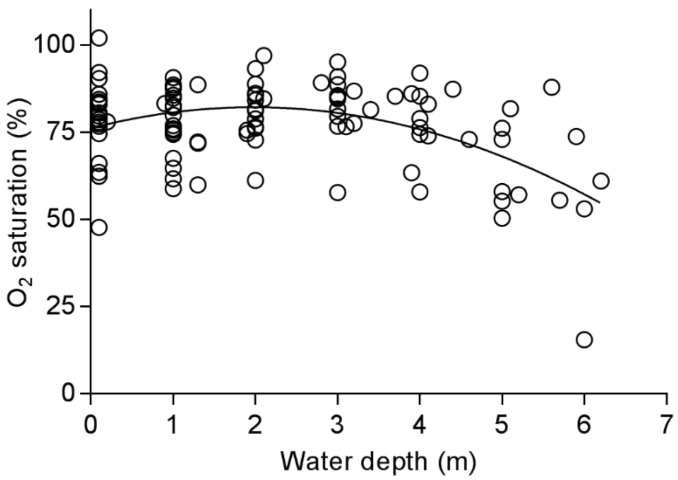

Figure S1. Change in O_2 saturation with depth in boreholes known to have subterranean beetles at the Sturt Meadows aquifer (*N*=22 boreholes, *N*=107 samples). Second order polynomial regression, y = 76.11+6.079x+-1.539x^2. Data collected April 2015, courtesy William (Bill) Humphreys, Western Australian Museum.

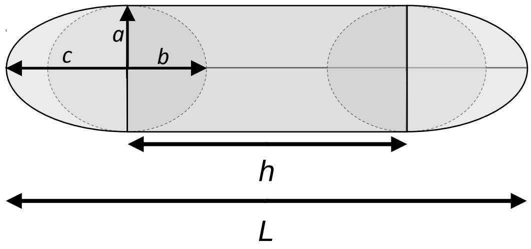

Figure S2. 3D geometric lozenge shape model that approximates the shape of subterranean diving beetles and allows estimation of surface area of the beetle. Axis a<*b, b*=*c*, *L* = total length, and h = elliptical cylinder length (*L* – 2*c*). Axis b is perpendicular to the plane of the page.

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Table S1. A mathematical model based on Fick's general diffusion equation to calculate PO_2 at different points within the O_2 cascade of subterranean diving beetles to determine where limitations to cutaneous respiration may exist. The model calculates the PO_2 at the surface of the beetle and on the inside of the cuticle representing O_2 diffusion through the boundary layer and cuticle, respectively. The model has the following assumptions: (1) surface area for gas exchange (*A*) is defined as the surface area of a lozenge (ellipsoid and elliptical cylinder, Fig. S2), (2) conductance of O_2 is constant through the water or cuticle and is the product of the Krogh's coefficient of O_2 in each medium and the quotient of *A* and boundary layer (X_w) or cuticle thickness (X_c), (3) water is well-mixed and in equilibrium with atmospheric PO_2 (20.6 kPa) at 25°C, and (4) diffusion to and into the surface of the beetle is linear. Variables and constants used in the model are shown in Table S2, and O_2 consumption rate values are those measured experimentally in the water-only chambers (Table 2).

	Equation	*Description*
(1)	$\dot{M}O_2 = KO_2 \times A/X \times \Delta PO_2$	Fick's diffusion equation used to describe O_2 diffusion through water to a respiratory surface (Rahn and Paganelli, 1968). Where $\dot{M}O_2$ (pmol s^{-1}) is the rate of O_2 consumption, KO_2 (pmol s^{-1} kPa^{-1} cm^{-1}) is the Krogh's coefficient of diffusion, the product of capacitance and diffusivity, *A* (cm^2) is the surface area for gas exchange, *X* (cm) is the boundary layer thickness, a fluid layer above a respiratory surface deficient in O_2 that provides resistance to O_2 diffusion, and ΔPO_2 (kPa) is the PO_2 difference between the surrounding water and respiratory surface.
(2) (3)	$PO_{2,s} = PO_{2,w} - ((\dot{M}O_2 \times X_w)/(KO_{2,w} \times A))$ $PO_{2,in} = PO_{2,s} - ((\dot{M}O_2 \times X_c)/(KO_{2,c} \times A))$	Rearrangements of Eq. 1 allow the calculation of PO_2 at the surface of the beetle (Eq. 2, $PO_{2,s}$, kPa) and on the inside of the cuticle (Eq. 3, $PO_{2,in}$, kPa), representing diffusion through the boundary layer and cuticle respectively. Where $PO_{2,w}$, is the PO_2 of the bulk surrounding water, $\dot{M}O_2$ is the experimentally determined O_2 consumption rate, X_w is the O_2 boundary layer surrounding the surface of the beetle, X_c is the cuticle thickness, $KO_{2,w}$ is the Krogh's coefficient for water, $KO_{2,C}$ is the Krogh's coefficient for the cuticle, and *A* is the surface area for gas exchange.

(4)	$A = 4\pi((ab)^{1.6}+(ac)^{1.6}+(bc)^{1.6}) \div 3)^{1/1.6} + (2\pi\sqrt{(1/2(a^2 + b^2))} \times h)$	Surface area of the beetle (A) can be calculated assuming the beetles are a lozenge shape, the sum of an ellipsoid (Knud-Thomsen approximation, the two ends) and an elliptical cylinder (the middle) (Michon, 2015 [http://www.numericana.com/answer/ellipsoid.htm#thomsen]; Spiegel et al., 2013), according to Eq. 4. Semi-axes a, b and c, and length (h) of the elliptical cylinder are shown in Fig. S2. Axis b (half of beetle width) is determined for each species from the diagrams given in Watts and Humphreys (2006), with the total length to width ratio (L:2b) being 1:2.5 for *P. macrosturtensis*, 1:2.97 for *L. palmulaoides*, and 1:2.45 for *P. mesosturtensis*. Axis c equals axis b. Axis a is determined for each species in the model by producing a linear regression of the mass of the lozenge, where mass (mg) $M_b = V \times 1078.4$ mg cm^{-3} (Density value determined for backswimmers, (Matthews and Seymour, 2008)) with an increasing value for a. Volume (V, cm^3) is determined with Eq. 5, the sum of the volume of an ellipsoid and elliptical cylinder (Spiegel et al., 2013). The linear mass regression is then rearranged to calculate a from the predicted wet mass of each species. The model can be adjusted to calculate P_{O_2} with increasing size by taking the average ratios between L and axes a, b and c (a = 1:10.3, b,c = 1:5.3) in the three beetle species and maintaining the ratios of axes with increasing length. The size range used was 0.04 – 4.82 mg (L = 0.1 – 5 mm).
(5)	$V = 4/3\pi abc + \pi abh$	Calculation for the volume of the lozenge shape.

Table S2. Variables and constants used in the mathematical model to calculate the PO_2 at the surface of the beetle and on the inside of the cuticle (Table S1).

Variable	Value	Units	Reference
$PO_{2,w}$	20.6	kPa	1
X_w	0.010 (circulated water, all species), 0.075 (*P. macrosturtensis* in stagnant water), 0.070 (*L. palmulaoides* in stagnant water), 0.045 (*P. mesosturtensis* in stagnant water)	cm	2, 3
X_c	0.00076 *P. macrosturtensis*, 0.00078 *P. mesosturtensis*, 0.000645 *L. palmulaoides*	cm	3
$KO_{2,w}$	0.290	pmol s^{-1} kPa^{-1} cm^{-1}	4
$KO_{2,c}$	0.010	pmol s^{-1} kPa^{-1} cm^{-1}	5, 6
a	0.042 *P. macrosturtensis*, 0.027 *P. mesosturtensis*, 0.032 *L. palmulaoides*	cm	3
b, *c*	0.08 *P. macrosturtensis*, 0.039 *P. mesosturtensis*, 0.086 *L. palmulaoides*	cm	3, 7
L	0.4 *P. macrosturtensis*, 0.23 *P. mesosturtensis*, 0.42 *L. palmulaoides*	cm	7
Mb	2.72 *P. macrosturtensis*, 0.55 *P. mesosturtensis*, 2.32 *L. palmulaoides*	mg	3

References: 1, Dejours, 1981; 2, Seymour *et al.*, 2015; 3, present study; 4, Seymour, 1994; 5, Krogh, 1919; 6, Bartels, 1971; 7, Watts and Humphreys, 2006; Symbols: X_w = O_2 boundary layer thickness, X_c = cuticle thickness, $KO_{2,w}$ = Krogh's coefficient of diffusion for O_2 in water at 25°C, $KO_{2,c}$ = Krogh's coefficient for chitin used for the cuticle, adjusted to 25°C with a Q_{10} of 1.1 according to Bartels (1971) , *a* = semi-axis *a* of the lozenge shape used to estimate surface area of the beetles, *b* = semi-axis *b* of lozenge, *c* = semi-axis *c* of lozenge shape, *L* = total length of lozenge shape, and total length of each beetle species (See Fig. S2), Mb = beetle body mass.