Geographic variation in desiccation tolerance in southern African Trogidae (Coleoptera)

by

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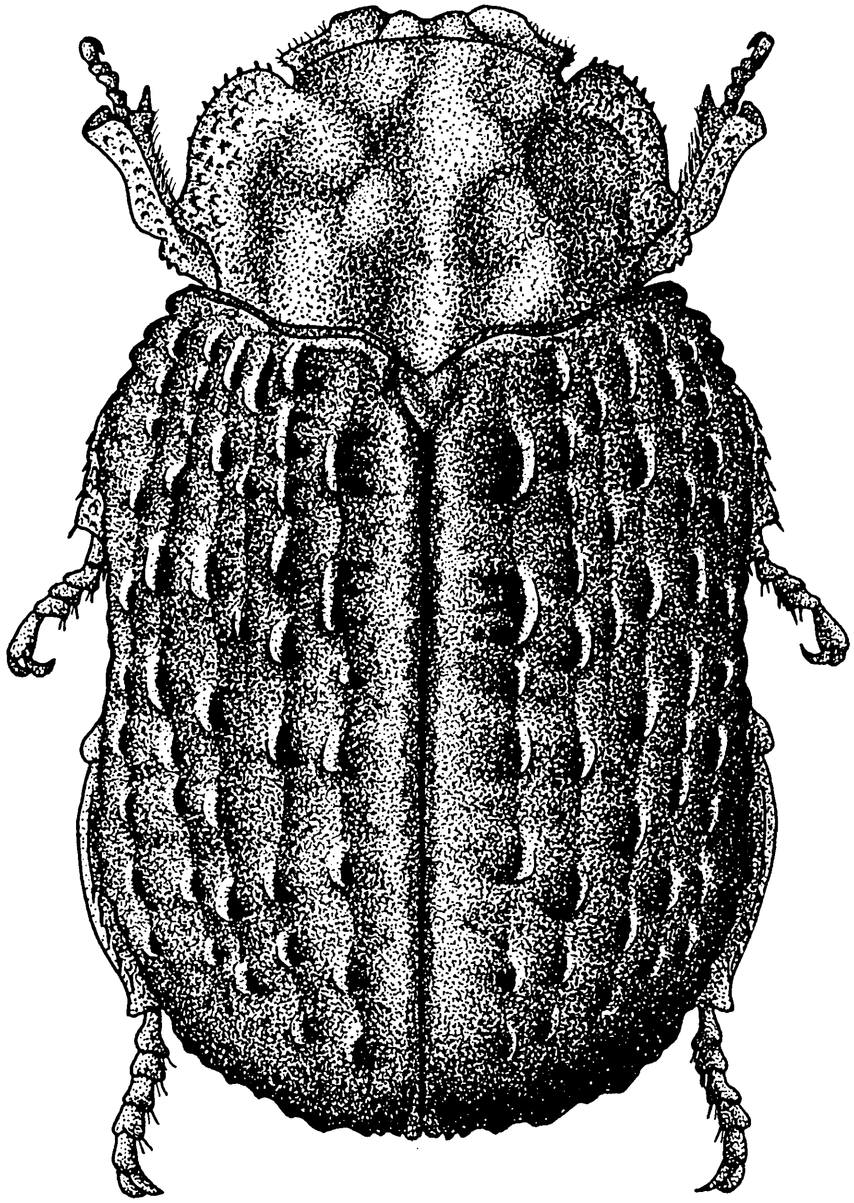

This thesis is dedicated to my husband Joël, for all his love, understanding and inexhaustible patience, and in memory of my father, Marie Gerard d'Hotman De Villiers St Pol for giving me an appreciation for nature and teaching me to persevere at all cost.

Abstract/Samevatting

The distributional patterns of taxa are influenced by a great many factors, both biotic and abiotic. Physiological tolerance of climatic extremes is certainly one such factor and is the subject of this study. In this thesis, desiccation tolerance was utilized as a tool to determine the influence of physiology on the distribution patterns of keratin beetles (Trogidae). Since nothing is known of water balance in these beetles, this thesis commenced with a study of water balance in the keratin beetles. The keratin beetles' body water and lipid content, when fully hydrated, their maximum water loss tolerance, their rate of water loss and survival time under desiccation conditions, were found to be comparable to those of other similar-sized beetles from similar habitats. With the exception of two arid dwelling species, keratin beetles were not able to replenish lost water through the catabolism of lipids. Like most other adult beetles, none of the keratin beetles were able to obtain water through cuticular absorption of atmospheric water vapour but they were able to restore a positive water balance by drinking free water. Surprisingly, none of the keratin beetles studied were able to osmoregulate. At half their LT_{50} the arid dwelling beetles' haemolymph osmolalities were found to be in excess of 1000 mOsm, without noticeable adverse effect to the beetles. In order to ascertain physiological differences in desiccation tolerance of beetles from different climatic areas, interspecific desiccation tolerance studies were conducted using eleven keratin beetles species from six localities, as well as intraspecific studies using five species from a variety of habitats. Both on an intra- and interspecific level, beetles from arid areas were generally found to be morphologically larger and physiologically more tolerant to desiccation than their more mesic counterparts. Being larger, these arid dwelling beetles also had a higher body water content, were able to loose more water and thus, were able to survive desiccation for significantly

longer than the more mesic dwelling beetles. However, the arid dwelling beetles were found to be more sensitive to desiccation temperatures. It was speculated that this sensitivity enables these beetles to survive unpredictable long dry (winter) seasons. A significant positive correlation was apparent between the beetles' body size, their survival time during desiccation and the degree of habitat aridification. Thus, although physiological tolerance to desiccation is an important factor in determining the distribution patterns of the keratin beetles, doubtlessly body size plays a major contributing role.

Die verspreidingspatroon van taksa word deur beide bioties en abiotiese faktore beinvloed. Fisiologiese toleransie teen klimaatuiterstes is een van hierdie faktore en is die onderwerp van hierdie studie. In hierdie tesis is gepoog om 'n verwantskap tussen desikasietoleransie en verspreidingspatrone van keratienkewers (Trogidae) te ondersoek. Aangesien geen inligting oor hierdie kewers se waterbalans beskikbaar was nie, is hulle waterbalans eers bestudeer. Daar is bevind dat die water- en lipiedinhoud, wanneer ten volle gehidreerd, die maksimum waterverlies toleransie, die tempo van waterverlies en oorlewingstyd gedurende dehidrasie vergelykbaar is met die van ander kewers met soortgelyke grootte en habitat. Met die uitsondering van twee spesies wat in dor gebiede leef, is daar gevind dat keratienkewers nie die vermoeë het om te kompenseer deur lipied katabolisme vir waterverlies nie. Soos die meeste ander volwasse kewers, kon geen van die keratienkewers water verkry deur kutikulêre absorpsie van atmosferiese waterdamp nie, maar kon 'n positiewe waterbalans verkry deur vry water te drink. Geen van die keratienkewers wat bestudeer is, was in staat tot osmoregulering nie. Alhoewel die hemolimf osmolariteite van kewers in dor streke hoër was as 1000 mOsm by die helfte van die LT_{50}, was die kewers blykbaar nie nadelig geaffekteer nie. Om fisiologiese verskille in desikasietoleransie van kewers van verskillende klimaatstreke te bepaal, is interspesies desikasietoleransie studies onderneem op elf keratienkewer spesies verkry van ses lokaliteite, asook intraspesies studies op vyf spesies van 'n verskeidenheid van habitatte. Op beide intra- en interspesies vlak is oor die algemeen gevind dat kewers van dor streke morfologies groter en fisiologies meer weerstandbiedend was teen desikasie as die kewers wat in meer matige gebede voorkom. Dit kan gedeeltelik toegeskryf word aan die feit dat die kewers van dor streke groter is, 'n hoër liggaamswaterinhoud het, en dus meer water kan verloor. Dit bring mee dat hulle ontwatering beter kan oorleef vir betekenisvol langer periodes as die kewers wat in meer matige klimaatsomstandighede leef. Daar is egter bevind dat die kewers van die dor streke meer sensitief is vir die temperatuur waarby dehidrasie plaasvind. Daar word gespekuleer dat hierdie sensitiwiteit die spesifieke kewers instaat stel om dor streke tydens matige temperature, te oorleef. 'n Betekenisvolle positiewe korrelasie is gevind tussen die kewers se liggaamsgrootte, oorlewingstyd tydens desikasie en die mate van dorheid van die habitat. Al is fisiologiese toleransie teen ontwatering dus 'n belangrike faktor wat die verspreidingspatrone van keratienkewers bepaal, speel liggaamsgrootte onteenseglik 'n groot bydraende rol.

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All praise and honour to You my Lord Jesus Christ. If You had not stood by me, I would never have had the courage to complete this thesis.

I shall sing to Yahweh all my life,
make music for my God as long as I live.
May my music be pleasing to Him,
for Yahweh gives me joy.
May sinners vanish from the earth,
and the wicked exist no more!

Bless Yahweh my soul.
(Psalm 105: 33-35)

Contents

Chapter 2: Desiccation resistance and water balance in southern African keratin beetles (Coleoptera, Trogidae): the influence of body size and habitat

2.2 Material and Methods

  3.2.2 Experimental procedures

2.3 Results

Chapter 3: Habitat-associated variation in desiccation tolerance of southern African keratin beetles (Coleoptera, Trogidae) : the role of temperature, allometry and phylogeny

Chapter 4: Intraspecific variation in desiccation tolerance in five South African keratin beetles species (Coleoptera, Trogidae)

Chapter 5: Conclusion

Chapter 1: Introduction

Geographical range of species and the influence of physiological tolerance on species distributional patterns

The geographical range of a species, defined as the broad geographical area in which a species may be found more or less permanently (Andrewartha and Birch 1954; Gaston 1991), is determined by ecological interactions between niche requirements and the biotic and abiotic characteristics of the environment (Brown 1995). Although much information is available on species' local and regional distributional ranges, and on the potential factors limiting these ranges (for range limiting factors see Andrewartha and Birch 1954; Brown and Gibson 1983; Gaston 1990; Cox and Moore 1993; Begon, *et al.* 1996), assessing the size and properties of the global geographical range of a species is still problematic. Sampling efforts are often restricted to local areas and global distributional ranges are often inaccurate and available for only a few taxa (Gaston 1990, 1996). There is some evidence that, provided study areas are large enough, global distributions for species may be extrapolated from regional studies (Williams 1988). However, insufficient evidence of this exists to justify its generalized use.

For many taxa, the frequency distribution of range size is right skewed, with many species occupying small ranges and few species having large ranges (Schoener 1987; Gaston and Lawton 1990; Brown 1984; Gaston 1996) but the factors limiting species' geographical ranges are still widely debated. The outer boundary of a species' distributional range occurs where environmental conditions cause the sum of death and emigration to exceed the sum of birth and immigration (Gaston 1990; Brown 1995). A wide variety of factors, both biotic and abiotic have been identified as limiting species range size (eg. Andrewartha and Birch 1954;

Brown and Gibson 1983; Cox and Moore 1993). History is no doubt a major contributor (Brown and Gibson 1983; Taylor and Gotelli 1994; Brown 1995; Begon, *et al.* 1996), i.e. the species' history (rate of speciation, cline formation and colonization ability) as well as the environmental history (plate tectonics, volcanic activity, climatic changes, etc.) which may either result in the eradication of suitable habitats or the formation thereof. Range limitation due to biotic interactions, such as inter- and intraspecific competition, parasitism, predation, etc. has been the focus of a great many recent studies (eg. Brown and Maurer 1989; Currie 1990; Hanski, *et al.* 1991; Pacala and Weiner 1991; Pacala and Tilman 1994; Hurtt and Pacala 1995; Marquet, *et al.* 1995; Begon, *et al.* 1996). It is generally agreed that multiple and complex interactions act as limiting factors in determining geographical range and since these factors vary across time and space (Brown and Gibson 1983; Brown 1984) it is virtually impossible to identify a single factor as the species range limiting factor. Caughley, *et al.* (1988) proposed a technique for determining range limiting factors by examining characteristics of populations, such as birth and death rates, body condition, etc. across the species' geographical range. By identifying factors which limit the "well being" of the population, Caughley, *et al.* (1988) suggested that the range limiting factors may more easily be identified. According to Gaston (1990), this technique is probably of minimal value where more than one limiting factor is involved, which is the rule rather than the exception.

It has been suggested that the distributional range of a species is greatly influenced by the species' migratory, dispersal and colonization abilities (Hansen 1980; Hanski and Zhang 1993; Begon, *et al.* 1996; Gaston 1996) which in turn are closely related to the organisms' body size (Lawton 1990; Cambefort 1994). Large organisms are generally more mobile than smaller ones and in some taxa have been found to be more widely distributed (Brown and Maurer 1987; Cambefort 1994; Brown 1995; Gaston and Blackburn 1996a). Much controversy surrounds the relationship between body size and range size (Gaston and Lawton 1988a, 1988b; Gaston and Blackburn 1996a, 1996b). Some authors speculate that this relationship is largely due to energy constraints (Wright 1983; Brown and Maurer 1989; Lawton 1989; Brown, *et al.* 1993; Currie and Fritz 1993; Cambefort 1994; Basset 1995; Brown 1995; also see Schmidt-Nielsen 1984 and Kozłowski 1996, for the relationship between energy utilization and allometry). Large bodied organisms usually control the greatest portion of natural resources and often outcompete smaller organisms (Schmidt-Nielsen 1984; Brown and Maurer 1986; Cambefort 1994; Basset 1995; Brown 1995; Blackburn and Gaston 1996a). However, because large organisms require high net energy intake per capita, they also require larger distributional ranges in order to meet their nutritional requirements (Brown and Maurer 1989; Cambefort 1994; Basset 1995; Brown 1995). Furthermore, since digestion is retarded in large organisms, nutrients can be extracted from poorer quality resources (Brown 1995) allowing the organisms to become more generalistic in their food requirements and enabling them to achieve larger distributional ranges (Brown 1995; Blackburn and Gaston 1996a). But being large and requiring high energy input limits the number of individuals that a given geographical area can sustain (Brown 1995; Gaston and Blackburn 1996b). Thus, large organisms, possibly with wide geographical ranges, often attain smaller population sizes (Brown and Maurer 1989; Lawton 1989; Stork and Blackburn 1993; Brown 1995; Gaston and Blackburn 1996b).

There is a definite relationship between body size, geographical range size and population density (Brown 1984, 1995; Brown and Maurer 1989; Blackburn and Gaston 1996b; Gaston and Blackburn 1996b) but the exact nature of this relationship is very controversial. In many taxa a positive correlation is evident between body size and distributional range size (Brown and Gibson 1983; Brown and Maurer 1987; Gaston and

Blackburn 1996a), and between population abundance and the size of the species' geographical range (Hanski 1982; Brown 1984; Gaston 1988; Brown and Maurer 1989; Gaston and Lawton 1990; Lawton 1990; Currie and Fritz 1993) However, in other taxa these factors have been found to be negatively correlated (Schoener 1987; Gaston and Lawton 1988a, 1988b) and in some organisms no relationship between body size, population density and/or range size are apparent (Cotgreave, *et al.* 1993). Once again, the relationship between body size, population density and range size can partially be explained in terms of energy constraints and reproductive potential. Smaller organisms have a higher intrinsic rate of increase (Remmert 1981; Schmidt-Nielsen 1984; Lawton 1989; Brown, *et al.* 1993), require less food per individual (Brown 1995) and can thus achieve a high population density in a given area. High population densities increase the potential for expanding the species' range and protect the species from local extinction (Lawton 1989; Remmert 1981; Gaston and Blackburn 1996a; Hanski, *et al.* 1996). The species that are able to maintain peak rates of increase over large areas are likely to have the largest geographical distributions (Gaston 1990). However, as the organisms' body size decrease so do their tolerance to environmental variations, i.e. abiotic factors (Remmert 1981; Willmer 1982; Schmidt-Nielsen 1984; Lawton 1989; Blackburn and Gaston 1996a), making the species more susceptible to local extinction should unfavourable environmental conditions prevail. Yet despite the organisms' small body size, if they are able to adapt physiologically, behaviourally or otherwise in order to resist/tolerate these harsh conditions, theoretically, the species should still be able to successfully colonize these less than favourable areas (see Slobodchikoff 1983; Schultz and Hadley 1987; Kaspari 1993; Zachariassen and Einarson 1993; and Sømme 1994 for examples). Ultimately, the factors limiting most species' distributional range is believed to be abiotic, mainly climatic (Myers and Giller 1988; Brown 1995).

Climate has long been recognized as a limiting factor on distributional range (Andrewartha and Birch 1954; Mayr 1956; Stevens 1989; Cushman, *et al.* 1993). The species with the widest distributional range must ultimately be the one whose tolerance to environmental variation is superior (Stevens 1989; Brown 1984). Body size buffers against environmental variation (Roff 1981; Willmer 1982) but the organisms' physiological tolerance is no doubt a major contributing factor. The role of physiological adaptation in species distribution patterns has received considerable attention recently (eg. Brown and Lee 1969; Arad, *et al.* 1989, 1992, 1993; Lighton and Feener 1989; Parmenter, *et al.* 1989; Block, *et al.* 1990; Arad 1990, 1993, 1995; Ring, *et al.* 1990; Chown and van Drimmelen 1992; Chown 1993; Crafford and Chown 1993) but many of these comparative ecophysiological studies have focused on very heterogenous taxa. Thus, conclusions reached are often limited by the heterogeneity of the taxa compared. Differences in phylogeny, life history, biology and morphology severely complicate physiological comparisons (see Coddington 1988 and Leroi, *et al.* 1994 for further discussion).

Keratin beetles (Trogidae, Scarabaeoidea) are ideally suited for comparative physiological studies. This is a small, monophyletic family with a cosmopolitan distribution (Scholtz 1980, 1986, 1993). The family is represented by only two African genera, *Trox* Fabricius and *Omorgus* Erichson. The most plesiomorphic of the two genera, *Trox* has a more temperate distribution and is morphologically smaller than *Omorgus*, which occurs predominantly in arid areas (Scholtz 1980, 1986; Scholtz and Caveney 1988). It is believed that all keratin beetles have very homogeneous biologies. Both larvae and adults are facultative necrophages and feed on a variety of keratinous matter, such as skin and hair of mammalian carcasses, carnivore excrement, owl pellets, and even old carpets and felt hats (Scholtz 1986, 1993). The adult beetles are active at dawn and dusk when the ambient temperature is mild and the relative humidity favourable. They avoid unfavourable climatic conditions by entering into a state of diapause or quiescence buried in the soil below the food source (Scholtz and Caveney 1988, 1992; Friedländer and Scholtz 1993). Keratin beetles are usually the last in a succession of decomposers to utilize a carcass and remain at the carcass as long as it remains a viable food source (for several successive generations, Scholtz 1986; Scholtz and Caveney 1988). Despite the trogids' seemingly homogeneous biology and morphology, differing only in body size (Chown, unpublished data), these beetles have very varied geographical distributional ranges. Some of the species are restricted to specific climate regimes while others occur both in mesic and arid areas (see Scholtz 1980 for species' distributional maps). Thus, it is possible that these beetles' physiological tolerance to temperature and desiccation plays a vital role in determining their distribution patterns.

For the purpose of this study, desiccation tolerance will be used as an indicator of the role played by physiology as a possible factor limiting the distributional patterns of keratin beetle species. Water balance and desiccation tolerance of several trogid species from a variety of habitats will be examined in order to determine whether arid dwelling beetles are physiologically more tolerant to desiccation than their mesic counterparts. These comparative studies will be conducted at both intra- and interspecific levels. Furthermore, desiccation trials will be carried out at various temperatures so as to ascertain the temperature sensitivity of the beetles from the various habitats. Since *Trox* and *Omorgus* have different general distributions (*Trox* occurring predominantly in temperate areas and *Omorgus* in arid regions), the influence of phylogeny on desiccation tolerance will be investigated by comparing tolerance at a generic, species and population level. As yet, nothing is known of keratin beetles' physiology. Thus, this study will first of all ascertain the physiological basis of water balance in the keratin beetles, i.e. the body water and lipid content of hydrated beetles, the maximum amount of water loss that they are able to tolerate during desiccation, their survival time under desiccating conditions, the rate at which they lose water, their ability to utilize metabolic water to compensate for water loss, their osmoregulatory abilities and their ability to replenish their body water content through drinking free water, and absorbing atmospheric water. Body size plays a significant role in distributional patterns in many taxa (see discussion above). This study will thus also examine the correlation between keratin beetles' body size and the degree of aridification of the their habitats. It is speculated that with increasing aridification of the habitat the species body size should increase, with possible concomitant increases in their physiological tolerance to desiccation.

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To fasten your attention on Wisdom is to gain perfect understanding.
If you look for her, you will soon find peace of mind,
because she will be looking for those who are worthy of her.

Wisdom of Solomon 6:15-16

Chapter 2

Desiccation resistance and water balance in southern African keratin beetles (Coleoptera, Trogidae): the influence of body size and habitat

2.1 Introduction

Temperature and water availability play major roles in determining species' distributional patterns both directly, by affecting their physiological performances, and indirectly, by influencing population dynamics and species interactions (Kingsolver 1989; Dunson and Travis 1991; Chown 1993; Cox and Moore 1993). This is true also of insect ectotherms. Consequently, many studies have focused on the physiological response of these organisms to geographic variations in temperature, humidity and water regimes (for reviews see Edney 1977; Hadley 1994a), often demonstrating physiological equilibria between the organisms and their environments (e.g. Chown 1993). However, the conclusions of many comparative studies may be limited by the broad range of taxa they include. If species differ not only in their habitat associations, but also in their life histories, body size and shape, and extent of phylogenetic propinquity, interpretation of the physiological data may be confounded by the presence of uncontrolled variables (see Harvey and Pagel 1991; Garland and Adolph 1994; Ward and Seely 1996). Hence conclusions concerning physiological adaptations in such studies may be unsubstantiated at best, and spurious at worst (see additional discussion in Coddington 1988; and Leroi, *et al.* 1994).

In this context, keratin beetles (Scarabaeoidea: Trogidae) are physiologically interesting. They are a small, monophyletic family (Scholtz 1986, 1993), with a homogeneous biology (Scholtz and Caveney 1988, 1992), and limited morphological variation (Chown, unpublished data), but with a distributional range that extends over a large range of climatic zones (for species distributional ranges see Scholtz 1980). In addition, the family comprises only three genera, of which two are African. Species in the genus *Trox* Fabricius occur in more temperate areas, while those in *Omorgus* Erichson, prefer more arid regions (Scholtz 1980, 1986, 1993; Scholtz and Caveney 1988).

Both larval and adult trogids are facultative necrophages and feed on a great variety of keratinous matter (Scholtz 1986, 1993). Hence they are usually the last in a series of decomposers to utilize a vertebrate carcass, and may remain at a single site for many successive generations (Scholtz 1986; Scholtz and Caveney 1988). In the arid, and other regions of southern Africa, adult *Omorgus* species have a biphasic, crepuscular activity pattern. The sunset activity phase coincides with decreasing ambient temperature, and according to Scholtz and Caveney (1992), relatively high temperatures may be necessary for social interaction. Activity declines during the middle of the night, but is resumed at dawn when most beetles feed. Temperatures are lowest at this time, but humidity reaches its highest daily level. Scholtz and Caveney (1992) suggested that because these beetles may be under water stress, feeding when humidity is high could contribute to a positive water balance if food is saturated and could also contribute to a lower rates of transpiratory water loss.

The biphasic activity pattern persists for several weeks after rain, but as conditions dry out activity declines and the adults eventually enter quiescence or diapause (Scholtz and Caveney 1988, 1992; Friedländer and Scholtz 1993). In arid areas, the long-lived adults may remain in this state for a considerable period (at least nine months may pass between rainfall events in the Kalahari, Rasa pers comm) experiencing very dry soil conditions. Larvae show similar responses and may also encounter relatively dry soils, although it seems unlikely that they can survive very dry conditions (Scholtz and Caveney 1988). Only the short-lived, egg and pupal stages are especially vulnerable to desiccation.

Therefore, it appears that keratin beetles, and particularly their adult stages, must be subject to a considerable range of water stresses, ranging from those associated with carcasses in the arid Kalahari desert to those found at more mesic tropical sites. Indeed, at the conclusion of their behavioural studies, Scholtz and Caveney (1988, 1992) implied that trogids from arid regions show a range of physiological adaptations, including elevated lipid contents during quiescence, to cope with dry conditions. Similarly, Scholtz (1981) suggested that water stress is an important environmental variable selecting for aptery in xeric trogids.

Hence keratin beetles may make particularly useful subjects to investigate geographic variation in desiccation resistance, and its importance in delimiting species geographic ranges (for an introduction to the importance of such ecophysiological studies see Andrewartha and Birch 1954; Johnston and Bennett 1996). However, no studies of the water relations of trogid beetles have been undertaken. Therefore, I examined desiccation resistance and water balance of the adults of seven trogid species from a variety of habitats in southern Africa, as part of a larger study of the interactions between geographic distribution, water balance and morphology of the species in this family. My aims were to determine what desiccation resistance strategies are employed by these beetles (see Edney 1977; Hadley 1994a for discussion), whether they are able to control haemolymph osmolality during desiccation, and whether these strategies vary geographically (i.e. with habitat water status).

2.2 Material and Methods

2.2.1 Study sites and animals

All beetles were collected between November 1991 and March 1995 from ungulate carcases and skins. Based on the availability of specimens, the following species were utilized in the study: *Omorgus asperulatus* (Harold) and *O. freyi* (Haaf) which occur in the western semi-desert to desert regions of southern Africa (the latter species is endemic to the Kalahari desert), *O. radula* (Erichson) and *Trox consimilis* Haaf which occur predominantly in arid savanna regions, *O. melancholicus* (Fahraeus) and *T. squamiger* Roth which are moist savanna to temperate species, and *O. squalidus* (Olivier), a eurytopic species widely encountered throughout southern African habitats (see Scholtz 1980). Details of the biomes and climatic conditions at the collection sites are provided in Table 1. All beetles were returned to the laboratory within one week of collection and acclimated for two to four weeks at 27±1.0°C (12L:12D) with free access to food and water.

Table 1. Biomes and climatic data at species collection sites.

Collection Site	Species	Latitude/Longitude	Biome
Twee Rivieren, Kalahari Gemsbok Park, Northern Cape	*O. asperulatus* *O. freyi*	26°28'S 20°37'E	Kalahari thornveld
Boekenhoutskloof, Gauteng	*O. melancholicus* *O. squalidus* *T. consimilis*	25°35'S 28°29'E	Sourish mixed bushveld
Suikerbosrand Nature Reserve, Gauteng	*T. squamiger*	26°49'S 27°26'E	Cymbopogon-Themeda veld
Skukuza, Kruger National Park, Mpumalanga	*O. radula*	24°59'S 31°36'E	Lowveld savanna

Collection site	Mean annual rainfall	Mean July min temp	Mean January max temp
Twee Rivieren, Kalahari Gemsbok Park, Northern Cape	210.0 mm	1.1°C (can drop to below -10°C)	36.4°C (temp. often exceeds 45°C)
Boekenhoutskloof, Gauteng	621.6 mm	1.1°C	29.7°C
Suikerbosrand Nature Reserve, Gauteng	531.5 mm	0.4°C	29.4°C
Skukuza, Kruger National Park, Mpumalanga	564.2 mm	6.7°C	32.9°C

All climatic data, averaged over at least five years, were obtained from The Institute of Soil, Climate and Water, Department of Agriculture, Pretoria, RSA and The Weather Bureau, Pretoria, RSA. Biomes taken from Acocks (1988)

2.2.2 Experimental procedure

2.2.2.1 Desiccation resistance

Desiccation resistance was determined gravimetrically (Ahearn 1970; Naidu and Hattingh 1986, 1988; Chown 1993). Prior to experimentation, beetles were starved for 24 h in moist soil in order to clear their digestive tracts. These were individually numbered and weighed on a Sartorius electronic microbalance accurate to 0.1 mg (fresh mass, FM). Body water content (g and % FM), was determined by drying the beetles to constant mass at 60°C (dry mass, DM) and calculating the difference between DM and FM. For determination of lipid content, a second group of beetles, used for an examination of lipid catabolism (see below) was treated in a similar fashion. However, following drying, individual specimens were subsequently cut into three sections (the three tagmata) to increase solvent penetration, and lipids extracted using three rotations (24 h each) of a 2:1 methanol-chloroform solution at room temperature (Naidu and Hattingh 1985). Lipid-free dry mass was then subtracted from DM to provide a measure of lipid content (g and % DM). The live beetles were placed in desiccation chambers over silica gel (r.h. < 5%, determined using a Novasina electronic thermohygrometer), at 27±1.0°C (L:D 12:12) and weighed at 24 h intervals until 100% mortality of the sample. Maximum water loss tolerated before death (g and % FM), time to maximum water loss (h), and rate of water loss (g h^{-1} and % FM h^{-1}), were calculated using the values recorded in the time interval immediately prior to the death of each individual. For each species, time to 50% (LT_{50}) and 100% (LT_{100}) mortality of the sample were also determined. Since no excretion of solid waste was observed during the course of the experiment, it was presumed that mass loss was equal to respiratory and incidental water loss (see Wharton 1985).

To investigate differences among species, these data were subjected to single classification analyses of variance (Sokal and Rohlf 1995) and multi-species comparisons were made using 95% Tukey HSD intervals. However, because initial differences in body mass may confound comparisons of absolute rates and tolerances (e.g. Packard and Boardman 1988; Chown 1993), least-squares linear regression analyses were used to determine whether there were significant relationships among species, between fresh mass and initial water content (g) and lipid content (g), and fresh mass (g) and maximum tolerable water loss (g), time taken to maximum water loss (h), and rate of water loss (g h^{-1}). Where the coefficient of determination (r^2) of these regressions exceeded 30%, the data were corrected for body mass, using covariance analysis, and the corrected means compared using Tukey's HSD intervals (see Packard and Boardman 1987, 1988).

2.2.2.2 Osmoregulation, rehydration and the production of metabolic water

Osmoregulatory ability, change in body lipid content over time, and the ability of beetles to replenish body water, were investigated using methods similar to those outlined above. The following species were studied: *O. asperulatus* (n = 144), *O. freyi* (n = 102), *O. squalidus* (n = 49) and *O. radula* (n = 55). *Omorgus melancholicus*, *T. consimilis* and *T. squamiger* were not utilized because of a shortage of specimens. Beetles were acclimated as above. Prior to the trial, five to ten beetles were removed from the sample and individually marked. They were weighed and a haemolymph sample (5-10 ul) was extracted from the coxal cavity of each specimen using a micro-haematocrit tube. Haemolymph osmolality was then determined using a Wescor 5120 B vapour pressure osmometer. Beetles, from which haemolymph had been extracted, were killed by freezing at -12°C, and their water and lipid contents determined. The beetles' body water content was not corrected for lost haemolymph since the volumes removed were extremely small (5-10 ul). The remaining beetles were then placed in desiccation chambers at 27°C (see below). At 24 h intervals, a group of dividuals of each species was removed from the chambers, weighed, subjected to haemolymph extraction for osmolality determinations, dried to constant mass and then subjected to lipid extraction (procedures given above). This was done until each species had reached approximately half the LT_{50} value determined in the desiccation resistance trials. (In some cases, small haemolymph volumes necessitated the pooling of samples to ensure osmometer accuracy). The surviving beetles were then divided into two groups and placed in humidity chambers (r.h. 97±2.0%). In addition to the high humidity, the first group of beetles was given free access to drinking water while the second group was not. The beetles were weighed at regular intervals and remained in the chambers until they reached a stable mass. Unfortunately, beetles gained access to condensed water in the “high humidity” chambers thus compromising the experimental design. Although repetition of the experiment was desireable, this could not be done because of a lack of available specimens.

Changes in the beetles' water content are expressed as a percentage of the initial body water content. The beetles' osmoregulatory abilities were determined using a linear least-squares regression analyses of percentage body water loss and haemolymph osmolality. The regression was plotted together with the predicted osmolality determined using Hadley's (1994a) formula, $\pi_e = (\pi_i \text{ x } V_i)/V_e$. Where π is osmolality, V is body water content, *i* is initial and *e* is experimental. The ability of beetles to produce metabolic water via lipid metabolism was investigated using a least-squares linear regression of body lipid content (% DM, and % lipid-free DM) on percentage body water loss (Naidu and Hattingh 1988; Chown, *et al.* 1995). An additional experiment, using all seven species, was undertaken to determine the ability of the beetles to use lipids for metabolic water production during desiccation. Beetles were weighed individually and divided randomly into two groups. The first group of beetles was killed by freezing at -12°C and served as a control group. The second group (experimental group) was dehydrated at 27°C, as described above, until 100% mortality. At the end of the trial, body lipid content was determined for the control and the experimental groups, and compared using ANOVA. Lipid content (g) was mass-adjusted prior to the ANOVA (see above) in *O. asperulatus*, *O. radula* and *T. squamiger*, due to significant differences in fresh mass between the control and experimental groups.

2.3 Results

2.3.1 Water balance

When data for all species were pooled, significant relationships (and with $r^2 > 30\%$) were found between fresh mass and initial body water and lipid contents (g), and between fresh mass (g) and maximum tolerable water loss (g), time to maximum water loss (h), and the rate of water loss (g h^{-1}) (Table 2).

Table 2. Relationship between fresh mass (g) and body water content (g), lipid content (g), maximum tolerable water loss (g), time taken to maximum water loss (h) and rate of water loss (g h^{-1}), of pooled data for *Omorgus asperulatus*, *O. freyi*, *O. melancholicus*, *O. squalidus*, *O. radula*, *Trox consimilis* and *T. squamiger* at 27°C. The regression equations are y = b mass + a. n = 88 and df = 87 for all regressions except lipid content, where n = 110 and df = 109.

	Slope±SE	Intercept±SE	r^2	F	P
Water content	0.58±0.009	-0.006±0.003	97.8%	3924.5	<0.0001
Lipid content	0.12±0.008	-0.002±0.002	66.7%	216.2	<0.0001
Max. water loss	0.41±0.013	-0.01±0.004	92.2%	1028.0	<0.0001
Time to max water loss	477.5±70.8	118.8±20.6	34.3%	45.5	<0.0001
Rate of water loss	0.00094±0.0001	0.0002±0.00004	34.5%	45.8	<0.0001

Summary statistics for initial fresh mass and water and lipid contents, and maximum tolerable water loss, time taken to maximum water loss, and the rate of water loss, and their mass-adjusted values are provided for each of the seven species in Tables 3 and 4. Both fresh mass (F = 185, P < 0.0001, df = 88, Fig. 1a) and body water content (F = 143, P < 0.0001, df = 88, Fig. 1b) were significantly greater in *O. asperulatus*, *O. freyi*, and *O. squalidus* than in the other species, which showed similar values. Furthermore, *O. freyi*, had significantly higher body lipid contents than the other species (F = 97.1, P < 0.0001, df = 109, Fig. 1c) even after adjusting lipid content for fresh mass (F = 53.9, P < 0.0001, df = 109). No significant differences were found in the trogids' mass-adjusted body water content (F=1.37, P = 0.236, df = 88).

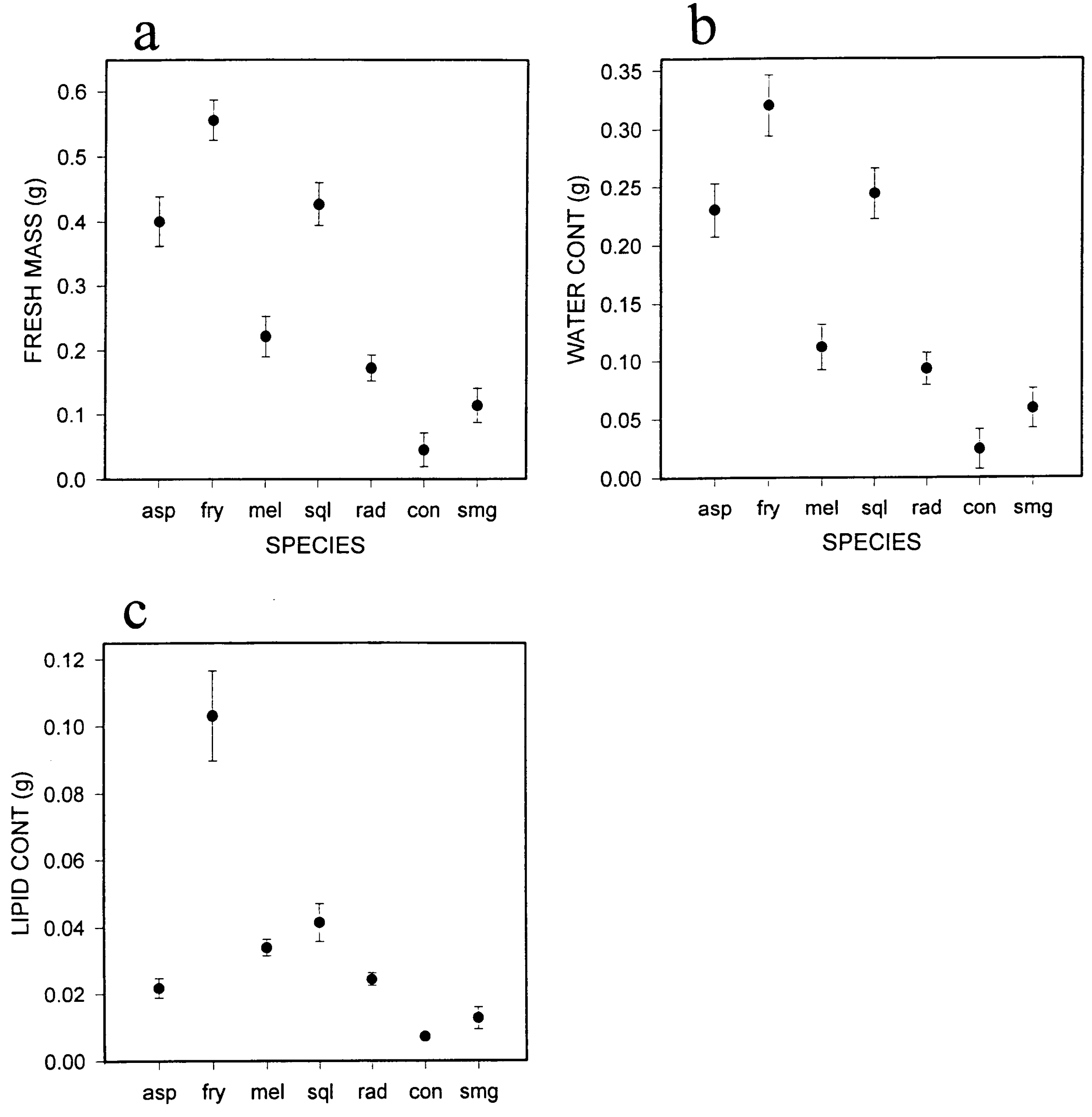

Figure 1 a-c Mean and 95% Tukey HSD intervals of (a) fresh mass (b) body water content; (c) body lipid content; for the seven trogid species, *Omorgus asperulatus* (asp); *O. freyi* (fry); *O. melancholicus* (mel); *O. squalidus* (sql); *O. radula* (rad); *Trox consimilis* (con); *T. squamiger* (smg).

Table 3. Mean ± SE of fresh mass (FM, g), water (g and % FM), lipid content (g and % DM), and adjusted (ADJ) water and lipid content (g) for each species. In column n (= sample sizes) values with an asterisk refer to lipid content assessments.

Species	FM (g)	Water (g)	Water (%)	Lipid (g)	Lipid (%)	ADJ water	ADJ lipid	n
O. asperulatus	0.40±0.015	0.23±0.009	58.2±1.2	0.022±0.002	15.9±0.75	0.14±0.005	0.008±0.002	8 or 18*
O. freyi	0.56±0.037	0.32±0.025	56.4±1.7	0.104±0.010	31.4±0.75	0.13±0.009	0.060±0.003	10 or 6*
O. melancholicus	0.22±0.007	0.11±0.004	51.1±1.9	0.034±0.002	29.0±0.77	0.12±0.004	0.037±0.002	10 or 15*
O. squalidus	0.43±0.014	0.24±0.008	57.5±1.9	0.041±0.003	20.7±1.4	0.13±0.008	0.019±0.003	9 or 14*
O. radula	0.17±0.005	0.09±0.004	53.5±1.1	0.025±0.002	25.1±1.67	0.13±0.002	0.028±0.002	23 or 17*
T. consimilis	0.05±0.002	0.02±0.0009	54.8±1.4	0.007±0.0002	29.3±0.76	0.14±0.0006	0.030±0.0002	15 or 16*
T. squamiger	0.11±0.006	0.06±0.004	51.8±0.6	0.013±0.001	21.8±1.15	0.13±0.0006	0.028±0.0008	14 or 24*

Maximum tolerable water loss showed a pattern almost identical to that of fresh mass and water content; *O. asperulatus*, *O. freyi* and *O. squalidus* were able to tolerate significantly greater water losses than the other four species (F = 99.65, P < 0.0001, df = 88, Fig 2a), with *O. freyi* having the highest tolerance. *Omorgus squalidus* had a much shorter time to maximum water loss than did either of the xeric species *O. asperulatus* and *O. freyi*, and fell within the same range as the more mesic *O. melancholicus*, *O. radula*, *T. consimilis* and *T. squamiger* (F = 50.89, P < 0.0001, df = 88, Fig. 2b). The significant differences between species in rates of water loss (F = 67.37, $P < 0.0001$, df = 88, Fig. 2c), was clearly habitat related. The *Omorgus* species showed a trend in increasing water loss rates with a decrease in aridity and the same pattern was evident in the *Trox* species. However, the *Trox* species tended to have much lower water loss rates than those of *Omorgus*.

Table 4. Mean ± SE of maximum water loss tolerated (MAX, g and % FM), maximum dehydration time tolerated (TIME, h), the rate of water loss (RATE, g h^{-1} and % FM h^{-1}), and the mass adjusted values (ADJ) of each species.

Species	MAX (g)	MAX (%FM)	TIME (h)	RATE (g h^{-1})
O. asperulatus	0.23±0.006	41.3±1.8	467.8±31.1	0.00036±0.00002
O. freyi	0.23±0.021	40.4±1.6	440.9±39.4	0.00053±0.00004
O. melancholicus	0.06±0.007	27.3±3.3	86.6±10.4	0.0007±0.00002
O. squalidus	0.15±0.011	36.2±3.1	165.6±11.6	0.00093±0.00005
O. radula	0.06±0.003	35.7±1.1	236.6±9.7	0.00026±0.000009
T. consimilis	0.02±0.001	35.6±2.1	210.2±16.2	0.000081±0.000006
T. squamiger	0.03±0.002	27.0±0.8	108.1±16.2	0.00039±0.000006

Species	RATE (% h^{-1})	ADJ MAX	ADJ TIME	ADJ RATE
O. asperulatus	0.09±0.004	0.96±0.007	391.4±35.6	0.00021±0.00002
O. freyi	0.10±0.008	0.95±0.008	287.9±31.3	0.00023±0.00004
O. melancholicus	0.32±0.009	0.07±0.007	93.8±11.2	0.00071±0.00002
O. squalidus	0.22±0.02	0.07±0.013	74.5±12.4	0.00075±0.00006
O. radula	0.16±0.007	0.09±0.002	266.5±8.7	0.00032±0.00001
T. consimilis	0.18±0.02	0.95±0.001	301.6±16.0	0.00026±0.000007
T. squamiger	0.35±0.05	0.08±0.001	166.3±16.0	0.0005±0.00006

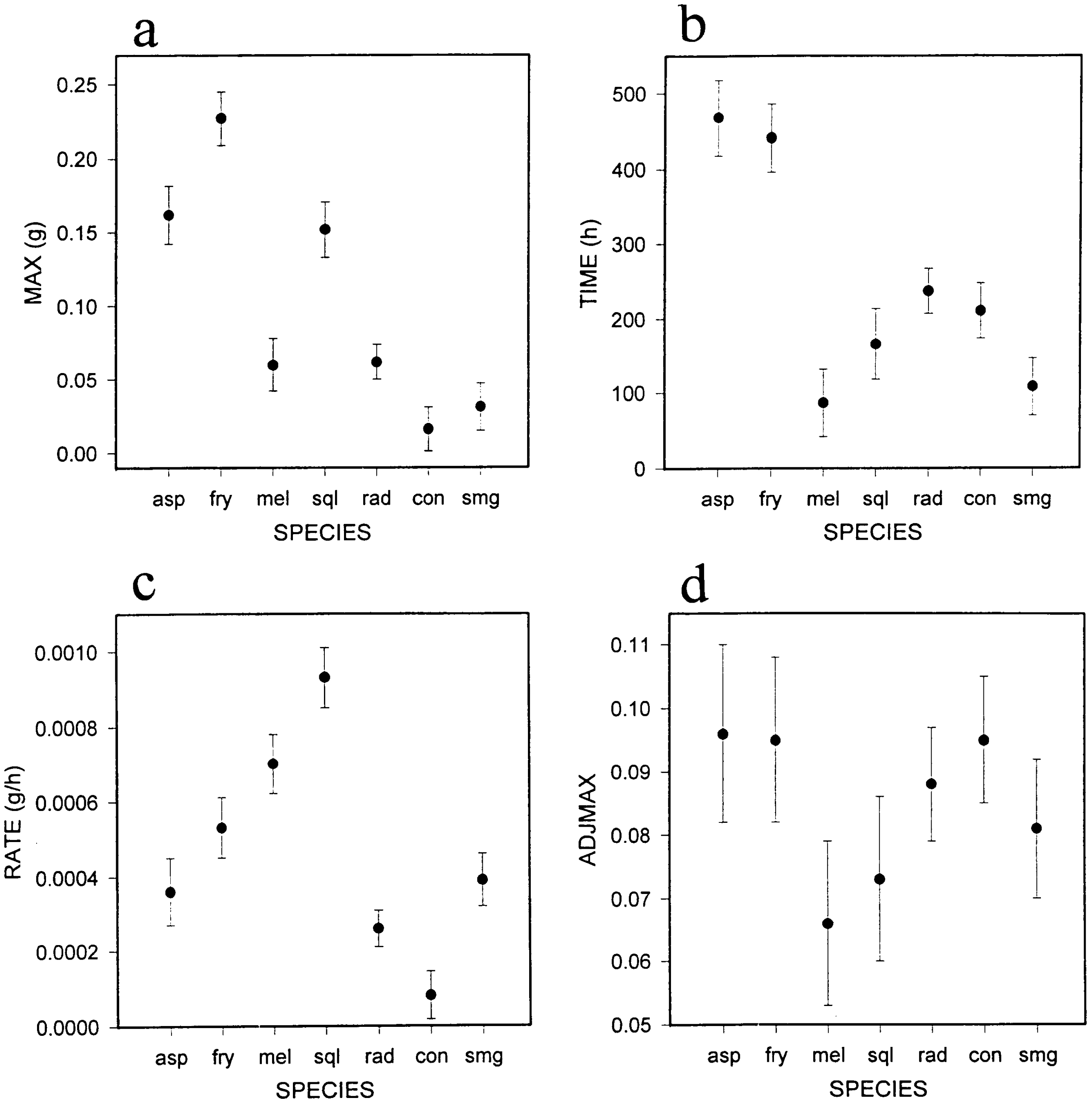

Figure 2 a-d Mean and 95% Tukey HSD intervals of (a) maximum water loss; (b) time to maximum water loss; (c) dehydration rate; (d) adjusted maximum water loss, for the seven trogid species, *Omorgus asperulatus* (asp); *O. freyi* (fry); *O. melancholicus* (mel); *O. squalidus* (sql); *O. radula* (rad); *Trox consimilis* (con); *T. squamiger* (smg).

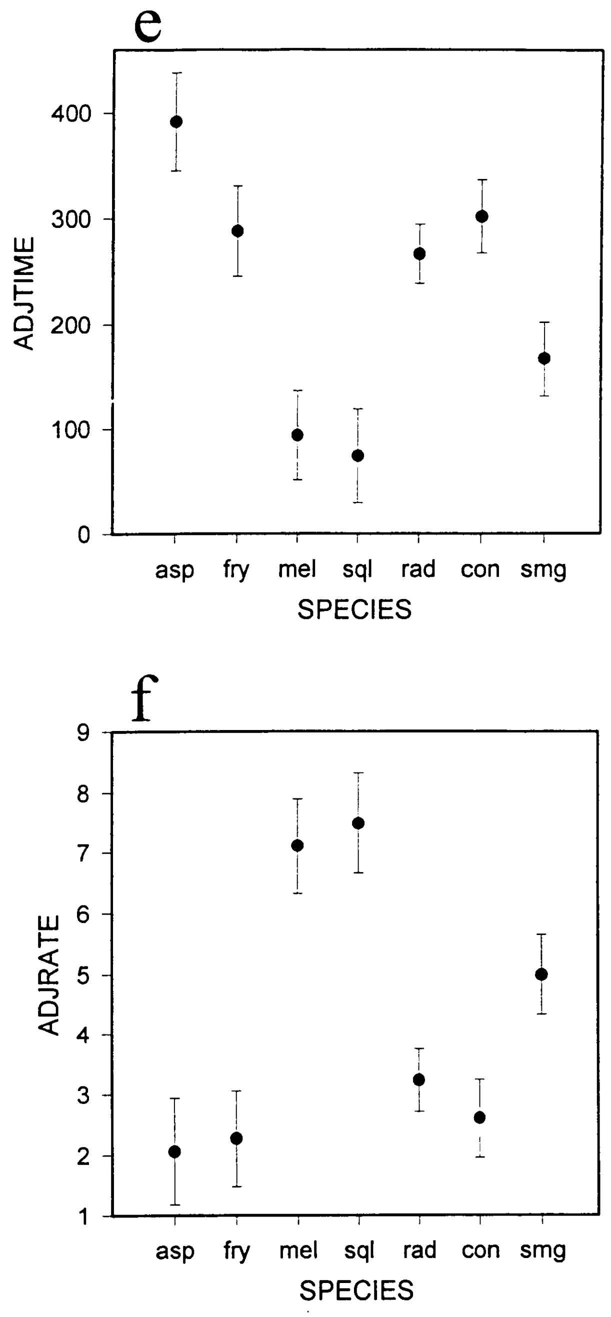

Figure 2 e-f Mean and 95% Tukey HSD intervals of (e) adjusted maximum dehydration time; (f) adjusted dehydration rate, for the seven trogid species, *Omorgus asperulatus* (asp); *O. freyi* (fry); *O. melancholicus* (mel); *O. squalidus* (sql); *O. radula* (rad); *Trox consimilis* (con); *T. squamiger* (smg).

Mass-adjusted maximum tolerable water loss was generally very similar in all seven trogid species although there were some between-species differences (F = 4.04, P = 0.0013, df = 88, Fig. 2d). However, there were significant differences in both adjusted time to maximum water loss (F = 34.62, P < 0.0001, df = 88) and rate of water loss (F = 40.0, P < 0.0001, df = 88), in which the species showed inverse, but almost identical groupings (Figs 2e and 2f). The predominantly arid dwelling *O. asperulatus*, *O. freyi*, *O. radula* and *T. consimilis* formed the first group with low water loss rates and long times to maximum water loss. The second, intermediate group comprised a single species, the moist-savanna *T. squamiger*, while the third group, with the highest water loss rates and shortest times to maximum water loss contained the moist savanna *O. melancholicus* and the eurytopic *O. squalidus*. Similar patterns were evident in the LT_{50} values, but the trend was less clear for LT_{100} (Table 5).

Table 5. The LT_{50} and LT_{100} for the trogid beetles desiccated at 27°C.

Species	LT_{50} (h)	LT_{100} (h)
O. asperulatus	504	600
O. freyi	480	1056
O. melancholicus	108	167
O. squalidus	168	336
O. radula	272	321
T. consimilis	216	264
T. squamiger	96	216

2.3.2 Osmoregulation, rehydration and the production of metabolic water

The mean change in body water content, expressed as a percentage of the initial water content, over time for each species, is shown in Figs 3a-d. After 192 h, *O. asperulatus* had lost 51% and *O. freyi* 43% of their initial body water content (26% FM and 24% FM, respectively). *Omorgus squalidus* lost 26% of its initial body water (14% FM) after 72 h of dehydration and *O. radula* lost 44% (initial body water 25% FM) after 120 h of desiccation. During rehydration, those beetles with access to drinking water replenished their body water to a significantly greater extent than those without (Figs 3a-d). Observations suggested that those beetles without access to free water were in fact utilizing droplets formed by condensation on the sides of the humidity chamber to replenish lost water.

Initial haemolymph osmolalities were high in all species (*O. asperulatus*: 635±15 mOsm, n = 10; *O. freyi*: 568.5±11.49 mOsm, n = 10; *O. squalidus*: 722.1±20.12 mOsm, n = 8; *O. radula*: 670.0±11.0 mOsm, n = 7), and increases during the course of dehydration in each of the four species are shown in Figs 4a-d. In all the species, the actual and predicted increases in haemolymph osmolality during desiccation were very similar (using an ANCOVA *O. asperulatus*: P = 0.38, F = 0.81, df = 65; *O. freyi*: P = 0.11, F = 2.59, df = 93; *O. squalidus*: P = 0.57, F = 0.35, df = 43; *O. radula*: P = 0.57, F = 0.34, df = 39). At maximum water loss, haemolymph osmolalities in all four species were extraordinarily high (Mean ± SE, *O. asperulatus*: 1082±57 mOsm; *O. freyi*: 1059±145 mOsm; *O. squalidus*: 939±35 mOsm; *O. radula*: 820±43 mOsm).

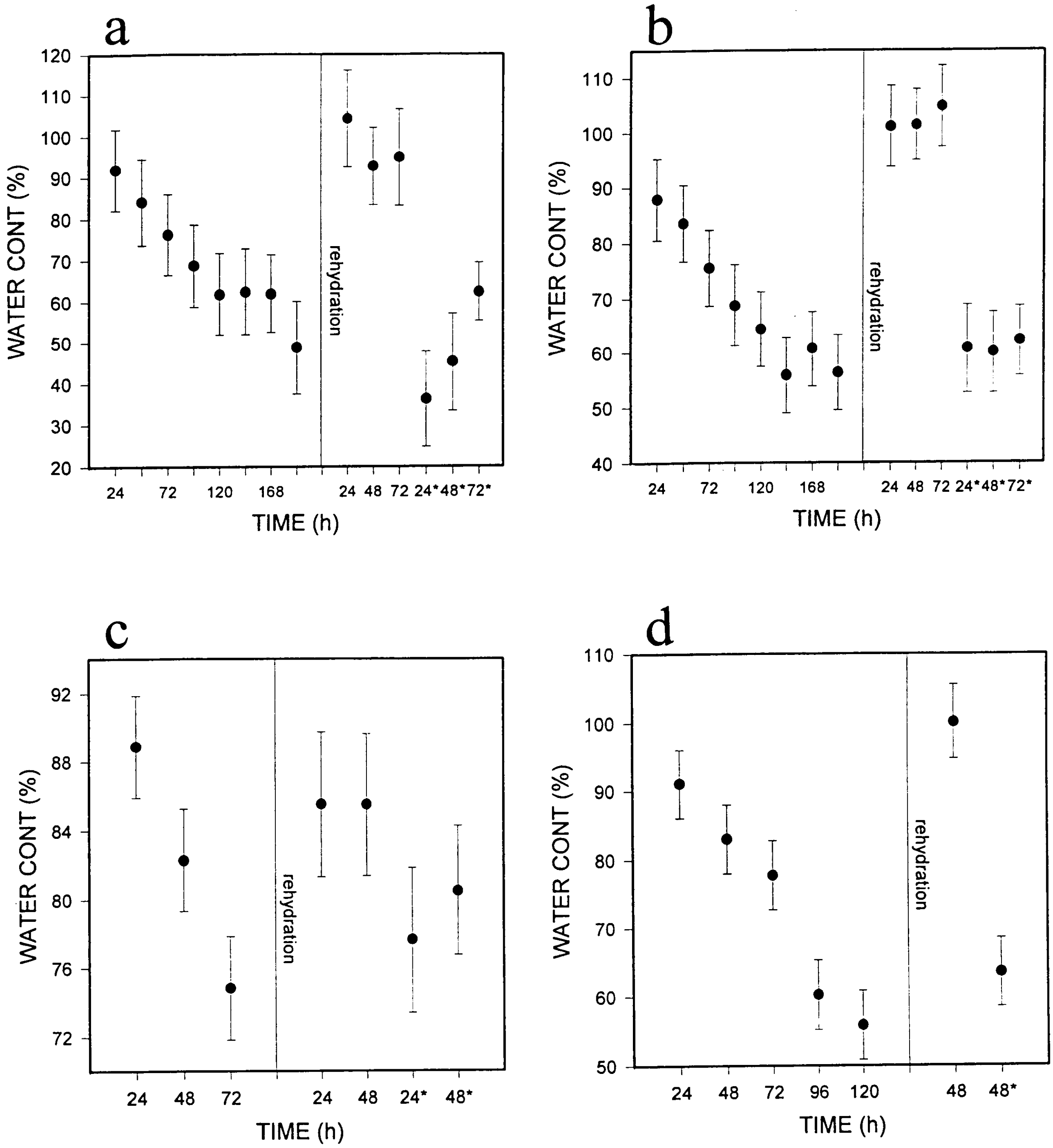

Figure 3 a-d Mean ± SE water content expressed as a percentage of initial body water content over the experimental time for (a) *Omorgus asperulatus*; (b) *O. freyi*; (c) *O. squalidus*; (d) *O. radula* (* beetles rehydrated without access to drinking water).

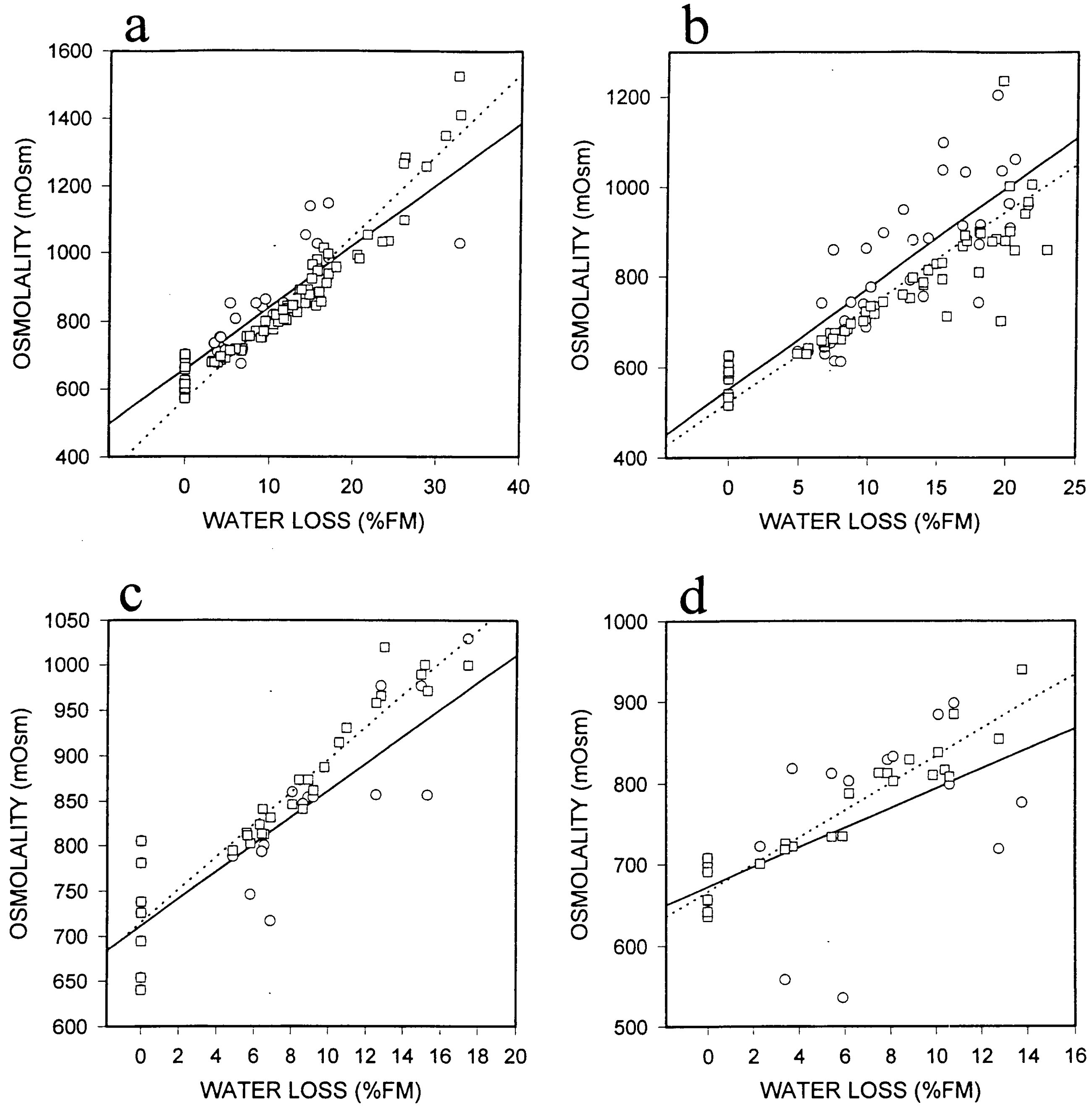

Figure 4 a-d Haemolymph osmolality (____ ; ○) and expected osmolality (······ ; □) in mOsm as a function of percentage water loss for (a) *Omorgus asperulatus* (haemolymph osmolality 0.72 = 659.9 + 18.1x, expected osmolality 0.91 = 550.7 + 26.3x where x = % water loss); (b) *O. freyi* (haemolymph osmolality 0.76 = 551.0 + 22.3x, expected osmolality 0.85 = 522.0 + 21.1x); (c) *O. squalidus* (haemolymph osmolality 0.74 = 712.0 + 145.0x, expected osmolality 0.89 = 715.8 + 8.0x); (d) *O. radula* (haemolymph osmolality 0.33 = 672.3 + 12.2x, expected osmolality 0.91 = 666.3 + 16.8x). The lines are linear regression lines, regression equations are y = a + b % water loss.

No significant relationships were found between lipid content (% DM and % lipid-free DM) and water loss (% FM) in any of the species, with the exception of *O. freyi*, where a significant, but poor relationship was found (Table 6).

Table 6. The relationship between body lipid content expressed as % dry mass (% DM) and total water loss (% FM), and body lipid content expressed as % lipid-free dry mass (% LFDM) and total water loss (% FM). The regression equations are y = a + b % water loss.

Species	Slope±SE	Intercept±SE	df	r^2	P
O. asperulatus					
%DM	-0.09±0.07	23.9±1.30	84	1.8%	0.22
%LFDM	-0.15±0.12	32.3±2.10	84	1.8%	0.22
O. freyi					
%DM	-0.16±0.08	23.5±1.21	64	6.2%	0.04
%LFDM	-0.23±0.12	30.8±1.93	64	5.4%	0.06
O. melancholicus					
%DM	0.13±0.15	25.1±1.40	29	2.3%	0.42
%LFDM	0.26±0.30	33.9±2.70	29	2.6%	0.4
O. squalidus					
%DM	-0.09±0.20	24.2±1.65	31	0.8%	0.63
%LFDM	-0.13±0.33	32.4±2.94	31	0.5%	0.7
O. radula					
%DM	-0.04±0.13	21.9±1.92	41	0.2%	0.77
%LFDM	-0.08±0.23	29.4±3.24	41	0.3%	0.73

In the second lipid metabolism experiment, the control and experimental groups in most species showed no difference in lipid content, irrespective of the way this was expressed (Table 7). However, differences in lipid content were found between control groups, and those subject to desiccation in *O. freyi* and the two *Trox* species. Nonetheless, consistent and strong evidence for lipid metabolism was found only in *O. freyi*, and to some extent in *T. consimilis*.

Table 7. Mean ± SE of lipid content, expressed in grams (g), as % dry mass (% DM) and as % lipid-free dry mass (% LFDM) at the end of the experimental period, for the control (hydrated) and experimental (dehydrated) beetles. * indicates mass-adjusted lipid content (lipid content was mass-adjusted due to significant differences in fresh mass of experimental and control beetles).

Species	Experimental	Control	Sig. level
O. asperulatus	n=8	n=18	df=25
lipid (g)	0.03±0.003	0.02±0.002	P=0.25 F=1.4
lipid (%DM)	17.4±1.41	15.86±0.75	P=0.3 F=1.15
lipid (%LFDM)	21.3±2.0	19.0±1.09	P=0.29 F=1.18
O. freyi	n=10	n=6	df=15
lipid (g)	0.04±0.006	0.107±0.01	P=0.0001 F=33.0
lipid (%DM)	16.1±2.18	31.44±0.75	P=0.0001 F=27.8
lipid (%LFDM)	19.9±3.30	45.9±1.58	P<0.0001 F=34.7
O. melancholicus	n=10	n=15	df=24
lipid (g)	0.029±0.003	0.034±0.002	P=0.13 F=2.5
lipid (%DM)	26.26±1.66	28.99±0.773	P=0.11 F=2.77
lipid (%LFDM)	36.2±2.92	41.06±1.52	P=0.12 F=2.6
O. squalidus	n=9	n=14	df=22
lipid (g)	0.035±0.006	0.041±0.003	P=0.3 F=1.12
lipid (%DM)	18.37±1.75	20.65±1.41	P=0.3 F=1.02
lipid (%LFDM)	23.0±2.76	26.22±2.07	P=0.36 F=0.91
O. radula	n=23	n=17	df=39
lipid (g)	0.019±0.002	0.022±0.002	P=0.18 F=1.9
lipid (%DM)	20.95±1.47	25.11±1.67	P=0.07 F=3.46
lipid (%LFDM)	27.5±2.43	34.5±2.8	P=0.07 F=3.6
T. consimilis	n=15	n=16	df=30
lipid (g)	0.005±0.0005	0.007±0.0002	P=0.0001 F=21.0
lipid (%DM)	22.16±1.85	29.33±0.76	P=0.001 F=13.45
lipid (%LFDM)	29.44±2.94	41.7±1.56	P=0.0008 F=14.2
T. squamiger	n=24	n=24	df=47
lipid (g)	0.01±0.0005	0.01±0.0008	P=0.06 F=3.7
lipid (%DM)	18.25±0.91	21.77±1.15	P=0.02 F=5.77
lipid (%LFDM)	22.66±1.31	28.45±1.86	P=0.01 F=6.5

2.4 Discussion

Body water content, maximum tolerable water losses and rates of water loss in the trogid species studied here were towards the lower end of the range found in insects (Edney 1977; Zachariassen, *et al.* 1988). Indeed, rates of water loss obtained here compared well with those of the adult beetles studied by Zachariassen, *et al.* (1988: Fig. 2) and were almost identical to the latter authors' single value for the Trogidae. Because no values for metabolic rate are available, it is not possible to determine whether the trogids support Zachariassen, *et al.*'s (1987a) hypothesis concerning the relationships between metabolic and cuticular water loss, and habitats in the Coleoptera. However, the very thick cuticle and high incidence of flightlessness in these species (Scholtz and Caveney 1988) suggests that cuticular transpiration may contribute considerably less to total water loss than respiratory transpiration (see Zachariassen 1991 for rationale). This aspect merits further study given the debate concerning the contribution of different water loss pathways to total transpiration in insects (see Quinlan and Hadley 1993; Hadley 1994b; Lighton 1994; Zachariassen, *et al.* 1987a, 1988).

Unlike the situation in most other insects (Sutcliffe 1963; Hadley 1994a), haemolymph osmolality values found in the trogids prior to desiccation (*c.* 650 mOsm.kg^{-1}) were high (verified by an independent laboratory, Department of Medical Physiology, Witwatersrand University). Generally, beetles in positive water balance from arid and mesic areas maintain haemolymph osmolalities in the region of 400 to 500 mOsm.kg^{-1} (Nicolson 1980; Riddle 1986; Zachariassen, *et al.* 1987b; Naidu and Hattingh 1988). Likewise, osmolality values at the end of the dehydration period were exceptionally high (*c.* 1000 mOsm.kg^{-1}) compared with most other desert-dwelling species. However, they were similar to the values found in the desert tenebrionid beetle, *Pimela thomsoni* (Gehrken and Sømme 1994). In that species, and the trogids studied here, the high osmolality values were recorded when body water content had declined to almost 50% of its original value. Although these high osmolality values are not the highest that have been recorded for insect haemolymph (haemolymph osmolalities may be very high in cold hardy insects during diapause, Block 1995), they certainly are amongst the highest recorded for non cold-hardy insects (Sutcliffe 1963). The trogids were also unusual in that they showed no haemolymph osmoregulatory ability during desiccation. Rather, haemolymph osmolality was very similar to that predicted solely on the basis of water loss. In most arid-adapted beetles, the adults are capable of regulating haemolymph osmolalities within a narrow range of values (e.g Nicolson 1980; Zachariassen, *et al.* 1987b; Naidu and Hattingh 1988). However, exceptions to this trend have been identified in tenebrionid beetles from the Atlas mountains (Gehrken and Sømme 1994), and in some Coleoptera from mesic habitats (Riddle 1986), but only a few insects are capable of tolerating widely varying haemolymph osmolalities (Riddle, *et al.* 1976; Punzo 1991), a strategy apparently more common in scorpions. Maintenance of high haemolymph osmolality could also be construed as an adaptation to xeric conditions because this may reduce the difference in water potential between beetles and their environment (see Ludheim and Zachariassen 1993). Because species such as these, and those studied here, do not actually conform to the “osmolality” of the external environment as do some aquatic arthropods, I suggest that they be termed osmotolerant.

The trogids also seemed to differ from many other adult beetle species (e.g Nicolson 1980; Naidu and Hattingh 1988) by virtue of their inability to catabolize lipids during dehydration, although this response may not be unusual in insects from more mesic climates where water is freely available (Chown, *et al.* 1995; Klok and Chown 1997). In addition, the utility of lipid catabolism as a means to provide a source of water during dehydration may also be rather more limited than has previously been supposed, and is likely to be traded off against water loss associated with respiration (Zachariassen, *et al.* 1987b). If respiratory water loss is considerably greater than that of cuticular water loss, then lipid catabolism is liable to play a minor role in water balance. However, the desert endemic, *O. freyi*, did show some ability to metabolize lipids during desiccation, and the large lipid contents found in this species (see Fig. 1c) have been thought of as a means to produce water during dry conditions (Scholtz and Caveney 1988). If the difference in lipid content between experimental and control beetles, provided in Table 7, is used as an estimate of lipid catabolism, and if it is assumed that 1 g of lipid leads to the production of 2 g of free water (Wharton 1985), then *O. freyi* would produce 0.12 g of water, which would amount to approximately 50% of water lost during dehydration. Such an ability to catabolize lipids to provide metabolic water also appears to be the case in *T. consimilis*, which by virtue of its small body size, has a large surface area to volume ratio, which means potentially high rates of water loss. However, using the same calculation as above, water produced by lipid catabolism amounts to only 20% of water loss in this species. Nonetheless, in both cases it seems far more likely that lipids accumulated, and catabolized, by these and the other trogid species have more to do with maintaining the ability to resume reproduction rapidly after quiescence. Friedländer and Scholtz (1993) demonstrated that after quiescence, resumption of gametogenesis was extremely rapid in *Omorgus* species from the Kalahari. They imply that the energy required for this was unlikely to have been supplied by immediate feeding, and was more likely to have been derived from the beetles' extensive lipid reserves.

Differences in water content, and maximum tolerable water loss and to a lesser extent lipid content, between the seven species were all clearly related to body size variation. Larger species could tolerate greater water losses than smaller ones simply because more water was "available" to be lost. Rates of water loss were, however, not as clearly associated with body size. In both *Omorgus* and *Trox* rates of water loss tended to be lowest in species from arid habitats, and highest in those from more mesic areas, but rates were consistently lower in the latter genus. The combination of body size and habitat-related variation in rates and tolerances (the mechanisms underlying desiccation resistance) led to the highest survival times (the ultimate test of resistance) in the two, large desert species (*O. asperulatus* and *O. freyi*), and very similar, but much lower, survival times in the remaining species. Therefore it appears that both changes in body size, as a determinant of water content and hence loss that can be tolerated, and in rates of water loss, contribute to differences in desiccation tolerance in these trogid species (see also Block, *et al.* 1990). These two mechanisms of desiccation resistance were reviewed by Edney (1977) and Hadley (1994a) and have been found to contribute in varying degrees to desiccation resistance in all insects, although differences in rates contribute more significantly than differences in maximum tolerances. That body size is an important contributor to desiccation resistance has also been shown in a variety of insects (Lighton, *et al.* 1994; Chown, *et al.* 1995).

When the data were adjusted for body size, the findings changed substantially, with the exception of lipid content. There were no differences in water contents and maximum tolerable water loss between species. With regard to water loss rates, habitat played the largest role in determining between species differences. Those species from arid habitats had the lowest rates, whereas rates of water loss in the species from the mesic habitats were much higher. As a consequence of equal maximum tolerable water losses, but habitat-associated differences in water loss rates, species from arid habitats had longer survival times than those from mesic habitats. Thus, phylogeny did not appear to play a significant role in desiccation tolerance, with the mesic *T. squamiger* having a lower rate of loss and a longer survival time than the mesic *Omorgus* species. Changes in rates at the lower end of the size range for these beetles may be affected overtly by the rapidly increasing surface area to volume ratio (see also Zachariassen, *et al.* 1987a; Chown, *et al.* submitted ms). In this context it is also of interest that *Omorgus* species, which are generally arid species (Scholtz 1980) are on average much larger than the more mesic *Trox* (see below). Of equal interest was the fact that the eurytopic *O. squalidus* showed a desiccation resistance pattern in keeping with the more mesic species. This supports Scholtz and Caveney's (1988) idea that *O. squalidus* tolerates aridity through behavioural rather than physiological regulation. This species never feeds on the surface of carcasses as do other *Omorgus* in arid areas. Rather, it is always found at the soil:carcass interface.

In conclusion, it appears that trogids studied here are unlike many other beetles because they showed very little osmoregulatory ability and no capacity to catabolize lipids for metabolic water production (with a one or two exceptions). Nonetheless, they are similar to most adult Coleoptera by virtue of the fact that they could efficiently replenish lost water by drinking (but not by atmospheric absorption, see Hadley 1994a), and had largely similar water contents, rates of water loss and water loss tolerances. In addition, like most other insects, the species from dry habitats had much lower water loss rates than those from more mesic habitats. More importantly, however, it appeared that the elevated water contents associated with large body size contributed considerably to the enhanced desiccation resistance of the xeric species. Absolute survival times were a function both of water loss rates and initial water contents of the species. Therefore, because large size confers high water contents and low surface area to volume ratios on a species (see also Schmidt-Nielsen 1984; Chown, *et al.* 1995; Chown, *et al.* submitted ms.), it may be strongly selected for in arid-adapted insects. Indeed, Scholtz (1980) and Scholtz and Caveney (1988) have shown that

Omorgus species from arid habitats are much larger than congenerics from more mesic areas (see above). This has considerable implications for the evolution of body size. In the current debate concerning the mechanisms underlying species-body size frequency distributions and geographic variation in body size (e.g. Brown, *et al.* 1993; Blackburn and Gaston 1996), the explanatory variables that have attracted most attention are metabolic rates and temperature (Atkinson 1994; Chown and Steenkamp 1996; Van Voorhies 1996; Chown and Gaston 1997). This study suggests that desiccation resistance may play an important role too.

2.5 References

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2.6 Addendum

It is common practice to acclimate field collected insects to laboratory conditions prior to experimentation (Nicolson 1980; Slobodchikoff 1983; Naidu and Hattingh 1986, 1988; Punzo 1989; Chown, *et al.* 1995). The effect of laboratory acclimation on experimental results has however, to my knowledge, never been quantified. Therefore, the purpose of this brief study is to quantify the effects of prior laboratory acclimation of trogids on the outcome of water balance trials. *Omorgus asperulatus* (Harold) (n = 15) was collected at the Kalahari Gemsbok Park, returned to the laboratory, in Pretoria, and immediately subjected to desiccation over silica gel (r.h. < 5%), at 27°C. A second group of *O. asperulatus* (n = 10), collected at the same site and at the same time as the first group, was allowed a two-week laboratory acclimation period prior to desiccation at 27°C. During the two-week acclimation period, the beetles were given free access to an abundance of water and food.

The results of the desiccation experiments revealed that the acclimated beetles had a significantly higher fresh mass (g, F = 41.8, $P < 0.0001$, df = 22), initial body water (g, F = 73.3, $P < 0.0001$, df = 22) and lipid content (g, F = 5.5, P = 0.02, df = 22), than the non-acclimated group. Having a higher body water content, the acclimated beetles were capable of tolerating more water loss than the non-acclimated ones (g, $F = 135.5$, $P < 0.0001$, $df = 22$) and since rate at which the beetles lost water was very similar in both groups (g h^{-1}, $P = 1.6$, $P = 0.217$, $df = 22$), the acclimated beetles were able to tolerate desiccation for significantly longer than the non-acclimated beetles, (h, $F = 42.5$, $P < 0.0001$, $df = 22$). The acclimated trogids had an LT_{50} of 498 h and an LT_{100} of 602 h. The non-acclimated beetles had an LT_{50} and an LT_{100} of 278 h of 398 h respectively.

Thus, laboratory acclimation of insects prior to experimentation had a significant influence on experimental results. Field collected specimens, being totally dependant on environmental conditions, are in various states of hydration when collected. Laboratory acclimation offers all individuals equal opportunity to reach optimum hydration and to accumulate body lipid reserves. Theoretically, acclimation should result in a more uniform group of experimental subjects and thus, producing more reliable and replicatable experimental results. Laboratory acclimation appears to be a justified and necessary practice in water balance trials.

2.6.1 References

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Reverence for the Lord is an education in itself.
You must be humble before you ever receive honours.

Proverbs 15:33

Chapter 3

Habitat-associated variation in desiccation tolerance of southern African keratin beetles (Coleoptera, Trogidae) : The role of temperature, allometry and phylogeny

3.1 Introduction

Although much is known about habitat-associated variation in desiccation tolerance of insects and other invertebrates (Edney 1977; Hadley 1994), past studies have focused mainly either on single species (e.g. Cooper 1982; Naidu and Hattingh 1986, 1988; Weissling and Gilblin-Davis 1993; Roberts, *et al.* 1994) or on heterogenous groups of organisms from single biomes (eg. Ahearn 1970; Slobodchikoff 1983; Arad 1993; Gehrken and Sømme 1994; but see Zachariassen, *et al.* 1987). Conclusions regarding habitat-related differences in water balance and desiccation tolerance have therefore largely been based on compilations of data for species which differ considerably in their behaviour, morphology, and physiology (e.g. Edney 1977; Hadley 1994). Attributing "adaptation" to any of these interspecific physiological differences is thus likely to be bedevilled, at least to some extent, by differences in the behaviour and morphology of the species concerned (Bennett 1987; Schmidt-Nielsen 1984), and by their phylogenetic placement (see Garland and Adolph 1994 for discussion). Few studies have examined desiccation tolerance in closely-related species (preferably in monophyletic taxa), that share similar behaviours and morphologies, across a variety of habitats (but see Chown 1993; Ward and Seely 1996). Therefore, conclusions concerning the full extent of habitat-related, physiological differences in desiccation resistance (and whether some of these differences can be considered adaptations) will remain open to question, until such investigations are undertaken.

In this context, keratin beetles (Coleoptera; Trogidae) are of considerable interest. They belong to a small, systematically well-known, cosmopolitan family, comprising only three genera (Scholtz 1980, 1986, 1993; Scholtz and Peck 1990). The biology of these beetles, which is thought to be homogeneous throughout the family, has been investigated (Scholtz and Caveney 1988, 1992), and a recent study has elucidated the basics of their water balance physiology (Le Lagadec, *et al.* in press). In addition, with the exception of size (the 66 African species vary from 3.8 to 21 mm in length) and elytral sculpturing (see Scholtz 1980), these beetles show rather limited interspecific morphological variation (S.L. Chown, J. Harrison, T. Joffe and C.H. Scholtz, unpublished data). As a consequence of their monophyly (Scholtz 1986), broad distribution, and limited interspecific morphological variation, keratin beetles thus make ideal candidates for examining the effects of phylogeny and habitat association on desiccation resistance, and the contribution that body size makes to this variation. The form of the relationship between body size and desiccation resistance is of particular significance given that the causal factors underlying both intra- and interspecific clines in body size are currently the subject of an intense debate, important to life history theory, macroecology and conservation biology (e.g. Cushman, *et al.* 1993; Kaspari and Vargo 1995; Van Voorhies 1996; Atkinson and Sibly 1997; Chown and Gaston 1997; Mousseau 1997; Partridge and Coyne 1997). Therefore, the aim of this study is to examine the form of the relationship between desiccation resistance and habitat association, and the way in which this relationship is influenced by body size and phylogenetic placement.

3.2 Material and Methods

3.2.1 Study sites and animals

In Africa, Trogidae is represented by two genera: *Trox* Fabricius comprises mostly smaller-bodied species (mean body length = 9.8 mm), while those of *Omorgus* Erichson tend to be larger (mean body length = 14.1 mm) (Scholtz 1980, 1993). Scholtz (1986) demonstrated that the family is monophyletic, having originated in Pangaea. He suggested that subsequent cladogenesis resulted in a tropical lineage, initially restricted to Gondwanaland, and ultimately giving rise to *Omorgus,* and a temperate one, initially restricted to Laurasia, and later giving rise to *Trox*. *Trox* is thought to have subsequently invaded Africa along the temperate, mountain chain formed by the East African highlands. Scholtz (1980, 1986) showed that modern *Trox* species occur predominantly in temperate African biomes (but with a few apomorphic species having dispersed into the drier regions of the country), while *Omorgus*, the more derived genus, is thought to have invaded arid regions from its more topical, mesic habitats, subsequent to aridification in Africa.

For this study, all beetles were collected between November 1991 and March 1995 from ungulate carcases and skins. The following species were utilized: arid region species: *Omorgus asperulatus* (Harold), *O. elevatus* (Harold), *O. freyi* (Haaf); arid savanna species: *O. radula* (Erichson), *O. rusticus* (Fahraeus), *Trox consimilis* Haaf; moist savanna species: *O. melancholicus* (Fahraeus), *O. tuberosus* (Klug), *O. zumpti* (Haaf), *T. squamiger* Roth; ubiquitous species: *O. squalidus* (Olivier). Details of the collection localities and their climates are given in Table 1.

Table 1. Climatic data for the keratin beetle collection sites in southern Africa.

Collection site	Species	Mean annual rainfall	Mean January max. temp.	Mean July min. temp.
Twee Rivieren, Kalahari Gemsbok Park (26°28'S 20°37'E)	*Omorgus asperulatus* *Omorgus freyi* *Omorgus rusticus*	210 mm	36.4°C (temperature often exceeds 45°C)	1.1°C (can drop to below -10°C)
Holoog, Namibia (27°17'S 17°54'E)	*Omorgus elevatus*	119.6 mm	35.6°C	7.1°C
Skukuza, Kruger National Park (24°59'S 31°36'E)	*Omorgus radula* *Omorgus melancholicus* *Omorgus tuberosus* *Omorgus squalidus*	564.2 mm	32.9°C	6.7°C
Pafuri, Kruger National Park (22°27'S 31°21'E)	*Omorgus zumpti*	413.1 mm*	32.1°C*	12.7°C*
Pearson, Eastern Cape (32°40'S 25°17'E)	*Trox consimilis*	514.4 mm	29.3°C	6.2°C
Giant's Castle Nature Reserve (29°14'S 29°28'E)	*Trox squamiger*	1035 mm	23.7°C	3.5°C

* Conditions in the cave in which *O. zumpti* occurs are very constant, relative humidity varies between 64-99% and temperature between 25.9-29.0°C (Braak 1989)

3.2.2 Experimental procedures

Only adult beetles were used in this study. All beetles were acclimated at 27±1.0°C (12L:12D) with free access to food and water for two to four weeks. Desiccation resistance was determined using the methods of Naidu and Hattingh (1986, 1988) and Chown (1993). Prior to experimentation, the beetles were starved for 24 h in moist soil to clear their digestive tracts. At the start of the desiccation experiments beetles were individually numbered and weighed on a Sartorius electronic balance accurate to 0.1 mg (fresh mass, FM), and placed in desiccation chambers over silica gel (r.h. < 5%, determined using a Novasina electronic thermohygrometer). The desiccation chambers were maintained in constant environment rooms at 27±1.0°C (12L:12D). The beetles were weighed at 24 h intervals until 100% mortality. At the end of the desiccation trials, the beetles' initial body water content, (g and % FM) was determined by drying the beetles to constant mass at 60±1.0°C (dry mass, DM) and subtracting the final dry mass from the initial fresh mass. Because trogids do not metabolize significant amounts of lipids during desiccation (Le Lagadec, *et al.* in press), the beetle's body lipid content was determined at the end of the desiccation period by extraction with three changes (24 h intervals) of a 2:1 methanol:chloroform solution at room temperature (Le Lagadec, *et al.* in press). Body lipid content was expressed in g and % DM. Maximum water loss tolerated (g and %FM), time taken to maximum water loss (h) and rate of water loss (g h^{-1} and % FM h^{-1}) were calculated using the values recorded in the time interval immediately prior to death. Because no excretion of solid waste was observed during the course of the experiment, it was presumed that mass loss was equal to incidental and respiratory water loss.

Linear least-squares regression analysis was used to establish the relationship between the fresh mass of the individual beetles and their initial body water, and lipid contents (g), maximum tolerable water loss (g), time taken to maximum water loss (h), and rate of water loss (g h^{-1}). Where the coefficient of determination (r^2) of these regressions exceeded 30%, the data were corrected for body mass, using analysis of covariance (ANCOVA), as suggested by Packard and Boardman (1987, 1988). Both the raw data and the corrected data were subjected to one way analyses of variance (ANOVA), and multiple comparisons were made using 95% Tukey HSD intervals.

Where numbers of specimens permitted, the desiccation experiments were repeated at 15, 20 and 30°C. The following species were used: *O. asperulatus*, *O. freyi*, *O. melancholicus*, *O. radula*, *O. squalidus*, *O. zumpti*, and *T. squamiger*. Due to significant variation in the beetles' fresh mass, the maximum water loss tolerated, time to maximum water loss and rate of water loss were corrected for fresh mass using an analysis of covariance. Linear least-square regression analyses were used to determine the effect of temperature on the mass-adjusted maximum water loss tolerated, the mass-adjusted time taken to maximum water loss and $\log_{10}$ of mass-adjusted rate of water loss (the conventional format for rate-temperature, or R-T curves, see Cossins and Bowler 1987). The slopes of the regressions were compared using Gabriel's approximation for the GT2 method (Sokal and Rohlf 1995) for critical differences at the 95% level.

3.3 Results

3.3.1 Desiccation at 27°C

Statistically significant relationships were found between fresh mass and body water content (g), body lipid content (g), maximum tolerable water loss (g) and time taken to maximum water loss (h). The values were therefore adjusted for fresh mass (Tables 2 and 3). Although rate of water loss (g h^{-1}) was not found to be closely correlated to fresh mass, it was also corrected for fresh mass to faciliate comparison.

Table 2. Relationship between fresh mass (g) and body water content (g), lipid content (g), maximum water loss (g), time taken to maximum water loss (h), and rate of water loss (g h^{-1}) of the pooled data for the trogid species desiccated at 27°C. The regression equations are in the form y = b mass + a. n = 145 and df = 144 throughout.

	Slope±SE	Intercept±SE	r^2	F	P
Water content	0.59±0.007	-0.008±0.002	97.9%	6666.9	<0.0001
Lipid content	0.06±0.004	0.006±0.001	56.7%	187.4	<0.0001
Maximum water loss	0.45±0.009	-0.017±0.003	95.1%	2753.8	<0.0001
Time to maximum water loss	751.9±52.8	62.3± 17.5	58.7%	203.0	<0.0001
Rate of water loss	0.00058±0.00009	0.0003±0.00003	21.6%	39.5	<0.0001

Table 3. Mean ± SE of fresh mass (FM, g), water (g and % FM), lipid content (g and % DM), and mass-adjusted (ADJ) water and lipid content (g) for each group desiccated at 27°C.

Species	n	FM (g)	Water (g)	Water (%)	Lipid (g)	Lipid (%)	ADJ water (g)	ADJ lipid (g)
O. asperulatus	9	0.40±0.015	0.23±0.009	58.4±1.04	0.030±0.003	17.8±1.25	0.154±0.004	0.023±0.003
O. freyi	10	0.56±0.037	0.32±0.025	56.4±1.71	0.040±0.006	16.1±2.18	0.144±0.009	0.023±0.006
O. elevatus	15	0.66±0.021	0.38±0.015	57.7±1.24	0.042±0.005	15.0±1.31	0.149±0.008	0.020±0.005
O. radula	23	0.17±0.005	0.09±0.004	53.5±1.11	0.017±0.002	21.0±1.47	0.148±0.002	0.023±0.002
O. rusticus	13	0.20±0.011	0.11±0.010	55.4±2.19	0.019±0.002	21.4±1.55	0.152±0.005	0.023±0.002
O. zumpti	10	0.24±0.015	0.16±0.011	65.1±0.83	0.015±0.002	17.8±2.37	0.172±0.003	0.017±0.002
T. consimilis	8	0.056±0.003	0.03±0.002	57.6±0.83	0.004±0.0003	16.6±1.60	0.157±0.0004	0.016±0.0004
O. melancholicus	10	0.22±0.010	0.12±0.007	53.6±1.37	0.019±0.002	18.6±1.49	0.146±0.003	0.022±0.002
O. tuberosus	9	0.22±0.009	0.11±0.006	48.1±1.55	0.022±0.002	19.6±0.99	0.134±0.004	0.025±0.002
T. squamiger	15	0.12±0.005	0.06±0.003	48.8±1.25	0.017±0.001	27.3±0.95	0.145±0.002	0.025±0.001
O. squalidus	10	0.41±0.021	0.24±0.011	58.1±1.48	0.26±0.004	14.2±1.56	0152.±0.006	0.018±0.003

Summary statistics for maximum tolerable water loss (g, % FM), time taken to maximum water loss (h), rate of water loss (g h^{-1}, % h^{-1}) and their mass-adjusted values are provided in Table 4.

Table 4. Mean ± SE of maximum tolerable water loss (Max, g and % FM), time to maximum water loss (Time, h), rate of water loss (Rate, g h^{-1} and % FM h^{-1}), and the mass adjusted values (ADJ) for the species desiccated at 27°C.

Species	Max (g)	Max (% FM)	Time (h)	Rate (g h^{-1})	Rate (% h^{-1})	ADJ max (g)	ADJ time (h)	ADJ rate (g h^{-1})
O. asperulatus	0.17±0.007	41.7±1.66	479.7±29.9	0.00036±0.00002	0.09±0.004	0.11±0.007	378.8±34.9	0.00028±0.00002
O. freyi	0.23±0.021	40.4±1.55	440.9±39.4	0.00053±0.00004	0.10±0.008	0.10±0.008	223.7±30.9	0.00036±0.00004
O. elevatus	0.29±0.015	43.4±1.53	655.5±30.9	0.00046±0.00004	0.07±0.006	0.11±0.010	362.2±32.6	0.00023±0.00004
O. radula	0.06±0.003	35.7±1.13	236.6±9.7	0.00026±0.000009	0.16±0.007	0.10±0.002	306.7±8.4	0.00032±0.000009
O. rusticus	0.07±0.006	34.1±1.50	195.6±19.0	0.00038±0.00005	0.20±0.025	0.10±0.003	246.7±23.2	0.00042±0.00005
T. consimilis	0.02±0.001	42.1±0.91	398.5±25.8	0.00006±0.000003	0.11±0.007	0.12±0.0006	556.3±24.5	0.00018±0.000003
O. melancholicus	0.07±0.005	33.0±1.60	128.7±8.5	0.00056±0.00002	0.26±0.012	0.10±0.003	167.3±7.3	0.00059±0.00002
O. tuberosus	0.07±0.005	30.1±1.66	206.3±12.7	0.00032±0.00002	0.15±0.006	0.09±0.003	243.3±13.6	0.00035±0.00002
O. zumpti	0.11±0.010	43.6±3.11	127.1±14.5	0.00098±0.00002	0.40±0.054	0.12±0.004	162.8±8.0	0.00081±0.00004
T. squamiger	0.03±0.003	25.4±1.70	74.2±4.0	0.00042±0.00005	0.36±0.032	0.10±0.002	185.1±5.1	0.00051±0.00005
O. squalidus	0.17±0.014	41.7±2.72	199.0±17.2	0.00087±0.00006	0.22±0.009	0.11±0.010	94.4±23.8	0.00079±0.00005

Prior to body size correction, the significant differences in fresh mass (F = 156.57, P < 0.0001, df= 130, Fig. 1a), body water content (F = 112.78, P < 0.0001, df= 130, Fig. 1b), and maximum tolerable water loss (F = 88.22, P < 0.0001, df= 130, Fig. 1c) showed similar trends. *Omorgus asperulatus, O. freyi, O. elevatus* and *O. squalidus* had the highest fresh mass, body water content and maximum tolerable water loss, while these values were lowest in *Trox consimilis* and *T. squamiger*. Although significant interspecific differences in mass-adjusted body water content (F = 2.95, P = 0.0024, df= 130, Fig. 1e) and mass-adjusted maximum tolerable water loss (F = 2.86, P = 0.0032, df= 130, Fig. 1f) were apparent, no consistent and interpretable patterns were visible. Similarly, lipid content also differed significantly between species (F = 12.46, P < 0.0001, df= 130, Fig. 1d) but this difference did not persist once body size had been accounted for (F = 0.96, P = 0.48, df= 130).

Omorgus asperulatus, O. freyi, O. elevatus and *T. consimilis* were able to tolerate desiccation for a significantly longer period than were the other species (F = 82.62, P < 0.0001, df= 130, Fig. 1g), with *O. elevatus* having the highest time to maximum water loss. However, after adjusting for fresh mass, *T. consimilis* had the longest time to maximum water loss (F = 30.19, P < 0.0001, df= 130, Fig. 1h). From Fig. 1h it is also apparent that, with the exception of *O. freyi* and *T. consimilis*, there is a gradual decline in mass-adjusted time to maximum water loss from the arid dwelling *O. asperulatus,* to the mesic *T. squamiger*, and eurytopic *O. squalidus*.

Interspecific differences in rate of water loss showed similar patterns both prior to and after correction for fresh mass. *Omorgus zumpti* and *O. squalidus* had the highest rates of water loss, and a trend of decreasing rate with increasing habitat aridity was evident (rate, F = 32.92, P < 0.0001, df= 130, Fig. 1i; mass-adjusted rate, F = 33.83, P < 0.0001, df= 130, Fig. 1j).

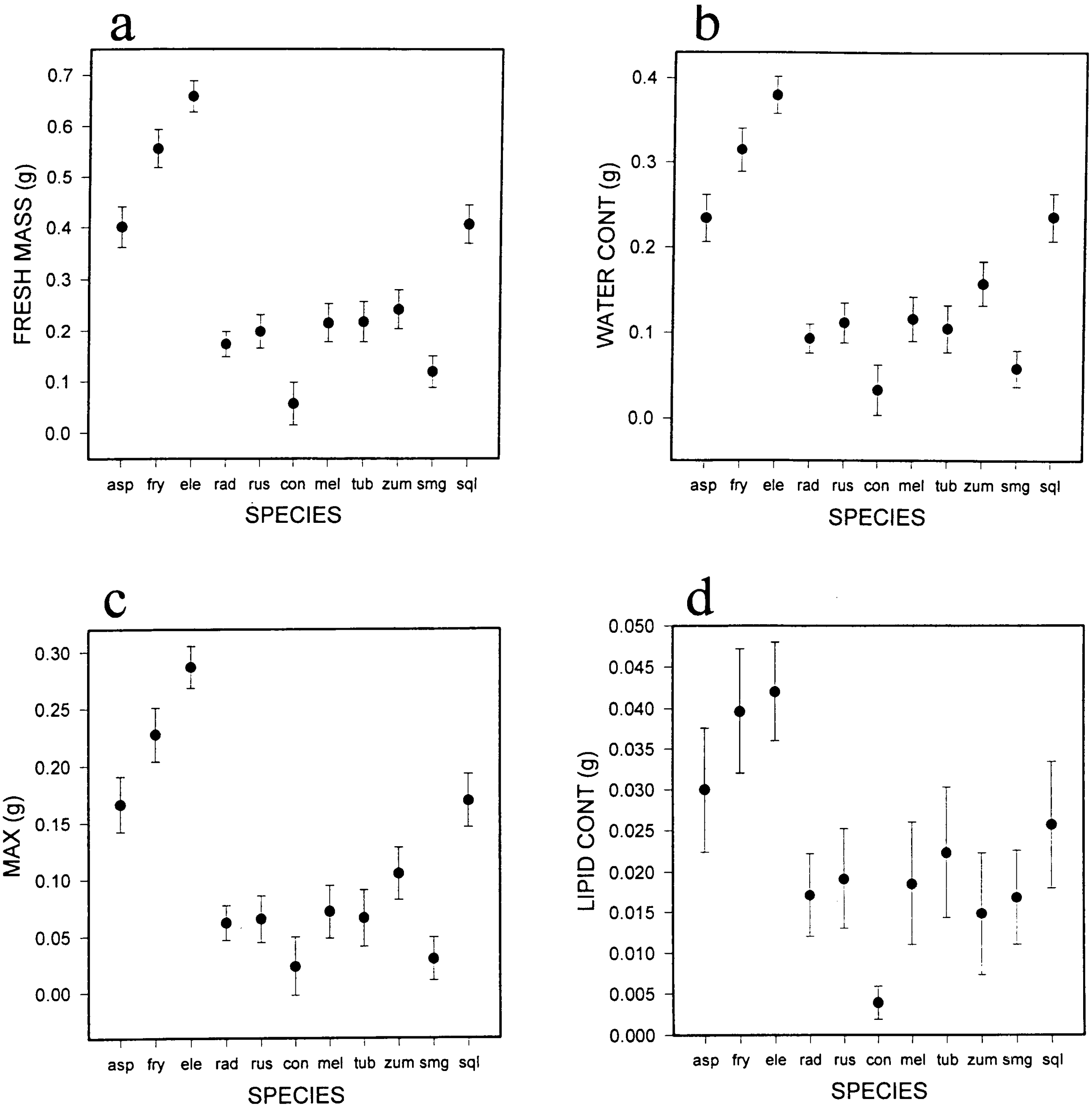

Figure 1 a-d Mean and 95% Tukey HSD intervals of (a) fresh mass; (b) body water content; (c) maximum tolerable water loss; (d) body lipid content, for *Omorgus asperulatus* (asp); *O. freyi* (fry); *O. elevatus* (ele); *O. radula* (rad); *O. rusticus* (rus); *Trox consimilus* (con); *O. melancholicus* (mel); *O. tuberosus* (tub); *O. zumpti* (zum); *T. squamiger* (smg); *O. squalidus* (sql).

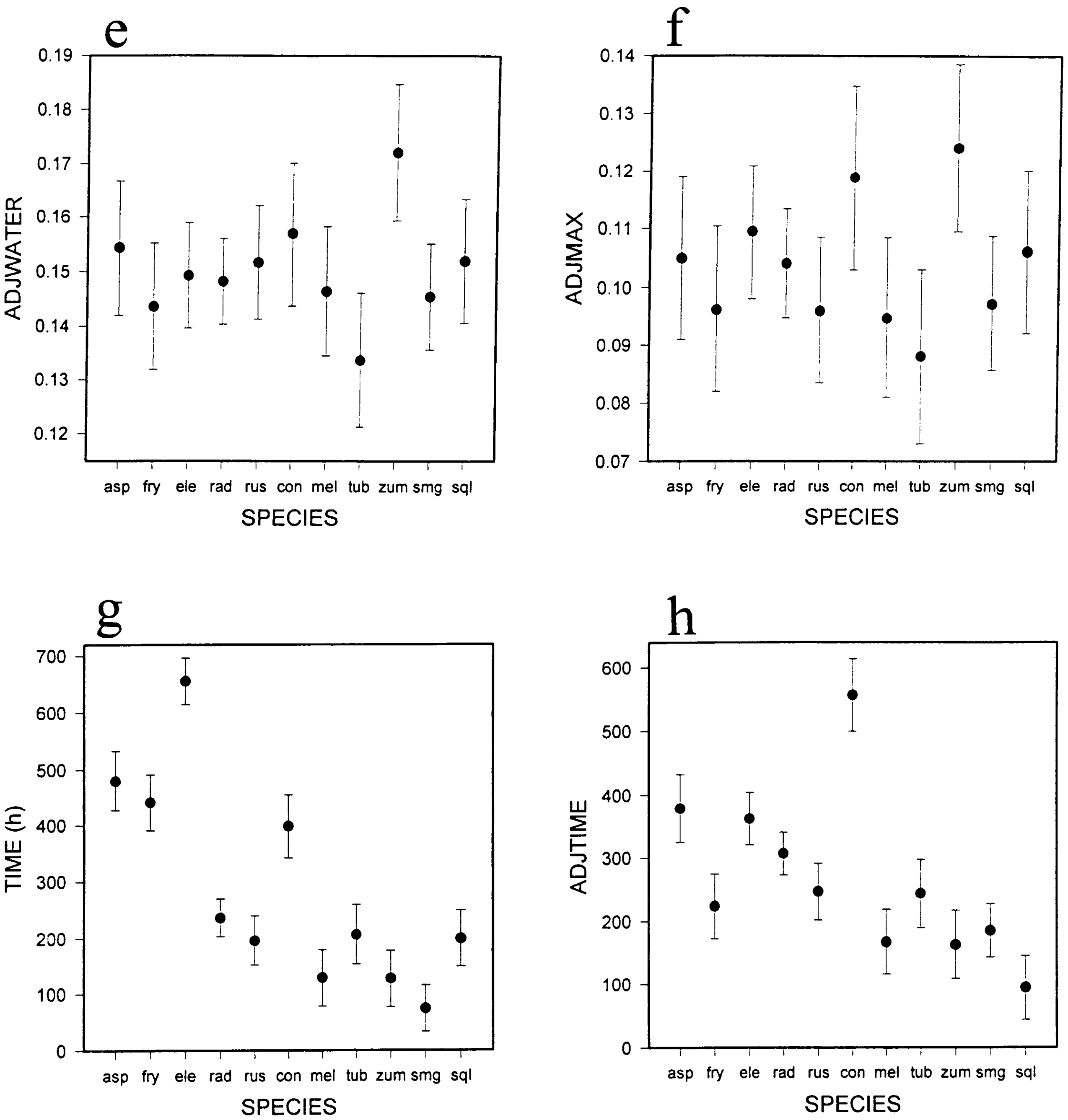

Figure 1 e-h Mean and 95% Tukey HSD intervals of (e) mass-adjusted body water content; (f) mass-adjusted maximum tolerable water loss; (g) time taken to maximum water loss; (h) mass-adjusted time taken to maximum water loss, for *Omorgus asperulatus* (asp)*; O. freyi* (fry)*; O. elevatus* (ele)*; O. radula* (rad)*; O. rusticus* (rus)*; Trox consimilus* (con)*; O. melancholicus* (mel)*; O. tuberosus* (tub)*; O. zumpti* (zum)*; T. squamiger* (smg)*; O. squalidus* (sql).

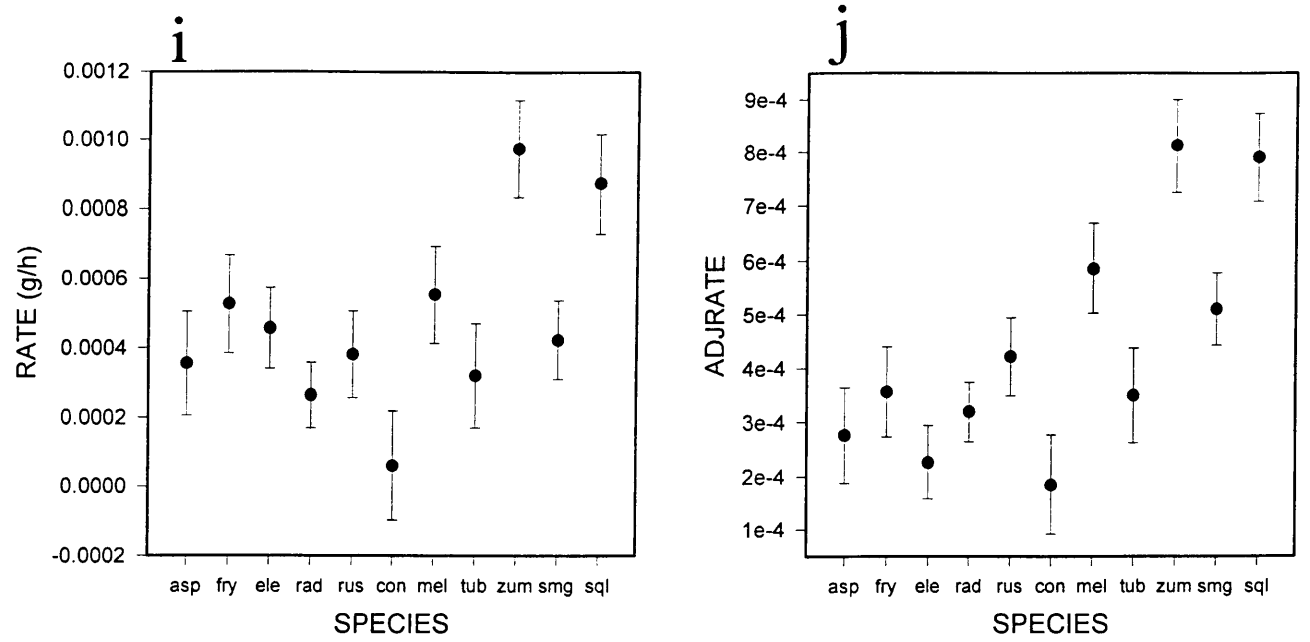

Figure 1 i-j Mean and 95% Tukey HSD intervals of (i) rate of water loss; (j) mass-adjusted rate of water loss, for *Omorgus asperulatus* (asp); *O. freyi* (fry); *O. elevatus* (ele); *O. radula* (rad); *O. rusticus* (rus); *Trox consimilus* (con); *O. melancholicus* (mel); *O. tuberosus* (tub); *O. zumpti* (zum); *T. squamiger* (smg); *O. squalidus* (sql).

3.3.2 The effect of temperature on desiccation

With the exception of *O. melancholicus* and *T. squamiger*, mass-adjusted maximum tolerable water loss was not significantly influenced by desiccation temperature in the species examined (Table 5). However, there was a significant, negative relationship between temperature and mass-adjusted time to maximum water loss in all of the species ($P < 0.0001$ throughout, Figs 2a-b). Large variations were apparent in the mass-adjusted time to maximum water loss at the lower temperatures, with *O. asperulatus, O. freyi, O. radula*, and *T. squamiger* surviving desiccation for significantly longer than *O. zumpti, O. melancholicus* and *O. squalidus* ($F = 25.9$, $P < 0.0001$, df = 69, Fig. 2a). However, at the higher desiccation temperatures, time to maximum tolerable water loss converged. Although the ANOVA revealed significant differences in the species' survival times at 30°C (F = 34.95, $P < 0.0001$, df = 58) this was due almost exclusively to the long survival time of *O. radula*.

A positive relationship was found between the log of mass-adjusted rate of water loss and desiccation temperature for all species ($P < 0.0001$ throughout, Figs 3a-b). *Omorgus asperulatus, O. freyi, O. radula* and *T. squamiger* had the lowest rates of water loss at both the lower (15°C), and higher (30°C), desiccation temperatures, although the regression lines tended to converge at the higher desiccation temperatures (Fig. 3a).

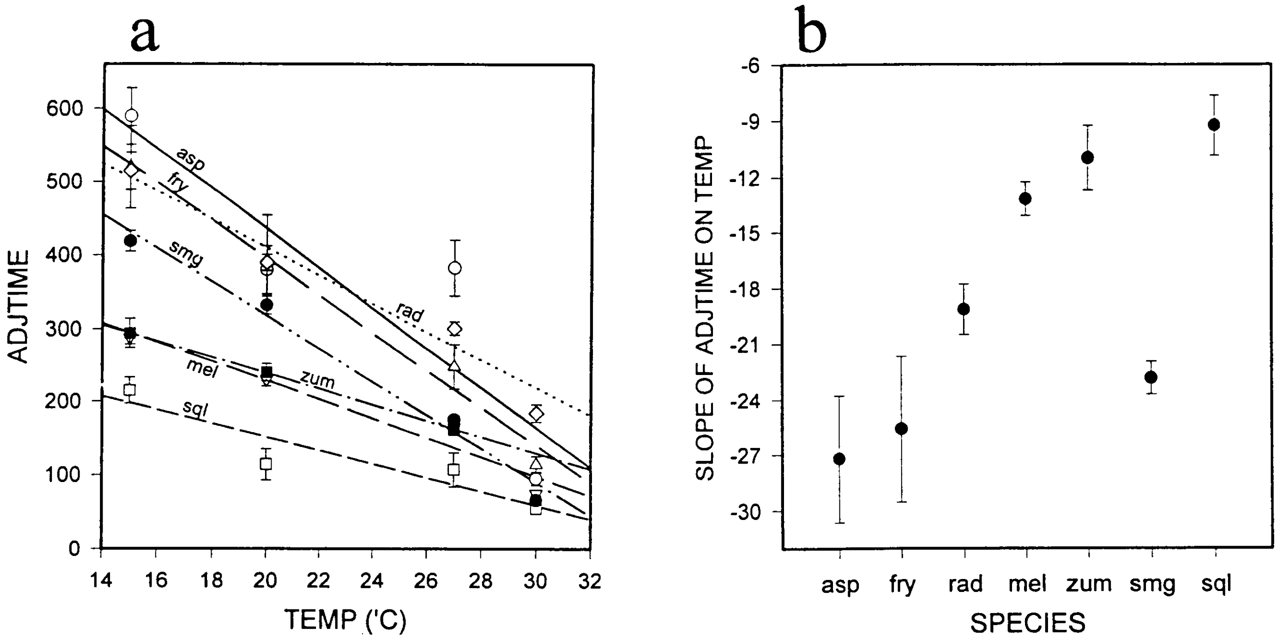

Figure 2 a-b The effect of desiccation temperature on mass-adjusted survival time; (a) the equations for the fitted regression lines are provided in Table 5; (b) mean and 95% MSD intervals of the slopes of the regression equations of mass-adjusted survival time, for *Omorgus asperulatus* (asp, ——— ○); *O. freyi* (fry, — — — △); *O. radula* (rad, ... ◇); *O. melancholicus* (mel, - - -▽); *O. zumpti* (zum, -·-·- ■); *Trox squamiger* (smg, -··-··- ●); *O. squalidus* (sql, ----- □).

Table 5. The relationship between desiccation temperature and mass-adjusted maximum water loss (ADJ MAX, g), mass-adjusted time taken to maximum water loss (ADJ TIME, h) and the log of mass-adjusted rate of water loss (ADJ RATE, g h^{-1}). Regression equations are in the form y = b temperature + a.

ADJ MAX						
Species	Slope±SE	Intercept±SE	F	P	r^2	df
O. asperulatus	-0.0014±0.0007	0.16±0.017	4.22	0.05	10.8%	36
O. freyi	-0.0006±0.0009	0.12±0.022	0.42	0.52	1.2%	37
O. radula	-0.0004±0.0002	0.12±0.006	2.59	0.12	4.4%	57
O. melancholicus	-0.0009±0.0003	0.13±0.007	8.95	0.004	15.7%	49
O. zumpti	-0.0002±0.0013	0.13±0.025	0.04	0.85	0.14%	28
T. squamiger	-0.0015±0.0002	0.15±0.006	43.0	0.0001	51.2%	42
O. squalidus	-0.0020±0.0014	0.17±0.033	2.13	0.15	5.1%	41

ADJ TIME						
Species	Slope±SE	Intercept±SE	F	P	r^2	df
O. asperulatus	-27.21±3.39	980.44±80.58	64.3	<0.0001	64.8%	36
O. freyi	-25.60±3.89	907.98±92.24	43.4	<0.0001	54.7%	37
O. radula	-19.15±1.37	793.04±33.08	196.5	<0.0001	77.8%	57
O. melancholicus	-13.18±0.91	492.69±20.91	210.9	<0.0001	81.5%	49
O. zumpti	-10.99±1.67	458.92±35.05	43.4	<0.0001	61.7%	28
T. squamiger	-22.82±0.88	774.27±21.34	674.6	<0.0001	94.3%	42
O. squalidus	-9.26±1.59	336.71±37.31	33.7	<0.0001	45.8%	41

LOG ADJ RATE						
Species	Slope±SE	Intercept±SE	F	P	r^2	df
O. asperulatus	0.041±0.0067	-4.32±0.160	36.6	<0.0001	51.1%	36
O. freyi	0.040±0.0068	-4.47±0.162	34.0	<0.0001	48.6%	37
O. radula	0.030±0.0026	-4.21±0.064	128.6	<0.0001	69.7%	57
O. melancholicus	0.029±0.0032	-3.87±0.073	87.2	<0.0001	64.5%	49
O. zumpti	0.021±0.0047	-3.68±0.099	20.7	<0.0001	43.4%	28
T. squamiger	0.044±0.0039	-4.39±0.095	128.9	<0.0001	75.9%	42
O. squalidus	0.031±0.0041	-3.75±0.097	54.4	<0.0001	57.6%	41

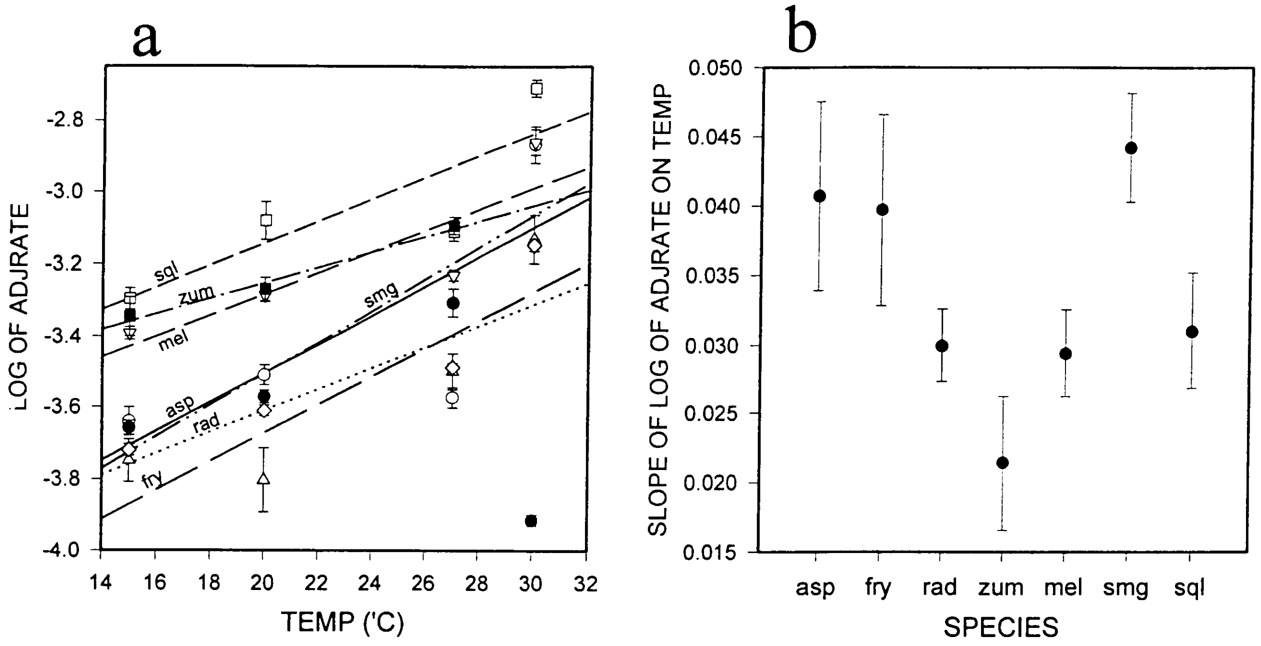

Figure 3 a-b The effect of desiccation temperature on log of mass-adjusted rate of water loss; (a) the equations for the fitted regression lines are provided in Table 5; (b) mean and 95% MSD intervals of the slopes of the regression equations of log of mass-adjusted rate of water loss, for *Omorgus asperulatus* (asp, —— o); *O. freyi* (fry, ——— ▵), *O. radula* (rad, ... ◇); *O. melancholicus* (mel, – – –▿); *O. zumpti* (zum, –···– ■); *Trox squamiger* (smg, –····– ●); *O. squalidus* (sql, ----- □).

3.4 Discussion

Because of considerable body-size associated variation, maximum tolerable water loss, body water content, and lipid content did not differ between species when the effects of body size were statistically removed. Such lack of variation in mass-adjusted maximum tolerable water loss is not surprising, given that both Edney (1977) and Hadley (1994) showed that in insects water loss tolerance has relatively narrow limits. Although body size had a considerable effect on maximum tolerable water loss, this is clearly not a consequence of metabolism of larger lipid reserves in these species because trogids tend not to utilise metabolic water to any large extent during short-term dehydration (Le Lagadec, *et al.* in press). Rather, it seems that this elevated tolerance is a consequence of the large body water reserves and reduced surface area to volume ratios characteristic of the larger species (see also Edney 1977; Willmer 1982; Schmidt-Nielsen 1984; Wright 1989; Hadley 1994; Chown, *et al.* submitted ms).

Rate of water loss and especially time to maximum water loss (the ultimate measure of desiccation resistance) showed considerable habitat-associated variation when examined both prior to and after correction for body mass. Nonetheless, body size variation still accounted for a considerable portion of the variation in both time and rate, and it seems likely that higher initial water contents and a reduced surface area to volume ratio are also responsible for this pattern. In this context it is important to note that the four species known to inhabit arid areas (*Omorgus elevatus*, *O. asperulatus*, *O. freyi* and *O. squalidus*) had the largest fresh masses of all the species examined. In addition, African species in the genus *Omorgus*, which are thought to have radiated into the arid biomes of Africa (Scholtz 1986, and above), are significantly larger than those in the genus *Trox*, which are mostly mesic species (for body length data in Scholtz 1980, $F = 40.03$, $P < 0.0001$, $df = 65$). Nonetheless, it is clear that physiological tolerances are involved, given that the mass-adjusted values continued to show considerable habitat-related variation, with species from arid regions showing the lowest rates and longest survival times.

There were two notable exceptions to these overall trends in desiccation resistance. *Trox consimilis* had a survival time equivalent to that of the larger species from more arid regions, despite its low fresh mass and body water content. This pattern became more pronounced once fresh mass had been taken into account. Therefore, it appears that physiological tolerance of desiccation is of considerable importance in this species, presumably because it cannot rely on the large water reserves usually associated with larger body size (see Table 2). On the other hand, *O. squalidus*, which had a high fresh mass and body water content, had a very high rate of water loss and a short survival time. Nonetheless, it is known to occur in arid regions such as the Kalahari desert (Scholtz 1986; Scholtz and Caveney 1988), and is in fact distributed throughout the mesic and arid regions of Africa (Scholtz 1980). Both Scholtz and Caveney (1988) and Le Lagadec, *et al.* (in press) have suggested that this species relies considerably on "non-physiological" means for inhabiting arid areas. These are thought to include both a larger body size (see below) and effective behavioural avoidance of high temperatures and low humidities.

The data presented here also suggest that phylogeny (in this case equivalent to generic placement, see Scholtz 1986) had a minimal effect on desiccation tolerance. Although species in the genus *Trox* are thought to inhabit more mesic areas than those in *Omorgus*, the two *Trox* species examined here had desiccation resistance characteristics that were influenced more by habitat association than by taxonomic propinquity. *Trox consimilis,* a species collected in the arid karoo biome, had a much longer survival time than did the mesic *T. squamiger*, which in turn was similar to the mesic *Omorgus* species. Similar, habitat-associated variation in desiccation resistance was evident in the *Omorgus* species. Thus it appears that habitat-related variation in desiccation resistance is greater than that associated with phylogeny, or, in other words, variance in desiccation resistance is partitioned at the species, rather than at the genus level in this family. Although variance in many life history variables is partitioned at higher taxonomic levels (Read and Harvey 1989; Harvey and Pagel 1991), variance in a number of physiological (e.g. cold tolerance strategy, Block 1982; Block 1990) and other variables (e.g. geographic range, Arita 1993; Brown 1995; Gaston in press) is partitioned at the species level. It appears that desiccation resistance is no exception. This may have profound consequences for the evolution of both geographic range and body size (see below).

As a consequence of changes in metabolic rate (Punzo and Huff 1989; Hadley 1994) and/or phase changes in cuticular lipids (Hadley 1994), desiccation resistance varies with temperature in many insects (Ahearn and Hadley 1969; Slobodchikoff 1983; Da Lage, *et al.* 1989; Lighton and Feener 1989; Parmenter, *et al.* 1989; Lighton, *et al.* 1994). Here, temperature had only a minimal effect on maximum tolerable water loss (as could be expected from studies on other species, see above), but rate of water loss and survival time were highly temperature dependent. The regression analyses indicated that the arid species (*O. asperulatus, O. freyi* and *O. radula*) had lower water loss rates but a higher temperature dependence of water loss rate than the mesic (*O. melancholicus, O. zumpti*) and eurytopic (*O. squalidus*) species, with the exception of the small *T. squamiger*. The habitat-associated differences in temperature dependence of survival time were even more pronounced. In addition, survival times for all species were similar at 30°C but diverged considerably at 15°C. Although higher temperature sensitivity in the arid species appears counterintuitive, it should be noted that keratin beetles are active only during the wet season (Scholtz and Caveney 1988), and spend dry periods in diapause or quiescence (Friedländer and Scholtz 1993). These dry spells tend to be extended and unpredictable in the arid regions (Rasa 1997), but less so in the more mesic areas of southern Africa (Tyson 1990). Hence there is a considerable premium on extending survival time to account for unpredictability of rainfall in the species from arid regions. Given that most southern African regions have a summer rainfall regime (Davis 1997), diapause or quiescence thus takes place over the cooler months of the year. This would account for both the long survival times at low temperatures, and for the considerable temperature dependence of survival time, in the xeric species.

The high temperature sensitivity of the mesic *T. squamiger* is clearly an exception to this general pattern. However, this is probably a consequence of its small body size. In the previous analysis I showed that smaller bodied species are considerably more sensitive to desiccation than the larger ones. Hence, increased sensitivity of survival time to temperature may also be important for restricting water loss during periods of quiescence in the small, *T. squamiger* and perhaps also in other small species. Further information on the temperature sensitivity of desiccation resistance in trogids is required to support this idea.

In sum, this study has demonstrated that variation in desiccation resistance in keratin beetles is partitioned mostly at the species level, that body size has a pronounced influence on this resistance, and that temperature can influence this relationship in a profound, but counterintuitive fashion. Although the former findings are not in themselves novel (see Edney 1977; Schmidt-Nielsen 1984; Yoder and Denlinger 1991; Arad 1993; Kaspari 1993; Hadley 1994; Lighton, *et al.* 1994; Chown, *et al.* 1995), the fact that I have demonstrated this in a monophyletic group, which shows considerable body size variation is significant (see also Chown 1993; Ward and Seely 1996). This is particularly the case in the context of debates on body size evolution.

Based on comparisons of a variety of species (beetles, ants and butterflies), a number of authors has suggested that resistance to either starvation or desiccation plays an important role in the evolution of interspecific body size gradients (Schoener and Janzen 1968; Remmert 1981; Cushman, *et al.* 1993; Kaspari and Vargo 1995). However, with the exception of the work done by Kaspari and Vargo (1995), all of these studies examined either a wide variety of relatively unrelated species, which may have rather different life history strategies, or did not include an examination of desiccation resistance *per se*. Thus, they did not provide an empirical test of the desiccation resistance hypothesis which they proposed as an explanation for clines in body size. Here I have demonstrated that in a small, monophyletic group of beetles, which have very similar life histories, desiccation resistance varies directly with habitat aridity and that this variation is closely related to body size variation. Thus, I have provided additional support for the resistance hypothesis for body size variation.

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Now we see only reflections in a mirror, mere riddles,
but then we shall be seeing face to face.
Now, I can know only imperfectly;
but then I shall know just as fully as I am myself known.

1 Corinthians 13:12

Chapter 4

Intraspecific variation in desiccation tolerance in five South African keratin beetle species (Coleoptera, Trogidae)

4.1 Introduction

Amongst the abiotic factors that are thought to have an influence on the position and size of species geographic ranges, temperature and water availability are undoubtedly the most important (Brown and Gibson 1983; Parmenter, *et al.* 1989; Cox and Moore 1993). Indeed, a large body of work now exists demonstrating that range positions are limited either by temperature or water availability (Rogers and Randolph 1986), or alternatively, that tolerance of these variables varies geographically (Arad, *et al.* 1989; Arad 1993a; Chown 1993). The latter is particularly true for insects, where many studies have demonstrated large interspecific differences in temperature tolerances (e.g. Klok and Chown 1997), the sensitivity of development to temperature (Honek 1996), and in desiccation resistance (Edney 1977; Hadley 1994). In many cases these interspecific differences are thought to represent adaptations of species to their particular environments because they reflect differences in the microhabitat distributions of the species concerned (Arad, *et al.* 1989; Arad 1990, 1993a, 1993b; Chown 1993; Bennett 1987).

However, that these kinds of demonstrations of interspecific differences in the tolerances of organisms is indicative of adaptation has also been criticized for a variety of reasons. These include reliance on two-species comparisons (Garland and Adolph 1994), lack of phylogeny-based comparative studies (e.g. Coddington 1988; Harvey and Pagel

1991), and the utilization of species that differ considerably not only in their tolerances, but also in their morphology and behaviour (Le Lagadec, *et al.* in press, submitted ms.). Furthermore, if adaptation is responsible for differences in the tolerances of species inhabiting different habitats, then presumably these differences should be most clearly reflected at the population level of the species concerned, given that this is the level at which selection acts (Mayr 1942, 1956; Endler 1986). Nonetheless, with a few exceptions (see Hoffmann 1991; Schultz, *et al.* 1992; Kimura, *et al.* 1994) few studies of insects have sought to quantify inter-population and hence intraspecific differences in the physiological tolerances of species, although this has been undertaken widely for other taxa (see e.g. Arad, *et al.* 1992, 1993a, 1993b; Garland and Adolph 1994). In addition, where such studies have been undertaken, these have generally involved comparisons of two or three populations (see e.g. Massion 1983; Hadley and Savill 1989; Hoffmann 1991) with little reference to the geographic extent of the species concerned, and no formal statistical assessment of the relationship between the tolerances documented and the characteristics of the populations' habitats.

The importance of geographic extent in this context is straightforward. If abiotic factors are responsible for limiting the distribution of restricted range species, pronounced differences in tolerance should be found between species, but only minor variation between populations of these species. However, species with wide ranges may maintain these either because they show pronounced inter-population differences in physiological tolerance, or because they utilize some other strategy to overcome variation presented by the very different environments they experience. These may include short-term behavioural responses (Bennett 1987), migration (Southwood 1977), and/or changes in morphology (Kaspari and Vargo 1995; Cushman, *et al.* 1993). In turn, these differing physiological responses to gradients in abiotic variables by populations (and species) is liable to exert an influence both on geographic range position and extent, and on latitudinal clines in both the physiological variables and in morphology. Understanding the causes and consequences of large scale variation in range size, body size and abundance of organisms is currently considered to an important component of current ecology (see Brown 1995; Brown, *et al.* 1996), largely because these patterns and the processes underlying them are of profound importance to species conservation in increasingly human-modified landscapes (Gaston 1994).

Hence the aim of this paper is to investigate intraspecific variation in desiccation tolerance and body size of five keratin beetle species that differ both in their geographic range position and range extent, and to determine whether this inter-population variation is related to differences in the abiotic conditions experienced by populations of these species. Keratin beetles (Coleoptera, Trogidae) were chosen for this study for two main reasons. First, they are ideally suited for comparative physiological studies because they are a small, monophyletic family with only two African genera, *Trox* Fabricius and *Omorgus* Erichson (Scholtz 1986, 1993). The members of the family generally have a rather homogeneous biology and are morphologically very similar, and their distribution throughout Africa has been reasonably well studied (Scholtz 1980, 1986, 1993; Scholtz and Caveney 1988, 1992; Scholtz and Peck 1990; Caveney and Scholtz 1993; Browne and Scholtz 1995). In addition, they are systematically well known, and the species differ considerably in both their geographic range extent and range position (see Scholtz 1980 for species distribution maps).

Second, interspecific differences in desiccation tolerance and water balance (and the effects of temperature on the former) of species from rather different habitats have recently been examined (Le Lagadec, *et al.* in press, submitted ms). Therefore, any intraspecific variation documented in this study can readily be interpreted in the broader context of interspecific differences in desiccation resistance found in these beetles.

4.2 Material and Methods

4.2.1 Study sites and animals

Five keratin beetle species, which differ considerably in their geographic range position and extent (Figs 1a-e), and which represent the two keratin beetle genera known from Africa were studied (distribution maps obtained from Scholtz 1980). These species and the sites from which they were collected are listed in Table 1, and information on the macroclimates and biomes of the collecting sites are provided in Table 2. All beetles were collected between November 1991 and March 1995 from ungulate carcases and skins.

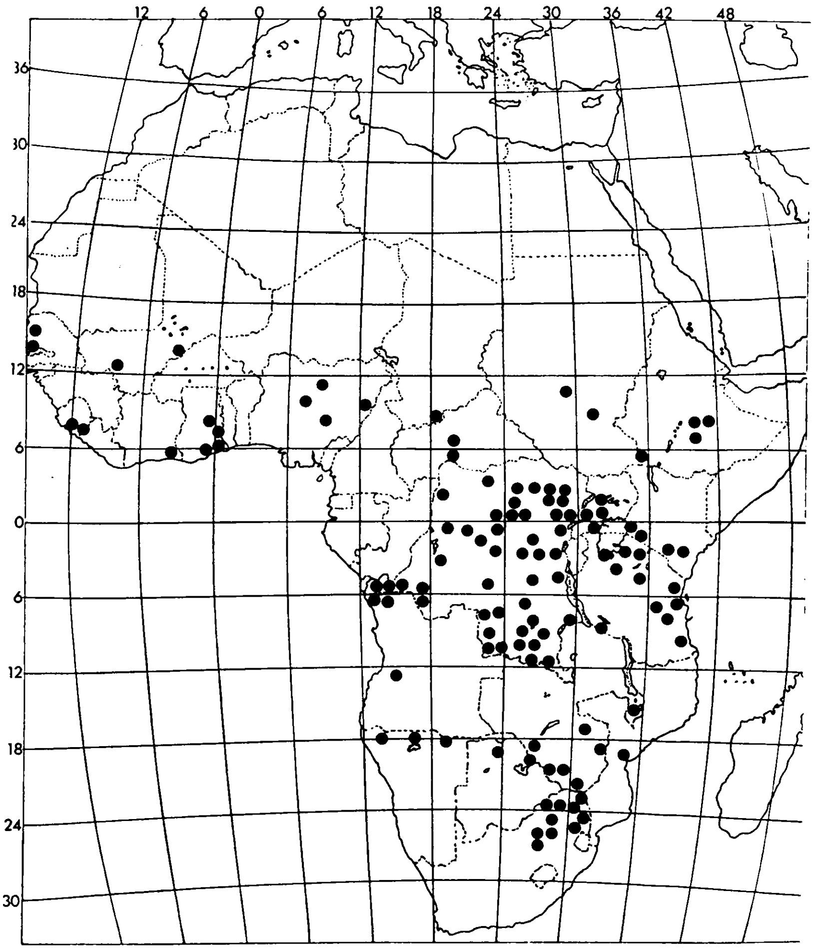

Figure 1 a Sub-Saharan distribution map of *Omorgus melancholicus* (from Scholtz 1980).

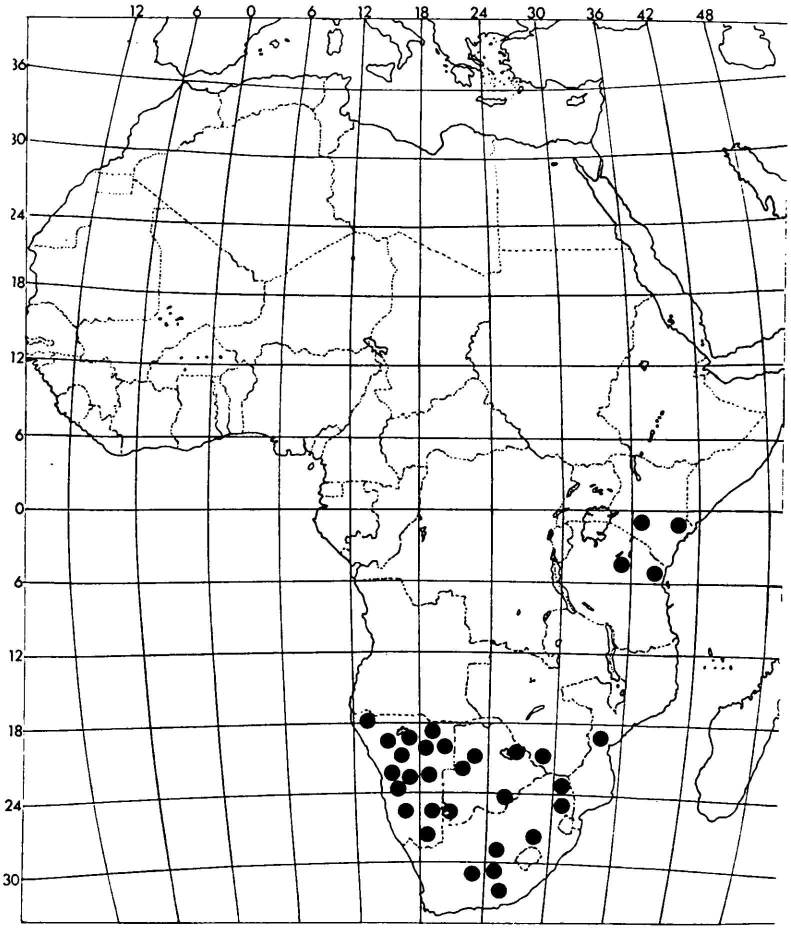

Figure 1 b Sub-Saharan distribution map of *Omorgus radula* (from Scholtz 1980).

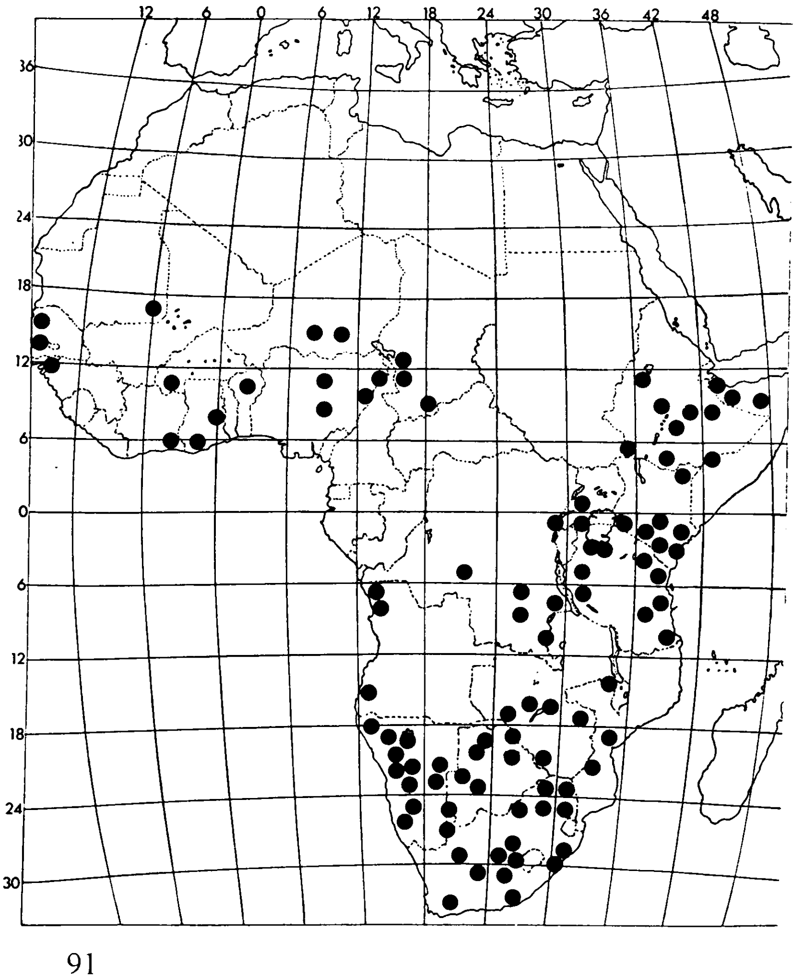

Figure 1 c Sub-Saharan distribution map of *Omorgus squalidus* (from Scholtz 1980).

Figure 1 d Sub-Saharan distribution map of *Trox consimilis* (from Scholtz 1980).

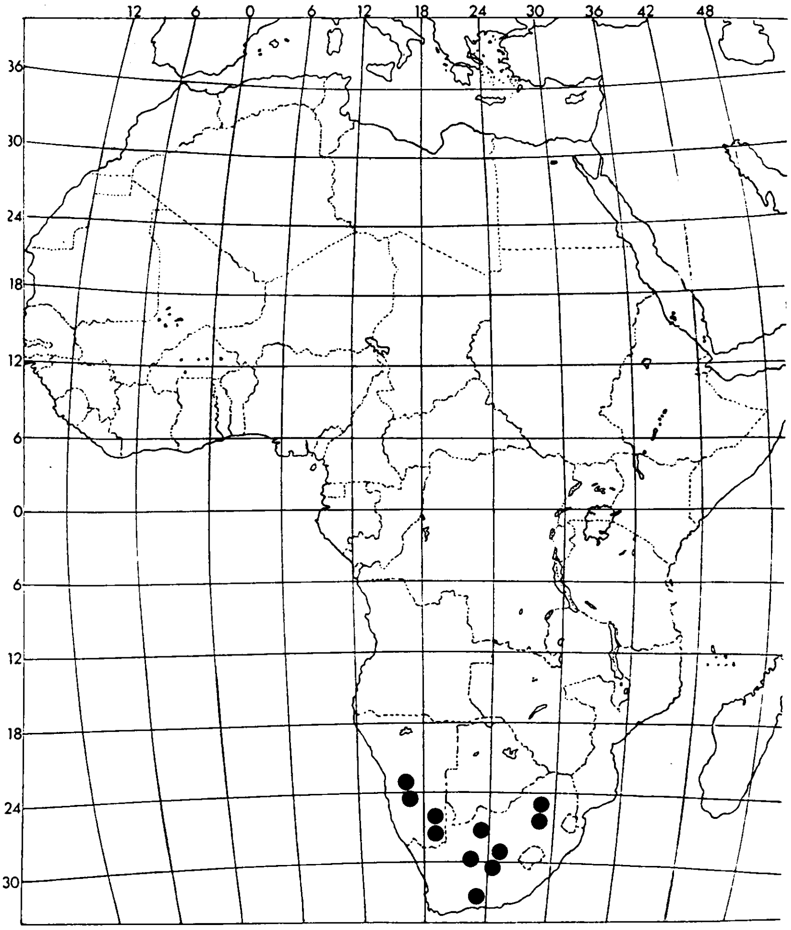

Figure 1 e Sub-Saharan distribution map of *Trox squamiger* (from Scholtz 1980).

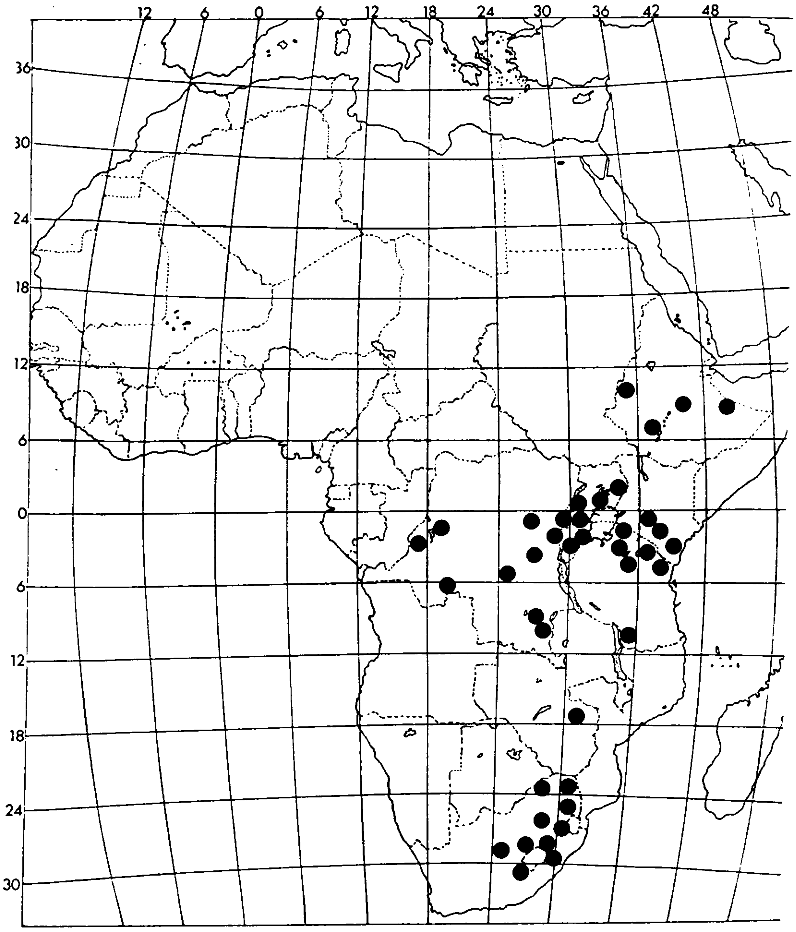

Table 1. Species used in the study and their collection sites.

Species	Collection Site
O. melancholicus (Fabraeus)	Boekenhoutskloof, Gauteng (25°35'S 28°29'E) Louis Trichardt, Northern Province (23°04'S 30°03'E) Rooiberg, Northern Province (24°37'S 27°24'E) Skukuza, Kruger National Park, Mpumalanga (24°59'S 31°36'E)
O. radula (Erichson)	Holoog, Namibia (27°17'S 17°54'E) Skukuza, Kruger National Park, Mpumalanga (24°59'S 31°36'E)
O. squalidus (Olivier)	Alldays, Northern Province (22°40'S 29°10'E) Boekenhoutskloof, Gauteng (25°35'S 28°29'E) Louis Trichardt, Northern Province (23°04'S 30°03'E) Rooiberg, Northern Province (24°37'S 27°24'E) Skukuza, Kruger National Park, Mpumalanga (24°59'S 31°36'E) Twee Rivieren, Kalahari Gemsbok Park, North Cape (26°28'S 20°37'E)
T. consimilis Haaf	Boekenhoutskloof, Gauteng (25°35'S 28°29'E) Pearson, Eastern Cape (32°40'S 25°17'E)
T. squamiger Roth	Boekenhoutskloof, Gauteng (25°35'S 28°29'E) Giant's Castle, Kwazulu-Natal (29°14'S 29°28'E) Louis Trichardt, Northern Province (23°04'S 30°03'E) Suikerbosrand Nature Reserve, Gauteng (26°49'S 27°26'E)

Table 2. Biomes and climatic data at species collection sites.

Collection Site	Rainfall	Mean Min temp	Mean Max temp	Biome
Alldays, Northern Prov.	324.2 mm	9.1°C	31.6°C	Sweet arid bushveld
Boekenhoutskloof, Gauteng	621.6 mm	1.1°C	29.7°C	Sourish mixed bushveld
Giant's Castle, Kwazulu-Natal	1035.0 mm	3.5°C	23.7°C	Dohne Sourveld
Holoog, Namibia	119.6 mm	7.1°C	35.6°C	Nama-Karoo
Louis Trichardt, Northern Prov.	370.4 mm	5.0°C	29.8°C	Mountain Sourveld
Pearson, Eastern Cape Prov.	514.4 mm	6.2°C	29.3°C	False Upper Karoo
Rooiberg, Northern Prov.	586.0 mm	1.4°C	33.0°C	Sourish mixed bushveld
Skukuza, Mpumalanga	564.2 mm	6.7°C	32.9°C	Lowveld savanna
Suikerbosrand, Gauteng	531.5 mm	0.4°C	29.4°C	Cymbopogon-Themeda veld
Twee Rivieren, Northern Cape Prov.	210.0 mm	1.1°C	36.4°C	Kalahari thornveld

All climatic data, averaged over at least five years, were obtained from The Institute of Soil, Climate and Weather, Department of Agriculture, Pretoria, RSA and The Weather Bureau, Pretoria, RSA. The rainfall figures represent mean annual rainfall, minimum temperatures were recorded in July and maximum temperatures in January. Biomes taken from Acocks (1988).

4.2.2 Experimental procedures

Only adult beetles were used in this study. All beetles were allowed a four week period of laboratory acclimation at 27±1.0°C (12L:12D) with free access to food and water. Desiccation resistance was determined as described by Le Lagadec, *et al.* (in press, submitted). Prior to experimentation, the beetles' digestive tracts were cleared by starving them for 24 h in moist soil. Beetles were individually numbered and weighed on a Sartorius electronic balance accurate to 0.1 mg (fresh mass, FM), and placed in desiccation chambers over silica gel (r.h. < 5%, determined using a Novasina electronic thermohygrometer). The desiccation chambers were maintained at 27±1.0°C (12L:12D) and the beetles were weighed at 24 h intervals until 100% mortality. At the end of the experimental period, their body water content, (g and % FM), was estimated by drying the beetles to constant mass at 60±1.0°C (dry mass, DM) and subtracting the dry mass from the fresh mass. Since these beetles do not metabolize significant amounts of lipids during desiccation (Le Lagadec, *et al.* in press), their body lipid content was determined at the end of the desiccation experiment by extraction with three rotations (24 h each) of a 2:1 methanol-chloroform solution at room temperature. Body lipid content was expressed in g and % DM. Maximum water loss (g and % FM), time to maximum water loss (h) and rate of water loss (g h^{-1} and % FM h^{-1}), were calculated using the values recorded in the time interval immediately prior to death.

The results obtained from the desiccation trials were analysed independently for each species. Linear least-squares regression analyses were used to establish the relationship between the fresh mass of the individual beetles and their initial water content (g), lipid content (g), maximum water loss tolerance (g), time to maximum water loss (h), as well as rate of water loss (g h^{-1}). Where the coefficient of determination (r^2) of these regressions exceeded 30%, the data were corrected for fresh mass, using analyses of covariance (ANCOVA), as suggested by Packard and Boardman (1987, 1988). Both the raw data and the corrected data were subjected to a one way analysis of variance (ANOVA) and multiple comparisons were made using 95% Tukey HSD intervals.

Where sample sizes permitted, the desiccation experiments were repeated at 15, 20 and 30°C. The effects of temperature on the maximum water loss tolerated (g), the time to maximum water loss (h), and the log of rate of water loss (g h^{-1}) were determined using least squares linear regression analyses. Rate of water loss was logarithmically transformed as this gave a better fit in the regression analyses and because this is in keeping with most studies of rate temperature interactions (e.g. Cossins and Bowler 1987). Where necessary, slopes of the regressions were compared using Gabriel's approximation for the GT2 method (Sokal and Rohlf, 1995).

For each species, multiple regression analyses were used to examine the relationship between rainfall, and summer maximum and winter minimum temperatures of the sites at which the populations of each species were collected, and population fresh mass and survival time (the ultimate measure of desiccation tolerance), respectively. Furthermore, the correlation between the populations' fresh mass and the latitude and longitude at which the beetles were collected was determined using least square linear regression analyses.

4.3 Results

Omorgus melancholicus

Summary statistics for the desiccation resistance at 27°C of four *O. melancholicus* populations are provided in Table 3. The fresh mass of the beetles from the various collecting sites did not differ significantly (F = 0.17, P = 0.92, df = 43), nor did their body water (F = 0.08, P = 0.97, df = 43) and lipid (F = 3.07, P = 0.08, df = 43) contents. Time to maximum water loss and rate of water loss were found to be inversely related. Beetles from Louis Trichardt and Skukuza survived desiccation for significantly longer than those from Boekenhoutskloof and Rooiberg (time to maximum water loss; F = 25.88, P < 0.001, df = 43, Fig. 2a) since they had the lowest rate of water loss (F = 16.46, P < 0.001, df = 43, Fig. 2b). The maximum water loss tolerated was not significantly different in the beetles from the four collection sites (F = 2.58, P = 0.067, df = 43).

Table 3. Summary statistics of *Omorgus melancholicus* from four collection sites desiccated at 27°C. Mean ± SE of fresh mass (FM, g), body water content (g and % FM), body lipid content (g and % DM), maximum water loss tolerated (MAX, g and % FM), time to maximum water loss (TIME, h) and the rate of water loss (RATE, g h^{-1} and % FM h^{-1}).

Locality	n	FM (g)	WATER (g)	WATER (%)	LIPID (g)	LIPID (%)
Boekenhoutskloof	10	0.23±0.007	0.11±0.004	51.1±1.9	0.03±0.003	26.26±1.66
Rooiberg	12	0.22±0.06	0.12±0.007	53.8±2.7	0.02±0.004	19.15±2.27
Skukuza	10	0.22±0.010	0.12±0.007	53.6±1.4	0.02±0.002	18.59±1.49
Louis Trichardt	12	0.21±0.006	0.11±0.004	53.9±0.9	0.02±0.002	21.22±1.14

Locality	n	MAX (g)	MAX (%)	TIME (h)	RATE (g^{-h})	RATE (%FM^{-h})
Boekenhoutskloof	10	0.06±0.007	27.3±3.3	86.6±10.4	0.00070±0.00002	0.32±0.009
Rooiberg	12	0.06±0.007	28.7±2.5	93.0±9.0	0.00068±0.00004	0.32±0.016
Skukuza	10	0.07±0.005	33.0±1.6	128.7±8.5	0.00056±0.00002	0.26±0.012
Louis Trichardt	12	0.08±0.004	37.6±1.4	174.0±3.6	0.00046±0.00003	0.22±0.01

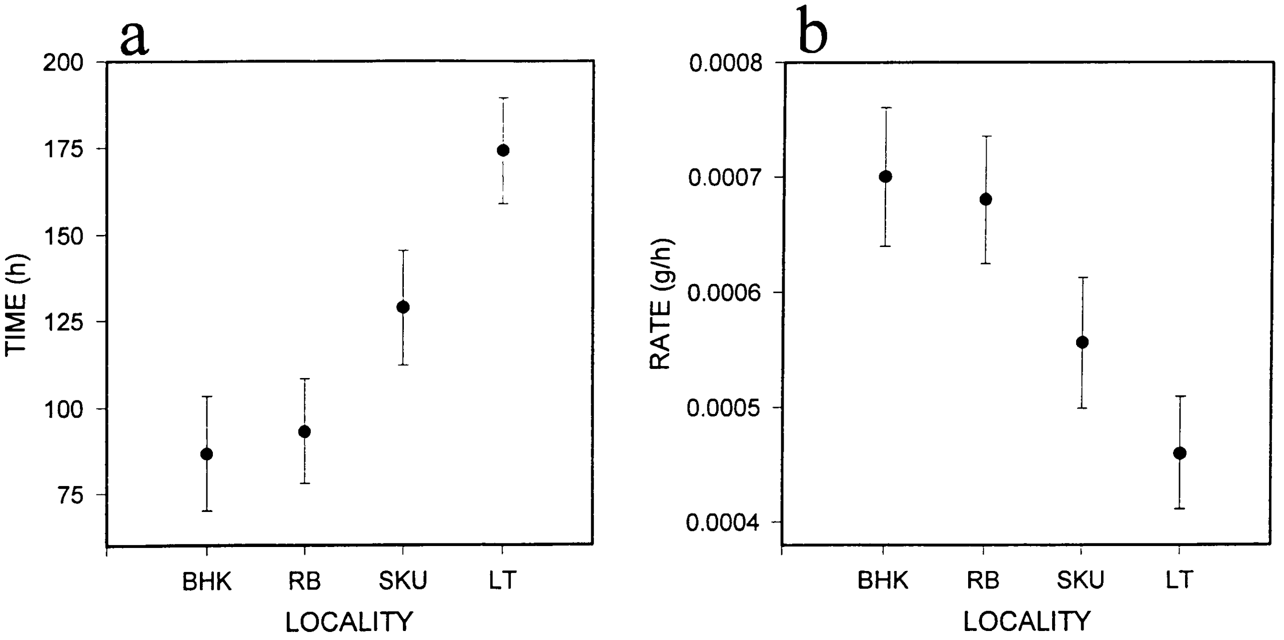

Figure 2 a-b *Omorgus melancholicus,* mean and 95% Tukey HSD intervals of (a) time to maximum water loss; (b) rate of water loss; for *O. melancholicus* from Boekenhoutskloof (BHK), Rooiberg (RB), Skukuza (SKU) and Louis Trichardt (LT).

Omorgus melancholicus from Boekenhoutskloof and Skukuza were desiccated at four temperatures. Maximum water loss tolerated by beetles from both collection sites was not significantly affected by the temperature at which they were desiccated ($P > 0.3$ throughout, Table 4). Time to maximum water loss and the log of rate of water loss were found to be inversely related and both were highly temperature dependent (Table 4, Figs 3a-c). From Figs 3a-b, it is apparent that the regression slope of time to maximum water loss was greatest for beetles from Skukuza. At low desiccation temperatures, time to maximum water loss differed greatly in beetles from the two collecting sites but the difference was not as apparent at higher temperatures. The regression slope of the log of rate of water loss in the two beetle groups was found not to differ significantly (Figs 3c-d).

Sixty six percent of the variance in survival time among the *O. melancholicus* populations was explained by variation in rainfall and winter minimum temperature of the sites at which they were collected (time = 250.15 - 0.276 rainfall + 5.175 minimum temperature), with both terms being highly significant ($t = -5.89$, $P < 0.0001$, $t = 2.58$, $P = 0.0137$, respectively, df = 43). No relationship between fresh mass and latitude ($F = 0.47$, $P = 0.498$, $r^2 = 1.1\%$, df = 43) or longitude ($F = 0.106$, $P = 0.75$, $r^2 = 0.3\%$, df = 43) was found in this species.

Table 4. The correlation between desiccation temperature and maximum water loss (MAX, g), time to maximum water loss (TIME, h) and log of rate of water loss (LOG RATE, g h^{-1}) for *Omorgus melancholicus* from Boekenhoutskloof and Skukuza. Regression equations are y = b temperature + a.

MAX Locality	Slope	Intercept	F	P	r^2(%)	df
Boekenhoutskloof	0.00031±0.0006	0.052±0.013	0.264	0.61	1.0	28
Skukuza	-0.00039±0.0004	0.084±0.009	0.987	0.33	2.5	39
TIME Locality	**Slope**	**Intercept**	**F**	**P**	**r^2(%)**	**df**
Boekenhoutskloof	-6.21±1.36	240.60±29.18	20.81	<0.001	43.5	28
Skukuza	-8.66±0.79	341.82±18.63	121.8	<0.001	76.2	39
LOG RATE Locality	**Slope**	**Intercept**	**F**	**P**	**r^2(%)**	**df**
Boekenhoutskloof	0.024±0.004	-3.76±0.086	36.78	<0.001	57.7	28
Skukuza	0.027±0.003	-3.86±0.077	68.20	<0.001	64.2	39

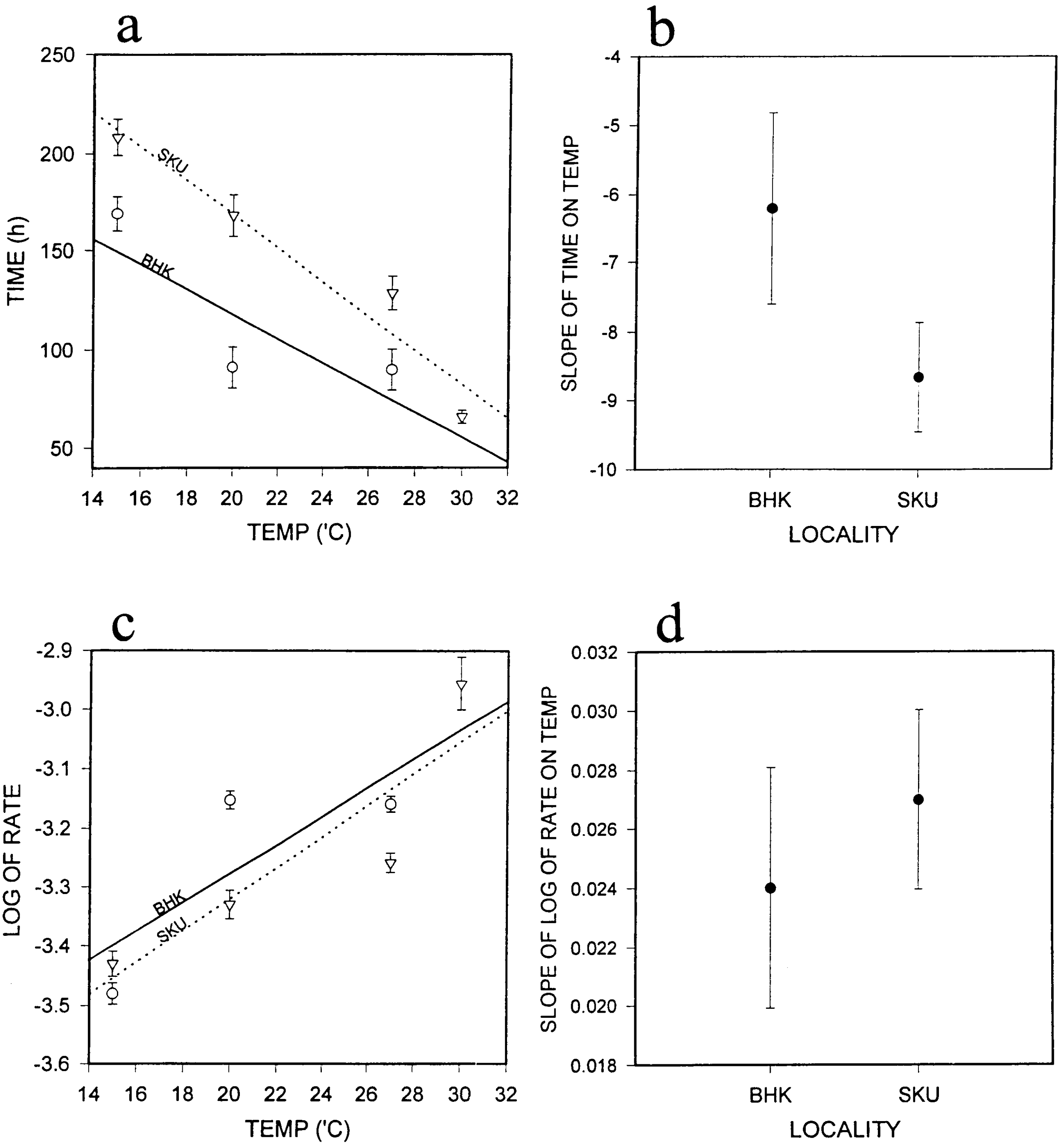

Figure 3 a-d *Omorgus melancholicus*, the effect of desiccation temperature on (a) time to maximum water loss. The regression equations are y = b temperature + a; (b) mean and 95% MSD intervals of the slopes of the regression equations of time to maximum water loss; (c) the log of rate of water loss. The regression equations are y = b temperature + a; (d) mean and 95% MSD intervals of the slopes of the regression equations of log of rate of water loss; for *O. melancholicus* from Boekenhoutskloof (BHK, ____) and Skukuza (SKU, ······).

Omorgus radula

Summary statistics for the two *O. radula* populations subjected to desiccation at 27°C are given in Table 5. *Omorgus radula* from Skukuza and Holoog differed significantly in their fresh mass ($F = 23.19$, $P < 0.0001$, df = 35), body water content ($F = 16.61$, $P = 0.0003$, df = 35) and lipid content ($F = 8.70$, $P = 0.0057$, df = 35). The population from Holoog had the highest values throughout. However, maximum water loss tolerated did not differ significantly in the beetles from the two collecting sites ($F = 3.90$, $P = 0.057$, df = 35). *Omorgus radula* individuals from Holoog displayed a higher time to maximum water loss ($F = 8.26$, $P = 0.0069$, df = 35), than did beetles from Skukuza but the rate of water loss did not differ significantly in the two groups ($F = 1.33$, $P = 0.26$, df = 35). A close correlation between the beetles' fresh mass and their body water content was evident (slope = 0.589±0.043, intercept = -0.0092±0.0083, $r^2 = 84.37\%$, $F = 183.57$, df = 35) and between fresh mass and maximum water loss tolerated (slope = 0.30±0.051, intercept = 0.0082±0.0097, $r^2 = 51.17\%$, $F = 35.63$, df = 35). Thus, body water content and maximum water loss tolerated was corrected for fresh mass. No significant differences were observed in the beetles' mass-adjusted body water content ($F = 0.034$, $P = 0.86$, df = 35) or mass-adjusted maximum water loss tolerated ($F = 1.13$, $P = 0.26$, df = 35).

Table 5. Summary statistics of *O. radula* from Skukuza and Holoog desiccated at 27°C. Mean ± SE of fresh mass (FM, g), body water content (g and % FM), lipid content (g and % DM), mass-adjusted water content (ADJ WATER), maximum water loss tolerated (MAX, g and % FM), time to maximum water loss (TIME, h), the rate of water loss (RATE, g h^{-1} and % FM h^{-1}), and mass-adjusted maximum water loss tolerated (ADJ MAX).

Locality	n	FM (g)	WATER (g)	WATER (%)	LIPID (g)	LIPID (%)	ADJ WATER
Skukuza	23	0.17±0.005	0.093±0.004	53.48±1.11	0.017±0.002	20.95±1.47	0.102±0.002
Holoog	13	0.22±0.007	0.117±0.004	54.44±0.62	0.024±0.002	24.40±1.05	0.101±0.001
Locality	**n**	**MAX (g)**	**MAX (%)**	**TIME**	**RATE (g^{-h})**	**RATE (%FM^{-h})**	**ADJ MAX**
Skukuza	23	0.062±0.0027	35.69±1.13	236.57±9.69	0.00026±0.000009	0.155±0.007	0.066±0.002
Holoog	13	0.071±0.0036	32.77±1.09	307.77±28.40	0.00024±0.00002	0.114±0.007	0.062±0.002

The results of the statistical analysis of *O. radula* desiccated at various temperatures are given in Table 6. *Omorgus radula* from Skukuza was desiccated at four temperatures while those from Holoog were desiccated at 20°C and 27°C. Although two desiccation temperatures is generally regarded as insufficient for a regression analysis, the analysis was carried out because of the large sample size used at each temperature. No significant correlation was evident between the maximum water loss tolerated and desiccation temperature in either of the trogid groups (refer to Table 6). A close correlation existed between time to maximum water loss and desiccation temperature, and the log of rate of water loss and desiccation temperature. The slope of the regression of time to maximum water loss was significantly greater in *O. radula* from Holoog than in beetles from Skukuza (Figs 4a-b). *Omorgus radula* from Holoog was able to tolerate desiccation for significantly longer at low temperatures than beetles from Skukuza. Once again, at higher desiccation temperatures the regression slopes for time to maximum water loss tended to converge. From Figs 4c-d it is apparent that the regression slope for the log of rate of water loss was significantly greater in beetles from Skukuza.

Due to the fact that only two populations were examined, regression techniques could not be used to investigate the relationship between survival time and the climatic variables recorded for the sites at which the populations were collected, or between population fresh mass and latitude and longitude of the collection sites. Nonetheless, it is instructive that the population from Holoog, the warmest, driest site, had a significantly longer survival time, and higher fresh mass, than the one from Skukuza.

Table 6. The relationship between desiccation temperature and maximum water loss (MAX, g), time taken to maximum water loss (TIME, h) and log of rate of water loss (LOG RATE, g h^{-1}) for *Omorgus radula* from Skukuza and Holoog. Regression equations are y = b temperature + a.

MAX Locality	Slope	Intercept	F	P	r^2(%)	df
Skukuza	0.000412±0.00034	0.049±0.008	1.44	0.24	2.5	57
Holoog	-0.0040±0.002	0.182±0.049	2.92	0.10	10.5	26
TIME Locality	Slope	Intercept	F	P	r^2(%)	df
Skukuza	-12.18±1.43	552.27±34.73	72.07	<0.001	56.3	57
Holoog	-20.80±4.75	869.32±112.15	19.21	<0.001	43.4	26
LOG RATE Locality	Slope	Intercept	F	P	r^2(%)	df
Skukuza	0.026±0.0018	-4.27±0.044	206.44	<0.001	78.7	57
Holoog	0.014±0.005	-4.006±0.129	6.73	0.016	21.2	26

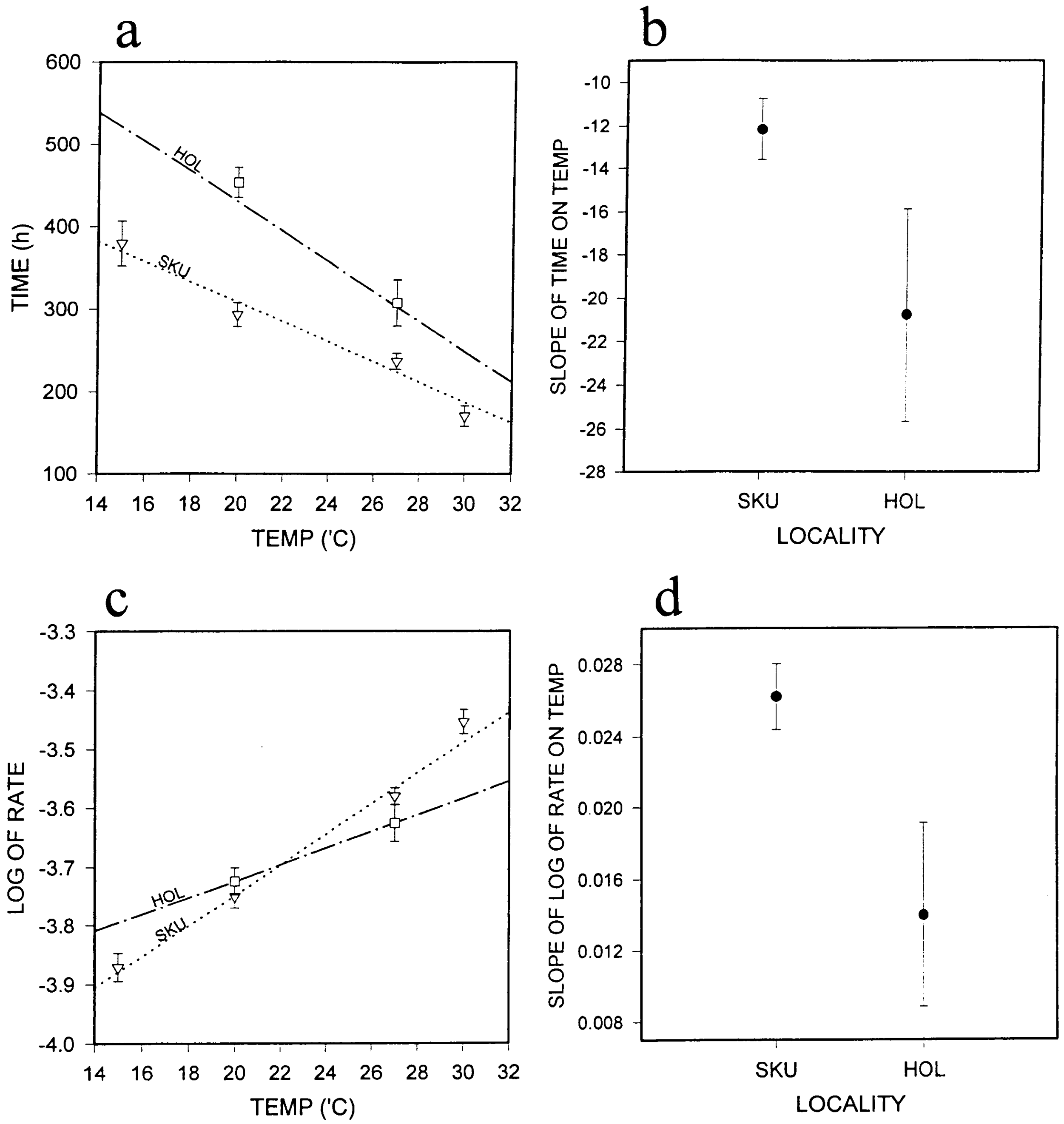

Figure 4 a-d *Omorgus radula*, the effect of desiccation temperature on (a) time to maximum water loss. The regression equations are y = b temperature + a; (b) mean and 95% MSD intervals of the slopes of the regression equations of time to maximum water loss; (c) log of rate of water loss. The regression equations are y = b temperature + a; (d) mean and 95% MSD intervals of the slopes of the regression equations of the log rate of water loss; for *O. radula* from Skukuza (SKU, ...) and Holoog (HOL, ·–·–·).

Omorgus squalidus

Summary statistics for *O. squalidus* at 27°C are given in Table 7. The population from Twee Rivieren had a significantly larger fresh mass than those from Louis Trichardt, Skukuza and Rooiberg ($F = 5.27$, $P = 0.0004$, $df = 71$, Fig. 5a) while *O. squalidus* from Boekenhoutskloof had an intermediate fresh mass. Although the body water content of the trogids from the various localities differed significantly ($F = 6.54$, $P = 0.0001$, $df = 71$, Fig. 5b), no clear patterns emerged. The beetles' body lipid content also differed significantly ($F = 13.07$, $P < 0.0001$, $df = 71$, Fig. 5c) with *O. squalidus* from Twee Rivieren and Louis Trichardt having the highest lipid content. Maximum water loss tolerated ($F = 6.74$, $P < 0.0001$, $df = 71$, Fig. 5d), time to maximum water loss ($F = 4.61$, $P = 0.001$, $df = 71$, Fig. 5e), and rate of water loss ($F = 6.45$, $P = 0.0001$, $df = 71$, Fig. 5f), of beetles from the various collection sites differed significantly but once again, due to considerable overlap, no clear patterns emerged. The beetles' body water content was closely related to their fresh mass (slope = 0.48 ± 0.043, intercept = 0.026 ± 0.018, $r^2 = 63.85\%$, $F = 123.63$, $df = 71$) and was thus, corrected for fresh mass. The mass-adjusted body water content of the keratin beetles from the various sites was found to differ significantly from each other ($F = 9.24$, $P < 0.0001$, $df = 71$, Fig. 5g). *Omorgus squalidus* from Louis Trichardt had a lower mass-adjusted body water content than those from the other collecting sites. The beetles' maximum water loss tolerated was also found to be closely correlated to their fresh mass (slope = 0.314 ± 0.051, intercept = 0.010 ± 0.021, $r^2 = 35.03\%$, $F = 37.74$, $df = 71$) and was corrected for fresh mass. The mass-adjusted maximum water loss tolerated differed significantly from each other ($F = 8.11$, $P < 0.0001$, $df = 71$, Fig. 5h) with *O. squalidus* from Skukuza having the highest mass-adjusted maximum water loss tolerated and *O. squalidus* from Louis Trichardt the lowest. However, once again, due to considerable overlap, no clear pattern emerged.

Table 7. Summary statistics of *Omorgus squalidus*, from six collection sites, desiccated at 27°C. Mean ± SE of fresh mass (FM, g), body water content (g and % FM), lipid content (g and % DM), mass-adjusted water content (ADJ WATER), maximum water loss tolerated (MAX, g and % FRESH MASS), time to maximum water loss (TIME, h), the rate of water loss (RATE, g h^{-1} and % FM h^{-1}), and mass-adjusted maximum water loss tolerated (ADJ MAX).

Locality	n	FM (g)	WATER (g)	WATER (%)	LIPID (g)	LIPID (%)	ADJ WATER
Boekenhoutskloof	9	0.43±0.014	0.24±0.0080	57.47±1.925	0.035±0.0057	18.37±1.754	0.237±0.0076
Rooiberg	11	0.38±0.016	0.21±0.0120	55.88±1.857	0.029±0.0053	16.49±2.222	0.227±0.0077
Skukuza	10	0.41±0.021	0.24±0.0109	58.14±1.483	0.26±0.0042	14.21±1.561	0.237±0.0051
Louis Trichardt	18	0.39±0.013	0.19±0.0069	48.93±0.614	0.055±0.0026	27.52±0.777	0.201±0.0024
Alldays	9	0.38±0.016	0.22±0.0098	58.46±1.022	0.019±0.0022	11.88±1.080	0.237±0.0040
Twee Rivieren	15	0.47±0.016	0.25±0.0083	52.25±1.132	0.058±0.0057	25.17±1.600	0.217±0.0051

Locality	n	MAX (g)	MAX (%)	TIME	RATE (g^{-h})	RATE (%FM^{-h})	ADJ MAX
Boekenhoutskloof	9	0.15±0.0108	36.15±3.081	165.56±11.58	0.00093±0.00005	0.220±0.0147	0.147±0.012
Rooiberg	11	0.13±0.0102	33.32±1.540	127.55±14.37	0.00011±0.000083	0.286±0.0258	0.138±0.006
Skukuza	10	0.17±0.0138	41.74±2.720	199.00±17.17	0.00087±0.00006	0.215±0.0094	0.171±0.011
Louis Trichardt	18	0.11±0.0044	29.00±0.564	162.79±6.97	0.00071±0.000032	0.183±0.0066	0.120±0.002
Alldays	9	0.14±0.0080	37.23±0.9.28	194.13±8.56	0.00074±0.000024	0.193±0.0064	0.152±0.004
Twee Rivieren	15	0.15±0.0052	32.20±0.916	175.67±9.52	0.00089±0.000054	0.313±0.0219	0.132±0.004

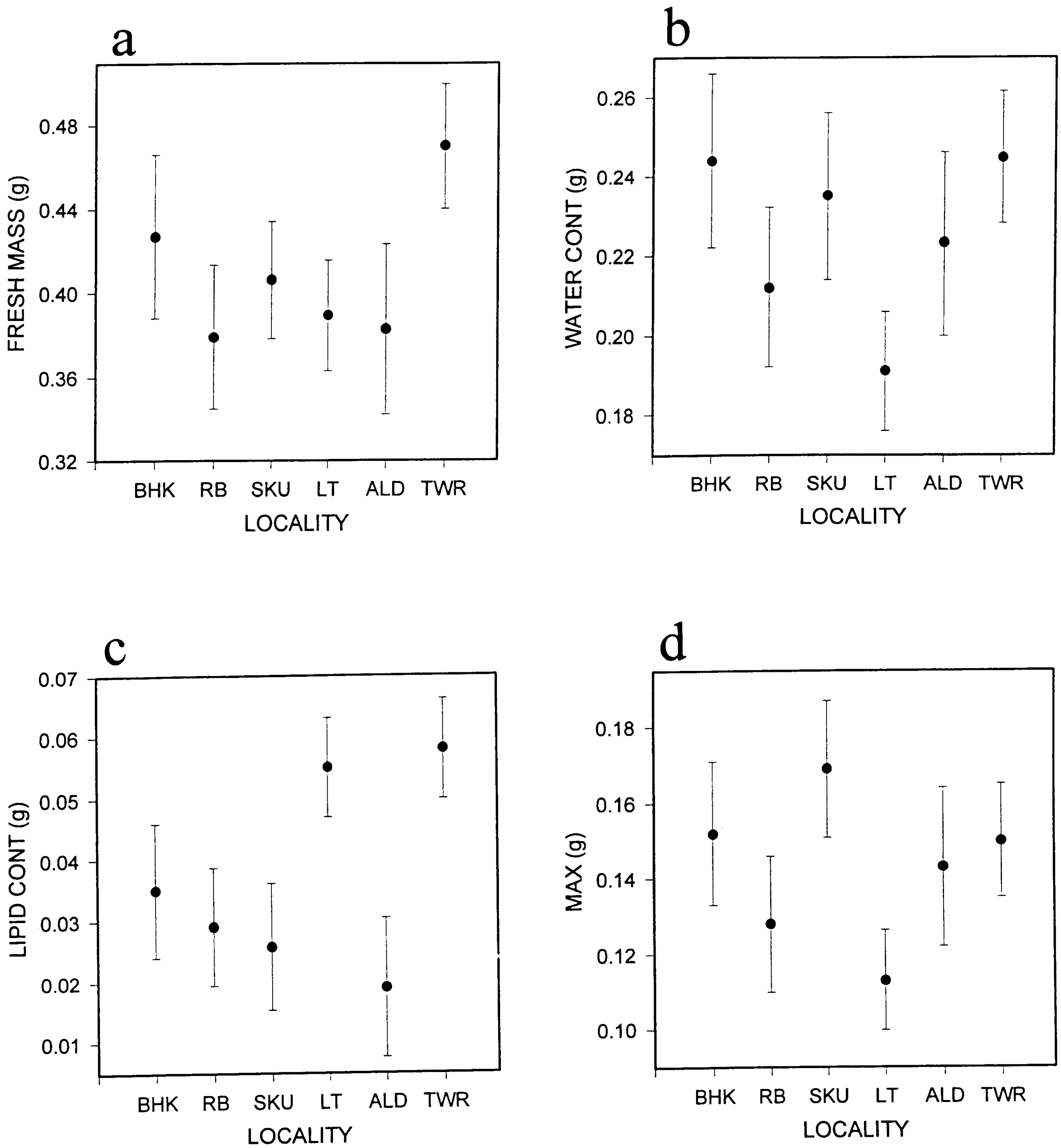

Figure 5 a-d *Omorgus squalidus*, mean and 95% Tukey HSD intervals of (a) fresh mass; (b) body water content; (c) body lipid content; (d) maximum water loss tolerance, for *O. squalidus* from, Boekenhoutskloof (BHK), Rooiberg (RB), Skukuza (SKU), Louis Trichardt (LT), Alldays (ALD) and Twee Rivieren (TWR).

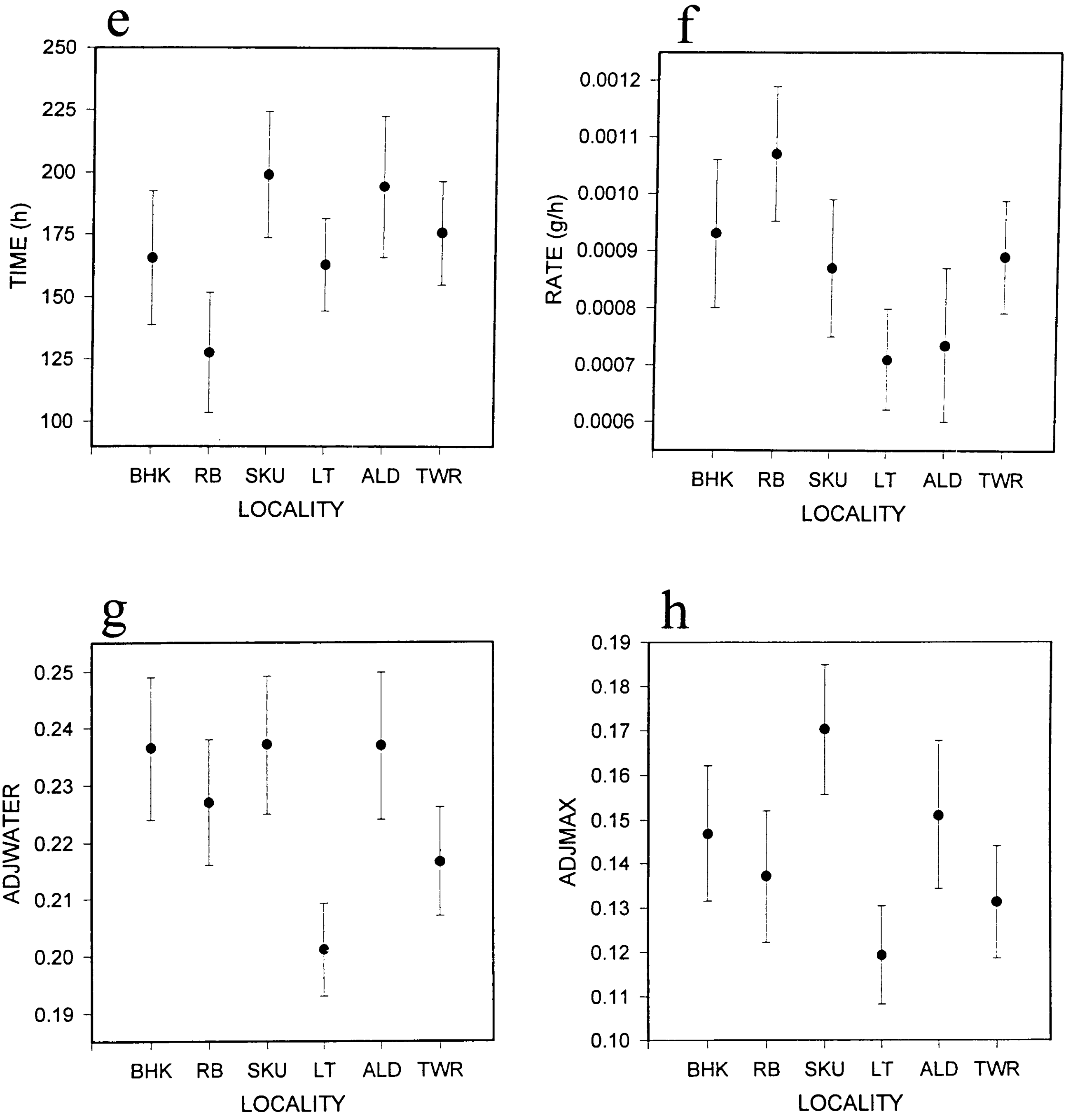

Figure 5 e-h *Omorgus squalidus,* mean and 95% Tukey HSD intervals of (e) time to maximum water loss; (f) rate of water loss; (g) mass-adjusted body water content; (h) mass-adjusted maximum water loss tolerated, for *O. squalidus* from, Boekenhoutskloof (BHK), Rooiberg (RB), Skukuza (SKU), Louis Trichardt (LT), Alldays (ALD) and Twee Rivieren (TWR).

Omorgus squalidus from Boekenhoutskloof, Skukuza and Twee Rivieren were desiccated at four temperatures. No significant correlation was found between maximum water loss tolerated and temperature at which desiccation occurred ($P > 0.1$, Table 8). However, both time to maximum water loss and the log of rate of water loss were greatly affected by desiccation temperature (Figs 6a-c). Time to maximum water loss tended to decrease as temperature increased, and the log of rate of water loss tended to increase with increasing temperature. The regression slope of time to maximum water loss was significantly steeper for *O. squalidus* from Boekenhoutskloof than for those from the other two collection sites (Figs 6a-b), indicating the population from Boekenhoutskloof 's heightened sensitivity to desiccation temperature. The log of rate of water loss, in *O. squalidus* from all three collecting sites were equally affected by desiccation temperature (the slopes of the regressions did not differ significantly from each other, Figs 6c-d).

The climatic variables explained only ten percent of the variance in survival time among the *O. squalidus* populations, with only minimum temperature being significant ($t = 2.53$, $P = 0.014$, df = 71, regression equation was: time = 168.18 - 0.038 rainfall + 4.29 minimum temperature). However inter-population variability in fresh mass was related to both latitude ($F = 18.5$, $P < 0.0001$, $r^2 = 21\%$, df = 70) and longitude ($F = 16.62$, $P = 0.0001$, $r^2 = 19\%$, df = 70).

Table 8. The relationship between desiccation temperature and maximum water loss (MAX, g), time taken to maximum water loss (TIME, h) and log of rate of water loss (LOG RATE, g h^{-1}) for *Omorgus squalidus* from Boekenhoutskloof, Skukuza and Twee Rivieren. Regression equations are y = b temperature + a.

MAX Locality	Slope	Intercept	F	P	r^2(%)	df
Boekenhoutskloof	-0.0012±0.0008	0.185±0.019	2.14	0.151	4.7	44
Skukuza	-0.003±0.0018	0.244±0.037	3.23	0.08	7.5	41
Twee Rivieren	-0.00010±0.0006	0.173±0.015	2.60	0.113	4.9	52

TIME Locality	Slope	Intercept	F	P	r^2(%)	df
Boekenhoutskloof	-19.38±1.16	679.46±27.6	277.16	<0.001	86.6	44
Skukuza	-15.67±1.64	566.53±38.39	91.2	<0.001	69.5	41
Twee Rivieren	-16.22±1.35	623.38±33.15	143.98	<0.001	73.8	52

LOG RATE Locality	Slope	Intercept	F	P	r^2(%)	df
Boekenhoutskloof	0.030±0.002	-3.84±0.052	176.38	<0.001	80.4	44
Skukuza	0.032±0.004	-3.73±0.099	56.11	<0.001	58.4	41
Twee Rivieren	0.027±0.002	-3.81±0.057	134.0	<0.001	72.4	52

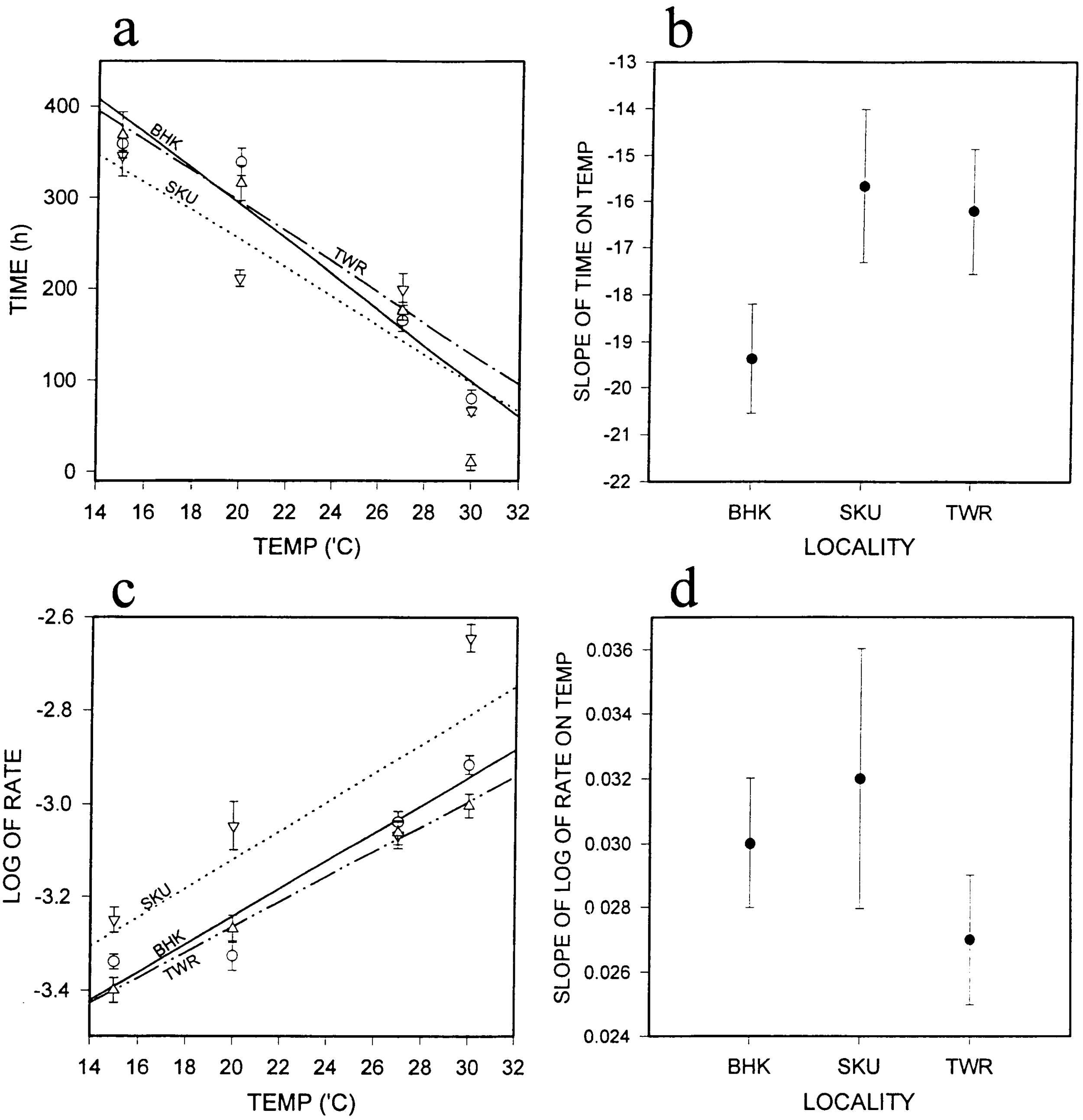

Figure 6 a-d *Omorgus squalidus*, the effect of desiccation temperature on (a) time to maximum water loss. The regression equations are y = b temperature + a; (b) mean and 95% MSD intervals of the slopes of the regression equations of time to maximum water loss; (c) log of rate of water loss. The regression equations are y = b temperature + a; (d) mean and 95% MSD intervals of the slopes of the regression equations of log of rate of water loss; for *O. squalidus* from Boekenhoutskloof (BHK, ____); Skukuza (SKU, ...) and Twee Rivieren (TWR, -··-··-··).

Trox consimilis

As can be seen in Table 9, the *T. consimilis* population collected at Pearson had a higher fresh mass (F = 16.29, P = 0.0006, df = 22), body water content (F = 19.01, P = 0.0003, df = 22) and maximum water loss tolerated (F = 22.45, P = 0.0001, df = 22) than did the one from Boekenhoutskloof. The body lipid content of *T. consimilis* from the two collecting sites was not significantly different (F = 3.90, P = 0.31, df = 22). Time to maximum water loss was higher in the beetles from Pearson (F = 41.93, P < 0.0001, df = 22) and the rate of water loss significantly lower (F = 12.85, P = 0.033, df = 22). A close correlation was found between the beetles' individual fresh mass and their body water content (slope = 0.62±0.052, intercept = -0.0031 ±0.0026, r^2 = 87.17%, F = 142.67, df = 22), maximum water loss tolerated (slope = 0.47±0.085, intercept = -0.0045±0.0042, r^2 = 59.68%, F = 31.1, df = 22) and time to maximum water loss (slope = 9912.5±1844, intercept = -206.8±91.1, r^2 =57.91%, F = 28.9, df = 22). Therefore, these were corrected for fresh mass. No significant differences were apparent in the mass-adjusted body water content (F = 0.89, P = 0.37, df = 22) and mass-adjusted maximum water loss tolerated (F = 2.53, P = 0.13, df = 22) of *T. consimilis* from the two collecting sites. However, their mass-adjusted time to maximum water loss differed significantly (F = 6.38, P = 0.02, df = 22), with the beetles from Pearson surviving desiccation for longer than those from Boekenhoutskloof.

Table 9. Summary statistics of *Trox consimilis* from Boekenhoutskloof (Boekenhout) and Pearson desiccated at 27°C. Mean ± SE of fresh mass (FM, g), body water (g and % FM),lipid content (g and % DM), mass-adjusted water content (ADJ WATER), maximum water loss tolerated (MAX, g and % FM), time to maximum water loss (TIME, h), rate of water loss (RATE, g h^{-1} and % FM h^{-1}), mass-adjusted maximum water loss tolerated (ADJ MAX), and mass-adjusted time to maximum water loss (ADJ TIME).

Locality	n	FM (g)	WATER (g)	WATER (%)	LIPID (g)	LIPID (%)	ADJ WATER	
Boekenhout	15	0.045±0.0015	0.024±0.0009	54.83±1.380	0.0047±0.0005	22.16±1.852	0.0269±0.0006	
Pearson	8	0.056±0.0029	0.033±0.0020	57.58±0.827	0.0039±0.0003	16.61±1.602	0.0278±0.0003	

Locality	n	MAX (g)	MAX (%)	TIME (h)	RATE (g^{-h})	RATE (%FM^{-h})	ADJ MAX	ADJ TIME
Boekenhout	15	0.016±0.0010	35.63±2.12	210.2±16.22	0.00008±0.000006	0.183±0.015	0.0178±0.001	250.56±16.85
Pearson	8	0.024±0.0012	42.07±0.911	398.5±25.84	0.00006±0.000003	0.109±0.007	0.020±0.0006	322.83±23.13

Trox consimilis from Boekenhoutskloof and Pearson were desiccated at 20°C and 27°C and summary statistics are presented in Table 10. Once again, despite there being only two experimental temperatures, regression analysis were carried out because of the relatively large sample sizes at each temperature. In both *T. consimilis* groups, no significant correlation was evident between the desiccation temperature and the maximum water loss tolerated ($P > 0.1$ throughout, Table 10). Time to maximum water loss was negatively correlated to desiccation temperature but no significant differences were observed in the regression slopes of the populations from the two collection sites (Figs 7a-b). However, *T. consimilis* from Pearson was able to tolerate desiccation for longer than the beetles from Skukuza both at low and high desiccation temperatures. A close, positive correlation was apparent between the desiccation temperature and the log of rate of water loss with *T. consimilis* from Boekenhoutskloof being significantly more sensitive to temperature than those from Pearson (Figs 7c-d).

Once again, in this case regression analyses could not be used to investigate differences between the populations of this species because only two populations were examined. However, the individuals from the drier, warmer site had longer survival times and higher fresh masses than did those from the more mesic site.

Table 10. The relationship between desiccation temperature and maximum water loss (MAX, g), time taken to maximum water loss (TIME, h) and log of rate of water loss (LOG RATE, g h^{-1}) for *Trox consimilis* from Boekenhoutskloof and Pearson. Regression equations are y = b temperature + a.

MAX Locality	Slope	Intercept	F	P	r^2(%)	df
Boekenhoutskloof	0.00011±0.00017	0.013±0.004	0.366	0.56	1.4	27
Pearson	0.00006±0.00026	0.022±0.006	0.044	0.84	0.3	16

TIME Locality	Slope	Intercept	F	P	r^2(%)	df
Boekenhoutskloof	-32.92±3.655	1098.95±87.73	81.12	<0.001	75.5	27
Pearson	-28.83±8.08	1177.0±190.37	12.73	0.003	45.9	16

LOG RATE Locality	Slope	Intercept	F	P	r^2(%)	df
Boekenhoutskloof	0.051±0.005	-5.49±0.12	110.30	<0.001	80.9	27
Pearson	0.026±0.005	-4.92±0.12	24.30	<0.001	61.8	16

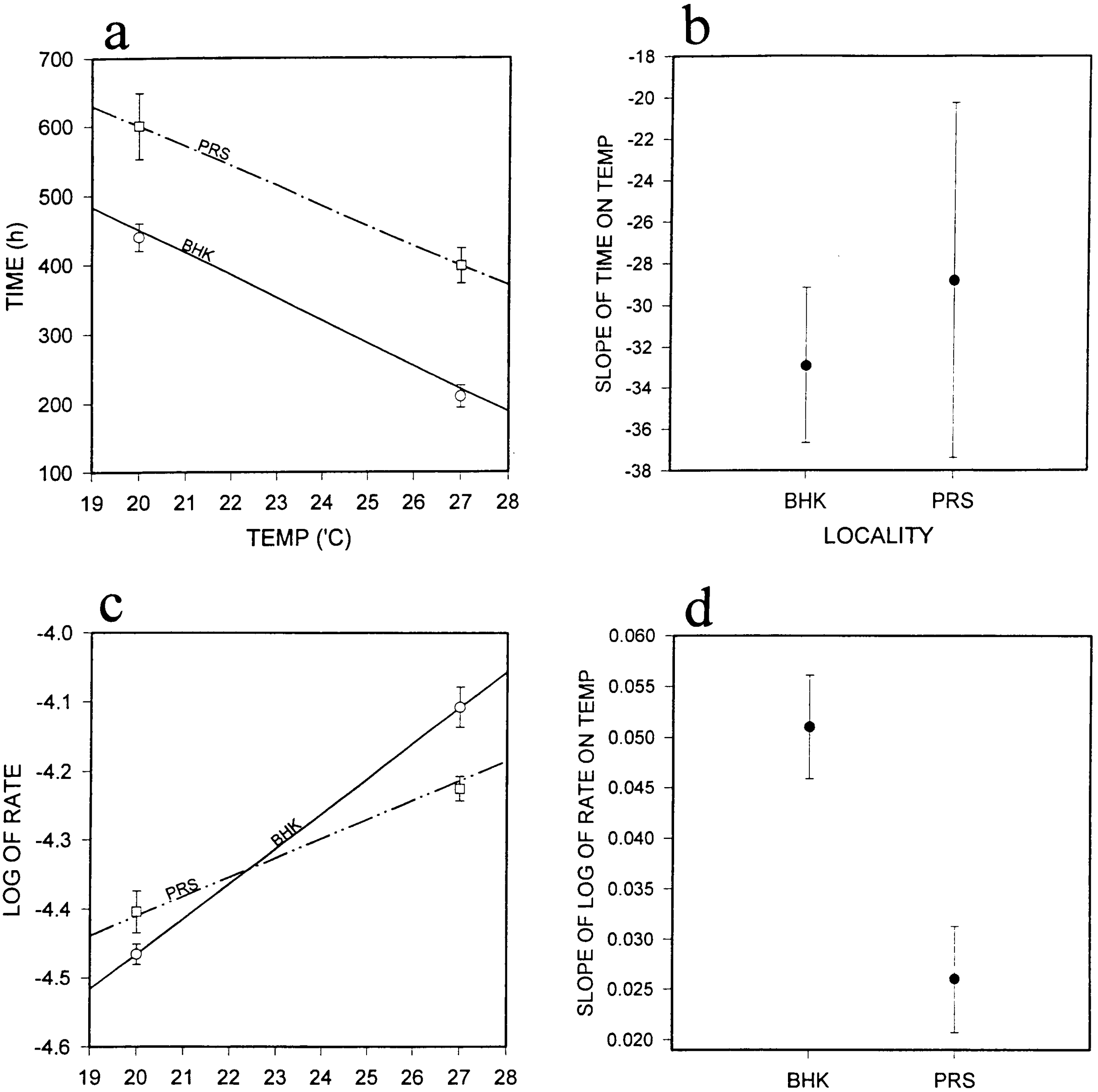

Figure 7 a-d *Trox consimilis*, the effect of desiccation temperature on (a) time to maximum water loss. The regression equations are y = b temperature + a; (b) mean and 95% MSD intervals of the slopes of the regression equations of time to maximum water loss; (c) log of rate of water loss. The regression equations are y = b temperature + a; (d) mean and 95% MSD intervals of the slopes of the regression equations of log of rate of water loss; for *T. consimilis* from Boekenhoutskloof (BHK, ____) and Pearson (PRS, -··-··-).

Trox squamiger

Summary statistics for *T. squamiger* desiccated at 27°C are given in Table 11. The beetles from Boekenhoutskloof were found to have a significantly higher fresh mass ($F = 5.46$, $P = 0.003$, df = 49, Fig. 8a) and body water content ($F = 3.16$, $P = 0.034$, df = 49, Fig. 8b) than those collected from the other three sites. *Trox squamiger* from Boekenhoutskloof were also found to have a significantly higher maximum water loss tolerance than those from Giant's Castle and Suikerbosrand ($F = 5.71$, $P = 0.002$, df = 49, Fig. 8c), while the Louis Trichardt trogids formed an intermediate group. The body lipid content of *T. squamiger* from Giant's Castle was higher than those from Suikerbosrand and Louis Trichardt but overlapped with the values obtained for beetles from Boekenhoutskloof ($F = 7.60$, $P = 0.0003$, df = 49, Fig. 8d). Significant differences in time to maximum water loss were also apparent ($F = 19.45$, $P < 0.0001$, df = 49, Fig. 8e). *Trox squamiger* from Boekenhoutskloof had the longest time to maximum water loss followed by beetles from Louis Trichardt. The Giant's Castle group proved to have the shortest time to maximum water loss. Once again, the rate of water loss was found to be the inverse of time to maximum water loss and differed significantly in the trogids from the four localities ($F = 4.34$, $P = 0.009$, df = 49, Fig. 8f). *Trox squamiger* from Giant's Castle and Suikerbosrand displayed higher rates of water loss than those from Boekenhoutskloof. A close correlation was apparent between the beetles' fresh mass and their body water content (slope = 0.49±0.031, intercept = 0.0018±0.0038, $r^2 = 84.47\%$, $F = 255.6$, df = 49) and maximum water loss tolerated (slope = 0.31±0.045, intercept = -0.0030±0.0056, $r^2 = 51.79\%$, $F = 46.19$, df = 49) and they were thus, corrected for fresh mass. The mass-adjusted water content were not significantly different in the beetles from the four sites ($F = 2.19$, $P = 0.102$, df = 49), whereas the mass-adjusted maximum water loss tolerated differed significantly ($F = 4.32$, $P = 0.01$, df = 49, Fig. 8g). *Trox squamiger* from Louis Trichardt had the highest maximum water loss tolerated and *T. squamiger* from Giant's Castle the lowest.

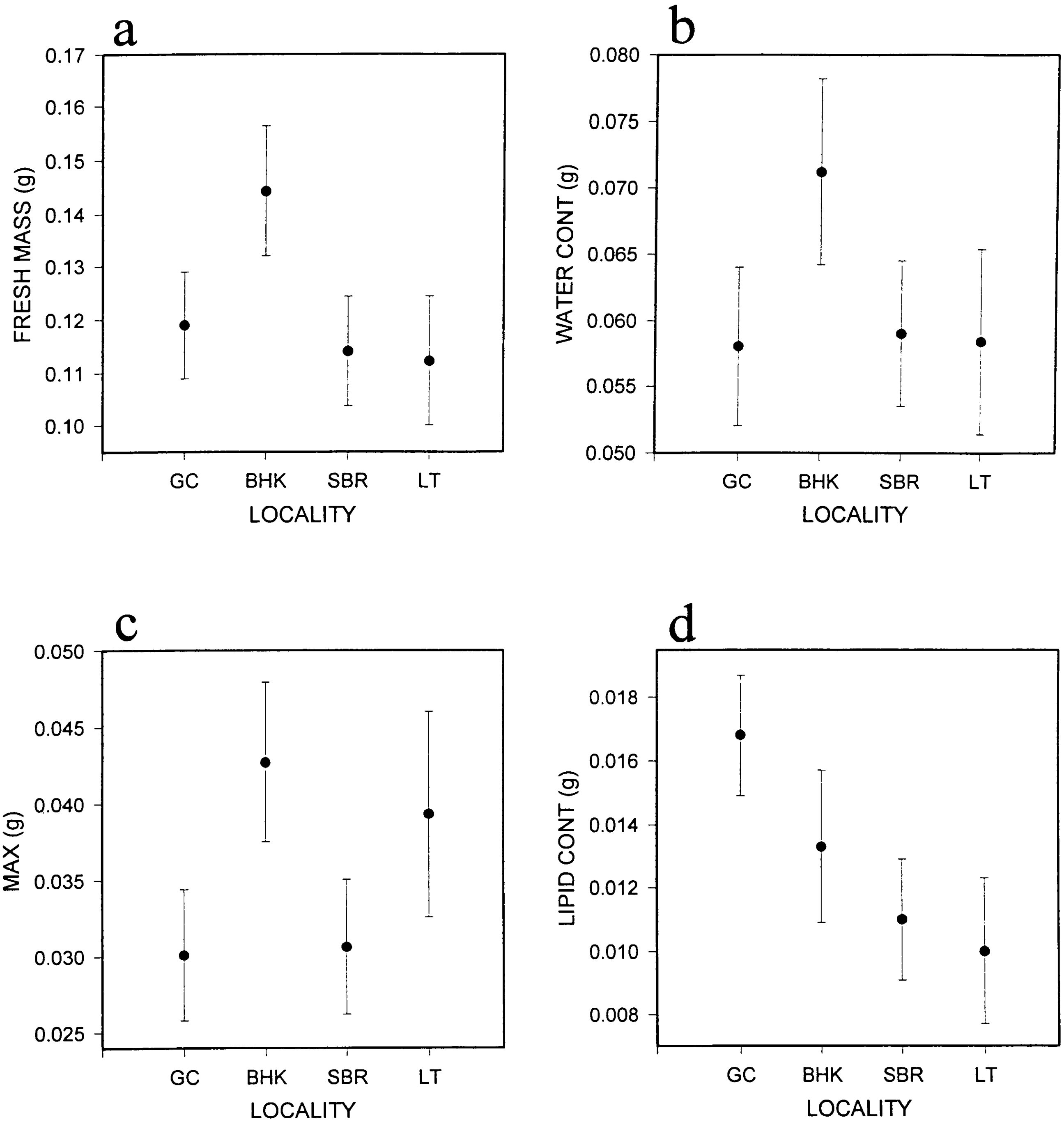

Figure 8 a-d *Trox squamiger* mean and 95% Tukey HSD intervals of (a) fresh mass; (b) body water content; (c) maximum water loss tolerance; (d) body lipid content, for *T. squamiger* from, Giant's Castle (GC), Boekenhoutskloof (BHK), Suikerbosrand (SBR) and Louis Trichardt (LT).

Table 11. Summary statistics of *Trox squamiger* from various collection sites desiccated at 27°C. Mean ± SE of fresh mass (FM, g), body water content (g and % FM), lipid content (g and % DM), mass-adjusted water content (ADJ WATER), maximum water loss tolerated (MAX, g and % FM), time to maximum water loss (TIME, h), the rate of water loss (RATE, g h^{-1} and % FM h^{-1}), and mass-adjusted maximum water loss tolerated (ADJ MAX).

Locality	n	FM (g)	WATER (g)	WATER (%)	LIPID (g)	LIPID (%)	ADJ WATER
Giant's Castle	15	0.12±0.0047	0.058±0.0027	48.83±1.254	0.017±0.0012	27.27±0.946	0.059±0.0016
Boekenhoutskloof	10	0.14±0.0087	0.071±0.0048	49.24±1.120	0.013±0.0012	18.13±1.15	0.060±0.0015
Suikerbosrand	14	0.11±0.0061	0.059±0.0035	51.81±0.619	0.011±0.0007	20.21±0.737	0.063±0.0008
Louis Trichardt	10	0.11±0.0032	0.058±0.0014	52.22±1.265	0.010±0.0015	17.96±1.876	0.063±0.0013

Locality	n	MAX (g)	MAX (%)	TIME (h)	RATE (g^{-h})	RATE (%FM^{-h})	ADJ MAX
Giant's Castle	15	0.030±0.0025	25.38±1.795	74.20±3.98	0.00042±0.000046	0.356±0.0322	0.031±0.0022
Boekenhoutskloof	10	0.043±0.0038	29.40±1.367	210.80±19.04	0.00021±0.000086	0.146±0.0089	0.036±0.0020
Suikerbosrand	14	0.031±0.0017	27.00±0.789	108.07±16.22	0.00039±0.00006	0.348±0.0544	0.033±0.0096
Louis Trichardt	6	0.039±0.0017	34.96±1.643	156.00±8.00	0.00026±0.00002	0.228±0.0189	0.041±0.0019

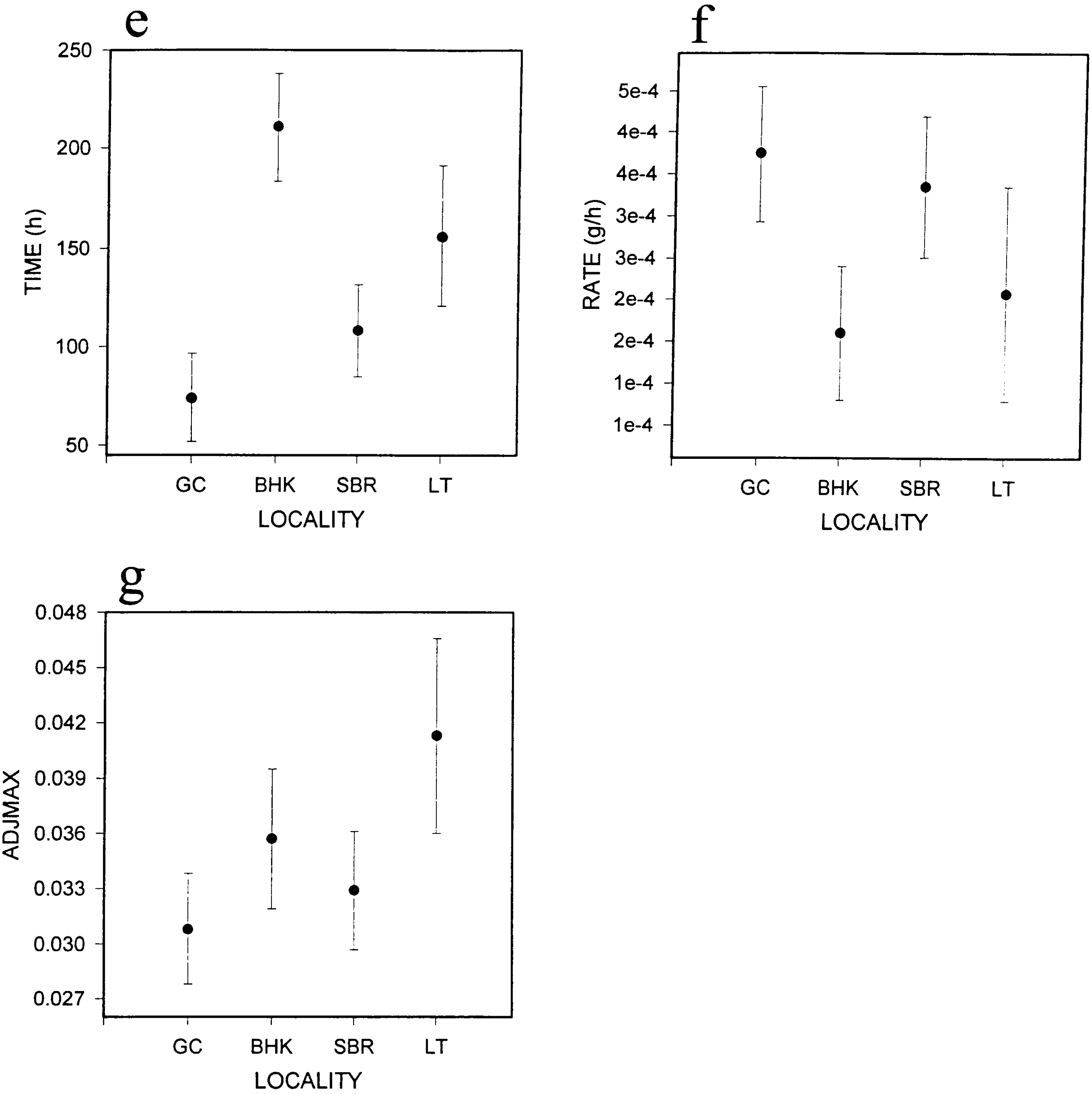

Figure 8 e-g *Trox squamiger* mean and 95% Tukey HSD intervals of (e) time to maximum water loss; (f) rate of water loss; (g) mass-adjusted maximum water loss tolerated, for *T. squamiger* from, Giant's Castle (GC), Boekenhoutskloof (BHK), Suikerbosrand (SBR) and Louis Trichardt (LT).

Summary statistics of *T. squamiger* from Giant's Castle, Boekenhoutskloof and Suikerbosrand desiccated at four temperature are given in Table 12. No significant correlation was found between the temperature at which desiccation occurred and the maximum water loss tolerated by beetles from the three localities ($P > 0.1$, refer to Table 12). Time to maximum water loss was found to be negatively correlated to desiccation temperature. This was most evident in *T. squamiger* from Boekenhoutskloof which displayed a significantly greater regression slope than those from the other two sites (Figs 9a-b). Once again, at high desiccation temperatures the beetles survival time was very similar where as at low temperatures, *T. squamiger* from Boekenhoutskloof tolerate desiccation for significantly longer than those from the other two collection sites. The log of rate of water loss was found to be positively correlated to increasing desiccation temperature. All three *T. squamiger* groups studied, displayed a similar temperature sensitivity to rate of water loss (Figs 9c-d).

The multiple regression analysis of survival time on the climatic variables for the sites at which each population was collected explained 56% of the variance in survival time of the populations with rainfall ($t = 5.098$, $P = 0.00001$), minimum temperature ($t = 4.25$, $P = 0.0001$) and maximum temperature ($t = 6.16$, $P = 0.00001$) being significant (regression equation: time = -2593.24 + 0.667 rainfall + 26.87 minimum temperature + 79.46 maximum temperature, df = 44). There was no relationship found between fresh mass and latitude ($F = 0.501$, $P = 0.48$, $r^2 = 1.2\%$, df = 44) or longitude ($F = 0.004$, $P = 0.949$, $r^2 = 0$, df = 44).

Table 12. The relationship between desiccation temperature and maximum water loss (MAX, g), time taken to maximum water loss (TIME, h) and log of rate of water loss (LOG RATE, g h^{-1}) for *Trox squamiger* from Giant's Castle, Boekenhoutskloof and Suikerbosrand. Regression equations are y = b temperature + a.

MAX Locality	Slope	Intercept	F	P	r^2(%)	df
Giant's Castle	-0.00011±0.0002	0.032±0.005	0.325	0.55	0.9	42
Boekenhoutskloof	-0.00044±0.00027	0.055±0.006	2.54	0.12	6.1	40
Suikerbosrand	-0.00027±0.00019	0.035±0.004	2.18	0.15	4.4	48

TIME Locality	Slope	Intercept	F	P	r^2(%)	df
Giant's Castle	-12.22±0.825	409.61±20.05	219.38	<0.001	84.3	42
Boekenhoutskloof	-25.48±1.790	878.30±42.09	201.91	<0.001	84.2	40
Suikerbosrand	-10.74±1.286	381.78±30.45	69.70	<0.001	59.7	48

LOG RATE Locality	Slope	Intercept	F	P	r^2(%)	df
Giant's Castle	0.044±0.004	-4.58±0.103	106.51	<0.001	72.2	42
Boekenhoutskloof	0.05±0.005	-4.89±0.119	98.02	<0.001	71.5	40
Suikerbosrand	0.041±0.005	-4.54±0.113	73.18	<0.001	60.9	48

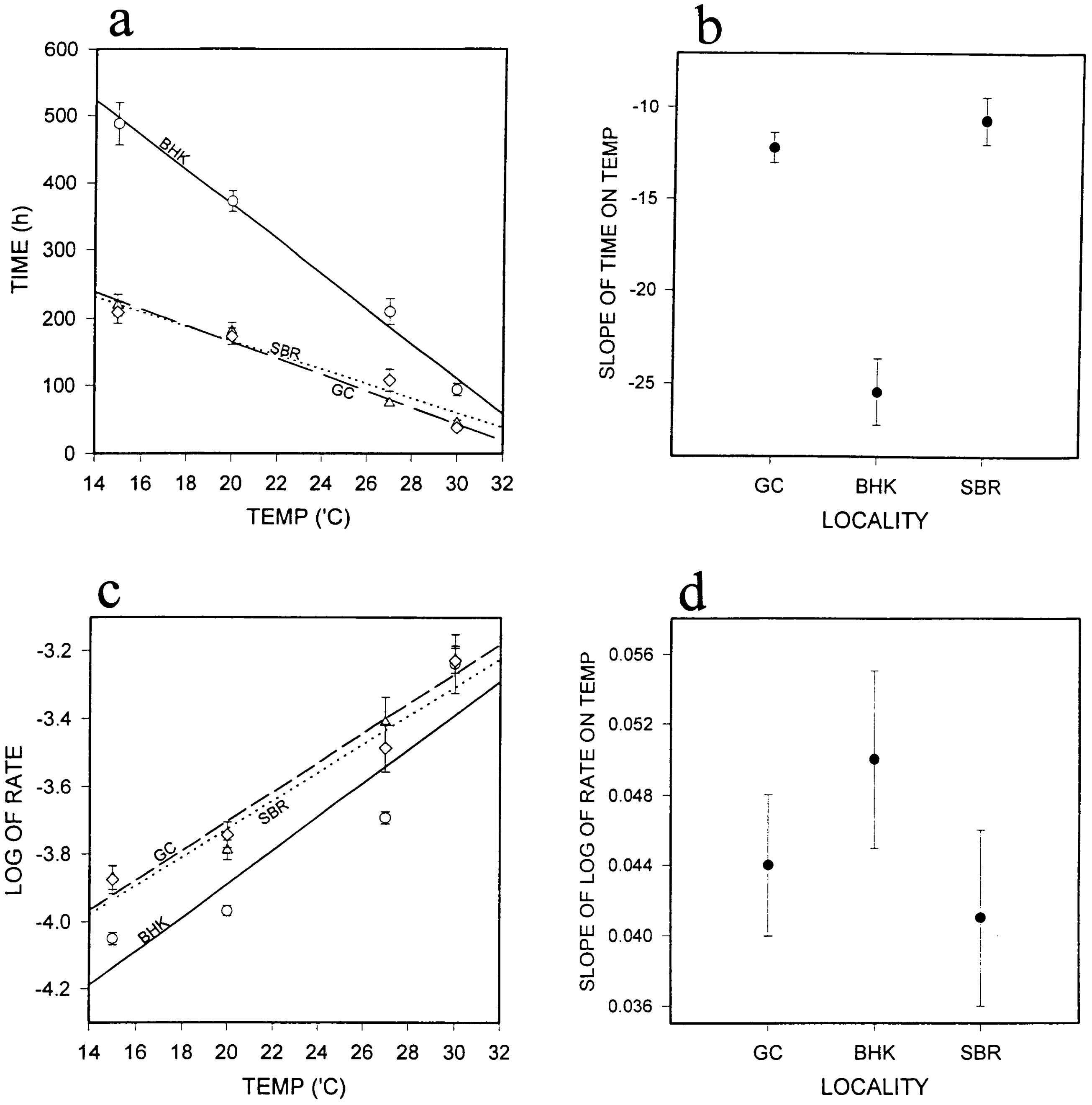

Figure 9 a-d *Trox squamiger*, the effect of desiccation temperature on (a) time to maximum water loss. The regression equations are y = b temperature + a; (b) mean and 95% MSD intervals of the slopes of the regression equations of time to maximum water loss; (c) log of rate of water loss. The regression equations are y = b temperature + a; (d) mean and 95% MSD intervals of the slopes of the regression equations of log of rate of water loss; for *T. squamiger* from Giant's Castle (GC, ---); Boekenhoutskloof (BHK, ___) and Suikerbosrand (SBR, ······).

4.4 Discussion

With the exception of the widely-distributed *O. squalidus*, survival time (the ultimate measure of desiccation tolerance, Le Lagadec, *et al.* in press, submitted ms) showed significant between population differences in the species examined here. Populations from the more arid sites had longer survival times than those from the more mesic sites and this was due both to enhanced physiological tolerances, and differences in fresh mass, and hence body water, content of the populations. Thus, habitat-associated differences in desiccation tolerance found at the interspecific level in keratin beetles are also present at the population level, suggesting that physiological adaptation to climatic conditions may well be important in this group. This finding is similar to that of Arad, *et al.* (1992, 1993a, 1993b) who have examined intraspecific differences in the desiccation tolerance of various land snail species, and supports the generalization that adaptation to desiccation is common in insects and related taxa (Edney 1977; Hadley 1994).

The effect of temperature on the survival time and desiccation rate of the populations examined was similar to that found at the interspecific level in this group of beetles (Le Lagadec, *et al.*, submitted ms). In general, as would be expected, desiccation rate increased with temperature (see Ahearn and Hadley 1969; Slobodchikoff 1983; Da Lage, *et al.* 1989; Lighton and Feener 1989; Parmenter, *et al.* 1989; Lighton, *et al.* 1994), while survival time decreased. However, the inter-population differences in the effect of temperature on survival time were similar to those found at the interspecific level (Le Lagadec, *et al.*, submitted ms). In general, populations from the drier, warmer areas, showed steeper regression slopes than did those from the more mesic sites. In other words, survival times converged at the higher temperatures, whereas they diverged dramatically at the lower temperatures. Le Lagadec, *et al.* (submitted ms) have argued that prolonged survival times (at the lower temperatures) in the more arid dwelling beetles ensures their survival during the unpredictably long, dry (winter) season. Thus, it appears that the survival strategies adopted by restricted range species from arid environments, as opposed to those from more mesic environments, are also characteristic of different populations of more widely distributed species. Few studies have demonstrated such correspondence of physiological variation at the inter and intraspecific levels.

Both with regard to between population differences in survival time, and the sensitivity of this measure to temperature, the widely-distributed *O. squalidus* was clearly an exception to the trends found for the remaining species. In addition, of the three species from which data from more than two populations were available, this was the only species which showed no relationship between among-population variability in survival time and the climate of the sites in question. Even for those species for which only two populations were examined (*O. radula, T. consimilis*), significant between site variation, associated with differences in the aridity of the sites was found. This suggests that *O. squalidus* is relying either on behavioural mechanisms (Scholtz and Caveney 1988; Le Lagadec, *et al.* in press, submitted ms) and/or on differences in morphology to overcome the environmental stress presented by the different habitats it occupies. Indeed, fresh mass was significantly related to both latitude and longitude in this species, and at least in the Kalahari population, *O. squalidus* is known to lead a slightly more protected life than other trogid species. Scholtz and Caveney (1988, 1992) found that *O. squalidus* emerges from the soil only when ambient temperatures are relatively low and always feeds in the interface between the soil and the carcass. Thus, during feeding, through behaviour, this species can avoid exposed to direct environmental conditions.

These results suggest that very different mechanisms may be operating which allow species to maintain wide ranges. In some cases this may be due to considerable physiological adaptation (e.g. *O. melancholicus*, which is widely distributed see Fig. 1a), whereas in others, this may be due to modulation of behaviour to suite the conditions within which the animals find themselves (*O. squalidus* see Fig. 1c). In fact the former strategy seems to be the one most commonly employed by the keratin beetles in this study, given that significant climate associated variation in desiccation resistance was found in populations of four, of the five species studied here, and that interspecific differences in desiccation resistance in these beetles showed similar, climate related variation (Le Lagadec, *et al.* in press, submitted ms). That fresh mass played a significant role in enhancing desiccation resistance (i.e. via significantly higher water contents) at both the population and species level, is also of considerable significance. Although this has been documented previously in a variety of taxa (Chown and van Drimmelen 1992; Chown 1993; Arad 1993a; Kaspari 1993; Lighton, *et al.* 1994; Chown, *et al.* 1995) particularly at the interspecific level (see Maurer, *et al.* 1992; Cushman, *et al.* 1993; Kaspari 1993; Kaspari and Vargo 1995), and indeed forms the basis of the "resistance hypothesis" for latitudinal body size clines, the importance of this variation at the intraspecific level has, until now not been recognized. Traditionally, latitudinal size variation within species (often known as Bergmann's rule, Mayr 1956) has been ascribed to differences in the temperatures at which the animals develop (Atkinson and Sibly 1997; Mousseau 1997; Partridge and Coyne 1997). However, the data on keratin beetles suggest that the ability of species to survive desiccation may also play an important role in determining intraspecific body size clines. Nonetheless, the importance of such variation in morphology seems to be linked to the ability of populations to show physiological adaptation to their surrounding environments, and to the distributional extent of the species concerned. For example, *O. melancholicus* showed significant between site variation in desiccation resistance, which was significantly related to both aridity and temperature. However, in this species, no significant variation in fresh mass was found. A similar situation was found in *T. squamiger*. On the contrary, *O. squalidus* showed little site related variation in desiccation tolerance, whereas significant variation in the body size of this species was apparent. This suggests that greater attention should be given to the role of desiccation resistance in determining both the position and extent of geographic ranges, and body size clines at both the intra- and interspecific levels.

In conclusion, I have shown that there is significant inter-population variation in desiccation tolerance of at least four fairly widely distributed keratin beetle species, that this variation is remarkably similar (in both strength and direction) to that found between more narrowly distributed species (see Le Lagadec, *et al.* in press, submitted ms); and that this variation is closely related to differences in climate between the sites at which the populations (and species) were collected. This provides strong evidence for physiological adaptation of the species to the environments they occupy. In addition, it suggests that greater attention should be given to the role of physiology in determining both the range size and extent of species, and in determining clinal variation in body size.

4.5 References

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> *Who can ever learn the will of God?*
> *Human reason is not adequate for the task,*
> *and our philosophies tend to mislead us,*
> *because our mortal bodies weigh our souls down.*
> *The body is a temporary structure made of earth,*
> *a burden to the active mind.*
> *All we can do is make guesses about the things on earth,*
> *we must struggle to learn about things close to us.*
>
> Wisdom of Solomon 9: 13-16

Chapter 5: Conclusion

5.1 Water balance in keratin beetles

Water balance and desiccation tolerance in keratin beetles is not unlike that of similar-sized beetles from similar habitats (see Chapter 2). Their body water content, rate of water loss during dehydration and time to maximum water loss proved to be very similar to those of arid dwelling tenebrionid beetles (Broza, *et al.* 1976; Gehrken and Sømme 1994). Furthermore, the keratin beetles' inability to catabolize significant amounts of lipids during desiccation, (with the exception of *Omorgus freyi* and possibly *Trox consimilis*), is not unusual for the class Insecta (e.g. Shaw and Stobbart 1972; Tucker 1977; Naidu and Hattingh 1986, 1988; Lighton and Bartholomew 1988; Hadley 1994). Lipids yield twice as much metabolic water during their catabolism as carbohydrates (Downer 1985; Wharton 1985). Lipid metabolism should, therefore, be an important source of metabolic water in insects (Edney 1977; Hadley 1994). However, if respiratory water loss is considerably greater than that of cuticular water loss (as argued in Chapter 2), then lipid catabolism is liable to play a minor role in water balance. It is of interest that the Kalahari endemic, *O. freyi*, metabolized significant amounts of lipids during desiccation and I believe that this phenomenon is related to both the species' habitat and its aptery. *Omorgus freyi* is the only species studied here that has a well-developed subelytral cavity (Scholtz and Caveney 1988). The subelytral cavity serves to reduce the rate of respiratory water loss (Cloudsley-Thompson 1964; Zachariassen and Einarson 1993), and according to Scholtz and Caveney (1988), is an adaptation to arid environments. Therefore, although respiratory rate is likely to increase during lipid metabolism, the subelytral cavity probably protects this species from excessive respiratory water loss. Furthermore, since this species is flightless, and rainfall in the Kalahari is very erratic (Rasa 1997), it is probable that *O. freyi* has developed specialized physiological characteristics, such as the production of metabolic water, to ensure their prolonged survival in this harsh habitat. Similarly, *T. consimilis* proved to have a very long survival time during desiccation (refer to Chapters 2 and 3), and it is feasible that this species has also developed the ability to catabolize lipids during desiccation. Given this species' low body water content (related to its small body size) and its success in colonizing arid habitats, the utilization of metabolic water in *T. consimilis*, could once again be an adaptation to arid conditions which may be lacking in most other arid-dwelling species possibly because of their reliance on their high body water content for their survival. In order to obtain more evidence concerning *O. freyi* and *T. consimilis*'s ability to utilize metabolic water during desiccation, it may be useful to determine the keratin beetles' metabolic rates while in a state of negative water balance and also to ascertain whether respiratory water loss does in fact exceed cuticular water loss during desiccation.

The keratin beetles' inability to osmoregulate, hence the term "osmotolerant", is relatively unique amongst the insects. With the exception of high altitude tenebrionids (Gehrken and Sømme 1994), no other incidences of such exceptionally high haemolymph osmolalities (during desiccation) have, to my knowledge, been recorded for insects. It is possible that the keratin beetles utilize such high haemolymph osmolality (even prior to desiccation) to restrict the rate of transpirational water loss. The solute concentration in a fluid compartment, e.g. haemolymph, determines the water activity in that compartment (Edney 1977; Wharton 1985). If the solute concentration is increased, relative to its surroundings, water will move into the compartment with the highest solute concentration. Since the haemolymph is thought to be the insect's largest water reservoir (Hadley 1994), by maintaining a high haemolymph osmolality, the beetles could be restricting the loss of water to the environment. Because the keratin beetles cannot absorb atmospheric water (refer to Chapter 2), high haemolymph osmolality would not facilitate the movement of water from the environment into the beetles but could restrict the rate of water loss to the environment and certainly increase water reabsorption from the keratin beetles' digestive tract. According to Gehrken and Sømme (1994), an increase in haemolymph osmolality leads to a proportional removal of intracellular water and thus, a reduction in cell volume, which is usually fatal (Zachariassen and Einarson 1993). How then do the keratin beetles cope with the problem of extra- and intracellular dehydration? There is considerable evidence that in cold-hardy insects, low temperatures trigger the production of cryoprotectants (usually carbohydrates) or ice nucleating agents (usually peptides), and possibly also extra- and/or intracellular dehydration, and subsequently diapause, during which time metabolic activity is minimal (Zachariassen 1980, 1982; Baust and Rojas 1985; Bale 1987; Block 1990; Storey and Storey 1991; Block, *et al.* 1992; Lundheim and Zachariassen 1993; Block and Convey 1995). Moreover, Holmstrup (1994); Holmstrup and Westh (1994), and Holmstrup and Zachariassen (1996) identified extracellular dehydration, induced by low temperatures, and subsequent diapause, as the key to cold-hardiness in lumbricid earthworm cocoons. Since it is well known that diapause or quiescence is a survival mechanism employed during unfavourable conditions (Denlinger, *et al.* 1991; Hadley 1994), it is feasible that in keratin beetles, water loss and increasing extracellular osmolality trigger quiescence or diapause accompanied by reduced metabolic rates and thus, prolonged survival during extended dry periods. These speculations warrant further investigation and promise to be extremely interesting.

5.2 The role of allometry and physiology in determining the keratin beetles' distribution patterns

To date, many comparative physiological studies have been undertaken (eg. Ahearn 1970; Slobodchikoff 1983; Arad 1993a, 1993b; Gehrken and Sømme 1994), but the uniqueness of this particular study lies in the monophyletic, homogeneous nature of the taxa examined. This is also one of the few studies that has ventured to explain distribution patters both on an inter- and intraspecific level using physiological tolerance as its limiting factor. The data presented in Chapters 3 and 4 show a very clear correlation between habitat aridity and body size (Figs 1a and 2). In general, arid dwelling trogid species proved to be morphologically larger, contained more body water, were able to lose more water (in absolute terms) and thus, were able to tolerate desiccation for significantly longer than their more mesic counterparts (Figs. 1a and 3). There were, however, two exceptions to these generalizations, namely *Trox consimilis* and the eurytopic *Omorgus squalidus*. *Trox consimilis,* despite their small body size, successfully inhabit relatively arid areas, possible due to their ability to restrict the rate of water loss during desiccation and conceivably, through their ability to utilize metabolic water through lipid catabolism (see above). On the other hand, *Omorgus squalidus,* despite their large body size and high water content, were unable to survive desiccation for longer than the more mesic beetles. This was due to the species' exceptionally high rate of water loss during desiccation (see Chapters 2 and 3), yet *O. squalidus* also successfully inhabit arid regions. Furthermore, on an intraspecific level, there was very little evidence that arid dwelling *O. squalidus* populations were physiologically more resistant to desiccation than the mesic populations. From studies conducted in the Kalahari (Scholtz and Caveney 1988) it can be concluded that this species avoids desiccation in arid habitats through behavioural rather physiological adaptations.

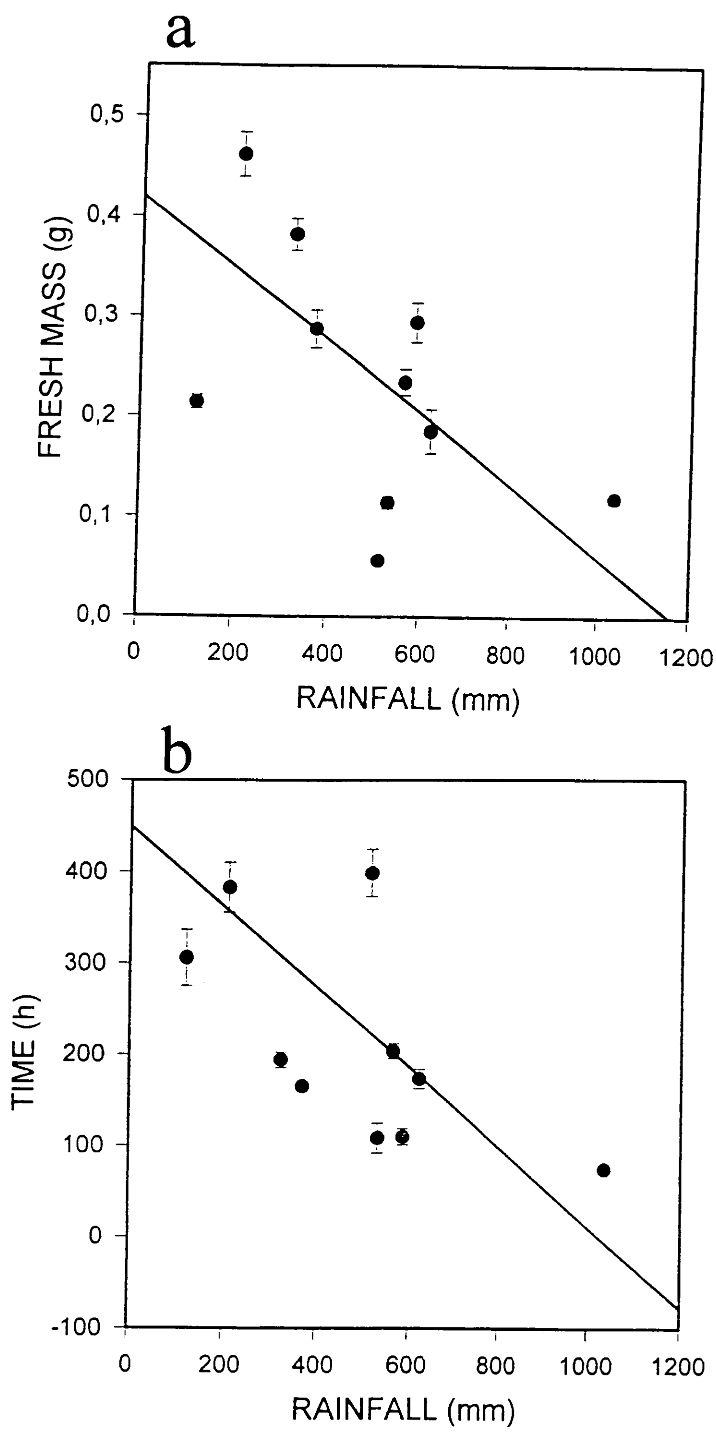

Figure 1 a-b Relationship between the average annual rainfall figure at the collection sites of keratin beetles, and (a) the keratin beetles' initial fresh mass (g, fresh mass = 0.422 - 0.0004 x rainfall, $r^2 = 23.4\%$, $P < 0.0001$, df = 220); and (b) survival time (h, time = 455.8 - 0.449 x rainfall, $r^2 = 36.4\%$, $P < 0.0001$, df = 220) when desiccated at 27°C (data derived from Chapters 3 and 4, the lines are linear regression lines, regression equations are y = a + b rainfall).

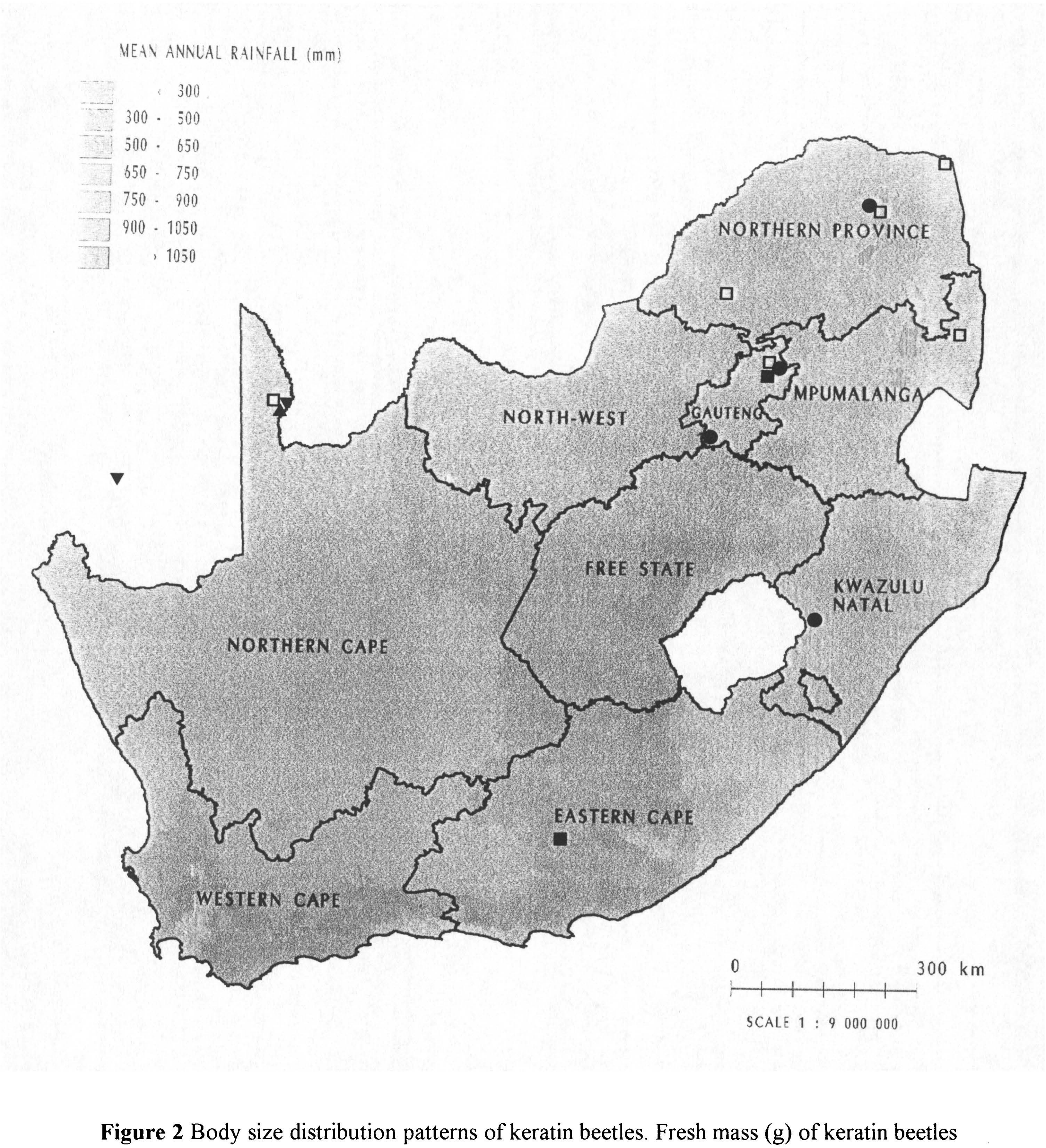

Figure 2 Body size distribution patterns of keratin beetles. Fresh mass (g) of keratin beetles plotted on an average annual rainfall map of South Africa (Fresh mass < 0.1= ■; 0.1-0.15 = ●; 0.16-0.25 = □; 0.35-0.5 = ▴; > 0.5 = ▾; *Omorgus squalidus* has been excluded from the figure due to its ubiquitous distribution).

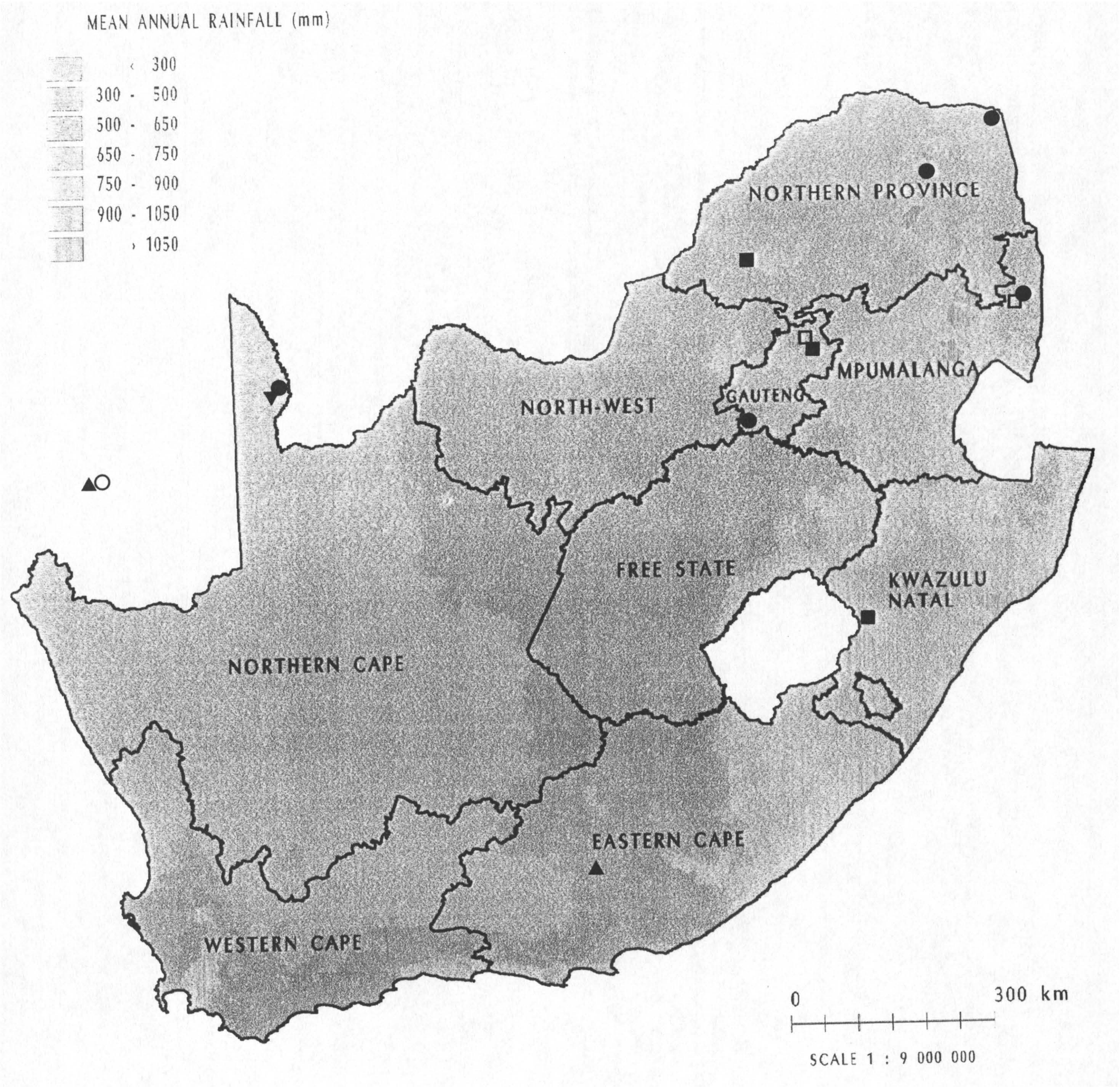

Figure 3 Survival time (h) of keratin beetles plotted on an average annual rainfall map of South Africa (Survival time < 100 = ■; 100-200 = ●; 201-300 = □; 301-400 = ▴; 401-500 = ▾; >500 = ○, *Omorgus squalidus* has been excluded from the figure due to its ubiquitous distribution).

Despite the two exceptions noted above, multiple regression analyses of both the intra- and interspecific desiccation tolerance studies (Chapters 3 and 4) revealed a close correlation between the beetles' desiccation survival time, and their fresh mass and the average annual rainfall figure at their collecting sites (time = 279.5 + 497.0 x fresh mass - 0.275 rainfall, $r^2 = 61.08\%$, $P < 0.0001$, df = 121 for data from Chapter 3; time = 263.6 + 455.7 x fresh mass - 0.283 rainfall, $r^2 = 52.7\%$, $P < 0.0001$, df = 219 for data from Chapters 3 and 4). By eliminating body size as a major contributing factor (by conducting an analysis of covariance, Packard and Boardman 1988), the physiological adaption to arid habitats was clearly evident. Arid dwelling beetles tended to have a lower rate of water loss and to be able to tolerate desiccation for longer than the more mesic beetles (refer to Chapters 3 and 4). These findings were confirmed when the keratin beetles were desiccated at various temperatures. The arid dwelling beetles, both intra- and interspecifically, showed a greater sensitivity to desiccation temperature in that they displayed markedly lower rates of water loss and longer survival times at the lower desiccation temperatures compared to those of the mesic beetles. It was speculated in Chapter 3 that these arid dwelling beetles are able to to tolerate climatically harsh environments because of reduced water loss rates and prolonged survival times at the low temperatures characteristic of the arid winter months.

This study has shown that in the Trogidae, phylogeny (equivalent to generic placement due to the monophyletic nature of the family, see Scholtz 1986) had a minimal effect on desiccation tolerance. Although the genus *Trox* is thought to be more temperate in distribution, and *Omorgus* more arid, *T. consimilis*'s distributional range extends into arid areas and the species proved to be more tolerant to desiccation than many of the mesic *Omorgus* species. Thus, variance in desiccation resistance is partitioned at the species or even population, rather than at the genus level in this family.

In conclusion, this study has shown that in the southern African keratin beetles, body size has a pronounced influence on desiccation resistance and that both allometry and physiological tolerance greatly influence the species distributional patterns. I would suggest that in view of the ongoing debate on body size evolution, more attention should be paid to the role of physiological tolerances. Furthermore, since the present study was conducted on a local scale, a comprehensive study, perhaps on a global scale, correlating body size to habitat aridification will serve to eliminate scepticism concerning the role of allometry in distributional patterns.

5.3 References

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Anyone who does not know God is simply foolish.
Such people look at the good things around them
and still fail to see the living God.
They have studied the things He made,
but they have not recognized the one who made them.
Instead they suppose that the gods who rule the world are
fire or wind or storms or the circling stars or
rushing water or the heavenly bodies.
People were so delighted with the beauty of these things
that they thought they must be gods,
but they should have realized that these things
have a master and that He is much greater than all of them,
for He is the creator of beauty, and He created them.

If they had enough intelligence to speculate about the nature of the universe,
why did they never find the Lord of all things?

Wisdom of Solomon 13:1-4, 9