Blood Physiology and Ecological Consequences in Weddell Sea Fishes (Antarctica)

Blutphysiologie und Ökologische Konsequenzen von Fischen des Weddellmeeres (Antarktis)

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Summary

The introduction focuses on the gas exchange, a primary function of blood in vertebrates. Since most of our knowledge on blood functions originates from mammals, important differences between haematology in fishes and mammals are outlined. Extensive studies on environmental adaptations in the respiratory system of fishes are briefly summarized. Emphasis is placed on the mode of life of fishes and its reflection in various haematological parameters.

Hydrographical isolation and constant low temperatures have led to a high degree of stenothermy and endemism in the Antarctic fish fauna. Consequently, a variety of adaptations in the oxygen transport system of these fishes to their environment has been developed.

Although blood of Antarctic fishes was of particular interest after the detection of haemoglobinless fish, no data are available for characteristic species, dominating the fish fauna in high-Antarctic waters. Our present knowledge on the blood physiology of Antarctic fishes is actually based on only few species. So far, it has not been tried to relate the ecology of a large range of different Antarctic fish species to their haematology in combination with structural and functional studies on their haemoglobins. Therefore, a detailed study of blood parameters and haemoglobins of Weddell Sea fishes was initiated. For most of the species it is the **first record** of haematology and haemoglobin properties. Special attention is paid to the mode of life and the evolution of these teleosts in relation to their habitat, in an attempt to find links with haematological characteristics.

Blood samples from 29 species of 20 genera and 6 families from the eastern and southeastern Weddell Sea were taken during 'Polarstern' expedition EPOS leg 3. Methods for the measurement of haematological parameters are briefly described. Investigated parameters comprise: pH, partial pressure of oxygen and carbon dioxide (PO_2, PCO_2), number of erythrocytes (RBC), haematocrit (Hct), haemoglobin content (Hb), mean corpuscular Hb concentration (MCHC), mean cellular Hb (MCH), oxygen carrying capacity (O_2CC) and plasma contribution (PC).

Structural and functional studies were carried out on haemolysates, including electrophoretical detection of Hb components, spectrophotometrical investigations of Root and Bohr effects, P_{50} estimation (the oxygen partial pressure required to achieve half-saturation) and complete sequencing of the α and ß chain of selected haemoglobins.

The results indicate on one hand a certain uniformity of blood parameters following the general trend of reduction of haematocrit, red blood cells and haemoglobin in all endemic fish species in Antarctica. When average values for haemoglobin content and red blood cell count for different families are compared, we find a decrease in the order Nototheniidae, Artedidraconidae, Bathydraconidae, Channichthyidae. It is concluded that this finding reflects the phylogeny of endemic Antarctic fish families.

The presence of distinct Bohr effects in most of the investigated species indicates a fine regulation of oxygen supply to the tissues. Active or moderately active species such as *Dissostichus mawsoni* or *Trematomus eulepidotus* were found to have a strong Bohr effect. Their Bohr coefficients were calculated to be close to -1. It is concluded that species which have only weak Bohr effects (e.g. *Aethotaxis mitopteryx*) have a limited scope of activity. High oxygen affinity of haemoglobin (i.e. low P_{50} value) was only found in active or moderately active species (*D. mawsoni, Pagothenia hansoni*).

The general absence of multiple haemoglobins is unusual in comparison with species from temperate areas and may reflect the stable physico-chemical conditions of high-Antarctic seas. Only in *Pleuragramma antarcticum* two haemoglobins in higher amounts were found.

On the other hand we find certain species with clear differences in their blood physiology, assigned to their mode of life and activity pattern. This is demonstrated in detail for *Aethotaxis mitopteryx*, a species with pelagic/benthopelagic **and** sluggish mode of life and for *Bathydraco marri*, a benthic species with large scope of activity. *A. mitopteryx* seems to have a poorly developed oxygen transport system, since all haematological characteristics were found to be low. Similar low values were measured in *P. antarcticum*, a fully pelagic species, repeatedly reported to be sluggish. More active species such as *D. mawsoni* or *T. eulepidotus* are characterized by significantly higher haematological values.

General conclusions about endemic Antarctic fish families are drawn with particular reference to links between evolution, physiological adaptations and abiotic preferences. The haematology of bathydraconids corresponds both to their preference for deep and cold areas and their taxonomic position, just below the channichthyids. Nototheniids, a family with great variation in morphology and mode of life, is assumed to be in a process of speciation. This is well reflected in a higher variance of haematological values, as compared to other endemic families. In this respect the exceptional role of haemoglobinless fishes is considered and the necessity for the red-blooded fishes to possess haemoglobin is discussed.

In a final remark it is suggested that environmental conditions in Antarctic waters have favoured the development of fishes with low activity level. This energy-saving 'strategy' is even followed by pelagic species.

Zusammenfassung

Die Einleitung konzentriert sich auf den Gasaustausch, eine der Hauptaufgaben von Blut in Vertebraten. Wichtige Unterschiede in der Hämatologie von Säugern und Fischen werden herausgestellt, da unsere Kenntnisse über Blutfunktionen hauptsächlich an Säugern gewonnen wurden. Eine kurze Zusammenfassung zahlreicher, ausführlicher Studien über Fischatmungsmechanismen und ihre Anpassungen an die Umwelt schließt sich an. Dabei werden verschiedene Lebensweisen und ihre Widerspiegelung in hämatologischen Parametern in den Vordergrund gestellt.

Hydrographische Isolation und konstant niedrige Temperaturen brachten eine Fischfauna hervor, die charakterisiert ist durch viele endemische und stenotherme Arten. Ferner entwickelten diese Fische eine Reihe von Anpassungen ihres Sauerstofftransportes an die Umgebung.

Das Blut antarktischer Fische war nach der Entdeckung von Fischen ohne Hämoglobin von besonderem Interesse. Dennoch sind kaum Daten vorhanden über Fischarten, die die hochantarktische Shelfmeerfauna dominieren. Unser heutiges Wissen über die Blutphysiologie antarktischer Fische stützt sich insgesamt nur auf wenige Arten. Dabei wurde bisher noch nicht versucht, die Ökologie einer Reihe möglichst verschiedener antarktischer Arten in Bezug zu ihrer Hämatologie unter gleichzeitiger Berücksichtigung der Struktur und Funktion ihrer Hämoglobine zu setzen.
Deshalb wurde eine detaillierte Studie an Blut und Hämoglobin von Fischen des Weddellmeeres durchgeführt. Die meisten Arten der vorliegenden Arbeit werden **zum ersten Mal** hinsichtlich ihrer Hämatologie und Hämoglobine untersucht. Im Vordergrund stehen dabei die Lebensweise und Evolution dieser Teleostier in Beziehung zu ihrem Habitat sowie die Verbindung mit hämatologischen Parametern.

Während der "Polarstern" Expedition EPOS Leg 3 wurden im östlichen und südöstlichen Weddellmeer Blutproben von 29 Arten aus 20 Gattungen und sechs Familien genommen. Die Methoden zur Messung hämatologischer Parameter werden kurz beschrieben. Die folgenden Parameter wurden untersucht: pH, Sauerstoff- und Kohlendioxyd-Partialdruck (PO_2, PCO_2), Anzahl der Erythrocyten (RBC), Hämatokrit (Hct), Hämoglobingehalt (Hb), mittlere korpuskuläre Hämoglobinkonzentration (MCHC), mittlerer zellulärer Hämoglobingehalt (MCH), Sauerstofftransportkapaziät (O_2CC) und Plasmabeitrag (PC).

An Hämolysaten wurden Untersuchungen zur Struktur und Funktion von Hämoglobin durchgeführt. Sie schließen den elektrophoretischen Nachweis der Hämoglobinkomponenten, die spektrophotometrische Untersuchung von Root und Bohr Effekt, die Bestimmung des P_{50} (der Sauerstoffpartialdruck, der nötig ist um 50% Sättigung zu erreichen) sowie die komplette Sequenzierung der α- und ß-Ketten ausgewählter Hämoglobine ein.

Einerseits zeigen die Ergebnisse eine gewisse Einheitlichkeit der Blutparameter. Sie folgen dem allgemeinen Trend der Reduzierung von Hämatokrit, Erythrocyten und Hämoglobingehalt, welcher in allen endemischen Fischarten der Antarktis vorhanden ist. Ein Vergleich der Hämoglobinkonzentrationen und Anzahl der Erythrocyten in verschiedenen Familien, führt zu einer Abnahme der Werte in der Reihenfolge Nototheniidae, Artedidraconidae, Bathydraconidae und Channichthyidae. Dies wird als Widerspiegelung der Phylogenie endemischer antarktischer Fischfamilien gedeutet.

Ausgeprägte Bohr Effekte in den meisten der untersuchten Arten deuten auf eine feine Regulation der Sauerstoffversorgung der Gewebe. Hämoglobine aktiver oder mäßig aktiver Arten haben alle einen starken Bohr Effekt, mit Bohrkoeffizienten nahe -1. Daraus wird abgeleitet, daß Arten mit schwachem Bohr Effekt (z.B. *Aethotaxis mitopteryx*) nur einen eingeschränkten Spielraum für Aktivität haben. Eine hohe Sauerstoffaffinität (also niedriger P_{50}) konnte nur in aktiven oder mäßig aktiven Arten festgestellt werden (*D. mawsoni, Pagothenia hansoni*). Das Fehlen multipler Hämoglobinkomponenten ist im Vergleich zu Arten gemäßigter und tropischer Breiten ungewöhnlich. Es wird als möglicher Ausdruck der stabilen physiko-chemischen Umgebung gedeutet. Nur in *Pleuragramma antarcticum* wurden zwei Hämoglobine in größeren Mengen gefunden.

Andererseits finden wir Arten mit deutlichen Unterschieden in ihrer Blutphysiologie, die auf verschiedene Lebensweise und Aktivität zurückführbar sind. Dies wird ausführlich gezeigt am Beispiel von *Aethotaxis mitopteryx*, einer Art mit pelagischer/benthopelagischer **und** träger Lebensweise sowie am Beispiel von *Bathydraco marri*, einer Art mit benthischer Lebensweise und großem Aktivitätsspielraum. *A. mitopteryx* scheint ein schwach entwickeltes Sauerstofftransportsystem zu haben, da alle hämatologischen Parameter niedrig sind. Ähnlich niedrige Werte wurden bei *P. antarcticum* gefunden, einer Art, die trotz ausschließlich pelagischer Lebensweise häufiger als träge charakterisiert wurde. Die aktiveren Arten (z.B. *D. mawsoni* und *T. eulepidotus*) zeichnen sich durch deutlich höhere hämatologische Werte aus.

Allgemeine Betrachtungen und Schlußfolgerungen über antarktische Fischfamilien schließen sich an. Dabei stehen Verbindungen zwischen Evolution, physiologischen Anpassungen und abiotischen Präferenzen im Vordergrund. Die Hämatologie der Bathydraconiden steht im Einklang sowohl mit ihrer Vorliebe für kalte und tiefe Gebiete als auch mit ihrer taxonomischen Stellung nahe den Channichthyiden. Die Nototheniiden sind eine Familie mit erstaunlicher Vielfalt bezüglich ihrer Morphologie und Lebensweise. Dies zeigt sich auch in einer, im Vergleich mit anderen endemischen Familien, hohen Varianz fast aller hämatologischen Parameter. In diesem Zusammenhang wird die besondere Stellung der Eisfische betrachtet und die Notwendigkeit des Besitzes von Hämoglobin diskutiert.

Eine Schlußbemerkung zeigt auf, daß die Umweltbedingungen antarktischer Meere offensichtlich die Entwicklung von trägeren Fischen begünstigt haben. Diese energiesparende 'Strategie' ist auch bei pelagischen Arten verwirklicht.

Introduction

BLOOD FUNCTIONS

"Blood is a very special liquid" (Goethe 1808). In the early 19th century blood had still a rather mystical meaning and Goethe could not know yet that his statement would once be 'to the point'. Due to the omnipresence of blood in a body it is an excellent carrier of information about nearly all metabolic processes. Therefore, extensive haematological investigations are nowadays routine procedures in clinical methodology. Blood has many different functions. According to Schmidt-Nielsen (1986) these are: transport of nutrients, metabolites, excretory products, gases, hormones and cells; heat exchange; transmission of force; coagulation; immune response and homeostasis. Obviously, the majority of these functions could be carried out by any aqueous medium. One of the primary functions of blood however, the gas exchange, is associated with highly complex biochemical properties of the blood components, at least in vertebrates. The transport of oxygen via haemoglobin, which is concentrated in red blood cells, is regarded as characteristic for vertebrates in zoophysiological textbooks (Penzlin 1980; Eckert 1986; Schmidt-Nielsen 1986). The size and shape of these cells, however, can vary markedly (Fig. 1.1).

Gas exchange covers oxygen delivery to the tissues, as well as carbon dioxide disposal from the tissues. The amount of oxygen transported by unit volume of blood depends on the partial pressure of O_2 in the blood, on the number of red blood cells per unit volume, on the amount of functional haemoglobin in the red cells and on the oxygen affinity of the haemoglobin(s). Carbon dioxide can be transported as molecular CO_2, bicarbonate, carbonate and carbamino compounds and red blood cells play a particular role in facilitating this transport.

FISH BLOOD

Most of our knowledge on haematology, oxygen transport and haemoglobins (Hb's) has been derived from research on mammals, particularly humans. When fish blood is investigated, there are a few important differences to be kept in mind for the interpretation of results.

Fish erythrocytes are nucleated and therefore more resistant to shear-induced shape changes (i.e. when flowing) than mammalian cells (Nikinmaa 1990). As a consequence a decrease in viscosity with increasing flow rate can be observed. Organic phosphates found in fish erythrocytes are mainly ATP and GTP in contrast to 2,3 DPG (diphosphoglycerate) in

mammals. In addition the red cells of some fish species contain UTP (uridosintriphosphate), IDP (inositoldiphosphate) and IPP (inositolpentaphospate).

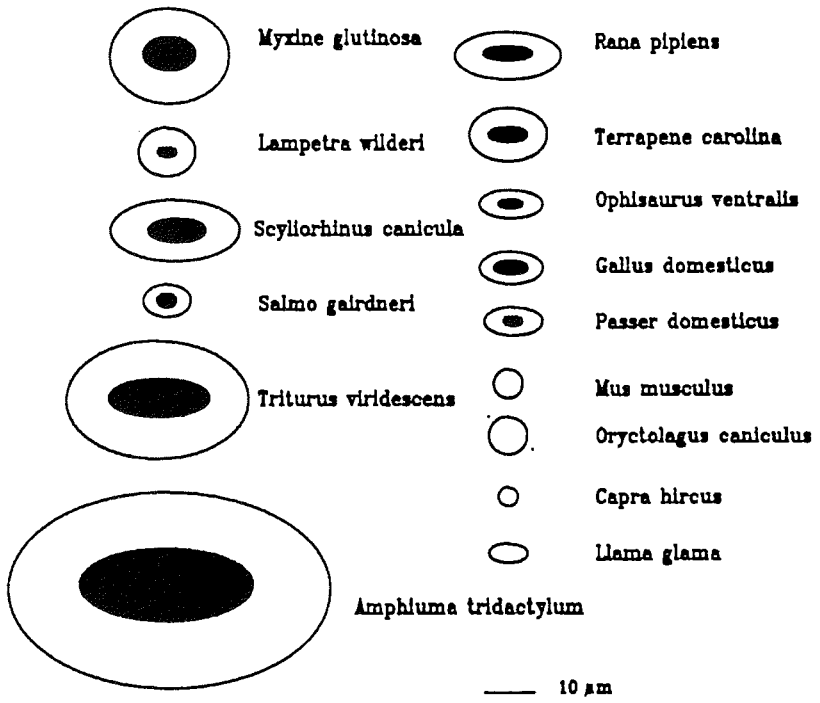


Fig. 1.1: Shape and size of some vertebrate red blood cells from fish, amphibians, reptiles, birds and mammals (from Nikinmaa 1990, modified).

Haematological adaptations

The respiratory system of fishes, and particularly their haemoglobins, constitute excellent models for studies on environmental adaptations (Powers 1980). These adaptations can be on morphological, physiological or molecular level. Most fish species regulate certain haematological parameters according to environmental conditions (Val et al. 1990). Studies on their erythrocytes are of great interest, particularly when correlated with activity and general ecology (Coburn & Fischer 1973). Erythrocytes of active fish species were found to be smaller and their viscosity was less shear-dependent (Wells & Baldwin 1990) in comparison to other species. The cells of active, tropical fishes were more densely packed with Hb (Wells & Baldwin 1990) and had more total Hb due to an increased number of red blood cells (Putnam & Freel 1978) than less active species. A positive correlation between haematocrit, total haemoglobin, total plasma proteins and activity was found in a number of temperate fishes (Larsson et al. 1976).

Multiple components

Many fishes have several haemoglobins, which can occur in quite different amounts. Active pelagic swimmers have multiple components with distinctly different functional properties, which guarantee sufficient oxygen supply to the tissues even under severe acidic conditions (Riggs 1970). Migrating species, such as eel and trout possess a set of functionally different Hb's and the appropriate one is synthesized on demand (Love 1980). Therefore Hb multiplicity has been regarded as an important adaptive strategy for fish to varying environmental conditions and/or changing metabolic requirements (Riggs 1970). In spite of this, there is no clear relation between the number of haemoglobin fractions and environmental stability (Val et al. 1990).

Respiratory properties of blood, especially the oxygen carrying capacity and the oxygen affinity respond to evolutionary selective pressure (Wells et al. 1989). In fishes we find special adaptations, which fit for a particular mode of life in a particular environment. The properties of fish haemoglobins reflect adaptations not only to the metabolic rate, but also to the prevailing external oxygen pressure (Riggs 1970). Active, pelagic fishes, for example, living in well-oxygenated waters, tend to have oxygen equilibria favouring unloading of oxygen to the tissues (Wells et al. 1989), i.e. their Hb's have a low O_2-affinity. Furthermore active fishes usually have Hb's with larger Bohr effects (see below). Sluggish, benthic fishes tend to have equilibria favouring oxygen uptake at the gills, which results in high oxygen affinities. The same applies to fishes living in oxygen-poor habitats (e.g. in Amazonian rivers), where in addition to high oxygen affinities facultative air-breathing was developed (Powers 1980).

Haemoglobin structure and function

Haemoglobin **structure** is an indispensable model for almost all fields of biochemistry, ranging from protein folding studies to molecular evolution. The analysis of the primary structure (amino acid sequence, AAS) reveals the evolutionary development of a molecule. The AAS causes a defined secondary and tertiary structure and is therefore responsible for the functional behaviour. This explains the need of structural studies for a full understanding of the functions.

Haemoglobins of all vertebrates are strikingly uniform in their subunit architecture and molecular weight, which is often close to 65000 (Riggs 1970). The primary functional unit of Hb is the haem group, which consists of a porphyrin ring system surrounding a central ferrous ion. The haem groups are placed in pockets formed by the characteristic folding of the globin chains. This arrangement enables a reversible reaction between the ferrous ion,

which remains in the reduced form, and the oxygen. In fishes the four globin chains (two α and two ß chains) have 142 (α) and 146 (ß) amino acids (AA) each. The complete sequences of these chains are known for a few temperate and tropical fish species only (Kleinschmidt & Sgouros 1987; Huber & Braunitzer 1989a, b). Recently the first sequences of Antarctic fish species were published (D'Avino & di Prisco 1988; D'Avino et al. 1989, 1990). Specific AA residues (see discussion) have been identified to be responsible for the various functional responses (Perutz et al. 1980; Perutz & Brunori 1982; Riggs 1988).

Haemoglobin plays a dual **functional** role in the transport of O_2 to the tissues. It must be able to
- bind O_2 effectively in the capillaries of the gas exchange organs, and
- unload O_2 at high partial pressures in order to maintain a large diffusion gradient between the tissue capillaries and the O_2-consuming structures.

This is achieved by a complex oxygen equilibria system. A representative oxygen equilibrium curve for fishes is presented in Fig. 1.2. The curve can be described by the following equation:

$$Y=\frac{K_A * P_{O2}^n}{1+K_A * P_{O2}^n} \qquad (1)$$

in which Y = fractional O_2 saturation of haemoglobin, KA = equilibrium constant for Hb:O_2 reaction, PO_2 = oxygen tension and n = Hill coefficient, which describes the degree of cooperativity (see below). Logarithmic transformation of equation (1) yields the Hill equation:

$$\log\frac{Y}{1-Y}=n*\log(PO_2)+\log(K_A) \qquad (2)$$

This is the common equation for a straight line (y=ax+b) and the Hill coefficient n and the equilibrium constant represent slope and y-axis intercept, respectively. At 50% oxygen saturation (fractional saturation Y = 0.5), log Y/(1-Y) is zero and the oxygen tension is the P_{50}. Thus, the Hill plot (e.g. Fig. 2.12 in Materials and Methods) gives several parameters directly. In the following section a brief description of these parameters and their meaning is given (illustrated by Fig. 1.2):

Values of n greater than one indicate that oxygen binding has positive cooperativity (i.e. the binding of the first O_2 molecule is more difficult than the binding of consecutive O_2 molecules). For n=1 we find no cooperativity and for n<1 the binding sites have different affinities for O_2. The oxygen tension at which Hb is 50% saturated with O_2 is called P_{50} (see (3) in Fig. 1.2), which is a direct indicator for the O_2-affinity of Hb (similar to K_m, the Michae-

lis-Menten constant in enzymes). The value of n at P_{50} is called n½.

The reaction between tetrameric Hb and O_2 can be expressed by the equilibrium reactions:

$$Hb+4O_2 \rightleftharpoons HbO_2+3O_2 \rightleftharpoons Hb(O_2)_2+2O_2 \rightleftharpoons Hb(O_2)_3+O_2 \rightleftharpoons Hb(O_2)_4 \qquad (3)$$

For a description of this cooperative O_2 binding of Hb several models have been established. The most commonly used theory is the allosteric model of Monod et al. (1965), which has been established for allosteric proteins (enzymes) in general. The Hb can exist in two conformations designated as T (tense) for deoxy-Hb and R (relaxed) for oxy-Hb. The binding of O_2 to a subunit of T-Hb induces a conformation change in the tertiary structure to R-Hb with an abrupt increase in affinity. An excellent summary of the complex mathematics of this model is given by Nikinmaa (1990). More complicated is the description of the oxygen equilibrium when heterotropic interactions are considered. The above mentioned cooperativity is a homotropic interaction, as one ligand (O_2) influences the binding of consecutive molecules of the same ligand. However, it is well known that other ligands such as organic phosphates, Cl^- ions, carbon dioxide and particularly protons can influence the O_2-affinity of haemoglobins (Riggs 1988).

The influence of protons on the O_2-affinity of Hb is called **Bohr effect.** In mammals two types of Bohr effect are distinguished; in this work the Bohr effect refers to the alkaline, usually negative Bohr effect (Riggs 1988), i.e. a rightward shift of the equilibrium curve with decreasing pH (cf. (1) in Fig. 1.2). The magnitude of the Bohr effect is usually described by the Bohr coefficient Φ ($\Delta \log P_{50}/\Delta pH$). The presence of organic phosphates and/or Cl^- can dramatically increase the Bohr effect (Riggs

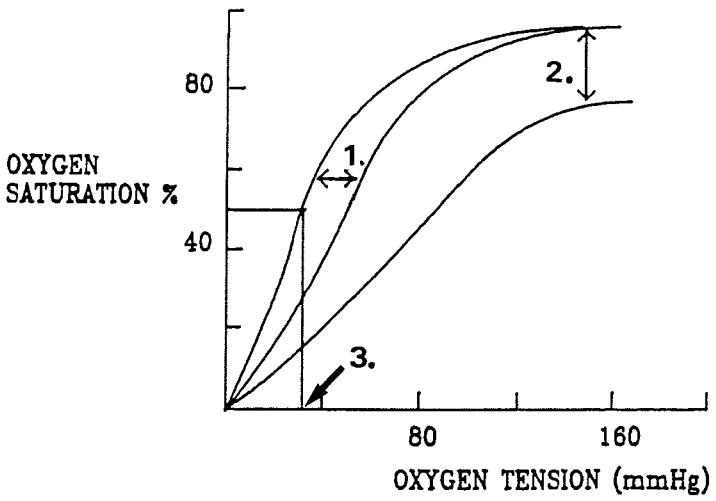


Fig. 1.2: Oxygen equilibrium curve of teleost haemoglobin. With decreasing pH the Bohr effect (1) and Root effect (2) is displayed. (3) = P_{50}.

1988). The fact that metabolizing tissues tend to decrease pH due to production of CO_2 and acids, underlines the biological significance. At low pH the oxygen equilibrium curve shifts to the right and O_2 unloading is eased. The Bohr effect helps to maintain a large gradient in PO_2 (O_2 partial pressure) between capillary blood and tissues.

The **Root effect** occurs essentially in fishes and describes the fact that individual haemoglobins cannot be fully saturated with oxygen at a low pH, even under oxygen pressure of several atmospheres (see (2) in Fig. 1.2). This is reflected in a large decrease of the O_2-affinity and cooperativity of the haemoglobin. The phenomenon was first observed by Root (1931). Since the Root effect is generally considered to be an extreme or extended Bohr effect, it can be increased by the presence of organic phosphates and/or Cl^- as well. In two reviews (Brittain 1987; Riggs 1988) data with a large number of fish species have been compiled. Many fish species show the Root effect in all haemoglobin components, whereas in others, only one or two components are sensitive to the decrease in pH. A direct link of the Root effect to the presence of either a swimbladder or a choroid rete mirabile in the eyes or both is assumed (Brittain 1987). The biological significance of the Root effect is O_2 secretion into the swimbladder through production of lactic acid in so-called 'gas gland cells' and/or keeping up high O_2 concentration in the choroid rete mirabile, which is required for effective diffusion and thus guarantees oxygen supply to the poorly vascularized fish eyes (Nikinmaa 1990).

ANTARCTIC FISHES AND THEIR ENVIRONMENT

The Antarctic Ocean hosts little more than 200 coastal fish species (Andriashev 1965, 1985). About 50% of these species are exclusively found in Antarctic waters and belong to the suborder Notothenioidei (De Witt 1970). The origin of this suborder dates back to the lower tertiary (~50 million years ago, Andersen 1984). Low temperatures have pervaded the Southern Ocean for some 40 million years (Kennett 1977). However, recent data suggest more or less constant low temperatures (+3°C to -2°C, Hellmer & Bersch 1985) only since about 13 million years ago (Eastman & Grande 1989), which have led to the high degree of stenothermy and endemism of the fish fauna (De Vries & Eastman 1981).

The high-Antarctic shelf seas are characterized by particularly low and constant temperatures (-1.6° to -2.1°C) and high oxygen contents of more than 95% saturation (Hellmer & Bersch 1985). Temperature has a direct influence on the solubility of gases (e.g. O_2 and CO_2) in liquids (e.g. water and plasma). Therefore, the low temperatures in polar oceans increase ambient O_2 (Table 1.1) as well as the solubility in the body fluids. However, a small rise in

temperature, especially in the range below zero, significantly decreases the solubility of oxygen. Additionally, due to the lack of regular exchange, the Warm Deep Water (WDW, Fig. 1.3) can contain as little as 5 ml O_2/l at +0.4°C in comparison to 8.5 ml/l at -1.9°C in the Ice Shelf Water (ISW, Arntz et al. 1990).

T [°C]	Freshwater [ml O_2/l]	Seawater [ml O_2/l]
0	10.29	7.97
10	8.02	6.35
15	7.22	5.79
20	6.57	5.31
30	5.57	4.46

Table 1.1: The temperature effect on the amount of oxygen dissolved in freshwater and in seawater in equilibrium with atmospheric air.

In the Weddell Sea we find some additional abiotic components, which govern fish life, such as a complex system of currents and ice-drift, summer stratification due to melting processes and advection and a specific distribution of water masses on the shelf (Hubold 1991, Fig. 1.3).

Research on the Antarctic fish fauna focused on the detection of physiological adaptations to 'highly unfavourable life conditions'. This is the reason, why we know much more about antifreeze, nervous function and muscle physiology than about life-cycles and ecology of these fishes. A comprehensive study on the ecology of the Weddell Sea fish fauna is presented by Hubold (1991). The concept of highly unfavourable life conditions is merely an anthropocentric point of view and only recently the idea was developed that conditions might not be bad at all, e.g. in terms of energetic requirements (Clarke 1990).

Antarctic notothenioids belong to the highly developed perciform fishes. They have adapted to the progressive, but slow cooling of their environment since the tertiary. A special pathway of evolution has brought forward a variety of adaptations in their oxygen transport systems (Wells et al. 1980). The general trend to reduce the number of erythrocytes and haemoglobin has been reported in all notothenioid families (Kooymann 1963; Hureau et al. 1977; Wells & Jokumsen 1982; Tetens et al. 1984). Wells et al. (1980) regard this as a mechanism of evolutionary adaptation; the family Channichthyidae, characterized by a complete lack of haemoglobin and by only few erythrocyte-like cells, would in this respect be the highest developed group of fishes in the Antarctic.

Channichthyids, in comparison to their notothenioid relatives, have significantly larger hearts, diameter of vessels and blood volumes, though gills are of similar size (Holeton 1976). Other major compensatory mechanisms are the increased heart stroke volume, the decreased blood viscosity and the considerably higher difference in partial pressure of oxygen (PO_2) between

blood and tissues (Macdonald et al. 1987). Thus, the missing haemoglobin is at least partially compensated by the increased solubility of oxygen in blood plasma. These fishes can reach respectable sizes (e.g. *Champsocephalus gunnari* up to 70 cm, Kock 1981) and fast growth is assumed also in Weddell Sea fishes (von Dorrien & Räke, unpublished; Hubold 1990).

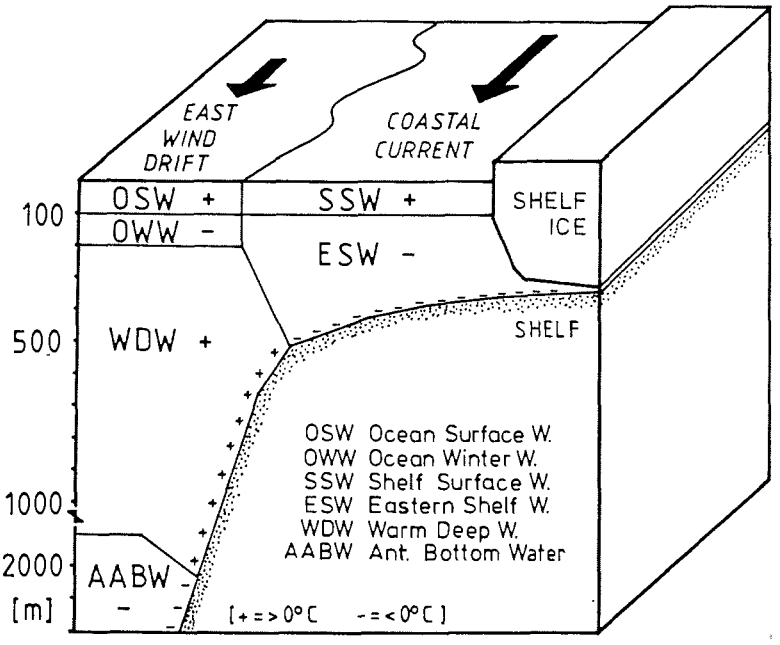


Fig. 1.3: Distribution of water masses. Cross-section at Vestkapp, Weddell Sea (from Hubold 1990).

Present knowledge in respiratory physiology

Investigations on the gill morphometrics of Antarctic fishes have shown small total gill areas (TGA) and therefore suggested a low activity level (Kunzmann 1987, 1990). A small gas exchange surface in combination with a long diffusion distance (water-blood distance, WBD) leads to low diffusion ratios and presumably low oxygen turnover. This should also be reflected in the oxygen capacity of the blood. According to De Jager & Dekkers (1975) there is a strong correlation between oxygen capacity and activity of fishes. Thus, highly active fishes have a large TGA, a short WBD and a low oxygen affinity of their haemoglobin.

Individual data on selected blood parameters are published on about 30 of the approximately

100 notothenioid Antarctic species (Grigg 1967; Everson & Ralph 1968; Hureau et al. 1977; Wells et al. 1980; Wells & Jokumsen 1982; di Prisco et al. 1988, 1990; D'Avino & di Prisco 1989; di Prisco & D'Avino 1989). Few papers have presented detailed data sets on blood oxygen-carrying capacity or functional properties. Most concentrate on comparisons between red-blooded and haemoglobinless species rather than on intrafamiliar studies or investigations of links to ecology and mode of life.

Nearly all investigated species are from sub-Antarctic areas or the Ross Sea, where so far (due to limited catching methods) only selected species from the entire Ross Sea fish community have been studied. No data are available for characteristic species such as *Pleuragramma antarcticum, Chionodraco myersi* or *Trematomus lepidorhinus*, dominating the fish fauna in high-Antarctic waters. By far most of the few investigated species belong to the family Nototheniidae. Species of the three remaining families of red-blooded notothenioids, namely Bathydraconidae, Artedidraconidae and Harpagiferidae, have only occasionally been investigated (Everson & Ralph 1968; Hureau et al. 1977; di Prisco 1988; di Prisco et al. 1988; di Prisco & D'Avino 1989).

In summary our present knowledge about the blood physiology of Antarctic fishes is based on only few species. Trends detected in mainly sub-Antarctic species have been generalized. Previous studies on blood usually focussed on only one of the subjects haematology, haemoglobin function or haemoglobin structure. Few authors have attempted to correlate either of these subjects with ecological parameters such as mode of life or activity pattern. This may be due to two major problems: we know very little about the ecology of most Antarctic fish species and those species of which blood physiological data are published do not cover a large range of different ecotypes.

When we want to gain insight into the principles which govern the development of adaptations in the oxygen transport system of Antarctic fishes to their environment we need to combine haematology with structural and functional properties of haemoglobin. And we need data from species with distinct differences in their mode of life. For instance, the blood physiology of *P. antarcticum*, the most abundant and the only fully pelagic fish species of high-Antarctic shelf systems (Hubold 1985), is completely unknown. Particularly in high-Antarctic seas we find a high diversity of *Trematomus* and *Pagothenia* species which represent various ecotypes (Schwarzbach 1988, Ekau 1988). From most of them we have no blood data. The same applies to bathydraconids such as *Bathydraco marri, B. macrolepis* or *Vomeridens infuscipinnis* which were found to prefer deep and cold water (Ekau 1988).

HYPOTHESES

From the observations and investigations cited above the following hypotheses and questions can be formulated:
As outlined on page 6 and 7, blood parameters reflect mode of life and/or activity pattern in temperate and tropical fishes. The major hypothesis of the present study is that this also applies to Antarctic fishes. Are environmental factors such as oxygen content or temperature also reflected in their blood physiology?

From findings in comparatively few species other authors postulated that all Antarctic fishes follow an evolutionary trend and have reduced haemoglobin concentrations and erythrocyte counts. Can this be confirmed for a large number of hitherto uninvestigated species? Is this evolutionary trend in haematology stronger than influence of mode of life or activity pattern? On which of the various haematological components do we find greatest influence?

In the present study it is postulated that structure and function of haemoglobins also respond to the physico-chemical environment and to oxygen requirements of Antarctic fishes and therefore permit conclusions on their activity. Both, blood parameters and haemoglobins may indicate evolutionary trends and may reflect a possible tree of origin of the Antarctic fish fauna.

We have only little information about the ecology of a large number of species. In this study it is assumed that most of the investigated links between blood physiology and ecology of temperate and tropical fishes also apply to Antarctic fishes. With the results of blood physiological studies it is attempted to predict (to a certain degree) the activity level of those species of which no other information is available.

A detailed study of blood parameters of high-Antarctic fishes and molecular structure and oxygen-binding properties of their haemoglobins is outlined in this thesis. So far, it has not been tried to relate the ecology of a large range of different Antarctic fish species to their haematology in combination with structural and functional studies on their haemoglobins. For most of the species it is the first record of haematology and haemoglobin properties. Emphasis is placed on a high number of different ecotypes and mainly high-Antarctic species are chosen. Special attention is paid to the mode of life and the evolution of these teleosts in relation to their habitat, in an attempt to find links with haematological characteristics.

Materials and Methods

COLLECTION OF FISHES

Fishes were caught by Agassiz Trawl, Bottom Trawl and Benthopelagic Trawl during PRV 'Polarstern' expedition 'EPOS III' (Jan - Mar 1988) in the eastern and southeastern Weddell Sea. Most of the fishes were captured at Kapp Norvegia, Vestkapp and Halley Bay (Fig. 2.1). A few specimens of sub-Antarctic species were collected around Elephant Island, in the vicinity of the Antarctic Peninsula. In the species list below they are marked with an asterisk. The exact positions, depths and duration of all hauls are given by Arntz et al. (1990).

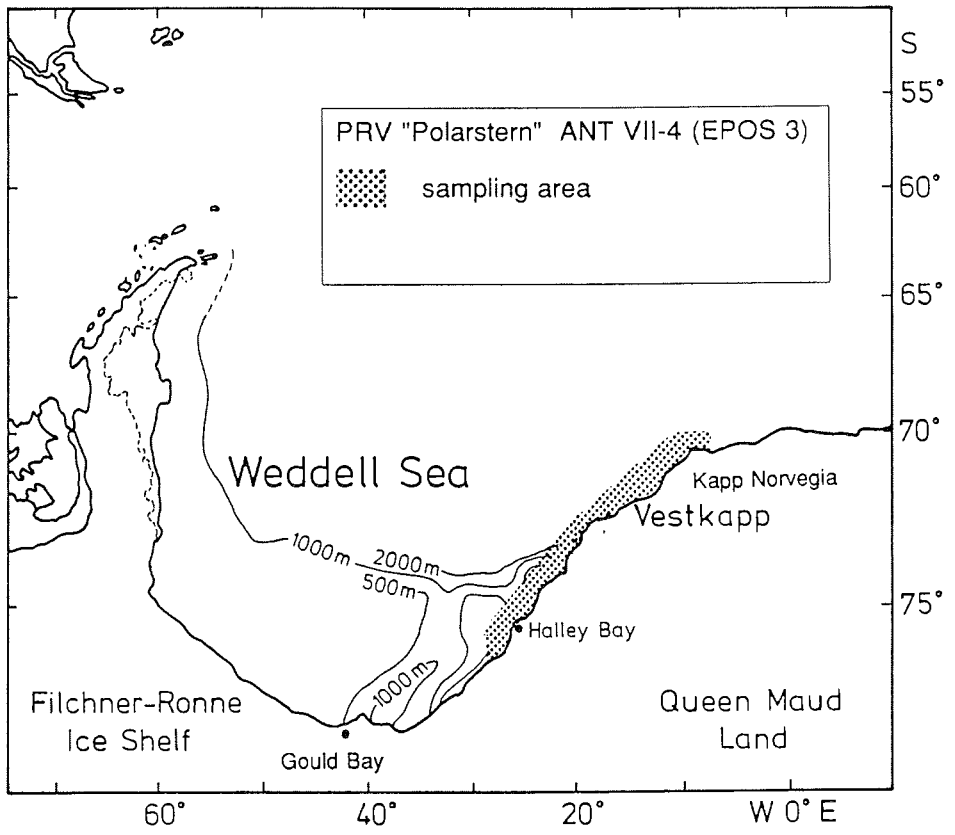


Fig. 2.1: Map of the Weddell Sea and the sampling area (from Hubold 1990, modified).

The following species were selected for blood investigations:

Fam. Nototheniidae: *Aethotaxis mitopteryx, Pleuragramma antarcticum, Dissostichus mawsoni, Trematomus centronotus, Trematomus eulepidotus, Trematomus lepidorhinus, Trematomus scotti, Pagothenia hansoni, Notothenia gibberifrons**

Fam. Bathydraconidae: *Bathydraco marri, Bathydraco macrolepis, Cygnodraco mawsoni, Gymnodraco acuticeps, Racovitzia glacialis, Gerlachea australis*

Fam. Artedidraconidae: *Pogonophryne species 1-3*

Fam. Channichthyidae: *Neopagetopsis ionah, Chionodraco myersi, Cryodraco antarcticus, Dacodraco hunteri, Pagetopsis macropterus, Pagetopsis maculatus, Chionobathyscus dewitti, Chionodraco rastrospinosus**, *Chaenocephalus aceratus**

Fam. Anotopteridae: *Anotopterus pharao*

Fam. Macrouridae: *Macrourus holotrachys*

Immediately after catch, the fishes were transferred to aquaria, where they were allowed to rest for at least 12 hours, in most cases 24 hours and more. Specimens of all species were kept alive onboard 'Polarstern' for several weeks, except for *Aethotaxis mitopteryx, Pleuragramma antarcticum, Anotopterus pharao* and *Macrourus holotrachys*. *A. mitopteryx* could only be maintained for up to three days and *P. antarcticum* never survived more than 48 hours. The two non-endemic species *A. pharao* and *M. holotrachys* died immediately after the catch. Some individuals of several species (Fig. 2.2 to Fig. 2.5) were brought back alive to Germany for subsequent research and are being maintained in aquaria for more than two years now. The number, size and weight range of investigated specimens is summarized in Table 2.1.

ANALYSIS OF BLOOD SAMPLES

Individual blood samples were drawn from the caudal vein of unanaesthetized specimens by means of heparinized syringes. The procedure was usually completed within 20 seconds from the first handling of a specimen. The general blood parameters were investigated immediately on board 'Polarstern' (Rankin et al. 1990) and comprised: pH, PO_2 and PCO_2 (partial pressure of oxygen and carbon dioxide), RBC (number of red blood cells), Hct (haematocrit), Hb (haemoglobin concentration), MCH (mean cellular haemoglobin) and MCHC (mean

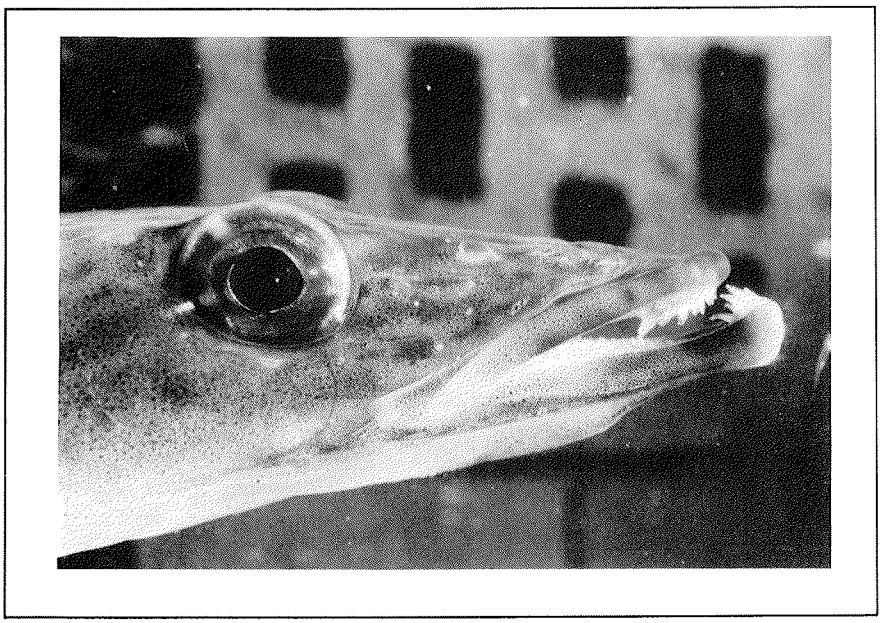


Fig. 2.2: *Gymnodraco acuticeps*, Bathydraconidae. Specimen of 29 cm length maintained at the Alfred-Wegener-Institut since March 1988.

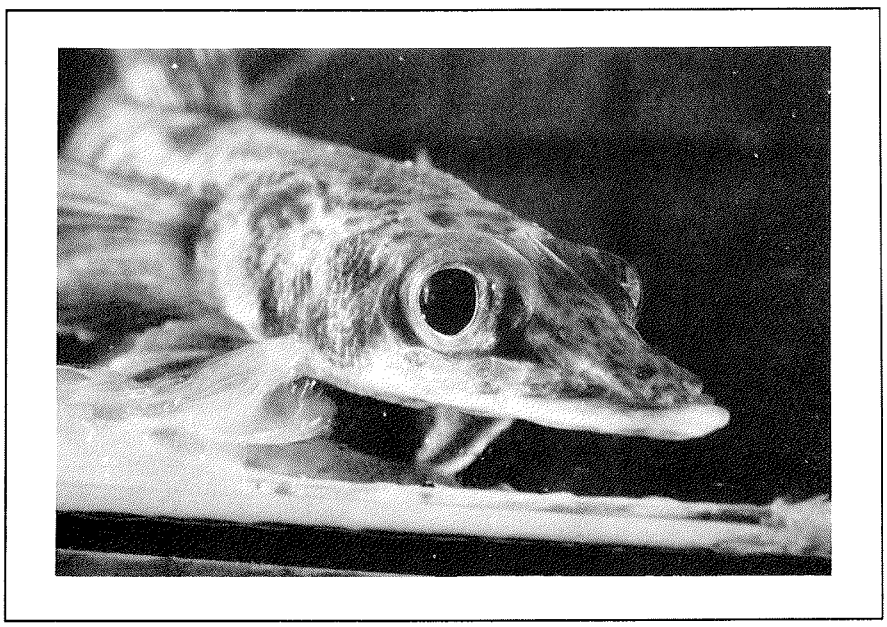


Fig. 2.3: *Bathydraco marri*, Bathydraconidae. Specimen of 21 cm length.

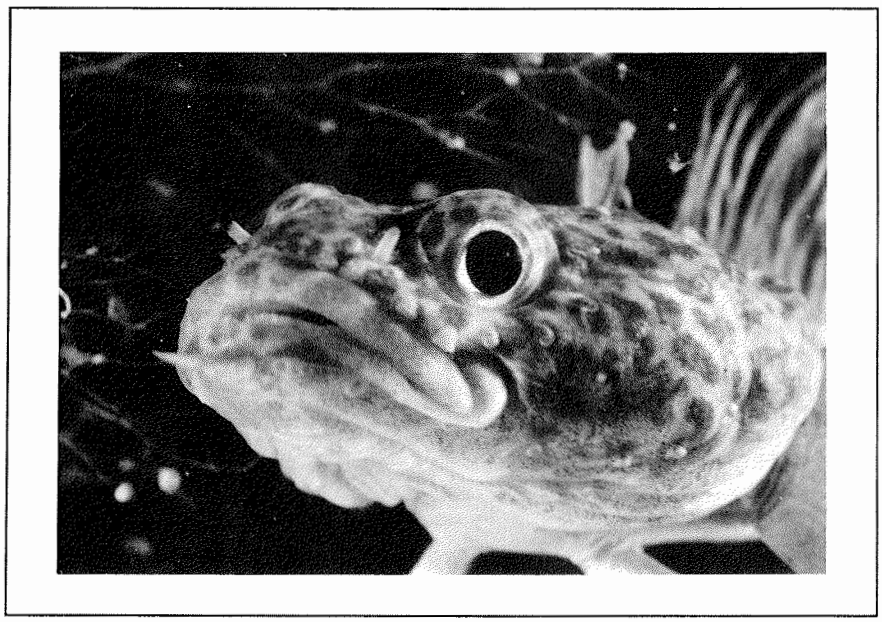


Fig. 2.4: *Pogonophryne sp.2*, Artedidraconidae. One of ten specimens maintained at the Institut für Polarökologie and the Alfred-Wegener-Institut since March 1988.

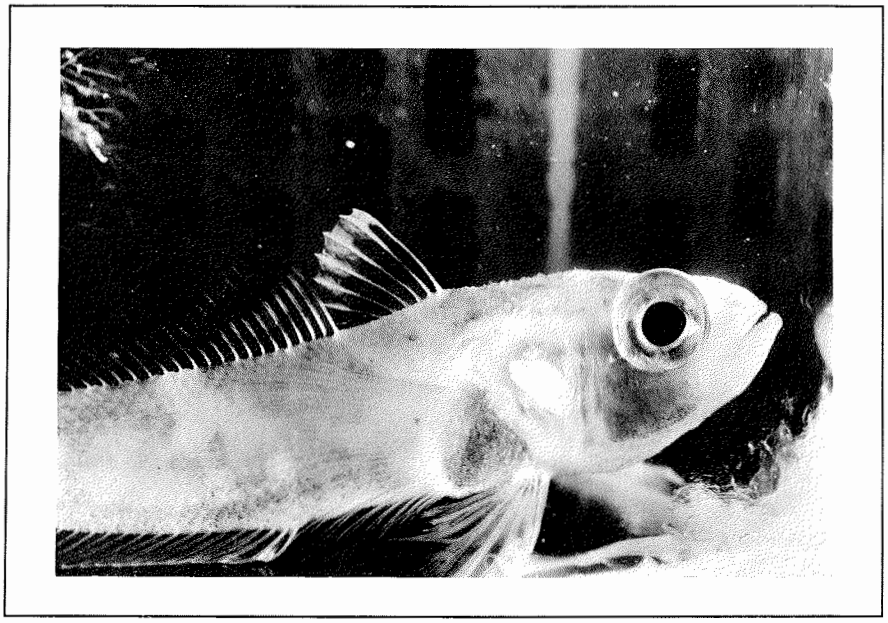


Fig. 2.5: *Trematomus eulepidotus*, Nototheniidae. Specimen of 20 cm length.

Table 2.1: Size and weight ranges of investigated specimens. N = specimen number, TL = total length, W = weight, max = maximum, min = minimum, / = not recorded.

Species	N	TL max [cm]	TL min [cm]	W max [g]	W min [g]
Nototheniidae	113				
Aethotaxis mitopteryx	11	42	32	310	213
Pleuragramma antarcticum	20	25.5	18	/	/
Dissostichus mawsoni	9	75	58	2250	5100
Trematomus centronotus	1	13	13	/	/
Trematomus eulepidotus	14	31	17	485	162
Trematomus lepidorhinus	27	33	19.5	158	72
Trematomus scotti	4	17	16.5	42	40
Pagothenia hansoni	7	22	39	780	400
Notothenia gibberifrons	20	37	24	/	/
Bathydraconidae	46				
Bathydraco marri	10	23	18		
Bathydraco macrolepis	1	28	28	/	/
Cygnodraco mawsoni	8	40	34.5	/	/
Gymnodraco acuticeps	7	29.5	28.5	/	/
Racovitzia glacialis	11	25	23	61	59
Gerlachea australis	9	26	18.5	80	70
Artedidraconidae	14				
Pogonophryne sp.1	2	27.5	21	/	/
Pogonophryne sp.2	10	21.5	18.5	/	/
Pogonophryne sp.3	2	22	21.5	/	/
Channichthyidae	65				
Neopagetopsis ionah	12	44	26		
Chionodraco myersi	22	38	22	350	146
Cryodraco antarcticus	6	59	37.5	1256	900
Dacodraco hunteri	5	28	23.5	120	100
Pagetopsis macropterus	1	21.5	21.5	/	/
Pagetopsis maculatus	9	19	16.5	42	40
Chionobathyscus dewitti	3	33	22.5	200	125
Chionodraco rastrospinosus	4	36	36	350	325
Chaenocephalus aceratus	3	62	32	1700	450
Anotopteridae	1				
Anotopterus pharao	1	89	89	/	/
Macrouridae	3				
Macrourus holotrachys	3	40.5	39.5	370	265

corpuscular haemoglobin concentration). For very small specimens of *P. antarcticum* and *B. marri* it was necessary to pool samples in order to have enough volume for all measurements.

Blood gases (PO_2 and PCO_2) and pH were measured at 0°C with a modified 'Eschweiler' blood-gas-analyzer System 2000, type 2031-02 ECO (Fig. 2.6). The samples were injected immediately after the collection. A great advantage of this system, which was developed for the clinical use on humans, is the small sample volume necessary (Fig. 2.7). The analyzer works successfully with a minimal volume of 40 µl (!). Due to the low temperature in the cooling container (-1°C), the recording cycles and the automatic calibration had to be adjusted. Each sample was measured in three consecutive cycles, results were averaged.

The number of red blood cells was counted with a 'Sysmex' CC-108 cell counter. For every species the adjustment for the cell size plateau was done individually. This was checked by counting the cells in a 'Thoma' chamber under a microscope (Romeis 1968; Hallmann 1980). For dilution the 'Hayem's' fluid was used. Each sample was counted threefold, results were averaged.

Haematocrit was estimated with 75 mm microcapillaries run at 12000 rpm in a 'Heraeus' microhaematocrit centrifuge according to DIN 58933 (Coburn & Fischer 1973; Hallmann 1980). Each sample was divided into three capillaries, results were averaged.

For the determination of the **haemoglobin concentration** the internationally standardized cyan-met-haemoglobin method was used (Coburn & Fischer 1973; Hallmann 1980). Triple measurements at 540 nm were conducted with each blood sample.

From the values for RBC, Hct and Hb the **mean cellular haemoglobin (MCH) and the mean corpuscular haemoglobin concentration (MCHC)** was calculated according to MCH = Hb/RBC and MCHC = Hb*100/Hct (Coburn & Fischer 1973; Hallmann 1980).

The mean size of erythrocytes of every species was computed with a 'Leitz' Microvid system using a 'Leitz' microscope and a 'Toshiba' laptop computer for relaying size bars and grids. Initial tests revealed that there is no size difference between fresh and preserved cells. At least 100 cells of every specimen were sized (Fig. 2.8 - Fig. 2.11). For comparison with data from other authors always the longest section was chosen. This is of minor importance for 'normal' cells (Fig. 2.10), but of great importance for cells shaped like in *R. glacialis* (Fig. 2.11). The results were averaged and are presented as mean erythrocyte length (MEL).

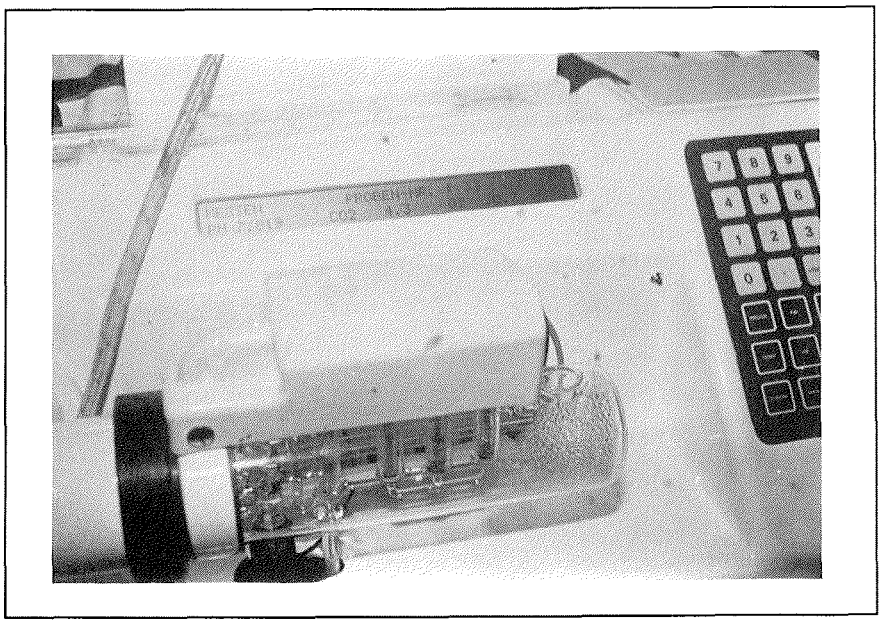


Fig. 2.6: 'Eschweiler' blood-gas-analyzer operated at -1°C in a cooling-container.

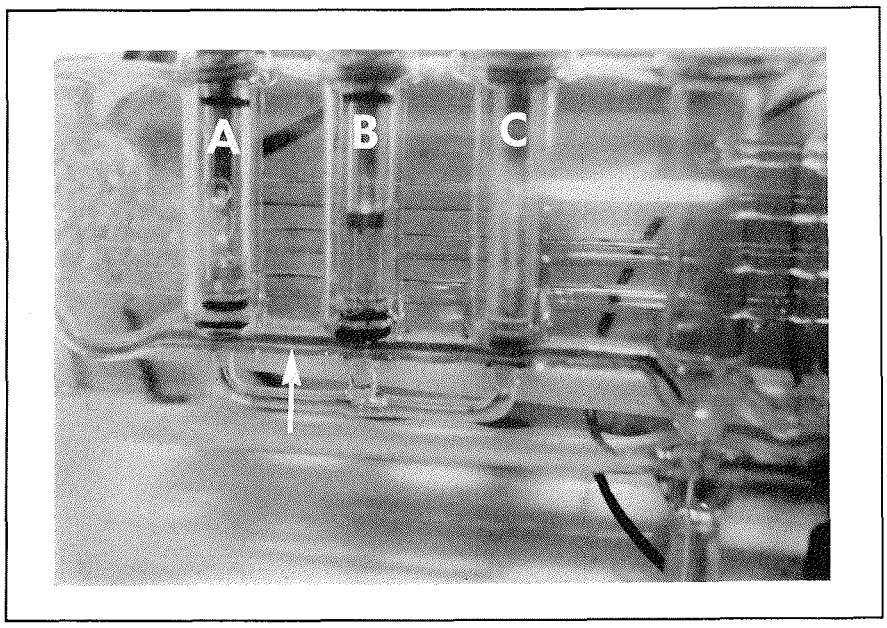


Fig. 2.7: Sample chamber of 'Eschweiler' blood-gas-analyzer with three electrodes (A,B,C). The volume of the visible blood sample is 50 µl (-->).

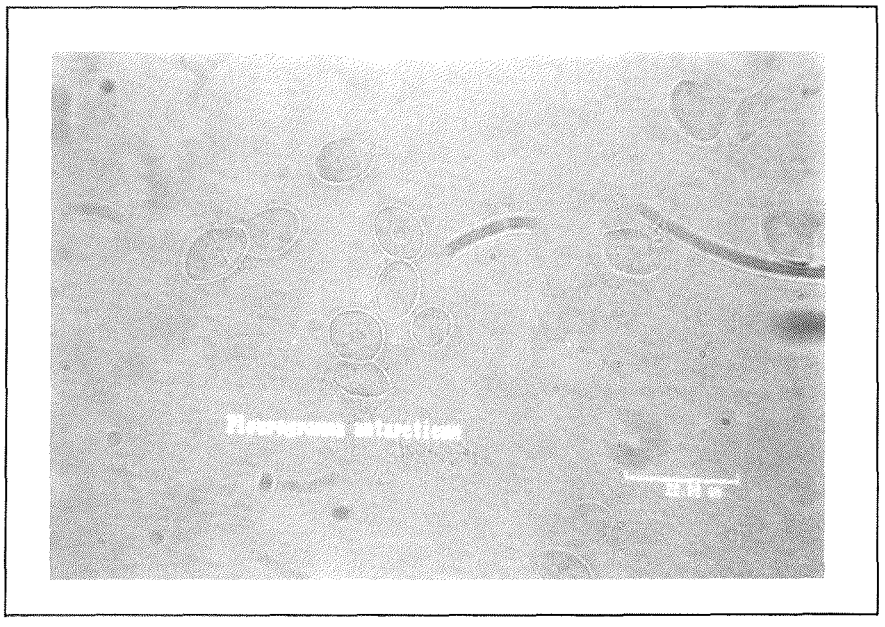


Fig. 2.8: *Pleuragramma antarcticum*, Nototheniidae. Red blood cells preserved with Hayem's fluid. Average diameter is 11.5 µm.

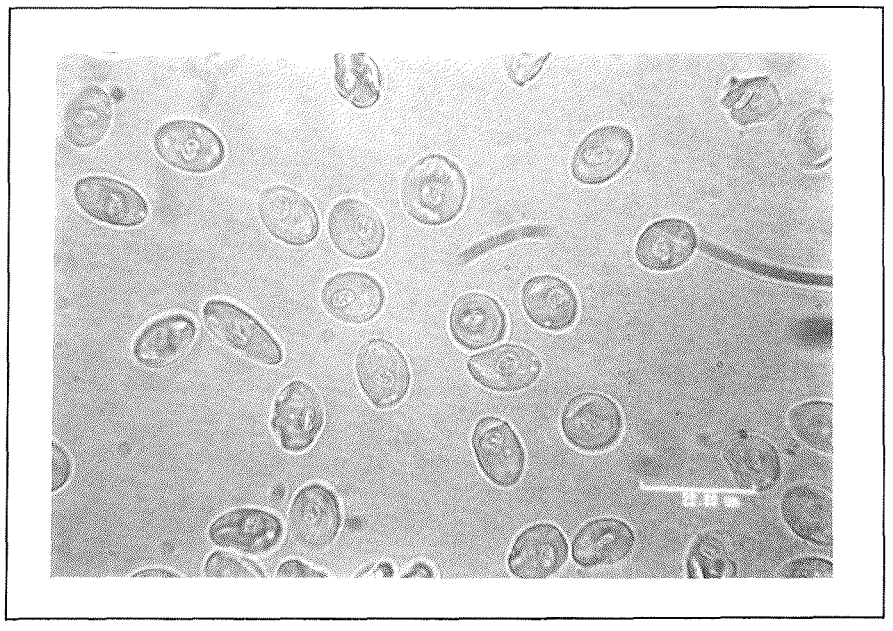


Fig. 2.9: *Aethotaxis mitopteryx*, Nototheniidae. Erythrocytes of fresh blood smear. Average diameter is 12.9 µm.

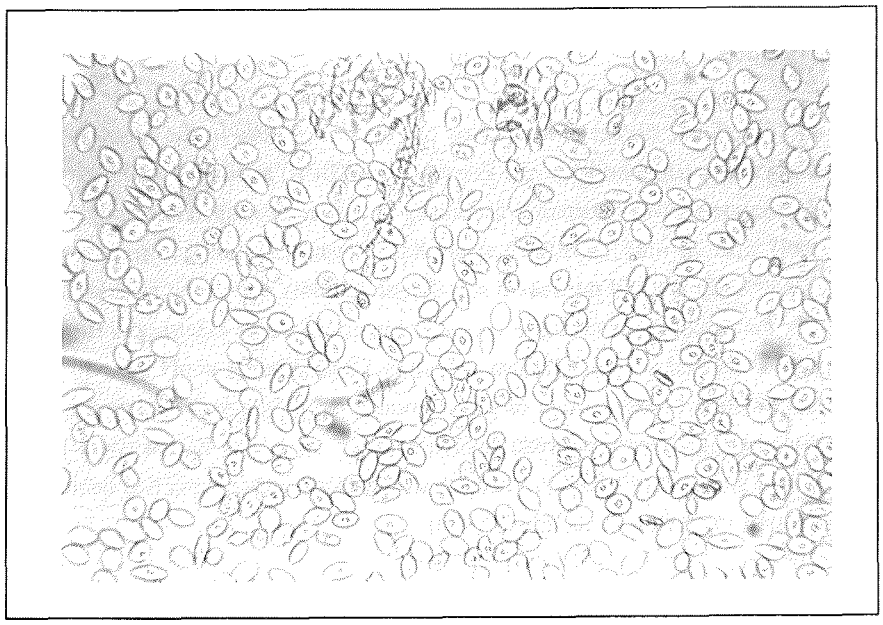


Fig. 2.10: *Bathydraco marri*, Bathydraconidae. Red blood cells at low magnification for overview. Average diameter is 12.6 µm.

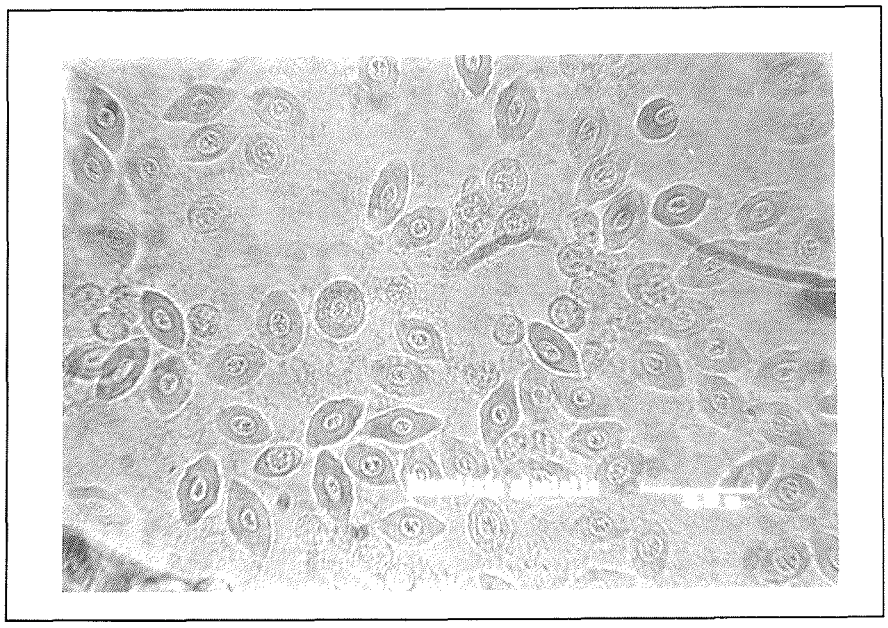


Fig. 2.11: *Racovitzia glacialis*, Bathydraconidae. Note the different shape of the red blood cells in comparison to the preceding micrographs.

The remaining blood was centrifuged and cells and plasma were frozen separately at -80°C. Some cells were preserved in 'Hayem's' solution for subsequent studies under the light microscope (Romeis 1968).

Structural and functional studies were carried out on frozen cells in the laboratory 'BP2' at the Institute of Protein Biochemistry and Enzymology (IBPE) at Naples in the group of Prof. di Prisco. Haemolysates were prepared according to earlier described methods (D'Avino & di Prisco 1988) with 20 mM TRIS/HCl at pH 8.0. Met-haemoglobin forming was checked spectrophotometrically and did not reveal significant amounts due to the freezing and storing procedure. For the detection of multiple haemoglobin components electrophoretic analysis of haemolysates was carried out on cellulose acetate in TRIS/Glycine at pH 9.0 with a chamber from 'Gelman Sciences'. Assessment of globin molecular weight was achieved by means of SDS-polyacrylamide gel electrophoresis, which was run on a 'Bio Rad' system PROTEAN II together with a standard of known molecular weight. Both procedures had been previously described in detail (D'Avino & di Prisco 1988).

STRUCTURAL STUDIES

Investigations on the primary structure (amino acid composition and sequence) of haemoglobins are very time-consuming and expensive and require a complete team to work on. Therefore these studies were carried out from the above-mentioned team 'BP2' in Naples. The studies are still in process and until now the haemoglobins of the two species *Bathydraco marri* and *Aethotaxis mitopteryx* could be sequenced completely.

The procedures of preparation, purification and proteolytic cleavage of globin chains, purification of tryptic peptides, amino acid analysis and sequencing were used according to earlier described methods (D'Avino & di Prisco 1988; D'Avino et al. 1989). Modifications were applied when necessary and the complete process applied in the case of *Bathydraco marri* haemoglobin is about to be published in detail (Caruso et al. in prep.). The following paragraph is a brief summarization:

The globin chain mixture was prepared by the acetone-acid method (Rossi Fanelli et al. 1958) and subsequently chromatographed on a high performance liquid chromatographer (HPLC) for separation and purification. The single globin chains were then digested with trypsin. The resulting peptide mixtures were dried by lyophilization. Protein cleavage at defined amino acids (Asp-Pro) was performed as previously described (Schininà et al. 1988). Tryptic peptides were purified by reversed-phase HPLC.

The amino acid analysis was accomplished on an 'Applied Biosystems' model 420A derivatizer-analyzer system. The sequencing was performed on a pulsed-liquid phase sequencer model 477A from 'Applied Biosystems' following the technique of automated repetitive Edman degradation.

FUNCTIONAL STUDIES

In the oxygen-binding studies some haemolysates were 'stripped', i.e. endogenous organic phosphates, which may act as effectors, were eliminated by treating the haemolysate with a mixed-bed ion-exchange resin. However, a comparison with 'unstripped' haemolysates revealed that, probably due to the long storage in the freezer, endogenous organic phosphates were no longer detectable; thus 'stripping' of 'old' haemolysates appeared unnecessary. In the study of Root and Bohr effects, oxygen saturation and P_{50} (the oxygen partial pressure required to achieve half-saturation) were measured spectrophotometrically at 20°C as described by Giardina & Amiconi (1981) and di Prisco et al. (1988). Oxygen equilibria were measured between pH 8.5 and 6.0 in steps of 0.5. Due to a shortage in material for some species the stepsize was increased to 1.0 in tests on influence of effectors. In order to investigate the influence of organic phosphates and Cl^--ions on the binding properties, NaCl, ATP and/or inositol hexaphosphate (IHP) were used throughout all experiments in saturating concentrations. Several initial tests revealed no difference in effect between IHP and ATP. Haemolysates were treated as follows:

For the Root effect study, the spectrum of oxygenated Hb was recorded between 600 and 500 nm in the absence and presence of organic phosphates. After treatment with sodium dithionite, the spectrum of the deoxygenated Hb was recorded again. The absorbance values at 540, 560 and 575 nm were used to calculate the degree of O_2-saturation at varying pH values (Giardina & Amiconi 1981). At these wavelengths maximal absorbance variation occurs in the oxy/deoxy transition.

For the Bohr effect analysis, Hb was deoxygenized in a special tonometer under constant rotation by means of a vacuum pump. The spectrum of fully deoxygenated Hb was recorded between 500 and 600 nm. Subsequently an exactly defined amount of oxygen (air) was added and the altered spectrum was recorded again. This procedure was repeated in steps until full saturation of the Hb was reached again. The oxygen saturation was then calculated as described above and used for the Hill plot (Riggs 1988). The Hill plot yields the oxygen partial pressure at 50% saturation (log P_{50}) and the Hill coefficient (n½), respectively: i.e. the x-axis intercept and slope of the derived straight lines. One example of all Hill plots generat-

ed is presented in Fig. 2.12 for *Bathydraco marri*. From the information of this plot the oxygen equilibrium curve (Fig. 3.6 in the Results section) was obtained.

When a second haemoglobin (Hb2) was detected in relatively large amounts, as was the case for *P. antarcticum* (see Results), the two components Hb1 and Hb2 had to be separated in order to study Root and Bohr effects individually. This was achieved by ion-exchange chromatography on a column of DE 52, equilibrated with 50 mM TRIS/HCl at pH 7.6. Hb2 was eluted with 100 mM TRIS. Due to the formation of met-haemoglobin the two components had to be reduced and reoxygenated. Unfortunately, these procedures decreased the stability of both haemoglobins at low pH. Due to met-Hb formation, the acidic values of 6.0 and 6.5 had to be omitted in both Root and Bohr effect studies. The inevitable loss of material during the separation and the limited amount of available samples only allows to consider preliminary results of the functional studies on *P. antarcticum* haemoglobins. The limited amount of samples is the reason for not presenting results on the Bohr effect of haemoglobins for some species of Table 2.1 (*T. scotti, B. macrolepis, G. australis, Pogonophryne sp.1 and 3, M. holotrachys, A. pharao*). For *P. hansoni, N. gibberifrons, G. acuticeps* and *C. mawsoni* results of functional studies have already been published (Wells & Jokumsen 1982; di Prisco et al. 1990).

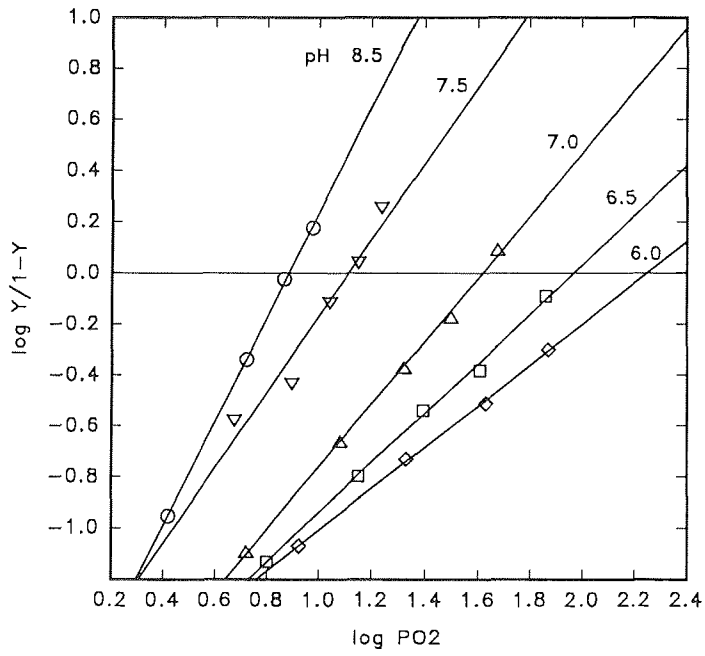


Fig. 2.12: Hill plot for *Bathydraco marri* haemoglobin (pH 6.0-8.5, without effectors). Y = fractional saturation. The log P_{50} and the Hill coefficient are given through x-axis intercept and slope of the regression lines

Results

BLOOD PARAMETERS

General observations

The red blood cells of some species (e.g. *Pleuragramma antarcticum, Racovitzia glacialis* and particularly of all examined *Pogonophryne* species) seem to be more fragile than others. Although the same treatment was used in each withdrawal of blood, a high level of haemolysis reduced the number of available samples for the above mentioned species. Moreover, the blood of *Pogonophryne* species was of a remarkable, lightly red colour and became 'slimy' independent of the heparin concentration used to prevent coagulation. The haemolized and 'slimy' samples were discarded. The blood of some *Pogonophryne* specimens and a few *Gerlachea australis* had a clearly visible component (2-3 Vol %; maybe a lipoprotein) after centrifugation in microcapillaries (see 'FAT' in Fig. 3.1).

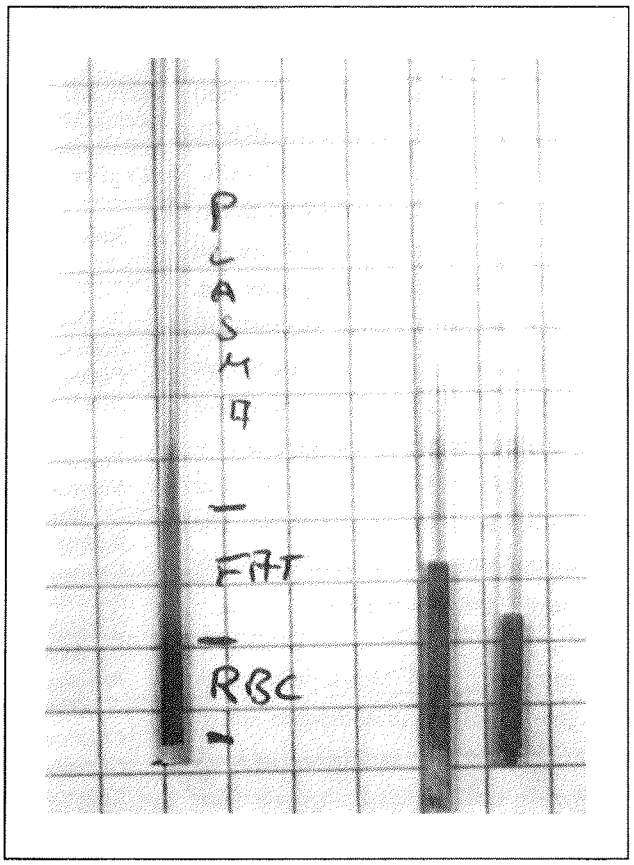


Fig. 3.1: Haematocrit capillaries with samples from *T. scotti* and *G. australis* (left). Note the fatty component on top of the packed cells (left) in comparison to the clear supernatant (right).

Red blood cell dimensions

When erythrocytes were measured and counted visually, the red blood cells of all investigated species but one were found to have the same shape. The regular ellipsoid to discoid contours are shown in Fig. 2.8-2.10. Only the cells of *Racovitzia glacialis* seem to be

stretched in longitudinal direction (Fig. 2.11). This is reflected in the mean erythrocyte length (MEL) in Table 3.1, where *R. glacialis* has the largest cells of all bathydraconids (13.5 µm). MEL ranges from 6.1 µm in *Trematomus scotti* to 13.6 µm in *Trematomus eulepidotus*. This also demonstrates the large variation even within the same family. MEL in channichthyids (7.2 µm) is clearly lower as compared to nototheniids (11.6 µm) and bathydraconids (11.9 µm).

Haematocrit and red blood cell counts

The two parameters haematocrit (Hct) and number of red blood cells (RBC), which express the contribution of cellular components to the total blood tissue, are summarized in Table 3.2 for all investigated high-Antarctic species (i.e. excluding channichthyids). Haematocrit varies between 12.4% in *Pagothenia hansoni* and 29.5% in *Pogonophryne sp.3*. The two non-endemic species *Macrourus holotrachys* and *Anotopterus pharao* are well within this range. The data do not seem to be related to activity level or taxonomic category.

Table 3.1: Red blood cell size of Weddell Sea fish species. MEL = mean erythrocyte length, SD = standard deviation, n = 100, mean values for families are also given, / = not applicable here.

Species	MEL [µm]	SD
Nototheniidae	11.6	
Aethotaxis mitopteryx	12.9	0.8
Pleuragramma antarcticum	11.5	1.0
Dissostichus mawsoni	10.0	1.3
Trematomus eulepidotus	13.6	1.8
Trematomus scotti	6.1	1.0
Notothenia gibberifrons	10.0	0.5
Bathydraconidae	11.9	
Bathydraco marri	12.6	1.4
Gymnodraco acuticeps	11.6	1.7
Racovitzia glacialis	13.5	0.8
Gerlachea australis	9.9	1.0
Artedidraconidae	/	
Pogonophryne sp.2	12.6	1.3
Channichthyidae	7.2	
Chionodraco myersi	8.0	1.2
Cryodraco antarcticus	6.5	0.4
Chionodraco rastrospinosus	7.3	1.2

The number of red blood cells varies between $0.39*10^{12}$/l in *Aethotaxis mitopteryx* and $0.99*10^{12}$/l in *Macrourus holotrachys*. In the families Bathydraconidae and Artedidraconidae the range of values is more narrow and their average number of erythrocytes is significantly lower ($0.51*10^{12}$/l and $0.66*10^{12}$/l, respectively) as compared to nototheniids ($0.72*10^{12}$/l).

Haemoglobin concentration and derived parameters

The values for the haemoglobin concentration (Hb) and all derived parameters, such as mean corpuscular haemoglobin concentration (MCHC) and mean cellular haemoglobin (MCH), are compiled in Table 3.2. From these values and the knowledge of oxygen solubility in plasma (0.8 vol %; Grigg 1967), the total oxygen carrying capacity of blood (O_2CC) was calculated. In addition the theoretical contribution of plasma to the total oxygen transport (PC) is presented in this table.

A comparison of all blood parameters with values known from temperate and tropical species is presented in the discussion.

Haemoglobin concentration varies between 20.1 g/l for *Gymnodraco acuticeps* and 47.1 g/l for *Dissostichus mawsoni*. Again we find highest variability within the family Nototheniidae (28-47 g/l) and again the average values for the families are highest in notothenilds (42 g/l) and lowest in bathydraconids (27 g/l). Other authors report a range of 23-43 g/l for Antarctic fishes (Wells et al. 1980, 1990).

Table 3.2: Blood parameters of red-blooded Antarctic fishes. Haematocrit (Hct), red blood cell count (RBC), haemoglobin content (Hb), mean corpuscular haemoglobin concentration (MCHC), mean cellular haemoglobin (MCH), blood oxygen carrying capacity (O_2CC) and plasma contribution to total oxygen transport (PC) for investigated species of five families. The number of individual specimens for a particular species is at least 10, except for species marked with *. For each of these samples triple measurements were conducted. For *T. centronotus, B. macropterus* and *A. pharao* only one sample was available. / = not recorded, SD = standard deviation.

Species	Hct [%] ±SD	RBC [10^{12}/l] ±SD	Hb [g/l] ±SD	MCHC [g/l]	MCH [pg]	O_2CC [%]	PC [%]
Nototheniidae							
A. mitopteryx	14.0±3.1	0.39±0.09	27.8±4.7	198.6	71.3	4.52	16.7
P. antarcticum	16.6±9.8	0.43±0.17	26.5±8.1	159.6	61.6	4.32	15.5
D. mawsoni	25.0±5.6	0.88±0.09	47.1±8.3	188.4	53.3	7.10	8.5
*T. centronotus**	20.0	0.44	/	/	/	/	/
T. eulepidotus	24.8±9.9	0.80±0.19	46.5±9.7	187.5	58.2	7.00	8.6
T. lepidorhinus	16.3±5.2	0.76±0.17	42.0±10.1	257.6	55.3	6.50	10.0
*T. scotti**	22.0±5.8	0.94±0.07	43.6±3.7	198.2	46.4	6.60	9.4
P. hansoni	12.4±5.3	0.47±0.19	27.9±12.3	135.4	59.4	4.55	15.3
Bathydraconidae							
B. marri	14.6±2.9	0.59±0.12	29.6±8.6	202.7	50.2	4.76	14.3
*B. macrolepis**	20.0	0.55	30.0	150.0	54.4	4.78	13.4
C. mawsoni	24.9±4.3	0.69±0.07	26.4±5.0	106.0	38.3	4.24	14.0
G. acuticeps	14.5±5.5	0.53±0.16	20.1±7.0	138.6	37.9	3.45	19.7
R. glacialis	14.0±4.6	0.51±0.14	30.0±9.2	214.3	58.8	4.83	14.3
G. australis	17.1±7.0	0.38±0.09	28.0±7.1	163.7	73.7	4.52	14.6
Artedidraconidae							
*Pogonophryne sp.1**	16.3±4.5	0.64±0.27	25.3±4.2	155.2	39.5	4.16	16.1
Pogonophryne sp.2	19.3±7.4	0.65±0.23	33.2±10.2	172.0	51.1	5.22	12.3
*Pogonophryne sp.3**	29.5±2.0	0.73±0.01	33.8±13.8	114.5	46.3	5.22	10.7
Anotopteridae							
A. pharao	22.5	0.59	32.0	142.2	54.2	5.17	14.7
Macrouridae							
M. holotrachys	18.0±1.0	0.99±0.05	39.0±10.0	216.7	39.4	6.14	12.3

The so-called 'erythrocyte indices' MCHC and MCH relate erythrocytes and haemoglobin to the efficiency of red cells for oxygen transport (Coburn & Fischer 1973). The variation of MCHC and MCH between species is considerably less than the variation in Hct, RBC and Hb (Table 3.2). A species with moderate Hb concentration values and rather low haematocrit,

such as *Aethotaxis mitopteryx,* can reach a MCHC (198 g/l) close to that of *D. mawsoni* (188 g/l). This is due to a rather high haematocrit of *D. mawsoni,* which also has much higher Hb concentration values. This means that in the same volume of red blood cells of both species we find approximately the same amount of haemoglobin. Provided the size of erythrocytes of both species does not differ considerably, this is reflected in the MCH. The average erythrocyte of *A. mitopteryx* carries more Hb (71.3 pg) than the average erythrocyte of *D. mawsoni* (53.3 pg).

Although the more active species such as *D. mawsoni, Trematomus eulepidotus* or *G. acuticeps* have low MCH values, there is no clear relation of this parameter to the activity level of species. A few inactive species also have low MCH values.

When ecological significance is considered, then the important factor is the total oxygen carrying capacity (O_2CC) of the blood and not so much the haematocrit, erythrocyte number or haemoglobin concentration *per se.* In Table 3.2 we find high values of O_2CC for active species such as *D. mawsoni* (7.2%) and *T. eulepidotus* (7.0%). As a consequence the contribution of plasma to the total oxygen transport (PC) in these two species is low (≈8.5%). The PC can reach values of up to 16-19%, which means more than 1/6 of the oxygen transport relies on the plasma alone. Published values for the O_2CC in Antarctic fishes are in the range 4.5-6.5% (Macdonald et al. 1988) and from Arctic teleosts a range of 3.4-8.4% is reported (Scholander & van Dam 1957). This subject together with a comparison with haemoglobinless species and species from lower latitudes is resumed in the discussion.

The results of the counts of 'red-blood-cell-like' cells in the channichhtyids are summarized in Table 3.3. The number of cells is one to two orders of magnitude lower than for red-blooded Antarctic species and ranges from $0.56*10^{10}$/l in *Neopagetopsis ionah* to $3.99*10^{10}$/l in *Chaenocephalus aceratus.* The few values available from published sources are well in line with the values presented here (Hureau et al. 1977; Wells et al. 1990). According to these data *N. ionah* has the lowest number of cells ever observed in a fish species.

Table 3.3: Count of erythrocyte like cells ('RBC') of channichthyids. For *P. macropterus* only one sample was available. SD = standard deviation.

Species	'RBC' [10^{10}/l] ±SD
Channichthyidae	
N. ionah	0.56±0.19
C. myersi	2.33±1.57
C. antarcticus	3.26±0.97
D. hunteri	1.20±0.37
P. macropterus	1.10
P. maculatus	1.74±0.94
C. dewitti	3.10±0.10
C. rastrospinosus	3.58±1.25
C. aceratus	3.99±1.52

PH and blood gases

Table 3.4 summarizes the results of measurements with the blood-gas-analyzer, i.e. pH, PCO_2 and PO_2 (partial pressures of O_2 and CO_2) of venous blood. These values together are a good indicator of the actual oxygen conditions in the fish prior to sampling and to a certain extent reflect stress conditions. Usually the blood PCO_2 immediately after capture was rather high (> 10 mm Hg). Specimens of several taxa were able to eliminate their initially high PCO_2 nearly completely after several days in the aquaria. Some of them lowered PCO_2 amazingly fast within a few hours (*Racovitzia glacialis, Aethotaxis mitopteryx, Gymnodraco acuticeps*).

Table 3.4: Oxygen conditions in blood of Antarctic fishes. pH, PO_2 and PCO_2 (partial pressures of O_2 and CO_2) measured with a blood-gas-analyzer. The number of individual specimens for a particular species is at least 10, except for species marked with *. For each of these samples triple measurements were conducted. For *T. centronotus* only one sample was available. From species marked with x mainly samples from stressed specimens had to be used. SD = standard deviation.

Species	pH±SD [1]	PO_2±SD [mm Hg]	PCO_2±SD [mm Hg]
Nototheniidae			
A. mitopteryx	7.85±0.18	43.5±14.6	1.9±1.4
P. antarcticum	7.66±0.07	29.6± 8.9	3.0±1.6
D. mawsoni	7.74±0.40	30.7±13.1	3.5±3.1
*T. centronotus**	7.54	43.6	2.7
T. eulepidotus	7.77±0.13	42.7± 7.7	1.8±0.9
T. lepidorhinus	7.76±0.23	46.3±18.9	1.8±1.8
*T. scotti**	7.65±0.11	24.5± 8.2	3.1±1.9
P. hansoni	7.64±0.33	29.2±13.8	4.8±2.6
N. gibberifrons	7.69±0.19	23.2± 9.9	4.6±2.1
Bathydraconidae			
B. marri	7.73±0.26	58.5±19.8	3.8±1.9
*B. macrolepis*x*	7.52±0.02	10.9± 1.4	6.4±0.7
C. mawsoni	7.82±0.16	65.4±29.9	4.3±2.1
G. acuticeps	7.82±0.19	54.4±16.7	1.3±1.2
R. glacialis	7.68±0.15	51.1±14.3	2.0±1.8
G. australis	7.47±0.11	35.3± 7.8	4.0±2.1
Artedidraconidae			
Pogonophryne sp.2	7.99±0.05	40.4±20.2	2.6±1.5
*Pogonophryne sp.3**	8.14±0.04	77.9±31.0	1.7±1.3
Channichthyidae			
N. ionah	7.56±0.42	73.7±23.9	2.6±1.9
C. myersi	7.48±0.22	61.6±16.0	2.4±1.9
C. antarcticus	7.62±0.20	59.0±24.7	3.8±2.0
D. hunteri	7.61±0.10	58.7± 8.2	0.9±0.3
*P. macropterus*x*	7.24±0.17	61.8±10.8	6.3±2.0
*C. rastrospinosus**	7.45±0.31	75.9±17.4	5.5±1.7

Values for PCO_2 were usually measured in the range 1-4 mm Hg. Unfortunately, of some species (marked with x, Tab. 3.4) mainly samples from stressed specimens had to be used. Therefore, the mean of these samples is high (up to 6.4 mm Hg). PO_2 usually varied between 25 and 50 mm Hg. Obviously channichthyids are able to maintain a higher PO_2 than members of other families. Their average PO_2 of 68 mm Hg is significantly higher ($P<0.02$) than PO_2 for nototheniids (36.3 mm Hg) or bathydraconids (53.6 mm Hg).

The measured pH varies only little and is usually in the range of 7.6-7.8, except for artedidraconids, where the pH is slightly higher at 8.0-8.1. For other Antarctic species a pH of 7.3-8.3 was found (Qvist et al. 1977).

HAEMOGLOBIN STRUCTURE

Haemoglobin multiplicity

Electrophoretic analysis of haemolysates of notothenioids shows a general pattern, indicating the presence of a single haemoglobin. When two components are found, one of these components accounts for 90-95% of the total (di Prisco 1988; D'Avino & di Prisco 1988, 1989; di Prisco et al. 1990). Fig. 3.2 presents the results of cellulose acetate electrophoresis (CAE). The number of components is clearly visible. Most investigated species have only one component. *T. eulepidotus, T. lepidorhinus, T. scotti* and *P. hansoni* have a second component in minor amounts. *P. antarcticum* is the only species, where the second component is present in higher amounts (20-25%).

The two non-endemic species *M. holotrachys* and *A. pharao* have a higher multiplicity (three and four components, respectively), as most non-Antarctic fishes have. SDS-polyacrylamide gel electrophoresis of the globin mixture from these haemoglobins reveals that the polypeptide chains (α and ß) of the different components have slightly different molecular weights, close to 16000 (Fig. 3.3). The two haemoglobins of *P. antarcticum* have three polypeptide chains only, because the ß chain is in common.

It should be kept in mind that one band on the gel can contain more than one globin chain, because of very similar molecular weights. *Anotopterus pharao* for example (no. 13 in Fig. 3.3), has three bands, although with CAE electrophoresis (Fig. 3.2) four components were found. This means that either globin chains are in common or one band refers to more than one globin chain.
Table 3.8 at the end of the Results section and table 4.4 in the Discussion section show a comparison of number of haemoglobin components with all data available so far.

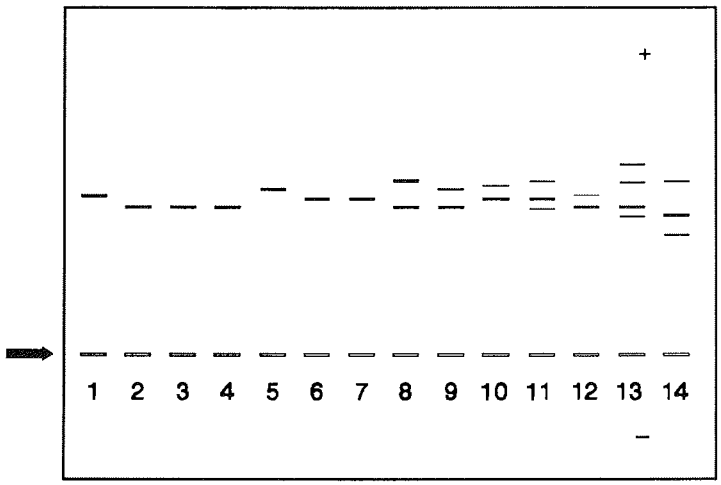


Fig. 3.2: Sketch of cellulose acetate electrophoresis of Antarctic fish haemolysates. The arrow indicates origin, + and - refer to polarity.
1 *B. marri*; 2 *B. macrolepis*; 3 *R. glacialis*; 4 *G. australis*; 5 *Pogonophryne sp.2*; 6 *D. mawsoni*; 7 *A. mitopteryx*; 8 *P. antarcticum*; 9 *T. lepidorhinus*; 10 *T. eulepidotus*; 11 *T. scotti*; 12 *P. hansoni*; 13 *A. pharao*; 14 *M. holotrachys*

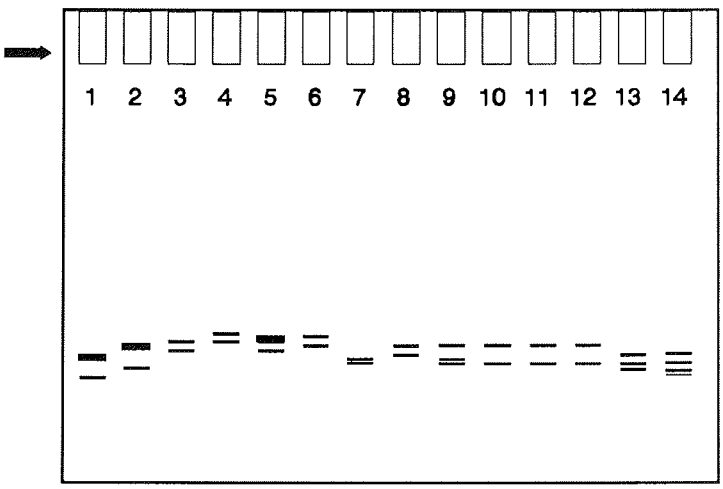


Fig. 3.3: Sketch of SDS-polyacrylamide gel electrophoresis of globin components of Antarctic fishes. The arrow indicates origin. The molecular weights of the α and ß chain of Hb1 of *Notothenia coriiceps neglecta* were used as standards.
1 *N. coriiceps neglecta* Hb1; 2 *B. marri*; 3 *B. macrolepis*; 4 *G. australis*; 5 *R. glacialis*; 6 *Pogonophryne sp.2*; 7 *D. mawsoni*; 8 *A. mitopteryx*; 9 *P. antarcticum*; 10 *T. lepidorhinus*; 11 *T. eulepidotus*; 12 *P. hansoni*; 13 *A. pharao*; 14 *M. holotrachys*

Amino acid sequence of the globin chains

The primary structure of the haemoglobins investigated here has so far been elucidated for the two species *Bathydraco marri* and *Aethotaxis mitopteryx* and is submitted for publication (Kunzmann et al.; Caruso et al.) or in preparation (Kunzmann et al.; D'Avino et al.).

Table 3.5: Number of individual amino acids in α and ß chains of *Aethotaxis mitopteryx* and *Bathydraco marri* haemoglobin.

Amino acid	*A. mitopteryx* α chain	*A. mitopteryx* ß chain	*B. marri* α chain	*B. marri* ß chain
Ala	13	16	15	17
Arg	6	4	3	4
Asn	4	7	3	8
Asp	11	10	11	11
Cys	1	2	1	2
Gln	2	4	2	4
Glu	4	7	3	4
Gly	8	10	6	10
His	6	6	6	8
Ile	11	10	11	12
Leu	13	13	14	15
Lys	13	11	14	9
Met	3	3	3	2
Phe	5	8	6	7
Pro	7	3	6	3
Ser	13	4	16	8
Thr	4	5	6	6
Trp	2	2	2	2
Tyr	4	6	3	4
Val	12	13	11	10
Σ	142	146	142	146

The amino acid sequences of α and ß chains of *B. marri* haemoglobin are shown in Fig. 3.4. The total number of amino acids (AA) in the α and ß chain are 142 and 146, respectively. AA occurring most frequently in the α chain are Serine, Alanine, Leucine and Lysine with approximately 10% each (Table 3.5). Cysteine occurs only once. In the ß chain most frequent amino acids (AA) are Alanine, Leucine and Isoleucine. Here Cysteine occurs twice. The molecular weights, calculated from the sequence (15552 and 16048, respectively) are in good agreement with those determined by SDS-polyacrylamide gel electrophoresis (Fig. 3.3).

The AA sequences of α and ß chains of *A. mitopteryx* are shown in Fig. 3.5. The increase in number of acidic (Asp/Glu) and basic (Arg/Lys) AA in both chains is evident in comparison to *B. marri* (Table 3.5). The molecular weights, calculated from the sequence, are 15702 and 16291. Note the different end-terminal AA 'Val' in the ß chain of *A. mitopteryx* in comparison to 'His' in *B. marri*. Two other AA known so far to be responsible for the Bohr and Root effect (i.e. 'Ser' and 'Glu' in position 93 and 94 of the ß chain) are present in both species.

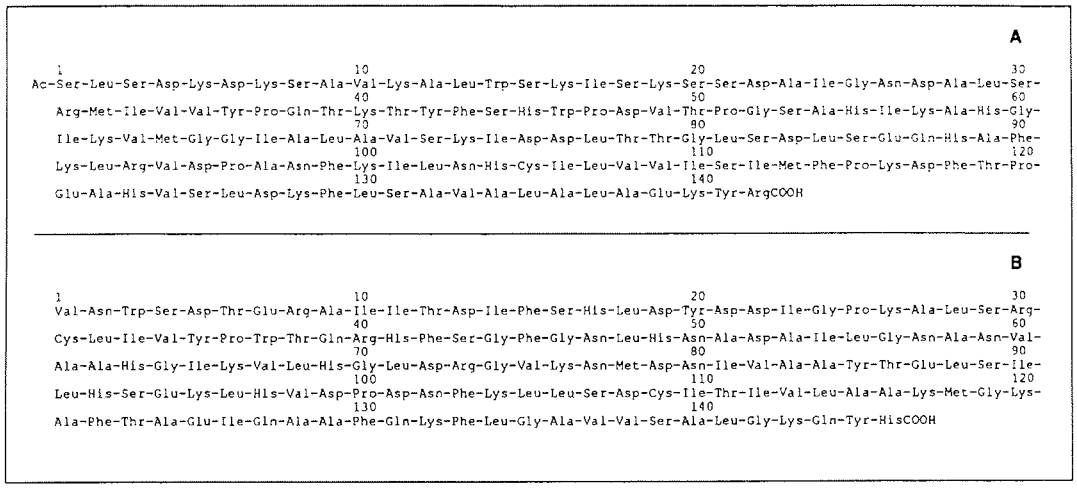


Fig. 3.4: Amino acid sequence of the α (panel A) and ß (panel B) chain of *B. marri* haemoglobin.

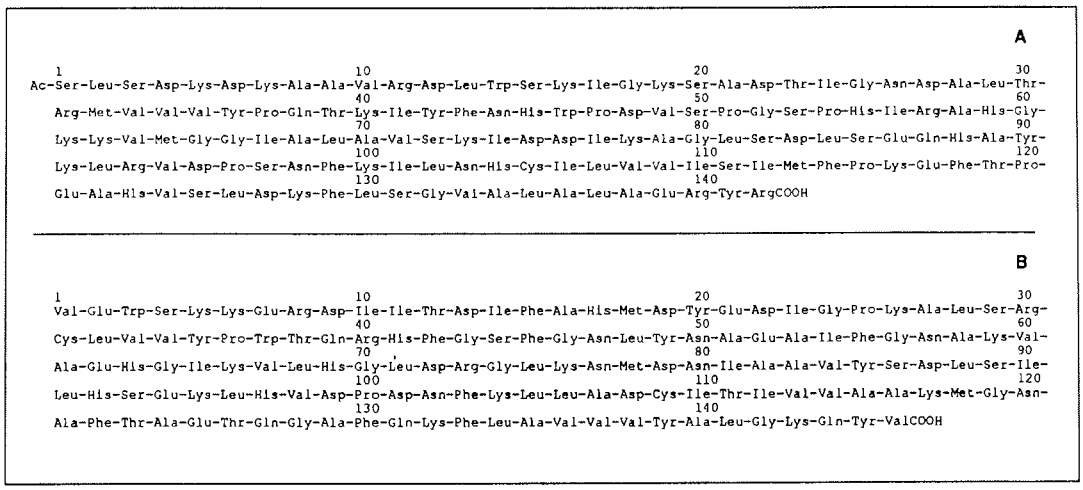


Fig. 3.5: Amino acid sequence of the α (panel A) and ß (panel B) chain of *A. mitopteryx* haemoglobin.

Table 3.6 shows the degree of sequence identity among the α and ß chains of *B. marri* and *A. mitopteryx* haemoglobin and some of the few available sequences of fishes living under totally different environmental conditions (carp, *Cyprinus carpio*; trout, *Salmo irideus*; bluefin tuna, *Thunnus thynnus*), as well as the sequence of the Antarctic teleost *Notothenia coriiceps neglecta* (D'Avino et al. 1989). Apparent is the fact that sequence identities of haemoglobins (only Hb1) are always higher among Antarctic fishes, whether they have a Root effect or not (*A. mitopteryx* haemoglobin has no Root effect, see functional studies).

Table 3.6: Sequence identity (%) of the α and ß chain of fish haemoglobins from Antarctic* and non-Antarctic fishes (from Kunzmann et al. submitted and D'Avino pers. comm.)

SPECIES	*T. thynnus*	*C. carpio*	*S. irideus* HbIV	*B. marri**	*N. coriiceps neglecta* Hb2*	*N. coriiceps neglecta* Hb1*
α-chains						
A. mitopteryx	75	62	59	84	85	83
N. cor. negl. Hb1*	73	59	57	84	63	
N. cor. negl. Hb2*	68	63	63	71		
*B. marri**	79	65	63			
S. irideus HbIV	60	63				
C. carpio	66					
ß-chains						
A. mitopteryx	67	58	60	80	82	82
N. cor. negl. Hb1,Hb2*	66	57	63	90		
*B. marri**	66	57	61			
S. irideus	61	73				
C. carpio	60					

HAEMOGLOBIN FUNCTION

Oxygen-binding properties

The oxygen binding properties of haemoglobins were investigated in the pH range 6.0-8.5, the physiologically possible range. As presented in Table 3.4 in the blood gas paragraph (page 31) the normal *in vivo* pH is around 7.8. Experiments were performed in the presence and absence of saturating concentrations of organic phosphates and Cl^- ions. In most cases particularly the organic phosphates clearly increased both Root and Bohr effects. In a few cases the Cl^- ions accounted for up to 50% of the increase in effect. The results of the studies on Root and Bohr effects are displayed in individual plots. Fig. 3.6 to Fig. 3.13 show Bohr effects and Fig. 3.14 to Fig. 3.28 Root effects of all investigated species.

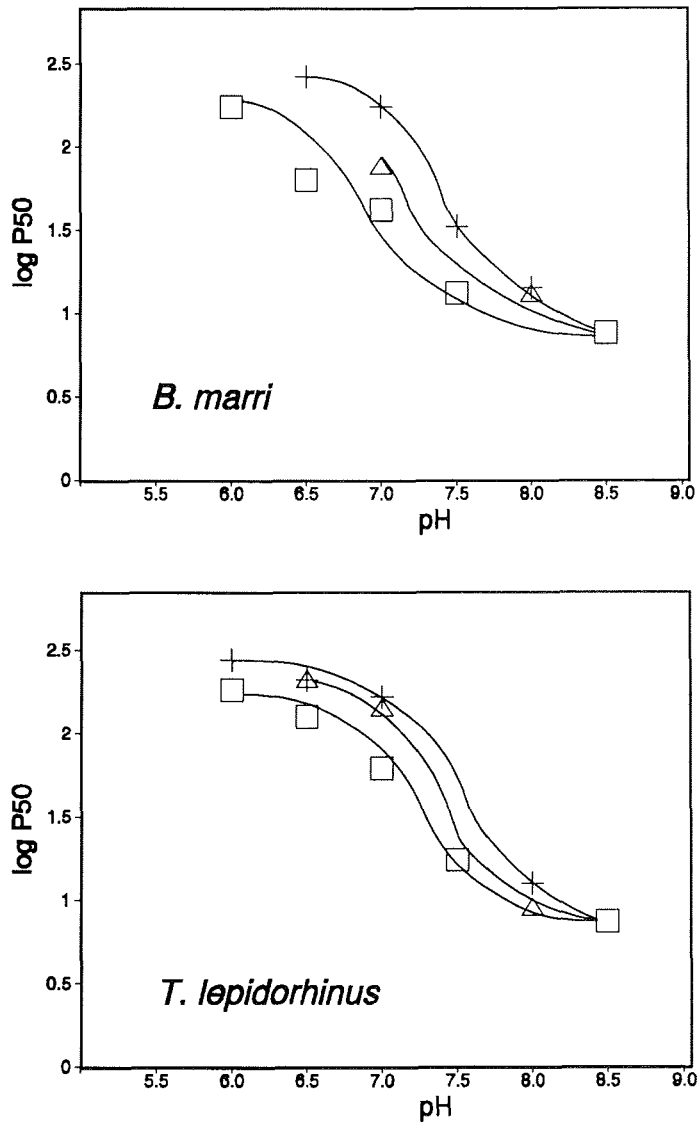
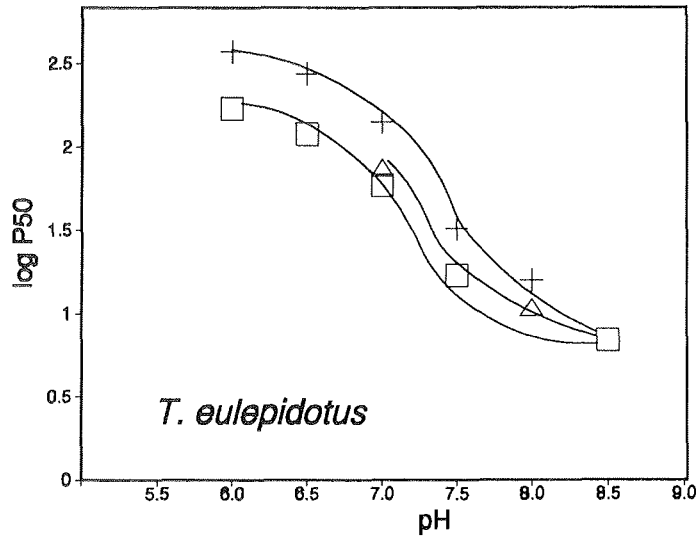


Fig. 3.6 and **Fig. 3.7**: Oxygen affinity of haemoglobin as a function of pH (Bohr effect). Haemolysate in the absence (□) or presence (△) of 0.1 M NaCl and (+) of 0.1 M NaCl, 3 mM inositol hexaphosphate (IHP).

As indicated in the Materials and Methods section, the Bohr effect studies could only be completed for some of the investigated species (Fig. 3.6 to Fig. 3.13). Particularly in the case of *Pleuragramma antarcticum*, there was not sufficient material available after separation of the two haemoglobins. The presented plot for Hb2 (Fig. 3.12) is therefore only preliminary, but nevertheless well-suited to indicate trends. The same applies to the graph of *Pogonophryne sp.2* (Fig. 3.13), where possible trends are shown in dotted lines.

As can be seen from the graphs, each haemoglobin responds very individually to changes in pH, oxygen saturation and concentration of effectors. Since it is the intention of this work to focus on ecological significance, I will not discuss each individual plot in great detail. Instead I selected two representative examples (*B. marri* and *A. mitopteryx*) in order to present those results which will be considered again in the Discussion and Conclusions.



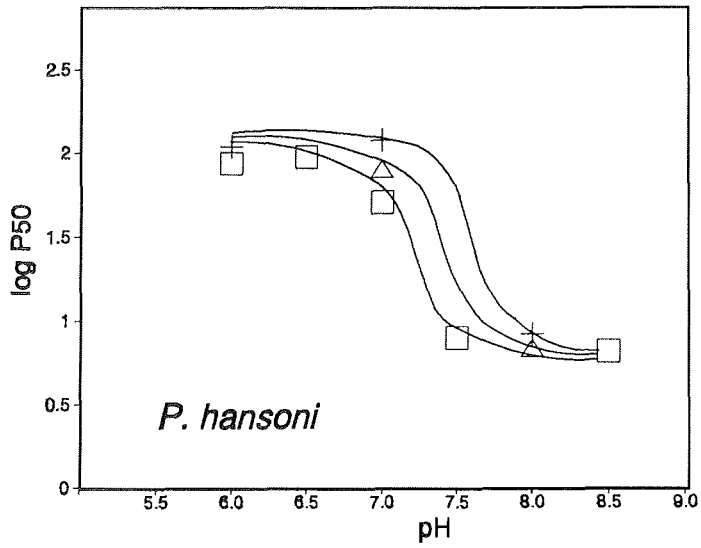


Fig. 3.8 and **Fig. 3.9**: Oxygen affinity of haemoglobin as a function of pH (Bohr effect). Haemolysate in the absence (□) or presence (△) of 0.1 M NaCl and (+) of 0.1 M NaCl, 3 mM inositol hexaphosphate (IHP).

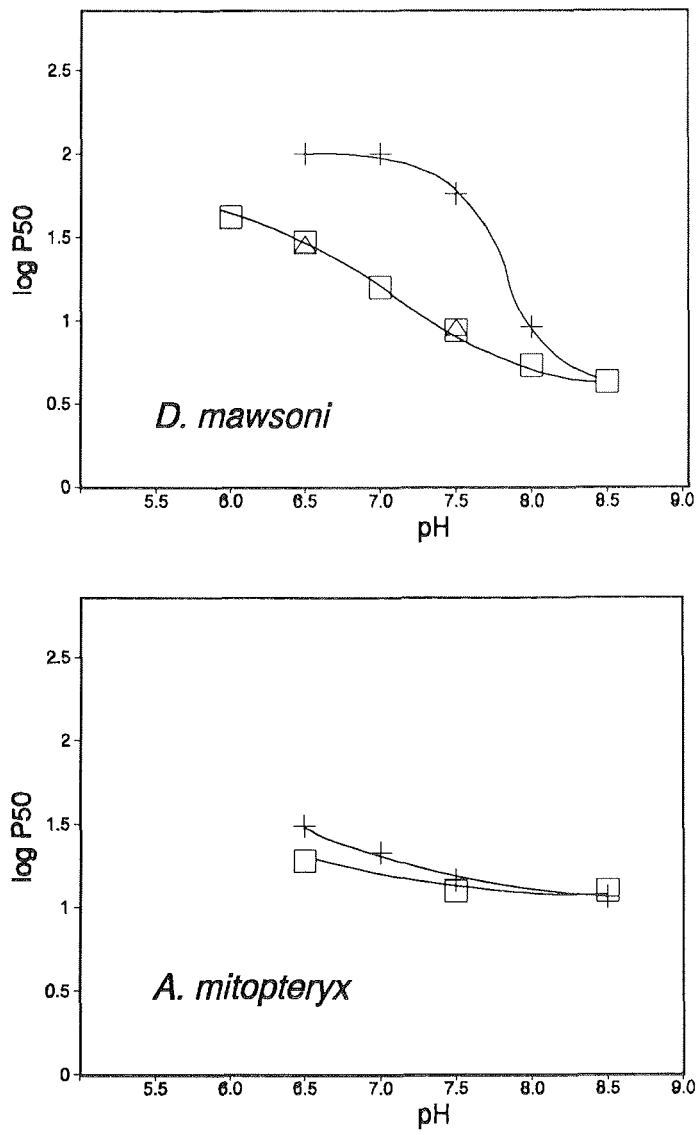
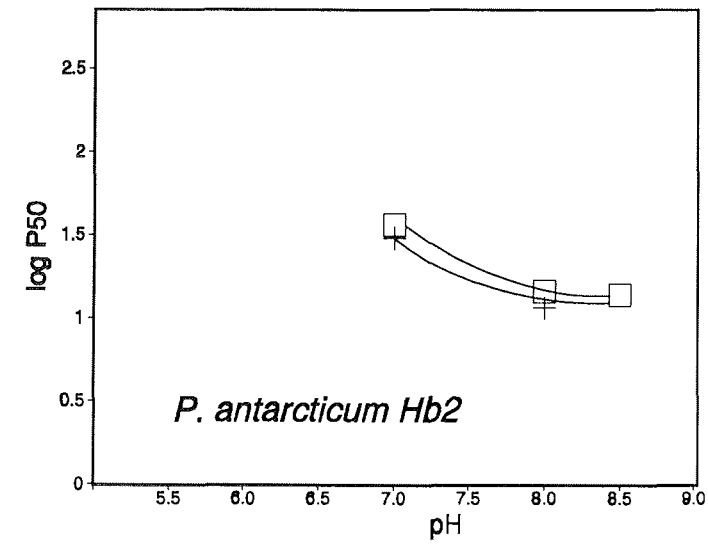


Fig. 3.10 and **Fig. 3.11**: Oxygen affinity of haemoglobin as a function of pH. Haemolysate in the absence (□) or presence (▵) of 0.1 M NaCl and (+) of 0.1 M NaCl, 3 mM IHP. Note a very weak Bohr effect in the lower plot.

Bohr effect studies

When measuring the effect of pH on the oxygen equilibrium curve of *B. marri* haemoglobin, a large, negative, alkaline Bohr effect was observed (Fig. 3.6). Both effectors (organic phosphates and Cl^- ions) clearly increased the extent of pH regulation. Between pH 7 and 8 at 20° C, the maximum Bohr coefficient Φ ($\Delta \log P_{50}/\Delta pH$) was -1.0. In the presence of Cl^-

and organic phosphates the coefficient increased considerably to -1.1 and -1.4, respectively. It is worth noting that the physiological pH is 7.7 (Table 3.4); the maximum Φ is also around this pH.



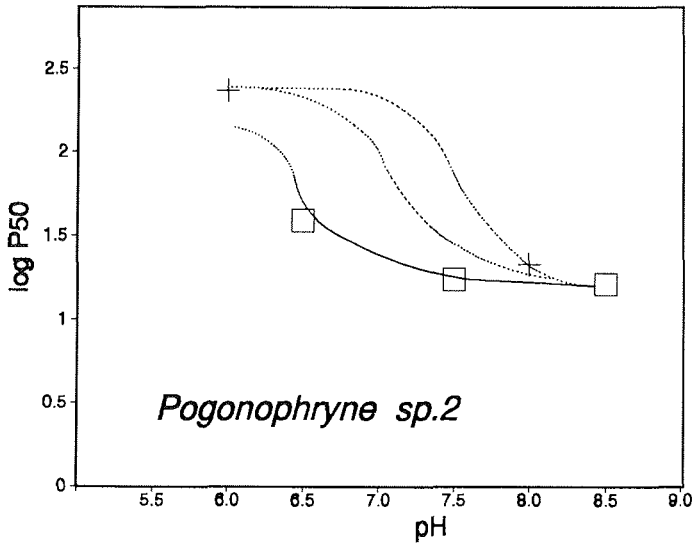


Fig. 3.12 and **Fig. 3.13**: Oxygen affinity of haemoglobin as a function of pH (Bohr effect). Haemolysate in the absence (□) or presence (△) of 0.1 M NaCl and (+) of 0.1 M NaCl, 3 mM IHP. The dotted lines are predictions.

The Hill coefficient (n½) decreased from 2 at pH 8.5 to 1 at pH 6.0, indicating disappearance of subunit cooperativity at low pH. The apparent oxygen affinity at pH 7.5 and 20°C was relatively low, with a P_{50} value of 13.2 mm Hg. In the presence of the effectors, the affinity decreased considerably to a P_{50} value of 33.1 mm Hg at pH 7.5 and 20°C. ΔH (i.e. apparent heat of oxygenation) values of -56 kJ/mol (Tetens et al. 1984) and -24 kJ/mol (Wells & Jokumsen 1982) have been reported for other Antarctic species; if we assume the ΔH of *B. marri* to fall in this range, it may be inferred that P_{50} at *in situ* temperature of -1.5°C, calculated from the van't Hoff equation, would be in the range of 2.1 - 6.4 mm Hg (Table 3.7).

For haemoglobins of *T. lepidorhinus, T. eulepidotus* and *P. hansoni* (Fig. 3.7 to Fig. 3.9) the situation is very similar. For Hb of *D. mawsoni* we find a distinct influence of organic phosphates already between pH 8.0 and 7.5 and hardly any influence of Cl^- (Fig. 3.10). For Hb of *Pogonophryne sp.2* the affinity can only be predicted and is indicated with dotted lines (Fig. 3.13).
For the narrow pH range which could be investigated in both haemoglobins of *P. antarcticum* (e.g. Hb2 in Fig. 3.12) it seems that effectors have no influence.

The only real exception from the above outlined general pattern is found in *A. mitopteryx*. When measuring the effect of pH on the oxygen equilibrium curve of this haemoglobin, only a weak Bohr effect can be detected (Fig. 3.11). Both effectors, organic phosphates and Cl^- ions, only slightly change the affinity. The Hill coefficient, indicating cooperativity, remains at values around 1.2 between pH values of 8.5 to 6.0, which means that cooperativity is very low. The apparent oxygen affinity at pH 7.5 and 20°C is moderate with a P_{50} value of 12.6 mm Hg (Tab. 3.6). With effectors the affinity decreases slightly to a P_{50} value of 14.4 mm Hg at pH 7.5 and 20°C. Corresponding values at *in situ* temperature of -1.5°C, assuming two different ΔH values (see above) are 2.05 mm Hg and 6.4 mm Hg, respectively.

The individual P_{50} values, Hill coefficient (n½) and Bohr coefficient ($\Phi = \Delta \log P_{50} / \Delta pH$) values for all investigated haemoglobins are summarized in Table 3.7. The highest affinity for oxygen (i.e. the lowest value for P_{50}) was found for *D. mawsoni* and *P. hansoni*.

Table 3.7: List of P_{50} values in [mm Hg], Bohr factors ($\Phi = \Delta\log P_{50}/\Delta pH$) and Hill coefficients ($n_{1/2}$) of haemoglobins. Upper row of values is obtained without effectors, the lower row is obtained under influence of 3 mM IHP and 0.1 M NaCl. The P_{50} values were originally obtained at 20°C and pH 7.8. For the Hill coefficient the change with decreasing pH is presented. The maximum Bohr factor usually occurred between pH 8.0 and 7.0.

[a] = transformation under the assumption ΔH = -21 kJ/mol (Grigg 1967; di Prisco et al. 1988).

[b] = transformation under the assumption ΔH = -56 kJ/mol (Tetens et al. 1984), see text.

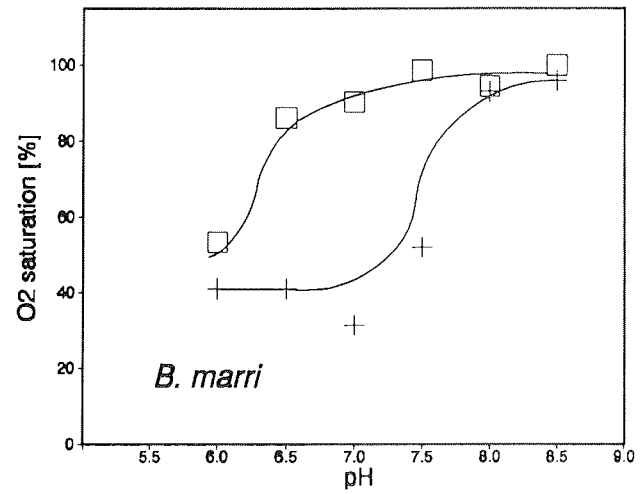
Species	P_{50} (20°C)	P_{50} [a] (-1.5°C)	P_{50} [b] (-1.5°C)	$n_{1/2}$	Φ
Aethotaxis mitopteryx	12.6	6.4	2.05	1.0-1.4	-0.18
	14.4	7.3	2.35	1.4-1.7	-0.28
Pleuragramma antarcticum	19.9	10.1	3.24	2.1-1.5	-0.20
	25.0	12.7	4.08	1.5	-0.74
Dissostichus mawsoni	6.3	3.2	1.03	2.4-1.9	-0.53
	57.0	28.8	9.30	1.9-1.2	-1.04
Pagothenia hansoni	7.9	4.0	1.29	1.9-1.0	-1.08
	31.6	16.0	5.15	1.9-0.7	-1.16
Trematomus eulepidotus	12.6	6.4	2.05	1.4-1.2	-0.85
	32.4	16.4	5.30	2.0-0.9	-0.93
Trematomus lepidorhinus	10.0	5.1	1.63	1.5-0.7	-0.86
	30.2	15.3	4.90	1.7-0.9	-0.84
Bathydraco marri	12.6	6.4	2.05	2.1-0.8	-0.68
	33.1	16.7	5.40	1.7-0.8	-1.14
Pogonophryne sp.2	17.4	8.8	2.84	1.5-0.9	-0.35
	21.4	10.8	3.48	1.6-0.9	-0.99

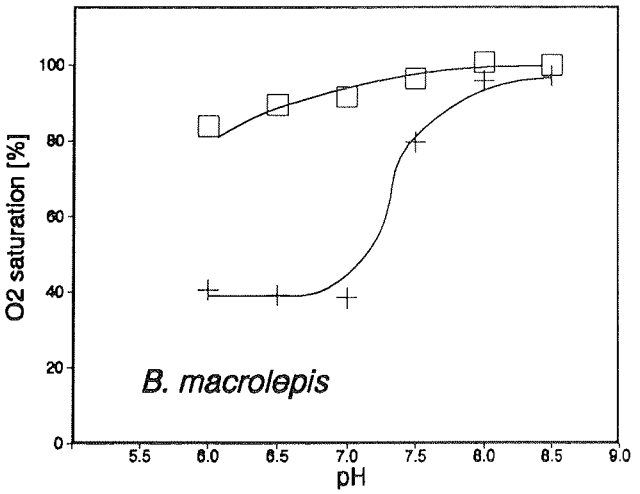
Root effect studies

In *B. marri* haemoglobin the effect of pH on the oxygen saturation indicated the presence of a strong Root effect, i.e. a large decrease of the O_2-affinity and cooperativity at low pH values (Fig. 3.14). A dramatic fall to 50% was observed between pH 8 and pH 7; the minimum (approx. 40 - 45%) was reached at pH 7.5 in the presence of organic phosphates and at pH 6.0 in their absence. The inflection point occurred around the physiological pH of 7.7.

A similarly strong Root effect can be observed in the closely related species *Bathydraco macrolepis* and *Racovitzia glacialis* and in the nototheniid species *Dissostichus mawsoni* (Fig. 3.15 to Fig. 3.19).

All other species (except one) displayed a less strong, but still clearly expressed, response to pH changes in their haemoglobins.





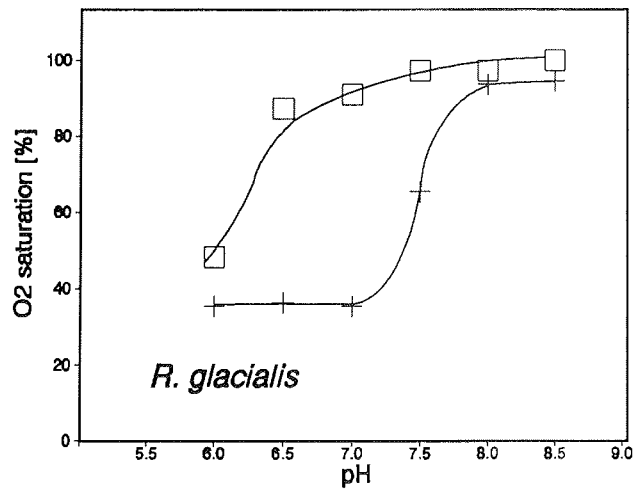
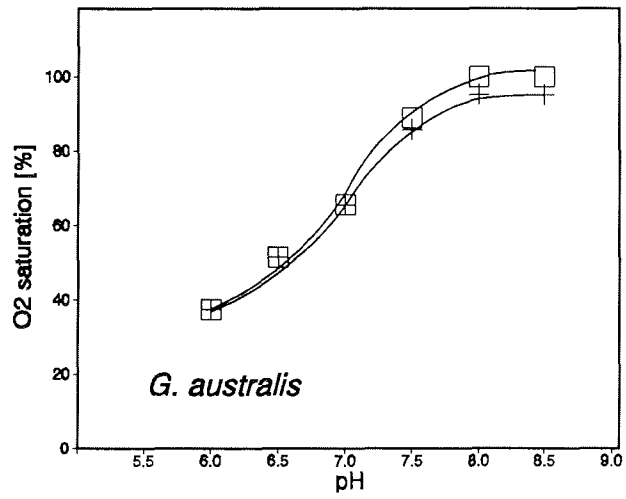


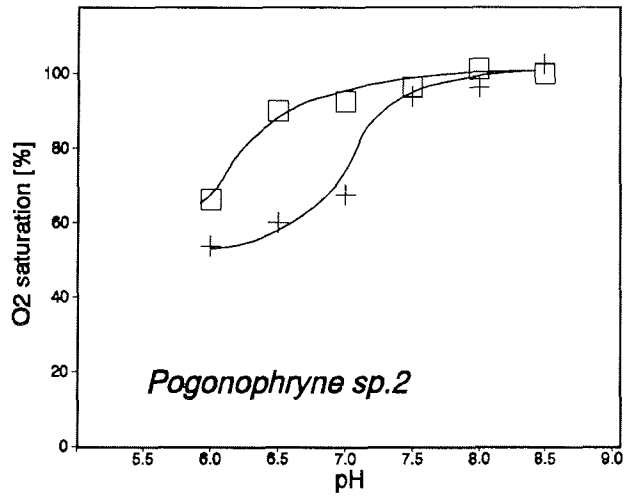
Fig. 3.14 to **Fig. 3.16**: Oxygen saturation of haemoglobin as a function of pH (Root effect). Haemolysate in the absence (□) or presence (+) of 3 mM IHP.

Again we find the exception in the single haemoglobin of *A. mitopteryx*. When the influence of pH on the oxygen saturation of *A. mitopteryx* haemoglobin is recorded, no Root effect is present; i.e. no change of the O_2-affinity and cooperativity at all pH values investigated (Fig. 3.26). The O_2-saturation remains at 100% over a pH range of 8.5-6.5.

There is a slight decrease down to 95/90% at pH 6, probably due to an instability of the haemoglobin molecule. Organic phosphates have no effects.

Most of the studied haemoglobins display a decrease in oxygenation at pH 6.0 down to 50/60% without influence of effectors and down to 40/45% under the influence of effectors. A particularly strong response to effectors can be observed in *D. mawsoni* and *R. glacialis* haemoglobin (Fig. 3.19 and Fig. 3.16), whereas the influence is very weak in haemoglobin of *Gerlachea australis* (Fig. 3.17).





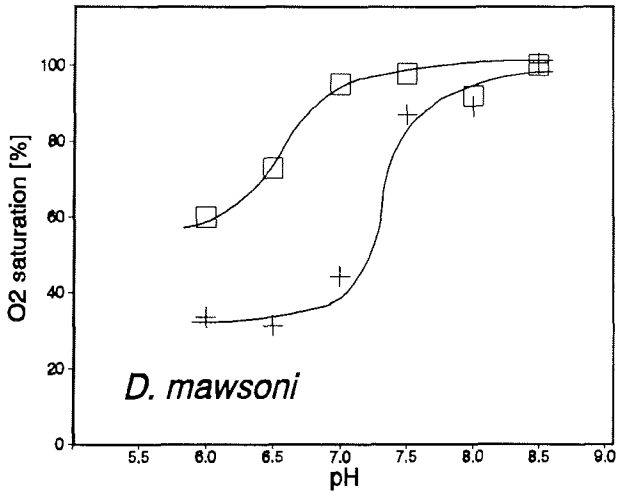


Fig. 3.17 to **Fig. 3.19**: Oxygen saturation of haemoglobin as a function of pH (Root effect). Haemolysate in the absence (□) or presence (+) of 3 mM IHP.

When the plots of the two haemoglobins of *P. antarcticum* are compared it is obvious that the decrease in saturation with decreasing pH is more distinct in Hb1, where values as low as 40% are reached at pH 6.0 (Fig. 3.24). In Hb2 the saturation at the same pH still reaches 60-65% (Fig. 3.25). This confirms the necessity to separate the two haemoglobins and study their oxygen binding properties individually.

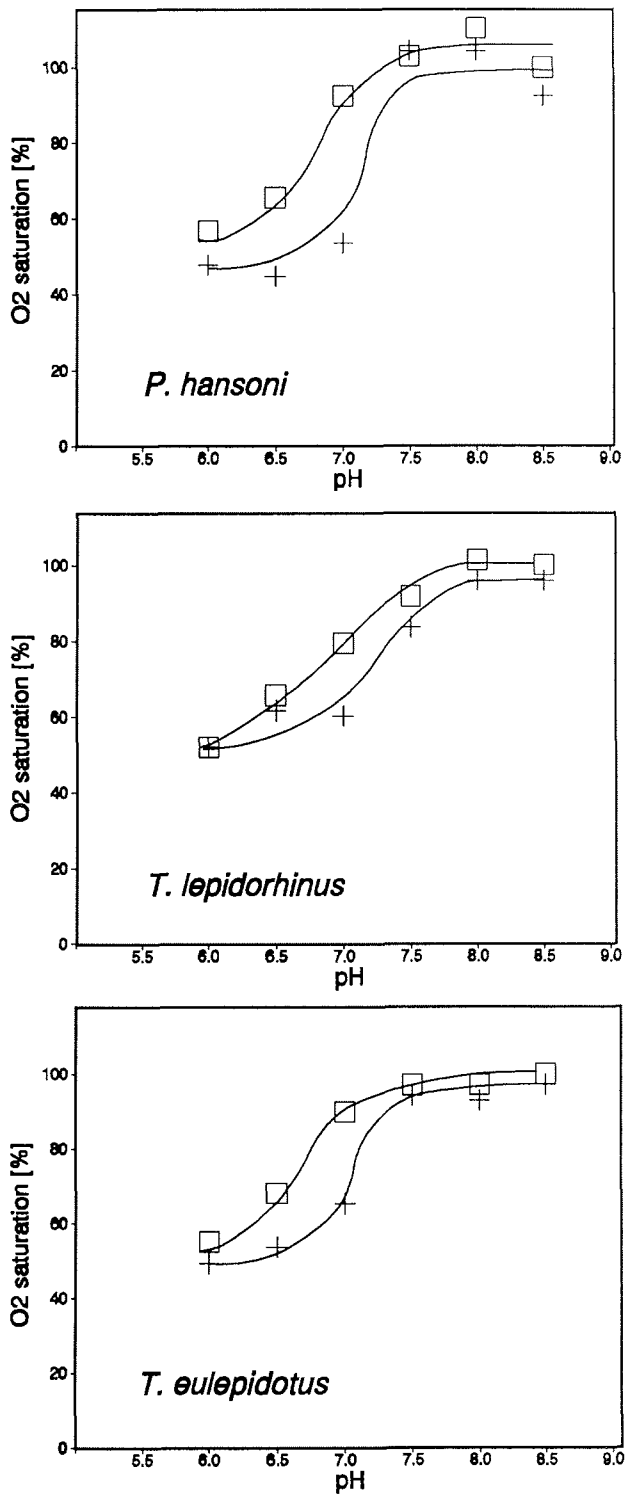


Fig. 3.20 to **Fig. 3.22**: Oxygen saturation of haemoglobin as a function of pH (Root effect). Haemolysate in the absence (□) or presence (+) of 3 mM IHP.

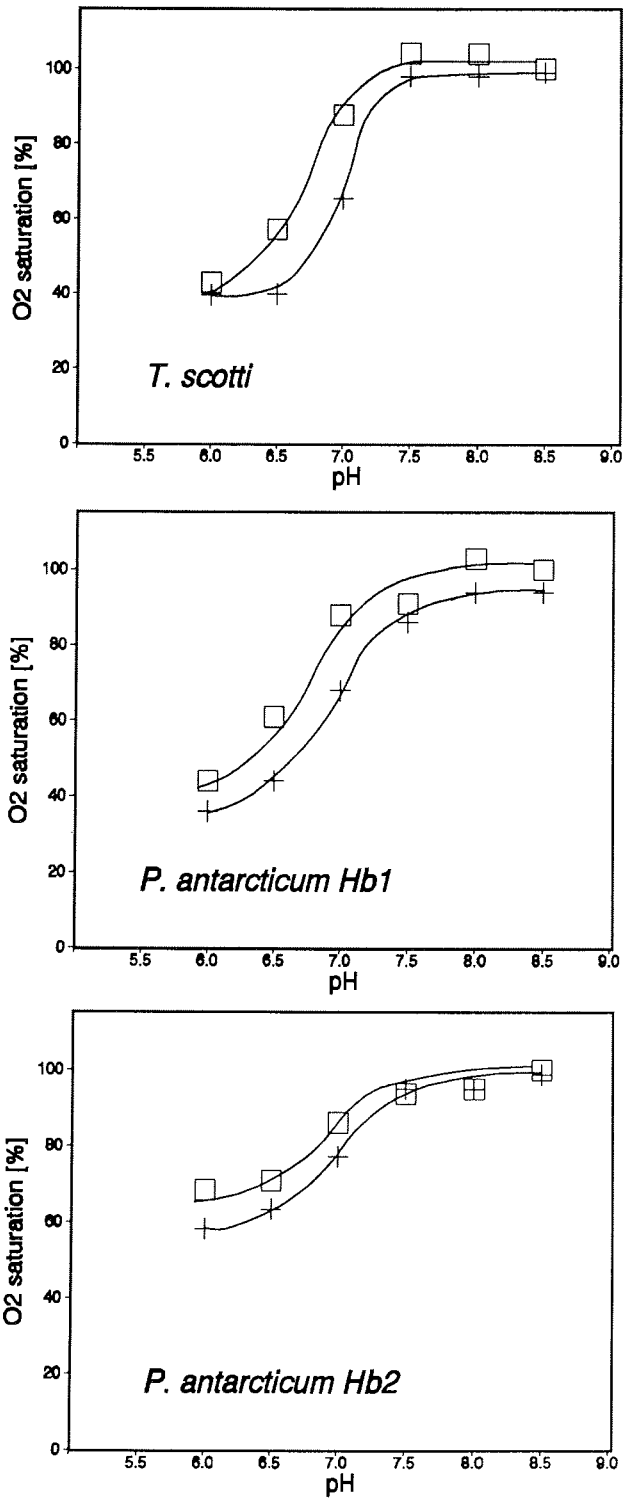


Fig. 3.23 to **Fig. 3.25**: Oxygen saturation of haemoglobin as a function of pH (Root effect). Haemolysate in the absence (□) or presence (+) of 3 mM IHP.

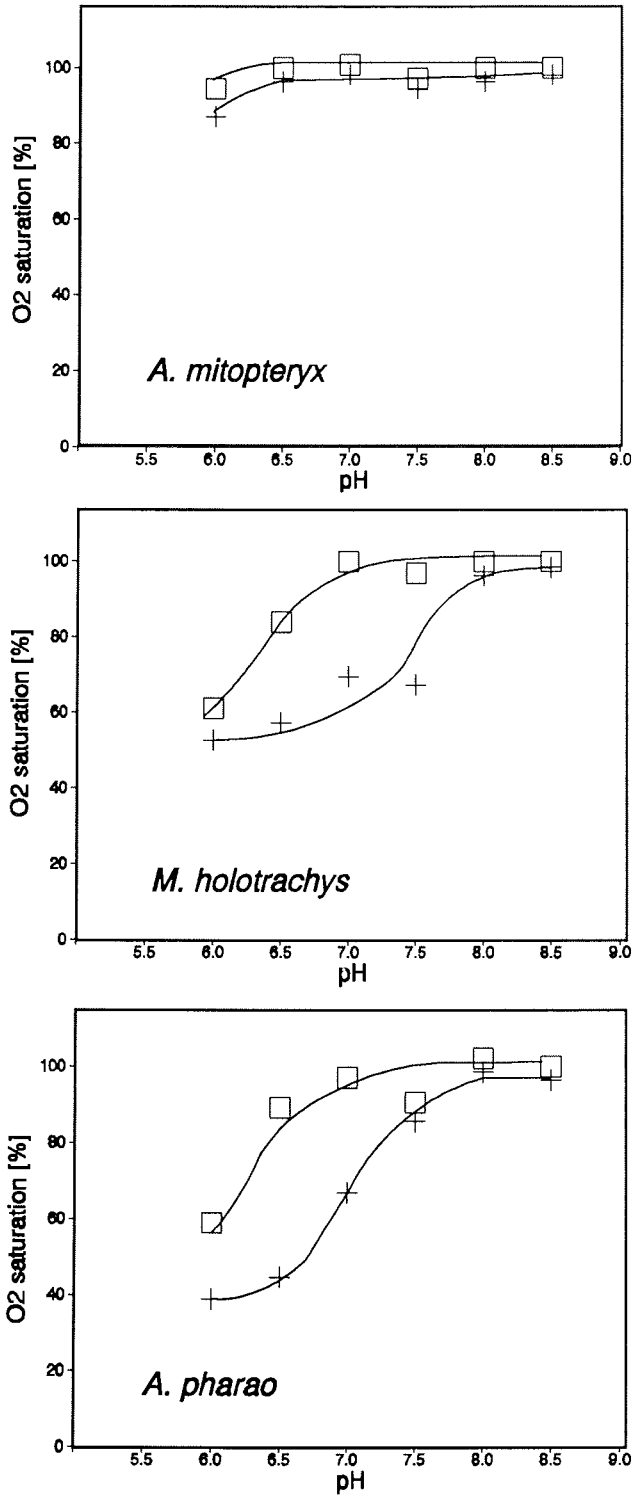


Fig. 3.26 to **Fig. 3.28**: Oxygen saturation of haemoglobin as a function of pH. Haemolysate in the absence (□) or presence (+) of 3 mM IHP. Note the Root effect absence in the upper panel.

A brief summary of the most important results concerning structural and functional studies is given in Table 3.8. For comparison published results are summarized and presented in tables in the Discussion. Selected data on blood parameters from other members of the family Bathydraconidae are presented in Table 4.2 and from other members of the family Nototheniidae in Table 4.3. All available data on haemoglobin components and function of species not investigated in this thesis, are compiled in Table 4.4.

Table 3.8: Haemoglobins of Antarctic fishes from the Weddell Sea. Oxygen binding and structural components (partly from Kunzmann & di Prisco 1990).

* CAE = Cellulose Acetate Electrophoresis, (relation Hb1/Hb2 in brackets)

** PAGE = Polyacrylamidgelelectrophoresis, SDS = Sodiumdodecylsulfate

Hb = haemoglobins, / = not investigated, - = no effect, + = slight effect, ++ = normal effect, +++ = strong effect.

Species	Root effect	Bohr effect	Hb CAE*	Globins PAGE-SDS**
Nototheniidae				
Aethotaxis mitopteryx	-	+	1	2
Pleuragramma antarcticum	+++	++	2 (75/25)	3
P. antarcticum Hb1	++	++	1	2
P. antarcticum Hb2	++	++	1	2
Dissostichus mawsoni	+++	+++	1	2
Trematomus eulepidotus	+++	+++	2	2-3
Trematomus lepidorhinus	++	+++	2 (90/10)	2
Trematomus scotti	+++	/	3 (85/10/5)	3-4
Pagothenia hansoni	++	+++	2	2
Bathydraconidae				
Bathydraco marri	+++	+++	1	2
Bathydraco macrolepis	+++	/	1	2
Gerlachea australis	+++	/	1	2
Racovitzia glacialis	+++	/	1	2
Artedidraconidae				
Pogonophryne sp.1	++	/	1	2
Pogonophryne sp.2	++	++	1	2
Pogonophryne sp.3	++	/	1	2
Macrouridae				
Macrourus holotrachys	+++	/	3	4
Anotopteridae				
Anotopterus pharao	++	/	3-4	3-4

Discussion and Conclusions

It was the intention of this thesis to find links between haematological characteristics of extremely well cold-adapted fishes and their evolution and ecology. Therefore, it was chosen to focus on high-Antarctic species, on a broad range of different ecotypes and on blood samples drawn from alive, rested specimens. A careful selection of specimens was necessary to meet these requirements. Catch, maintenance in aquaria and blood sampling cause unavoidable disturbance for any fish specimen. These problems are discussed in the next paragraph.

STRESS

Stress in general influences most haematological parameters (Blaxhall & Daisley 1973; Coburn & Fischer 1973), increases metabolism (Davison et al. 1988) and can even cause death of fishes. According to Wells et al. (1990) the most common haematological reactions on stress are a rapid and marked increase in haematocrit, erythrocyte swelling and increase in plasma viscosity.

As outlined in the Methods section, the catch causes a considerable amount of stress for fish. The most careful ways to collect fish are most likely by Scuba diving or with baited traps or hook and line. Fishes caught by trawls are usually not in good condition and mortality during or right after the catch is high. Losses can be considerably decreased, when the haul duration and particularly the 'out of water' period is kept short for the fishes. Since most of the species investigated in this study cannot be attracted by bait, there was not much choice in catching methods. More than 30 specimens have been kept in aquaria for several years now and feed and grow. This demonstrates that catch and the subsequent handling and maintenance was tolerable at least for most of the specimens used in this study.

A recovery period of 48 h is regarded to be sufficient by most authors (e.g. Wells et al. 1984; Davison et al. 1988). Others argue that even 72 h are not enough for fish to return to their normal resting metabolism (Wells et al. 1990). Metabolism of Antarctic fish maintained in tanks or aquaria will most likely never recover totally. In this study a recovery period of at least 48 hours was allowed, in most cases more (except for a few specimens, see Materials and Methods section). It is assumed that this may allow an approximation of what is called 'routine metabolism' and stable haematological values.

It is worth to stress that most of the published data on blood parameters (including the data

from this study) were obtained on blood samples drawn by acute techniques. Thus, a corresponding stress effect (which cannot be quantitatively assessed) on blood parameters cannot be excluded. Wells et al. (1990) argue that the only reliable means of sampling blood from resting, undisturbed fish is from a chronically implanted cannula. However, laboratory experiments reveal increases in blood pressure as response to simply touching a cannulation tube, even when the tube-end is far away from a fish kept in complete darkness (Rankin, pers. comm.). This demonstrates that stress is not avoidable with presently known techniques.

Avoidable sources of error in the collection and treatment of blood samples were taken into account, also concerning anaesthesia (Baumgarten-Schuhmann & Piiper 1968; Wells et al. 1984), anticoagulation (Blaxhall 1973; Barham et al. 1979; Hille 1982; Korcock et al. 1988) or routine haematology (Blaxhall & Daisley 1973; Coburn & Fisher 1973). All above mentioned authors claim that stress in general increases values for haematocrit, haemoglobin and red blood cell number. Since in this thesis the low values are emphasized in some cases, it should be kept in mind that these values would even be lower in totally undisturbed fish.

BLOOD PARAMETERS

In the extensive compilation of (non-Antarctic) fish data in Coburn & Fischer (1973) and in data for other vertebrates (Schmidt-Nielsen 1986) we find correlations between adaptations in the respiratory physiology and environmental conditions and/or ecology of the investigated species. Various haematological parameters can directly be correlated with factors such as activity of a species or ambient oxygen contents. The parameters of the present study were used to test the hypothesis that these correlations also exist in high-Antarctic fish species.

A general problem of these correlations is that we know only little about the mode of life or activity of most Antarctic fish species. Authors frequently limit their descriptions to statements like 'sluggish, benthic, scavenger' or 'active, fish predator' (e.g. Tetens et al. 1984; Macdonald et al. 1987). Therefore, we have to rely on indirect information for instance about food (Schwarzbach 1988) or muscle physiology (Johnston 1989) or on own observations from aquaria or remote camera vehicles. An overview of the few available data on the mode of life of species investigated in this study is presented in Table 4.1.

Red blood cell dimensions

Red blood cells of active non-polar fishes were found to be smaller in comparison to more sluggish species (Wells & Baldwin 1990). Data of this study (Table 3.1) suggest that this

Table 4.1: Summary of available data on mode of life based on activity, habit, food and lipid incorporation from published sources. Own observations from aquaria and/or remote video camera vehicles are included. + fish = including fish; 1 = Macdonald et al. 1987; 2 = Tetens et al. 1984; 3 = Johnston et al. 1989; 4 = Hubold 1991; 5 = Schwarzbach 1988; 6 = Ekau 1988; 7 = Kunzmann 1990; 8 = di Prisco pers. comm.; 9 = Wells et al. 1980; 10 = aquaria + video observations; 11 = Hureau et al. 1990; 12 = Eastman & De Vries 1982; 13 = Daniels 1982; 14 = Permitin & Tarverdiyeva 1978

Species (reference)	Activity	Habit	Indirect information (food)	Aquaria Video
Nototheniidae				
Aethotaxis mitopteryx (4, 6, 10, 12)	sluggish ?	pelagic, bentho-pelagic, >500 m	pelagic food, lipid deposits	extremely sluggish
Pleuragramma antarcticum (3, 4, 7, 10, 12)	sluggish	holopelagic, <500 m	pelagic food, lipid deposits seasonal migrations	sluggish
Dissostichus mawsoni (2, 4, 8, 10, 12)	active moderate	benthopelagic, benthic, 300-500 m	fish predator, lipid deposits caught by handlines	moderately active
Trematomus centronotus (1, 4, 5, 10)		benthic, shallow, 30-300 m	caught in traps	moderately active
Trematomus eulepidotus (4, 5, 10)	active	pelagic, 200-500 m	pelagic food	active
Trematomus lepidorhinus (4, 5, 10)		benthic, 200-500 m	benthic food	sluggish
Trematomus scotti (5, 13)		200-500 m	benthic food, generalist	sluggish
Pagothenia hansoni (4, 9, 10)	active	benthic, 100-500	predator, caught in traps food generalist	moderately active
Bathydraconidae				
Bathydraco marri (5, 10)		benthic, >700 m	prefers deep and cold water	potential for activity
Bathydraco macrolepis (5, 10)		benthic, >700 m	prefers deep and cold water	potential for activity
Cygnodraco mawsoni (5)			large benthic prey + fish	
Gymnodraco acuticeps (1, 9, 10)		cryopelagic anchor-ice	crustacean predator caught by handlines	moderately active
Racovitzia glacialis (5, 10)		benthic	motile benthic food	sluggish
Gerlachea australis (5, 10)		benthic	motile benthic food	sluggish
Artedidraconidae				
Pogonophryne sp. (4, 5, 10)	sluggish	benthic	benthic food	sluggish
Channichthyidae				
Neopagetopsis ionah (14)		deep	pelagic food	
Chionodraco myersi (5, 6, 10)			pelagic food + fish	sluggish
Cryodraco antarcticus (5, 10)			pelagic food + fish	sluggish
Dacodraco hunteri (5, 10)	active		pelagic food + fish	moderately active
Pagetopsis macropterus (1, 5)		benthic, shallow	pelagic food + fish	
Pagetopsis maculatus (5)			pelagic food	
Chionobathyscus dewitti (11)		> 500 m, down to 2000 m		

correlation does not apply to Antarctic fishes, since both active and sluggish species have a similar red blood cell size. Data from Tyler (1960), Everson & Ralph (1968) and Hureau et al. (1977) indicate, however, that high-Antarctic species might have smaller red blood cells (RBC) than sub-Antarctic species. The average RBC size for seven red-blooded sub-Antarctic species is reported to be about 15 µm (Everson & Ralph 1968). In the present study most high-Antarctic species indeed have smaller cells.

A clear difference found in the present study is the in general smaller size (average = 7.2 µm) of 'erythrocyte-like' cells in haemoglobinless fishes. This is confirmed by data from Hureau et al. (1977), who report an average size of 9 µm (including some sub-Antarctic icefish species).

Values known from temperate teleost species are between 5 µm and 18 µm (Coburn & Fischer 1973) and all Antarctic species (including the haemoglobinless ones) are within this range. Therefore, it is concluded that the red blood cell size does not have any zoogeographical significance.

Haematocrit (Hct)

Although haematocrit is the most frequently used parameter in fish haematology and pathology, it is still the most difficult parameter to draw conclusions from (e.g. Coburn & Fischer 1973). This is mainly due to the following facts, which should be kept in mind for the interpretation:

a) variability in Hct is large in various fish species from all latitudes
b) applied methods differ considerably (particularly before critical reviews on this subject were published)
c) the number of circulated red blood cells can easily be increased due to stress by releasing cells from the spleen
d) red blood cell swelling increases haematocrit
e) response to stress is very individual
f) in Antarctic fishes the difference in activity between very active and very sluggish species is not as pronounced as e.g. between a tuna and a toadfish.

In spite of these difficulties, some clear tendencies were identified. In very active species, such as tuna and mackerel (50-53%, Coburn & Fischer 1973; Larsson et al. 1976) or horse-mackerel (46-50%, Larsson et al. 1976; Putnam & Freel 1978) we find high haematocrits. The haematocrit of temperate fishes is reportedly close to 29 for inactive and 39-43 for active species (Love 1980).

Such a clear relationship between Hct and activity have not been found in data from polar fishes. Results of the present study show a broad range of haematocrit values (Table 3.2), but do not exceed 25%, except for a single, rather stressed specimen of *Pogonophryne*. Some active species, such as *Dissostichus mawsoni*, have a high haematocrit (Table 3.2 and 4.3), but other active species such as *Pagothenia hansoni* do not. The same applies vice-versa to the sluggish species of this study, where two species of similar low activity level, *Bathydraco marri* and *B. macrolepis* have low and high haematocrits, respectively (Table 3.2 and 4.2).

Table 4.2: Blood parameters and oxygen binding properties of members of the family Bathydraconidae. / = not investigated; Hct = haematocrit; RBC = number of red blood cells; Hb = haemoglobin concentration; Hb comp. = haemoglobin components

Species (reference)	Hct [%]	RBC [10^{12}/l]	Hb [g/l]	Hb comp.	Root effect	Bohr effect
V. infuscipinnis (Hureau 1977)	/	0.36	/	/	/	/
P. charcoti (Hureau 1977; di Prisco et al. 1988, 1990)	/	0.44	/	1	+	+
P. georgianus (Everson & Ralph 1968)	/	0.21	8.0	/	/	/
G. acuticeps (Wells et al. 1980; di Prisco et al. 1990)	24	0.78	22.4	1	-	-
C. mawsoni (di Prisco et al. 1990)	24.9	0.69	26.4	2	+	+
G. australis	17.1	0.38	28.0	1	+	/
R. glacialis	14.0	0.51	30.0	1	+	/
B. macrolepis	20.0	0.55	30.0	1	+	+
B. marri	14.6	0.59	29.6	1	+	+

Other authors report values in the range 19-38% for Antarctic fishes (Kooyman 1962; Grigg 1967; Everson & Ralph 1968; Rakusa-Suszczewski & Zukowski 1980; Wells et al. 1980). From temperate and tropical teleosts a haematocrit range of 15-53% is reported (Coburn & Fischer 1973; Love 1980). Apart from the observation that haematocrit in Antarctic fishes in general is low and with respect to the influence of stress, it is concluded that this parameter does not allow any conclusions on the ecology of Antarctic fishes.

Number of red blood cells (RBC)

The number of red blood cells, besides haematocrit, is one of the most frequently investigated parameters in blood physiology of fish. A range of $1\text{-}2*10^{12}$/l is reported from temperate teleosts (Love 1980). Active pelagic species can reach values as high as $4*10^{12}$/l (Coburn & Fischer 1973), which is close to values known for humans ($5*10^{12}$/l, Hallmann 1980). In contrast to that, less active species have lower RBC numbers. The validity of this relationship has previously been tested for Antarctic fishes, but only for a very limited number of different ecotypes.

The RBC values presented in this study (Table 3.2) are within the range of $0.4\text{-}1.2*10^{12}$/l, reported for Antarctic fishes by other authors (Tyler 1960; Kooyman 1962; Everson & Ralph 1968; Hureau et al. 1977; Rakusa-Suszczewski & Zukowsky 1980; Wells et al. 1980). Only considering the 'active' fishes in Table 3.2, such as *Dissostichus mawsoni* or *Trematomus eulepidotus*, we find them at the upper end of the range. This is in good agreement with the above mentioned relationship. Two species with little (*Macrourus holotrachys)* or unknown activity level (*Trematomus scotti*) have rather high values, too. However, since *M. holotrachys* is a non-endemic, bathypelagic species (often caught dead) and only a few samples of stressed *T. scotti* were available, these data are not suited for comparison.

On the other hand we find two sluggish species, *Pleuragramma antarcticum* and *Aethotaxis mitopteryx*, at the lower end of the range (Table 3.2). Species of little or moderate activity also have low RBC numbers (Table 3.2). Other authors (Grigg 1967; Hureau et al. 1977) confirm these values for some additional species such as *Trematomus centronotus* or *Pagothenia bernacchii* (Table 4.3).

These findings demonstrate that, although a general, evolutionary trend of reduction in cell count and haemoglobin content is expressed in all endemic Antarctic fishes (see Introduction), the relationship between red blood cell number and activity still holds.

Haemoglobin content (Hb)

In temperate and tropical species, values of 62-73 g/l have been found in inactive fish and 104-127 g/l in some active scombrids (Love 1980). This has led to the hypothesis of a positive relationship between haemoglobin and activity, i.e. increasing haemoglobin content with increasing activity (Love 1980). This hypothesis seems also to apply to polar fishes. Arctic species, for instance cover a range of 28 g/l for a most probably inactive liparid, to 68 g/l for reasonably active species (Scholander & Van Dam 1957).

Table 4.3: Compilation of blood parameters and oxygen binding properties of members of the family Nototheniidae.
/ = not investigated; +- = preliminary data; * = at environmental temperature of -1.8°C; # = originally measured at 20°C, adjusted with ΔH=-56/-21kJ/mol, see text.
Hct = haematocrit; RBC = number of red blood cells; Hb = haemoglobin concentration; MCHC = mean corpuscular haemoglobin concentration; MCH = mean cellular Hb; O_2CC = carrying capacity for O_2; P_{50} = O_2-pressure for 50% saturation of Hb; Φ = Bohr coefficient Δlog P_{50}/ΔpH; R = Root effect; B = Bohr effect
1 = Hureau et al. 1977, 2 = Everson & Ralph 1968, 3 = Wells et al. 1980, 4 = Grigg 1967, 5 = Wells & Jokumsen 1982, 6 = Tetens et al. 1984, 7 = D'Avino & di Prisco 1988, 8 = Kunzmann & di Prisco 1990, 9 = di Prisco et al. 1988, 10 = Qvist et al. 1977)

Species (reference)	**mode of life**	**Hct** [%]	**RBC** [10^{12}/l]	**Hb** [g/l]	**MCHC** [g/l]	**MCH** [pg]	**O_2CC** [%]	**Hb comp.**	**P_{50}*** [mmHg]	**Φ** [*-1]	**R**	**B**
T. centronotus (3, 4, 5, 7, 8, 9)	benthic, sluggish	19-22	0.44-0.55	28.3-29.7	95-156	43-55	3.3-5.2	2	1.3-7.8	/	/	/
P. bernacchii (1, 2, 3, 4, 5, 6, 7, 8)	benthic, sluggish	13-21	0.5-0.88	21-50	143.2	33	5.3	2	2.6-13.5	0.59-0.7	+	+
P. hansoni (1, 3, 4, 5, 7, 8)	benthic, active	31-34	0.5-0.76	28-56	108-135	47-59	7.7	1-2	3.7-10.8	0.87	+	+
P. borchgrevinki (3, 4, 5, 6, 7)	cryo-pelagic, active	13-32	0.9	30-48	133.5	53	6.6	2	8.2-22	0.26-0.6	+	+
D. mawsoni (3, 5, 6, 7, 8, 9, 10)	bentho-pelagic, active	17-27	0.88-0.99	33-64	160-188	44-53	7.1	1	6.5-14.4	0.49-0.7	+	+
A. mitopteryx	pelagic, sluggish	14	0.39	28	198.6	71	4.5	1	2.05-6.4 (12.6)#	≈0.2	-	-
P. antarcticum	pelagic, sluggish	17	0.43	27	159.6	62	4.3	2	3.2-10.1 (19.9)#	?	+	+?

However, data for Antarctic species had hitherto been limited to a few species and are in some cases contradictive. Several authors (e.g. Grigg 1967; Everson & Ralph 1968; Qvist et al. 1977; Wells et al. 1980; Wells & Jokumsen 1982; Tetens et al. 1984) investigated haemoglobin concentrations. In the group of active and/or moderately active species we find values from 33 to 64 g/l for *Dissostichus mawsoni*, 28 to 56 g/l for *Pagothenia hansoni* and 30 to 48 g/l for *Trematomus borchgrevinki* (Table 4.3). In this range, there is not much of a difference to sluggish species, such as *Pagothenia bernacchii* and *Trematomus centronotus*, where values as high as 50 g/l were found and lower limits are in the range 21-30 g/l (Table 4.3). In this context it should be kept in mind that active (or facultative active) species seem to have the capability to mobilize additional erythrocytes (and thus haemoglobin) and enhance oxygen delivery on demand, while their resting haemoglobin values are comparatively low (Wells et al. 1990).

The results of the present study (Table 3.2) clearly differentiate between active species, such as *D. mawsoni* and *Trematomus eulepidotus*, with high haemoglobin contents around 47 g/l and sluggish species, such as *Pleuragramma antarcticum* and *Aethotaxis mitopteryx*, with low values around 27 g/l. Species of moderate activity have values in between. Moreover, these results show less variability in haemoglobin content between species than values for red blood cell number and haematocrit.

These findings confirm the above mentioned hypothesis of a positive relationship between haemoglobin content and activity. Due to their low haemoglobin content it could be concluded that bathydraconids are the least active group amongst red-blooded species. Their haemoglobin values (20-30 g/l, Table 3.2) are clearly lower than values in other families. Unfortunately, no reliable observations on activity are published for these species. Only indirect information, like "pelagic and motile food" (Schwarzbach 1988), and own observations from aquaria give hints about their activity.

Obviously, this haemoglobin-activity relationship has limits. Channichthyids, without haemoglobin, can still be relatively active (e.g. Kock 1985).

Derived parameters (MCHC/MCH, O_2CC, PC)

The erythrocyte indices 'mean corpuscular haemoglobin concentration' (MCHC) and 'mean cellular haemoglobin' (MCH) have been identified as the most reliable blood parameters in vertebrates (Coburn & Fischer 1983). Few authors have attempted to correlate MCHC of temperate (Larsson et al. 1976), tropical (Putnam & Freel 1978) and Antarctic (Wells et al. 1980) fishes with activity and have not found a correlation. Instead, Wells et al. (1989) found a correlation of MCHC with latitude. They hypothesized that reduction of MCHC is the prin-

cipal mechanism to reduce haemoglobin, rather than through reduction in haematocrit and therefore may be an adaptation to low temperatures. When their data from nine Antarctic species are combined with results of the additional thirteen species studied here (Table 3.2), the correlation is still valid, except for the upper limit which has to be extended from 160 to 200 g/l for polar species. However, it is still well below the upper limits given for temperate (300 g/l) and tropical (370 g/l) species.

What is behind the 'strategy' to reduce haemoglobin via reduction of MCHC? A possible explanation could be that thus the probability to find a red blood cell at a given time and place in a body is much higher and a more even distribution of oxygen to the tissues is ensured. This indeed makes more sense than decreasing haematocrit and retaining only few cells densely packed with haemoglobin.

Channichthyids indirectly confirm this 'strategy'. They have completely reduced their haemoglobin (MCHC = 0), but still possess erythrocytes in significant amounts as the present study could demonstrate in so far uninvestigated species (Table 3.3). This raises questions about other possible functions of erythrocyte-like cells in these fishes. A reasonable explanation takes enzymatic functions into consideration, like for instance the maintenance of an appropriate acid-base balance in the blood via carbonic anhydrase (Wells et al. 1980). Another example is a recent investigation which revealed a significantly higher glucose-6-phosphate dehydrogenase (G6PD) activity in icefish blood cells, so that due to their increased blood volume the total G6PD activity in icefish blood is similar to that in red-blooded fishes (di Prisco 1985; di Prisco & D'Avino 1989).

In this context it is interesting to look into oxygen transport in haemoglobinless fishes, where the plasma contribution (PC) reaches 100%. Channichthyids can only partially compensate the low oxygen carrying capacity (O_2CC) of their blood. Although they have a higher volume of blood (2-4 fold; Hemmingsen & Douglas 1970) in combination with an increased heart stroke volume (9-15 fold; Johnston et al. 1983) the oxygen transported per unit of time still does not reach that of red-blooded Antarctic species. The consequences and the role of icefishes in general are resumed again later in a paragraph about evolutionary significance.

The values for O_2CC and PC found in this study for their red-blooded relatives (Table 3.2) clearly reveal that active species, such as *Dissostichus mawsoni* and *Trematomus eulepidotus*, rely mainly (>90%) on haemoglobin for their oxygen supply. They have the highest O_2-carrying capacities (7.1 and 7.0%) and lowest plasma contributions (8.5 and 8.6%) observed among the 19 red-blooded species studied.

Blood gases

Fishes in general have very low partial pressures of CO_2 (PCO_2), when compared to e.g. mammals. Mean arterial and venous ranges observed in non-polar fishes are 1.3-2.9 mm Hg and 2.0-4.6 mm Hg, respectively (Piiper & Schuhmann 1967). Except for two species, of which only highly stressed specimens were available, these limits are not exceeded in this study (Table 3.4).

However, several venous PO_2 values of this study (up to 78 mm Hg) exceed the range reported from literature for non-polar fishes (10-30 mm Hg) and reach arterial values (50-110 mm Hg; Hughes 1964; Piiper & Baumgarten-Schuhmann 1968). A possible explanation for this deviation and the high variance observed in all data on blood partial pressures (including those of this study) is proposed by Piiper & Schuhmann (1967). They refer to regulatory processes, such as varying water shunt flow across gill filaments, which substantially changes arterial partial pressures or varying blood shunt flow in tissues, which considerably changes venous partial pressures. Therefore, much more data on blood gases are needed, particularly in combination with measurements in gills and tissues.

Unfortunately, hardly any data are available on blood gases of red-blooded Antarctic fishes. The data of the present study are a first step. Since they were obtained on caudal venous blood only, they represent only part of the oxygen conditions. Arterial values and additional mixed venous measurements of blood entering the gills are needed. These measurements would require much more experimental effort on each specimen. The intention of this thesis, however, is to investigate a broad range of different ecotypes.

The oxygen transport system of icefishes is not so efficient as in red-blooded fishes; they cannot withstand exposure to environmental oxygen levels below 40-50 mm Hg (Holeton 1972), they rely heavily on additional cutaneous respiration (up to 40-50%; Wells 1987) and they have to maintain a high diffusion gradient (i.e. difference in partial pressure) to guarantee sufficient O_2 supply to the tissues. The results of this thesis (Table 3.4) indicate that they can maintain higher venous PO_2 values (60-75 mm Hg) in comparison to their red-blooded relatives (30-60 mm Hg). The highest arterial PO_2 observed in an icefish is 134 mm Hg (Holeton 1972), which allows a high diffusion gradient even when venous PO_2 is as high as found in this study. According to tissue needs the venous PO_2 can drop to 6 mm Hg, the lowest value ever reported for an icefish (Holeton 1972).

HAEMOGLOBIN STRUCTURE AND EVOLUTION

Structural studies on haemoglobin are necessary for a full understanding of its functional behaviour. Therefore, studies on haemoglobin multiplicity and molecular weight were performed on most of the investigated species. A detailed analysis of the primary structure (amino acid sequences) reveals the evolutionary development of the haemoglobin molecule and thus allows conclusions on the species level. As outlined in the Materials and Methods section, the amino acid sequence has so far only been established for two species, *Aethotaxis mitopteryx* and *Bathydraco marri*.

Haemoglobin multiplicity

Including results of this study (Table 3.2 and 3.8), the haemoglobin multiplicity has been investigated in seven bathydraconid species: six have one haemoglobin and only one has two (Table 4.2 and 4.4). Many species of the family Nototheniidae are characterized by the presence of two haemoglobins, one major and one minor component (di Prisco 1988; D'Avino and di Prisco 1988; di Prisco et al. 1990). The latter is suggested to be an evolutionary remnant. The evolutionary trend, from multiple haemoglobins in temperate species via one single or major haemoglobin in Antarctic red-blooded fishes to a total lack of haemoglobin in channichthyids, has been discussed previously by Wells et al. (1980). If we assume that channichthyids are indeed the most advanced group within the Notothenioids then bathydraconids, which in general have only one haemoglobin, would be one step below. This conclusion is strongly supported by haematological data of the present study (cf. page 67) and morphological investigations by Iwami (1985). The average number of red blood cells and the average haemoglobin contents were found to decrease in the order nototheniids - artedidraconids - **bathydraconids** - channichthyids (Table 3.2 and 3.3). In a phylogenetic tree by Iwami (1985) based on morphological investigations, bathydraconids are in fact one 'branch' before channichthyids (Fig. 4.1).

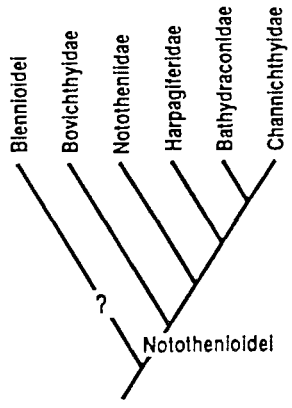


Fig. 4.1: Cladogram of notothenioid evolution from Iwami (1985) and Eastman & Grande (1989). Note bathydraconids and channichthyids in the top branch.

Primary structure (amino acid sequence)

The large number of published amino acid sequences of various haemoglobins, including a few non-polar fish species (e.g. Kleinschmidt & Sgourus 1987) indicates that in this protein

the primary structure is highly conserved during evolution. Conservation is most expressed in domains of structural importance, including residues known to be invariant in vertebrate globin chains (Dickerson & Geis 1983).

Results of the two species of the present study (*Bathydraco marri* and *Aethotaxis mitopteryx*; Fig. 3.4 and 3.5) and investigations on five additional Antarctic species (D'Avino & di Prisco 1988; di Prisco et al., in press) suggest that the above mentioned findings apply to Antarctic fishes as well. All invariant residues were found to be present (see Results section). The similarity in residues between Antarctic and non-polar fishes with completely different mode of life and taxonomic category, such as trout or carp, still reaches some 60% (Table 3.6). This clearly demonstrates the conservative nature of haemoglobin structure.

Comparing sequences within one taxonomic category reveals higher similarity between species than comparing sequences of different taxonomic categories. Even a tuna and the Antarctic species of this study, which all belong to the taxonomic order Perciformes, have higher similarities (75 and 79%, Table 3.6) than e.g. trout and the two Antarctic species (59 and 63%). Data from five additional species (di Prisco et al., in press) confirm these findings.

Although the two species of this study (*B. marri* and *A. mitopteryx*) belong to different families, the similarity in sequence of their haemoglobins is much more pronounced (84% for the α chain and 80% for the ß chain; Table 3.6) than between either of the two and any non-polar fish of a different family. This is an indication that the evolutionary distance between different fish families is less pronounced within the endemic Antarctic fish fauna.

Although the structural results of this study can be discussed more detailed, particularly the role of individual residues, this would lead beyond the frame of this thesis. These aspects (including results of this study) are discussed in two papers, which are in preparation (Caruso et al.; D'Avino et al.) and in an excellent reviewing discussion by di Prisco et al. (in press), where the replacement of particular residues, their relevance for functional effects and the role of the minor haemoglobin components are elucidated in detail. The more general conclusions drawn from results of the present study are summarized below.

The high degree of sequence similarity within Antarctic species may be a sign of the strong evolutionary pressure to which Antarctic fishes have been exposed south of the Antarctic Convergence. It could also be that it is a result of no or only weak evolutionary pressure on all Antarctic fish species after the separation of Antarctica. The low degree of identity with non-Antarctic species and the high degree of identity among Antarctic fishes of different families reflect the long isolation of the endemic Antarctic fish fauna. Only when more

sequences of polar fish haemoglobins are known, we shall hopefully gain insight into the molecular basis of cold adaptation and the phylogeny of these fishes.

HAEMOGLOBIN FUNCTION AND LINKS TO ECOLOGY AND EVOLUTION

Functional properties of haemoglobin respond to evolutionary selective pressure (Wells et al. 1989) and reflect adaptations to the metabolic rate and/or to the ambient oxygen pressure (Riggs 1970). Active, pelagic fishes, for example, have haemoglobins with a low O_2-affinity and large Bohr and Root effects (see Introduction). Low ambient oxygen pressures correspond to high oxygen affinities and vice-versa. Due to the above mentioned sensitivity of functional properties of haemoglobin to environment or mode of life, we would expect adaptations in the species investigated in this study.

Bohr and Root effects

Briefly summarized the Bohr effect is the influence of protons on the O_2-affinity of haemoglobin, i.e. a shift of the oxygen equilibrium curve with changing pH (see Introduction). It is generally accepted that its biological significance is to permit a fine regulation of oxygen supply to the tissues, thus enabling a wide scope of activity. This leads to the conclusion that species relying only on haemoglobins with no or weak Bohr effect have a limited scope of activity.

In this study normal or strong Bohr effects were found in all investigated species, except in *Aethotaxis mitopteryx*, where the Bohr effect was only very weak (Table 3.8). Strong Bohr effects were found in haemoglobins of five species (*Dissostichus mawsoni, Pagothenia hansoni, Trematomus eulepidotus, T. lepidorhinus, Bathydraco marri*); their Bohr coefficients, particularly under the influence of effectors, are all below or close to -1 (Table 3.7). These findings are in line with what is known about the ecology of the species. Three species (*D. mawsoni, P. hansoni, T. eulepidotus*) are known to be active (Wells et al. 1980, Hubold 1991), *T. lepidorhinus* is poorly described, presumably of moderate activity (Hubold 1991) and the ecology of *B. marri* is described in more detail below. Due to their activity these species rely on a well developed regulatory system of oxygen supply to tissues and have a great scope for the additional supply of oxygen on demand. Moreover their capacity to withstand or recover from hypoxia is large. This is supported by the haematological results of this study (Table 3.2): their values for the oxygen carrying capacity are at the upper end of the range.

All species with normal Bohr effect (*Pogonophryne spec.2, Pleuragramma antarcticum*; Table 3.8) or only weak Bohr effect (*Aethotaxis mitopteryx*; Fig. 3.11) are known or assumed to be sluggish (Ekau 1988, Kunzmann 1990, Hubold 1991). This is in good agreement with haematological data in Table 3.2. These species have comparatively low numbers of red blood cells, haemoglobin concentration and oxygen carrying capacity. The relation of blood characteristics to the ecology of *A. mitopteryx* is described in more detail below.

Another important information from Bohr effect studies is the oxygen affinity of haemoglobin (i.e. P_{50} values). Oxygen affinities can to a certain degree reflect ambient oxygen concentrations. Since in Antarctic waters oxygen concentrations are generally assumed to be high, the oxygen affinities of Antarctic fish haemoglobins should be uniformly low. The results of this study (Table 3.7) and of other authors (Macdonald et al. 1987) indicate indeed low oxygen affinities for most of the investigated species in comparison with known values for species well adapted to low oxygen concentrations. This marks a certain dependency on well-oxygenated waters (Macdonald et al. 1987). However, oxygen affinity values of individual species can be comparatively high, as for instance in the moderately active predator *Dissostichus mawsoni* (Table 3.7) with a very low P_{50} (i.e. high O_2-affinity). This species is known for vertical migrations, where different water bodies with considerably less oxygen (see Introduction) can easily occur. In this case a haemoglobin with high oxygen affinity would be of advantage.

In contrast to the Bohr effect, the functional relevance of the Root effect (in fact an exaggerated Bohr effect) is still unclear. Since all Antarctic notothenioids lack a swimbladder, the only remaining oxygen secreting structure known so far is the choroid rete in the eye. The eye usually has a very high rate of O_2 consumption like the brain of which it is an extension (Riggs 1979). But unlike mammalian eyes, retinal tissue in fish eyes is poorly vascularized and depends on the diffusion of O_2 over substantial distances. It has been postulated that Root effect haemoglobins in fishes without swimbladder are always associated with the occurrence of a choroid rete.

This hypothesis is confirmed by all 16 Antarctic species of the present study. As shown in the results section of this study (Table 3.8), the Root effect is fully operative in all investigated Antarctic species, except for *Aethotaxis mitopteryx*. This corresponds well with findings on a number of additional species (Table 4.4), which all show Root effects in their haemoglobins, except for *Gymnodraco acuticeps* (di Prisco et al. 1990). An extensive study on ocular morphology (Eastman 1988 and pers. comm.) revealed that all investigated species have a well developed choroid body, except the two species without Root effect haemoglobins, *A. mitopteryx* and *G. acuticeps*.

Table 4.4: Summary of haemoglobin characterization of Antarctic fishes (from di Prisco et al. 1990 and unpublished). Hb = haemoglobins, CAE = Cellulose Acetate Electrophoresis, AP = Antarctic Peninsula, RS = Ross Sea, / = not investigated

Species	Origin	Hb CAE	Bohr effect	Root effect
Nototheniidae				
Notothenia coriiceps neglecta	AP	2	+	+
Notothenia rossii	AP	2	+	+
Notothenia gibberifrons	AP	2	+	+
Notothenia nudifrons	AP	2	/	/
Notothenia larseni	AP	2	/	/
Pagothenia bernacchii	RS	1	+	+
Pagothenia borchgrevinki	RS	2	/	/
Trematomus newnesi	RS	2	+/-	+/-
Trematomus nicolai	RS	2	+	+
Trematomus centronotus	RS	2	+	+
Trematomus loennbergi	RS	2	+	+
Bathydraconidae				
Parachaenichthys charcoti	AP	1	+	+
Gymnodraco acuticeps	RS	1	-	-
Cygnodraco mawsoni	RS	2	+	+
Harpagiferidae				
Harpagifer antarcticus	AP	1	/	/
Artedidraco skottsbergi	AP	1	/	/
Harpagifer velifer	RS	1	/	+
Zoarcidae				
Lycenchelys nigripalatum	AP	4	/	/
Rigophila dearborni	RS	4	/	-,+
Austrolycichthys brachycephalus	RS	5	/	-,+

Although the present study clearly supports the existence of a correlation between Root effect and choroid rete, it is difficult to explain why most Antarctic fishes should rely so heavily on visual perception. This is even more surprising when considering the light situation under closed ice and/or the presence of icefishes (with no haemoglobin, therefore no Root effect and without choroid rete) in the same habitat. Maybe the release of small amounts of oxygen via Root effect is needed elsewhere, for instance into other hollow organs such as intestine or fat tissue, thus dramatically increasing the buoyancy, as proposed by di Prisco et al. (1988; in press).

Functional aspects and activity level

For a detailed discussion of functional properties of haemoglobins and its ecological signifi-

cance it is convenient to select representative examples. In the present case, where functional results of 16 species (Table 3.8) need interpretation it is appropriate to choose two extremes. Therefore, the haemoglobins of *Bathydraco marri* with a strong Root and a well-pronounced Bohr effect and of *Aethotaxis mitopteryx* with no Root and very weak Bohr effect were selected.

Bathydraco marri: Haemoglobin of *B. marri* has strong Root and Bohr effects (Fig. 3.6 and 3.14) with a large degree of cooperativity (n½, Table 3.7). The oxygen affinity (P_{50}, Table 3.7) is amongst the lowest values found in this study. Haematology reveals large erythrocytes (Table 3.1) and moderate values for the erythrocyte count, haemoglobin content and oxygen carrying capacity (Table 3.2). Results for other bathydraconids of this study are very similar. How are these results related to the mode of life and activity of *B. marri* ?

In the following, the few data available from published sources on distribution and activity and from own observations are combined in the attempt to formulate a hypothesis about the possible lifestyle and evolution of bathydraconids.

Although 'dragon fishes' play only a minor role in the eastern Weddell Sea (e.g. at Vestkapp 6% by numbers), their occurrence in catches reaches 16-29% by numbers in the more southern Gould Bay. During 'EPOS leg 3' they were caught in deeper water (>700 m), but in more northerly regions around Halley Bay (Hureau et al. 1990, and own observations). *B. marri* and *B. macrolepis* are known to occur at least down to 1150 m (Ekau 1988) and Andriashev (1965) states that some members of the genus *Bathydraco* occur even down to 2600 m. The percentage of bathydraconids in catches from the eastern and southern Weddell Sea increases with increasing depth below 500 m. 60% by numbers of all fishes caught below 700 m are bathydraconids and 50% by numbers of all bathydraconids caught, occur below 700 m (Schwarzbach 1988).

With the help of cluster analysis Schwarzbach (1988) found that bathydraconids are characteristic for the area of the Filchner depression, which is a deep trench running from the Filchner ice shelf to the continental slope. The prevailing water body is Ice-Shelf-Water (ISW) with temperatures as low as -2.2° C and salinities of 34.6-34.7‰ (Hellmer & Bersch 1985). The relatively high abundance of bathydraconids in the Gould Bay, which is adjacent to the Filchner depression, could be due to the extremely low temperatures of -2.0 to -2.2° C, as proposed by Ekau (1990), or to the high pressure or a combination of both.

How far the influence of high pressure is involved, is difficult to assess. In a recent publication on hydrostatic pressure as selective factor Somero (1990) points out that conformation

changes in multisubunit proteins (such as many enzymes and haemoglobin) are much more dependent on pressure as generally is assumed. 'Normal' proteins are hampered in their function by pressures above 500-600 m depth and need special adaptations. As indicated above bathydraconids prefer deeper areas. Pressure adaptations in their haemoglobin are therefore probable.

In summary these findings demonstrate a preference of bathydraconids for high latitudes, great depths and extremely low temperatures. The low temperature and therefore high oxygen content of such water bodies is reflected in the low oxygen affinity of *B. marri* haemoglobin, which is amongst the lowest values found in this study (Table 3.4). Accordingly low values for haematocrit and erythrocytes were found (Table 3.2). Hence, *B. marri* seems to be confined to high environmental oxygen contents. Values for other bathydraconids are similar, particularly for those species which are also known to prefer deep and cold waters (*Bathydraco macrolepis, Gerlachea australis, Racovitzia glacialis*).

These species have also the presence of pronounced Bohr and Root effects in common, i.e. a strong dependence of oxygen binding on small changes in pH. The only bathydraconid species without Bohr and Root effects is *Gymnodraco acuticeps* (Wells & Jokumsen 1982; di Prisco et al. 1990; Table 4.4), a species which so far has not been caught in deep and cold waters.

Strong Bohr and Root effects are often found in active species. Own observations on *Bathydraco marri* and *Bathydraco macrolepis* indicate a generally low level of routine activity. For instance *B. marri* sits most of the time motionless on the bottom of the aquarium. However, disturbances can cause impressively active reactions. It swims by means of fast bursts of the tail in a zig-zag-like manner. These bursts can last up to 3-4 minutes, making it difficult to capture a specimen in a large tank. This behaviour still exists after more than two years in captivity.

Gymnodraco acuticeps, another bathydraconid species we keep since more than two years, does **not** show such active reactions. Instead, it remains in position and only slightly increases movements with pectoral and pelvic fins, even upon severe disturbance. The single haemoglobin of *G. acuticeps* does **not** have a Root and Bohr effect (Wells & Jokumsen 1982; di Prisco et al. 1990). To date, only one other Antarctic species, *Aethotaxis mitopteryx*, has been found to have a single haemoglobin **not** displaying Root and Bohr effects (D'Avino et al. 1990). In good accordance also *A. mitopteryx* is **not** active at all (own observations during 'EPOS leg 3' and see below).

From these observations on activity of bathydraconids it is concluded that the presence of pronounced Root and Bohr effects in the single haemoglobin indicates their potential for activity.

Aethotaxis mitopteryx: In this species blood physiological results differ distinctly from those found in *B. marri*. Haemoglobin of *A. mitopteryx* does not show a Root effect and hardly any Bohr effect. The Bohr coefficient found in this study (Φ, Table 3.7) does not exceed -0.28 and cooperativity ($n_{1/2}$, Table 3.7) is almost absent. Haematology is marked by very low values for the number of red blood cells, haemoglobin content and oxygen carrying capacity (Table 3.7). What do these features of *A. mitopteryx* blood suggest for its mode of life ?

The very weak (nearly absent) Bohr effect does not allow a fine tuning of oxygen delivery to the tissues and indicates a limited scope of activity. The absence of the Root effect, a rather unusual feature in Antarctic fishes investigated so far, seems to exclude a particular role of visual perception. This, of course, would also exclude certain fast moving food organisms.

The low haematological values suggest a very sluggish mode of life. Although *A. mitopteryx* is regularly caught in the Warm-Deep-Water (WDW), which can have considerably less oxygen content (see Introduction), its haemoglobin has only a moderate oxygen affinity. This strongly suggests that optimized oxygen uptake is not very important for this species, which is an argument in favour of a sluggish mode of life. The majority of blood characteristics found in this study for *A. mitopteryx* also apply to *Pleuragramma antarcticum*, a closely related species known to be sluggish (Johnson 1989; Kunzmann 1990; Hubold 1991) and entirely pelagic.

These assumptions on the ecology of *A. mitopteryx* are confirmed by several authors. De Witt (1970) underlines the close affinities to the pelagic *Pleuragramma antarcticum* in body shape and general appearance and assumes a pelagic lifestyle at the continental slope. Subsequent studies of various authors suggested a rather pelagic lifestyle, too (Eastman 1981, De Vries & Eastman 1981, Eastman & De Vries 1982, Andriashev 1985, Miller 1985, Ekau 1988), although repeated occurrence in Agassiz Trawls (Hubold pers comm.) rather indicates a benthopelagic lifestyle. Authors agree on a very sluggish mode of life, mainly based on buoyancy adaptations (Eastman & De Vries 1982) and food observations (Eastman 1985). Own observations in aquaria also confirm this assumption.

A. mitopteryx and *P. antarcticum* are the only species with very low haematological parame-

ters combined with a sluggish **and** pelagic or benthopelagic mode of life. Both species are good examples for adaptation to an environment, which has only recently (in evolutionary terms) been occupied by fishes. According to Iwami (1985) *A. mitopteryx* and *P. antarcticum* are 'modern' species and the colonization of the pelagic realm has repeatedly been reported to be a rather recent process, too (Eastman 1985; Hubold 1990). Hubold (1990) suggests that the colonialization of the pelagic niche has great advantages, at least during parts of the year, due to the increased productivity of the water column. The extreme seasonality in light, ice and primary production is buffered by incorporation of considerable lipid deposits (Eastman 1985; Hagen 1988) and the low energy consuming mode of life, which is reflected in the extremely slow growth of adults (Ekau 1988).

GENERAL CONCLUSIONS ABOUT FAMILIES AND THEIR EVOLUTION

Bathydraconidae, Artedidraconidae

When haematological parameters are considered on a family level, it is obvious that the variability is always greatest in nototheniids. In bathydraconids and artedidraconids there is in general less variance (except for a few haematocrit values). At the same time values for Hb and RBC are decreasing in the order nototheniids, artedidraconids, bathydraconids and finally channichthyids. Keeping in mind the overall evolutionary trend to reduce blood viscosity in all Antarctic species, this decreasing order could be interpreted as an indirect reflection of evolutionary processes, where bathydraconids and channichthyids represent the most advanced groups.

Bathydraconids and artedidraconids are usually referred to as the 'typical high-Antarctic' species (De Witt 1970) and, as outlined above, bathydraconids clearly prefer deep and cold areas (Ekau 1988; Schwarzbach 1988). Thus, their haematology reflects both, extremely stable environments as well as their taxonomic position, just below the channichthyids (Fig. 4.1). This hypothesis is strongly supported by the absence of haemoglobin multiplicity in this family (table 3.8) and by morphological data from other authors (Iwami 1985; Eastman & Grande 1989; cf. page 59).

Nototheniidae

The geographical distribution of nototheniids does not show such clear preferences and many species are also found in sub-Antarctic areas. The variation in morphology and lifestyle is also much more pronounced in this family, as a comparison between *Notothenia gibberifrons*, *Dissostichus mawsoni* and *Pleuragramma antarcticum* easily demonstrates. There are also many hints from other fields of investigation that particularly this family seems to be in a

process of speciation (Hubold 1991). This would explain higher variance of haematological values, as compared to bathydraconids or artedidraconids.

20-25 million years ago the family Nototheniidae split into three groups. In Miocene, i.e. less than 10 million years ago, the most advanced group amongst them, the Pleuragrammiini, divided into three species *Pleuragramma antarcticum, Cryothenia peninsulae* and *Aethotaxis mitopteryx* (Andersen 1984). All three species are more or less confined to a pelagic/benthopelagic mode of life. There are indications that some special adaptations (e.g. neutral buoyancy, antifreeze, blood characteristics) may be of relatively recent origin (Andersen 1984), and could be assigned to recent changes in mode of life.

Antarctic notothenioids derived from primarily benthic perciform fishes. The increasing pelagization of species may be seen as a fairly recent process (Eastman 1985). Only these pelagic/benthopelagic species show peculiarities in haematological parameters and oxygen binding properties (Table 4.3). *P. antarcticum* and *A. mitopteryx* do not follow expected patterns known from temperate species. Their low values for haematocrit, red blood cell number and haemoglobin concentration are close to those of sluggish, benthic species, such as *Trematomus centronotus* or *P. bernacchii*. In fact, as outlined above, both *P. antarcticum* and *A. mitopteryx* seem to be sluggish.

However, the only moderate oxygen affinities of their haemoglobins rather resemble the more active species, such as *Pagothenia borchgrevinki* or *Dissostichus mawsoni*. The low pH sensitivity of *A. mitopteryx* haemoglobin is also unusual. This is apparently related to the unique mode of life, i.e. pelagic **and** sluggish. Usually, pelagic species are the most active ones. The only other Antarctic species, which has similar haematological values and a pH insensitive haemoglobin, is the bathydraconid *Gymnodraco acuticeps* (Wells et al. 1980; di Prisco et al. 1990). It is not surprising that it is the only bathydraconid species with a cryopelagic mode of life.

On the other hand *P. antarcticum* has in contrast to *A. mitopteryx* two haemoglobins in higher amounts (Fig. 3.2 and Table 3.8). As outlined in the Materials and Methods section (page 30) available samples were not sufficient for a detailed functional study on both haemoglobins (Hb1, Hb2) of *P. antarcticum*. Therefore, we do not know yet if Hb1 and Hb2 are functionally different haemoglobins. However, from temperate and tropical fishes we know that ratios between multiple haemoglobins can vary seasonally and synthesis on demand is possible (Love 1980). This is particularly interesting in *P. antarcticum*, because the species is known for seasonal migrations. During migrations it is likely that different water masses are crossed and a functionally different second haemoglobin could be helpful

when environmental oxygen varies. Since morphological data suggest that both *P. antarcticum* and *A. mitopteryx* are modern species (Iwami 1985) it is not likely that the second haemoglobin (Hb2) of *P. antarcticum* is only an evolutionary remnant. However, this would mean Hb2 is needed and therefore may differ functionally from Hb1.

Channichthyidae

In the same environment we find on one hand red-blooded fishes with a complex machinery for oxygen transport and on the other hand fishes totally lacking haemoglobin. This raises a number of intriguing questions, such as: What is the advantage or disadvantage of haemoglobinless fishes in Antarctic fish communities? Do Antarctic fishes need haemoglobin at all?

The following considerations should be kept in mind when answers are sought. At sub-zero temperatures, metabolic demand for oxygen is reduced, while the solubility of oxygen in blood plasma is increased. Thus, the blood of Antarctic fishes does not have the same transport requirements as that of more active fishes living in warmer waters (Wells 1987). Moreover, fish are not nearly as dependent upon the quantity of functioning haemoglobin as are mammals and birds. Many sluggish fish species can loose most of their haemoglobin without any immediate disastrous effects (Steen & Berg 1966; Holeton 1972). However, experiments with active or moderately active fish of temperate regions (including perciform species) have shown that these fishes cannot loose more than 50% of their haemoglobin (di Prisco, pers. comm.).

Recent experiments by di Prisco et al. (in press) with carbon monoxide treatment totally blocked the oxygen binding site in haemoglobin of the Antarctic species *Pagothenia bernacchii*. The results did not reveal any discernible effects on the vital functions. Induced reduction of the haematocrit to less than 1-2% had no apparent effect either (Wells et al. 1990). These experiments demonstrate that *P. bernacchii* can carry the routinely needed oxygen just physically dissolved in plasma. Unfortunately, *P. bernacchii* is only moderately active. It would be interesting to see how active Antarctic fishes perform. From very active temperate and tropical fishes it is known that they die immediately without haemoglobin.

It is therefore concluded, that as long as Antarctic fishes (including icefishes) swim normally, their tissue can get all oxygen it needs from the dissolved part in the plasma alone. However, as soon as activity over longer periods (more than a few seconds) is needed this can most likely only be performed with haemoglobin (Love 1980). From earlier investigations it is known that resting icefishes use already up to 60-70% of their oxygen capacity (Holeton 1970). Therefore, drastic enhancement of oxygen delivery to support escape reactions is simply not possible. In contrast, resting red-blooded fishes use only about 20-30% of their

capacity, although the resting oxygen consumption of red-blooded and haemoglobinless species is similar (Holeton 1970).

Thus, one consequence for channichthyids seems to be a reduced scope of activity and a reduced ability to withstand sustained hypoxia (Macdonald et al. 1987). There is no evidence, however, that icefish are disadvantaged in any way. As in red-blooded families we find relatively active species, such as *Dacodraco hunteri* and species with respectable size, such as *Champsocephalus gunnari* (Kock 1981). Wells et al. (1990) even point out, that channichthyids are hardy species surviving the rigours of capture, anaesthesia, handling, exercise and hypoxia just as well as red-blooded nototheniids.

FINAL REMARKS

For the following considerations it should be kept in mind that the routine activity level of a sluggish, boreal species such as *Zoarces viviparus*, is still two orders of magnitude higher than that of a sluggish, Antarctic species (Hubold 1991). Antarctic fishes described as active should therefore not be compared with active, tropical fish.

In an ecological sense the midwaters of the Southern Ocean seem to be underutilized by fish (Eastman & Grande 1989). Consequently, the above mentioned process of pelagization of species is expanding and most expressed in channichthyids (Schwarzbach 1988). Our common concept about pelagic fish, actively swimming and therefore relying on efficient oxygen transport systems, should be extended. Antarctic notothenioids, including the pelagic species, seem to rely on low activity, small gas exchange areas, low oxygen capacity and low metabolic rates. Apparently, power and performance of gills, circulatory systems and/or muscles are not selective factors. Notothenioids simply can afford these energy saving strategies, because of facilitating factors, such as:
- high ambient oxygen contents, enabling a poorly developed oxygen transport system
- absence of food-competition and predatory pressure, facilitating a sluggish mode of life
Therefore, even pelagic species can rely on the 'sit-(swim)-and-wait strategy'.

Most key adaptations of Antarctic notothenioids to the cold environment are based on biochemical adjustments (Eastman & Grande 1989) in metabolic pathways or important cell structures. Some notable examples are: increased protein synthesis, low activation energies and enhanced low temperature activities in various enzymes, poor developed glycolytic pathways combined with an increased role of the pentosephosphate cycle, lipid storage in muscles instead of glycogen deposits, increased membrane fluidity, high conduction velocities

and fully compensated synaptic events in the nervous system and finally cold-stable microtubules (for a review see Macdonald et al. 1987). Some of these adjustments directly influence respiration. As previously assumed and confirmed by the results of this study, evolutionary adaptations in the respiratory system seem to be directional (directive) and follow the line icefishes went along. This does not necessarily mean that only haemoglobin-less fishes will survive. But due to the fact that oxygen transport is not a crucial factor in Antarctic ecosystems, evolution may be directed towards less variance in respiratory characteristics.

Various fields of physiology regard Antarctic fishes as exceptional with respect to their adaptations. A final question is therefore: Is the respiratory physiology of Antarctic fishes also different from that of other fishes?

Regarding oxygen uptake Antarctic fishes seem to be disadvantaged. Red-blooded species have smaller gills, less erythrocytes and less haemoglobin than their relatives from temperate areas. Heart size and blood volume are similar to other fishes. They usually rely on only one haemoglobin with a low oxygen affinity, which marks a dependency on well-oxygenated waters. However, the blood viscosity of red-blooded Antarctic fishes is lower than for instance in Arctic fishes. In energetic terms this is a slight advantage. During evolutionary progress more respiratory characteristics have been modified. In channichthyids, the most advanced group amongst Antarctic fishes, we find already significantly reduced blood viscosity, no haemoglobin and only very few 'erythrocyte-like' cells. Moreover, their blood volume, diameter of vessels, heart size and heart stroke volume is increased. Their gill size seems larger than in their red-blooded relatives and is thus of similar size to that of temperate and tropical fishes. Scales are completely reduced and cutaneous respiration is important. Thus, the missing haemoglobin in channichthyids is at least partly compensated.

Adaptations in oxygen delivery or consumption are as important as adaptations in oxygen uptake. In channichthyids we find a considerably increased difference in oxygen partial pressure between blood and metabolizing tissues. This greatly facilitates oxygen delivery. It is suggested that an in general less active and energy-saving (thus O_2-saving) mode of life is a key adaptation which reduces O_2-consumption. Thus, channichthyids can cope without haemoglobin and red-blooded Antarctic fishes manage with less haemoglobin. It is suggested that Antarctic fishes in general are less active than fishes from temperate and tropical areas. This 'strategy' could only be developed because of high ambient O_2 tensions and the absence of food-competition and predatory pressure. Even pelagic species follow this 'strategy' with the help of large lipid deposits to maintain buoyancy.

References

Andersen, N.C. 1984 Genera and subfamilies of the family Nototheniidae from the Antarctic and Sub-Antarctic. Steenstrupia 10(1): 1-34.

Andriashev, A.P. 1965 A general review of the Antarctic fish fauna. Monographiae Biologicae 25: 491-550.

Andriashev, A.P. 1987 A general review of the Antarctic bottom fish fauna. Proc. V Congr. Europ. Ichthyol. Stockholm, pp. 357-372.

Arntz, W.; Ernst, W.; Hempel, I. 1990 The expedition Antarktis VII/4 (EPOS leg 3) and VII/5 of RV "Polarstern" in 1989. Rep. Polar Res. 68: 1-213.

Barham, W.T.; Smit, G.L.; Schonbe, H.J. 1979 The effect of heparin concentration on certain blood parameters of the rainbow trout *Salmo gairdneri* Richardson. Comp. Biochem. Physiol. 63C: 369-371.

Baumgarten-Schuhmann, D.; Piiper, J. 1968 Gas exchange in the gills of resting unanaesthetized dogfish (*Scyliorhinus stellaris*). Resp. Physiol. 5: 317-325.

Blaxhall, P.C. 1973 Error in haematrocrit value produced by inadequate concentration of ethylendiamine tetra-acetate. J. Fish Biol. 5: 767-769.

Blaxhall, P.C.; Daisley, K.W. 1973 Routine haematological methods for use with fish blood. J. Fish. Biol. 5: 771-781.

Brittain, T. 1987 The Root effect (Minireview). Comp. Biochem. Physiol. 86B(3): 473-481.

Caruso, C.; Kunzmann, A.; di Prisco, G. in prep. Primary structure and oxygen binding properties of the single haemoglobin of the Antarctic teleost *Bathydraco marri*.

Clarke, A. 1990 Temperature and evolution: Southern Ocean cooling and the Antarctic marine fauna. In: Kerry, K.R.; Hempel, G. (eds.) Antarctic ecosystems: change and conservation. Springer, Berlin, pp. 9-22.

Coburn, C.B.; Fischer, B.A. 1973 Red blood cell hematology of fishes: a critique of techniques and a compilation of published data. J. Mar. Science 2(2): 37-58.

Daniels, R.A. 1982 Feeding ecology of some fishes of the Antarctic peninsula. Fish. Bull. 80: 575-588.

D'Avino, R.; Fago, A.; Kunzmann, A.; di Prisco, G. in prep. Amino acid sequence and functional behaviour of the single haemoglobin of the Antarctic teleost *Aethotaxis mitopteryx*.

D'Avino, R.; Caruso, C.; Schinina', M.E.; Rutigliano, B.; Romano, M.; Camardella, L.; Bossa, F.; Barra, D.; di Prisco, G. 1989 The amino acid sequence of the α- and ß-chains of the two haemoglobins of the Antarctic fish *Notothenia coriiceps neglecta*. FEBS Letters 250: 53-56.

D'Avino, R.; di Prisco, G. 1988 Antarctic fish haemoglobin: an outline of the molecular structure and oxygen binding properties. - I. Molecular structure. Comp. Biochem. Physiol. 90B:

579-584.

D'Avino, R.; di Prisco, G. 1989 Haemoglobin from the Antarctic fish *Notothenia coriiceps neglecta*. 1. Purification and characterization. Eur. J. Biochem. 179: 699-705.

D'Avino, R.; Fago, A.; Kunzmann, A.; di Prisco, G. 1990 Structure and function of the haemoglobin of the Antarctic teleost *Aethotaxis mitopteryx*. Ital. biochem. Soc. Trans., Minerva Biotecnologica (Suppl.) 2: 315.

Davison, W.; Forster, M.E.; Franklin, C.E.; Taylor, H.H. 1988 Recovery from exhausting exercise in an Antarctic fish, *Pagothenia borchgrevinki*. Polar Biol. 8: 167-171.

De Jager, S.; Dekkers, W.J. 1975 Relations between gill structure and activity in fish. Neth. J. of Zool. 25(3): 276-308.

De Vries, A.L.; Eastman, J.T. 1981 Physiology and ecology of notothenoid fishes of the Ross Sea. J. Royal Soc. of New Zealand 11(4): 329-340.

De Witt, H.H. 1970 The character of the midwater fish fauna of the Ross Sea, Antarctica. In: Holdgate (ed.) Antarctic Ecology, Vol 1, pp. 305-314, Academic Press, London.

di Prisco, G. 1985 Antarctic fishes and cold-adaptation Atli 1 Simposio di Biochimica marina. Editoriale Grasso, Bologna, pp. 51-86.

di Prisco, G. 1988 A study of haemoglobin in Antarctic fishes: purification and characterization of haemoglobins from four species. Comp. Biochem. Physiol. 90B: 631-637.

di Prisco, G.; D'Avino, R. 1989 Molecular adaptation of the blood of Antarctic teleosts to environmental conditions. Antarctic Science 1: 119-124.

di Prisco, G.; D'Avino, R.; Camardella, L.; Caruso, C.; Romano, M.; Rutigliano, B. 1990 Structure and function of haemoglobin in Antarctic fishes and evolutionary implications. Polar Biol. 10: 269-274.

di Prisco, G.; Giardina, B.; D'Avino, R.; Condo', S.G.; Bellelli, A.; Brunori, M. 1988 Antarctic fish haemoglobin: an outline of the molecular structure and oxygen binding properties. - II. Oxygen binding properties. Comp. Biochem. Physiol. 90B: 585-591.

Dickerson, R.E.; Geis, I. 1983 Haemoglobin: structure, function, evolution and pathology. Benjamin/Cummings Publishing Co., Menlo Park, CA.

Dorrien, C.F. von; Räke, P. unpubl. Altersanalysen an *Pagetopsis maculatus* (Barsukov & Permitin) (Channichthyidae). Semesterarbeit 1987, IfM, Universität Kiel, 22 pp.

Eastman, J.T. 1981 Morphological specializations in Antarctic fishes. Ant. J.U.S. 16(5): 146-147.

Eastman, J.T. 1985 The evolution of neutrally buoyant Notothenioid fishes: their specialisations and potential interactions in the Antarctic marine food web. In: Siegfried, R.W.; Condy, P.R.; Laws, R.M. (eds.): Antarctic nutrient cycles and food webs. Springer, Berlin, pp 430-436.

Eastman, J.T. 1988 Ocular morphology in Antarctic notothenioid fishes. J. Morphol. 196: 283-306.

Eastman, J.T.; De Vries, A. L. 1982 Buoyancy studies of notothenioid fishes in Mc Murdo Sound,

Antarctic. Copeia: 385-393.

Eastman, J.T.; Grande, L. 1989 Evolution of the Antarctic fish fauna with emphasis on the recent notothenioids. In: Crame, J.A.(ed.) Origins and evolution of the Antarctic biota. Geol. Soc. Special Publ. 47: 241-252.

Eckert, R. 1986 Tierphysiologie. Thieme, Stuttgart etc., 697 pp.

Ekau, W. 1988 Ökomorphologie nototheniider Fische aus dem Weddellmeer, Antarktis. Ber. Polarforsch. 51: 1-140.

Ekau, W. 1990 Demersal fish fauna of the Weddell Sea, Antarctica. Antarctic Science 2: 129-137.

Everson, I.; Ralph, R. 1968 Blood analyses of some Antarctic fish. Br. Antarct. Surv. Bull. 15: 59-62.

Fischer, W.; Hureau, J.C. 1985 FAO Species identification sheets for fishery purposes. Southern Ocean (Areas 48, 58, 88). Vol.2, 252 pp.

Giardina, B.; Amiconi, G. 1981 Measurement of binding of gaseous and nongaseous ligands to hemoglobin by conventional spectrophotometric procedures. Methods Enzymol. 76: 417-427.

Goethe, J.W. 1808 Faust I. Goethe's Werke - Achter Band. Cottasches Handschriftenarchiv, Schiller National Museum, Marbach.

Grigg, G.C. 1967 Some respiratory properties of the blood of four species of Antarctic fishes. Comp. Biochem. Physiol. 23: 139-148.

Hagen, W. 1988 On the significance of lipids in Antarctic zooplankton. Rep. Polar Res. 49: 1-129.

Hallmann L. 1980 Klinische Chemie und Mikroskopie. Thieme, Stuttgart, 754 pp.

Hellmer, H.H.; Bersch, M. 1985 The Southern Ocean. Rep. Polar Res. 26: 1-115.

Hemmingsen, E.A.; Douglas, E.L. 1970 Respiratory characteristics of the hemoglobinfree fish *Chaenocephalus aceratus*. Comp. Biochem. Physiol. 33(4): 733-744.

Hille, S. 1982 A literature review of the blood chemistry of rainbow trout *Salmo gairdneri* Richardson. J. Fish. Biol. 20: 535-569.

Holeton, G.F. 1970 Oxygen uptake and circulation by a haemoglobinless fish (*Chaenocephalus aceratus*) compared with three red-blooded Antarctic fish. Comp. Biochem. Physiol. 34: 457-471.

Holeton, G.F. 1972 Gas exchange in fish with and without hemoglobin. Resp. Physiol. 14(1/2): 142-150.

Holeton, G.F. 1976 Respiratory morphometrics of white and red-blooded Antarctic fish. Comp. Biochem. Physiol. 54A: 215-220.

Huber, F.; Braunitzer, G. 1989a The primary structure of electric ray hemoglobin (*Torpedo marmorata*) - Bohr effect and phospate interaction. Biol. Chem. Hoppe-Seyler 370: 831-838.

Huber, F.; Braunitzer, G. 1989b The primary structure of the hemoglobin of the electric eel (*Electrophorus electricus*). Biol. Chem. Hoppe-Seyler 370: 245-250.

Hubold, G. 1985 The early life-history of the high-Antarctic silverfish *Pleuragramma antarticum*. In: Siegfried, W.R.; Condy, P.R; Laws, R.M. (eds.): Antarctic nutrient cycles and food webs, pp. 445-451, Berlin, Springer.

Hubold, G. 1990 Ecology of notothenioid fishes in the Weddell Sea. II Int. Conf. Biol. Ant. Fishes, Ravello, 30.5.-1.6.1990.

Hubold, G. 1991 Zur Ökologie der Fische im Weddellmeer. Habilitationsschrift, Math.-Nat. Fakultät, Universität Kiel, pp.

Hughes, G.M. 1964 Fish respiratory homeostasis. Symp. Soc. Exp. Biol. 18: 81-107.

Hureau, J.C.; Balguerias, E.; Duhamel, G,; Kock, K.-H.; Ozouf-Costaz, C. 1990 Fish fauna of the eastern Weddell Sea. Rep. Polar Res. 68: 130-138.

Hureau, J.C.; Petit, D.; Fine, J.M.; Mameux, M. 1977 New cytological, biochemical, and physiological data on the colorless blood of the Channichthyidae (Pisces, Teleosteans, Perciformes). In: Llano, G.A. (ed.): Adaptations within Antarctic ecosystems. Gulf Publ. Co, Houston, pp. 459-477.

Iwami, T. 1985 Osteology and relationships of the family Channichthyidae. Mem. Natl. Inst. Polar Res. Ser. E No.36: 1-69.

Johnston, I.A. 1989 Antarctic fish muscles - structure, function and physiology. Antarctic Science 1(2): 97-108.

Johnston, I.A.; Fitch, N.; Zummo, G.; Wood, R.E.; Harrison, P.; Tota, B. 1983 Morphometric and ultrastructural features of the ventricular myocardium of the haemoglobinless icefish *Chaenocephalus aceratus*. Comp. Biochem. Physiol. 76A(3): 475-480.

Kennet, J.P. 1977 Cenoroic evolution of Antarctic glaciation, the circumantarctic ocean and their impact on global paleoceanography. J. Geophys. Res. 82: 3843-3876.

Kleinschmidt, T.; Sgouros, J.G. 1987 Hemoglobin sequences: Mini review. Biol. Chem. Hoppe-Seyler 368: 579-615.

Kock, K.-H. 1981 Fischereibiologische Untersuchungen an drei antarktischen Fischarten: *Champsocephalus gunnari* Lönnberg, 1905, *Chaenocephalus aceratus* (Lönnberg, 1906) und *Pseudochaenichthys georgianus* Norman, 1937. Mitt. Inst. Seefischerei 32: 1-226.

Kock, K.-H. 1985 Antarctic fish. In: Bonner, M.W.; Walton, D.W.H. (eds.): Key environment, Antarctica, Pergamon Press, Oxford etc., pp. 173-192.

Kooyman, G.L. 1963. Erythrocyte analysis of some Antarctic fishes. Copeia 1963: 457-458.

Korcock, D.E.; Houston, A.H.; Gray, J.D. 1988 Effects of sampling conditions on selected blood variables of rainbow trout, *Salmo gairdneri* Richardson. J. Fish. Biol. 33(2): 319-330.

Kunzmann, A. 1987. Gill morphometrics of an Antarctic fish, *Pleuragramma antarcticum*. Proc. V Congr. europ. Ichthyol., Stockholm, pp. 467-468.

Kunzmann, A. 1990 Gill morphometrics of two Antarcic fish species: *Pleuragramma antarcticum* and *Notothenia gibberifrons*. Polar Biol. 11: 9-18.

Kunzmann, A.; Caruso, C.; di Prisco, G. (in press) Haematological studies on a high-Antartic fish: *Bathydraco marri* Norman. J. exp. mar. Biol. Ecol.

Kunzmann, A.; di Prisco, G. 1990 On the blood physiology of Weddell Sea fishes. 2nd Int. Conf. Biol. Ant. Fishes, Ravello, 30.5-1.6.90.

Kunzmann, A.; di Prisco, G.; Fago, A.; D'Avino, R. in prep. Blood and haemoglobin analysis in a high-Antarctic fish: *Aethotaxis mitopteryx* De Witt.

Larsson, A.; Johansson-Sjöbeck, M.L.; Fänge, R. 1976 Comparative study of some hematological and biochemical blood parameters in fishes from the Skagerrak. J. Fish Biol. 9: 425-440.

Love, R.M. 1980 The chemical biology of fishes. Vol 2, Advances 1968-77. Academic Press, London, 943 pp.

Macdonald, J.A.; Montgomery, J.C.; Wells, R.M.G 1987 Comparative physiology of Antarctic Fishes. Advances in Mar. Biol. 24: 321-388.

Macdonald, J.A.; Montgomery, J.C.; Wells, R.M.G. 1988 The physiology of McMurdo Sound fishes: Current New Zealand research. Comp. Biochem. Physiol. 90B(3): 567-578.

Miller, R.G. 1987 Origins and pathways possible for the fishes of the Antarctic Ocean. Proc. V Congr. europ. Ichthyol., Stockholm, pp. 373-380.

Monod, J.; Wyman, J.; Changeaux, J.P. 1965 On the nature of allosteric transitions: a plausible model. J. molec. Biol. 12: 88-118.

Nikinmaa, M. 1990 Vertebrate red blood cells. Springer, Berlin etc., 262 pp.

Penzlin, H. 1980 Lehrbuch der Tierphysiologie. Fischer Verlag, Stuttgart, New York, 550 pp.

Permitin, Y.Y.; Tarverdiyeva, M.I. 1978 Feeding of fishes of the families Nototheniidae and Channichthydae in the South Orkney islands. Sov. J. Mar. Biol. 4: 619-622.

Perutz, M.F.; Brunori, M. 1982 Stereochemistry of cooperative effects in fish and amphibian haemoglobins. Nature 299: 421-426.

Perutz, M.F.; Kilmartin, J.V.; Nishikura, K.; Fogg, J.H.; Butler, P.J.G.; Rollema, H.S. 1980 Identification of residues contributing to the Bohr effect in human haemoglobin. J. molec. Biol. 138: 649-670.

Piiper, J.; Baumgarten-Schuhmann, D. 1968 Effectiveness of O_2 and CO_2 exchange in the gills of the dogfish (*Scyliorhinus stellaris*). Resp. Physiol. 5: 338-349.

Piiper, J.; Schuhmann, D. 1967 Efficiency of O_2 exchange in the gills of the dogfish *Scyliorhinus stellaris*. Resp. Physiol. 2: 135-148.

Powers, D.A. 1980 Molecular ecology of teleost fish hemoglobins: strategies for adapting to changing environments. Am. Zool 20: 139-162.

Putnam, R.W.; Freel, R.W. 1978 Hematological parameters of five species of marine fishes. Comp. Biochem. Physiol. 61A: 585-588.

Qvist, J.; Weber, R.E.; De Vries, A.L.; Zaphol, W.M. 1977 pH and haemoglobin oxygen affinity in blood from the Antarctic cod *Dissostichus mawsoni*. J. exp. Biol. 67: 767-788.

Rakusa-Suszczewski, S.; Zukowski, C. 1980 Blood of Antarctic fishes: *Notothenia rossii marmorata* Fischer and *Notothenia neglecta* Nybelin. Pol. Polar Res. 1(1): 103-108.

Rankin, J.C.; Johnson, T.P; Kunzmann, A.; Wöhrmann, A.P.A. 1990 Physiological studies on teleost fish. Rep. Polar Res. 68: 144-151.

Riggs, A. 1970 Properties of fish hemoglobins. In: Hoar, W.S.; Randall, D.J. (eds.) Fish Physiology Vol. IV: 209-246, Academic Press, London.

Riggs, A. 1979 Studies of the hemoglobins of Amazonian fishes: an overview. Comp. Biochem. Physiol. 62A: 257-272.

Riggs, A.F. 1988 The Bohr effect. Ann. Rev. Physiol. 50: 181-204.

Romeis, B. 1968 Mikroskopische Technik. Oldenburg Verlag, München, 757 pp.

Root, R.W. 1931 The respiratory function of blood in marine organisms. Biol. Bull. 61: 427-456.

Rossi Fanelli, A.; Antonini, E.; Caputo, A. 1958 Studies on the structure of hemoglobin. I Physicochemical properties of human hemoglobin. Biochim. Biophys. Acta 30: 608-615.

Schininà, M.E.; De Biase, D.; Bossa, F.; Barra, D. 1988 *In situ* treatment of proteins prior sequence analysis. J. Prot. Chem. 7: 284-286.

Schmidt-Nielsen, K. 1986 Animal physiology - adaptation and environments. Cambridge University Press, Cambridge, 619 pp.

Scholander, P.F.; Van Dam, L. 1957 The concentration of hemoglobin in some cold water Arctic fishes. J. cell. comp. Physiol. 49: 1-4.

Schwarzbach, W. 1988 The demersal fish fauna of the eastern and southern Weddell Sea: geographical distribution, feeding of fishes and their trophic position in the food web. Rep. Polar Res. 54: 1-94.

Somero, G.N. 1990 Life at low volume change: hydrostatic pressure as a selective factor in the aquatic environment. Amer. Zool. 30: 123-135.

Steen, J.B.; Berg, T. 1966 The gills of two species of hemoglobinfree fishes compared to those of other teleosts. Comp. Biochem. Physiol. 18: 517-526.

Tetens, V.; Wells, R.M.G.; De Vries, A.L. 1984 Antarctic fish blood: respiratory properties and the effects of thermal acclimation. J. exp. Biol. 104: 269-288.

Tyler, J.C. 1960 Erythrocyte counts and haemoglobin determinations for two Antarctic nototheniid fishes. Standford Ichthyol. Bull. 7: 199-201.

Val, A.L.; Almeida-Val, V.M.F.; Affonso, E.G. 1990 Adaptive features of Amazonian fishes:

haemoglobins, haematology, intraerythrocytic phosphates and whole blood Bohr effect of *Pterygolichthys multiradiatus* (Siluriformes). Comp. Biochem. Physiol. 97B(3): 435-444.

Wells, R.M.; Ashby, M.D.; Duncan, S.J.; Macdonald, J.A. 1980 Comparative study of the erythrocytes and haemoglobins in nototheniid fishes from Antarctica. J. Fish Biol. 17: 517-527.

Wells, R.M.; Jokumsen, A. 1982 Oxygen binding properties of hemoglobins from Antarctic fishes. Comp. Biochem. Physiol. 71B: 469-474.

Wells, R.M.G. 1987 Respiration of Antarctic fish from Mc Murdo Sound. Comp. Biochem. Physiol. 88A(3): 417-424.

Wells, R.M.G.; Baldwin, J. 1990 Oxygen transport potential in tropical reef fish with special reference to blood viscosity and hematocrit. J. exp. mar. Biol. Ecol. 141: 131-143.

Wells, R.M.G.; Grigg, G.C.; Beard, L.A.; Summers, G. 1989 Hypoxic responses in a fish from a stable environment: blood oxygen transport in the Antarctic fish *Pagothenia borchgrevinki.* J. exp. Biol. 141: 97-111.

Wells, R.M.G.; Macdonald, J.A.; di Prisco, G. 1990 Thin-blooded Antarctic fishes: a rheological comparison of the haemoglobin-free icefishes *Chionodraco kathleenae* and *Cryodraco antarcticus* with a red-blooded nototheniid, *Pagothenia bernacchii.* J. Fish Biol. 36: 595-609.

Wells, R.M.G.; Tetens, V.; De Vries, A.L. 1984 Recovery from stress following capture and anaesthesia of Antarctic fish: haematology and blood chemistry. J. Fish Biol. 25: 567-576.

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