Oxygen availability and thermal tolerance investigated by MR imaging & spectroscopy in the Antarctic eelpout *Pachycara brachycephalum*

Felix Mark, Christian Bock & Hans-O. Pörtner

Alfred-Wegener-Institut für Polar- und Meeresforschung

Introduction

Stenothermal polar ectotherms show a restricted range of thermal tolerance. According to earlier work in crustaceans and fish (1, 2), thermal limitation becomes effective first by a drop in aerobic scope at pejus temperatures T_p and, then, by the onset of anaerobic mitochondrial metabolism, which is expressed in the critical temperature T_c. Oxygen limitation may therefore characterize the first line of thermal intolerance.

To study the role of oxygen in vertebrate thermal tolerance, we investigated the effects of hyperoxia and temperature on energy metabolism, blood flow and tissue oxygenation in the Antarctic eelpout *Pachycara brachycephalum* using *in vivo* MR methods in combination with oxygen consumption measurements.

Experimental procedure

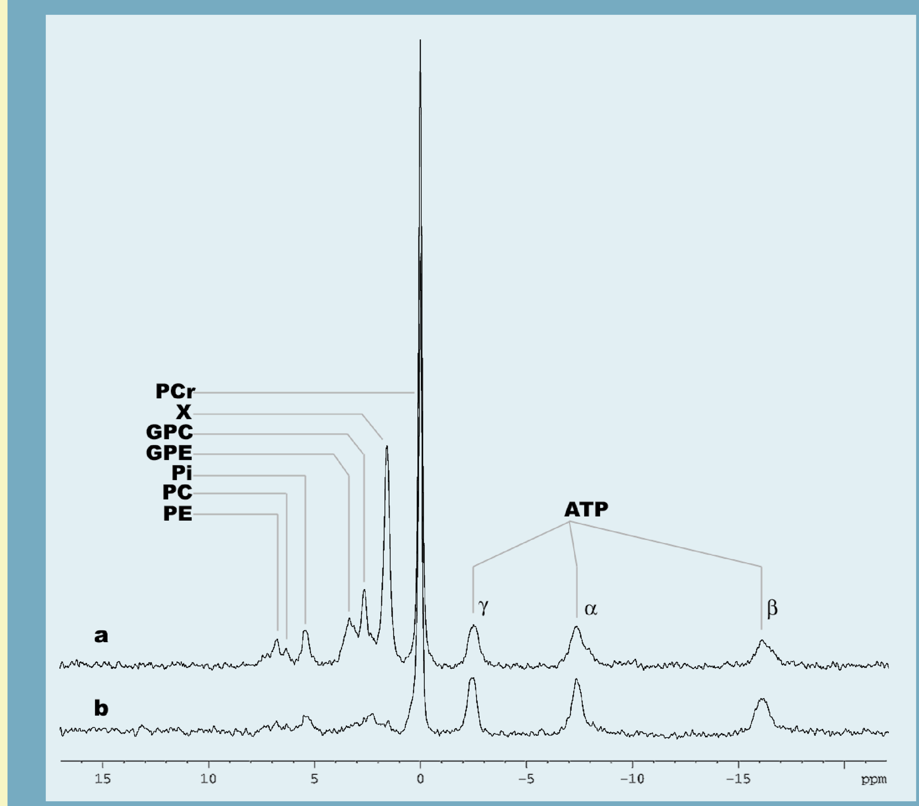

Figure 1: *in vivo* ^{31}P-NMR spectrum of the musculature of the Antarctic eelpout *Pachycara brachycephalum* **(a)** and the North Sea eelpout *Zoarces viviparus* **(b)**.
Abbreviations: α-, β-, γ-ATP: adenosintriphosphate; GPC: glycerophosphocholine; GPE: glycerophosphoethanolamine; Pi: free inorganic phosphate; PCr: phosphocreatine; PDE: phosphodiester; PME: phosphomonoester; X: unidentified phosphodiester compound

Two experimental series were carried out, one under normoxia and one under hyperoxia (PO_2: 45kPa). Temperature in both series was increased by 1°C * 12 hrs^{-1} between 0 and 15°C. MR experiments were conducted using a 4,7T Magnet with a 40cm diameter bore. Inside the magnet, unanaesthetized animals were placed in a flow-through perplex chamber in which they could move without restraint. Water flow through the chamber (up to 2l * min^{-1}) was maintained by hydrostatic pressure and could be controlled to ± 1ml min^{-1}. Fluoroptic sensors continuously monitored the temperature inside the animal chamber. For respiration measurements, optodes were used to determine oxygen concentration in both in- and outflowing water. Blood flow and tissue oxygenation were measured by using flow weighted (Fig. 2) and T_2* weighted MR imaging methods, respectively. To monitor energy metabolism and intracellular pH, we applied *in vivo* ^{31}P-NMR-spectroscopy (Fig. 1).

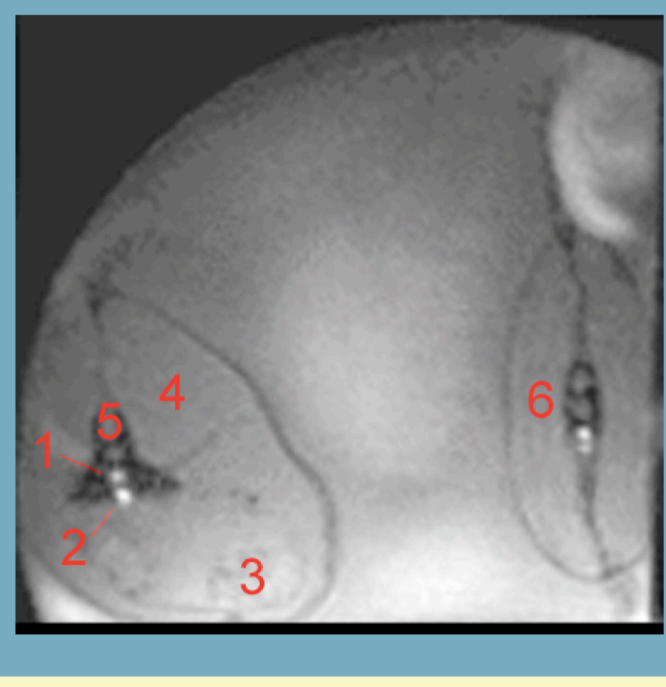

Figure 2 (left): Flow weighted transversal MR image of the trunk region of *P. brachycephalum* (as indicated in Fig. 3).
1: Aorta dorsalis;
2: Vena cardinalis posterior;
3: stomach;
4: dorsal musculature;
5: spine; 6: tail

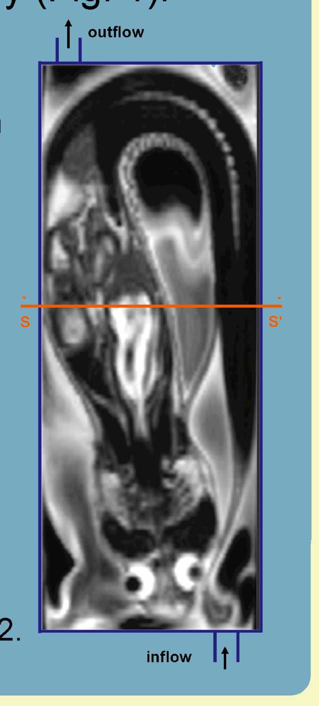

Figure 3 (right): Schematic view onto the fish's position in the perplex chamber.
S-S': orientation of the transversal image in Fig.2.

Results

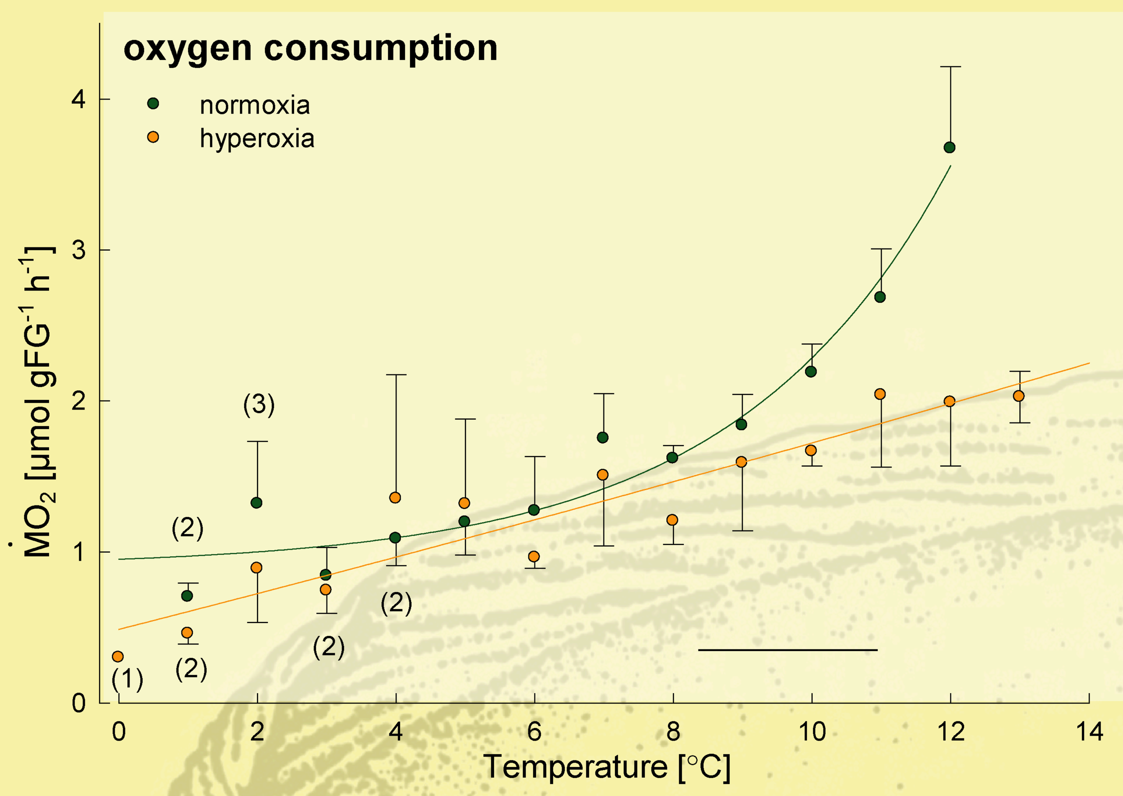

Figure 4: Oxygen consumption under normoxia and hyperoxia at rising temperatures.
Exposure to hyperoxia and warmer temperatures resulted in a linear increase in oxygen consumption; the typical exponential increment of oxygen uptake was eliminated.
Data are given as MEANS±SE. N=4-7, unless indicated otherwise.
--: significantly different between the two experimental series.

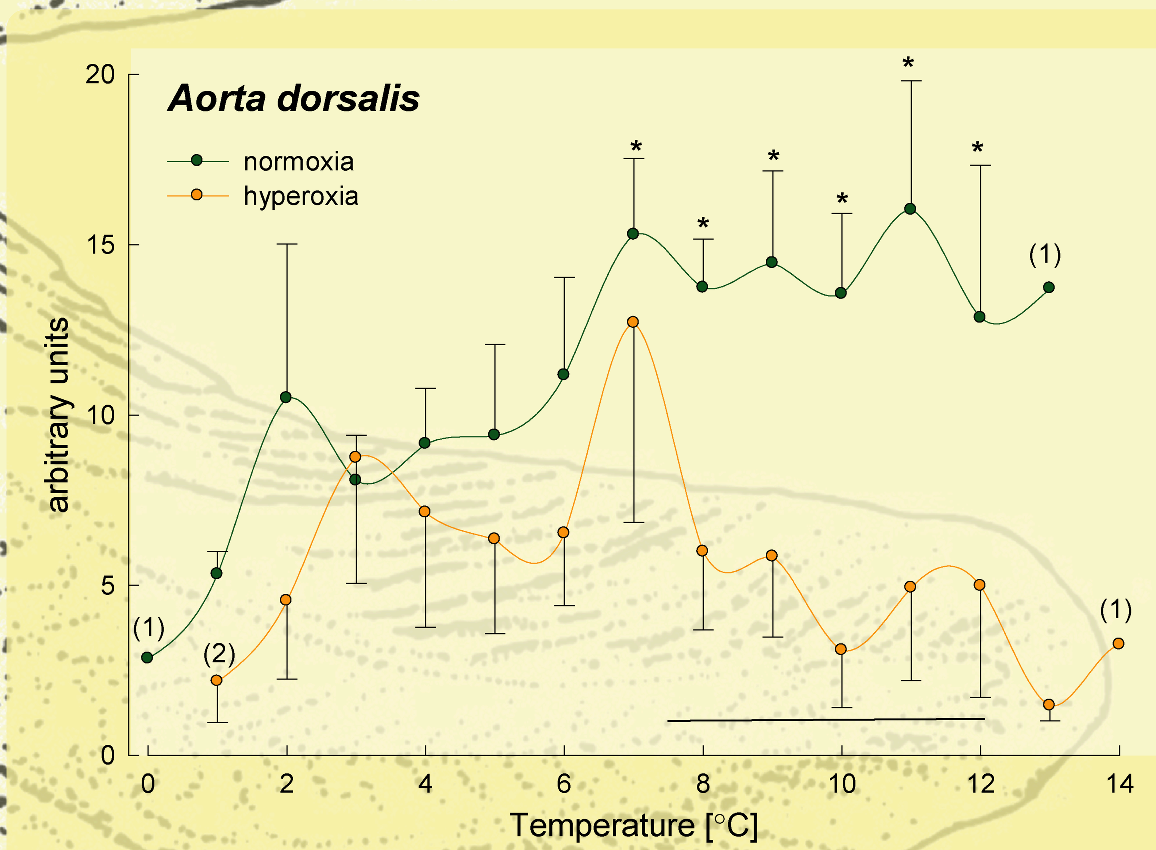

Figure 5: Blood flow in the Aorta dorsalis under normoxia and hyperoxia at rising temperatures.
Blood flow increased significantly in normoxic animals and reached a new level above 6°C. Similarly, blood flow increased in hyperoxic animals, however, not to the same extent.
Data are given as MEANS±SE. N=3-6, unless indicated otherwise.
*: significantly different compared to values at lower temperatures.
--: significantly different between the two experimental series.

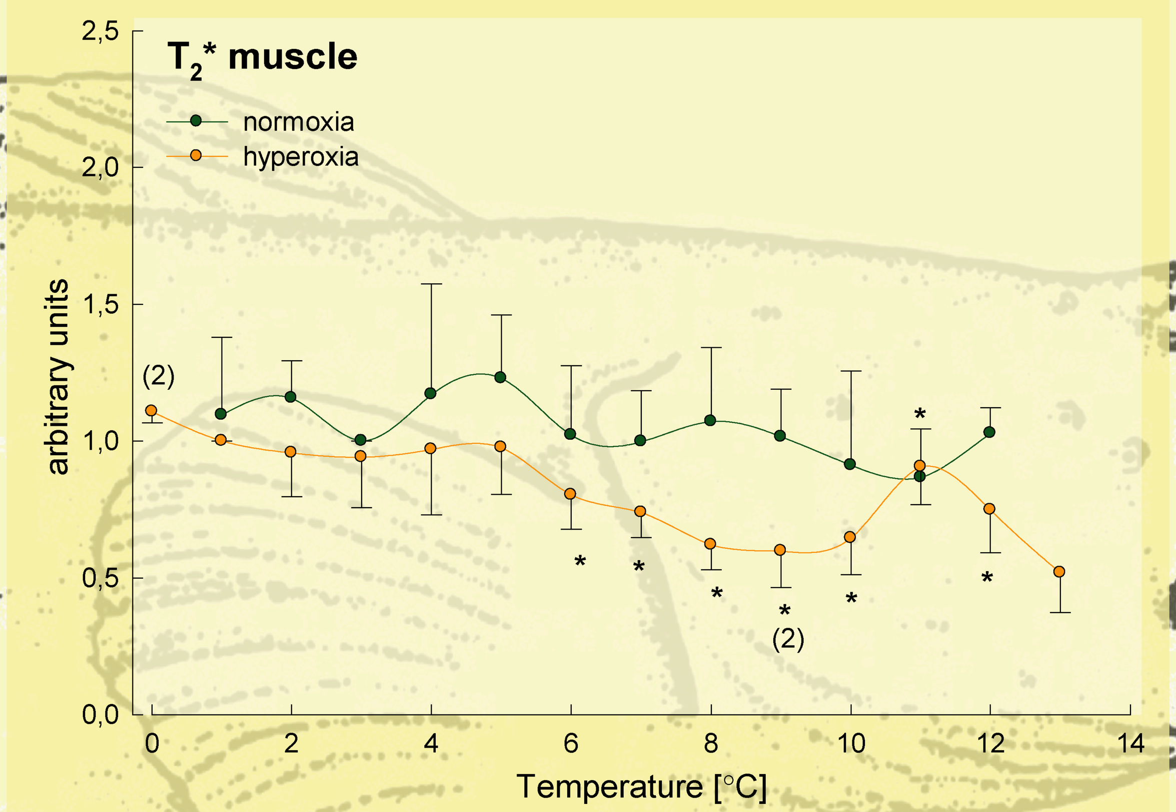

Figure 6: White muscle oxygenation under normoxia and hyperoxia at rising temperatures.
Tissue oxygenation showed a slight decline above 5°C in both groups, being more prominent under hyperoxia.
Data are given as MEANS±SE. N=3-5, unless indicated otherwise.
*: significantly different compared to values at lower temperatures.

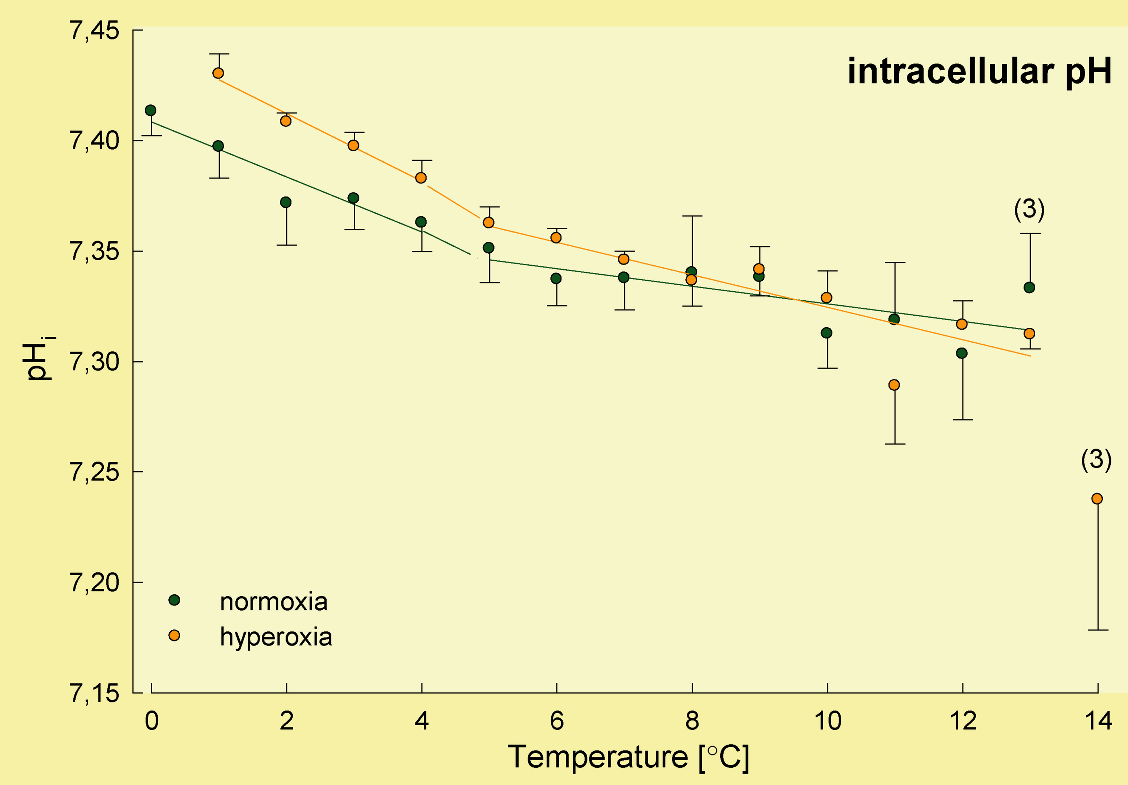

Figure 7: Intracellular pH under normoxia and hyperoxia at rising temperatures.
From 0-5°C, pHi decreased with rising temperature (normoxia: -0,012 units * °C^{-1}; hyperoxia: -0,015 units * °C^{-1}), following the pattern suggested by the α-stat hypothesis (-0,017 units * °C^{-1},(3)). Above 5°C a different regulatory pattern with a smaller decrease in pHi with temperature could be observed (normoxia: -0,004 units * °C^{-1}; hyperoxia: -0,007 units * °C^{-1}).
Data are given as MEANS±SE. N=4-7, unless indicated otherwise.

Conclusions

- Improved oxygen availability may diminish the effects of thermal stress by reducing the energy cost of oxygen uptake and distribution.
- A putative reduction of the aerobic scope, which is reflected in the drop in tissue oxygenation and a break in pHi regulation, can be made out between 6 and 7°C.
- Once the oxygen limitation of thermal tolerance has been eliminated, further restrictive mechanisms become effective.
- Thus, an increased supply of oxygen does not widen the thermal tolerance range to a large extent. It mainly improves the fish's physical conditions within the normoxic range of tolerance.

References
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Correspondence:
F. Mark
AG Ökophysiologie
Alfred-Wegener-Institut für
Polar- und Meeresforschung
27568 Bremerhaven, Germany
email: fmark@awi-bremerhaven.de