrows in nature to escape high temperatures, and the latter reduces energy expenditure by not digging deep burrows. This indicates that behavioural strategies are also very different between the two species.

Behavioural adaptations by scorpions to avoid extreme temperatures may involve the use of nocturnal surface activity or the use of burrows (Hadley 1974, 1990). Depending on depth within a burrow, a certain measure of temperature regulation is attained in *Hadrurus arizonensis* (Hadley 1970b). *Parabuthus villosus* is not known to dig deep burrows (Lamoral 1979), and none were found in the present study; this species will therefore be exposed to larger variations in temperature. From pilot experiments in the dunes, we conclude that *O. flavescens* probably experiences a relatively high constant temperature only when burrowed in the sand. It can thus avoid extremes. Further dune experiments were not possible because of the logistics required and were beyond the scope of the present study.

Hadley (1970b) points out that the burrow can act as a

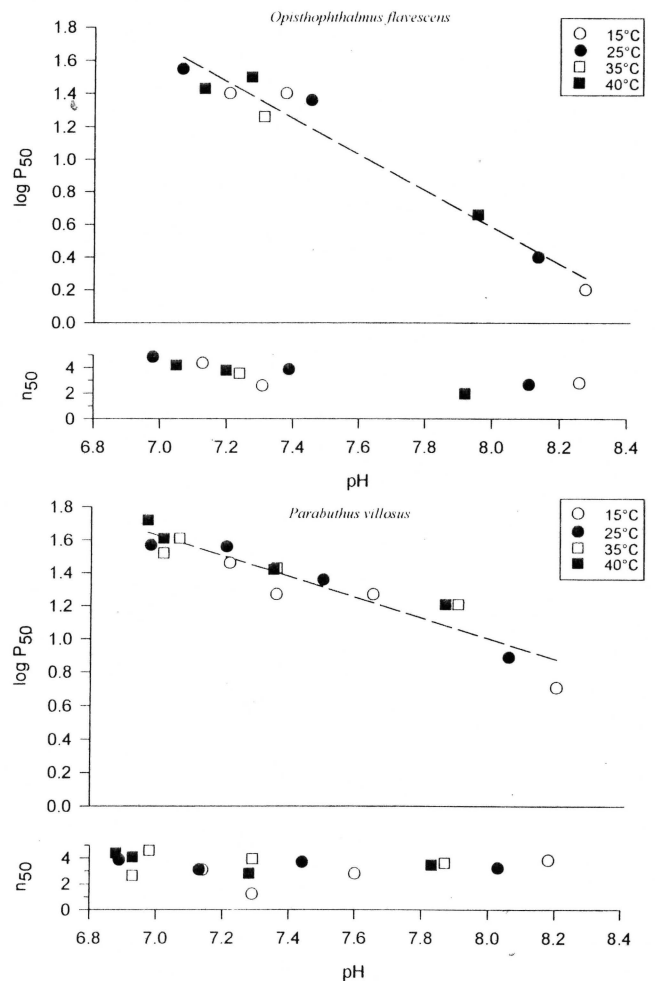


Figure 7. Dependence of oxygen affinity (log P₅₀) on pH for the haemocyanin of *Opisthophthalmus flavescens* (top) and *Parabuthus villosus* (bottom) at 15°, 25°, 35°, and 40°C. Regression for *O. flavescens*: log P₅₀ = -1.06 pH + 8.98 ($r = 0.979$). Regression for *P. villosus*: log P₅₀ = -0.59 pH + 5.78 ($r = 0.943$). The pH dependence of cooperativity measured at n₅₀ is shown in the lower part of each panel.

retreat from suboptimal temperatures at night. Similarly, in the present study, the large stones probably acted as a heat reservoir. The T_b of both species dropped by 2°–5°C when the scorpions left their rock cover at night (Fig. 3). The temperature reverted rapidly to previous levels when the animals returned to cover, thus indicating a behavioural means of maintaining a higher T_b . Rock cover, however, is inadequate to provide complete protection from heat or cold, and considerable tolerance to temperature change must exist in both species.

Field measurements of T_b in other desert arthropods such as tenebrionid beetles (Hadley 1970b; Seely et al. 1988; Roberts et al. 1991) have, as in the present study, reported high T_b 's (29°–39°C). These were correlated with the aseasonality of the temperatures of the Namib environment and the presence of thermal refuges. These were provided by the slip faces of the dunes into which the beetles burrowed to avoid high temperatures.

Heart Rate

In general, it is difficult to make conventional measurements of heart rate in organisms with high haemolymph pressures within the circulatory system, since bleeding occurs when the vessels are damaged by sensor implantation. Mostly optical or noninvasive methods have therefore been used in scorpions or spiders to measure heart rate (Carrel 1987). The present study represents one of the first measurements of heart rate in free-moving scorpions under field conditions. The range of heart rate observed, from 5 to 152 beats min^{-1} in *O. flavescens* and 4 to 178 beats min^{-1} in *P. villosus*, is extreme for an arthropod. Farley (1987) reported values as high as 120 beats min^{-1} in *Paruroctonus mesaensis*, and Carrel (1987) gives comparative values for spiders that range from less than 10 and to greater than 120 beats min^{-1} . Transient bradycardia and tachycardia are associated with various external stimuli in scorpions (Farley 1985), and both species in the present study showed a similar response.

Two main differences are evident in the present study in the response of heart rate of each species to temperature change. The first of these is the hysteresis of heart rate during warming then cooling in *O. flavescens* (Fig. 5A), compared with the opposite effect in *P. villosus* (Fig. 5B). These two pattern types were observed in every experiment ($n = 12$) and therefore do not represent an experimental artifact.

Bartholomew and Lasiewski (1965) reported a much more pronounced effect on heart rate of the marine iguana during aquatic heating and cooling. Heart rate increases rapidly on heating and decreases more rapidly on cooling as in *P. villosus*. They suggested that this bradycardia, which occurred during diving, is adaptive in maintaining T_b . Heat loss would be lowered because of decreased perfusion when external temperatures were falling. Using this interpretation for the two scorpion species would indicate that in *P. villosus* this effect could

be used for maintaining T_b , and in *O. flavescens*, hysteresis would facilitate cooling. This may be correlated with the different habitats of the two species. *Opisthophthalmus flavescens* normally living under relatively constant thermal conditions in a burrow (32°C) may need to cool down. *Parabuthus villosus* inhabits a more variable temperature regime and may need to maintain T_b as night approaches and T_a decreases. This hypothesis, which still has to be proven, assumes that heat loss can be controlled by peripheral circulation in an animal with a cuticle. The hysteresis effect and its opposite may, however, be unconnected with temperature regulation and reflect changes in the gas-exchange system, as discussed below.

The second major difference between the two species was the temperature dependence of heart rate. A Q_{10} of 1.69 in *O. flavescens* indicates that heart rate has a low temperature sensitivity. This may be adaptive in a species that experiences little temperature variation in situ and therefore will not be subjected to increased oxygen demands through temperature increase. It should also be noted that even at high temperatures, heart rate is low in this species compared with that of *P. villosus*. In *P. villosus*, heart rate is linearly correlated with temperature, having a Q_{10} of 2.18. This is a typical value found for many biological processes; similar values are shown for the relationship between temperature and heart rate in spiders (Carrel 1987). Animals exposed to thermal extremes in general may have higher Q_{10} values for metabolism. Cossins and Bowler (1987) point out that deviations from the general values of Q_{10} between two and three may indicate regulatory control of the process. The regulatory control in this case may involve other adaptations that serve to maintain oxygen transport to the tissues during increased demand.

Oxygen Consumption

\dot{V}_{O_2} 's for *P. villosus*, *O. flavescens*, and other scorpions are compared in Table 1. Basal \dot{V}_{O_2} in scorpions is low compared with that of other arthropods (van Aardt 1991), and for some are amongst the lowest rates measured for nonsedentary invertebrates. This may be correlated with their mode of life, where reduced metabolism and restricted periods of activity may accommodate an irregular food supply (Riddle 1978). \dot{V}_{O_2} data from Robertson et al. (1982) for *P. villosus* from the same area are very similar to those in the present study (Table 1). Comparative \dot{V}_{O_2} values for *O. flavescens* are not available but were much higher in *Opisthophthalmus latimanus* (van Aardt 1991).

\dot{V}_{O_2} was temperature-dependent in both species, which correlates with findings in other species. In Table 1, Q_{10} values vary from 1.14 to 4.03 amongst the various scorpion species, and Crawford (1981) has suggested that low Q_{10} values may be associated with large desert arthropods. Care should be taken in interpreting the values, as similar experimental conditions were not used (feeding states, measuring equipment),

and the temperature ranges considered may be different in each study (Table 1). It is clear, however, that animals coming from differing thermal habitats can show metabolic changes similar to temperature increase.

Upon comparing Q_{10} values for heart rate and $\dot{V}O_2$, a slight mismatch of 13% is apparent in *P. villosus* (2.18 compared with 2.50), and a more marked mismatch (45%) is apparent in *O. flavescens* (1.69 compared with 3.61). This implies that increased heart rate alone can almost satisfy oxygen transport to the tissues during temperature stress in *Parabuthus* but plays a much more minor role in *Opisthophthalmus*. Stroke volume changes may increase to offset the rate mismatch, but we were unable to measure this parameter in the present study in the field. Farley (1990) has suggested that heart movements in scorpions may help book lung ventilation by forcing blood to the heart and agitating blood and book lung lamellae, thereby aiding gas exchange. Blood flow through the book lungs is also under neuronal control (Farley 1990), and these mechanisms may be used to improve gas exchange during temperature stress. The hysteresis effect on heart rate seen in *O. flavescens* may help to compensate for temperature stress by maintaining a higher perfusion rate during recovery.

Haemocyanin-Oxygen Affinity and Implications for Gas Transport

Table 2 summarises some of the available data on scorpion haemocyanin-oxygen affinity from the literature. It can be seen that P_{50} values vary between and within species (Decker 1990; Pfeffer-Seidl 1991). The intraspecific difference may be due to the use of Tris buffers to dilute whole haemolymph in the

former study, since Tris buffers have recently been reported to increase oxygen affinity in spider haemocyanin (Paul et al. 1994a; Sterner et al. 1994). A similar effect may also be present in scorpion haemocyanin, although careful ion substitution experiments are still required to confirm the mechanism of the Tris effect. In spiders and scorpions, a temperature effect on oxygen affinity has been shown in other species (Bridges 1988; van Aardt 1991). Significantly, in both *O. flavescens* and *P. villosus*, haemocyanin oxygen affinity shows no intrinsic temperature effect, with oxygen affinity remaining relatively constant with increasing temperature.

In both species during temperature stress, metabolism rises and $\dot{V}O_2$, which is supplied by the product of cardiac output and arteriovenous oxygen content difference, increases. Cardiac output is dependent on heart rate and stroke volume, and the arterial oxygen content can be influenced by book lung ventilation and haemocyanin-oxygen affinity. The former determines the efficiency of gas exchange, that is, arterial loading, and the latter determines arterial saturation at a given oxygen tension. Venous oxygen content should passively follow the oxygen needs of the tissues, decreasing as more and more oxygen is required. The magnitude of the decrease depends on the oxygen affinity of the haemocyanin.

Haemocyanins with a temperature sensitivity, that is, decreased oxygen affinity with rising temperature, could be disadvantageous for *Opisthophthalmus* and *Parabuthus*, since both species may lack mechanisms for temperature regulation and are dependent mainly on behavioural adaptations. A better means of oxygen affinity control would be through the pH sensitivity of the haemocyanin, the Bohr effect. Changes in haemolymph pH are controlled through

Table 1: Comparison of $\dot{V}O_2$ and Q_{10} values in scorpions

Species	Temperature (°C)	$\dot{V}O_2$ ($\mu\text{L O}_2 \text{ g}^{-1} \text{ h}^{-1}$)	Temperature Range (°C)	Q_{10}	Source
<i>Centruroides sculpturatus</i>	20	75.0	10–20	2.18	Hadley and Hill 1969
<i>C. sculpturatus</i>	20–30	1.96	Hadley and Hill 1969
<i>C. sculpturatus</i>	30–40	2.24	Hadley and Hill 1969
<i>Hadrus arizonensis</i>	25	70.0	25–40	1.14 ^a	Hadley 1970b
<i>H. arizonensis</i>	25–45	3.57 ^a	Hadley 1970b
<i>Opisthophthalmus latimanus</i>	25	95.6	10–25	4.03	van Aardt 1991
<i>O. latimanus</i>	30–40	1.54	van Aardt 1991
● <i>Opisthophthalmus flavescens</i>	20 ●	21.1 ●	16–40	3.06	Present study
<i>Paruroctonus utahensis</i>	20	36.4	19–29	2.28 ^a	Riddle 1978
<i>Padinus imperator</i>	20	14.5	20–30	3.0	Paul et al. 1989
● <i>Parabuthus villosus</i>	20 ●	27.5 ●	15–35	2.46 ^a	Robertson et al. 1982
● <i>P. villosus</i>	20 ●	30.9 ●	16–25	2.50	Present study

^a Calculated from authors' data.

Table 2: Comparison of oxygen-transporting properties of scorpion haemocyanins

Species	Temperature (°C)	pH	P ₅₀ (Torr)	n ₅₀	Bohr Coefficient ($\Delta \log P_{50} / \Delta \text{pH}$)	Temperature Effect	Blood Treatment	Source
<i>Pandinus imperator</i>	20	7.6	8	>6	-1.3	nd	+Tris	Decker 1990
<i>P. imperator</i>	25	7.5	38	3.8	-.24 to -.52	Only at pH 7.0	wb + CO ₂	Pfeffer-Seidl 1991
<i>Opisthophthalmus</i> <i>latimanus</i>	25	7.3	43	4.3	nd	Significant	wb + CO ₂	van Aardt 1991
<i>Opisthophthalmus flavescens</i>	25	7.5	11	3.6	-1.06	Not significant	wb + CO ₂	Present study
<i>Parabuthus villosus</i>	25	7.5	20	3.5	-.59	Not significant	wb + CO ₂	Present study

Note. nd, not determined; wb, whole blood or haemolymph.

acid-base regulation, which is rapid in scorpions (Paul et al. 1994b). In both species, haemolymph pH temperature sensitivity averaged 0.0125 pH units °C⁻¹ at relatively constant P_{CO₂}. A change in temperature from 15° to 40°C would give rise to a 0.3-unit change in haemolymph pH. This would cause a decrease in P₅₀ from 10 to 25 Torr in *Opisthophthalmus*, with its high initial oxygen affinity and large Bohr coefficient. In *Parabuthus*, the corresponding changes would be smaller, from 20 to 28 Torr, since the Bohr coefficient is smaller and initial oxygen affinity lower. These properties may be adaptive in *O. flavescens*, since heart rate does not change with temperature but $\dot{V}O_2$ does. A decrease in haemocyanin-oxygen affinity with a shift of the equilibrium curve to the right will enable more oxygen to be unloaded from the haemocyanin at the same venous oxygen partial pressure, thereby satisfying the increased oxygen demand of the tissues. As long as arterial oxygen tension values are maintained or increased during temperature stress, then no deleterious effects will be seen in oxygen loading. Unfortunately, to date these measurements are lacking in scorpions, although in spiders arterial oxygen partial pressure values increase with activity (Paul et al. 1994a). In *Parabuthus*, both cardiac rate changes and flexibility in the oxygen equilibrium curve of the haemocyanin may be used to meet the increased metabolic demands. The normal thermal environment may dictate the extent to which flexibility in the equilibrium curve in the one species and cardiac rate changes in the other play a major or minor role in temperature response of each species.

From this study, it is clear that bothids and scorpionids differ not only their behaviour and lifestyles but also in their ecophysiological adaptations to temperature stress. Further work in the field on free-ranging scorpions is now necessary to elucidate these adaptations on a wider scale.

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