

Spicer & Morley:

Will giant polar amphipods be first to fare badly in an oxygen-poor ocean? Testing hypotheses linking oxygen to body size.

Supplementary materials 2

Respirometry

A. Comparing closed and semi-open respirometer techniques

For the closed respirometry which was carried out at Rothera, Antarctic Peninsula and also as part of the comparison of techniques at the University of Plymouth glass bottles (volumes = 100, 60, 25, 11, 5 and 1.8 mL), each fitted with a gas-tight lid and an oxydot were used as respirometers. The size of the bottle was matched to the size of the individual such that the time course of the rate of O₂ decline with time in each bottle was broadly comparable. Each bottle contained an appropriately scaled section of plastic mesh inserted (5 cm x 5 cm, 4 cm x 4 cm, 3 cm x 3cm, 4cm x 2 cm or 2 cm x 1 cm respectively, mesh size = 1 mm in each case) to which the amphipod could adhere throughout the experiment. Providing a substrate to which amphipods can adhere or shelter within substantially reduces amphipod activity as shown previously for temperate [1] and Antarctic [2] species. Each bottle was also fitted with a small Teflon covered metal stirring bar, separated from the amphipod by plastic mesh. The actual volume of water within each bottle was calculated by subtracted the volume of water displaced by the animal, the stirring bar, and the plastic mesh.

Bottles without lids were submerged in jacketed water baths (22 x 40 x 75 cm) each containing constantly aerated sea water maintained at the appropriate test temperature ($T = -1.0 \pm 0.1^{\circ}\text{C}$) using thermostatically controlled water baths that pumped cooled water through the jacket (Grant LT D20G). One amphipod was carefully placed in each bottle the night before measurements commenced. Gauze was secured across the mouth using a cable tie. This prevented the individual from escaping while allowing free exchange of dissolved gases with water in the bottle. The next morning the gauze was removed and replaced with a gas-tight lid, fitted with an extra layer of aluminium foil. The whole operation was carried out with the bottles and lids submerged, taking care not to introduce air bubbles. Eleven amphipods were run at any one time. One empty bottle was included to quantify background respiration and taken into account when calculating the rate of O₂ uptake for individuals.

This closed technique for measuring oxygen uptake of Antarctic amphipods was compared with a semi-open technique used previously for crustaceans, including amphipods. The reason for this was that open and semi-open techniques are often seen as superior to closed bottle techniques for a number of reasons (e.g. the animal is more settled, and the water is not fouled so quickly using a semi-flow through technique) but the closed bottle technique is easier to use and so was preferred for use in Antarctica.

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The semi-open respirometry technique run alongside the closed bottle technique at the University of Plymouth in September 2016 was set up as follows. Single amphipods were placed into the same specially constructed Perspex respirometer (vol. = 21 mL) as used by Agnew and Taylor [1] when investigating the effect of declining oxygen tensions on the oxygen uptake of the intertidal amphipods, *Echinogammarus pirloti* and *E. obtusata*. Each respirometer had an inlet and outlet pipe which was connected *via* gas-impervious tubing (Tygon) to a pump situated in a reservoir (vol. = 2L) of filtered, continuously aerated, sea water maintained at $T = 0^{\circ}\text{C}$ ($S = 35$). Reservoir water was gently pumped through the respirometer supplying the amphipod with clean flowing sea water. Each respirometer was fitted with an enclosed magnetic stirring bar. The amphipod was protected from the bar by a piece of plastic gauze to which it often adhered during its time in the respirometer. Instead of an O_2 electrode (ES046 Radiometer, Denmark) being inserted into the chamber to measure oxygen depletion the same oxydot system as was used in the closed bottle respirometers was used. Amphipods were left overnight (for the same period as those amphipods in the closed system experienced) before the experiment was started. At the end of this period the pump was stopped and the amphipod was left to deplete the O_2 in the chamber as a result of its own respiration. Controls consisting of respirometers without animals were run simultaneously. The rate of O_2 reduction inside the respirometer was recorded and the rates of O_2 uptake and Respiratory Index (RI) calculated exactly as outlined for the individuals in the closed bottle set up. The pH of the sea water in both the closed bottle and the semi-open respirometer decreased < 0.3 pH units during the course of the experiment. However, in line with the checks carried out by Agnew and Taylor [1] during exposure of amphipods to declining O_2 , we frequently flushed the chamber with water at the same O_2 tension (prepared using a set of Wostoff precision gas mixing pumps, Bochum, Germany) as recorded in the chamber just before the flushing. The respirometer was then sealed again and the O_2 decline produced by the respiration by the amphipod, continued. This made no difference to the measurements obtained.

The VO_2 s of *Prostebbingia brevicornis* and *Paraceradocus miersi* were measured under conditions of declining PO_2 s concurrently using closed bottle and semi-open respirometry. The amphipods were collected at Rothera on the Western Antarctic Peninsula (See Supplementary materials 1) during the austral summer (2005-2006) and returned to the UK by ship in specially constructed thermostatically controlled shipping aquaria. They were kept at $T = -1$ to 0°C in aquaria at the British Antarctic Survey buildings in Cambridge for a couple of months before they were transported by road, in thermostatically controlled containers, to the University of Plymouth. Here they were kept ($T = 0^{\circ}\text{C}$, $S = 35$), unfed for at least 96 h, before used in the technique comparison trials.

Presented in Figure S2.1 is the VO_2 response to acutely declining O_2 tensions for one individual of *P. brevicornis* and one individual of *P. miersi*, together with an example of perfect regulation ($\text{RI} = 1$) and an example of perfect oxyconforming ($\text{RI} = 0$).

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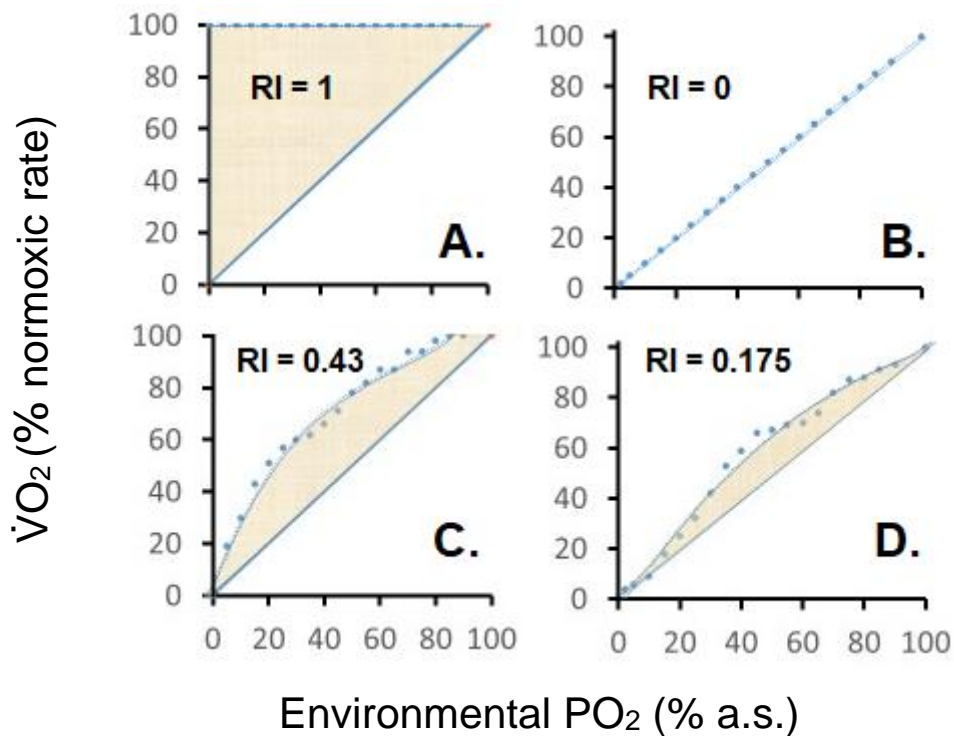


Figure S2.1 Changes in $\dot{V}O_2$ (blue circles) with acutely declining environmental PO_2 for (A) Hypothetical animal with perfect oxyregulation (B) Hypothetical animal which is an oxyconformer (C) *Prostebbingia brevicornis* and (D) *Paraceradocus miersi*. The Respiratory Index (RI) is represented by shaded areas, delineated by the oxyconforming line (blue line) and the $\dot{V}O_2$ (blue circles with fitted blue line).

Presented in Figure S2.2 are mean RI and $\dot{V}O_2$ values for both species measured using closed and semi-open respirometry.

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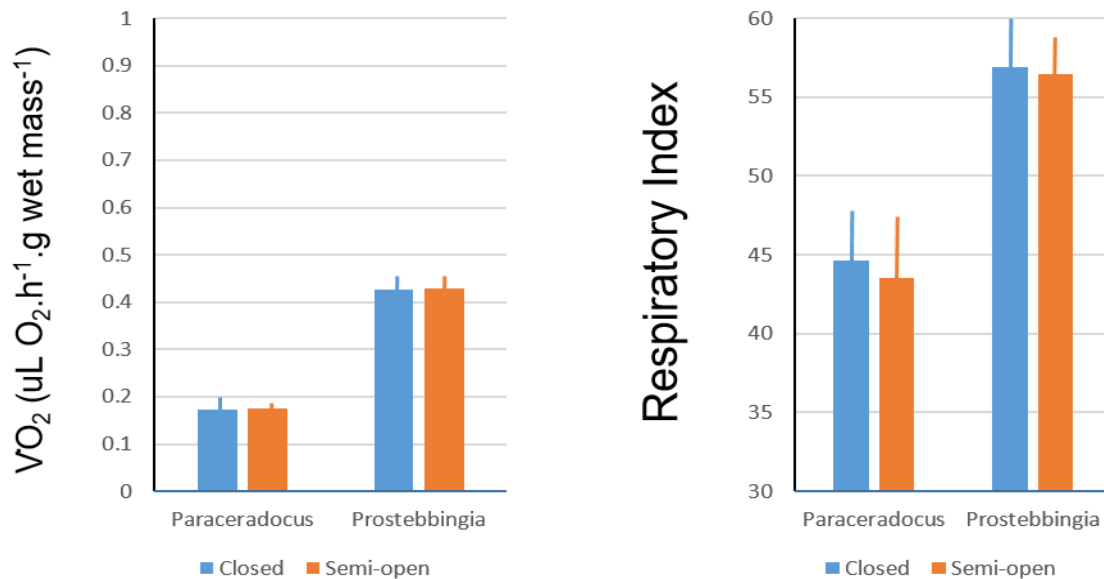


Figure S2.2 in $\dot{V}O_2$ and Respiratory Index for *Paraceradocus miersi*. and *Prostebbingia brevicornis* measured using different respirometry techniques. Values are means ± 1 . S.D.

There was no significant difference in the calculated RI for individuals run in the closed bottle system compared with those run in the semi-open system ($t \leq 0.67$, $P \geq 0.522$, $df = 7$). The normoxic $\dot{V}O_2$ of *P. brevicornis* was significantly greater than that of *P. miersi*. However, there was no difference as a result of the two test conditions, semi-open or closed respirometry ($t \leq 0.65$, $P \geq 0.534$, $df = 7$).

B. $\dot{V}O_2$ and body mass

The experiments reported in this paper carried out in Rothera, Western Antarctic Peninsula set out to control for, but not investigate, the effect of body mass on mass-specific rates of O₂ uptake ($\dot{V}O_2$). In the main paper we showed that when comparing species of similar body masses, *P. brevicornis* had a significantly greater $\dot{V}O_2$ than either *S. gracilis* or *P. miersi*. However, because the size ranges do not overlap we could not include *P. ovata* in the formal comparison. However, because there were inter and intra-specific mass differences in the individuals used, it is possible to build a supporting circumstantial case for *P. brevicornis* being the only one of the four species to have an elevated $\dot{V}O_2$.

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Presented in Figure S2.2 is $\dot{V}O_2$ expressed as a function of body mass for individuals of all four species investigated.

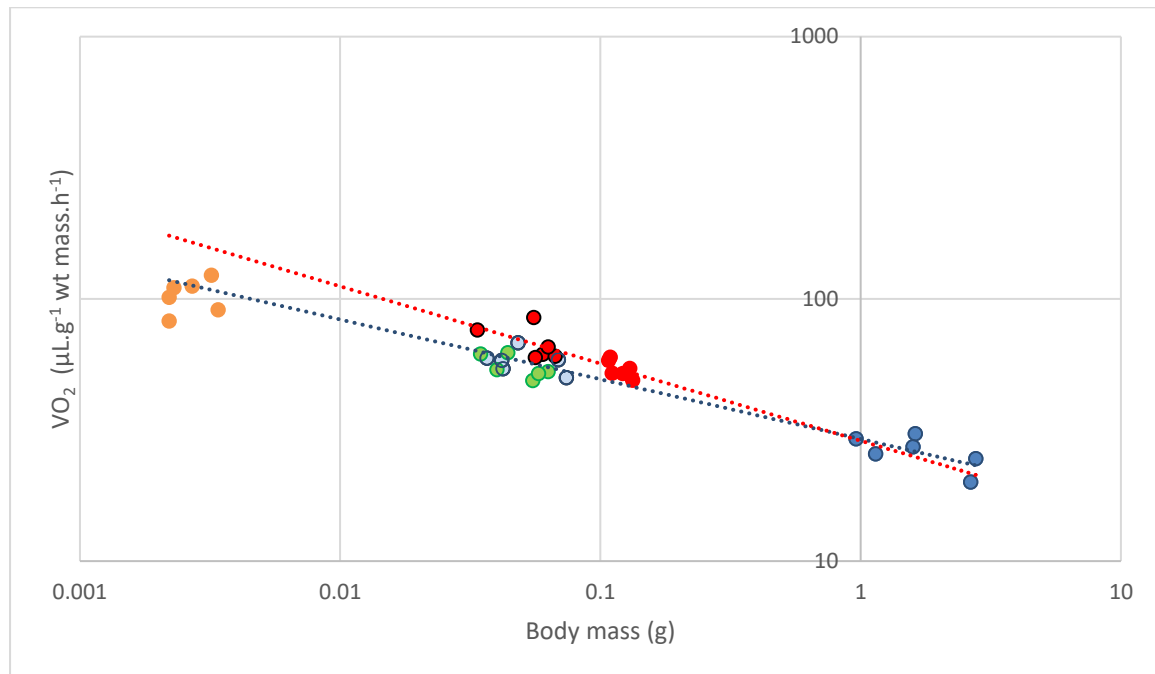


Figure S2.2. $\dot{V}O_2$ s for *P. miersi* large (dark blue) and small (light blue, with black border), *P. brevicornis* large (red) and small (red with black border), *S. gracilis* (green) and *P. ovata* (orange). Each point represents a single individual. Dashed blue line = line of best fit for all values of *P. miersi* ($\log_{10} y = 1.466 - \log_{10} 0.228x$; $r^2 = 94.6\%$, $F_{1,11} = 175.45$, $P < 0.001$) Dashed red line = line of best fit for all values for *P. brevicornis* ($\log_{10} y = 1.460 - 0.294 \log_{10} x$; $r^2 = 66.3\%$, $F_{1,11} = 19.71$, $P < 0.001$). There were no significant relationships for *S. gracilis* or *P. ovata* on their own ($F_{1,5} \geq 3.76$, $P \geq 0.125$ in each case).

There were significant relationships detected for *P. miersi* and *P. brevicornis* but not the other two. The line of best fit for *P. miersi* when extrapolated bisected the cloud of *P. ovata* points. Furthermore correlation which included *P. miersi*, *S. gracilis* and *P. ovata*, improved the r^2 calculated for *P. miersi* on its own from 94.6 to 95.2 %. The relationship between mass and all species with the exception of *P. brevicornis* could be described by the equation $\log_{10} y = 1.468 - 0.212 \log_{10} x$ ($F_{1,23} = 437.39$, $P < 0.001$). There was a significant difference between *P. brevicornis* and all of the other species taken together (ANCOVA $F_{1,35} = 23.19$, $P < 0.001$).

In conclusion, the relationship between $\dot{V}O_2$ and body mass for *P. ovata* fits well with all of the other species except *P. brevicornis*. This together with the formal ANCOVA comparison between *P. brevicornis* and the remaining three species provides strong circumstantial evidence for the statement that *P. brevicornis* has a greater $\dot{M}O_2$ than the other three species investigated, not just the two where the overlap in mass range allowed a straight forward formal comparison.

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References

- [1] Agnew DJ, Taylor AC 1985. The effect of oxygen tension on the physiology and distribution of *Echinogammarus pirloti* (Sexton & Spooner) and *E. obtusatus* (Dahl) (Crustacea: Amphipoda). *J. Exp. Mar. Biol. Ecol.* **87**, 169-190.
- [2] Chapelle G, Peck LS 1995. The influence of acclimation and substratum on the metabolism of the amphipods *Waldeckia obesa* (Chevreux 1905) and *Bovallia gigantea* (Pfeffer 1888). *Polar Biol.* **15**, 225-232.

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