

## ANTARCTIC FISH BLOOD: RESPIRATORY PROPERTIES AND THE EFFECTS OF THERMAL ACCLIMATION

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### SUMMARY

1. The effects of thermal acclimation on whole blood oxygen affinity were examined in the antarctic fish *Pagothenia borchgrevinkii*.

2. 4.5 °C-acclimated fish had a  $P_{50}$  value of 26.7 mmHg at pH 8.1, compared to 20.7 mmHg for -1.5 °C-acclimated fish. The apparent heat of oxygenation,  $\Delta H = -26.7 \text{ kJ mol}^{-1}$ , is comparable to values for temperate species.

3. Warm-acclimation was followed by an increased ATP:Hb<sub>4</sub> molar ratio, resulting in an augmentation of the thermal effect on oxy-haemoglobin affinity. This may be considered adaptive in a constantly well oxygenated environment, where oxygen loading at the gills is secured. Unloading to the tissues is thereby enhanced, supporting an elevated rate of aerobic metabolism at higher temperatures.

4. *In vivo* blood pH was high, between 8.10 and 8.25 at -1.5 °C. Astrup titration revealed arterial CO<sub>2</sub> tensions of less than 0.8 mmHg, indicating relative hyperventilation and low oxygen extraction efficiency in antarctic fish.

5. Blood oxygen affinities of four antarctic nototheniid species were low ( $P_{50}$  between 11.9 and 20.7 mmHg at pH 8.1 and -1.5 °C) in comparison with the temperate species *Notothenia angustata* ( $P_{50} = 10.8 \text{ mmHg}$ ). The zoarcid *Rhigophila dearborni* had a high blood oxygen affinity ( $P_{50} = 4.3 \text{ mmHg}$ ). Blood oxygen-binding properties are discussed in relation to the polar environment, mode of life, and the concept of cold adaptation.

### INTRODUCTION

Fishes inhabit environments that vary widely in physical and chemical properties, particularly with respect to temperature and oxygen availability. The respiratory properties of blood have been shown to respond appropriately to these environmental

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factors and to the metabolic requirements of fishes with very different activity levels as reviewed by Weber (1982).

The marine environment in McMurdo Sound, Antarctica, is characterized by an extremely stable temperature of  $-1.8 \pm 0.1$  °C, and constant salinity. All year round, the water column is nearly saturated with oxygen although half-saturated bottom oxygen contents have been observed beneath the ice shelf (Littlepage, 1965). A circum-antarctic oceanic circulation has isolated the fauna during the last 25 million years (Kennet, 1977). Several families of fish have evolved during this isolation, and physiological specializations have been developed to permit exploitation of this unique marine environment (DeVries & Eastman, 1981). The Nototheniidae comprises about 100 species, of which most are endemic to antarctic waters (DeWitt, 1971). The Zoarcidae range widely into all latitudes, and seven species are found in the antarctic seas (Andriashev, 1965).

Few nototheniid species have been studied extensively. They show extreme stenothermia, only allowing life within the  $-2.5$  to  $6.0$  °C range (Somero & DeVries, 1967). An elevated routine metabolic rate relative to temperate and tropical species, acclimated to the same low temperature, was claimed by Wohlschlag (1960, 1964), but was later questioned by Holeyton (1974) and Clarke (1980). The antarctic zoarcid *Rhigophila dearborni* did not show this phenomenon of 'cold adaptation' (Wohlschlag, 1963). The blood oxygen-binding properties of several antarctic species have been studied previously and low oxygen affinities were noted (Grigg, 1967; Qvist, Zapol & DeVries, 1975; Qvist, Weber, DeVries & Zapol, 1977). Moreover, it was found that antarctic species showed a pronounced effect of temperature on blood oxygen affinity (Grigg, 1967).

The present study was accomplished in order to establish the haematological and respiratory properties of blood from five antarctic fish species under precisely defined conditions encompassing a physiological pH range. In addition, specimens of the nototheniid *Pagothenia* (= *Trematomus*) *borchgrevinki* were acclimated to  $4.5$  °C in order to study the haematological effects of warm-acclimation and to determine the *in vivo* temperature sensitivity of the blood oxygen-binding equilibrium. The results are discussed in relation to the habits of the species and the environments of the antarctic waters.

#### MATERIALS AND METHODS

##### *Maintenance of animals and blood collection*

Fish were caught during November 1982 at fishing stations sited over holes in the 1–2 m thick sea ice of the McMurdo Sound, Antarctica ( $77^{\circ}\text{S}$ ,  $166^{\circ}\text{E}$ ). The pelagic *Pagothenia borchgrevinki* Boulenger and the benthic species *Trematomus bernacchii* Boulenger and *T. lonnbergi* Regan (all specimens weighing 100–200 g) were captured on hand lines with lures in shallow water. Specimens of the large (16–58 kg) midwater species *Dissostichus mawsoni* Norman were hooked on baited lines 300–500 m below the sea ice. All these species belong to the family Nototheniidae. Small specimens (approx. 100 g) of the benthic zoarcid species *Rhigophila dearborni* DeWitt were caught in baited traps set on the sea floor at 500–600 m depth. Specimens of the

sedentary benthic species *Notothenia angustata* Hutton (Nototheniidae) found in cold-temperate and sub-antarctic waters were transported by plane to Antarctica from Dunedin, New Zealand (46°S).

Captured specimens were transported in insulated containers with aerated sea water to the Eklund Biological Center aquarium at the McMurdo Station. Here they were kept in large tanks circulated with fresh sea water at  $-1.5 \pm 0.2$  °C for at least 3 days prior to experiments.

Eight specimens of *P. borchgrevinki* were kept in a 150 l tank with aerated sea water. The water temperature was raised gradually to 4.5 °C over a period of 2 days. The fish were acclimated to this temperature for 8–13 days before blood sampling.

Blood to be used for haematological and oxygen binding studies was collected into heparinized syringes by caudal venepuncture of unanaesthetized specimens. Approximately 1.6 ml blood was taken within 30 s and each specimen was bled only once. A few specimens were successfully cannulated to validate measurements taken following acute venepuncture. Smaller samples from *T. bernacchii*, *T. lonnbergi* and *R. dearborni* were pooled. Blood from *D. mawsoni* was collected *via* indwelling caudal catheters, using PE-50 polyethylene tubing (Intramedic, Clay-Adams). The cannulations were performed on specimens anaesthetized with MS-222. Gills were continuously irrigated during the operation. Two to three days recovery from capture and anaesthesia were allowed prior to blood sampling. All samples were analysed without storage or delay, except for the determination of oxygen-binding curves, where storage for up to 3–4 h was an inevitable consequence of the technique.

#### *Haematological measurements*

Haematocrit and haemoglobin concentration were determined according to Dacie & Lewis (1975). The met-cyanide haemoglobin derivative was centrifuged to remove nuclear debris before spectrophotometry. Adenosine triphosphate (ATP) and guanosine triphosphate (GTP) were determined by thin-layer chromatography (Johansen, Lykkeboe, Weber & Maloij, 1976) using ATP and GTP standards (Sigma Chemicals, St. Louis, Missouri, U.S.A.) as controls. *P. borchgrevinki* showed no detectable erythrocyte GTP, and subsequent ATP determinations on this species were done using a nonspecific enzymatic method for determination of total trinucleoside phosphate (Sigma Bulletin 366-UV, 1974). The two methods gave identical results. Blood lactate was assayed using Sigma enzymatic test chemicals (Sigma Bulletin 826-UV, 1976). Spectrophotometric measurements were made using a Perkin Elmer 552 recording spectrophotometer.

#### *Oxygen content and blood pH*

Blood samples were divided into 80  $\mu$ l aliquots in disposable microtonometer tubes (Radiometer, Copenhagen, Denmark) and placed in water at  $-1.5$  °C until required. Blood samples were equilibrated for 20 min in a Radiometer BMS-2 Blood Micro System, and oxygen content was determined by the method of Tucker (1967). The Tucker chamber was thermostatted and calibrated at 35 °C to improve the response time of the oxygen electrode (Radiometer E5046/0 connected to a PHM 71 meter). Appropriate mixtures of air, nitrogen and carbon dioxide (NZIG, New Zealand) were delivered to the tonometers by cascaded gas mixing pumps (Wösthoff, Bochum,

Germany). The measured oxygen contents were corrected for physically dissolved oxygen using Bunsen solubility coefficients of 0.0060 and 0.0053 vol% O<sub>2</sub> mmHg<sup>-1</sup>, for -1.5 °C and 4.5 °C respectively (Christoforides & Hedley-Whyte, 1969). Blood pH was measured using Radiometer equipment (BMS-2 and PHM 71) calibrated with precision phosphate buffers.

Tonometry of blood and pH measurement were done at controlled temperatures, appropriate for the experiment (-1.5 °C and 4.5 °C).

Oxygen affinity (expressed by the oxygen tension at half saturation, P<sub>50</sub>) was calculated assuming that the oxygen content of blood equilibrated with a gas mixture of the desired carbon dioxide fraction, balanced with air, corresponded to 100 % saturation. No apparent Root effect was discerned. The Hill coefficient, *n* was determined as the slope of the Hill plot at saturation levels between 20 and 80 %. The CO<sub>2</sub> Bohr factor,  $\Phi$  ( $\Delta \log P_{50} / \Delta \text{pH}$ ) was determined by fitted linear regression of  $\log P_{50}$  on pH.

## RESULTS

### *Changes during tonometry of blood*

The changes in blood pH, haematocrit and haemoglobin-bound oxygen content occurring after the start of blood tonometry are shown in Fig. 1, for blood from

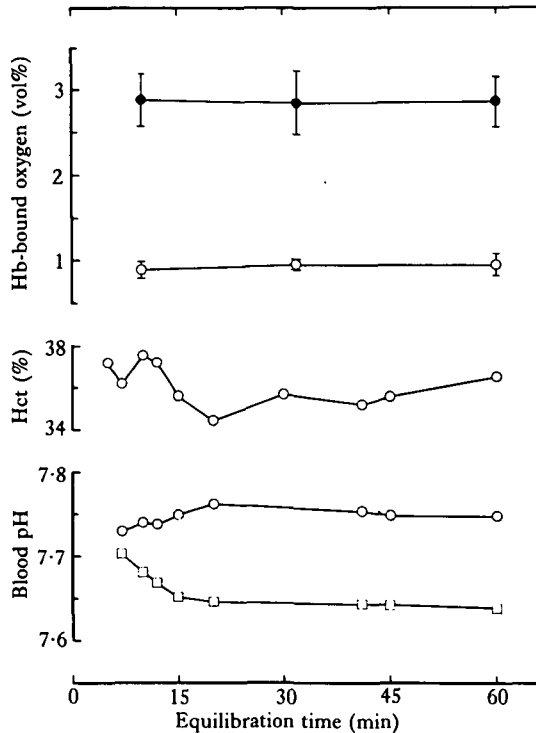


Fig. 1. Time course of blood pH, haematocrit (Hct) and haemoglobin-bound oxygen of blood from *Pagothenia borchgrevinki* during equilibration at -1.5 °C with controlled gas mixtures: P<sub>O<sub>2</sub></sub> = 150.4 mmHg, P<sub>CO<sub>2</sub></sub> = 2.3 mmHg (●); P<sub>O<sub>2</sub></sub> = 15.8 mmHg, P<sub>CO<sub>2</sub></sub> = 2.3 mmHg (○); P<sub>O<sub>2</sub></sub> = 15.8 mmHg, P<sub>CO<sub>2</sub></sub> = 4.6 mmHg (□).

*P. borchgrevinki* at  $-1.5^{\circ}\text{C}$ . The haematocrit for this specimen was the highest recorded in our study, but agrees with values reported by Kooyman (1963) and Wells, Ashby, Duncan & MacDonald (1980), indicating some individual variation. Blood pH, haematocrit, and oxygen content at two levels of oxygen saturation were stable following equilibration for about 20 min.

#### In vivo pH and blood acid-base status

The *in vivo* blood pH of resting fish at  $-1.5^{\circ}\text{C}$ , measured on blood samples taken by acute venepuncture or *via* indwelling catheters, gave values between 8.10 and 8.25, irrespective of the sampling method. Blood pH tended to be slightly higher for *D. mawsoni* than for *P. borchgrevinki*.

No data exist on the plasma  $pK'$  value and solubility of  $\text{CO}_2$  at  $-1.5^{\circ}\text{C}$  and at these very high pH values. However, since temperature and pH act in opposite ways on plasma  $pK'$ , and the  $\text{CO}_2$  solubility coefficient of plasma ( $\alpha\text{CO}_2$ ) does not change dramatically with temperature, an extrapolation of tabulated values (Severinghaus, 1965) should not introduce significant errors. Using extrapolated values ( $\alpha\text{CO}_2 = 0.073 \text{ mmol l}^{-1} \text{ mmHg}^{-1}$ ;  $pK' = 6.20$ ) at pH 8.2 we calculated an *in vivo* plasma bicarbonate concentration of  $6.7 \text{ mmol l}^{-1}$  for one unanaesthetized and resting specimen of *D. mawsoni*. The arterial  $\text{CO}_2$  tension of  $0.8 \text{ mmHg}$  for this fish was determined by the Astrup method (Fig. 2) (Astrup, 1956).

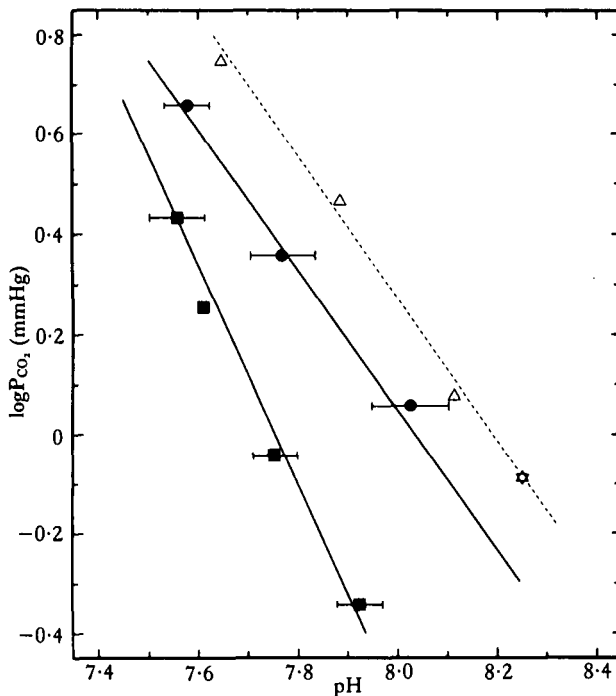


Fig. 2. Astrup lines illustrating the *in vitro* relationship between pH and  $\log P_{\text{CO}_2}$  of blood from *Pagothenia borchgrevinki* acclimated to and measured at  $-1.5^{\circ}\text{C}$  (●) and  $4.5^{\circ}\text{C}$  (■). Each point represents the mean value, with  $\pm 1$  s.d. indicated by horizontal bars. Astrup titration at  $-1.5^{\circ}\text{C}$  of blood from one completely recovered and resting *Dissostichus mawsoni*, cannulated *via* the efferent vessel of the second gill arch ( $\Delta$ ). *In vivo* arterial pH of this specimen ( $\star$ ).

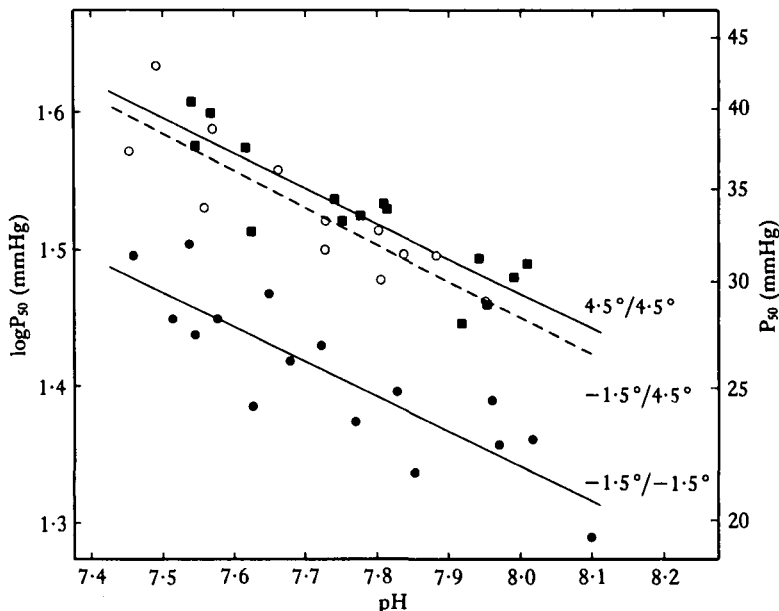


Fig. 3.  $\log P_{50}$  values and their pH dependence in blood from *Pagothenia borchgrevinki*:  $-1.5^{\circ}\text{C}$ -acclimated fish measured at  $-1.5^{\circ}\text{C}$  (●) and  $4.5^{\circ}\text{C}$  (○), and  $4.5^{\circ}\text{C}$ -acclimated fish measured at  $4.5^{\circ}\text{C}$  (■). Regression lines:  $\log P_{50} = -0.26\text{pH} + 3.39$  ( $r = -0.87$ ) (●);  $\log P_{50} = -0.27\text{pH} + 3.61$  ( $r = -0.87$ ) (○); and  $\log P_{50} = -0.26\text{pH} + 3.53$  ( $r = -0.88$ ) (■).

#### Effect of temperature

Subsequent  $P_{50}$  values are expressed at pH 8.1, calculated by use of the Bohr factor found for the appropriate group of fish.

The blood oxygen affinity of *P. borchgrevinki* was relatively low, with a  $P_{50}$  value of  $20.7 \pm 1.4$  mmHg (Table 1, Fig. 3), despite a low temperature of  $-1.5^{\circ}\text{C}$  and the high pH. Changing the measuring temperature to  $4.5^{\circ}\text{C}$  resulted in a significant ( $P < 0.001$ ) rightward shift of the oxygen-binding curves ( $P_{50} = 26.7 \pm 1.5$  mmHg). Blood from  $4.5^{\circ}\text{C}$ -acclimated fish showed a  $P_{50}$  value of  $27.6 \pm 1.4$  mmHg, when measured at this temperature (Table 1, Fig. 3). There was no significant difference between  $P_{50}$  values of blood warmed *in vitro*, and blood from warm-acclimated fish. The effect of temperature on  $P_{50}$  was quantified from the van't Hoff relationship by calculating the apparent heat of oxygenation,

$$\Delta H = 0.0192 \left[ \frac{T^1 \cdot T^2}{T^2 - T^1} \right] \log \left[ \frac{P_{50}^1}{P_{50}^2} \right] \text{ kJ mol}^{-1}$$

(Wyman, 1964). The apparent heat of oxygenation determined *in vitro* was  $-26.7$  kJ mol $^{-1}$  ( $-6.39$  kcal mol $^{-1}$ ).

The  $\text{CO}_2$  Bohr factor was  $-0.26$  for both acclimation groups. The Hill coefficient was 1.92 at  $-1.5^{\circ}\text{C}$  and 1.98 at  $4.5^{\circ}\text{C}$ .

Acclimation of *P. borchgrevinki* to  $4.5^{\circ}\text{C}$  for 8 days resulted in several haematological changes (Table 1). There was a significant rise in blood haemoglobin concentration from  $0.39$  to  $0.58$  mmol l $^{-1}$ , corresponding to an increase in blood oxygen capacity from  $3.5$  to  $5.2$  vol%  $\text{O}_2$ . This was accomplished by an increase in haematocrit

Table 1. *Respiratory and haematological data of blood of Pagothenia borchgrevinki acclimated to and measured at -1.5 °C and 4.5 °C*

	Acclimation group		Significance* of difference
	-1.5 °C	4.5 °C	
P <sub>50</sub> at pH 8.1 (mmHg)	20.7 ± 1.4 (9)	27.6 ± 1.4 (8)	0.001
Bohr coefficient ( $\Delta \log P_{50} / \Delta \text{pH}$ )	-0.26 (9)	-0.26 (8)	
Hill coefficient ( <i>n</i> )	1.92 (9)	1.98 (8)	
Haematocrit (%)	15.1 ± 2.5 (10)	22.2 ± 5.7 (8)	0.01
Haemoglobin† (mmol tetramer l <sup>-1</sup> blood)	0.39 ± 0.08 (10)	0.58 ± 0.16 (8)	0.02
MCHC† (mmol tetramer l <sup>-1</sup> red cells)	2.56 ± 0.46 (10)	2.45 ± 0.45 (8)	N.S.
ATP (mmol l <sup>-1</sup> red cells)	0.70 ± 0.27 (10)	1.80 ± 0.48 (8)	0.001
ATP:Hb <sub>4</sub>	0.29 ± 0.15 (10)	0.76 ± 0.24 (8)	0.001
Lactate (mmol l <sup>-1</sup> blood)	0.24 ± 0.17 (10)	0.44 ± 0.08 (8)	0.01

Mean ± 1 s.d. Brackets = number of fish.  
 \* *P*-values from Students *t*-test (N.S. = not significant).  
 † Calculated on the assumption of a molecular weight of haemoglobin tetramer of 65 000 g mol<sup>-1</sup>.

whereas the mean red cell haemoglobin concentration (MCHC) did not change, indicating no changes in erythrocyte volume. Red cell ATP concentration, as well as the molar ratio of ATP to haemoglobin tetramer, were significantly increased, although the absolute values were small compared to the values from a range of species from lower latitudes (cf. Bartlett, 1978*a,b*).

There was a small but significant increase in blood lactate concentration following warm-acclimation, but the values were still within the range of resting and routinely active rainbow trout (Black, Commor, Lam & Chiu, 1962; Driedzic & Kiceniuk, 1976). By visual means we found that the ventilatory frequency of 4.5 °C-acclimated fish was  $32.3 \pm 7.8 \text{ min}^{-1}$  (*N* = 8) compared to  $18.8 \pm 4.6 \text{ min}^{-1}$  (*N* = 10) for fish at -1.5 °C.

Acclimation to 4.5 °C resulted in an altered acid-base status of the blood, as shown by the *in vitro* relationships between pH and logP<sub>CO<sub>2</sub></sub> (Fig. 2). pH measurements done without delay after quick sampling from both acclimation groups did not reveal any significant change in the *in vivo* blood pH. Warm-acclimation was therefore possibly accompanied by a metabolically compensated respiratory alkalosis. The change in the slope of the pH-logP<sub>CO<sub>2</sub></sub> line indicated an increased buffer capacity of the blood as a result of a higher blood haemoglobin concentration (Table 1).

#### *Haematological and respiratory properties of antarctic fish blood*

Table 2 displays haematological data of one zoarcid and five nototheniid species.

Table 2. *Haematological data of pooled blood from five antarctic fish species, acclimated to  $-1.5^{\circ}\text{C}$ , and the sub-antarctic *Notothenia angustata*, acclimated to  $4.5^{\circ}\text{C}$*

Species	N	Haema- tocrit (%)	Haemo- globin*†	MCHC†‡	ATP*	ATP:Hb <sub>a</sub>	GTP*	GTP:Hb <sub>a</sub>
<i>Rhizophila dearborni</i>	5	15.0	0.63	4.20	0.77	1.22	0.12	0.19
<i>Pagothenia borchgrevinki</i>	5	13.0	0.46	3.54	0.19	0.41	0	0
<i>Trematomus bernacchii</i>	5	13.5	0.38	2.81	0.33	0.86	0.03	0.08
<i>Trematomus lonnbergi</i>	2	8.0	0.29	3.63	0.21	0.72	0.03	0.11
<i>Dissostichus mawsoni</i>	3	17.5	0.98	5.60	0.80	0.81	0.10	0.10
<i>Notothenia angustata</i>	2	18.5	0.76	4.11	1.17	1.54	0.11	0.15

Organic phosphates were determined by thin-layer chromatography. N = number of fish.

\* mmol l<sup>-1</sup> blood.

† mmol l<sup>-1</sup> red cells.

‡ Assuming a molecular weight of haemoglobin tetramer of 65 000 g mol<sup>-1</sup>.

Table 3. *Blood oxygen-binding properties of five antarctic fish species, acclimated to and measured at  $-1.5^{\circ}\text{C}$*

Species	N	P <sub>50</sub> (mmHg)	Bohr factor, $\Delta\log P_{50}/\Delta\text{pH}$	Hill coefficient, n	Remarks on habits
<i>Rhizophila dearborni</i>	5	4.3	-0.90	1.36	Sluggish benthic scavenger (>200 m).
<i>Pagothenia borchgrevinki</i>	9	20.7	-0.26	1.92	Cryo-pelagic, feeding on zooplankton and amphipods (0-50 m).
<i>Trematomus bernacchii</i>	5	13.5	-0.59	1.45	Sedentary benthic scavenger (50-200 m).
<i>Trematomus lonnbergi</i>	2	11.9	-0.73	1.75	Benthic-pelagic scavenger (>450 m).
<i>Dissostichus mawsoni</i>	4	14.4	-0.71	1.60	Active predator, feeding from all levels in water column.
<i>Notothenia angustata</i> *	2	10.8	-0.74	1.33	Sedentary benthic.

P<sub>50</sub> values at pH 8.1

The oxygen-binding properties were determined on pooled blood samples except for *P. borchgrevinki* and *D. mawsoni*.

N = number of specimens.

\* Acclimated to and measured at  $4.5^{\circ}\text{C}$ . P<sub>50</sub> value corrected to  $-1.5^{\circ}\text{C}$  assuming a  $\Delta\text{H}$  value of  $-30\text{ kJ mol}^{-1}$ .

The specimens of *P. borchgrevinki* were caught one month later than the group used for the acclimation experiment, which may account for the small difference in the haematological data between the two groups, the latter one showing lower red cell concentration of haemoglobin and ATP.

Blood haemoglobin concentrations agreed favourably with an earlier study by Wells *et al.* (1980), except for *T. lonnbergi*, but were somewhat lower than values reported by Kooyman (1963) and Grigg (1967). Haematocrit values were for all species lower than previously reported, whereas the mean red cell haemoglobin concentration was



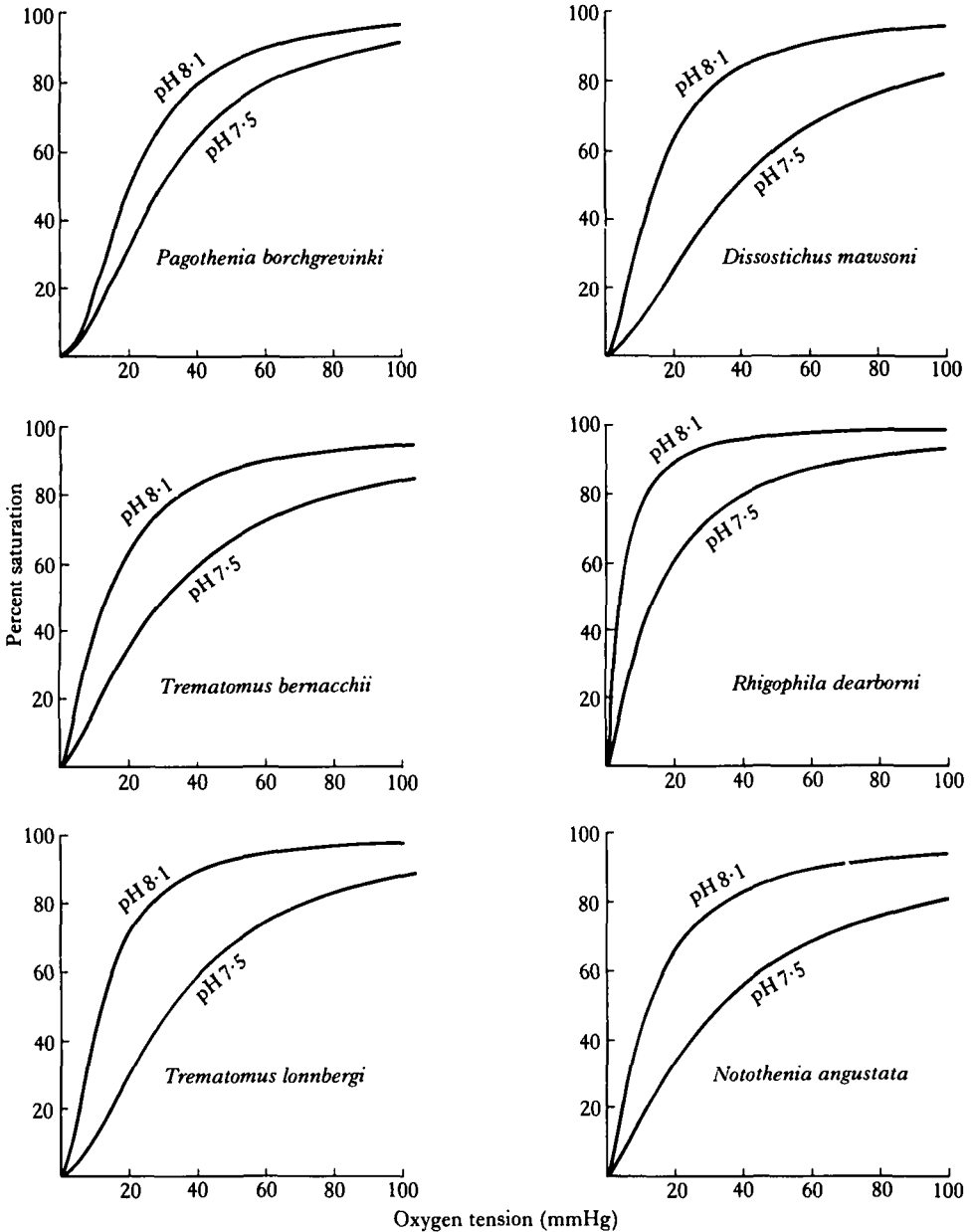


Fig. 4. Blood oxygen binding curves at  $-1.5^{\circ}\text{C}$  for four nototheniid and one zoarcid (*Rhigophila dearborni*) species confined to antarctic waters. The temperate and sub-antarctic nototheniid *Notothenia angustata* was acclimated to and measured at  $4.5^{\circ}\text{C}$ , and the oxygen-binding curve corrected to  $-1.5^{\circ}\text{C}$  assuming a  $\Delta\text{H}$  value of  $-30\text{ kJ mol}^{-1}$ .

higher. These differences may be accounted for by handling stresses in the earlier studies, as demonstrated for salmonid fish (Soivio, Westman & Nyholm, 1974; Soivio & Nikinmaa, 1981). There was a marked difference between the species in the blood concentration of nucleoside triphosphates. Generally, there were only trace amounts

of GTP. The molar ratios of ATP to haemoglobin tetramer in *D. mawsoni* were lower than those found by Qvist *et al.* (1977). The bottom-dwelling *R. dearborni* and *N. angustata* both had ATP:Hb<sub>4</sub> ratios above 1:1, while the other species had ratios below 1:1.

Blood respiratory properties showed a wide variation among species. *P. borchgrevinki* had the lowest oxygen affinity ( $P_{50} = 20.7$  mmHg) and Bohr factor ( $\Phi = -0.26$ ), but displayed pronounced cooperative oxygen binding of the blood ( $n = 1.92$ ), as shown in Table 3 and Fig. 4. At the other extreme we found a very high oxygen affinity ( $P_{50} = 4.3$  mmHg) and Bohr factor ( $\Phi = -0.90$ ), and one of the lowest  $n$  values of 1.36 for the zoarcid *R. dearborni*. The temperate and sub-antarctic *N. angustata* was acclimated to 4.5 °C, and the oxygen-binding curves were determined at that temperature. The  $P_{50}$  value was then corrected to -1.5 °C assuming a  $\Delta H$  value of -30 kJ mol<sup>-1</sup>. At this temperature *N. angustata* manifested a higher blood oxygen affinity and greater Bohr factor than antarctic nototheniid species, but showed low cooperativity ( $n = 1.33$ ).

## DISCUSSION

### *Comments on methods*

Some of the previous studies on the blood oxygen-binding properties of antarctic fish suffer from potentially erroneous results due to the use of the mixing technique (see Haab, Piiper & Rahn, 1960; Scheid & Meyer, 1978) for the determination of oxygen dissociation curves, although our results compare favourably with those found for *D. mawsoni* by Qvist *et al.* (1977) using this method. The long response time of 20 min (Qvist *et al.* 1977) of the oxygen electrode at sub-zero temperatures could allow changes in  $P_{O_2}$  to occur during the course of the measurement. Furthermore, the complete deoxygenation of blood necessary for this method may result in measured values of  $P_{50}$  and  $n$  that are lower than the true values, due to the organic phosphate metabolism of nucleated red cells (Tetens & Lykkeboe, 1981). The use of the Tucker method eliminates these problems, since the oxygen electrode is kept at 35 °C and complete deoxygenation of blood does not occur.

Our preliminary study on the changes occurring during tonometry of blood indicated that complete equilibration between blood and gases was attained within 20 min at the low temperature of -1.5 °C. It was therefore concluded that tonometry with continuously flowing gases was essentially complete within 20 min and subsequently remained stable. The main source of error from the Tucker method is in the measurement of sample volumes, as is apparent from the bigger standard deviation for determinations of the blood oxygen capacity (at  $P_{O_2} = 150.4$  mmHg; see Fig. 1).

Grigg (1967) reported that storing of chilled blood was unsuccessful because the plasma turned opaque. We did not observe this phenomenon and generally found the blood very stable at -1.5 °C. Duplicate determinations before and after 4 h storage repeatedly gave identical oxygen contents and pH of blood equilibrated to equal gas mixtures.

### *Blood acid-base status*

The measured *in vivo* blood pH values confirm the observation of Qvist *et al.* (1977) that antarctic fish at sub-zero temperatures follow the pH-temperature line of

ther species (Rahn & Baumgardner, 1972). The predicted blood pH at  $-1.5^{\circ}\text{C}$  should be in the range of 8.1 to 8.4 to which all our data pertain.

Dejours & Armand (1983) have recently questioned the thermal basis for the rule of constant relative alkalinity, and they showed that the relatively hyperoxic state of water at low temperatures might be expected to result in a respiratory acidosis due to hypoventilation. However, our findings of a high blood pH and low arterial  $\text{P}_{\text{CO}_2}$  for *D. mawsoni* and *P. borchgrevinki* (Fig. 2) conform to predicted values according to Rahn & Baumgardner (1972). This emphasizes the strategy of relative hyperventilation and low oxygen extraction in antarctic fish.

The change in blood acid-base status resulting from warm-acclimation of *P. borchgrevinki* follows observations from temperate elasmobranchs (Albers & Pleschka, 1967) and teleosts (Randall & Cameron, 1973). Increased water temperature resulted in nearly a doubling of the ventilatory rate, possibly also of the gill ventilation volume. However, no significant change in blood pH was observed, despite a lowered plasma bicarbonate concentration (cf. Fig. 2) and slightly elevated blood lactate (Table 1). A cautious interpretation would be that *P. borchgrevinki* showed a metabolically balanced respiratory alkalosis following warm-acclimation, and that this species apparently did not conform to the concept of constant relative alkalinity.

#### *Effect of temperature*

A prominent feature of our study was the observation that the antarctic fish *P. borchgrevinki* did not have an exceptionally high temperature sensitivity of the blood oxygen affinity.

Grigg (1967) reported an extremely high  $\text{P}_{50}$  value of blood from this species when measured at  $4.5^{\circ}\text{C}$ , relative to the  $\text{P}_{50}$  value at  $-1.5^{\circ}\text{C}$ . The absolute value at  $-1.5^{\circ}\text{C}$  and pH 7.5 compares favourably with our  $\text{P}_{50}$  value, but at a physiological blood pH of 8.1. As shown by Fig. 2, the blood has to be equilibrated at  $\text{P}_{\text{CO}_2} = 5\text{--}6$  mmHg to obtain a blood pH of 7.5, which is an order of magnitude higher than stated by Grigg (1967). The observed temperature effect on blood  $\text{P}_{50}$  ( $\Delta\text{H} = -26.7$  kJ mol $^{-1}$ ) is significantly smaller than that of  $-45$  kJ mol $^{-1}$ , calculated from the data of Grigg (1967). However, our  $\Delta\text{H}$  value compares well with findings by Qvist *et al.* (1977) for the antarctic nototheniid *D. mawsoni* ( $\Delta\text{H} = -30.3$  kJ mol $^{-1}$ ), and with recent reports on blood from temperate fish species like rainbow trout (Weber, Wood & Lomholt, 1976), the Australian blackfish (Dobson & Baldwin, 1982) or the European eel (Laursen, Andersen & Lykkeboe, 1984). Human blood has a  $\Delta\text{H}$  value of  $-34.3$  kJ mol $^{-1}$  (Reeves, 1980). Wells & Jokumsen (1982) reported a  $\Delta\text{H}$  value of  $-56.13$  kJ mol $^{-1}$  for 'stripped' haemolysate of blood from *P. borchgrevinki*. The difference in apparent heat of oxygenation between 'stripped' haemolysate and whole blood is expected because of the exothermic binding of organic phosphates to haemoglobin (Benesch, Benesch & Yu, 1969).

The small difference in  $\text{P}_{50}$  between *in vitro* heated blood and blood from warm-acclimated fish may be a result of either a change in the relative proportions of various haemoglobin components or an alteration of the intraerythrocytic environment of the haemoglobin.

The latter possibility was exploited in the thermo-acclimatory response of *P. borchgrevinki*. The increased red cell ATP concentration and unchanged mean cellular

haemoglobin concentration of warm-acclimated fish (Table 1) should result in both a direct right-shift of the oxyhaemoglobin dissociation curve, as shown by Wells & Jokumsen (1982) for *P. borchgrevinki*, and an indirectly increased  $P_{50}$  value because of an altered Donnan distribution of ions across the red cell membrane (Duhm, 1971; Wood & Johansen, 1973). Both effects, however, would be relatively small because of the low sensitivity of the haemoglobin to ATP (Wells & Jokumsen, 1982) and the small Bohr factor (Table 1).

The observed augmentation of the right-shift of the oxygen-binding curve by an increased ATP: Hb<sub>4</sub> molar ratio following warm-acclimation is contrary to the general theory for water breathers, as outlined by Wood & Lenfant (1979), Powers (1980) and Weber (1982). The normal response to warm-acclimation of teleost fish appears to be a decrease in red cell organic phosphate (NTP), thereby diminishing the *in vivo* change in  $P_{50}$ . Such a response was reported for the antarctic *D. mawsoni* (Qvist *et al.* 1977) following acclimation to 4 °C. The acclimation period in their study was only 4 days, which might be too short to reveal a new steady-state level of ATP: Hb<sub>4</sub> (see Greaney & Powers, 1977). However, an increase in the molar ratio of NTP to Hb<sub>4</sub> has recently been shown for the Australian blackfish (Dobson & Baldwin, 1982), and for the European eel at low temperatures (Laursen *et al.* 1983). Dobson & Baldwin (1982) interpreted this response as being adaptive for fish with a high  $P_{50}$  value and living in well-oxygenated waters, thereby facilitating the oxygen delivery to the tissues. Wohlschlag (1964) found that antarctic fish have a slightly greater metabolic response to small temperature increases than do temperate species. Small temperature increases of antarctic waters are more likely to cause problems with the tissue oxygen supply rather than insufficient oxygen loading at the gills. The epochs of isolation in constantly well-oxygenated waters might have conceived this response of increased red cell ATP in *P. borchgrevinki* to support an elevated rate of oxygen-dependent metabolism at higher temperatures.

*P. borchgrevinki* is possibly exceptional among antarctic nototheniid fish in displaying haemoglobin multiplicity (Wells *et al.* 1980). Thermal acclimation has been shown to induce changes in the relative concentrations of individual haemoglobin components of both relatively stenothermal species like the rainbow trout (Houston & Cyr, 1974; Weber *et al.* 1976) and the eurythermal carp and goldfish (Houston, Mearow & Smeda, 1976; Houston & Rupert, 1976; Houston & Smeda, 1979). A higher proportion of haemoglobin components with low oxygen affinity or high affinity for the allosteric modifier ATP could explain some of the observed  $P_{50}$  change following warm-acclimation.

#### *Blood oxygen affinity of antarctic fish*

All species examined in our study possessed erythrocytic ATP and GTP, except *P. borchgrevinki* where GTP was absent. However, the only species with high concentrations of NTP (essentially ATP) were the two bottom-dwelling species, *R. dearborni* and *N. angustata*, which live in potentially oxygen-depleted environments. The high red cell NTP concentrations of these species probably reflect a potential mechanism for regulated increase of blood oxygen affinity during hypoxic situations, as in temperate fish (Wood & Johansen, 1972; Wood, Johansen & Weber, 1975; Tetens & Lykkeboe, 1981).

The high blood oxygen affinity of the zoarcid *R. dearborni* can be considered adaptive to low water oxygen contents, securing adequate oxygen loading at the gills. Oxygen unloading to the metabolizing tissues will thus be promoted *via* the high Bohr factor. The nototheniid species *T. bernacchii* and *T. lonnbergi* have relatively low  $P_{50}$  values, in accord with their benthic mode of life. The very low blood oxygen affinity and Bohr factor and the highly cooperative oxygen binding of *P. borchgrevinki* would seem adaptive to an epipelagic mode of life in well-oxygenated waters, with effective blood oxygen delivery to the tissues along the steep part of the oxygen binding curve. The high Bohr factor of the pelagic midwater predator *D. mawsoni* will be physiologically adaptive for securing adequate tissue oxygenation during bursts of swimming activity when hunting.

The range of  $P_{50}$  values of blood from antarctic nototheniid fish at  $-1.5^{\circ}\text{C}$  and an *in vivo* blood pH of 8.1 is similar when directly compared with a range from temperate species at higher temperatures and lower *in vivo* pH. Extrapolation to  $-1.5^{\circ}\text{C}$  and pH 8.1 of  $P_{50}$  values from a number of temperate species (using the appropriate  $\Delta\text{H}$  values and Bohr factors) however, clearly gives  $P_{50}$  values nearly an order of magnitude lower than the values presented in this paper. The low blood oxygen affinities of antarctic nototheniid species could be an adaptation to constantly well-oxygenated waters, and to a higher resting metabolism than temperate fish adapted to relatively high temperatures and acclimated to low temperatures, as observed by Wohlschlag (1960, 1964). The dispute about cold adaptation, defined as a higher resting metabolism among species from low latitudes relative to temperate and tropical species, has not yet been settled (see Houlihan & Allan, 1982). However the interpretation described above is supported by the very high blood oxygen affinity of the zoarcid *R. dearborni*, which is claimed not to have an elevated resting metabolism (Wohlschlag, 1963). Our finding of a  $P_{50}$  value from the sub-antarctic nototheniid *N. angustata* lower than the endemic antarctic species of the same family further supports the theory of cold adaptation.

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