

METABOLIC EVOLUTION OF SUBTERRANEAN DIVING BEETLES FROM THE CALCRETES OF WESTERN AUSTRALIA

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Subterranean estuaries formed within carbonate deposits in the paleodrainage systems of Western Australia are habitats for an great diversity of stygiobitic beetles, some of which are sympatric sister species. This study examines the metabolism of stygiobiont beetles using respiration and key metabolic enzymes to determine whether sympatric sister species differ in energy allocation and respiration.

Key words: Dytiscidae, calcretes, metabolism, lactate dehydrogenase, citrate synthase

INTRODUCTION

Subterranean estuaries occur in >10 m thick carbonate deposits in the arid Yilgarn region of Western Australia. These groundwater calcretes are deposited from groundwater flow immediately upstream of salt lakes that represent regional base level and possibly date from the mid-Tertiary (HUMPHREYS, 2001).

The Yilgarn calcretes, each harboring a unique species assemblage, provide habitat for the richest diversity of subterranean diving beetles in the world (BALKE *et al.*, 2004). There are more than 100 species of stygiobitic diving beetles within the calcretes, the majority of species evolving from the dispersal of epigeal ancestors. Molecular clock analyses (COOPER *et al.*, 2002) suggest that the early epigeal ancestors made the transition to a subterranean existence during the Miocene, 8–5 mya. Beetle species in the calcretes often occur as groups of sister species suggesting speciation may have occurred in sympatry (LEIJS *et al.*, 2012). Single calcretes can contain up to four sympatric species separated morphologically by size (LEYS *et al.*, 2003).

Current debate surrounds the driving force for the colonization of subterranean habitats (LEIJS *et al.*, 2012), however, regardless of the mechanism driving evolution, each hypothesis requires species to have pre-adaptation to the subterranean environment. For members of the Dytiscidae, there is evidence of a potential preadap-

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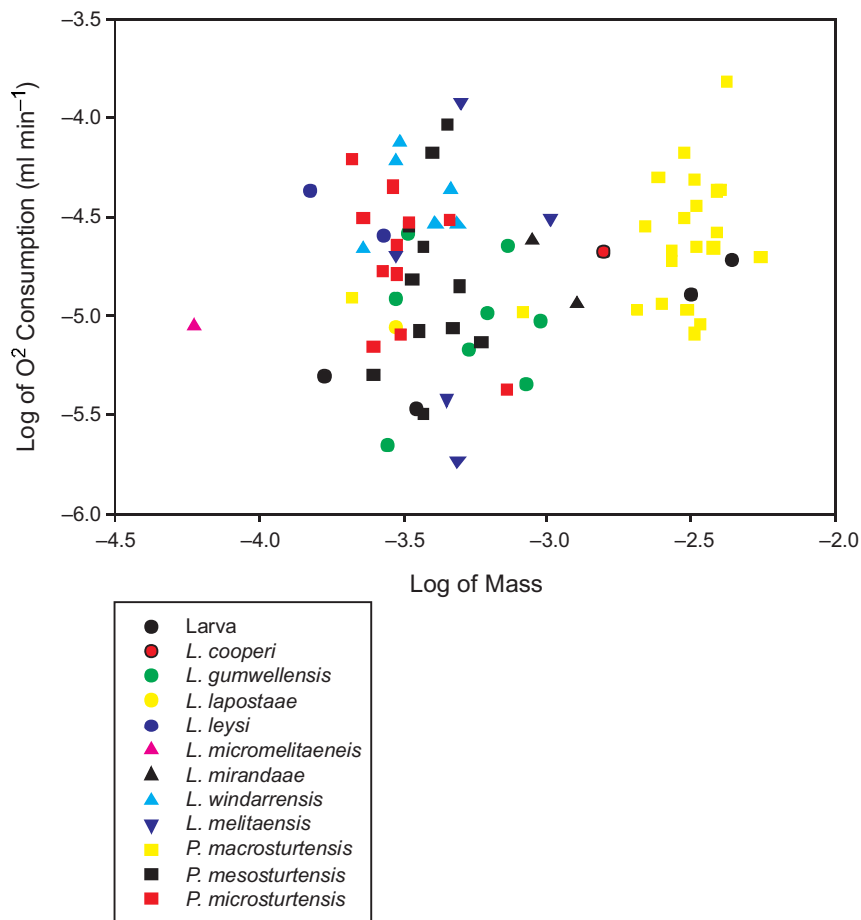


Fig. 1

tation. Oxygen uptake by the elytra was assumed by SMRŽ (1981). KEHL & DETNER (2009) demonstrated that oxygen was taken up by submerged *Deronectes aubei*, a rheophilic species of beetle. They examined the elytral surface and observed tracheated setae which act as gills. This information combined with the presence of groups of sister species in the calcretes leads us to several questions:

1. Are the species of subterranean diving beetles capable of oxygen consumption submerged as anticipated by KEHL & DETNER (2009)? If so, how do the rates compare with those of epigean as well as hypogean, aquatic dwelling species?

2. What is driving the speciation occurring in the calcretes? Can the evolutionary strategy be observed in the metabolic rates of the co-occurring beetle species?

MATERIALS & METHODS

Diving beetles were collected from bore holes in seven different calcretes within about 130 km of Sturt Meadows pastoral station in Western Australia by means of small (65 mm diameter, 250 μm mesh size) plankton nets (ALLFORD *et al.*, 2008).

Oxygen consumption measurements were conducted on individual beetles using microcathode, polarographic oxygen electrodes. At the completion of the incubations (6–12h), specimens were removed from the respirometers and frozen immediately in liquid nitrogen for enzyme analyses. Citrate synthase (CS) and lactate dehydrogenase (LDH), activities were subsequently determined.

RESULTS AND DISCUSSION

Paroster (n = 102) specimens were collected from the Sturt Meadows and *Limbo-*
dessus (n = 60) from calcretes in the surrounding area (detailed in WATTS & HUMP-
HREYS, 2009). All species examined demonstrated submerged oxygen consumption
(Fig. 1).

Although collected from dysoxic systems ($0.3\text{--}3\text{ mg O}_2\text{ l}^{-1}$) (TYSON & PEARSON,
1991) all beetles were aerobically poised, with CS activities /LDH activities >1 .
Subterranean beetles evolved from multiple colonization events by epigeal species,
therefore, the biochemical heritage is predominantly aerobic. Unlike other subterra-
nean species from anchialine regions e.g. copepods, ostracods, amphipods, isopods
and remipedes, which are anaerobically poised (BISHOP *et al.*, 2004), calcrete species
must function with a heavy reliance on aerobic processes.

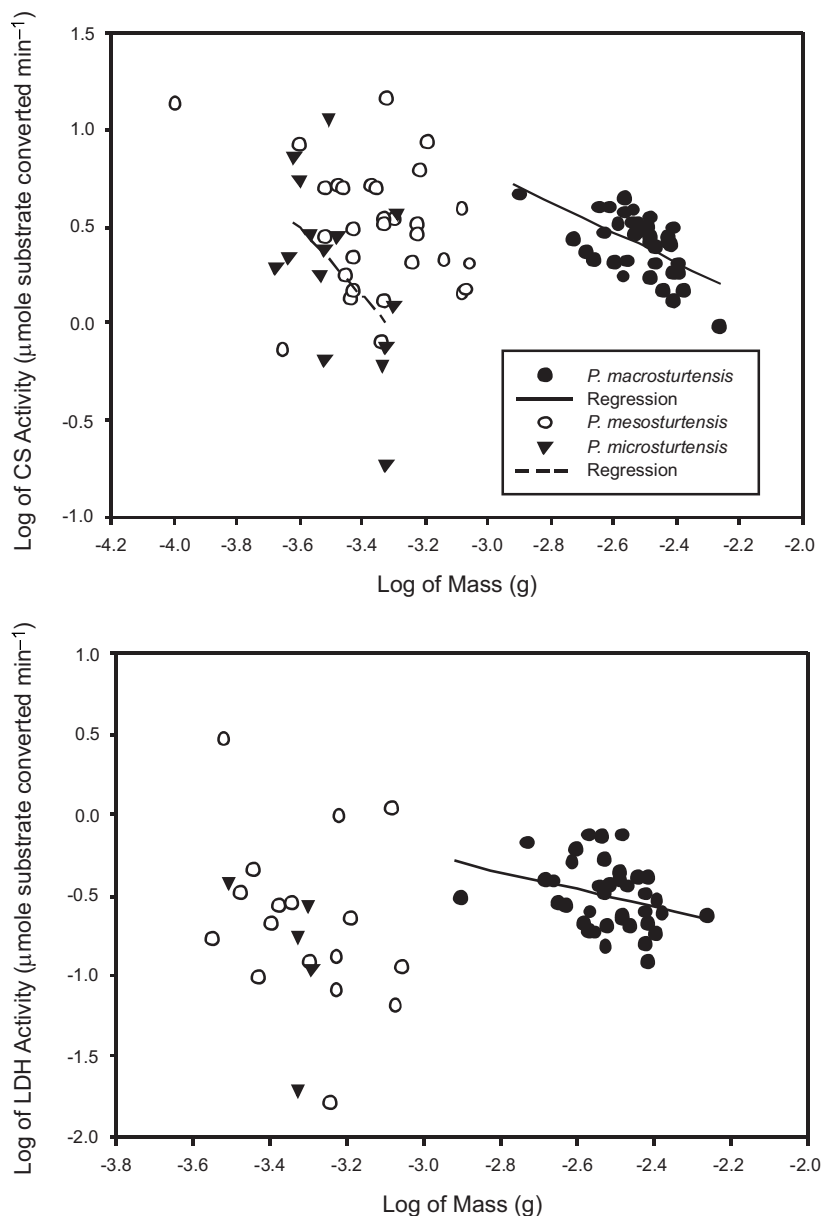


Fig. 2

To examine species differences within a calcrete, we focused on the sister species *P. microsturtensis*, *P. mesosturtensis* and *P. macrosturtensis* from Sturt Meadows. There were no significant differences in oxygen consumption, and CS and LDH activities (μmol substrate converted min^{-1}) between the three species. *P. macrosturtensis* (mean wet mass = $3.02 \pm 0.152 \mu\text{g}$, $n = 41$), although significantly larger, does not require greater energy than either *P. mesosturtensis* (mean wet mass = $0.48 \pm 0.071 \mu\text{g}$, $n = 41$) or *P. microsturtensis* (mean wet mass = $0.35 \pm 0.034 \mu\text{g}$, $n = 20$).

There are substantial physiological and ecological benefits to greater mass without the metabolic expense of having to maintain larger size e.g. reduced predation, mate attraction, starvation resistance (CUSHMAN *et al.*, 1993), increased female reproductive performance (MCCABE & PARTRIDGE, 1997), and even increased tissue synthesis (SIBLY & CALOW, 1986).

For the subterranean beetles, the larger body size does come at a cost. Within each sister species, enzyme activities, both CS and LDH, declined with increasing mass (Fig. 2). Citrate synthase, located at the beginning of the Krebs's citric acid cycle, is an indicator of an organism's maximum aerobic potential. In *P. macrosturtensis* and *P. microsturtensis*, the decline in CS activity with increasing mass was significant ($p < 0.05$). Lactate dehydrogenase is the terminal enzyme in glycolysis and serves as indicator of glycolytic potential. With regard to adaptation, scaling of glycolytic power is more plastic than that of aerobic processes (CHILDRESS & SOMERO, 1990) therefore, adaptation to a dysoxic system should be evident in LDH activities. The significant decline in LDH with increasing mass in *P. macrosturtensis* indicates a decline in burst swim potential in larger organisms. Smaller beetles in each species are using glycolytic potential to escape predation while larger beetles are sacrificing that potential for increased mass.

CONCLUSIONS

In this study, we demonstrated that subterranean diving beetles in the genera *Paroster* and *Limbodessus* consume oxygen when submerged. However, the oxygen consumption rate does not increase with increasing mass as would be expected. Across sister species groups, we did not observe a significant difference in CS or LDH activities between species but for each species CS and LDH activities decreased with increasing beetle mass. From this result, we can conclude that within each genus burst swim potential declines as individuals sacrifice glycolytic potential for increased mass. By extrapolating across the 100 or so subterranean dytiscid species, the results provide preliminary evidence that this phenotype (consumption of oxygen when submerged) has evolved independently numerous times from surface ancestral species.

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