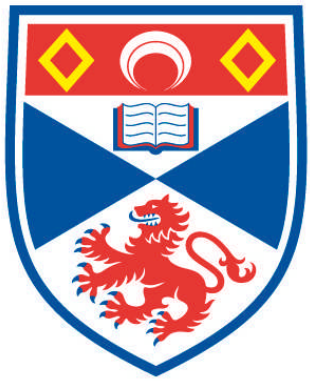
ASPECTS OF THE PHYSIOLOGY AND ANATOMY OF CARDIAC AND SKELETAL MUSCLES IN SOUTH POLAR NOTOTHENIOID FISH

Paul Harrison

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



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ASPECTS OF THE PHYSIOLOGY AND ANATOMY OF CARDIAC AND SKELETAL MUSCLES IN SOUTH POLAR NOTOTHENIOID FISH

A thesis submitted to the University of St. Andrews for the degree of Doctor of Philosophy

by

Paul Harrison

Department of Biology
and Pre-clinical Medicine,
Gatty Marine Laboratory,
University of St. Andrews.

October 1988.

DECLARATION FOR THE DEGREE OF PH.D.

I Paul Harrison hereby certify that this thesis has been composed by myself, that it is a record of my own work, and that it has not been accepted in partial or complete fulfilment of any other degree of professional qualification.

Signed [redacted] Date 10/10/88

I was admitted to the Faculty of Science of the University of St. Andrews under Ordinance General No 12 in October, 1982 and as a candidate for the degree of Ph.D. in October, 1982.

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For my fiancee, Hazel.

ACKNOWLEDGEMENTS

I am grateful to many people who have helped me on the long road leading up to the presentation of this thesis.

Primarily I would like to express my appreciation for the constant help, guidance and enthusiasm provided by my supervisor, Professor Ian Johnston.

My thanks are also due to my friends and colleagues in the Physiology Department and the Gatty Marine Laboratory in the University of St. Andrews; in particular to Drs. Colin Nicol and John Altringham for advice and help with practical work.

Similarly, I must thank my fellow winterers who saw me through 1984 on Signy Island, especially Rick Price and Steve Clarke. Martin White of the British Antarctic Survey has been extremely helpful throughout providing information on the taxonomy and ecology of antarctic fish and advice on fishing techniques. He also took considerable trouble to look after Hazel while I was in the antarctic for which I'll always be grateful.

Final thanks to Hazel for typing the manuscript and for her hard work, support and encouragement.

CONTENTS

ABSTRACT

1. Aspects of the physiology and anatomy of cardiac and skeletal muscle in south polar notothenioid fish have been investigated.

2. Relative ventricle weights for the haemoglobinless Chaenocephalus aceratus (family Channichthyidae) were approximately three times greater than for sympatric 'red-blooded' species. The ventricle in the channichthyid was 'sac-like' in shape and had an entirely trabecular myocardium: these characteristics were associated with low maximum myocardial power outputs (1.46mW(g ventricle weight)$^{-1}$). The blood supply to the ventricle was through the venous lacunary circuit and vessels in the subepicardium.

3. The myoglobin-poor ventricular myocardium in C.aceratus was composed of myocytes and granulated non-contractile cells. Myocardial cytochrome oxidase activity (34μmoles $g^{-1}min^{-1}$) at 0°C was similar to that of warmer-water species with myoglobin-rich ventricles. This suggests that the channichthyid has achieved compensation for the rate depressing effects of the low temperatures and low myoglobin concentrations on oxidative metabolism. Morphometric analyses of the myocytes in C.aceratus indicated that they had higher mitochondrial (0.43) and lower myofibrillar (0.31) volume densities than other teleosts. It was proposed that the proliferation of mitochondria serves to maintain high aerobic capacities by reducing oxygen diffusion distances between the lacunae and the myocytes.

4. The anatomy of the pectoral fin muscles in N.neglecta (family Nototheniidae) has been described. Six muscles (2.49% of total body weight) articulated the fin blade. These were composed of a core of small diameter fibres (18-99μm) which stained intensely for markers of aerobic metabolism. Overlying these was a layer of larger diameter fibres (24-156μm) which stained poorly for these markers. The two fibre types were also differentiated on the basis of their mechanical properties: demembranated preparations of the larger diameter fibres generated approximately twice the tensions and had twice the unloaded contraction velocities of the small diameter fibres. Despite these differences however, the fibres could not easily be differentiated on the basis of the pH stabilities of their myosins.

5. At 1°C the maximum isometric tension (Po) generated by both pectoral fibre types in N.neglecta demonstrated incomplete temperature compensation compared to published values for homologous fibres in warm-water teleosts. Unloaded contraction velocity (Vmax) was not temperature compensated. In addition to Po, adaptive modifications in the curvature of the force-velocity curves were identified in the notothenid which served to enhance power output at low temperatures. Between -5 and +10°C Po was relatively temperature independent (R_{10} = approximately 1.2) whereas the temperature coefficent of Vmax was high (Q_{10} = approximately 2). Following activations in excess of 12°C both fibre types failed to relax completely. This suggests that adaptations in the contractile proteins which conferred high power outputs at low temperatures were

associated with a limitation in the temperature range over which they could maintain function.

6. Simultaneous measurements were made of force generation and myofibrillar ATP hydrolysis during isometric contractions in demembranated white trunk fibres isolated from teleosts adapted to different thermal environments. ATP hydrolysis was quantified using a novel technique which allowed the fibres to be activated in the presence of an ATP regenerating system based on phosphocreatine and creatine phosphokinase. ATP activity was determined by measuring the increase in free creatine in the activating solution. The results obtained indicated that the economy of contraction (tension/ATP hydrolysed) was substantially higher in the fibres from Notothenia neglecta than those from warmer-water species at their preferred body temperatures.

CHAPTER 1

General Introduction.

INTRODUCTION

The experimental work presented in this thesis is concerned with aspects of the physiology and anatomy of cardiac and skeletal muscles in fish of the suborder Notothenioidei - the dominant group of fish in the inshore waters of the Southern Ocean.

In spite of the obvious logistical problems associated with research in the antarctic, these animals have been the subjects of considerable scientific investigation since the mid-nineteenth century (see reviews by Andriashev, 1965, 1987). In recent years the emphasis in inshore antarctic fish biology has shifted from studies of taxonomy and distribution towards a diverse range of physiological and biochemical studies. Interest in the latter stems largely from the extreme conditions in the Southern Ocean, an environment characterized by low, stable temperatures, the presence of ice and extreme seasonality in primary production.

The aim of this introductory chapter is to relate the evolutionary history of the Notothenioidei to the geological development of their environment and, against this background, to review the literature concerning their physiological characteristics.

THE GEOLOGICAL DEVELOPMENT OF THE ANTARCTIC MARINE ENVIRONMENT

Antarctica in Gondwanaland.

The former existence of one or more land masses, formed by close association of the present-day continents, was first postulated 300 years ago, but won wide acceptance only in this century following the comprehensive theories of continental drift proposed by Wegener (1912) (translated into English, 1929) and Du Toit (1937) (see Hume (1948) for the history of the theory of continental drift). Recently, a combination of geological, geomagnetic and paleontological data (reviewed by Craddock, 1982) have shown clearly that Antarctica, South America, Africa, Madagascar, Sri Lanka, India, Australia and New Zealand were once connected to form a protocontinent, now referred to as 'Gondwanaland'.

The origin and early geological history of Gondwanaland is subject to considerable uncertainty due to the scarcity of Precambrian rock outcrops in the southern hemisphere; however, paleomagnetic studies indicate that it had certainly formed by the Ordovician Period (500 m.y. (million years) ago) (McElhinny, 1973). The geometric arrangements of the modern continental land masses in Gondwanaland are shown in Fig.1:1a. Antarctica occupied a southerly position, flanked by South America to the west and Australia to the east.

The break up of Gondwanaland.

The present positions of the southern continents are the result of fragmentation of Gondwanaland and the accretion of crustal material along mid-ocean ridges between the resulting tectonic plates - a process known as 'sea-floor spreading'. The first geological event associated with the fragmentation of Gondwanaland is thought to have occurred 180 m.y. ago (Kyle et al., 1981). However, accurate dating of the separation of one land mass from another has proved difficult. One interpretation of the break up of the protocontinent (after Norton and Sclater (1979)) is shown in Fig.1:1. According to this scheme the first separation occurred between West Gondwanaland (South America-Africa) and East Gondwanaland (India-Antarctica-Australia) (Fig.1:1b). Later, as India became isolated, Antarctica and Australia moved south to higher latitudes (Fig.1:1c). The geographical isolation of Antarctica became complete only after the separation of Australia 53-55 m.y. ago (Sclater and Fisher, 1974; Molnar et al., 1975; Veevers and McElhinny, 1976) and the opening of the Drake Passage between South America and the Antarctic Peninsula 22 m.y. ago (Barker and Burrell, 1977).

Prior to the fragmentation of Gondwanaland antarctic vegetation was predominantly temperate (Kennett, 1977). The change in climatic conditions leading to the current glaciation was the result of the tectonic events described above. However, the high latitude position adopted by Antarctica following the fragmentation of Gondwanaland did not, in itself, initiate climatic cooling: as late as

the Early Eocene (55 m.y. ago), when Antarctica had a definite polar position (Fig.1:1c), the climate remained temperate and surface sea-water temperatures were relatively warm (Kennett, 1977). The decline in sea temperature followed the development of a deep, circumpolar current which occurred after the separation of Australia and South America from Antarctica. This effected the thermal isolation of Antarctica by acting as a barrier to heat flow from sub-tropical warm-water currents.

Marine paleotemperatures have been determined by examining the ratio of ^{18}O to ^{16}O in marine microfossils (Hudson, 1977; Savin, 1977). Fig.1:2 shows antarctic surface water temperatures since the Early Tertiary. The graph illustrates a gradual cooling from approximately 15°C in the Tertiary to recent freezing temperatures. This is reflected in the species composition of benthic core samples: warm-water species occurred in deposits formed 38 million years ago but were replaced in the late Oligocene by cold temperate and cosmopolitan species (Ocean Drilling Program, Leg 114 shipboard scientific party, 1987). It is apparent that cold water (arbitrarily taken to be less than 5°C (Clarke, 1983)) has been a characteristic of the Southern Ocean for only 15 to 20 million years. In geological terms therefore, the present south polar marine environment may be considered to be relatively recent.

The present antarctic ice-cap is thought to have originated in advance of the decline in sea temperatures. Bentley and Ostenso (1961) and Mercer (1973) have suggested

that it may have begun as a temperate ice-sheet at high altitudes on East Antarctica. There is a diversity of opinion concerning the timing of the growth of the ice-cap down to sea-level (see review by Mercer, 1983): the traditional view holds that significant expansion coincided with the thermal isolation of the continent (25 m.y. ago); more recently however, Matthews and Poore (1980) have suggested that an ice-cap, similar in size to the current one, may have existed prior to the Eocene-Oligocene boundary (38 m.y. ago).

THE PRESENT ANTARCTIC MARINE ENVIRONMENT

Extent and bathymetric characteristics of the Southern Ocean.

Strictly defined, the Southern Ocean is bounded by the coastline of Antarctica to the south and the Antarctic Convergence (see below) to the north (Herdman et al., 1956). However, many of the salient hydrographical and bathymetric characteristics of the seas around the continent can be traced much further north and practical definitions of the Southern Ocean usually include most of the region south of the coastal waters of South America, South Africa and Australia.

Essentially, the Southern Ocean is a deep water environment with an average depth of 3700m. The bathymetric features of the region are dominated by three deep basins which surround the continent: the Atlantic-Indian Basin, the

Southern Indian Basin and the South-East Pacific Basin (Fig.1:3). Areas of shallow water are limited to the shelf around Antarctica and to a series of ridges which form the boundaries between the basins (Fig.1:3). Except in the region of the ice shelves (see below) the continental shelf around Antarctica tends to be very narrow (500 to 900m) and, as a result of isostatic subsidence caused by the weight of the continental ice-cap ($24x10^6 km^3$ of ice), it is unusually deep: the break in slope of the continental shelf occurs at 400-500m compared to 200m around other continents (Andriashev, 1965; Denton et al., 1971). The ocean ridges break the surface in places to form the antarctic and sub-antarctic islands. These have provided important dispersal routes for shallow-water organisms but, like the continental shelf, constitute only a very small proportion of the total oceanic area (Fig.1:3).

Horizontal patterns of water movement.

In broad terms, the horizontal pattern of water movement in the Southern Ocean (Fig.1:4) can be correlated with prevailing wind patterns, though density effects may be important locally.

In the narrow region between the continent and about 60°S easterly winds drive the Antarctic Coastal Current which flows from east to west. Further north, westerlies prevail resulting in the Circumpolar Current which flows in the opposite direction, west to east. The Circumpolar Current is generally slow ($15-20 cms^{-1}$) except through the constriction of the Drake Passage. It does extend deep into

the water column however, and transports a greater volume of water than any other oceanic current system. Between the Antarctic Coastal Current and the Circumpolar Current a number of gyres and eddies occur. Most of these are transient in nature, though a major gyre exists in the Weddell Sea which is a constant feature and is responsible for the transport of a significant amount of water (Carmack and Foster, 1975).

Sea-water temperatures and vertical patterns of water circulation.

The thermal characteristics of the Southern Ocean are dominated by low and seasonally stable temperatures (Fig.1:5). Mean surface temperatures range from -1.9°C at McMurdo Sound (78°S) (Littlepage, 1965) to +3°C at South Georgia (54°S) (Everson, 1977). At Signy Island, in the South Orkney group (61°S), the site from which the fish used in the present study were obtained, mean sea-water temperatures range from approximately -2°C in winter to +1°C in summer (British Antarctic Survey, unpublished records).

The vertical patterns of water circulation in the Southern Ocean (Fig.1:6) have been studied in considerable detail because of the relationship between Antarctic Surface Water and the Bottom Water found in other ocean systems (see below). Surface isotherms around Antarctica are not equally spaced: two ocean fronts have been discerned where surface temperatures change steeply with increasing latitude. The most northerly of these is the Antarctic Convergence where cold Antarctic Surface Water sinks beneath less dense

Sub-Antarctic Water to form Antarctic Intermediate Water. The position of the Convergence is not fixed and it may form twists and loops or even isolated rings (Mackintosh, 1946). However, its mean position is well established and occurs between 45 and 60°S (Fig.1:7). The convergence acts as a natural barrier to migration and has a profound effect on the distribution of shallow water organisms at high southern latitudes.

Further south, near the continent, a second front occurs. In this region Antarctic Surface Water flows down the continental slope forming 'Antarctic Bottom Water', which is distributed to the other oceans. Unlike the Antarctic Convergence, this southern front is not a circumpolar phenomenon but is limited to the western Weddell Sea and, to a lesser extent, to the Ross Sea (Carmack, 1977).

Sandwiched between the Antarctic Bottom and Intermediate Water masses is a large body of southerly flowing water called 'Circumpolar Deep Water'. This is characterized by relatively high temperatures, high salinity and high nutrient contents. Circumpolar Deep Water originates in the North Atlantic and its upwelling is responsible for the seasonally high primary productivity at south polar latitudes (see below). In general it does not reach the surface as a single mass but diverges north and south, mixing with the Surface Water and contributing to the Intermediate and Bottom Water masses.

Ice.

Ice occurs in the Southern Ocean as sea-ice and anchor-ice (both of which are frozen sea-water) and glacier-ice (frozen fresh-water of terrestrial origin).

The general pattern and seasonal variation in sea-ice cover has been studied recently using satellite imagery. The ice-front extends north from the continent rapidly in the austral autumn and retreats in the austral spring: minimum ice-cover occurs in late February and reaches a maximum in September. The progression of the ice-front is irregular, particularly in the Weddell Sea where it is influenced by the Weddell Gyre. The presence of sea-ice acts as an insulator to heat transfer across the surface of the ocean and, during formation, it has local affects on salinity and the precipitation of organic material from the water column. From the ecological point of view however, its main influence is as a barrier to light penetration. The northerly extension of the ice-front in the autumn corresponds with the marked decline in incident solar radiation which is a characteristic of high latitudes (El-Sayed, 1971). The combination of sea-ice with snow cover almost entirely blocks the penetration of this limited radiation into the water column. Under these conditions primary production is negligible and only redistribution of carbon to other trophic levels takes place. However, many species of antarctic phytoplankton can survive the darkness associated with seasonal ice-cover in an inactive state. As a result of upwelling, nutrient concentrations in the Southern Ocean do not limit algal growth (El-Sayed, 1985)

and phytoplankton blooms occur directly with the retreat of the ice in spring. Primary production during these blooms is very high: values of $3.6gCm^{-2}day^{-1}$ have been recorded off the Antarctic Peninsula (El-Sayed, 1985). However, since the blooms are limited to periods of open water, the annual primary production is not particularly high: for example, the mean production rate for the Southern Ocean ($0.134gCm^{-2}day^{-1}$) is less than half that in the Gulf of Mexico ($0.289gCm^{-2}day^{-1}$) (El-Sayed, 1985).

Anchor-ice forms beneath sea-ice by aggregation of large platelets of ice crystals on hard substrates in the top 33m of the water column. This can form massive structures which are important habitats for several notothenioid species and many shallow-water invertebrates.

Glacier-ice from the Polar ice-cap flows beyond the coastline of Antarctica to form the Ronne, Ross and Filchner ice-shelves. These are considerably thicker than sea-ice, varying between 50m at the seaward end to 800m at the hinge with the shore. Despite the thickness of the ice-shelves, there is a limited degree of primary production in the underlying water. This is achieved by algae occupying cracks in the ice which reach up to the photic zone. The water pressure under the shelves is greater than in the open sea and the resulting depression of freezing point leads to the presence of very cold water (-2.15°C) (Foster, 1978). The ice-shelves expand seawards by 1m each day. Weathering causes the edges to break off forming icebergs. These may be

up to 100km long and 300m thick. Icebergs and smaller pieces of glacier-ice are often frozen in amongst sea-ice forming rough areas referred to as pack. On average, icebergs take four years to melt and in this time may reach latitudes of 50-60°S where they lower the temperature and decrease the salinity of surface water. However, the principal ecological influence of icebergs occurs when they become grounded in shallow water and destroy the benthic environment.

ORIGINS AND DISPERSAL OF NOTOTHENIOID FISHES

Ancestral notothenioids are represented in the fossil record by a single vertebral centrum discovered in Early Tertiary deposits on Seymore Island off the north-east tip of the Antarctic Peninsula (Smith-Woodward, 1908). In the absence of a broad base of paleontological data, interpretations of the origins and dispersal routes of these fish are based largely on their current distribution and are necessarily speculative.

Extant notothenioid species are mainly benthic in habit (Marshall, 1964): it seems likely therefore that the ancestral stock was also bottom-living (DeWitt, 1971). Miller (1987) has suggested that they evolved along with, or parallel to, emerging percoid fish on the temperate southern coast of Gondwanaland during the Late Cretaceous (approximately 100 m.y. ago). The centrum referred to above indicates that they had diversified from other percoids by

the Early Tertiary (55 m.y. ago). During the Tertiary the fragmentation of Gondwanaland was well established, but Australia and South America were still connected to Antarctica (Fig.1:1c). In view of the fact that there are few notothenioid species in Australia, Andersen (1984) has suggested that, prior to the complete geographical isolation of Antarctica, notothenioids were limited to the Pacific Western and Pacific South American regions of the continent. Dispersal from these sites may have been largely passive since circumcontinental distribution of notothenioids corresponded with the establishment of the circumpolar current systems (Andersen, 1984). However, active swimming (Andriashev, 1965) and tectonic activity (Miller, 1987) may also have been important agents in their dispersal. The diverse notothenioid fauna in New Zealand, for example, originated by rifting of the Campbell Plateau from West Antarctica approximately 80 million years ago (Andersen, 1984). The dispersal of notothenioids to the antarctic and sub-antarctic islands depended on two factors: their distance from the sites inhabited by the ancestral population and their geological age. Thus the South Orkney Islands, which were formed 25 million years ago and lie close to the Antarctic Peninsula have more notothenioid species than Bouvet Island which is approximately 1 million years old and more distant (see Fig.1:7).

Fossil evidence indicates that ancestral notothenioids were part of a diverse antarctic ichthyofauna (see review by Grande and Eastman, 1986). The climatic events associated with the thermal isolation of the continent had a

catastrophic effect on many of these groups: as a result of the decline in sea-water temperatures most of the non-notothenioid fish inhabiting the coastal waters of the antarctic during the Early Cenozoic became extinct. Shallow-water competition to the Notothenioidei in the Southern Ocean has never been re-established, primarily due to the presence of the Antarctic Convergence which represents a formidable thermal barrier to immigration.

In the absence of competition, notothenioids diversified widely and extant species occupy a range of ecological niches (Targett, 1981). Most notably, some species have adopted a secondarily pelagic habit. Two selection pressures appear to have influenced the evolution of pelagism in these fish: the reduction in available shallow-water regions caused by isostatic subsidence and the evolution of large zooplankton populations in the water column. The principal adaptations associated with mid-water swimming in notothenioids are changes in the structure of the caudal fin (Andersen, 1984), improvements in streamlining and colouration (DeWitt and Hopkins, 1977) and buoyancy adaptations. The latter have been investigated extensively by DeVries and Eastman (1978), Eastman and DeVries (1981a, 1981b, 1982, 1985) and by Clarke et al. (1984). In the absence of a swim-bladder most notothenioids are negatively buoyant. Pelagic species have achieved near-neutral buoyancy through reductions in the mineralisation of bones and scales and through the presence of large lipid deposits, particularly in subcutaneous and muscular sites.

PRESENT DAY NOTOTHENIOIDEI: PHYLOGENETIC RELATIONSHIPS AND BIOGEOGRAPHY

Species composition.

The recent antarctic ichthyofauna is dominated by the Notothenioidei which account for 53% of the total species present (Andriashev, 1987). Many notothenioids are endemic to the region (97% for species and 85% for genera). However, the species diversity at high southern latitudes is low and, according to DeVries and Eastman (1981), available niches in the Southern Ocean are under utilized by fish. For example, there are 32 fish families in the arctic compared to 18 in the antarctic (Llano, 1978). Since both of these environments are approximately the same age (Clarke, 1983), other factors must account for the poor species diversity and unique species composition in the antarctic. With regard to the inshore environment, four of these have been identified: (a) the lack of shallow water connections; (b) the lack of south flowing oceanic currents; (c) the extreme environmental conditions; (d) the establishment of the Antarctic Convergence.

Taxonomic relationships.

There are 5 notothenioid families: Bovichthyidae, Harpagiferidae, Bathydraconidae, Channichthyidae (icefish) and Nototheniidae. The first of these occurs mainly outside the Antarctic Biogeographical Region (see below). The last two are of particular interest here since they provided most

of the experimental subjects used in chapters 2 to 6.

There are 10 genera and 16 species of channichthyid. Their distribution ranges from the Patagonia/Falkland Islands region in the north to the coastline of Antarctica. They have attracted considerable physiological interest because they lack haemoglobin (Ruud, 1954); in addition, they possess myoglobin only in low concentrations (Douglas et al., 1985).

The Nototheniidae is the largest and most diverse notothenioid family. It includes 3 subfamilies, 7 genera and some 50 species.

Biogeography.

The first account of the biogeography of inshore antarctic fish (Regan, 1914) has been revised several times to account for the discovery of new species and taxonomic revisions (Norman, 1938; Nybelin, 1947; Andriashev, 1965, 1987). According to the most recent scheme, the Antarctic Region can be sub-divided as follows (Fig.1:8):

Antarctic Region:

I Glacial Subregion:

1. East Antarctic (or Continental) Province.
2. West Antarctic (or Graham) Province.
3. South Georgia Province.

II Kerguelen (transitional) Subregion:

1. Indian-Island Province.

    A. Kerguelen-Heard District.

    B. Marion-Crozet District.

2. Macquarie Province.

The Glacial Subregion includes the coastline of Antarctica, the islands of the Scotia Arc (excluding the Falkland Islands) and Bouvet Island, all of which are within the extreme limits of the pack-ice. Endemic to this subregion are the notothenioid family Bathydraconidae and the genera Trematomus and Artedidraco. The three provinces in this subregion are disparate in terms of their geographical extent and thermal characteristics. The East Antarctic Province encompasses most of the coastline of the continent: it is a high latitude environment, characterized by particularly low temperatures and the presence of permanent ice. In contrast, the South Georgia Province is much less extensive and has less severe environmental conditions (it has higher temperatures and shorter periods of sea-ice formation). The West Antarctic Province has intermediate characteristics. These three provinces have been accorded equal ranking, despite differences in their geographical areas, because 2/5 of the East Antarctic ichthyofauna (by species) is common to West Antarctica and 2/5 of that of West Antarctica is common to the South Georgia Province.

The South Orkney Islands, including Signy Island, the base for much of the work described in this thesis, is

included in the West Antarctic Province. It has a diverse notothenioid fauna encompassing approximately 17 species, of which five are abundant (Fitch, 1986).

The Kerguelen Subregion includes a number of island groups to the north-west and south-west of the continent. They enjoy comparatively mild conditions: mean sea temperatures at 50m depth are between 3 and 6°C (Andriashev, 1965) and they lie outside the influence of sea-ice. As a result there is a high proportion of species of northern origin in this subregion. Biogeographically the Kerguelen Subregion is identified largely by negative characteristics: impoverished representation of Harpagiferidae and Bathydraconidae and an absence of the genus Trematomus. Several species are endemic to particular islands in this subregion but the significance of their distribution is not fully understood (Andriashev, 1987).

PRESENT DAY NOTOTHENIOIDEI - PHYSIOLOGICAL CHARACTERISTICS

With the exception of the study by Ruud (1954) on the haemoglobinless condition in channichthyids (see below), most physiological investigations on the fish of the Southern Ocean have been carried out since the International Geophysical Year of 1957-58. This multi-national initiative was concerned mainly with the atmospheric and earth sciences, however it led to the establishment of many research stations in the antarctic, providing a range of facilities, such as the means to maintain live marine

animals, which had not been available previously.

The literature concerning the physiology of the Notothenioidei published over the past 30 years encompasses a wide range of investigations from whole animal studies of locomotory performance (Montgomery and Macdonald, 1984; Archer and Johnston, 1988) to adaptations in genome organization associated with low environmental temperatures (Haschemeyer, 1985). Three aspects, relevant to the experimental work described in this thesis, are reviewed below: (a) adaptations to low temperatures, (b) oxygen uptake and blood gas transport in the channichthyidae, (c) locomotion and muscle physiology.

ADAPTATIONS TO LOW ENVIRONMENTAL TEMPERATURES

The environmental temperatures experienced by high latitude notothenioids probably represents the extreme low end of the thermal range at which vertebrate ectotherms remain active. The literature concerning the physiology of these fish is dominated by considerations of the effects of low temperature and the mechanisms by which they are overcome.

Two categories of adaptive adjustments to changes in environmental temperature have been defined by Precht (1958). Modifications in the thermal tolerance of physiological processes, including the survival limits of

the whole animal, are referred to as 'resistance adaptations'. 'Capacity adaptations' apply to compensation of rate processes at altered temperatures.

Resistance adaptations.

Many resistance adaptations have been described in notothenioids which allow survival at low temperatures. Some of these, such as reduced haematocrits and structural modifications in proteins and membranes, are discussed elsewhere in this chapter. Resistance adaptations in thermal tolerance and the mechanisms of freezing prevention are considered below.

Thermal tolerance.

The narrow range of temperatures in the Southern Ocean is reflected in relatively narrow ranges of thermal tolerance for the indigenous notothenioids. High latitude species from McMurdo Sound (78°S) can be acclimated to only 4°C (Somero and DeVries, 1967) and relatively low latitude species, such as Notothenia rossii from Signy Island (61°S) can be acclimated to 9°C (O'Neill, 1987). In the wild, the lower lethal temperature for notothenioids is determined by the freezing point of sea-water. In the laboratory however, some species have been supercooled to -6°C (Eastman and DeVries, 1986). Thus only 10°C separate the upper and lower lethal temperatures of high latitude species - a degree of stenothermy which is comparable to that of many endotherms.

Freezing prevention.

Aqueous solutions with the same organic salt

composition as teleost plasma are hypo-osmotic to sea-water and freeze at -0.8 to -1°C. Since even brief exposure to tissue ice formation can prove fatal to fish (Scholander et al., 1957; DeVries and Lin, 1977) this presents a major problem to most antarctic teleosts which experience environmental temperatures considerably lower than the equilibrium freezing points of their body fluids.

Two south polar notothenioids rely on freezing avoidance and have no resistance to tissue freezing. The distribution of Lepidonothen kempi in the Antarctic Region is limited to a subsurface water layer near the Balleny Islands formed by an intrusion of relatively warm sub-antarctic water (DeVries and Lin, 1977). The other species, Trematomus lonnbergi exists in a supercooled state at depths below 500m where there is no environmental ice to initiate tissue freezing (DeVries, 1974). These strategies are not available to most antarctic notothenioids. They depend on the synthesis of glycopeptide antifreeze molecules to prevent tissue ice formation. Eight antifreeze glycopeptides, ranging in size from 2600 to 33700 daltons, have been identified in the cryopelagic nototheniid Pagothenia borchgrevinki (DeVries and Lin, 1977).

The presence of these glycopeptides exerts a profound effect on the physical properties of the body fluids. For example, in P.borchgrevinki they double the freezing point depression of the plasma caused by low molecular weight organic solutes and organic salts (Fig.1:9) (DeVries and Lin, 1977). Two factors suggest that they influence freezing

point in a non-colligative fashion: they occur at relatively low concentrations (up to 4% weight/volume) (DeVries et al., 1970) and they do not affect melting points (DeVries and Lin, 1977).

Raymond and DeVries (1977) have suggested that the glycoproteins act by a mechanism of 'absorbtion/inhibition'. The proteins are thought to bind to developing ice-crystals inducing growth-fronts which are highly curved. The resulting increase in surface-area to volume ratio renders the crystals highly unstable so that they lose water molecules to the bathing medium and thus stop growing. Recent evidence (Eastman and DeVries, 1986) suggests that spontaneous ice formation does not occur in notothenioids until temperatures reach -6°C. The main role of the glycoproteins therefore may be to prevent ice-crystals from the environment seeding tissue ice formation across the integument, gills etc. This would explain the absence of antifreeze molecules from body fluids such as vitreous humour (DeVries, 1978; Ahlgren et al., 1988) which are protected from exposure to external sources of ice.

Capacity adaptations.

Patterns of capacity adaptations.

The responses of biological rate processes to changes in temperature are most often classified according to schemes devised by Precht (1958) and Prosser (1973). Both of these were originally developed in order to describe short-term (acclimatory) adaptations but are also useful for

describing modifications which occur over an evolutionary timescale. Representations of these schemes, modified for application to evolutionary temperature compensation, are shown in Figs.1:10a and b.

Compensation of enzyme activity.

Many of the biochemical reactions which occur in warm-water fish could not proceed at rates sufficient to support life at polar temperatures. It is clear therefore that the activities of those enzymes which catalyse certain critical metabolic pathways in notothenioids must demonstrate a degree of temperate compensation. Such adaptations have been shown to occur in brain acetylcholinesterase (Baldwin, 1971) and in the following muscle enzymes: glyceraldehyde-3-phosphate dehydrogenase (Greene and Feeney, 1970); lactate dehydrogenase, cytochrome oxidase (Feeney and Osuga, 1976; Johnston and Harrison, 1985); carnitine palmitoyl transferase, 3-hydroxyacyl-CoA dehydrogenase (Johnston and Harrison, 1985); creatine phosphokinase, adenylate kinase, AMP deaminase (Dunn and Johnston, 1986); myofibrillar ATPase (Johnston and Walesby, 1977, 1979; Johnston et al., 1975a, 1977b) and sarcoplasmic reticulum ATPase (McArdle and Johnston, 1980a).

The mechanisms which confer high activities on cold adapted enzymes are not fully understood; however, three factors have been identified which may be important in this context.

(a) Increases in enzyme-substrate (E-S) affinity:E-S affinity is inversely related to Km, the substrate concentration at half maximal enzyme activity. The activities of most enzymes are sub-maximal *in vivo* due to low substrate concentrations. Under these conditions increases in E-S affinity could profoundly enhance reaction rates. This is thought to be an important adaptive strategy in eurythermal species exposed to acute temperature changes (Somero, 1969). The role of increased E-S affinity in evolutionary cold-adaptation is less certain: only two notothenioid enzymes, acetylcholinesterase (Baldwin, 1971) and pyruvate kinase (Somero and Hochachka, 1968) have been shown to have E-S affinities which are comparable to those measured in warmer-water species. Selection for high E-S affinities results in enzyme saturation at relatively low substrate concentrations. Somero (1969, 1978, 1983) has suggested that this is disadvantageous in that sudden increases in substrate concentrations cannot be met by increases in enzyme activity. There is, therefore, a tendency towards constancy of E-S affinity in enzymes from species adapted to different temperatures; that is, in most cases, the regulatory role of enzyme function is selected for rather than its catalytic role.

(b) Adaptive modifications in enzyme thermodynamics and molecular structure:Myosins isolated from cold-adapted fish readily undergo side-by-side aggregation (Connell, 1961). The denaturation kinetics

of myofibrillar ATPase from teleost white fibres have been investigated by Johnston and Walesby (1977): at 37°C the average half life of thermal denaturation of ATPase activity ranged from 1.5 minutes in south polar notothenioids to over 500 minutes in fresh-water species adapted to temperatures in excess of 40°C. The SH groups of myosins from the notothenioids also react with 5,5'-dithiobis-2-nitrobenzoic acid significantly faster than those of warmer-water species (Johnston et al., 1975a). These results suggest that the myosins in cold-adapted fish have a more open tertiary structure, stabilised by fewer weak bonds, than those of warmer-water species.

These adaptive modifications in the molecular structure of myosins in notothenioids are associated with favourable thermodynamic characteristics of myofibrillar ATPase. The absolute energy barrier (the Gibbs' free energy of activation, $\Delta G^{\ddagger}$) to the reaction catalysed by this enzyme is slightly lower in south polar notothenioids than warm-adapted species (Johnston and Walesby, 1977). These differences serve to enhance reaction rates in the cold adapted species. However, probably more important than these are interspecific differences in the contributions of enthalpy ($\Delta H^{\ddagger}$) and entropy ($\Delta S^{\ddagger}$) to $\Delta G^{\ddagger}$. These terms are related as follows:

$$\Delta G^{\ddagger} = \Delta H^{\ddagger} - T\Delta S^{\ddagger}$$

where T is absolute temperature. $\Delta H^{\ddagger}$ is the contribution of the kinetic energy of the enzyme molecule to Gibbs' free energy and is directly related to temperature. $\Delta S^{\ddagger}$ is a measure of the energy required to achieve conformational changes in molecular structure during the formation of the E-S complex. Johnston and Walesby (1977) showed that, in cold-adapted species, the contribution of $\Delta H^{\ddagger}$ to $\Delta G^{\ddagger}$ was relatively low whereas that of $\Delta S^{\ddagger}$ was relatively high. Low values of $\Delta H^{\ddagger}$ are clearly adaptive in systems in which environmental temperature is low. Similarly, minimising the changes in molecular structure involved in the reaction (associated with an increased entropic contribution) may also be considered advantageous at low temperatures.

(c) Variations in the physico-chemical environment of the enzymes: The activities of enzymes may be influenced by changes in their local environment rather than, or in addition to, direct changes in their structure. There is some evidence that changes in pH and membrane fluidity are important regulators of enzyme activity in south polar notothenioids.

The substrate binding and catalytic characteristics of many enzymes are influenced by the charge status of ionizing groups within the molecules. In particular, the charge borne by imidazole groups of histidine residues appear to be particularly important in this respect (Hazel et al., 1978). At constant pH,

decreasing temperature increases the dissociation of imidazole. Many ectotherms are thought to regulate the pH of their body fluids to decrease pH by 0.015 to 0.02 pH units per degree centigrade. This opposes the dissociation of imidazole, maintaining constant net protein charges, a process known as 'alphastat control' (Reeves, 1972). The high internal pH apparent in antarctic notothenioids (Qvist et al., 1977) suggests that they employ this strategy. This is supported by data from Yancy and Somero (1978) which indicate that increasing pH is an important mechanism in P.borchgrevinki which serves to maintain values of Km for lactate dehydrogenase which are similar to those of warmer-water species.

The activities of membrane bound enzymes vary with temperature because of changes in the viscosity of the membrane lipid fraction (see Hazel and Prosser, 1974). Compensation for the decrease in viscosity at low temperatures is achieved by increasing the proportion of unsaturated fatty acids in the lipids. This phenomenon, known as 'homeoviscous adaptation' (Sinensky, 1974), occurs in fish acclimated to low temperatures (Cossins, 1977) and over evolutionary time periods (Cossins and Prosser, 1978). The presence of lipids with high unsaturated fatty acid contents in notothenioids (Patton, 1975; Morris and Schneider, 1969; Meyer-Rochow and Pyle, 1980) suggests that it may play an important role in cold-adaptation in these fish.

Metabolic cold adaptation.

Krogh (1916) observed that, in general, ectotherms adapted to warm environments became torpid when exposed to very low temperatures. He reasoned that, if torpor was induced in these animals by a depression of metabolic rate, cold-adapted species must have higher metabolic rates to compensate for the effects of the cold. This concept, known as metabolic cold adaptation (MCA), was investigated by Scholander *et al*. (1953) and Wohlschlag (1960, 1963, 1964) who measured routine oxygen uptake rates at low temperatures in arctic and antarctic teleosts respectively. They compared their results with data obtained for a single goldfish, *Carassius auratus*, acutely exposed to a range of temperatures between 0 and 28°C, referred to as 'Krogh's normal curve' (Krogh, 1914). These comparisons indicated that, in all but one very sluggish antarctic species (*Rhigophila dearborni*) (Wohlschlag, 1963), metabolic rates in cold-adapted teleosts were indeed upwardly displaced compared to data from the goldfish (Fig.1:11). The support which these studies gave to the concept of MCA was largely responsible for its general acceptance.

Holeton (1973, 1974) called the data obtained for cold-adapted species into question when he measured the rates of oxygen consumption in twelve arctic teleosts. With the exception of the arctic cod, *Boreogadus saida*, which had relatively high rates of oxygen uptake, all of Holeton's experimental subjects had substantially lower routine metabolic rates than those previously reported for polar

species (Fig.1:11). In his experiments Holeton took particular care to minimise handling and hypoxic stress. He suggested that the high metabolic rates reported by Scholander et al. (1953) and Wohlschlag (1960, 1964) were artefactual, and were a direct result of these stresses. In support of his results, Holeton (1974) emphasised the energetic advantage to polar ectotherms of incurring low metabolic maintenance costs and suggested that these could be associated with increased metabolic scopes for activity. This argument has been elaborated recently by Clarke (1980, 1983) (see Chapter 7).

More recent studies on channichthyids (see Fig.1:11) and a range of notothenioids from South Georgia (Morris and North, 1984) and McMurdo Sound (Wells, 1987) indicate that there is a wide variation in routine metabolic rates in cold-adapted species including some values which are similar to those obtained by Wohlschlag in his early studies. Measurements of routine oxygen uptake include periods of spontaneous activity. Interspecific differences in the routine oxygen consumption rates of notothenioids appear to be related to activity patterns (Clarke, 1983; Morris and North, 1984). Thus active species may have longer or more frequent bouts of spontaneous activity inside the respirometer resulting in higher rates of oxygen consumption. Future attempts to validate MCA must therefore compare species from different thermal environments with similar activity patterns. Until these experiments are performed MCA cannot be discounted.

Protein Synthesis.

Measurements of protein synthetic rates are usually expressed in terms of the proportion of total protein synthesized per unit time (k_s) or the polypeptide elongation rate (amino acid residues per ribosome per second).

Smith and Haschemeyer (1980) have determined k_s for liver and white trunk muscle in several notothenioids at -1.5°C. The values obtained were lower than for warmer-water species measured at their preferred body temperatures, but higher than expected by extrapolation of these data to low temperatures (Fig.1:12). k_s is dependent on the number of active ribosomes and so does not necessarily provide information on the fundamental mechanisms of protein synthesis in the way that elongation rate does. Elongation rates in hepatocytes from the notothenioid Trematomus hansonii at 0°C are 0.3 residues per ribosome per second (Smith and Haschemeyer, 1980). This value is substantially less than for the toadfish, Opansus tau, near its acclimation temperature of 22°C (1.4 residues per ribosome per second) but higher than the rate for this eurythermal species extrapolated to 0°C (0.04 residues per ribosome per second) (Clarke, 1983). Clarke (1983) has argued that the incomplete compensation of elongation rates and k_s in notothenioids does not indicate that rates of protein synthesis cannot be adjusted for the effects of low temperature. He suggested that protein stability is favoured at polar temperatures and that the apparently low rates of protein synthesis in cold-adapted species simply reflect a reduction in synthesis required to replace degraded protein.

However, protein synthesis in white muscle is generally considered to be related to growth rather than replacement: the low rates of k_s in the muscles of fed notothenioids therefore (Fig.1:12) suggest that there may be some limitation to protein synthesis in notothenioids at low temperatures.

One might expect selection pressures to act on certain proteins to ensure rapid synthesis. This is likely to be the case, for example, with heat-shock proteins which serve to protect cells from acute changes in temperatures (see Schlesinger et al., 1982). Comparison of the rates of synthesis of proteins such as these are required in order to determine unequivocably whether or not complete temperature compensation can be achieved by notothenioids.

OXYGEN UPTAKE AND TRANSPORT IN THE CHANNICHTHYIDAE

The sixteen species in the notothenioid family Channichthyidae (icefish) are unique amongst adult vertebrates in that they lack haemoglobin (Ruud, 1954; Holeton, 1970; Douglas et al., 1973; Hureau et al., 1977). In addition, the respiratory characteristics of these animals are influenced by low intracellular concentrations of myoglobin: the concentrations of this haemprotein in the ventricles of the icefish investigated by Douglas et al. (1985) were approximately 35 times less than in those of the rainbow trout, Salmo gairdneri.

On isolation, icefish blood is translucent with a faint yellow/cream colouration. In the absence of haemoglobin, oxygen is transported in physical solution in the plasma. As a result, the oxygen carrying capacity of icefish blood is only 0.67 vol% compared to approximately 6 vol% for sympatric nototheniids (Ruud, 1954) and 12-17 vol% for temperate teleosts (Prosser, 1973). In spite of these differences, available evidence suggests that the routine metabolic rates of channichthyids are comparable to those of nototheniids (Holeton, 1970) and the low oxygen carrying capacity of their blood has not prevented the diversification of these animals into the pelagic environment: analyses of gut contents indicate that several species, such as Champsocephalus gunnari and Neopagetopsis ionah, consume planktonic euphausiids (Permitin and Tarverdieva, 1978) and must therefore have relatively high metabolic scopes for activity. How then do these animals secure an adequate supply of oxygen for their tissues?

Environmental factors, in particular the high oxygen solubilities in the Southern Ocean, undoubtedly play some part in overcoming the effects of the low oxygen carrying capacity of the channichthyid blood. However, compensation for this depends mainly on cardiovascular adaptations associated with large blood volumes, high blood flow rates and, to a lesser extent, on adaptations which enhance oxygenation of the plasma at the respiratory surfaces.

Cardiovascular characteristics of channichthyids.

Table 1:1 compares the cardiovascular characteristics

of the channichthyid Chaenocephalus aceratus with some other teleost species. Resting cardiac output in the icefish is several times higher than for most other teleosts (Twelves, 1972; Hemmingsen and Douglas, 1977). This is achieved at modest heart rates by means of large stroke volumes (Hemmingsen and Douglas, 1977) which can be directly related to a substantial cardiomegaly: the relative ventricle weights of icefish are typically three times greater than in other south polar species (Everson and Ralph, 1970; Holeton, 1970; Twelves, 1972; Holeton, 1974; Feller et al., 1983; Johnston et al., 1983). In spite of the high cardiac outputs in these fish however, the peripheral resistances, manifested as low ventral aorta blood pressures (Holeton, 1970; Hemmingsen and Douglas, 1977), are less than in other species due to low blood viscosities (Twelves, 1972) and the presence of capillaries with large cross-sectional areas (Steen and Berg, 1966; Fitch et al., 1984).

Adaptations associated with loading of arterial blood with oxygen.

The oxygen saturation of arterial blood in C.aceratus surpasses that of many teleosts with normal blood haemoglobin levels (Holeton, 1970). This is achieved by high branchial flow rates and countercurrent flow patterns between water and blood in the gills (Holeton, 1970). The gills themselves do not appear to be elaborated: the weight specific gill areas in the channichthyid C.aceratus (Hughes, 1966; Steen and Berg, 1966; Holeton, 1976), and Channichthyis rhinoceratus (=C.rugosus) (Jakubowski and Byczkowska-Smyk, 1970) are similar to those of 'red-blooded'

species with similar activity patterns. However, there is evidence that the branchial transfer of respiratory gases in channichthyids is augmented by cutaneous respiration: Hemmingsen and Douglas (1970) have shown that the posterior region of the trunk of C.aceratus consumes oxygen at an appreciable rate. The transfer of blood gases across the channichthyid integument is facilitated by extensive dermal vascularization (Walvig, 1960; Jakubowski and Byczkowska-Smyk, 1970) and a reduction in scales.

In theory, the evolution of these mechanisms to maximise the oxygen saturation of arterial blood may go some way towards offsetting the reduction in absolute oxygen carrying capacity of the blood. However, in C.aceratus at least, this is negated by the maintenance of relatively high venous oxygen tensions (Holeton, 1970). The primary advantage of the high oxygen saturation of the blood in channichthyids therefore may be associated with the development of high blood/tissue oxygen gradients (Holeton, 1970).

LOCOMOTION AND MUSCLE PHYSIOLOGY

Swimming characteristics.

The propulsive mechanisms employed by notothenioids depend on swimming speed. During sustained, low speed swimming, synchronous movements of their pectoral fins are used in a drag-based, labriform mode of locomotion (Robilliard and Dayton, 1969; Twelves, 1972; Montgomery and

Macdonald, 1984; Archer and Johnston, 1988). Burst speeds are achieved by adduction of the pectoral fins and recruitment of the segmental myotomal muscles in subcarangiform swimming (Montgomery and Macdonald, 1984; Archer and Johnston, 1988).

Comparison of pectoral and caudal fin propulsion in fish with similar activity patterns suggests that the former is the least efficient (Webb, 1975a). Pectoral fin swimming is, therefore, usually limited to species inhabiting regions, such as coral reefs and kelp forests, in which manoeuvreability has a greater selective advantage than speed (Blake, 1981). As with many aspects of their biology, the reliance of notothenioids on labriform locomotion must be related to the characteristics of the ancestral stock, though it is not inconsistent with the benthic habits of most extant species. Those notothenioids which have adopted pelagism are an interesting, and possibly unique, group of fish, in which labriform locomotion is employed in the mid-water environment. Given the energetic disadvantages of this swimming mode compared to caudal fin propulsion (Webb, 1975a), it seems unlikely that pelagic notothenioids could have evolved in the presence of competition. Thus the diversification of these animals into the water column can be related directly to the extinction of other teleost groups in the shallow areas of the Southern Ocean during the Early Cenozoic.

With these considerations in mind, it is interesting to note that in the most well-developed pelagic

notothenioid, Pleurogramma antarcticum, there appears to be a tendency towards a reduced contribution of pectoral fin propulsion. Casual examination of this species reveals a reduction in pectoral fin area and a greater development of the trunk musculature than in benthic species such as Notothenia neglecta. In addition, Andersen (1984) has described modifications in the caudal skeleton of P.antarcticum which are consistent with a greater reliance on the tail as a propulsive surface than in most other notothenioids.

Quantitative analyses of the swimming performance of notothenioids are limited to studies on the adult stage of the cryopelagic species P.borchgrevinki (Montgomery and Macdonald, 1984) and the adult (benthic) and juvenile (pelagic) stages of N.neglecta (Archer and Johnston, 1988). In the adults of both species there was a distinct two-geared system with steady low speeds achieved in the labriform mode and steady high speeds in the subcarangiform mode. Intermediate speeds were recorded only during periods of acceleration or deceleration. In the juveniles of N.neglecta however, the two propulsive mechanisms almost overlapped at steady speeds of 2 body lengths per second (Ls^{-1}). The range of length specific steady swimming speeds over which subcarangiform swimming was used in the juveniles was three times greater than in adult N.neglecta. As in P.antarcticum, the greater reliance of the juveniles on subcarangiform propulsion may reflect the energetic advantage of caudal swimming over pectoral swimming in the pelagic environment.

The maximum speeds recorded for adult P.borchgrevinki and N.neglecta in both the labriform and subcarangiform modes (approximately 1.5 and $5Ls^{-1}$ respectively) were comparable to available data for warmer-water species measured at their preferred body temperatures. However, burst swimming in juvenile N.neglecta did not appear to be temperature compensated. Low burst speeds in these fish were associated with low tail beat frequencies (Archer and Johnston, 1988) suggesting that this may be due to incomplete thermal adaptation in the properties of the myotomal muscles.

Muscle fibre types.

Classification of locomotory muscle fibre types in fish.

Early anatomical studies on fish defined two groups of locomotory muscle fibres, termed 'red' and 'white', solely on the basis of their visual appearance (for example, Lorenzini in 1678, quoted in Bone, 1978). These categories are still recognised as the main locomotory fibre types, though a third, 'pink', fibre type has been identified in some species. The major characteristics of red, pink and white fibres are summarised in Table 1:2. Essentially they are differentiated on the basis of their aerobic capacities (demonstrated by enzyme assays or histochemical staining for specific enzymes and substrates) and speed of shortening. Shortening speeds can be measured directly (for example, see Altringham and Johnston, 1982) or indirectly by biochemical determination of myofibrillar ATPase activity (Barany, 1967). There are, in addition, histochemical techniques

which can differentiate fast and slow fibre types (Guth and Samaha, 1969; Johnson *et al.*, 1974).

Complete characterization of the electrophysiological properties of red and white teleost muscle fibres has been achieved only recently. Red fibres are multiply innervated by small diameter myelinated nerve fibres with *en grappe* endings (Bone, 1964). It was thought that they produced only local junction potentials in response to depolarizing pulses (Stanfield, 1972) and were, therefore, similar to the tonic fibres described in other vertebrates (Morgan and Proske, 1984). Tonic red fibres do occur in teleosts (Kilarski and Kozlowski, 1983), however, Altringham and Johnston (1988a) have shown that the bulk of teleost red fibres do, in fact, produce overshooting action potentials and are capable of twitch responses. White fibres are focally innervated in primitive teleosts and multiply innervated in higher groups (Bone, 1964; Bone and Ono, 1982). They are activated by action potentials (Altringham and Johnston, 1988a) and respond to direct stimulation with all-or-none twitch responses (Altringham and Johnston, 1988b).

On the basis of the characteristics outlined in Table 1:2 it appears that the red, pink and white fibres in teleosts are functionally identical to the slow twitch oxidative, fast twitch oxidative glycolytic and fast twitch glycolytic fibres described in higher vertebrates (Peter *et al.*, 1972). However, classification of teleost fibres based on colour persists in the literature and is adhered to in this thesis.

To date, most of the studies on the distribution of locomotory fibre types in fish have concentrated on the axial musculature. In the trunk, red, pink and white fibres are spatially separated. In transverse section (Fig.1:13) red fibres can be seen to occupy a wedge shaped region around the lateral line (the lateral line triangle) and, in some species, a superficial layer encompassing the whole of the trunk. White fibres form the bulk of the musculature with pink fibres, in those species which possess all three fibre types, interposed between the other two. The main exception to this pattern is in scombroids in which substantial internalization of red muscle occurs (Carey and Teal, 1966).

The discrete organization of the fibres in the axial musculature has enabled their pattern of recruitment to be investigated using electromyography. Initial studies on a range of elasmobranchs indicated that the red fibres were responsible for bending movements of the trunk during sustained swimming and that white fibres were recruited at higher speeds which could only be sustained for brief periods (Bone, 1966). These observations can easily be related to the metabolic characteristics of the two fibre types shown in Table 1:2. Red fibres are capable of supporting continuous exercise because they derive ATP from aerobic pathways using both lipids and carbohydrates as fuel, whereas white fibres are anaerobic in character, dependent on anaerobic glycogenolysis, and are limited in the duration of their activity by finite glycogen stores.

The greater bulk of white compared to red axial muscle (Fig. 1:13) reflects the fact that the power required for swimming increases with (velocity)^3 (Webb, 1975b). More recent experiments with teleosts have confirmed that red fibres are recruited at slower swimming speeds than white fibres, though, in these fish, there is some overlap. At the highest sustained speeds in teleosts, white fibres make a significant contribution to bending movements of the trunk (Hudson, 1973; Johnston et al., 1977a; Johnston and Moon, 1980a, b). These observations are consistent with the results of experiments with the saith, Pollachius virens, which showed that exercise training at moderate sustained speeds resulted in hypertrophy of both white and red axial fibres (Greer-Walker, 1971) and with biochemical studies which showed that glycogen was depleted and lactate accumulated in teleost white trunk fibres during sustained swimming (Pritchard, et al., 1971; Johnston and Goldspink, 1973a, b). Recruitment of pink fibres at intermediate swimming speeds has been demonstrated in the carp, Cyprinus carpio (Johnston et al., 1977a).

Fibre types in notothenioids.

Differentiation of notothenioid muscle fibre types on the basis of their colour is difficult because aerobic fibres contain low concentrations of myoglobin, the pigment which confers the red colour on these fibres in other groups (Hamoir and Gerardin-Otthiers, 1980; Walesby et al., 1982). This is particularly marked in the family Channichthyidae in which myoglobin appears to be completely absent from locomotory muscles (Walesby et al., 1982). The problem of

fibre differentiation in these fish is further exacerbated by an apparent inability to identify fast and slow isotypes of myosin ATPase in cold adapted species using standard histochemical techniques (Davison and Macdonald, 1985; Harrison, et al., 1987; Dunn, et al., 1988). However, a growing body of literature, summarised below, suggests that both characteristic red and white fibres do occur in notothenioids.

(a)Red fibres: The aerobic character of notothenioid red fibres has been demonstrated in a number of studies. They have high oxygen uptake rates (Lin et al., 1974; Johnston, 1987b); high aerobic enzyme activities (Walesby and Johnston, 1980; Walesby et al., 1982; Johnston and Harrison, 1985; Johnston, 1987b); stain intensely for histochemical markers of aerobic metabolism (Walesby and Johnston, 1980; Walesby et al., 1982; Davison and Macdonald, 1985; Dunn et al., 1988) and are well supplied by capillaries and have high mitochondrial volume densities (Fitch et al., 1984; Johnston and Camm, 1987).

Johnston (1987b) has compared the mechanical characteristics of isolated, live bundles of red and white fibres from the channichthyid C.aceratus. Red fibres developed only approximately half of the tension and half of the contraction velocities of white fibres.

Both red and white fibres in notothenioids have

larger diameters than homologous fibres from warmer-water teleosts with similar activity patterns (Smialowska and Kilarski, 1981). It has been suggested that this may serve to reduce the energy requirements necessary to maintain electrochemical gradients across the sarcolemmae (Smialowska and Kilarski, 1981). However, in combination with the influences of low environmental temperature and low myoglobin concentrations, large diameters are likely to adversely affect oxygen diffusion into red fibres and so limit aerobic metabolism. Comparison of published data concerning the quantitative ultrastructural characteristics of red fibres from notothenioids and warmer-water species suggests that compensation for this is achieved by increases in mitochondrial volume density (Dunn, 1988; Johnston, 1987b).

(b)White fibres:Notothenioid white fibres are anaerobic. They have a poorly developed vascular supply (Johnston and Camm, 1987), stain poorly for histochemical markers of aerobic metabolism (Walesby and Johnston, 1980; Walesby et al., 1982; Davison and Macdonald, 1985; Dunn et al., 1988) and have low mitochondrial volume densities (Walesby and Johnston, 1980; Johnston and Camm, 1987). The pattern of anaerobic energy supply in these fibres appears to be rather unusual. The activities of glycolytic enzymes in the white muscle of N.rossii, N.neglecta and C.aceratus are all lower than in other teleosts (Walesby and Johnston, 1980; Johnston and Harrison,

1985; Dunn and Johnston, 1986). In addition, both C.aceratus (Hemmingsen and Douglas, 1972) and P.borchgrevinki (Davison et al., 1988) have been shown to have a limited capacity to produce lactate. These observations suggest that the glycolytic capacity of white muscle in notothenioids is relatively low. Instead, ATP for burst work is obtained by the rephosphorylation of ADP from phosphocreatine (PCr) and, to a lesser extent, from the adenylate kinase (AK) reaction which converts 2ADP to ATP and AMP (Dunn and Johnston, 1986). Another enzyme 5'AMP deaminase (AMPD) converts AMP to IMP and so drives the AK reaction in the direction of ATP. The evolution of this pattern of energy supply serves to enhance the capacity for short term, high power output activity (Dunn and Johnston, 1986; Dunn, 1988). However, reliance on endogenous PCr stores imposes a limitation on burst endurance in these fish: Dunn and Johnston (1986) could elicit only 60-80 consecutive tail flips from specimens of N.neglecta and Johnston and Harrison (1985) calculated that the PCr stores in white axial muscle in notothenioids were sufficient for only 50-100 seconds of activity at maximum power output.

Following burst swimming, the intracellular concentration of inorganic phosphate (Pi) in notothenioid white fibres may be in excess of 30mM (Johnston, 1987a). Pi is known to inhibit tension generation in demembranated muscle fibres (Ruegg et al., 1971; Brandt et al., 1982). However, notothenioid

white fibres appear to be relatively insensitive to high concentrations of this metabolite, allowing the maintenance of high levels of contractile performance until PCr stores are exhausted (Altringham and Johnston, 1985b).

Adaptations of notothenioid neuromuscular systems to low temperatures.

Three stages can be discerned in the contraction of skeletal muscle fibres *in vivo*: neural stimulation, calcium release and uptake by the sarcoplasmic reticulum (S.R.), and interactions between the contractile proteins in the myofilament lattice. Macdonald and Montgomery (1982) have developed preparations of the oblique extraocular muscle of *P.borchgrevinki* which allows contractile responses to be investigated following direct or neural stimulation. The high levels of contractile performance demonstrated by these preparations at low temperatures indicate that some degree of thermal adaptation has been achieved in each of the three stages detailed above.

Neurophysiological characteristics of notothenioids.

Both resistance and capacity adaptations have been described in the conduction of impulses in notothenioid peripheral nerves (Macdonald and Wells, 1978). At polar temperatures, nerve conduction in temperate ectotherms is either very slow or blocked completely. In contrast, conduction in isolated, super-cooled nerves from notothenioids can be maintained down to -5°C. Conduction

velocities reported in these preparations at low temperatures demonstrate a high degree of temperature compensation compared to data for south temperate species. However, adaptation to function at low temperatures is associated with a reduction in the upper limit for neural conduction: heat failure in isolated nerve preparations from notothenioids occurs at 28°C compared to 40°C in temperate ectotherms (Macdonald and Wells, 1978). At the neuromuscular junction, partial temperature compensation of both the spontaneous (Macdonald and Montgomery, 1982) and evoked (Pockett and Macdonald, 1986) release of the neuromuscular transmitter acetylcholine are also thought to be important in maintaining neural control over muscle activity in antarctic fish.

Homeoviscous adaptations in nerve cell membranes are thought to underlie the thermal compensation of both conduction velocities and the release of neurotransmitters in notothenioids (Macdonald et al., 1987). This remains to be demonstrated directly, however Macdonald and Montgomery (1986) have shown that the electrical events associated with the release of acetylcholine in the neuromuscular junctions of P.borchgrevinki are characteristic of membranes with high fluidities.

Adaptations in the sarcoplasmic reticulum.

In fish muscles, the control of free Ca^{2+} concentration, and hence activation of the contractile proteins, is achieved largely by the sarcoplasmic reticulum (S.R.). This tubular membrane system sequesters calcium ions

by means of a Ca^{2+} dependent ATPase pump. The rate of relaxation of muscle fibres is largely governed by the activity of this ATPase.

The Ca^{2+} dependent ATPase in the sarcoplasmic reticulum of temperate teleosts has been shown to be highly temperature dependent (McArdle and Johnston, 1980b). In order to maintain contractile function therefore, it is clear that cold-adaptation must be associated with changes in the kinetics of the pump. McArdle and Johnston (1980a) have shown that this is the case: they showed that the activity of the enzyme in S.R. isolated from antarctic species at low temperatures is higher than in temperate and tropical species at their normal range of temperatures. The thermodynamic characteristics of the S.R. ATPase in notothenioids are similar to those described for myofibrillar ATPase (see above).

Adaptations in contractile proteins.

Two approaches have been used in the study of evolutionary adaptations of fish contractile proteins to low environmental temperatures: biochemical studies on the properties of the proteins in isolation (discussed above in relation to enzyme adaptation) and mechanical studies on demembranated muscle fibres. The latter are usually prepared by chemical disruption of the sarcolemma which leaves an intact myofibrillar lattice accessible to ions in the bathing medium. Direct Ca^{2+} activation of the contractile proteins allows their functional properties to be investigated in the absence of nerve and membrane effects.

The characteristics of demembranated fibres have been shown to differ from those predicted by the properties of the contractile proteins in isolation. For example, unloaded contraction velocities (Vmax) of vertebrate muscles are usually directly related to the activity of myofibrillar ATPase (Barany, 1967). However, Johnston and Sidell (1984) have shown that the ATPase activity of isolated myofibrills from the white muscle of Myoxocephalus scorpius has a different temperature dependence to Vmax of demembranated preparations of these fibres. It seems that the properties of the contractile proteins are affected by the constraints imposed by the organization of the myofibrillar lattice (Shriver, 1984). Demembranated fibres, therefore, probably reflect the in vivo properties of the contractile proteins more accurately than isolated preparations.

Isotonic release techniques have been developed which allow direct measurements of both isometric tension (force) and shortening velocity of demembranated fibres at different pre-set loads (Altringham and Johnston, 1982). The relationship between these parameters is usually expressed by Hill's (1938) equation which provides an accurate fit for data obtained for loads below 0.7 to 0.8 Po:

$$V(P + a) = b(Po - P)$$

where: P = tension; Po = maximum isometric tension; V = shortening velocity; a and b are constants.

Maximum power outputs for the fibres occur at tensions equivalent to $(a^2+a)^{\frac{1}{2}} - (a/Po)$ and are the product of tension and velocity. Studies on fibres from a number of south polar notothenioids (Johnston and Altringham, 1985; Johnston and Harrison, 1985) indicate that maximum power output demonstrates a high degree of temperature compensation compared to that of warmer-water species measured at their preferred body temperatures. For example, the maximum power output for demembranated white fibres from C.aceratus at 0°C is $23Wkg^{-1}$ (Johnston and Altringham, 1985), compared to $16Wkg^{-1}$ for the temperate cod, Gadus morhua, at 8°C (Altringham and Johnston, 1982) and 37 and $55Wkg^{-1}$ in the tropical marlin, Makaira nigricans, at 15 and 25°C respectively (Johnston and Salamonski, 1984).

The mechanisms through which notothenioids achieve cold-adaptation in contractile function are discussed fully in Chapter 5. However, they appear to depend principally on adaptations in the contractile proteins associated with force generation.

Table 1:1. Resting cardiovascular parameters in notothenioids and other teleosts.

SPECIES	BLOOD VOLUME (%)	BLOOD PRESSURE (mmHg) Dorsal aorta	BLOOD PRESSURE (mmHg) Ventral aorta	CARDIAC OUTPUT (ml kg/min)	HEART RATE (beats/min)
Salmo gairdneri (Rainbow trout)[a]	1.6	29-25	40-32	16	46
Gadus morhua (Atlantic cod)[b]	1.8	-	29-18	9	30
Katsuwomus pelamis (Tuna)[c]	-	85-80	-	80.	70
Notothenia gibberifrons[d]	-	10-7.	-	-	16
Notothenia neglecta[d]	5.7	-	-	-	15
Chaenocephalus aceratus[e]	9.2	17-11	20-12	66-104	17

References:

a. Stevens and Randall (1967).
b. Prosser (1973).
c. Stevens (1972).
d. Holeton (1970).
e. Hemmingsen and Douglas (1977).

Table 1:2. Distinguishing characteristics of red, pink and white twitch fibres in teleosts (see text for references).

	RED	PINK	WHITE
AEROBIC CAPACITY	High	Intermediate	Low
Myoglobin content	high	intermediate	low
Oxidative enzyme activity	high	intermediate	low
Glycolytic enzyme activity	low	intermediate	high
Mitochondrial content	high	intermediate	low
Capillary supply	extensive	?	low
Recruitment	frequent	intermediate	infrequent
Fatigue time	long	intermediate	short
CONTRACTION VELOCITY	slow	intermediate	fast
Myofibrillar ATPase activity	low	intermediate	high
Alkali stability of M. ATPase	labile	intermediate	stable
TENSION GENERATION	low	intermediate	high
Myofibrillar content	low	intermediate	high

Fig.1:1. Stages in the dispersal of the constituent continents within Gondwanaland. **a:** geometric organization of the land masses 180 m.y. ago, prior to fragmentation. **b:** 115 m.y. ago. **c:** 53 m.y. ago. **d:** 39 m.y. ago. **e:** present configuration of the southern continents. Labelling is shown in Fig.1:2e. Heavy lines show axes of mid-ocean ridges.

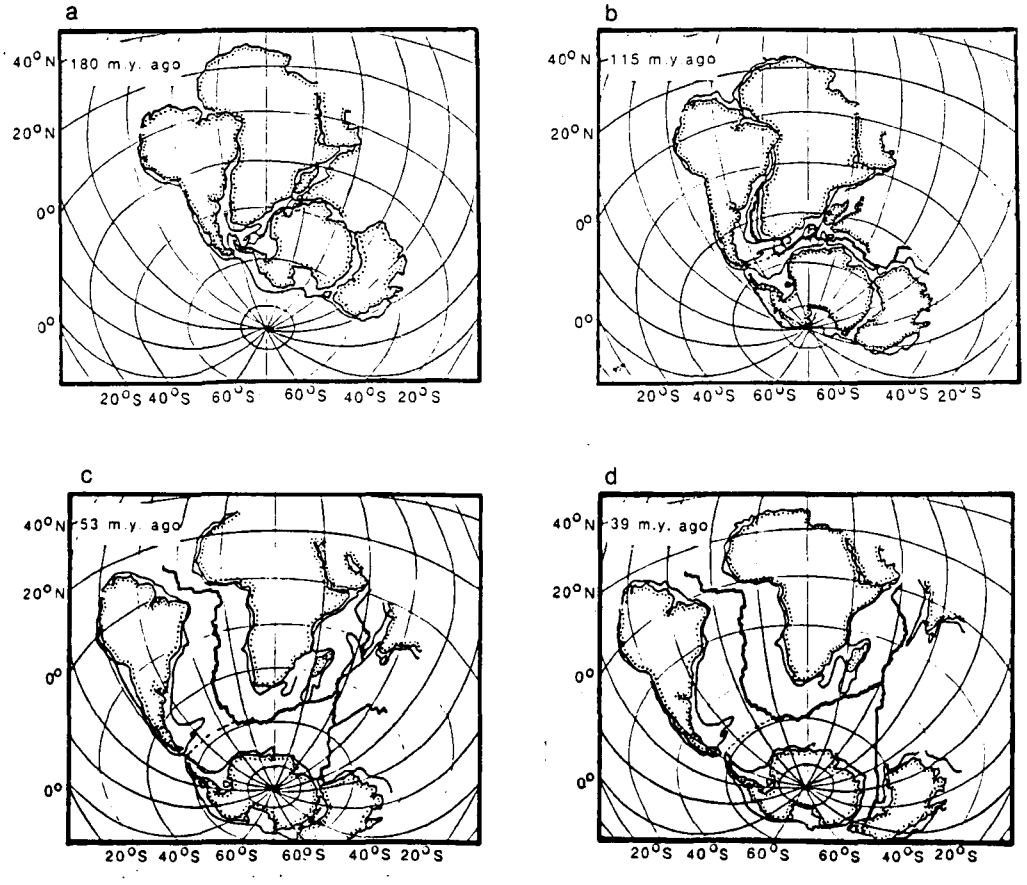

a
180 m.y. ago
40°N
20°N
0°
0°
20°S 40°S 60°S 60°S 40°S 20°S
b
115 m.y ago
40°N
20°N
0°
0°
20°S 40°S 60°S 60°S 40°S 20°S
c
53 m.y. ago
40°N
20°N
0°
0°
20°S 40°S 60°S 60°S 40°S 20°S
d
39 m.y. ago
40°N
20°N
0°
0°
20°S 40°S 60°S 60°S 40°S 20°S

Fig.1:1. Continued.

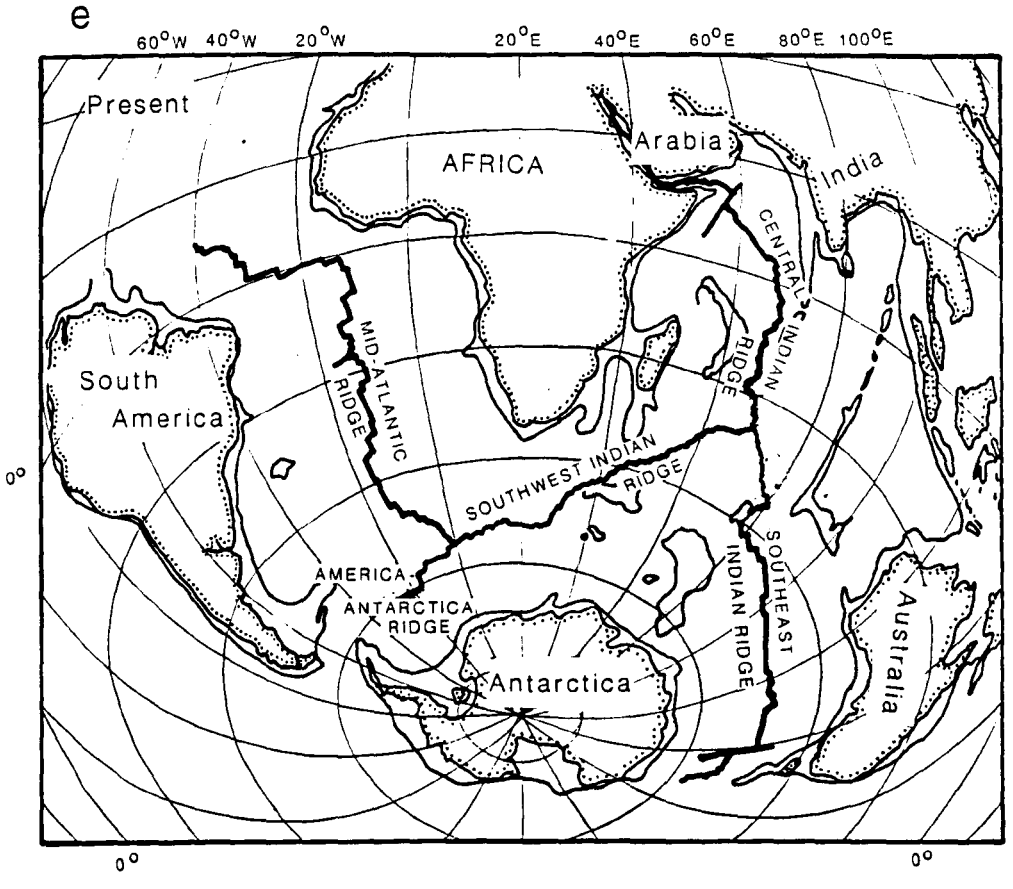

e
60°W 40°W 20°W
20°E 40°E 60°E 80°E 100°E
Present
AFRICA
Arabia
India
South America
MID-ATLANTIC RIDGE
CENTRAL INDIAN RIDGE
SOUTHWEST INDIAN RIDGE
SOUTHEAST INDIAN RIDGE
AMERICA-ANTARCTICA RIDGE
Antarctica
Australia
0°
0°
0°

Fig.1:2. Paleotemperatures in the Southern Ocean over the past 55 million years derived from oxygen isotope ratios in deep sea microfossil cores (after Shackleton and Kennet, 1975).

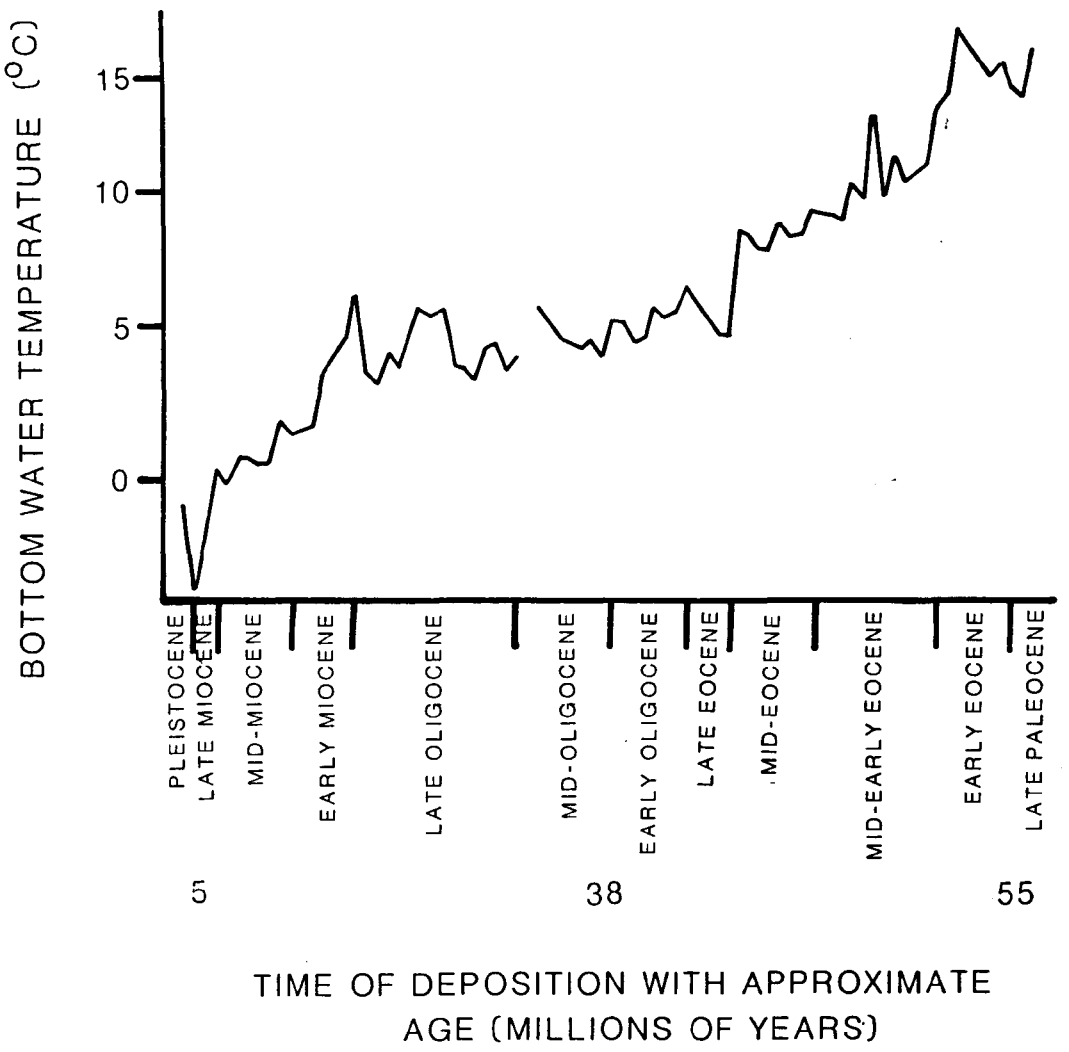

BOTTOM WATER TEMPERATURE (°C)
15
10
5
0
PLEISTOCENE
LATE MIOCENE
MID-MIOCENE
EARLY MIOCENE
LATE OLIGOCENE
MID-OLIGOCENE
EARLY OLIGOCENE
LATE EOCENE
MID-EOCENE
MID-EARLY EOCENE
EARLY EOCENE
LATE PALEOCENE
5
38
55
TIME OF DEPOSITION WITH APPROXIMATE
AGE (MILLIONS OF YEARS)

Fig.1:3. Principal bathymetric features of the Southern Ocean. Stippled region shows ocean ridges and continental shelves. I = Mid-Atlantic Ridge. II = Atlantic-Indian Rise. III = Prince Edward Island and Crozet Island. IV = Kerguelen-Gaussberg Ridge. V = Indian-Antarctic Ridge. VI = Pacific-Antarctic Ridge. VII = Scotia Arc.

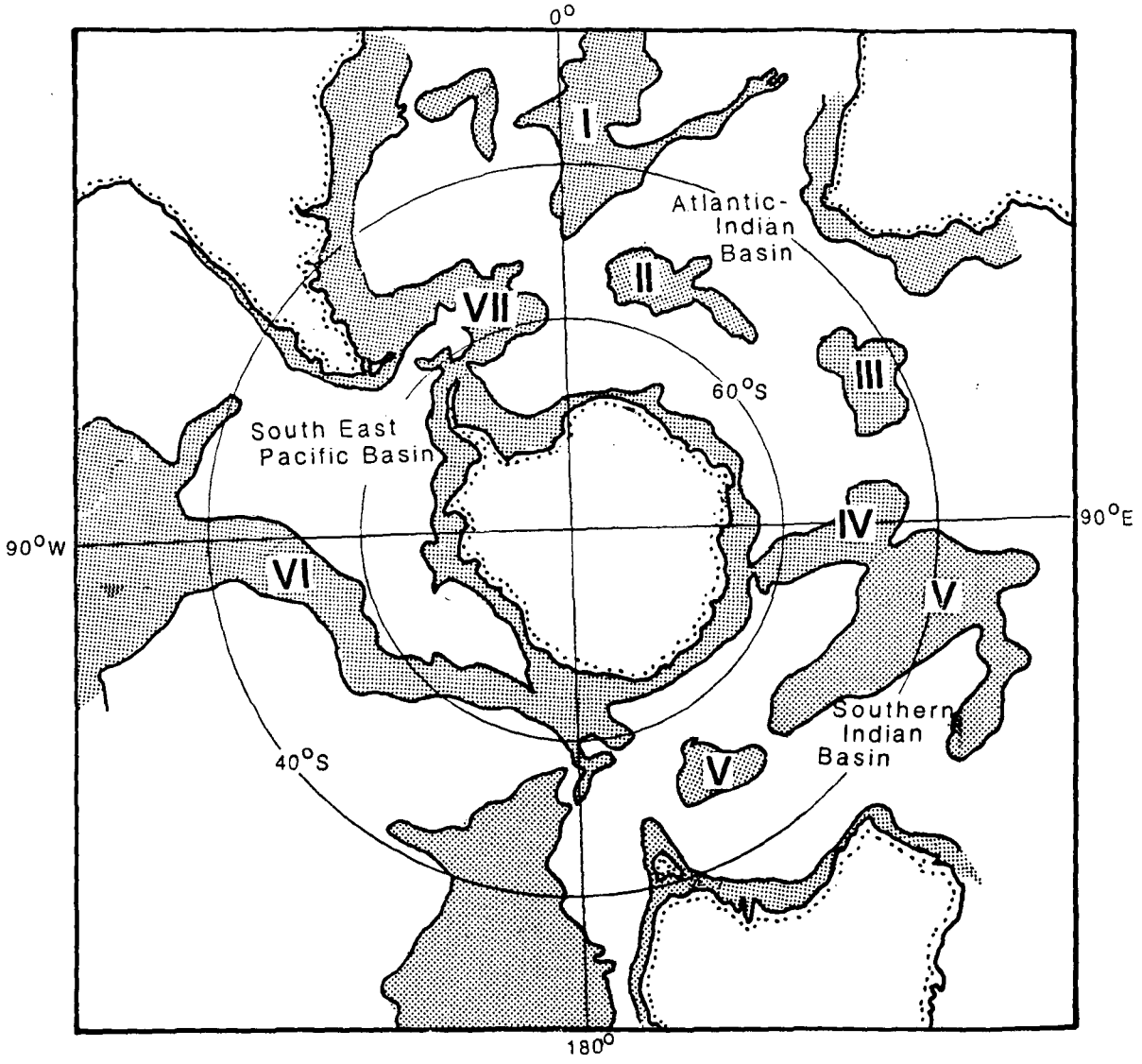

0°
I
Atlantic-
Indian
Basin
II
VII
III
60°S
South East
Pacific Basin
90°W
90°E
IV
VI
V
Southern
Indian
Basin
40°S
V
180°

Fig.1:4. Principal characteristics of the surface circulation of the oceans south of latitude 30°S. W.G. = Weddell Gyre. (After Pittock *et al.*, 1978).

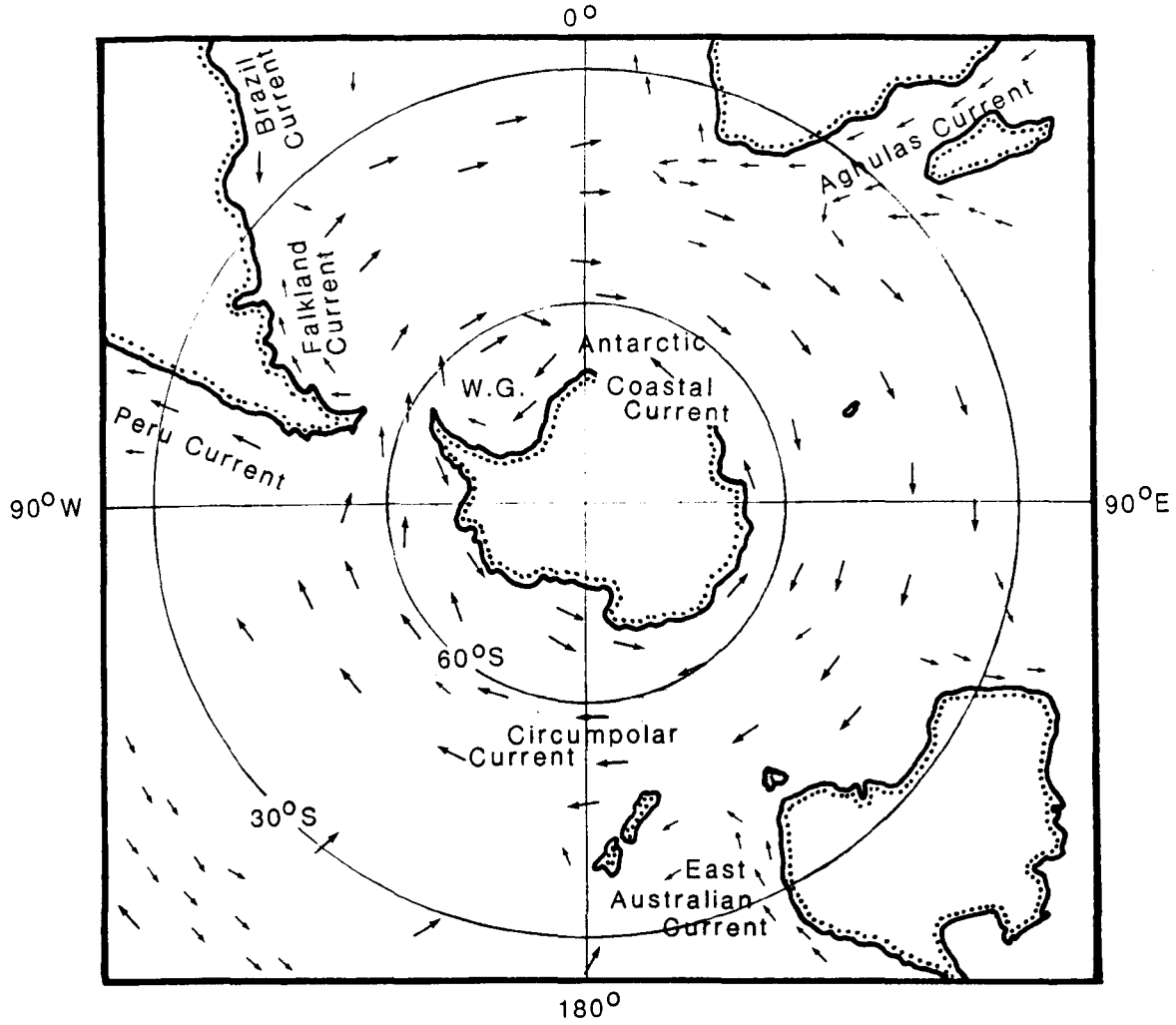

0°
Brazil Current
Falkland Current
Agulas Current
Antarctic Coastal Current
W.G.
Peru Current
90°W
90°E
60°S
Circumpolar Current
30°S
East Australian Current
180°

Fig.1:5. Surface sea-water temperatures in the Southern hemisphere. **a:** Average zonal temperature distribution (from Wust *et al.*, 1954). **b:** Annual temperature range (from Sverdrup *et al.*, 1942).

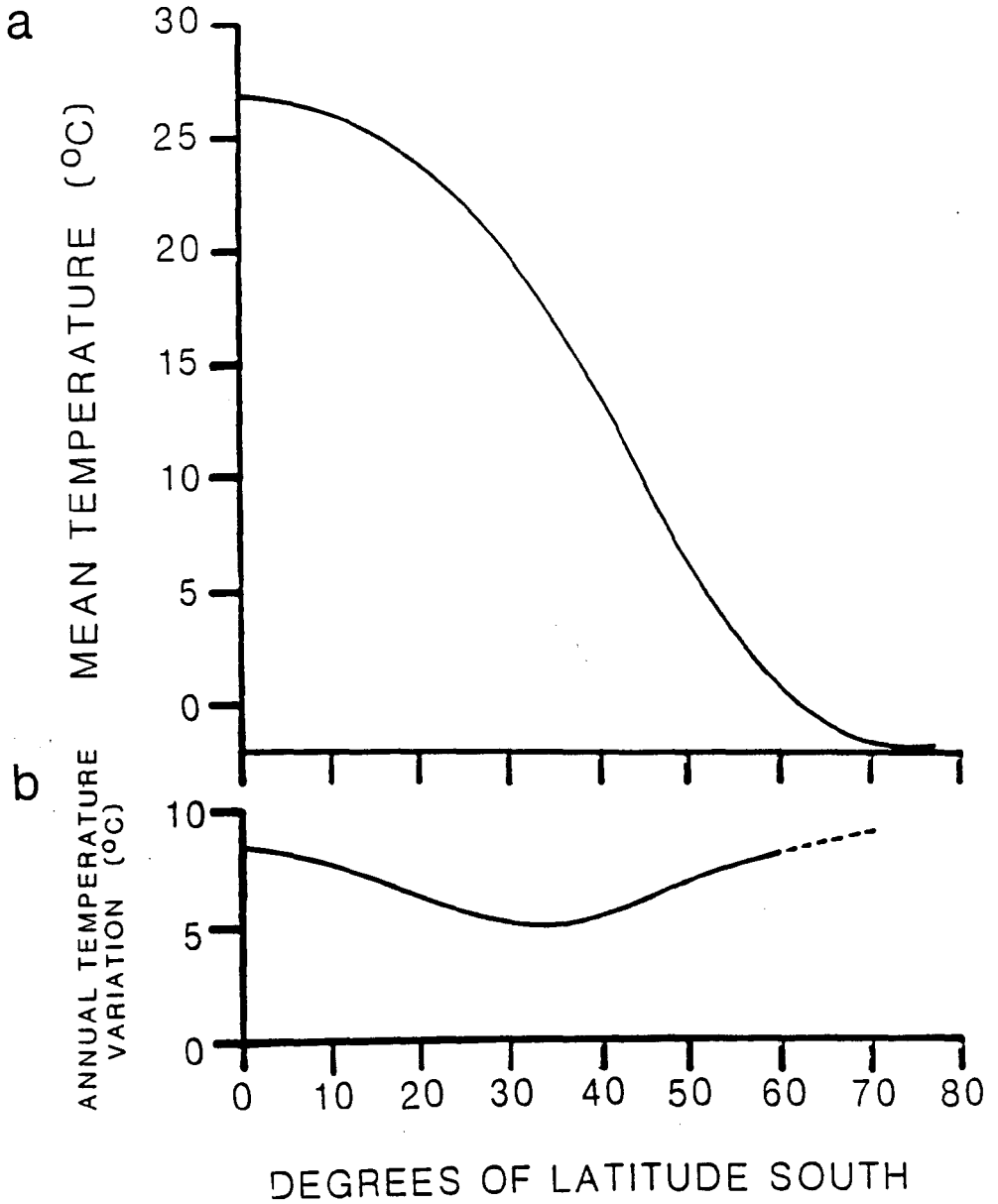

a
30
25
20
15
10
5
0
MEAN TEMPERATURE (°C)
b
ANNUAL TEMPERATURE VARIATION (°C)
10
5
0
0
10
20
30
40
50
60
70
80
DEGREES OF LATITUDE SOUTH

Fig.1:6. Schematic representation of the currents and water masses in the Southern Ocean. (From El-Sayed, 1985).

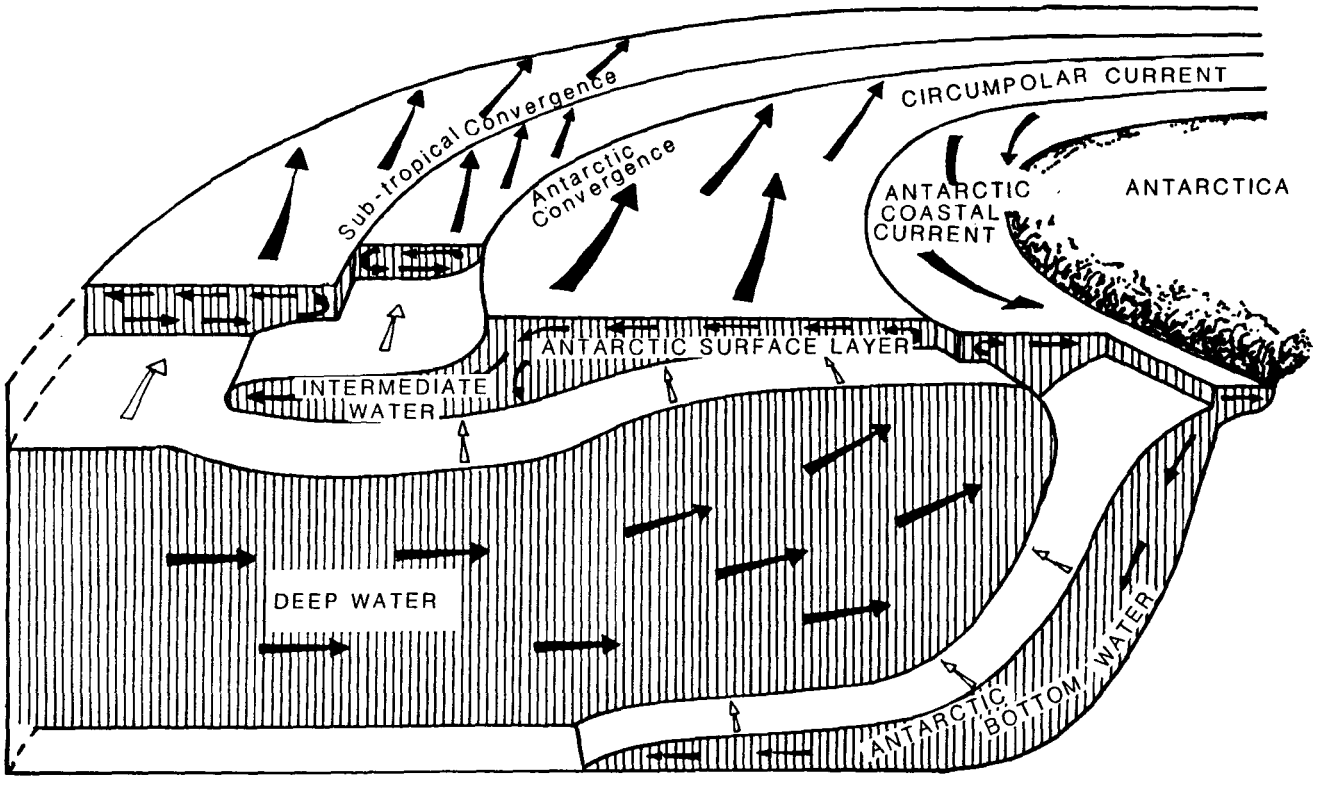

Sub-tropical Convergence
Antarctic Convergence
CIRCUMPOLAR CURRENT
ANTARCTIC COASTAL CURRENT
ANTARCTICA
ANTARCTIC SURFACE LAYER
INTERMEDIATE WATER
DEEP WATER
ANTARCTIC BOTTOM WATER

Fig.1:7. Geographic features of the Southern Ocean referred to in the text.

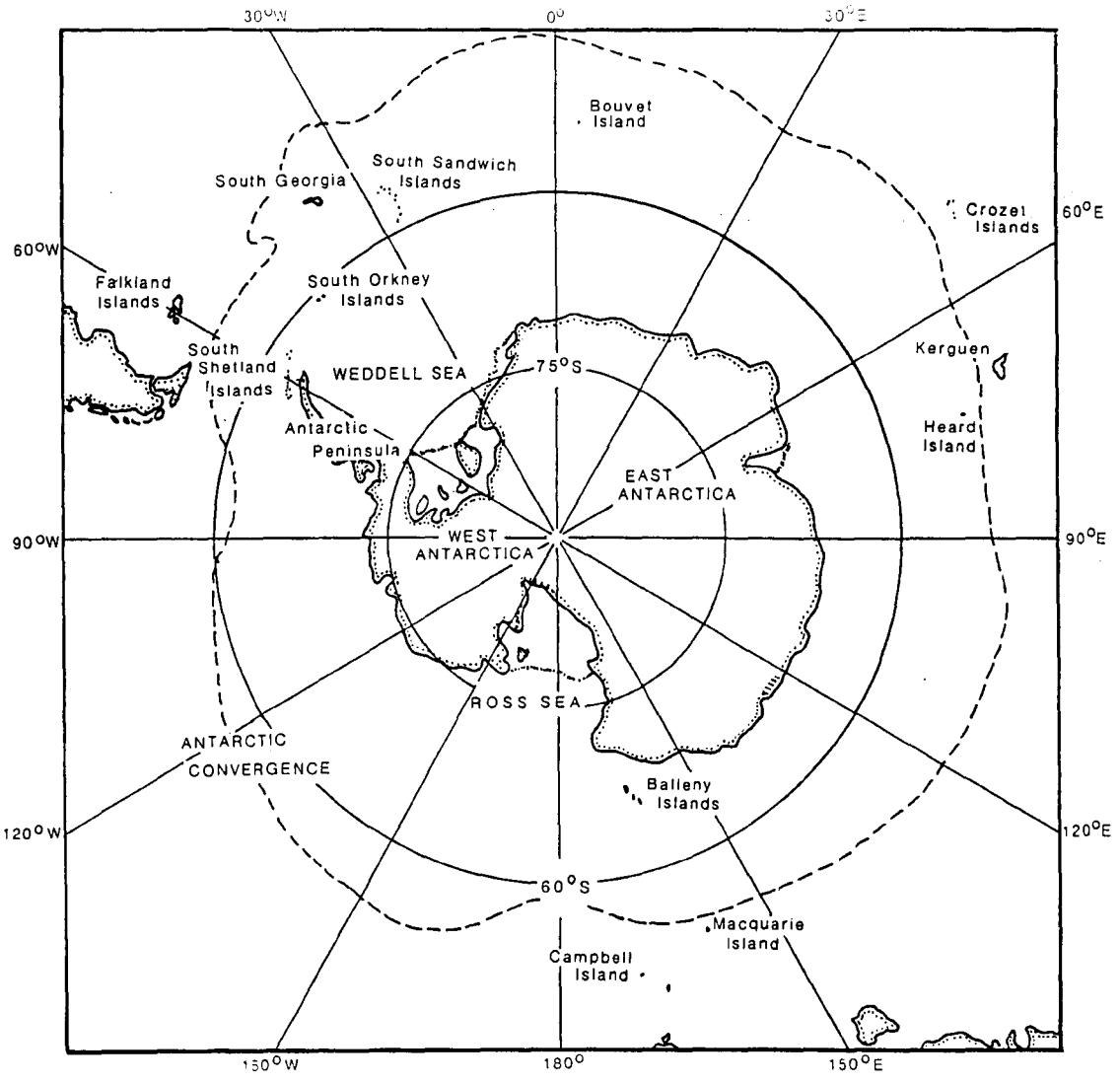

30°W
0°
30°E
Bouvet Island
South Sandwich Islands
South Georgia
Crozet Islands
60°E
60°W
Falkland Islands
South Orkney Islands
South Shetland Islands
WEDDELL SEA
75°S
Kerguen
Heard Island
Antarctic Peninsula
EAST ANTARCTICA
90°W
WEST ANTARCTICA
90°E
ROSS SEA
ANTARCTIC CONVERGENCE
Balleny Islands
120°W
120°E
60°S
Macquarie Island
Campbell Island
150°W
180°
150°E

Fig.1:8. Biogeographical distribution of inshore fishes in the Antarctic Region: I Glacial Subregion (1. East Antarctic Province; 2. West Antarctic Province; 3. South Georgia Province). II Kerguelen Subregion (4. Indian Island Province (A. Kerguelen- Heard District; B. Marion-Crozet District); 5. Macquarie Province). (From Andriashev, 1987).

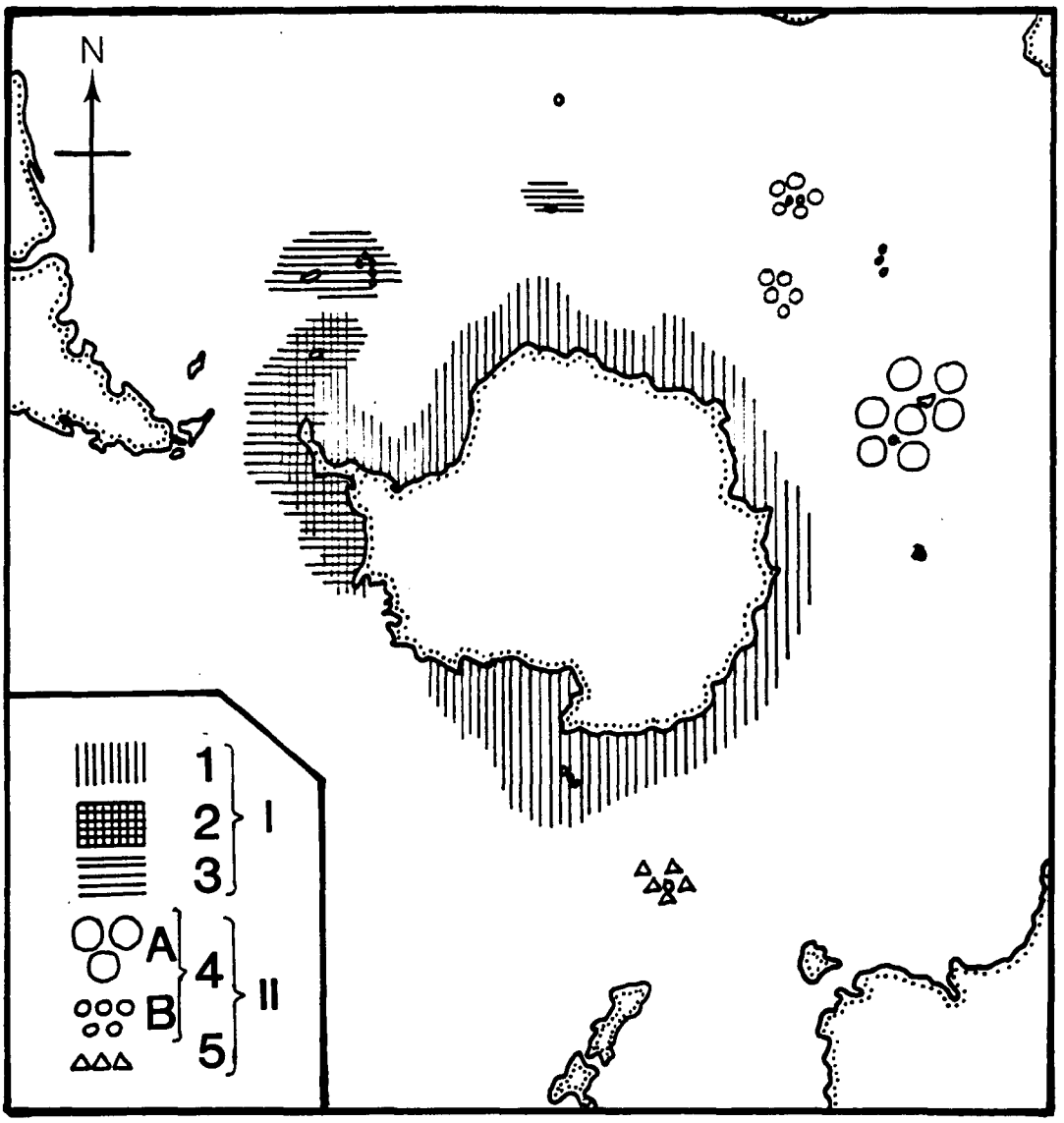

N
1
2
3
I
A
4
B
II
5

Fig.1:9. Contribution of various solutes to the depression of freezing points of blood plasma drawn from warm-water and south polar teleosts. The importance of glycopeptides in notothenioids is apparent. These have high molecular weights and account for the high freezing point depression in notothenioid plasma following dialysis. (From Eastman and DeVries, 1987).

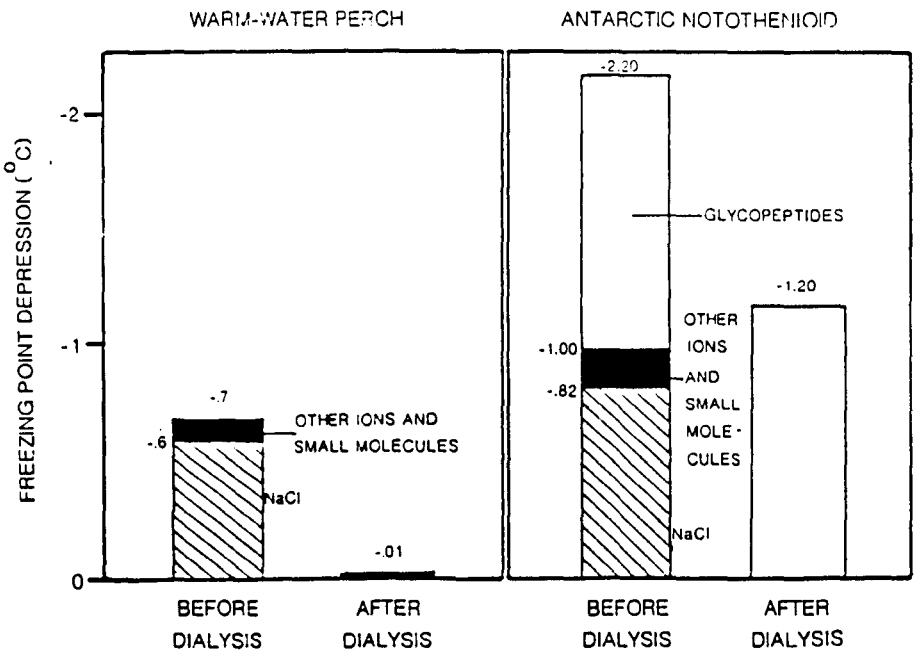
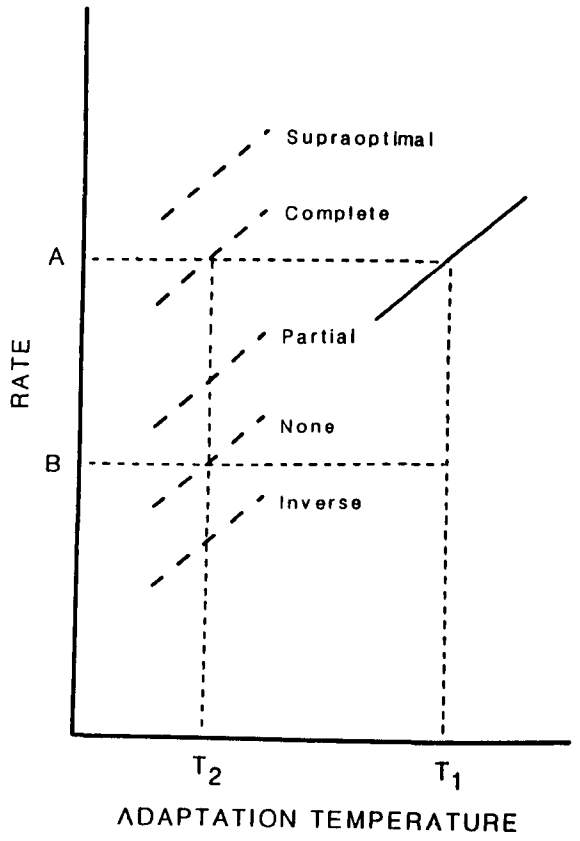

WARM-WATER PERCH
ANTARCTIC NOTOTHENIOID
FREEZING POINT DEPRESSION (°C)
-2
-1
0
-.7
-.6
OTHER IONS AND SMALL MOLECULES
NaCl
-.01
-2.20
GLYCOPEPTIDES
-1.20
-1.00
-.82
OTHER IONS AND SMALL MOLE-CULES
NaCl
BEFORE DIALYSIS
AFTER DIALYSIS
BEFORE DIALYSIS
AFTER DIALYSIS

Fig.1:10. Patterns of capacity adaptations in ectotherms. **a:** Scheme devised by Precht (1958) applied to evolutionary adaptation. The solid line represents the effect of temperature on a rate process of an ectotherm adapted to a mean temperature T_2. At T_2 the rate = A. The dashed lines show possible effects of temperature on the same rate process in an ectotherm adapted to a lower temperature T_2. If the rate in the second ectotherm at T_1 = B then adaptation is complete; if the rate at T_1 = A then there is no compensation. Other possibilities are shown. **b:** Scheme devised by Prosser (1976). The rate-temperature curves for cold- and warm-adapted ectotherms (c and w respectively) are shown.

a


Supraoptimal
Complete
A
Partial
RATE
None
B
Inverse
T_2
T_1
ADAPTATION TEMPERATURE

b

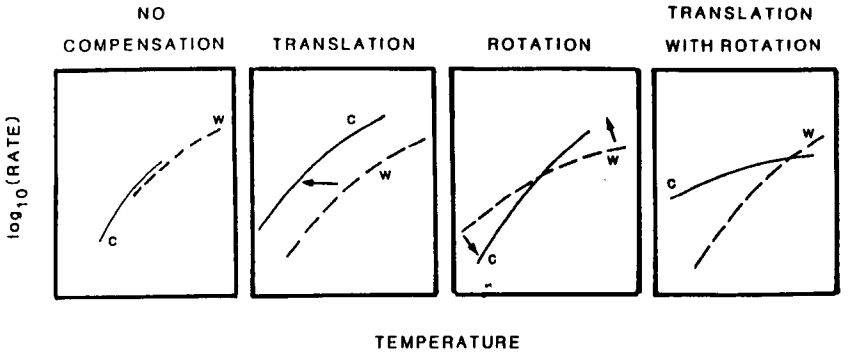

NO COMPENSATION
TRANSLATION
ROTATION
TRANSLATION WITH ROTATION
$\log_{10}$(RATE)
W
C
C
W
C
W
C
W
TEMPERATURE

Fig.1:11. Oxygen consumption rates in teleosts adapted to different environmental temperatures. The high values of oxygen uptake for cold-adapted species reported by Wohlschlag (1960, 1964) and Scholander *et al.* (1953) support the concept of metabolic cold adaptation. Hatched area A, shows scatter of data for the antarctic species *Trematomus bernacchii* reported by Wohlschlag. The data obtained for polar species by Holeton (1973, 1974) are similar to temperate species. Hatched ares B and C show respectively the scatter of data for south polar channichthyids (Ralph and Everson, 1968; Robilliard and Dayton, 1969; Holeton, 1970; Hemmingsen and Douglas, 1970, 1977; Hureau *et al.*, 1977) and the south polar zoarcid, *Rhigophila dearborni* (Wohlschlag, 1963). (Figure from Macdonald *et al.*, 1987).

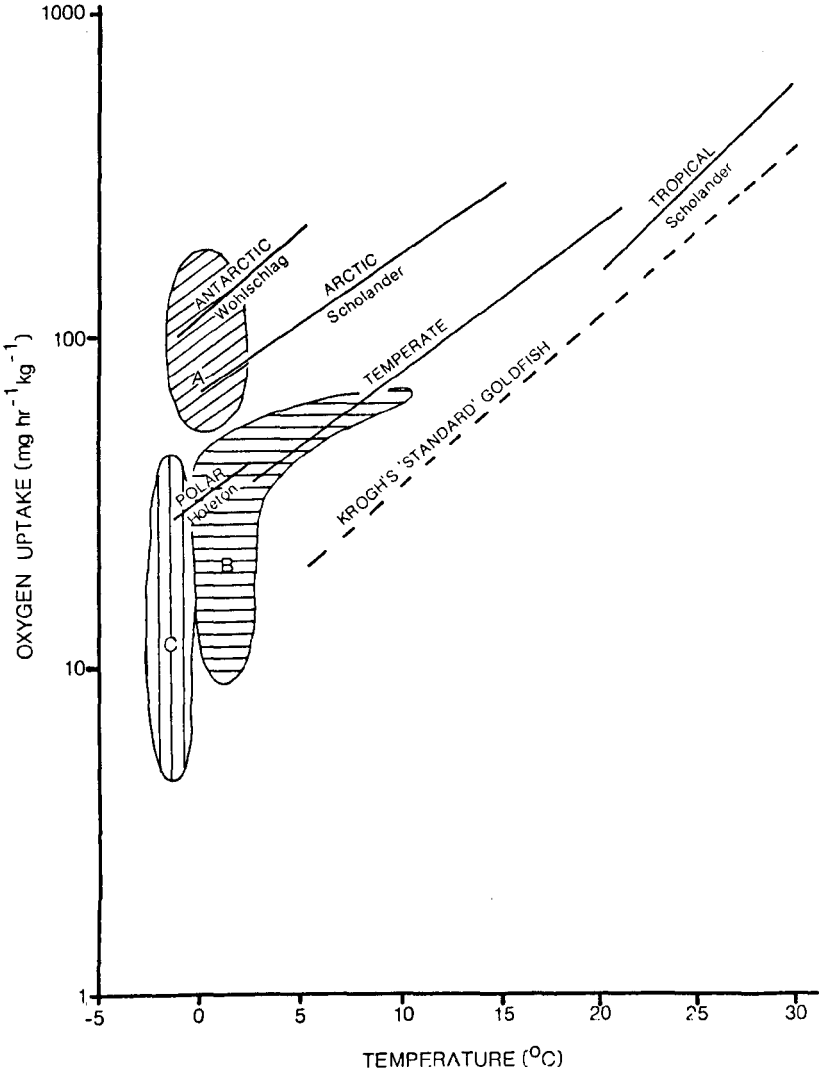

1000
100
10
1
OXYGEN UPTAKE (mg hr^{-1} kg^{-1})
-5
0
5
10
15
20
25
30
TEMPERATURE (°C)
ANTARCTIC
Wohlschlag
ARCTIC
Scholander
TEMPERATE
TROPICAL
Scholander
KROGH'S 'STANDARD' GOLDFISH
POLAR
Holeton
A
B
C

Fig.1:12. Protein synthetic rates (percentage of tissue protein synthesized per day, K_s) *in vivo*. Symbols on the upper part of the graph refer to measurements in liver; lower symbols refer to white epaxial muscle. Species are as follows: (□) = rat and mouse at 37°C (Haschemeyer, 1976; McNurlan *et al.*, 1979); (●) = brown triggerfish at 20°C and 30°C (Haschemeyer *et al.*, 1979); (○) = striped mullet at 26°C (Haschemeyer and Smith, 1979); (Δ) = rainbow trout at 12°C (Smith, in preparation); (▲) = Antarctic fishes at -1.5°C (Smith and Haschemeyer, 1980). Theoretical lines are shown for the temperature dependency of protein synthesis (Q_{10} = 2.5) based on studies in toadfish (Mathews and Haschemeyer, 1978). The data indicate incomplete temperature compensation of protein synthesis in both tissues of the notothenioids at low temperatures. (Figure from Smith and Haschemeyer, 1980).

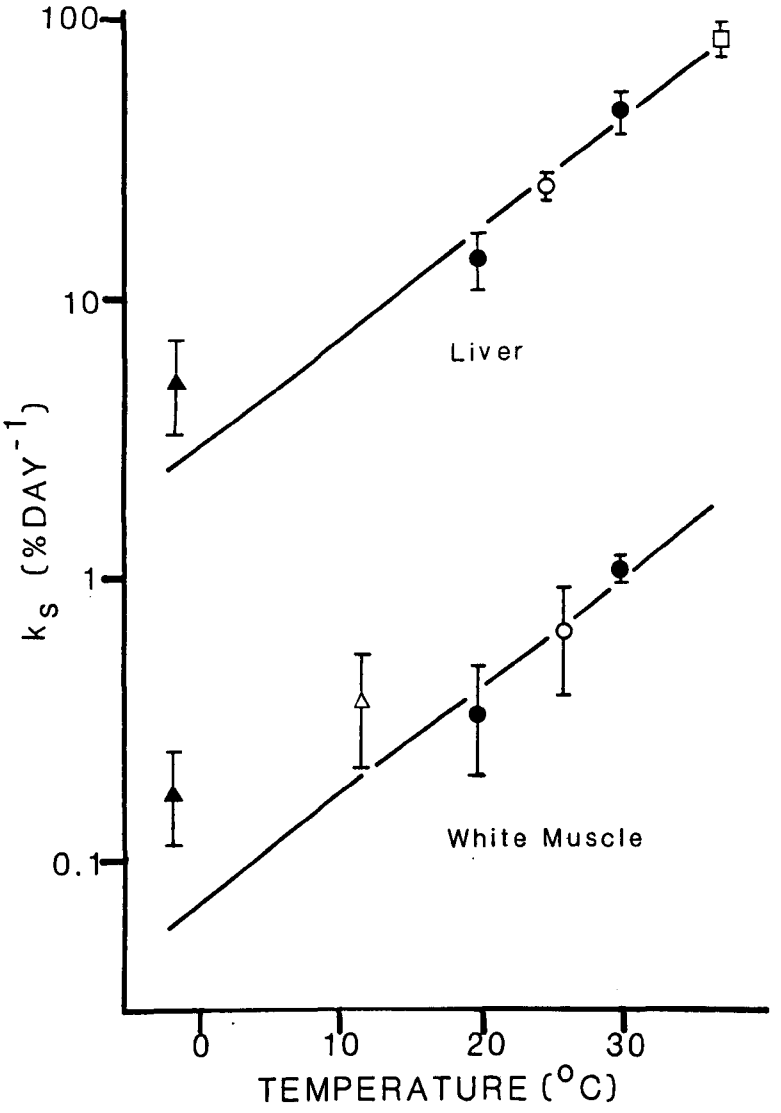
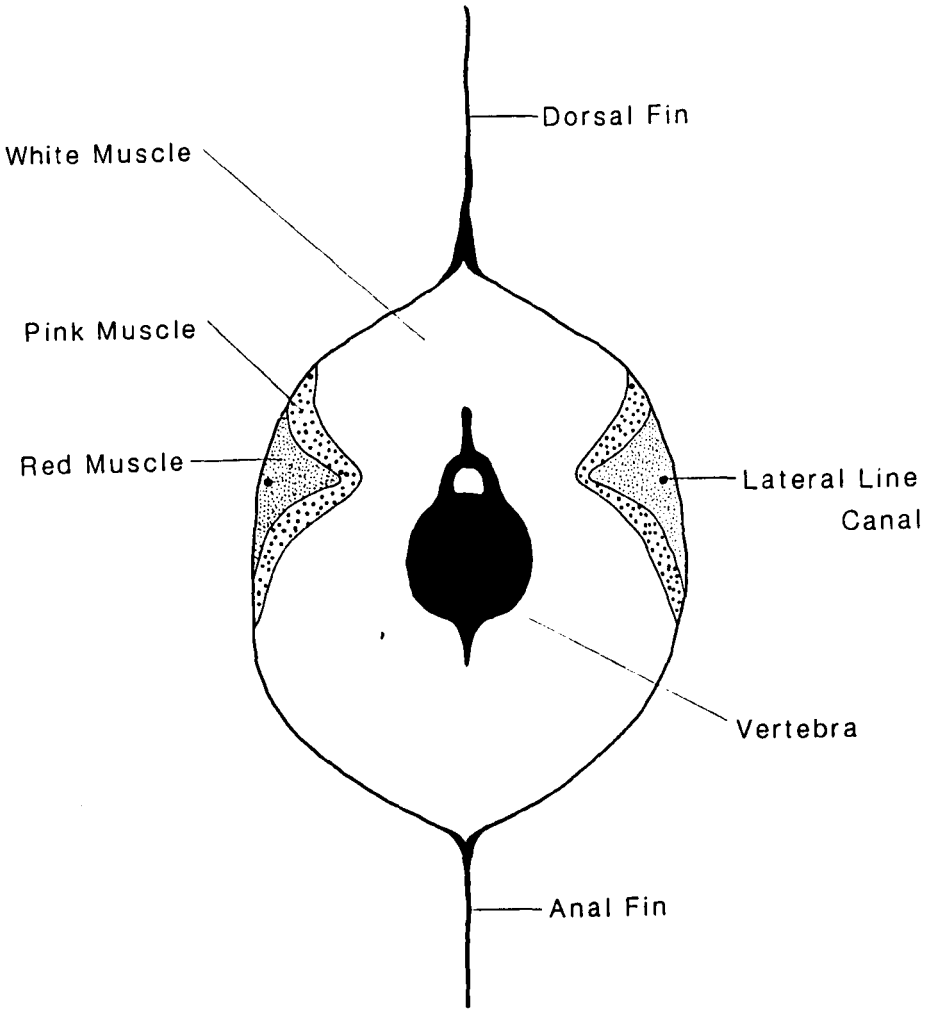

100
10
1
0.1
k_s (%DAY^{-1})
Liver
White Muscle
0
10
20
30
TEMPERATURE (°C)

Fig.1:13. Schematic diagram of a transverse section through the trunk of a teleost fish showing discrete distribution of muscle fibre types.


Dorsal Fin
White Muscle
Pink Muscle
Red Muscle
Lateral Line Canal
Vertebra
Anal Fin

CHAPTER 2

A Descriptive Study of the Ventricle in the Haemoglobinless Channichthyid *Chaenocephalus aceratus* Lonnberg.

INTRODUCTION

The ventricle is the main propulsive chamber of the teleost heart and, as such, it has been the subject of considerable anatomical investigation (for review see Santer, 1984). From these studies it is clear that there is substantial interspecific variation in both the gross morphology of the ventricle and the microscopic organization of the myocardium. In most teleosts the ventricle is either tubular or sac-like in shape (Santer et al., 1983) and the myocardium is entirely trabecular, forming a loose network of tissue, known as 'spongiosa', which projects from the subepicardium (Satchell, 1971). These spongy ventricles are supplied by venous blood perfusing the intertrabecular spaces from the lumen, and, in some species, by a caudally derived epicardial vascular network (Grant and Regnier, 1926; Poupa and Ostadal, 1969). In other species the ventricle is pyramidal in shape (Santer et al., 1983) and the myocardium is of the 'mixed-type', in which the spongiosa is surrounded by a cortical layer of densely packed myocytes - the 'compacta' (Satchell, 1971). The close apposition of myocytes in the compact layer precludes blood supply from the lumen: the evolution of the mixed-type of myocardium is therefore, associated with the development of an elaborate intramural coronary vascular network derived from the hypobranchial arteries (Grant and Regnier, 1926; Poupa and Ostadal, 1969; Tota et al., 1983).

Ostadal and Shiebler (1971; 1972) proposed that the presence of the compact layer in fish ventricles is

determined by high body weights and high heart weight to body weight ratios. More recently however, Santer and Greer-Walker (1980) surveyed the ventricular myoarchitecture in 107 teleost and elasmobranch species and concluded that the mixed-type of myocardium is a characteristic of those species with high metabolic rates, irrespective of body weight or relative heart weight. This is now widely accepted, though, as Emery et al. (1985) have pointed out, in practise it is difficult to separate these two ideas since, in general, active species have higher relative ventricle weights than more sedentary species (Clark, 1927; Poupa and Ostadal, 1969; Santer et al., 1983). Thus the sedentary plaice (Pleuronectes platessa) has a small, sac-like ventricle (Santer et al., 1983) and an entirely trabecular myocardium (Santer and Greer-Walker, 1980), whereas the pelagic mackeral (Scomber scombrus) has a large, pyramidal ventricle (Santer et al, 1983) and mixed-type myoarchitecture (Santer and Greer-Walker, 1980).

Santer and Greer-Walker (1980) suggested that the relationship between metabolic rate and ventricular anatomy is mediated by the requirement for increased blood flow to the tissues in active species. In this context, a number of features of the ventricles in active species can be related to their superior function as a pump compared to spongy, sac-like or tubular ventricles: for example, the cortical cuff of compact muscle in mixed-type ventricles is structurally well adapted to generate high systolic forces (Cimini et al., 1977; Tota 1983) and the pyramidal shape, according to Laplace's Law (see Tota, 1983), is dynamically

more favourable than rounded shapes. According to Farrell (1985), myocardial power output provides the best measurement of the heart's ability to overcome peripheral resistance and to provide sufficient blood flow to meet metabolic demands (Power Output = Cardiac Output x Ventral Aorta Blood Pressure). It may be assumed therefore that ventricular anatomy is related to myocardial power output, though this has not been demonstrated quantitatively.

The aim of the present study was to describe the structure of the ventricle and to determine the relationship between body weight and ventricle weight in the south polar notothenioid Chaenocephalus aceratus (family Channichthyidae). An understanding of channichthyid ventricular anatomy is of particular interest in view of their circulatory adaptations associated with the haemoglobinless condition (see Chapter 1). To date, detailed studies have been made of the cardiac anatomy in only one species of icefish, Channichthyis rhinoceratus (Feller et al., 1983; Feller et al., 1985; Feller, 1987). This species has a large ventricle which is sac-like in shape and has an entirely spongy myocardium. It is endowed with blood vessels in the subepicardium and with an extensive intratrabecular capillary network.

The results from the present study on C.aceratus are compared with those from C.rhinoceratus (Feller et al., 1983; Feller et al., 1985; Feller, 1987) and discussed in relation to the unique cardiovascular adaptations in icefish.

METHODS

Fish collection and maintenance.

Specimens of Chaenocephalus aceratus Lonnberg, Notothenia neglecta Nybelin, Notothenia rossii Fischer and Notothenia gibberifrons Lonnberg were caught using trammel nets in Normanna Strait and, in the case of N.neglecta and N.rossii, in Borge Bay, Signy Island, British Antarctic Territory between 1980 and 1985. Live fish were maintained in a recirculated sea-water aquarium between 0 and +2°C in the British Antarctic Survey's research station on Signy Island and fed every other day to satiation on fish and amphipods.

Scaling of ventricle weight to body weight.

The relationships between body weight and ventricle weight were determined in Chaenocephalus aceratus (24 fish; mean body weight $\pm$ S.E.M. = 1.20 $\pm$ 0.08kg), Notothenia neglecta (65 fish; 0.75 $\pm$ 0.06kg), Notothenia rossii (15 fish; 0.63 $\pm$ 0.10kg), and Notothenia gibberifrons (29 fish; 0.38 $\pm$ 0.05kg). Experimental animals were killed with a blow to the head, weighed and their hearts excised. The ventricles were separated from the other cardiac chambers, flushed with teleost ringer (Hudson, 1967) to remove blood from the lumen, dabbed dry with tissue and weighed.

Sample preparation for microscopy.

Small samples of ventricular tissue from five

specimens of C.aceratus (body weights between 0.5 and 1.5kg) were fixed in 3% gluteraldehyde in 0.15M phosphate buffer (pH 7.4 at 4°C) for 24 hours with a change to fresh fixative after the first 4 hours. Fixed tissue was washed twice in the buffer, post fixed for 1 hour in 1% osmium tetroxide in phosphate buffer (pH 7.4 at 4°C) and washed twice in distilled water. Samples were fully dehydrated in a series of alcohols and immersed in a 1:1 mixture of 100% ethanol and propylene oxide for 40 minutes before clearing in three 40 minute immersions in propylene oxide. The material was left for 16 to 18 hours in a 1:1 mixture of propylene oxide and Araldite CY212 resin (E.M. Scope, Ashford, Kent, England) to facilitate infiltration of the resin. Finally, the tissue was transferred to freshly prepared Araldite for 4 to 6 hours before embedding in a further batch of resin. The blocks were hardened in an oven at 60°C for 48 hours.

Light microscopy.

Semi-thin sections (0.5 to 1.0μm) of the Araldite embedded tissue were cut with a Reichert Ultracut ultramicrotome, transferred to glass slides, stained with toluidine blue and viewed and photographed through a Nikon Labophot microscope.

Electron microscopy.

Ultra thin sections (0.06 to 0.07μm) were cut with a Reichert Ultracut ultramicrotome, mounted on pyroxylin coated 150 mesh copper grids, stained with uranyl acetate and lead citrate and observations made with a Philips 400 electron microscope.

Statistical analyses.

Interspecific differences in the slopes and elevations of the regression lines for ventricle weights versus body weights were compared using a method described by Snedecor and Cochran (1972).

RESULTS

Scaling of heart weight and body weight.

The results are shown in Figs.2:1, 2:2, and 2:3. The large relative ventricle size of the icefish C.aceratus compared to a more typical teleost is illustrated in Fig.2:1 which shows hearts isolated from specimens of C.aceratus and rainbow trout, Salmo gairdneri, with similar body weights. Quantitative demonstrations of this are presented in Figs.2:2 and 2:3 which show the relationships between body weight and ventricle weight for C.aceratus and a range of other teleost species. Fig.2:2 shows the data obtained in the present study for C.aceratus and three 'red-blooded' notothenиid species. The equations for the regression lines are given in the legend. Interspecific comparisons of the regressions indicate that the slopes were not significantly different ($P>0.05$) but that there are significant differences between the elevations in all the species ($P<0.05$) except in the case of N.rossii and N.neglecta ($P>0.05$). The relative ventricle weights of the icefish were approximately three times greater than those of N.rossii and N.neglecta, and approximately five times greater than those

of N.gibberifrons.

Fig.2:3 shows the regression lines from Fig.2:2 together with a number of others taken from the literature. The additional data is from the Atlantic bluefin tuna, Thunnus thynnus, and from a range of north temperate 'sea-fish' (both from Poupa et al., 1981) and from two other south polar species: the channichthyid, C.rhinoceratus and the notothenid, Notothenia magellanica (Feller et al., 1983). There is considerable similarity in the relative ventricle weights of the tuna and both species of icefish. With the exception of N.gibberifrons, which has low relative ventricle weights, the relationships between body weight and ventricle weight for the notothenids are similar to that of the 'sea-fish'.

Anatomy of the ventricle.

Gross appearance and general organisation of the ventricular wall:The ventricle in C.aceratus was light in colour (Fig.2:4). Its shape corresponded to the sac-like classification described by Santer et al. (1983): it was rounded in outline, without a well defined apex, and the bulbus, which was separated from the atrium, was angled anteriosuperiorly (Fig.2:4).

The general organization of the ventricular wall is shown in Fig.2:5. In common with other teleosts (see Santer, 1984) it consisted of a myocardium sandwiched between outer epicardial and inner endocardial layers. The myocardium was entirely trabecular.

Epicardium and subepicardium: The epicardium consisted of a monolayer of mesothelial cells joined at lateral cell abutments by extensive desmosomes (Fig.2:5a and b). These cells contained large, centrally placed nucleii, vacuoles, pinocytotic vesicles and moderately electron-dense bodies of different sizes (Fig.2:6a and b). The underlying subepicardium was well developed: its thickness varied but was typically 90μm (Fig.2:5). It was composed of loose connective tissue and appeared to be stratified with cellular components, such as fibroblasts, predominating in the immediate subepicardial region and larger bundles of collagen closer to the myocardium (Figs.2:5 and 6a). Blood vessels were common within the collagenous region (Figs.2:5 and 2:6c). Two size classes of vessels could be distinguished: small diameter (approximately 15μm) and larger vessels (approximately 50μm). In most sections the latter could be seen to connect directly to the lumen through the myocardial and endocardial layers (Fig.2:5). It was not possible to determine whether these vessels were venous or arterial owing to continuity between their tunica adventitia and the subepicardial connective tissue.

Myocardium:The myocardium consisted of a heterogenous population of cells including both myocytes and non-contractile cells (Fig.2:7b). Numerically, myocytes were the main constituents. These were long, narrow cells, which were often ovoid in transverse section (Fig.2:7a). Within the myocytes, nucleii and myofibrils were peripherally located (Fig.2:7a). T-tubules were absent and elements of

sarcoplasmic reticulum were particularly common between the myofibrills and the sarcolemma (Fig.2:7c and e). A number of electron dense and moderately electron dense bodies occurred, randomly distributed, in the cytoplasm (Fig.2:7b). A remarkable feature of these cells was the particularly large mitochondrial compartment. These organelles occupied virtually all the cytoplasmic space not taken up by other organelles: there was little free cytoplasmic space (Fig.2:7). Two forms of mitochondrial cristae were apparent: tubular and lamellar (Fig.2:7c). Within the mitochondrial compartment multi-membrane bodies were common (Fig.2:7c).

The non-contractile cells were interposed between myocytes (Fig. 2:7b); no specialized topological relationship between these two cell types could be detected. The major characteristics of the non-contractile cells was the occurrence of many granules of different electron densities scattered within the cytoplasm (Fig.2:7b).

Endocardium and subendocardium:The endocardium constituted a lining to the lumenal surface of the myocardium which was continuous except where branches of the subepicardial vasculature were in confluence with the lacunae (Fig.2:5). Two cell types occurred in the endocardium. The most abundant of these were squamous mesothelial cells which had relatively flat nucleii (Fig.2:8a and c). Less common were cells with larger, irregularly shaped, nucleii and large perinuclear regions which projected into the lacunae (Fig.2:8a and b). The latter contained many electron dense and moderately electron dense bodies (Figs.2:8a and b).

Lateral endocardial cell-cell abutments were formed by interdigitations (Fig.2:8c).

The endocardium was separated from the myocardial region by a narrow subendocardial space which contained collagen bundles (Figs.2:7a and 2:8c) and fibroblasts.

Relationship between cardiac performance and ventricular anatomy.

With the exception of the unspecified species of tuna which was restrained (Jones and Brill personal communication, in Farrell, 1985), the data in Table 2:1 show the cardiovascular characteristics of some teleost species during maximum cardiac work. In the case of the cod (Gadus morhua) (Axelsson and Nilsson, 1986) and the trout (S.gairdneri) (Kiceniuk and Jones, 1977) the experimental animals were swimming at their maximum sustainable swimming speed. In the case of C.aceratus maximum cardiac output and ventral aorta blood pressure were calculated from data concerning resting specimens (see Table 1:1) based on the observation that, in closed respirometers, this species can increase its cardiac output by a maximum of 50% while its blood pressure does not change significantly (Hemmingsen et al., 1972). Maximum myocardial power output (M.P.O.) for each species was calculated according to the following equation:

$$M.P.O. = \text{Cardiac Output } (m^3s^{-1}) \times \text{Ventral Aorta B.P. } (Nm^{-2})$$

Despite the high cardiac output in C.aceratus, myocardial

power outputs were less than any of the other species due to the particularly low blood pressures.

There appears to be a close relationship between the heart's ability to generate power and the development of the compacta: both C.aceratus and the cod (1.46 and 3.30mW(g ventricle weight)$^{-1}$ respectively) have entirely spongy ventricles, whereas the trout and the tuna (6.29 and >12mW(g ventricle weight)$^{-1}$ respectively) have pyramidal ventricles with mixed-type myocardia.

DISCUSSION

Scaling of body weight and ventricle weight.

The relative ventricle weight in teleosts is subject to considerable interspecific variation: species with high metabolic rates generally have larger ventricles in relation to body size than more sedentary species (Poupa and Ostadal, 1969; Poupa et al., 1981). This reflects the more general situation in the vertebrate subphylum as a whole in which phylogenetic 'jumps' in the relationship between body weight and heart weight can be related to the evolution of physiological characteristics, such as homeothermy, which increase overall oxygen consumption (Poupa and Ostadal, 1969; Ostadal et al., 1970; Poupa et al., 1970). Larger ventricles are clearly better able to support metabolically active tissue with high rates of blood flow as a result of their greater muscle mass and larger stroke volumes.

The relationship between metabolic rates and relative ventricle size in teleosts is best illustrated by the tuna, T.thynnus, in which the combined extracirculatory loads of sustained high speed swimming and the maintenance of elevated red muscle and brain temperatures have resulted in massive ventricles (Fig.2:3), the relative weights of which approach those of small mammals (Poupa et al., 1981).

The cardiomegaly in the tuna is matched by the channichthyids C.aceratus and C.rhinoceratus (Fig.2:3). In contrast to the tuna, both of these are predominantly benthic species. Their high relative ventricle weights (Fig.2:3) are associated with high cardiac outputs which compensate for the low oxygen carrying capacity of the blood resulting from the haemoglobinless condition (Hemmingsen and Douglas, 1970; Holeton, 1970; Hemmingsen et al., 1972; Holeton, 1975; Holeton, 1976; Feller et al., 1983, Johnston et al., 1983). The cardiomegalies in icefish and tuna therefore, can be related to different selection pressures, and provide an example of convergent evolution at the organ level.

With the exception of N.gibberifrons, the relationship between body weight and ventricle weight for the notothenиid species shown in Fig.2:3 are similar to that of a composite line drawn for a range of 'sea-fish'. The significantly lower relative ventricle weights in N.gibberifrons compared to N.rossii and N.neglecta (Fig.2:2) is consistent with the lower metabolic rate in the former (Holeton, 1970).

Ventricular shape, myoarchitecture and pattern of blood supply.

The ventricle in C.aceratus appeared pale brown or yellow in colour (Fig.2:4) due to the presence of, lipids, mitochondria, flavoproteins, lipofuscin (Feller et al., 1985) and low concentrations of myoglobin (Douglas et al., 1985). In broad outline the gross ventricular shape corresponded to the 'sac-like' classification described by Santer et al. (1983) (Fig.2:4) and the myocardium was entirely trabecular (Figs.2:5 and 2:6d). The ventricular wall was supplied by venous blood perfusing the intertrabecular spaces from the lumen and through a subepicardial vascular network (Figs.2:5 and 2:6c). No attempt was made to determine the origin of these vessels and, in common with the observations of Feller et al. (1985) on C.rhinoceratus, arterial and venous profiles could not be distinguished owing to the continuity between the adventitia of the vessels and the connective tissue of the subepicardium. However, two distinct size classes of vessel were apparent with diameters of approximately 50 and 15μm (Fig.2:5). In many sections, the lumina of the larger vessels could be seen to be in confluence with the intertrabecular lacunae (Fig.2:5). Similar connections have been observed in the ventricles of plaice (Santer, 1976). Santer (1976) suggested that the subepicardial vessels in this species may be formed by infolding of the endocardium and that the connections represent regions in which this process is incomplete.

The observations made in the present study on the

shape and myoarchitecture of the ventricle in C.aceratus are broadly similar to those made by Feller et al. (1985) and Feller (1987) on C.rhinoceratus. However, these studies revealed fundamental differences in the patterns of ventricular blood supply in the two species: in contrast to C.aceratus, the myocardium in C.rhinoceratus is endowed with a highly developed intratrabecular microvasculature. Feller (1987) has suggested that these vessels may be microlacunae, though it seems most likely that they represent a capillary network derived from the hypobranchial arteries (Felleret al., 1985) and that they serve to enhance the delivery of oxygen to the myocytes.

Recently, Tota et al. (1983) have proposed a tentative classification of fish ventricles based on a combination of myoarchitecture and pattern of blood supply (Table 2:2). According to this scheme, type I ventricles are entirely trabecular and are either avascular (subtype a) or have subepicardial blood vessels (subtype b). Types II, III and IV ventricles are all of the mixed-type. In the type II ventricle only the compacta is vascularised, whereas in types III and IV the spongiosa is also endowed with blood vessels. These last two categories can be differentiated only by the relative thickness of their compacta.

On the basis of the observations made in this study the ventricle of C.aceratus should be classified as type I, subtype b. C.rhinoceratus also has a type I (i.e. spongy) ventricle, but, because of the presence of myocardial vessels in this species, it does not correspond to either of

the two subtypes.

The rather limited data in Table 2:1 is the first quantitative demonstration that there is a close relationship between maximum myocardial power outputs and the anatomy of the ventricle wall in teleosts. The type I ventricle in C.aceratus is consistent with the low power output which this species achieves. Interestingly, Greco et al. (1980) have reported the presence of a compact layer in the ventricle of another icefish, Champsocephalus gunnari. This is a more active, pelagic species than C.aceratus and the extracirculatory load associated with sustained swimming may have promoted the development of the compacta. Differences in ventricular myoarchitecture between such closely related species as C.aceratus and C.gunnari are not unprecedented; for example, Santer and Greer-Walker (1980) have observed similar variations in salmonid species. However, it should be noted that the material described by Greco et al. (1980) was poorly preserved and it is possible that the authors may have observed an artefactual 'false compact layer' (see Santer and Greer-Walker, 1980).

Histology and ultrastructure.

A range of cell types has been described in the epicardial layer of fish ventricles (for review see Santer, 1984). The epicardium in C.aceratus was composed of squamous cells which, like those of the Japanese medaka (Lemanski et al., 1975), were joined by desmosomes without any further elaboration of cell boundaries (Fig.2:6a and b). Santer (1984) has suggested that the complex interdigitations

between epicardial cells in other species may serve to protect against disruption during systole. The proliferation of connective tissue in the subepicardium of C.aceratus (Fig.2:5 and 2:6) is greater than that reported in most other species and may serve to minimise the lateral forces acting on the epicardium.

The myocardium in C.aceratus was similar to that in C.rhinoceratus (Feller et al., 1985). In both species the trabeculae were composed mainly of myocytes. The most notable ultrastructural feature of these cells in C.aceratus was the particularly large mitochondrial compartment. The proliferation of these organelles is thought to compensate for the influence of low environmental temperatures and, in particular, for the problems associated with maintaining oxygen uptake rates resulting from the low myocardial myoglobin contents (see Chapter 3). The myocytes in C.rhinoceratus had much lower mitochondrial volume densities (Feller et al., 1985; Feller, 1987). In this species, the supply of oxygen to the ventricle appears to be enhanced by the intratrabecular microvasculature. The large mitochondrial compartment in C.aceratus myocytes must result in high rates of membrane turnover in these cells. In this context, it was interesting to note that multi-membrane bodies were common in the myocytes (Fig.2:7c). Similar structures have been associated with mitochondrial breakdown in rat ventricular myocytes (Travis and Travis, 1972) and are common in the axial muscles of fish undergoing periods of starvation (Beardall and Johnston, 1983).

In common with C.rhinoceratus (Feller et al., 1985), both tubular and lamellar cristae were observed in the mitochondria of C.aceratus(Fig.2:7c). The former have not been reported previously in teleost myocytes. In other tissue, such as the adrenal cortex in mammals (Mazzocchi et al., 1982), tubular cristae are associated with lipid metabolism or steroidogenesis. This may be the case in channichthyid ventricles, since Feller (1987) has demonstrated relatively high activities and concentrations of enzymes and metabolites associated with lipid turnover in the ventricle of C.rhinoceratus.

The high mitochondrial volume densities in C.aceratus myocytes are achieved at the expense of space devoted to myofibrils (Chapter 3). These contractile elements are situated at the periphery of cells (Fig.2:7a and e). This is often the case in fish myocardial cells and is thought to facilitate excitation-contraction coupling in the absence of T-tubules (Santer, 1984). This interpretation is consistent with the prevalence of elements of sarcoplasmic reticulum adjacent to myofibrils in the immediate subsarcolemmal region in C.aceratus (Fig.2:7e).

In contrast to the epicardium, two cell types occurred in the endocardium of C.aceratus: squamous and more cuboidal cells which projected into the lumen (Fig.2:8a and b). These are similar to the 'type II' and 'type I' cells respectively described by Saetersdal et al. (1974) in the atrial endocardium of the cod. The prevalence of granules, particularly in the cuboidal cells, suggests that they may

have a secretory function.

In summary, the present study has confirmed previous reports of the large relative ventricle weight in the channichthyid C.aceratus, an adaptation which is related to the low oxygen carrying capacity of icefish blood. In addition, it has been shown that the myocytes in this species are characterized by large mitochondrial populations. In other respects the gross and ultrastructural characteristics of the ventricle are typical of other sedentary species such as the plaice (Santer and Cobb, 1972; Santer, 1976; Santer and Greer Walker, 1980; Santer et al., 1983).

Table 2:1. Relationship between maximum myocardial power output and development of the the compact layer in the ventricles of four species of teleost.

SPECIES	RELATIVE VENTRICLE WEIGHT (g ventricle/ kg body wt.)	TEMP.(°C)	CARDIAC OUTPUT ($ml.kg^{-1}.min^{-1}$)	VENTRAL AORTA B.P. (kPa)	MYOCARDIAL POWER OUTPUT ($mW(g\ vent.wt.)^{-1}$)	% COMPACTA
Chaenocephalus aceratus[a]	0.310	0.5-2.0	128	2.14	1.46	0
Atlantic cod[b] (*Gadus morhua*)	0.079	10-11	25.4	6.2	3.30	0
Rainbow trout[c] (*Salmo gairdneri*)	0.115	9.0-10.5	52.6	8.24	6.29	38.9
Tuna[d]	—	—	—	—	>12	>39.1

References:

a. Calculated from data for resting specimens (Hemmingsen and Douglas, 1977) assuming cardiac output increases 50% during activity (Hemmingsen *et al.*, 1972).
b. Axelsson and Nilsson (1986).
c. Kicenuik and Jones (1977).
d. Jones and Brill personal communication, in Farrel (1985).

Table 2:2. Classification of the organization of the ventricular wall in fish (from Tota *et al.*, 1983).

	TYPE I		TYPE II	TYPE III	TYPE IV
Representative species	Subtype (a) *Scorpaena*.	Subtype (b) *Pleuronectes*.	*Conger*.	*Scyllium*, *Torpedo*.	Sharks, Xiphoids, Scombroids.
Myoarchitecture					
Spongiosa	+	+	+	+	+
Compacta	-	-	+	+	+ +
(%) of compacta			19-21	22-24	31-42
Circulation					
Venous lacunary	+	+	+	+	+
Vascularisation of epicardium	-	+	+	+	+
Capillarisation of compacta	-	-	+	+ +	+ +
Capillarisation of spongiosa	-	-	-	+	+
Shunts	-	-	-	+	+

Symbols: - absent; + present; + + highly developed.

Fig.2:1. Hearts isolated from rainbow trout (Salmo gairdneri) (315g) and Chaenocephalus aceratus (320g) with similar body weights. The heart of C.aceratus is shown on the right with an intact gill array.

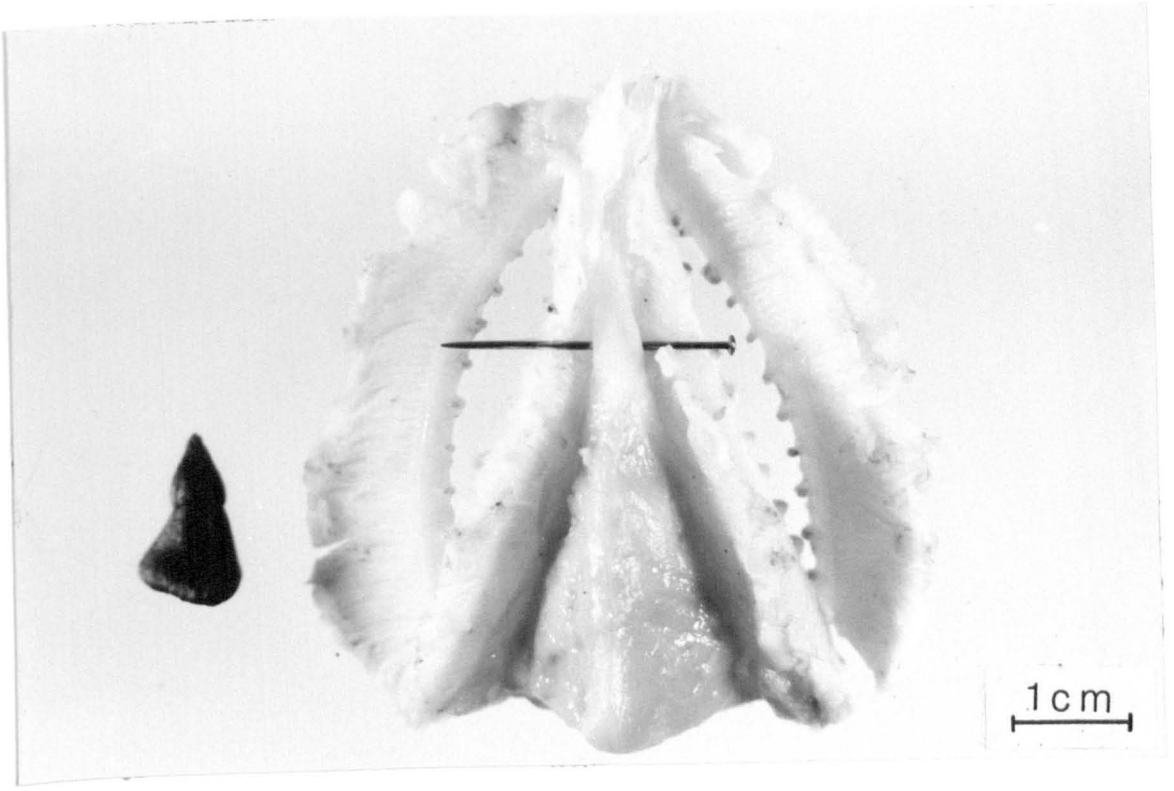

1cm

Fig.2:2. Relationship between ventricle weight and body weight for four species of south polar notothenioids: Notothenia gibberifrons(■), Notothenia neglecta, (●), Notothenia rossii (O) and Chaenocephalus aceratus (▲). The regression line equations were as follows: N.gibberifrons, $y = 1.01x - 0.20$ (n=29); N.neglecta, $y = 0.86x - 0.03$ (n=38); N.rossii, $y = 0.94x + 0.04$ (n=15); C.aceratus, $y = 0.84x + 0.49$ (n=24); where $y=\log_{10}$ ventricle weight in g and $x = \log_{10}$ body weight in kg.

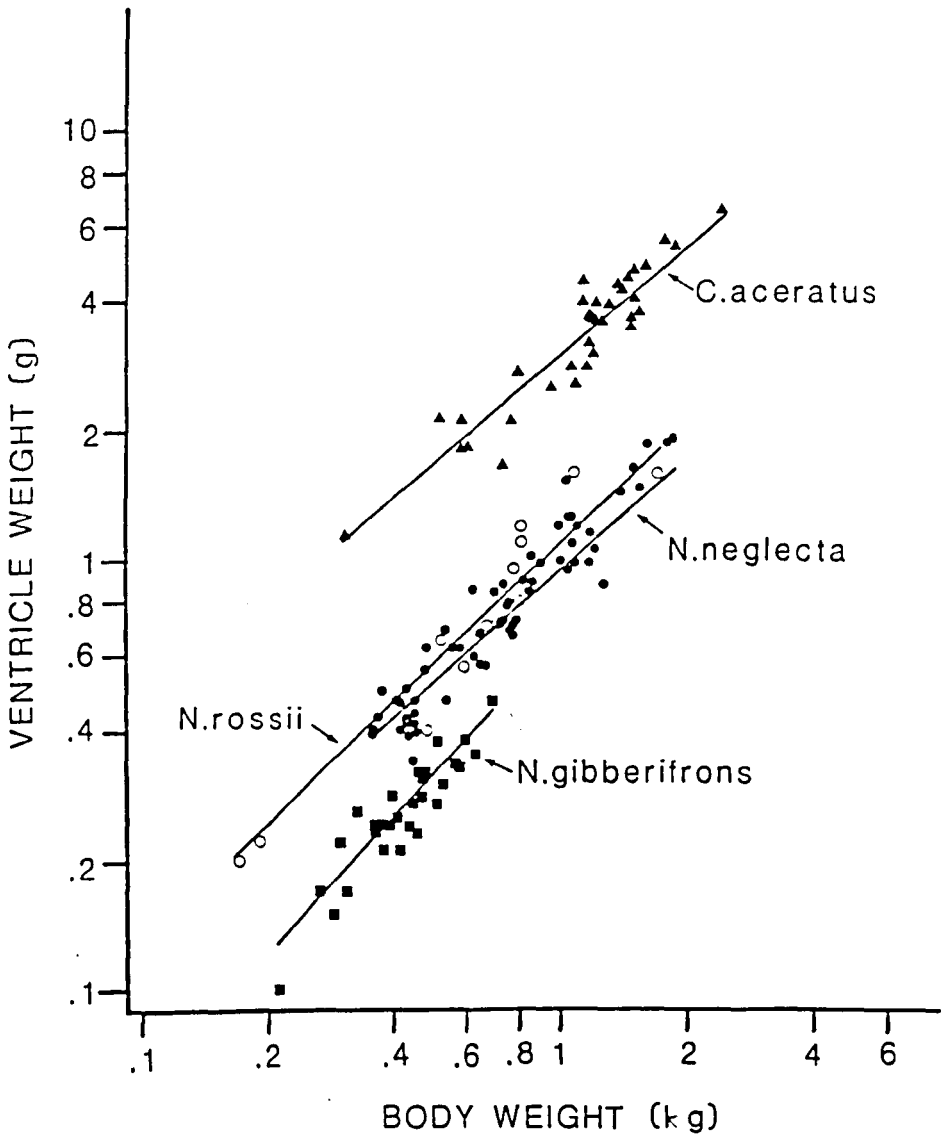

VENTRICLE WEIGHT (g)
10
8
6
4
2
1
.8
.6
.4
.2
.1
.1
.2
.4
.6
.8
1
2
4
6
BODY WEIGHT (kg)
C.aceratus
N.neglecta
N.rossii
N.gibberifrons

Fig.2:3. Relationship between ventricle weight and body weight for the species shown in Fig.2:2 together with data for other species taken from the literature: *Notothenia magellanica* and *Channichthyis rhinoceratus* (Feller *et al.*, 1983); *Thunnus thynnus* and a composite temperate sea-fish line (Poupa *et al.*, 1981).

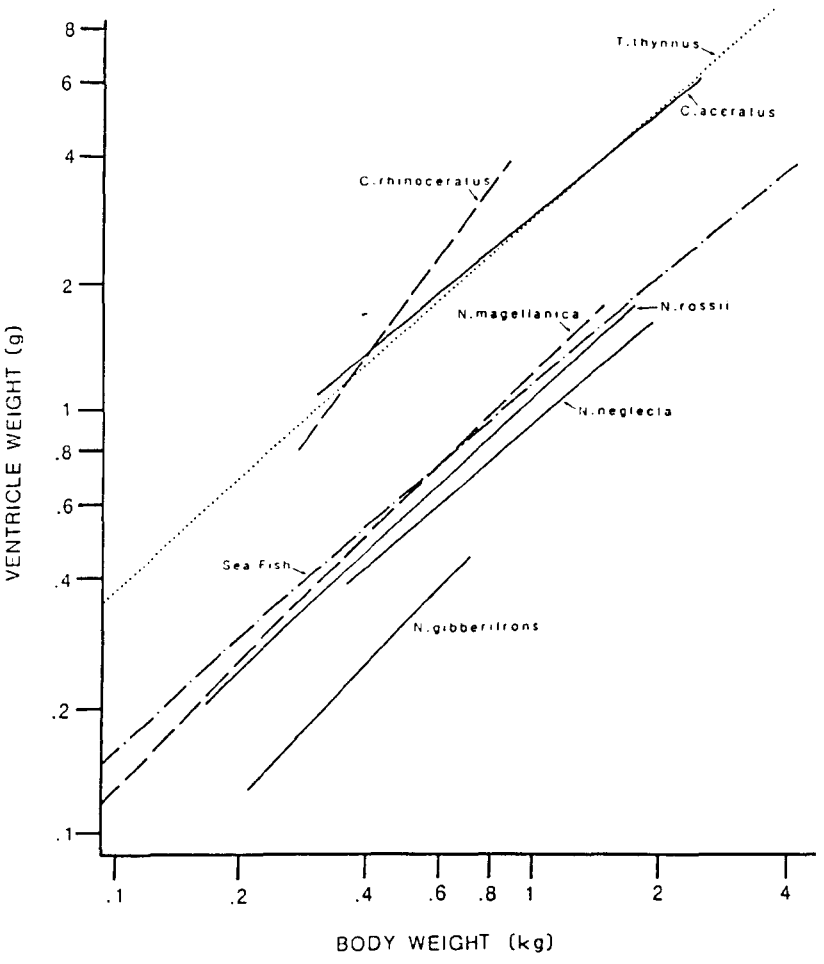

VENTRICLE WEIGHT (g)
8
6
4
2
1
.8
.6
.4
.2
.1
.1
.2
.4
.6
.8
1
2
4
BODY WEIGHT (kg)
T.thynnus
C.accratus
C.rhinoceratus
N.magellanica
N.rossii
N.neglecta
Sea Fish
N.gibberifrons

Fig.2:4. Lateral view of the ventricle of Chaenocephalus aceratus. Note the light colour and sac-like shape of the ventricle.

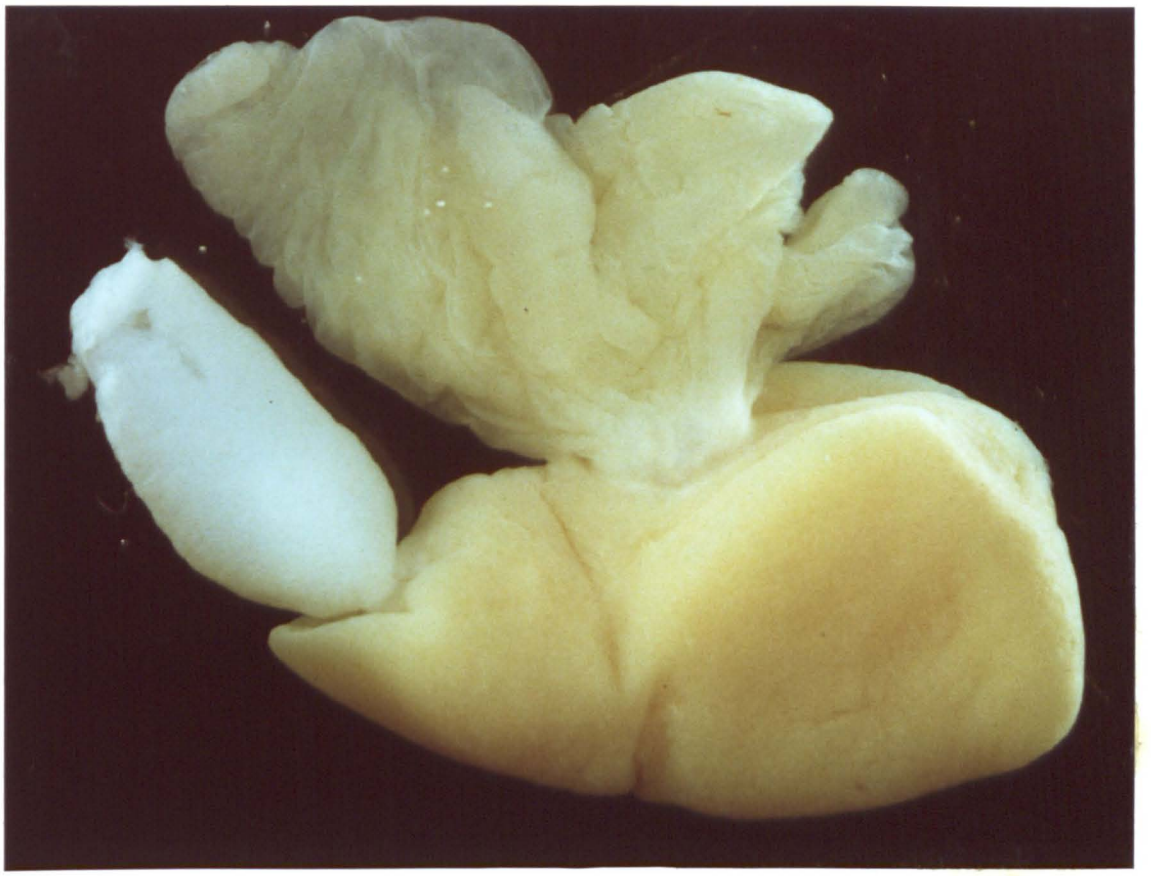


Fig.2:5. Toluidine blue stained, semi-thin section through the ventricular wall of Chaenocephalus aceratus. The ventricle is bounded on the pericardial (per) surface by the epicardium (single arrowhead). The stratified nature of the subepicardium (SEp) is shown clearly with a high density of fibroblasts in the immediate subepicardial region and collagen and blood vessels (BV) predominating closer to the myocardium. Two large blood vessels can be seen and one smaller vessel towards the right of the figure. The lumina of the former are in confluence with the intertrabecular lacunae (L). This figure also illustrates the trabecular nature of the myocardium (MC).

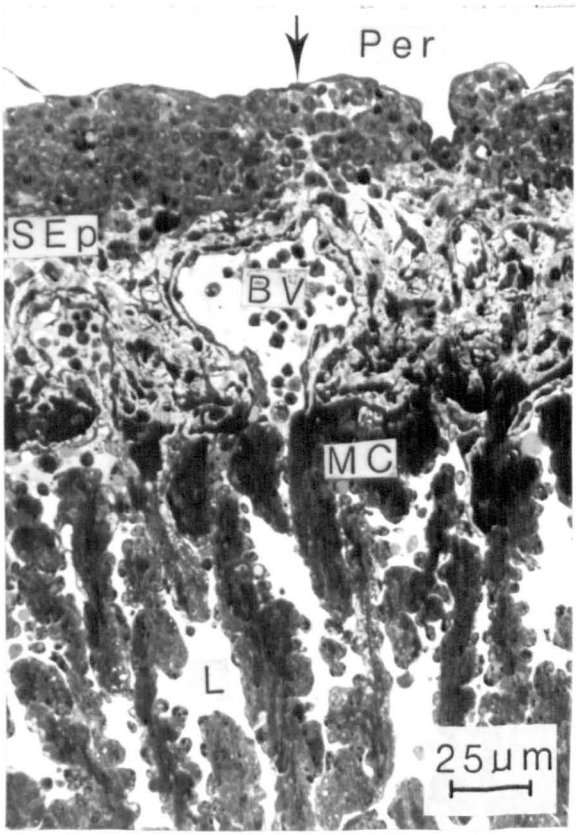

Per
SEp
BV
MC
L
25µm

Fig.2:6. Transmission electron micrographs showing the epicardial and subepicardial regions of the ventricle in Chaenocephalus aceratus. **a:** Epicardial cells (Ep) with large, central nucleii (Nuc) overlying numerous fibroblasts (F) in a collagenous matrix (C). Per = pericardial space. **b:** Detail of desmosome between two epicardial cells. Note the presence of a moderately electron dense body, numerous vacuoles and pinocytotic vesicles (arrow head) within the cytoplasm. **c:** Large blood vessel within the subepicardium. A leucocyte (Le) can be seen in the lumen of the vessel. C = collagen in the subepicardium. **d:** Junctional region between the subepicardium (SEp) and the myocardial trabeculae (T). Note the presence of some longitudinally orientated myocytes (arrowhead) in this region. L = lacunae.

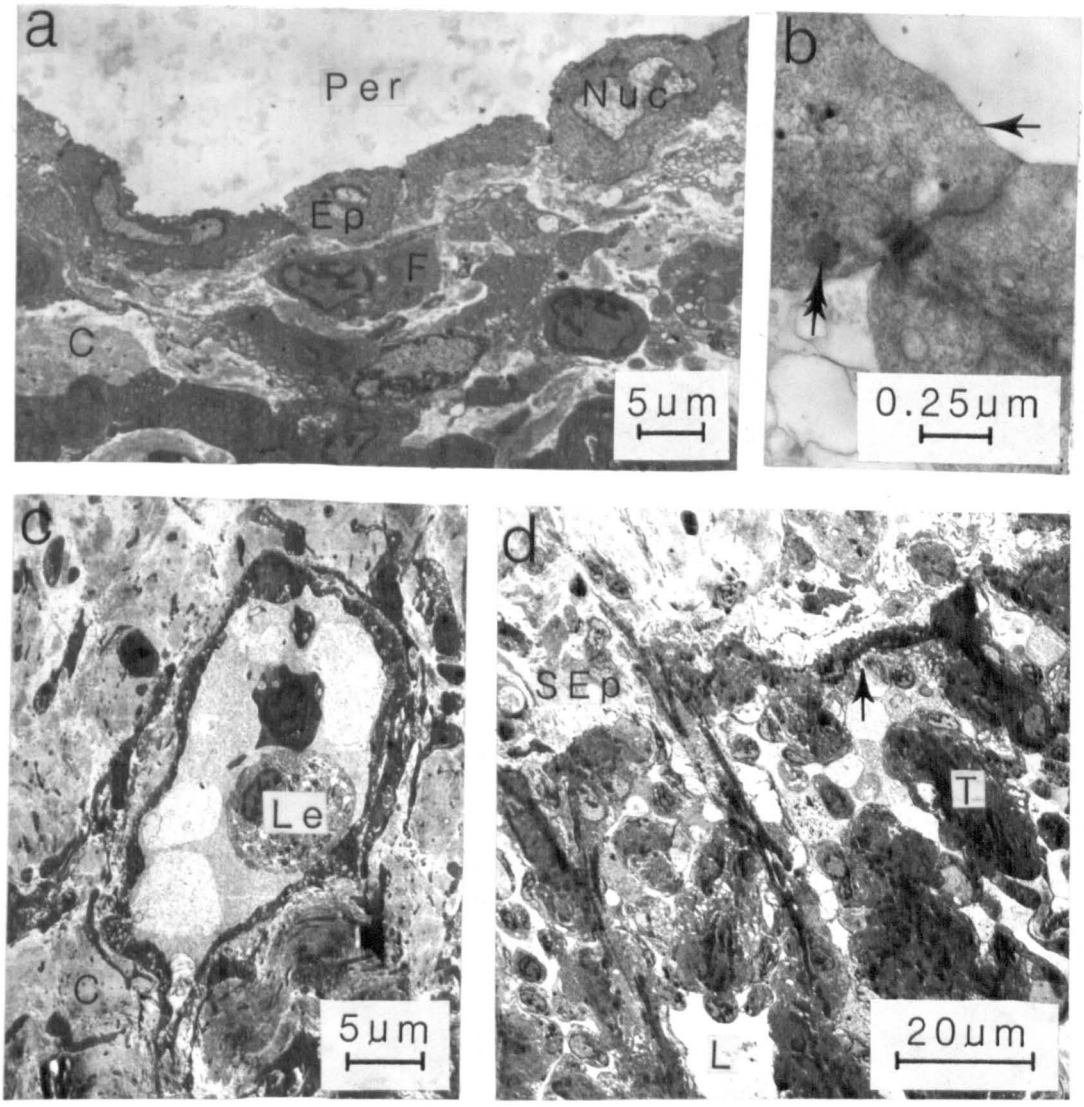

a
Per
Nuc
Ep
F
C
5µm
b
0.25µm
c
Le
C
5µm
d
SEp
T
L
20µm

Fig.2:7. Transmission electron micrographs showing detail of the ventricular myocardial layer in Chaenocephalus aceratus. **a:** Transverse section through a trabeculum. Endocardial cells (En) form a continuous boundary between the trabeculum and the lacunae (L). The endocardial cells contain numerous vacuoles (V). In places, a well developed subendocardium containing collagen bundles (C) is apparent between the endocardial cells and the myocytes. The myocytes contain myofibrills (My) and a particularly large population of mitochondria (Mt). **b:** Two non-contractile cells (NCC) between two myocytes (MC). Note the large nucleii (Nuc) in the non-contractile cells and the presence of many electron dense bodies. **c:** The mitochondrial compartment. The tubular structure of some of the mitochondrial cristae is apparent. A multi-lammellar body (arrowhead) can be seen amongst the mitochondria. double arrowhead = sarcoplasmic reticulum. **d:** Sarcomere banding pattern. **e:** Elements of sarcoplasmic reticulum (arrowheads) between myofibrills (My) and sarcolemma (double arrowhead). Mt = mitochondria.

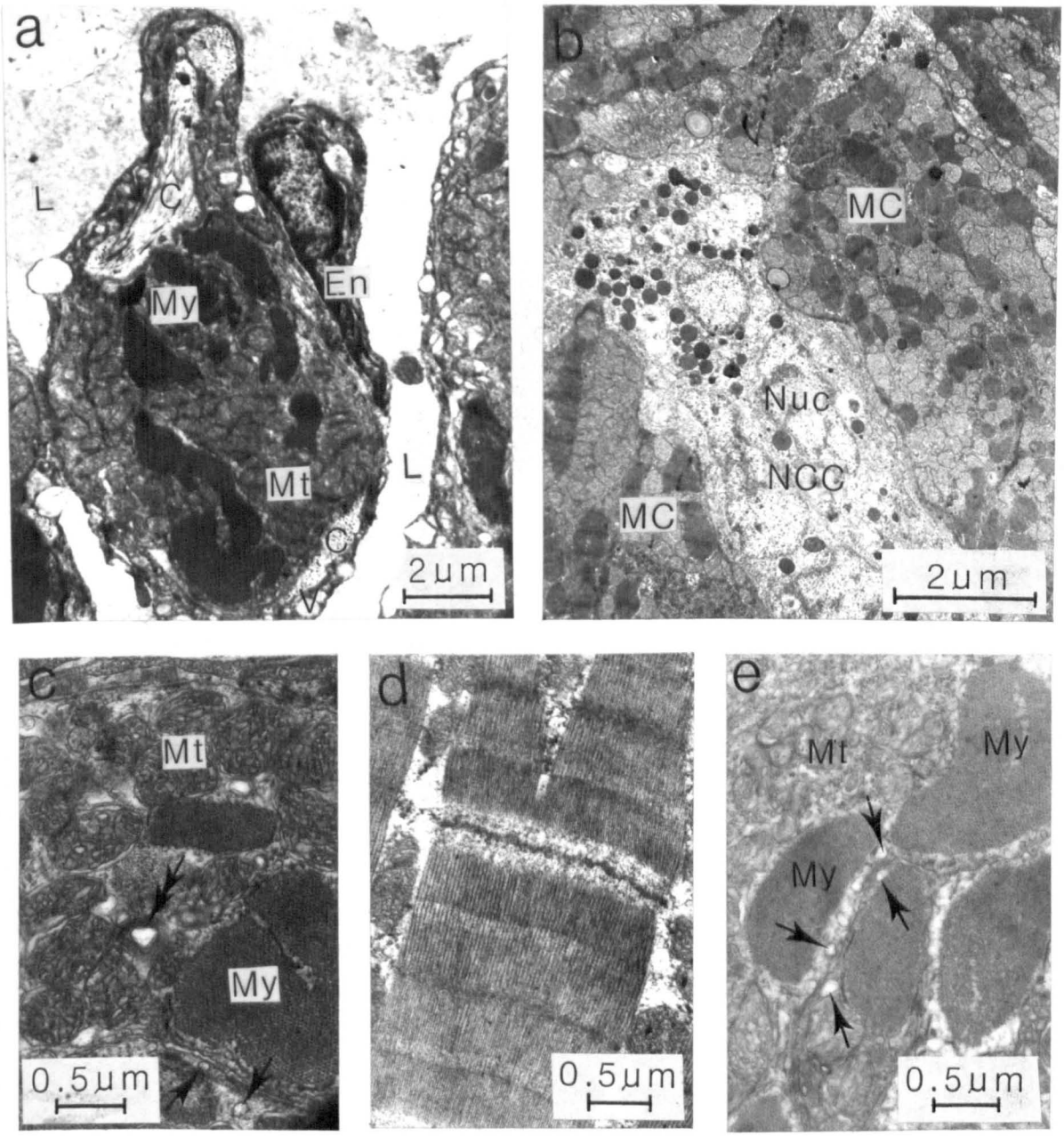
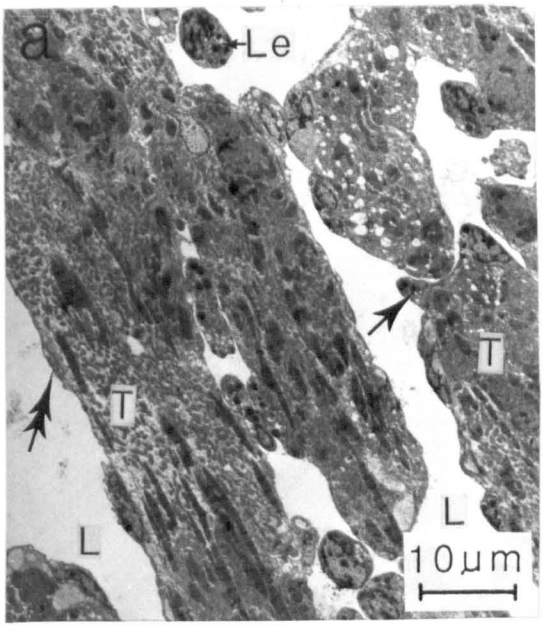
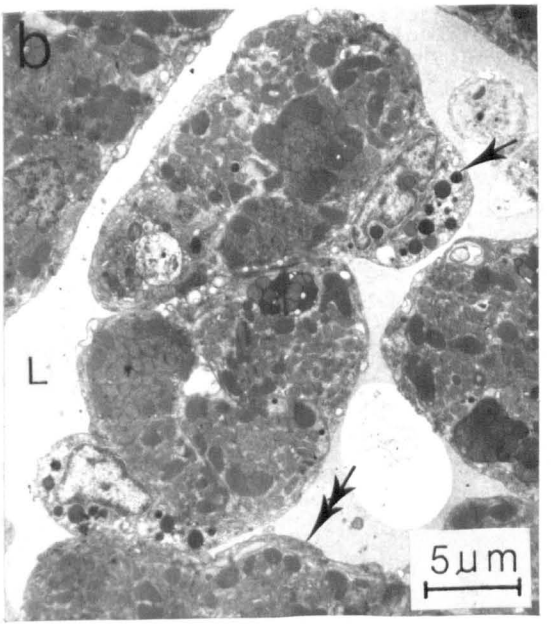
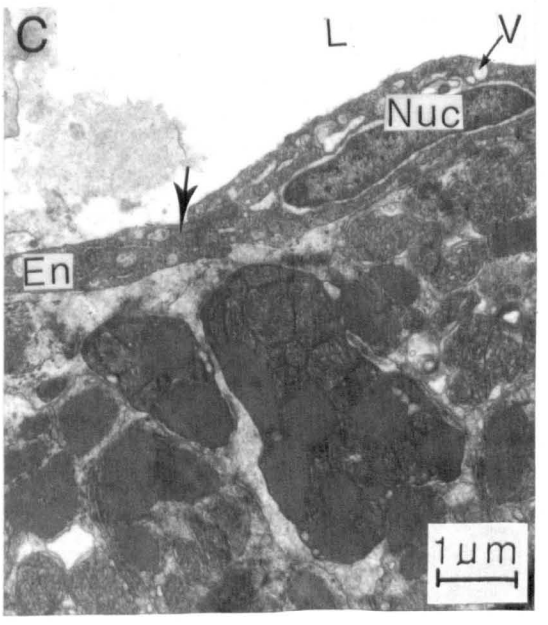

a
L
C
My
En
Mt
L
C
V
2µm
b
MC
Nuc
NCC
MC
2µm
c
Mt
My
0.5µm
d
0.5µm
e
Mt
My
My
0.5µm

Fig.2:8. Transmission electron micrographs showing details of the ventricular endocardium in Chaenocephalus aceratus. **a:** lining trabeculae (T). L = lacunae; Le = leucocyte. **b:** Section through trabeculae showing cuboidal (single arrowhead) and squamous mesothelial cells (double arrowhead). Note the electron dense bodies near the nucleus in the former. L = lacunae. **c:** Squamous endothelial cell (En). The complex interdigitation of adjacent cells can be seen (arrowhead), together with the cell nucleus (Nuc) and cytoplasmic vacuoles (V). L = lacunae.


a
Le
T
T
L
L
10 µm


b
L
5 µm


c
L
V
Nuc
En
1 µm

CHAPTER 3

The Influence of Low Temperatures and Low Myocardial Myoglobin Concentrations on the Ultrastructural Morphometric Characteristics of Ventricular Myocytes in South Polar Notothenioid Fish.

INTRODUCTION

There is an extensive literature concerning the ultrastructural morphometry of oxidative locomotory muscle fibres in teleost fish. Comparison of data from species with similar activity patterns suggests that in south polar notothenioids compensation for the rate depressing effects of low temperature on aerobic ATP synthesis is achieved partially by increases in mitochondrial volume density (Johnston, 1987b; Dunn, 1988). In one notothenioid family, the Channichthyidae, the influence of low temperature on aerobic processes is compounded by low intracellular myoglobin concentrations (Greco et al., 1980; Hamoir and Gerardin-Otthiers, 1980; Walesby et al., 1982; Douglas et al., 1985). Johnston (1987b) has shown that the mitochondrial packing in the oxidative fibres of one channichthyid, Chaenocephalus aceratus, is particularly dense and suggested that, in addition to offsetting the effects of low temperatures, this may serve to compensate for the myoglobin-poor condition.

Enzyme and metabolite profiles indicate that teleost myocardial tissue has a higher aerobic capacity than oxidative skeletal muscle (see Driedzic and Stewart, 1982). On this basis, one might expect that the combination of low myocardial myoglobin concentrations and low environmental temperatures would also promote high mitochondrial volume densities in channichthyid myocytes. This appears to be the case in C.aceratus: in a recent descriptive study on the ventricle in this species it was noted that the myocytes had

large mitochondrial compartments (Chapter 2). However, Feller et al. (1985) have shown that in the myocytes of another channichthyid, Channichthyis rhinoceratus, the packing of mitochondria is similar to that in a sympatric notothenioid with a myoglobin-rich ventricle.

The aim of the present study was to quantify the mitochondrial volume density in the myocytes of C.aceratus. Comparative data was obtained from Notothenia neglecta, a south polar notothenioid (family nototheniidae) with a myoglobin-rich ventricle, and two warmer-water teleosts. The activities of cytochrome oxidase in the ventricles of C.aceratus and N.neglecta were also measured to provide an index of oxidative capacity. The results from these investigations, together with additional data from the literature, enable a detailed discussion of the selection pressures which act on the mitochondrial compartments in channichthyid myocytes.

MATERIALS AND METHODS

Fish collection and maintenance.

The species used in this study were from south polar, north temperate, and tropical biogeographical regions. The south polar species, Chaenocephalus aceratus Lonnberg and Notothenia neglecta Nybelin, were caught using trammel nets around Signy Island, South Orkney Islands, British Antarctic Territory. The north temperate species, the lumpsucker (Cyclopterus lumpus L.), and the sculpin (Myoxocephalus

scorpius L.), were caught using gill nets in St. Andrews Bay off the east coast of Scotland. The tropical freshwater species, oscar cichlid (Astronotus oscellatus Cuvier), was obtained commercially (House of Pisces, Dundee, Tayside). The fish were maintained in tanks near their preferred body temperatures, i.e. 1, 7, and 24°C respectively, and fed every other day to satiation on squid and fish or, in the case of the cichlids, on proprietary fish flakes.

Electron microscopy and quantitative analysis.

The mitochondrial (Vv(mt,f)) and myofibrillar (Vv(mf,f)) volume densities in ventricular myocytes of the following species were determined: C.aceratus (eight fish, body weight ± S.E.M. = 0.83 ± 0.03kg), N.neglecta (six fish, 0.64 ± 0.06kg), lumpsuckers (six fish, 0.83 ± 0.07kg) and oscar cichlids (six fish, 8.3 ± 0.7g). Experimental animals were killed with a blow to the head followed by spinal cord transection. The hearts were excised and ventricular tissue processed for electron microscopy as described previously (see Chapter 2).

At least ten blocks, selected at random, from each species were sectioned and approximately sixty transverse sections of whole myocytes photographed at between X9100 and X15000. The resulting micrographs were projected onto sheets of plain A4 paper and the profiles of the myocytes, mitochondria and myofibrills traced with a sharp pencil. The areas of these profiles were measured using a digital planimeter interfaced to a microcomputer. The diameters of the myocytes were determined from the areas assuming

circularity and the mitochondrial and myofibrillar volume densities calculated as follows:

$$Vv \text{ (Volume density)} = \frac{\text{Sum Organelle areas}}{\text{Cell C.S. Area}}$$

Determination of myoglobin.

The concentrations of myoglobin in the ventricles of six specimens of N.neglecta (0.64 ± 0.04kg) and six specimens of sculpin (0.19 ± 0.01kg) were measured using a direct photometric technique (Sidell, 1980). Following excision the contents of the ventricular lumena were carefully washed-out with 0.1M phosphate buffer at 0°C to remove haemoglobin. The tissue was homogenised in 10 volumes of the buffer and the resulting homogenates centrifuged at 20,000g for thirty minutes. The pellets were extracted a further two times in 5 volumes of buffer. The absorbance spectra of the combined supernatants were determined over the range 490-600nm and the concentration of myoglobin was calculated from the absorbance at 581nm using a millimolar extinction coefficient of 12.8 (Hardman et al., 1966).

Determination of cytochrome oxidase activity (EC.1.9.3.1.).

The activity of cytochrome oxidase was measured in ventricles from six specimens of C.aceratus (0.93 ± 0.07kg) and six specimens of N.neglecta (0.55 ± 0.03kg). Tissue was homogenised at 0°C in 10 volumes of extraction buffer containing 100mM Tris (hydroxymethyl) aminomethane-HCl, 1mM ethylenediamine-tetraacetic acid (EDTA), 0.5mM dithioerythritol at pH 7.6. The cytochrome oxidase activity in the homogenates was determined by following the oxidation

of reduced cytochrome C in 50mM phosphate buffer (pH 7.6) at 550nm.

Statistics

Interspecific differences in the quantitative ultrastructural characteristics and the activities of cytochrome oxidase measured in the present study were compared using Student's t-test.

RESULTS

Mitochondrial and myofibrillar volume densities.

The ultrastructure of typical ventricular myocytes from each of the four species investigated in the present study are shown in Fig.3:1. The quantitative ultrastructural characteristics of these cells, together with comparative data from the literature, are presented in Table 3:1. With the exception of the tunas, Thunnus alalunga (Breisch et al., 1983) and Thunnus thynnus (Tota, 1978), all the species included in this table had Type I (i.e. spongy) ventricles as defined by Tota et al. (1983).

The mitochondrial volume density (Vv(mt,f)) was subject to considerable interspecific variation, with almost a three-fold difference between the oscar cichlid with the lowest (0.15) and the highest, in the channichthyid C.aceratus (0.43). In both C.aceratus and the lumpsucker, two species with white hearts, Vv(mt,f) was higher than in species with myoglobin-rich hearts from similar thermal

environments: specifically, the mean mitochondrial volume density in C.aceratus was significantly higher than in the south polar notothenid N.neglecta ($P<0.01$) and the mitochondrial compartments in lumpsucker myocytes were larger than in the sculpin. In C.aceratus, the proliferation of mitochondria was associated with a reduction in intracellular space devoted to contractile elements: the myofibrillar volume density in this species was significantly less than in N.neglecta ($P<0.01$). In contrast to C.aceratus, the mitochondrial and myofibrillar volume densities in another channichthyid, Channichthyis rhinoceratus, were similar to those of the notothenioids, N.neglecta and Notothenia rossii.

Considering the species in the 'red-hearts' section of Table 3:1, the highest mitochondrial volume densities occurred in the tunas, T.alalunga and T.thynnus. The other species demonstrated a trend towards increasing values of Vv(mt,f) with decreasing adaptation temperature (Fig.3:2).

Myocyte diameters.

In general, the myocytes from the south polar notothenioids had larger diameters than the warmer water species (Table 3:1). This was confirmed by statistical analyses of the results obtained in the present study. These showed that there was no significant difference between the mean myocyte diameters of C.aceratus and N.neglecta ($P>0.05$) or between those of the lumpsuckers and oscar cichlids ($P>0.05$); however, the south polar species both had significantly larger diameters than these tropical and

temperate species ($P<0.05$).

Cytochrome oxidase activities.

Table 3:2 shows the activities of cytochrome oxidase and the myoglobin contents of spongy ventricles from four sedentary teleost species. The data suggests that the activity of cytochrome oxidase is independent of both ventricular myoglobin content and environmental temperatures. The difference between the activities of this enzyme in the ventricles of C.aceratus and N.neglecta was not significant ($P>0.05$).

DISCUSSION

The results obtained in the present study confirm the extensive development of the mitochondrial compartments in the ventricular myocytes of C.aceratus suggested by the observations made in Chapter 2. The mean mitochondrial volume density in these cells (0.43) is higher than in any other teleost studied to date (Table 3:1): indeed, with the exception of the Etruscan shrew ($Vv(mt,f) = 0.45$) (Weibel, 1985) it is the highest reported in the myocytes of any vertebrate. The proliferation of these organelles appears to be at the expense of space devoted to force generating elements: the mean myofibrillar volume density in C.aceratus myocytes (0.31) is low compared to other teleosts (Table 3:1).

The comparative data presented in Table 3:1 indicate

that both low environmental temperatures and low myocardial myoglobin concentrations are promoters of high mitochondrial volume densities in teleost myocytes. The high values of Vv(mt,f) in C.aceratus myocytes may, therefore, be related to a combination of these factors.

Low environmental temperature.

The trend towards high values of Vv(mt,f) with decreasing adaptation temperature is demonstrated most clearly with data from sedentary species with myoglobin-rich ventricles (Fig.3:2). This adaptive strategy is analagous to that which has been reported in the skeletal muscle fibres of eurythermal teleosts during acclimation to low temperatures (Johnston and Maitland, 1980; Tyler and Sidell, 1984). These changes in morphology are thought to compensate for the rate depressing effects of low temperature on the activities of oxidative enzymes and on the diffusion of metabolites across mitochondrial membranes (Sidell, 1983). The adaptive significance of the high mitochondrial volume densities in the myocytes of the cold-adapted species (Fig.3:2) may also be related the maintenance of functional levels of aerobic ATP synthesis. Certainly, increases in the activity of mitochondrial enzymes in cold adapted species may be inferred from Table 3:2 which shows that the activity of cytochrome oxidase in ventricular tissue from N.neglecta at 0°C is similar to that in two temperate species at 10°C. The high mitochondrial volume densities in the tunas, T.alalunga and T.thynnus (Fig.3:2) are not associated with environmental temperature. Breisch et al. (1983) have related the anomolous values of Vv(mt,f) in these fish to

the high myocardial power outputs and high blood lactate concentrations which are characteristic of active species.

Low myocardial myoglobin concentrations.

The data concerning the influence of low myocardial myoglobin concentration on myocyte mitochondrial volume densities appears to be more equivocal than that of low temperatures. Table 3:1 includes data from three species with myoglobin-poor ventricles: two south polar channichthyids, C.aceratus and C.rhinoceratus, and the temperate lumpsucker. The mitochondrial volume density in the myocytes of C.aceratus (0.43) was considerably higher than in the south polar nototheniids, N.neglecta (0.25) and N.rossii (0.23) both of which have myoglobin-rich ventricles (Table 3:1). This disparity was not associated with differences in aerobic capacities since the activities of cytochrome oxidase in the ventricles of C.aceratus and N.neglecta were not significantly different (Table 3:2). This observation suggests that the proliferation of mitochondria in the myocytes of the channichthyid is, to some extent, related to diffusion limitations (see Sidell, 1983) and may well represent an evolutionary strategy serving to maintain functional rates of oxygen uptake. This is supported by the data from the lumpsucker in which the mean value of Vv(mt,f) (0.27) was considerably higher than in the sculpin (0.17) a largely sympatric species (Wheeler, 1978) with a red-heart (Table 3:1).

High mitochondrial volume densities may compensate for low myocardial myoglobin concentrations by reducing oxygen

diffusion path-lengths between the intertrabecular spaces and the mitochondria. This interpretation is consistent with the results of experiments using artificial systems in which reduced diffusion path-lengths resulted in a decreased contribution of facilitated diffusion to the total oxygen flux across membranes (Kreuzer and Hoofd, 1972). In the case of C.aceratus the problems of oxygen diffusion associated with the myoglobin-poor condition may be exacerbated by the large diameters of the myocytes (Table 3:1). However, it should be noted that in species with avascular myocardia, such as C.aceratus (Chapter 2), myocyte diameters may be less significant in terms of oxygen diffusion than the dimensions of the ventricular trabeculae. Unfortunately, as Santer (1984) has pointed out, stereological techniques have not yet been developed which can adequately describe the complex organization of the teleost spongy myocardium.

In contrast to C.aceratus and the lumpsucker, the mean mitochondrial volume density in the myocytes of C.rhinoceratus (0.24) was relatively low, and was in the range reported for south polar species with myoglobin-rich ventricles (Table 3:1). In common with C.aceratus, C.rhinoceratus is a sedentary, benthic icefish with a large ventricle (Feller et al., 1983; Feller et al., 1985). If the high mitochondrial volume densities in the myocytes of C.aceratus represent a compensatory adaptation associated with low myoglobin concentrations, how can myocardial function be maintained in C.rhinoceratus? The answer to this question may lie in consideration of the circumstances under which myoglobin mediated flux becomes important for oxygen

delivery to the myocardial mitochondria. Experimental evidence suggests that, in the absence of myoglobin, the mechanical performance and rates of ATP synthesis in cardiac tissue are impaired only under hypoxic conditions (Driedzic et al., 1982; Driedzic, 1983; Bailey and Driedzic, 1986; Taylor et al., 1986; Canty and Driedzic, 1987). Channichthyids are unlikely to be subjected to environmental hypoxia as a result of the high solubility of oxygen in the Southern Ocean (Chapter 1). However, Johnston and Harrison (1987) have suggested that they may experience low venous oxygen tensions during periods of maximal sustained activity. This is supported by experiments which have shown that C.aceratus can survive venous oxygen tensions of less than 1mmHg (Hemmingsen and Douglas, 1970). In C.aceratus this would certainly render the myocardium hypoxic since the ventricle is supplied largely through the venous lacunary circuit (Chapter 2). In C.rhinoceratus the myocardium is perfused by an elaborate vascular network (Feller et al., 1985). The nature of these vessels has not been established (see Chapter 2); however, they may serve to enhance blood supply to the ventricle and so protect the myocardium from exercise induced hypoxia. If this is the case then, in contrast to C.aceratus, extreme mitochondrial proliferation would not represent a selective advantage in this species.

Table 3:1. Quantitative ultrastructural characteristics of teleost ventricular myocytes.

SPECIES	HABITAT AND ACTIVITY PATTERN	MYOGLOBIN (nmoles per g wet wt.)	Vv(mt,f)	Vv(my,f)	Vv(mt,f) / Vv(my,f)	MYOCYTE DIAMETER (μm)	
'WHITE HEARTS'							
Chaenocephalus aceratus	Polar, marine. Sedentary, benthic.	1.02-1.51[a]	0.43±0.04[b]	0.31±0.03[b]	1.39[b]	5.95±1.92[b]	
Channichthyis rhinoceratus	Polar, marine. Sedentary, benthic.	nd[c]	0.24±0.04[d]	0.44±0.07[d]	0.54[d]	5.7±2.3[d]	
Lumpsucker (Cyclopterus lumpus)	Temperate, marine. Sedentary, benthic.	nd[e]	0.27±0.01[b]	0.42±0.02[b]	0.64[b]	2.17±1.00[b]	
'RED HEARTS'							
Notothenia neglecta	Polar, marine. Largely benthic.	19.6±0.1[b]	0.25±0.01[b]	0.43±0.02[b]	0.58[b]	5.25±1.51[b]	
Notothenia rossii	Polar, marine. Bentho/pelagic.	---	0.23±0.04[d]	0.45±0.02[d]	0.51[d]	4.3±1.3[d]	
Nezumia aequalis	Mesopelagic, marine. Bentho/pelagic.	---	0.20±0.07[f]	0.51±0.11[f]	0.39[f]	3.68±2.92[f]	
Cottus (Myoxocephalus scorpius)	Temperate, marine. Sedentary, benthic.	20.7±0.4[b]	0.17±0.06[f]	0.51±0.15[f]	0.33[f]	3.59±2.59[f]	
Thunnus alalunga	Tropical/Temperate, marine. Active, pelagic.	--- ---	0.24±0.02[g] 0.32±0.02	0.52±0.01[g] 0.55±0.02	0.50[g] 0.61	4-6[g] 2.5-5.0	Compacta Spongiosa
Thunnus thynnus	Tropical/Temperate, marine. Active, pelagic.	581-1175[h] 581-1175	0.39[i] 0.32	0.47[i] 0.49	0.62[i] 0.65	--- ---	Compacta Spongiosa
Oscar cichlid (Astronotus ocellatus)	Tropical, fresh-water. Pelagic.	---	0.15±0.01[b]	0.45±0.02[b]	0.33[b]	2.01±1.12[b]	

Abbreviations: Vv(mt,f)=mitochondrial volume density.
Vv(my,f)=myofibrillar volume density.
nd=not detected

References:

a.Calculated from Douglas et al. (1985) assuming the molecular weight of myoglobin=16600 and the ratio of ventricular dry weight:wet weight is 1:5.2.
b.Present study.
c.Feller (1987).
d.Feller et al (1985).
e.Driedzic and Stewart (1982).
f.Morris and Johnston (unpublished results).
g.Breisch et al. (1983).
h.Giovane et al. (1980).
i.Tota (1978).

Table 3:2. Myoglobin concentrations and activities of cytochrome oxidase in teleost red and white ventricles.

SPECIES	ASSAY TEMP. (°C)	MYOGLOBIN (nmoles/g wet weight)	CYTOCHROME OXIDASE ACTIVITY (µmoles/g wet wt./min.)
'WHITE HEARTS'			
Chaenocephalus aceratus	0	1.02±1.51[a]	33.8±3.9[b]
Ocean Pout (*Macrozoarces americanus*)	10	5.1±1.8[c]	29.8±4.8[c]
'RED HEARTS'			
Notothenia neglecta	0	19.6±0.1[b]	33.0±3.7[b]
Sea Raven (*Hemitriperus americanus*)	10	63.8±2.1[c]	35.8±8.0[c]

References:

a. Calculated from Douglas *et al.* (1985) assuming the molecular weight of myoglobin=16600 and the ratio of ventricular dry weight:wet weight is 1:5.2.
b. Present study.
c. Driedzic and Stewart (1982).

Fig.3:1. Transmission electron micrographs showing transverse sections through ventricular myocytes from four teleost species. **a**: Chaenocephalus aceratus, **b**: Notothenia neglecta, **c**: Lumpsucker, Cyclopterus lumpus, **d**: Oscar cichlid, Astronotus ocellatus. Note the particularly large mitochondrial (mit) and small myofibrillar (my) compartments in the cells in C.aceratus.

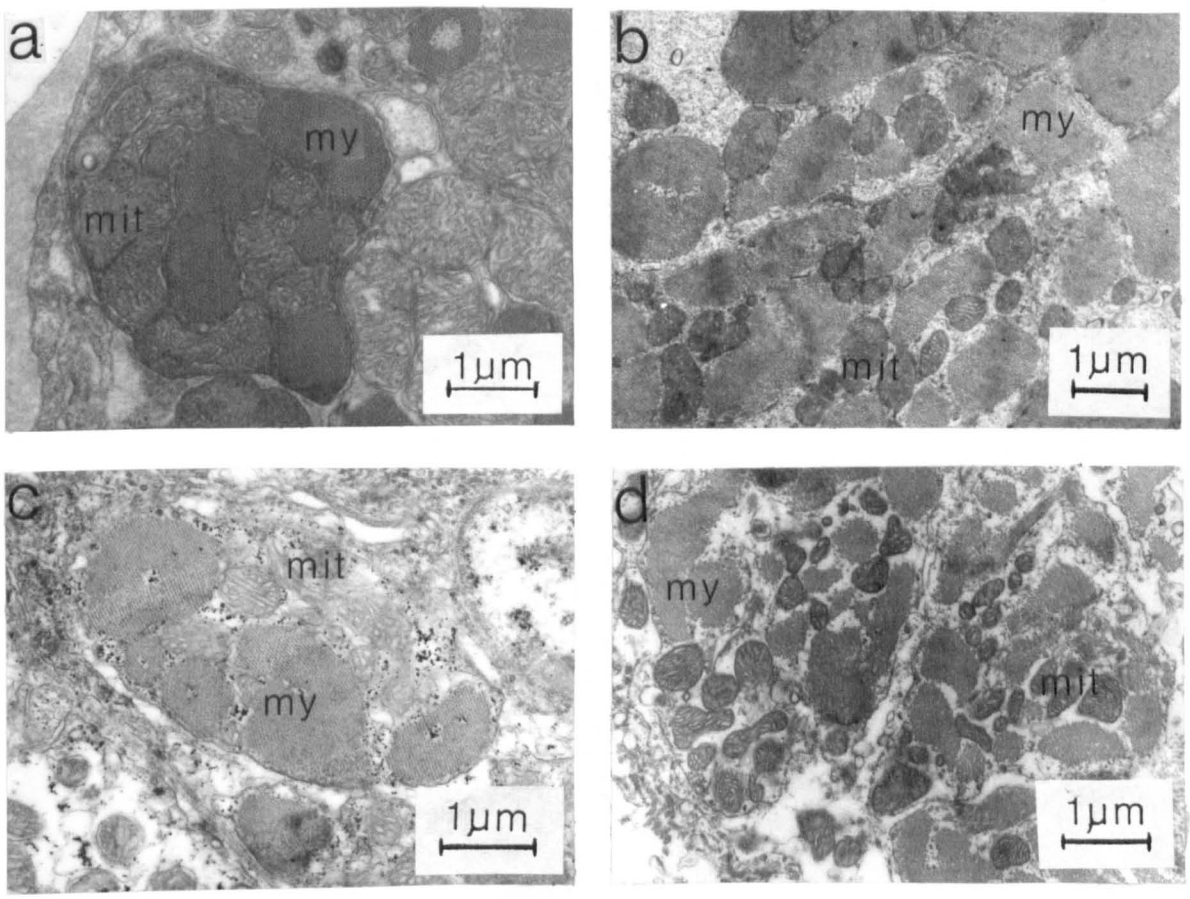
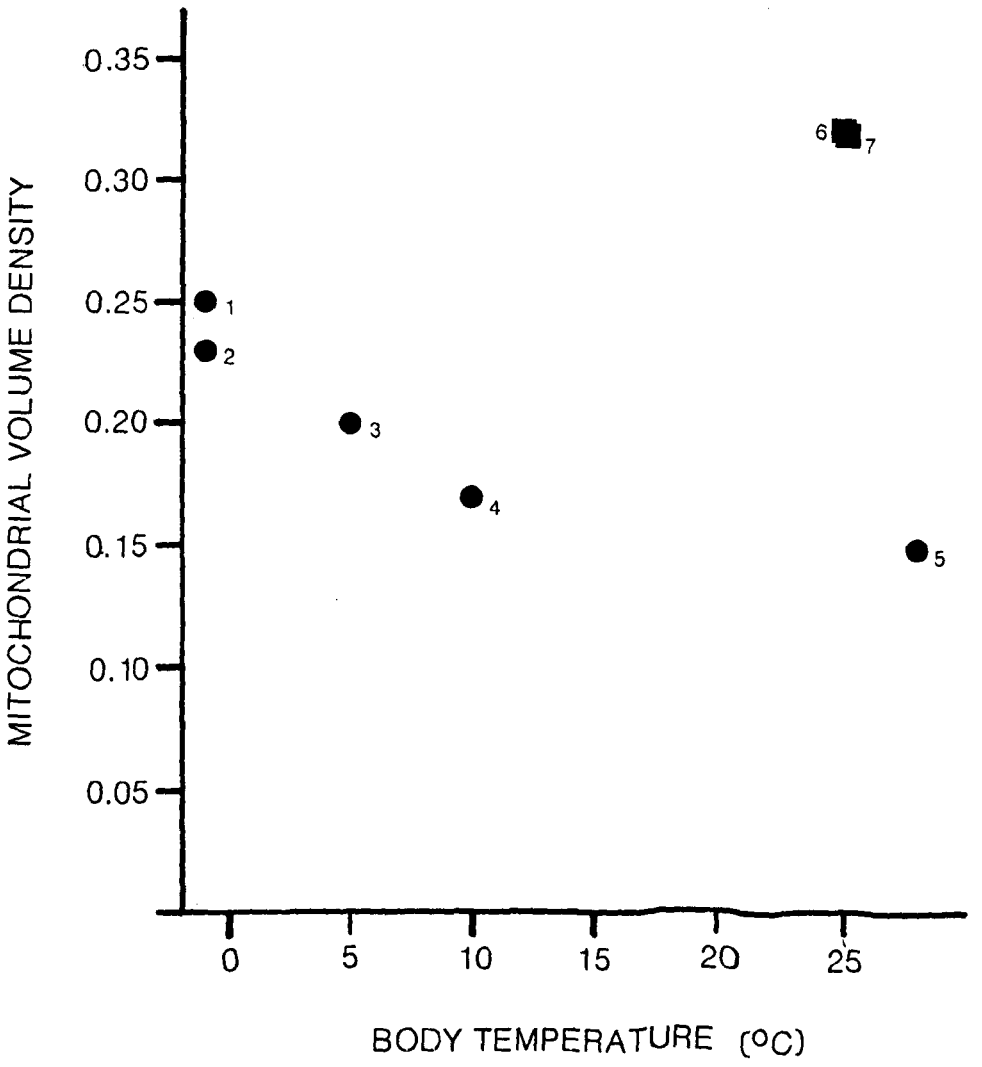

a
my
mit
1μm
b
my
mit
1μm
c
mit
my
1μm
d
my
mit
1μm

Fig.3:2. Relationship between body temperature and mitochondrial volume density in myocytes from the spongy ventricular myocardia of sedentary (●) and active (■) species of teleosts with myoglobin-rich hearts.

1. Notothenia neglecta (present study).
2. Notothenia rossii (Feller et al., 1985).
3. Nezumia aequalis (Morris and Johnston, unpublished results).
4. Myoxocephalus scorpius (Morris and Johnston, unpublished results).
5. Astronotus oscellatus (present study).
6. Thunnus alalunga (Breisch et al., 1983).
7. Thunnus thynnus (Tota, 1978).


MITOCHONDRIAL VOLUME DENSITY
0.35
0.30
0.25
0.20
0.15
0.10
0.05
1
2
3
4
5
6
7
0
5
10
15
20
25
BODY TEMPERATURE (°C)

CHAPTER 4

The Gross Morphology, Fibre Composition and Mechanical Properties of Pectoral Fin Muscles in the Benthic Nototheniid *Notothenia neglecta* Nybelin.

INTRODUCTION

Two distinct propulsive mechanisms are employed by notothenioid fish at different swimming speeds. Sustained, low speed swimming is achieved with large, fan-shaped pectoral fins in a drag-based, labriform mode of locomotion (Twelves, 1972; Montgomery and Macdonald, 1984; Archer and Johnston, 1988). Burst speeds involve adduction of the pectoral fins and recruitment of the segmental myotomal muscles to derive propulsion from undulatory movements of the trunk and caudal fin (Montgomery and Macdonald, 1984; Archer and Johnston, 1988).

A number of authors have reported that the pectoral fin muscles in these fish are entirely aerobic in character and related this to their role in sustained swimming (Lin et al., 1974; Smith and Haschemeyer, 1980; Eastman and DeVries, 1982). However, more detailed ultrastructural (Johnston and Camm, 1987) and histochemical (Walesby and Johnston, 1980; Walesby et al., 1982; Davison and Macdonald, 1985; Dunn et al., 1988) investigations have shown that, in most notothenioid species, there is a range of pectoral fibre types which differ in their aerobic capacities. Unfortunately, classification of these into the red pink or white categories defined in other teleosts (see Chapter 1) has proved difficult due to an inability to differentiate between fast and slow isotypes of myosin ATPase in cold-adapted species using standard histochemical techniques (Davison and Macdonald, 1985; Dunn et al., 1988).

The present study describes the muscle fibre composition in the pectoral muscles of the benthic notothenioid Notothenianeglecta. Constituent fibre types were differentiated initially on the basis of their size and histochemical staining for markers of aerobic metabolism. Full characterization was then achieved by determination of the contractile properties of demembranated preparations of these fibres. In addition, the gross anatomy of the pectoral fin muscles was described in some detail. The results form the most comprehensive account of the pectoral fin musculature in a notothenioid available to date and are important pre-requisites to the interpretation of electromyographic and kinematic studies of sustained swimming in N.neglecta currently in progress.

METHODS

Fish collection and maintenance.

Specimens of Notothenia neglecta Nybelin were caught using trammel nets in Borge Bay off Signy Island, South Orkney Islands, British Antarctic Territory. Live fish were maintained in a recirculated sea-water aquarium at 0 to +1°C and fed every other day on fish and amphipods. Experimental animals were killed with a blow to the head followed by spinal cord transection.

Whiting (Merlangius merlangus L.) were caught using beam trawls in the Firth of Forth off the East coast of Scotland. Freshly caught specimens were returned dead to the

laboratory.

Myology and anatomy of the pectoral fin skeleton.

Dissections of 18 freshly killed specimens of N.neglecta (between 0.32 and 0.87kg total body weight) were carried out under a binocular microscope. Drawings of the pectoral muscles in situ and of the pectoral fin skeleton were made with the aid of a camera lucida. Intact pectoral muscles were removed and weighed. In addition, the entire trunk musculature was dissected from the skeleton, skinned and weighed. Comparative muscle weights were obtained from six specimens of whiting (between 0.06 and 0.14kg total body weight).

Histochemistry.

Histochemical studies were carried out on the pectoral muscles from six specimens of N.neglecta (mean weight $\pm$ S.E.M. = 0.22 $\pm$ 0.04kg). Tissue samples, approximately $3mm^2$ and 5mm long, were dissected from deep and superficial regions of the muscles and mounted on chilled cryostal chucks in OCT embedding medium (Miles Scientific, Naperville, Illinois, USA). The tissue and chuck assembly was immediately frozen in isopentane cooled to near its freezing point by immersion in liquid nitrogen. 10μm transverse sections of the tissue were cut using a Bright cryostat cooled to -20°C and the ribbons mounted on dry cover slips prior to staining. All incubation and washing procedures were carried out at approximately 4°C unless otherwise specified.

Succinic Dehydrogenase (SDH): Staining for SDH was carried out using a method described by Nachlas et al. (1957). Sections were incubated for three hours at room temperature in 50mM phosphate buffer containing 70mM sodium succinate and 1.5mgml^{-1} nitro blue tetrazolium. The sections were washed for five minutes in distilled water, dried in air and mounted under glycerol-gelatin.

Glycogen: Sections were stained for glycogen using the PAS reaction (Bancroft and Stevens, 1972). The sections were incubated in 1% periodic acid for 60 minutes at room temperature, washed thoroughly in tap water and stained for 10 minutes in Schiff's reagent.

Myofibrillar ATPase: Sections were stained for myofibrillar ATPase using a modification of the method of Guth and Samaha (1969). Staining was carried out with and without acid preincubation. Acid preincubation, 20 minutes in 0.2mm acetate buffer (pH 4.8), was followed by immersion in the following medium for 1 hour: 2.5mM ATP and 25mM $CaCl_2$ in 0.2mM Tris buffer (pH 9.5). Sections which were not acid treated were incubated in the same medium for 10 minutes.

Force-velocity characteristics of demembranated muscle fibres.

The force-velocity characteristics of pectoral muscle fibres isolated from 22 specimens of N.neglecta (mean weight $\pm$ S.E.M. = 0.51 $\pm$ 0.03kg) were determined at 0°C. Experiments were carried out on large diameter fibres isolated from the the 'white region' of the M.AB.P. (see

Fig.4:5a) and on small diameter fibres from each of the six pectoral muscles.

Apparatus: An isotonic lever device, similar to that described in detail by Altringham and Johnston (1982), was used in these experiments. The apparatus consisted of two units which allowed independent measurement of force production and velocity of shortening. The muscle fibre preparations were attached to these units via a pair of fine glass hooks.

The hook associated with force measurement was attached directly to the silicon beam of an A.M.E. tension transducer (A.M.E., Horton, Norway) (sensitivity = $0.5mNV^{-1}$). The transducer was held firmly in a perspex block at an angle of approximately 50° to the horizontal. A screw enabled the transducer/block assembly to be moved to adjust the resting length of the muscle fibres.

A second hook was attached to the free end of a balsa-wood beam. The other end of the beam was fixed to the central spindle of a moving coil galvanonometer. Free movement of the beam under the influence of a contracting muscle fibre was impeded by a metal rod soldered to the armature of a miniature relay. Activation of the relay withdrew the rod and allowed the fibre to contract through approximately 500µm at which point the movement of the beam was arrested by a second stop. As it moved, an aluminium flag attached to the top of the beam passed through an L.E.D.-photodiode assembly and allowed the measurement of

displacement against time. The isotonic afterload on the beam could be varied between 0 and approximately 25mN by changing the current through the galvanometer coil.

The outputs from the force and velocity units were displayed on a dual beam storage oscilloscope.

Experimental solutions were contained in three 2ml perspex baths suspended beneath the hooks. The baths were mounted on a rail which allowed the fibre preparations to be immersed in each bath in turn. The temperature of the solutions was maintained at $0 \pm 0.1°C$ with a circulating solution of 20% ethanol in water.

Solutions: Relaxing solution had the following composition: 20mM imidazole, 110mM KCl, 3mM $MgCl_2$, 5mM EGTA, 2.5mM ATP, 10mM phosphocreatine and $20Uml^{-1}$ creatine phosphokinase (added to the solution just before each experiment). The pH of the solution was set to 7.56 at 0°C. Skinning solution had the same composition with the addition of 1% Brij 58 (polyoxyethylene-20-cetyl-ether). Activating solution was made by adding 4.8mM $CaCl_2$ to relaxing solution and had the following ionic composition: pCa 4.90, pMg 3.19, pMgATP 2.68, ionic strength 180mM (at 0°C and pH 7.56). Free concentrations of these ionic species were calculated using an iterative computer programme.

Fibre preparation: Strips of large and small diameter fibres were dissected from appropriate regions of the pectoral muscles identified by histochemistry and stored under

relaxing solution on ice. Bundles of approximately 10-20 fibres were removed from these strips, transferred to a glass slide on the cooled stage of a binocular microscope and covered with silicone fluid (B.D.H., Poole, Dorset). Single large diameter fibres or bundles of 2-3 small diameter fibres were teased from the muscle mass using fine, watch-makers' forceps; care was taken to avoid touching the fibres except at the extreme ends. The fibres were mounted on the apparatus between the glass hooks using plexiglass in acetone glue.

The fibres were demembranated by immersion in skinning solution for 20 minutes and subsequently transferred to relaxing solution where they were allowed to equilibrate for 5 minutes. The sarcomere length was measured by laser diffraction and set to 2.3μm. At this stage, the diameters and lengths of the fibres were measured through a binocular microscope mounted above the apparatus.

Force-velocity determination: Fibres were transferred to the final bath containing activating solution and the development of isometric tension monitored on a chart recorder. When this reached a steady level the fibres were given six or more step-tension releases at a series of increasing afterloads.

Shortening velocities were measured over the second 50ms of the displacement transients on the oscilloscope (Fig.4:1a) and expressed as muscle lengths per second. The corresponding relative tensions (tension after release (P)/

maximum tension (Po)) were also calculated from the oscilloscope traces (Fig.4:1a). Data from releases up to 0.6 Po for individual fibres were fitted to a linear form of the following equation (Hill, 1938) as shown in Fig.4:1c:

$$V(P+a) = b(Po-P)$$

Where V is shortening velocity, P is tension, Po is maximum tension and a and b are constants (see Fig.4.1b). The maximum contraction velocity (Vmax) and Hill's constants (a/Po and b) were determined as shown in Fig.4:1b.

Statistics: The maximum isometric tensions and maximum contraction velocities of different fibre types and of the same fibre types but from different muscles were compared using Student's t-test.

RESULTS

The pectoral fin skeleton.

The term 'pectoral fin skeleton' is used in the present study to describe skeletal elements associated with the muscles which articulate the pectoral fin blade, i.e. the entire primary shoulder girdle (the radials, scapula and coracoid) and the cleithrum (Figs.4:2a and b).

The primary shoulder girdle was in the form of a single plate with obvious sutures between the constituent elements. Three shield-like radials and the scapula formed

the posterior border. A prominent, round pectoral condyle on the posterior border of the scapula was associated with the position of the most dorsal fin ray. In this species most of the scapular foramen occurred outside the border of the scapula and mainly notched the coracoid. A second major foramen, the interosseous space, was separated from the scapular foramen by the transverse arm of the coracoid. Three smaller foramina occurred in the region of the most ventral radial (Fig.4:2b).

The cleithrum was attached to the primary shoulder girdle at three points: the dorsal region of the scapula, the transverse arm of the coracoid, and the anterior coracoid process. Part of the ventral border of the cleithrum, between the scapula and the ventral coracoid process, was extended further distally than the coracoid. This formed a ridge over the scapular foramen, the transverse arm of the coracoid and part of the interosseous space. This ridge was an important point of origin for the superficial abductor muscle and the M.ARR.V. (see below).

Figure 4:3 shows the position of the pectoral fin skeleton in the intact animal. The anterior border of the cleithrum formed the posterior border of the branchial chamber. The fin rays articulated around the posterior borders of the scapula and radials.

The fin rays.

Specimens of N.neglecta from Signy Island have 17 or 18 pectoral fin rays (Norman, 1938). Details of the bases of

some of these rays are shown in Fig.4:4. In the present study these are numbered dorso-ventrally. The rays consisted of two hemitrichia. With the exception of number 1, which was entirely fused, the hemitrichia were separated at the ray bases but fused distally. The bases of the hemitrichia were complex. They had dorsal and ventral processes which formed the points of insertion for the pectoral fin muscles (Fig.4:4b). The positions of the insertions of a given muscle varied on different hemitrichia: for example, the insertion of the adductor profundis muscle on the medial hemitrichia of fin ray 2 was near the base, whereas it was much more distal on ray 16 (Fig.4:4b).

The bases of the hemitrichia were arranged around the posterior border of the radials and part of the scapula, such that the medial and lateral hemitrichia fell on opposite sides of the shoulder girdle. Apart from number 1, which was in contact with the pectoral condyle, the fin rays did not articulate directly on the radials or the scapula but on a fibrocartilage pad interposed between these elements of the primary shoulder girdle and the bases of the rays.

Myology of the Pectoral Fin.

The organization of the pectoral fin muscles is shown in Fig.4:5. The nomenclature follows Winterbottom, (1974).

Abductor muscles.

M. Abductor Superficialis (M.AB.S., Fig.4.5a): Had extensive origin along lateral border of cleithrum.

There was no insertion of this muscle on ray 1 (Fig.4:4b). Insertions were tendinous at the dorsal processes of lateral hemitrichia 2-17 (or 18) (Fig.4:4b). The ventral region of this muscle was composed of a well defined, triangular 'white region'.

M. Adductor Profundis (M.AB.P., Fig.4:5b): Origin along lateral side of ventral border of coracoid. Insertions at the base of ray 1 and at the ventral processes of all lateral hemitrichia (Fig.4:4b).

M. Arrector Ventralis (M.ARR.V., Fig.4:5b): Lay dorsal to, and in same plane as the M.AB.P.. Extensive origin at cleithrum medial to origin of M.AB.S.. Fibres tapered to single insertion at ray 1 (Fig.4:4b).

Adductor muscles.

M. Adductor Superficialis (M.AD.S., Fig.4.5c): This muscle could be separated into two layers at the insertions. Both layers shared a common origin at the scapular border of the cleithrum. However, the origin of the deeper portion extended further in an anterior direction resulting in the two fibre orientations apparent in Fig.4:5c. The deeper portion had insertions at the base of ray 1 and at the dorsal processes of medial hemitrichia 2-6 (or, occasionally, 7) (Fig.4:4b). The superficial layer consisted of a large flat sheet with insertions at the dorsal processes of the remaining medial hemitrichia (Fig.4:4b).

M. Adductor Profundis (M.AD.P., Fig.4.5d): A large muscle originating at the anterior region of the

cleithrum around the border of the pelvic girdle. Tendinous insertions occurred at the ventral processes of medial hemitrichia numbers 2-18 (Fig.4:4b). There was no insertion at ray 1 (Fig.4:4b).

M. Arrector Ventralis (M.ARR.V., Fig.4:5d): A small muscle which lay beneath the dorsal region of the M.AD.P. and was closely associated with this larger muscle. It originated at the anterior coracoid process, and inserted tendinously at the base of ray 1 (Fig.4:4b).

Relative Muscle Weights.

Table 4:1 shows the relative trunk and pectoral muscle weights in N.neglecta and the whiting. In both species the trunk muscles comprised greater proportions of the total body weight than the combined pectoral muscle weights. The relative weight of the combined pectoral muscles in N.neglecta was almost five times greater than in the whiting. The principal pectoral muscle in N.neglecta was the main adductor muscle, the M.AD.P., whereas in the whiting an abductor muscle, the M.AB.P., had the largest relative weight.

Histochemistry.

The pectoral muscles comprised two fibre types: large diameter (24-156μm) fibres which stained poorly for SDH and glycogen and smaller fibres (10-99μm diameter) which stained intensely for SDH and had relatively high levels of PAS staining (Table 4:2). Both of these stained intensely for myosin ATPase following preincubation at pH9.5 (Table 4:2;

Fig.4:6b). No incubation at pH 10.3 was possible as the fibres were disrupted by even short exposure to this pH. Following acid preincubation both fibre types were poorly stained; however, they could be differentiated under these conditions as the smaller fibres were slightly less acid labile than the larger fibres (Fig.4:6c).

The organization of the two fibre types in the pectoral muscles is shown in Fig.4:7. With the exception of the ventral region of the M.AB.S., the bulk of the muscles was composed of the small diameter fibres. The large diameter fibres occurred as thin, superficial layers. In the ventral part of the M.AB.S. there was a 'white region' (Fig.4:5a) in which the large diameter fibres occurred without any underlying small diameter fibres (Fig.4:7b).

Contractile properties.

The two fibre types differentiated by means of their histochemical characteristics also differed in their mechanical properties. Maximum contraction velocities and maximum tensions for the fibres were approximately twice those of the small fibres ($P<0.001$) (Table 4:3). For example, Vmax and Po for fibres from the white region of the M.AB.S. were $0.43 Ls^{-1}$ and $123 kNm^{-2}$ respectively compared to $0.24 Ls^{-1}$ and $58 kNm^{-2}$ for those from the red region. There was no significant difference in the contractile properties of the small diameter fibres isolated from the six different pectoral muscles ($P>0.05$) (Table 4:3).

DISCUSSION

Regan (1912) and Norman (1938) used some salient morphological characteristics of the pectoral fin skeleton as distinguishing features in keys to the identification of antarctic notothenioids. In addition, a detailed description of this structure in one notothenioid, Notothenia tessellata, was included in Starks' (1930) comparative survey of the teleostean primary shoulder girdle. The observations made in the present study on the pectoral fin skeleton in N.neglecta (Figs.4:2 and 4:3) are in broad agreement with the descriptions given in these earlier studies. Of particular interest are the fusion of the elements of the primary shoulder girdle and the size and shape of the radials, in N.neglecta which, according to Starks (1930), may be considered typical of sedentary, benthic species.

In N.neglecta, six muscles, responsible for movements of the pectoral fin blade, have origins on each pectoral fin skeleton. The organization of these is typical of the general teleost plan described by Shann (1920) and Winterbottom (1974): two abductor and two adductor muscles are responsible for articulating the rays about the posterior border of the fin skeleton and two arrector muscles act to fan out the fin blade (Fig.4:5). Previous authors have described single pectoral adductor muscles in the notothenioids Notothenia rossii (Walesby and Johnston, 1980) Pagothenia borchgrevinki and Trematomus bernacchi (Davison and Macdonald, 1985). Whilst this arrangement is

not unusual in teleosts (Winterbottom, 1974) a more thorough investigation of the pectoral myology in these species may be worthwhile. For example, the 'posterior dilator muscle' described by Walesby and Johnston (1980) in N.rossii is almost certainly a misrepresentation of the M.AD.S..

Drag-based sustained swimming in N.neglecta involves a power stroke, during the course of which the pectoral fin is spread to a large area, and a recovery stroke in which it is feathered (Archer and Johnston, 1988). In another labriform swimmer, the angelfish, Pterophyllum eimekei, the recovery period requires only 5% of the force necessary for the power stroke (Blake, 1981). Differences of this kind are reflected in N.neglecta by the disparity in the relative weights of the abductor and adductor muscles: the latter account for 75% by weight of the fin musculature (Table 4:1). Comparative data concerning muscle weights in the whiting are also presented in Table 4:1. This species employs caudal fin propulsion at all swimming speeds. The pectoral muscle mass is correspondingly poorly developed, comprising only 0.53% of total body weight compared to 2.49% in N.neglecta. The pectoral fins in fish such as the whiting are used mainly to adjust trim (Webb, 1975b). In this role, the forces acting on the fin blade are imposed mainly on the lateral face. This is the reverse of the situation in N.neglecta and, in consequence, the pectoral abductor muscles in the whiting constitute a greater percentage of the total body weight than the adductor muscles.

Two fibre types were differentiated in the pectoral

muscles of N.neglecta on the basis of their size and histochemical staining properties: with the exception of the ventral region of the M.AB.S., each of the six muscles was composed of a core of small diameter fibres overlain by a thin, layer of larger diameter fibres (Fig.4:7). At the ventral border of the M.AB.S. a triangular 'white region' occurred (Fig.4:5a) in which there were no underlying small diameter fibres (Fig.4:7c). Histochemical investigations indicated that the large diameter fibres were anaerobic: they stained poorly for both SDH and glycogen (Table 4:2; Figs.4:6 and 7). In contrast, the small diameter fibres were aerobic in character: they stained intensely for SDH and moderately for glycogen (Table 4:2; Figs.4:6 and 7). These histochemical characteristics are consistent with the results of an ultrastructural study of the small diameter pectoral fibres in N.neglecta which revealed the presence of many glycogen granules and a well developed capillary supply (Johnston and Camm, 1987).

In common with histochemical studies on a number of other notothenioid species (Davison and Macdonald, 1985; Dunn et al., 1988) the fibre types in the pectoral fin muscles of N.neglecta could not be differentiated on the basis of the alkali stabilities of their myosin ATPases: all of the fibres stained intensely at pH 9.5 (Table 4:2; Fig.4:6b) which usually indicates fast types of myosin. No combination of pH or preincubation time produced a histochemical differentiation which corresponded to the measured differences in contractile properties (see below). Myosins from Antarctic fish are known to be unusual in that

they aggregate readily on isolation and are very susceptible to thermal denaturation (Johnston et al., 1975a). These structural characteristics can be correlated with adaptations in force production and the force-velocity relationship for function at low temperatures (Johnston and Brill, 1984; Johnston and Altringham, 1985). The lack of differentiation of fibres in N.neglecta muscles may be related to these specializations in myosin structure. A similar anomaly has been described in the neonatal rat in which foetal myosin from fibres with slow contractile properties has similar histochemical characteristics to adult fast myosin (Guth and Samaha, 1972).

In the absence of histochemical demonstration of fast and slow isotypes of myosin ATPase, demembranated preparations were used to investigate the contractile properties of the pectoral fibres in N.neglecta directly. The large diameter fibres had approximately twice the maximum tensions and unloaded contraction velocities of the small diameter fibres (Table 4:3). On the basis of these mechanical data and the histochemical characteristics shown in Table 4:2 the small and large diameter fibres may be confidently classified respectively into the red (slow) and white (fast) categories defined in other teleosts (see Chapter 1).

The histochemical properties of the two pectoral fibre types in N.neglecta are similar to those described by Davison and Macdonald (1985) in Trematomus bernachii and by Dunn et al. (1988) in a number of adult notothenioids.

However, in some other species the pectoral fibre types are all aerobic: in the adductor muscle of Pagothenia borchgrevinki for example, there is a single oxidative fibre type (Davison and Macdonald, 1985), while in Notothenia rossii (Walesby and Johnston, 1980) and Champsocephalus gunnari (Walesby et al., 1982) the adductor muscles are composed of two aerobic fibre types. These interspecific differences in pectoral fibre composition must be related to variations in locomotory habits. In N.neglecta swimming speed increases with pectoral fin beat frequency up to a maximum of approximately 2Hz (Archer and Johnston, 1988). In an earlier account of the present study (Harrison et al., 1987) we suggested that the small diameter fibres may be responsible for low frequency articulation of the fin blade with fast fibres recruited at higher frequencies. This seems unlikely since, as referred to above, there is only one fibre type in the adductor muscle of P.borchgrevinki (Davison and Macdonald, 1985), a species in which swimming speed also increases with fin beat frequency (Montgomery and Macdonald, 1984). A more likely interpretation has been proposed by Davison and Macdonald (1985). They considered two phases of low speed swimming in benthic notothenioids: an initial power stroke, providing lift to raise the fish from the substrate, followed by routine labriform swimming. They suggested that in species such as T.bernacchii and N.neglecta the large white fibres may be used to generate lift, with the aerobic fibres reserved for paddling movements in the water column. This is consistent with the single fibre type in the pectoral muscles of P.borchgrevinki since this is a secondarily pelagic species which swims

constantly off the bottom. The results of electromyographic studies, currently in progress, should define the functions of the pectoral fibre types in N.neglecta unequivocably.

Table 4:1. Relative trunk and pectoral muscle weights for *Notothenia neglecta*. Values refer to bilateral muscle weights.

SPECIES	WEIGHT OF TRUNK MUSCLE AS % OF BODY WEIGHT	WEIGHT OF COMBINED PECTORAL MUSCLES AS % OF BODY WEIGHT	WEIGHT OF INDIVIDUAL PECTORAL MUSCLES EXPRESSED AS % OF TOTAL BODY WEIGHT					
			M.AB.S.	M.AB.P.	M.ARR.V.	M.AD.S.	M.AD.P.	M.ARR.V.
Notothenia neglecta	30.39	2.49	0.38	0.43	0.04	0.42	1.19	0.06
Whiting (*Merlangius merlangus*)	46.80	0.53	0.13	0.17	0.04	0.11	0.08	0.01

Table 4:2. Diameters and histochemical staining properties of fibres from the pectoral abductor muscles of Notothenia neglecta.

	FIBRE DIAMETER (μm) Mean±SEM	DIAMETER RANGE (μm).	STAINING INTENSITIES SDH	PAS	ATPase (pH9.5)	ATPase (pH4.8)
M.AB.S.						
White region	88.8±1.8	35-156	+	+	+++	0
Red region	50.9±1.1	18-99	+++	+ +	+++	+
M.AB.P.						
White region	81.3±1.8	24-149	+	+	+++	0
Red region	35.6±0.8	10-74	+++	+	+++	+

0 background stain only; + lightly stained; +++ heavily stained.

Table 4:3. Force-velocity (P-V) characteristics of demembranated fibres isolated from the pectoral fin muscles of *Notothenia neglecta*. Figures represent means±S.E.M..

MUSCLE	MAXIMUM TENSION (KNm^{-2})	Vmax (MUSCLE LENGTHS sec^{-1})	a/Po	b
M.AB.S. (white region)	122.8±5.9	0.43±0.04	0.28±0.03	0.12±0.02
M.AB.S. (red region)	58.0±6.7	0.24±0.02	0.36±0.04	0.08±0.01
M.AB.P. (red)	60.9±1.5	0.35±0.04	0.31±0.03	0.10±0.01
M.ARR.V. (red)	56.6±3.5	0.27±0.03	0.39±0.04	0.11±0.02
M.ADD.P. (red)	61.1±5.5	0.32±0.04	0.38±0.03	0.12±0.02
M.ARR.D. (red)	53.6±5.2	0.29±0.05	0.39±0.04	0.10±0.02
M.ADD.S. (red)	63.2±2.8	0.24±0.00	0.39±0.03	0.09±0.01

Fig.4:1. Results for white fibres to illustrate the method for determining force-velocity characteristics. **a:** Isotonic velocity transients (L) following a series of step tension releases. Po = maximum isometric tension; 0 = zero tension baseline. **b:** Force-velocity curve showing combined data for seven white fibres. **c:** Data for individual fibres were analysed using a linear form of Hill's (1938) equation for muscle shortening. Regression lines were fitted using a least squares method. Hill's constant a/Po is obtained from the x intercept; 1/Vmax is given by the y intercept and 1/b by the slope of the line.

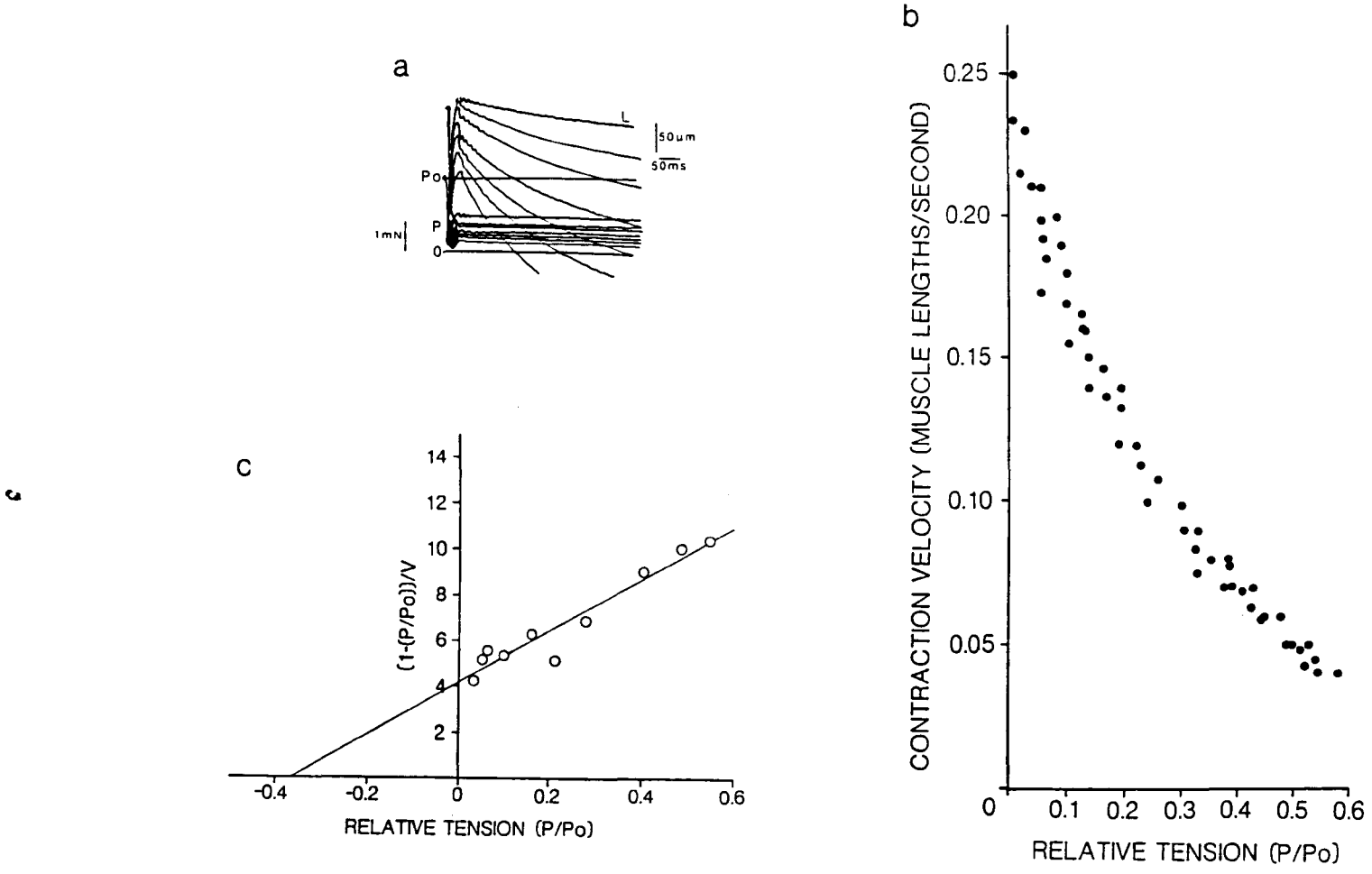
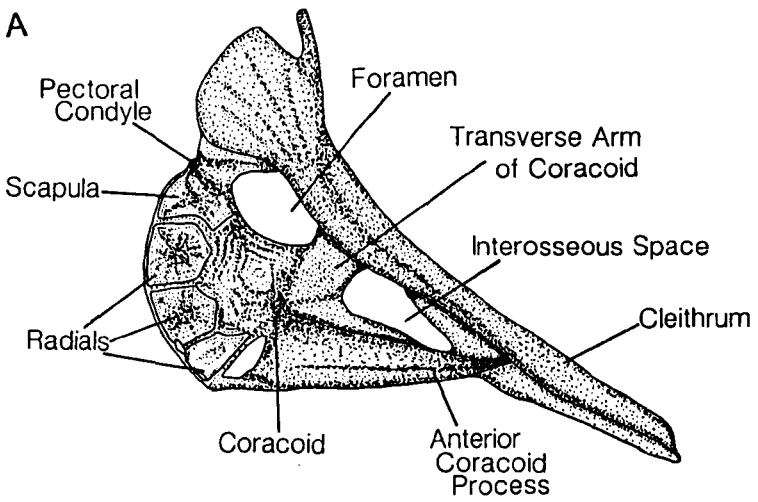

a
L
50 µm
50 ms
Po
P
1 mN
0
b
CONTRACTION VELOCITY (MUSCLE LENGTHS/SECOND)
0.25
0.20
0.15
0.10
0.05
0
0.1
0.2
0.3
0.4
0.5
0.6
RELATIVE TENSION (P/Po)
c
(1-(P/Po))/V
14
12
10
8
6
4
2
-0.4
-0.2
0
0.2
0.4
0.6
RELATIVE TENSION (P/Po)

Fig.4:2. The pectoral fin skeleton removed from the right side of Notothenia neglecta. a: Lateral view b: Medial view.


A
Pectoral Condyle
Foramen
Transverse Arm of Coracoid
Scapula
Interosseous Space
Cleithrum
Radials
Coracoid
Anterior Coracoid Process

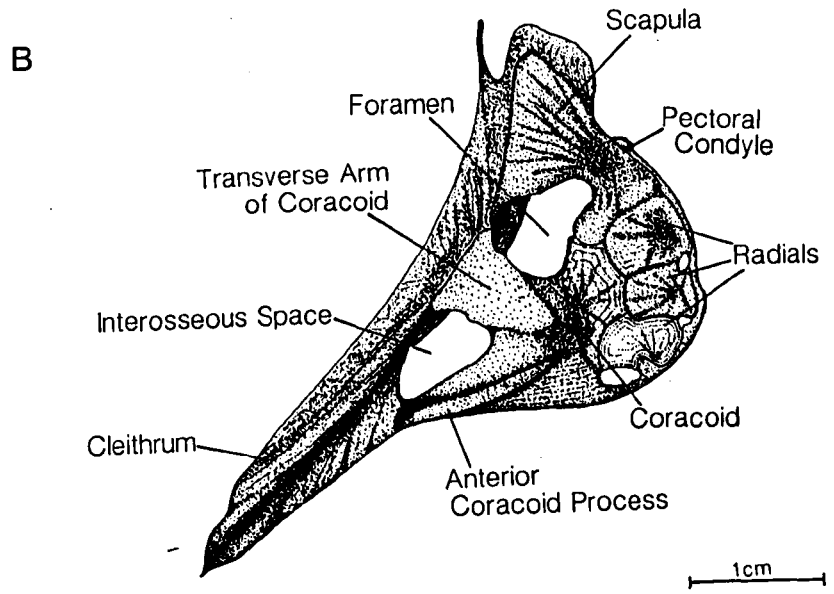

B
Scapula
Foramen
Pectoral Condyle
Transverse Arm of Coracoid
Radials
Interosseous Space
Coracoid
Cleithrum
Anterior Coracoid Process
1cm

Fig.4:3. Relationship between the pectoral fin skeleton and external features of Notothenia neglecta.

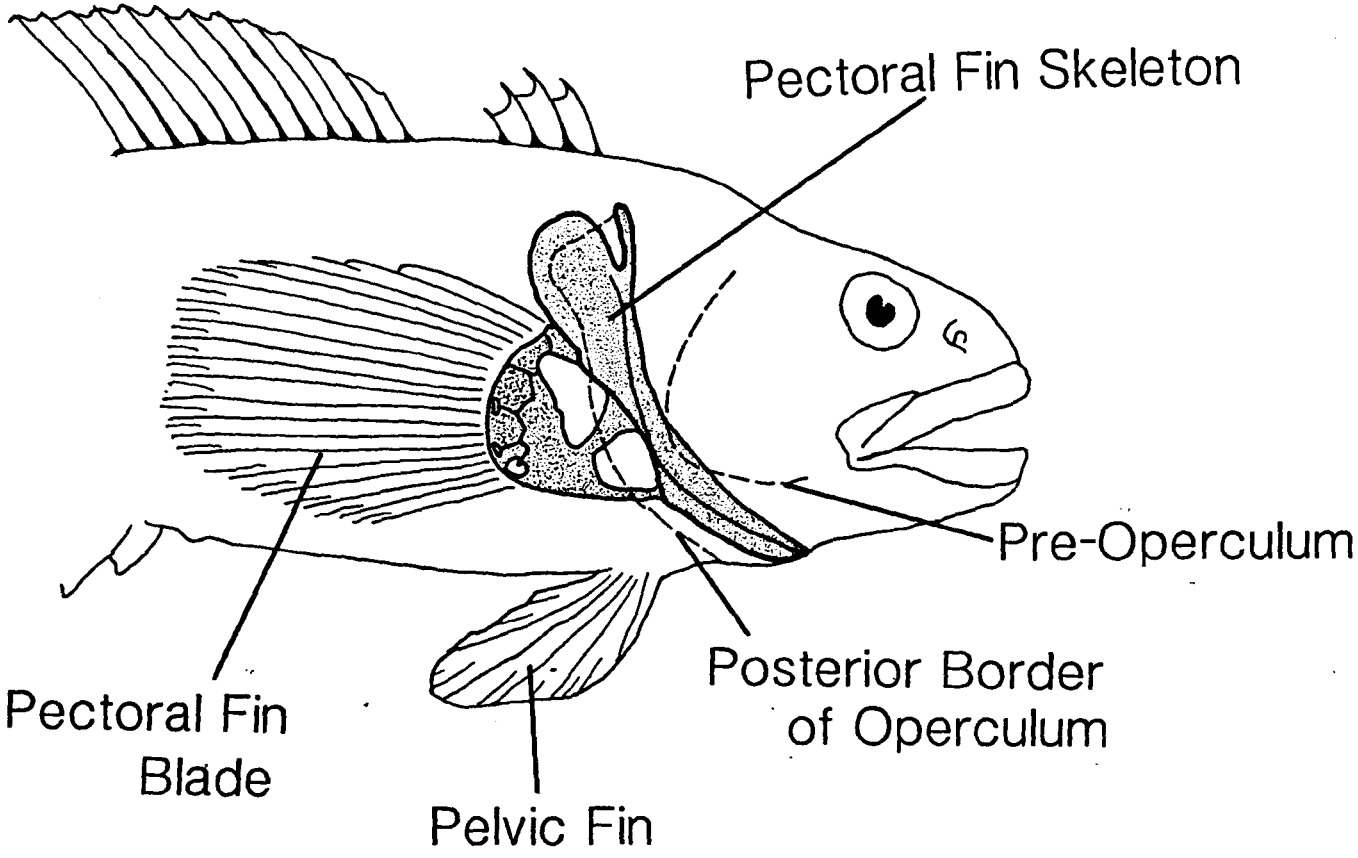

Pectoral Fin Skeleton
Pre-Operculum
Posterior Border
of Operculum
Pectoral Fin
Blade
Pelvic Fin

Fig.4:4. a: Detail of the bases of the selected pectoral fin rays in Notothenia neglecta. The rays are numbered dorso-ventrally. The hemitrichia of number 1 are entirely fused. The hemitrichia of the other rays are separate at the bases but fused distally. Note the processes which form the insertions for the pectoral muscles. **b:** Reproduction of the bases of the rays shown in (a) but including details of the positions of the insertions of the pectoral muscles. Note the distal movement of the insertion of the M.AD.P. with increasing fin ray number.

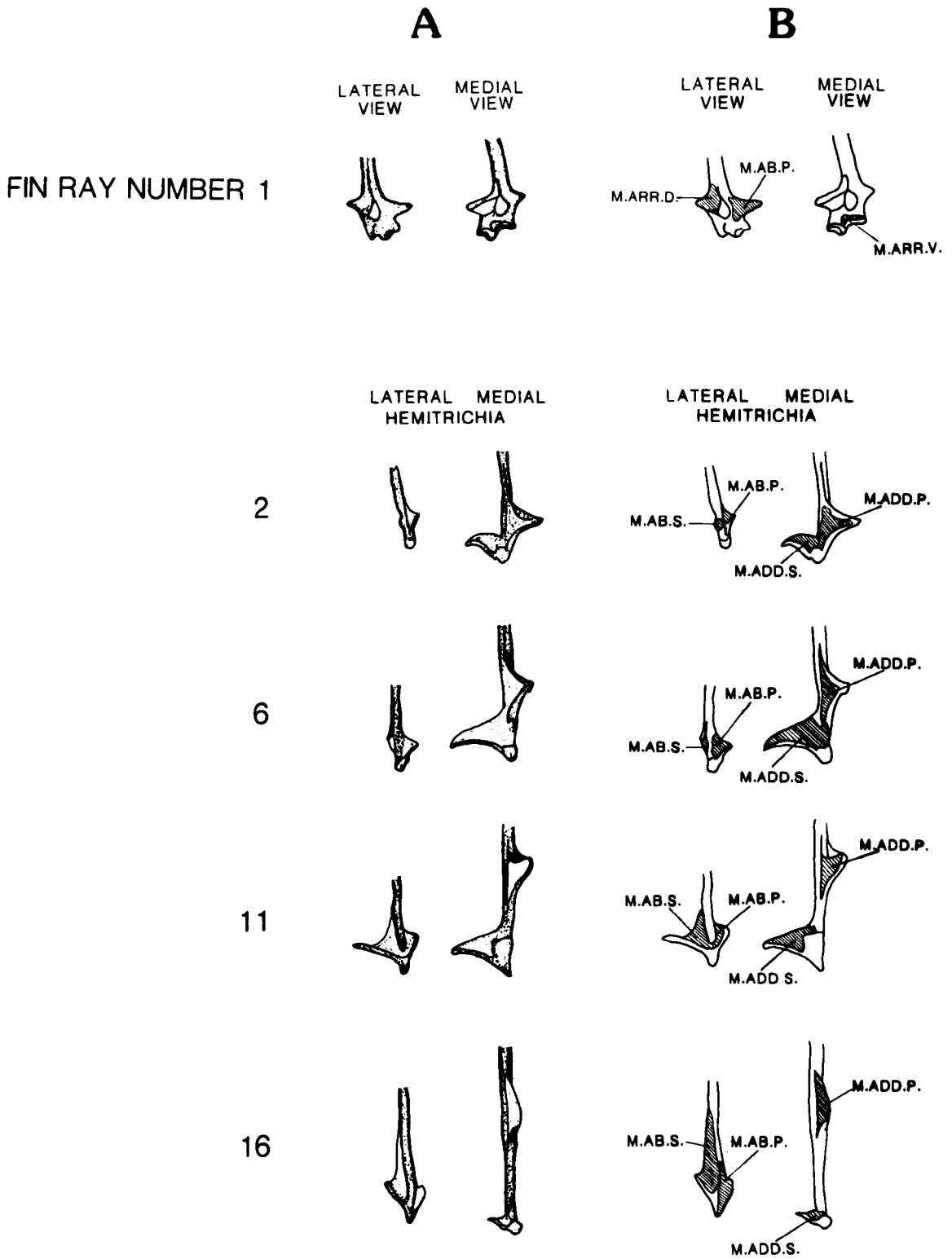

A
B
LATERAL VIEW
MEDIAL VIEW
LATERAL VIEW
MEDIAL VIEW
FIN RAY NUMBER 1
M.AB.P.
M.ARR.D.
M.ARR.V.
LATERAL MEDIAL HEMITRICHIA
LATERAL MEDIAL HEMITRICHIA
2
M.AB.P.
M.AB.S.
M.ADD.P.
M.ADD.S.
6
M.ADD.P.
M.AB.P.
M.AB.S.
M.ADD.S.
11
M.ADD.P.
M.AB.S.
M.AB.P.
M.ADD S.
16
M.ADD.P.
M.AB.S.
M.AB.P.
M.ADD.S.

Fig.4:5. Gross morphology of the pectoral muscles of Notothenia neglecta. **a:** Lateral view of the abductor muscles following removal of the skin. **b:** M. adductor profundis (M.AB.P.) and M. arrector ventralis (M.ARR.V.) following removal of M. abductor superficialis (M.AB.S.), lateral view. **c:** Medial view of adductor muscles. Note two fibre directions within the M. adductor superficialis(M.AD.S.). **d:** M. adductor profundis (M.AD.P.) and M. arrector dorsalis (M.ARR.D.) following removal of M.AD.S..

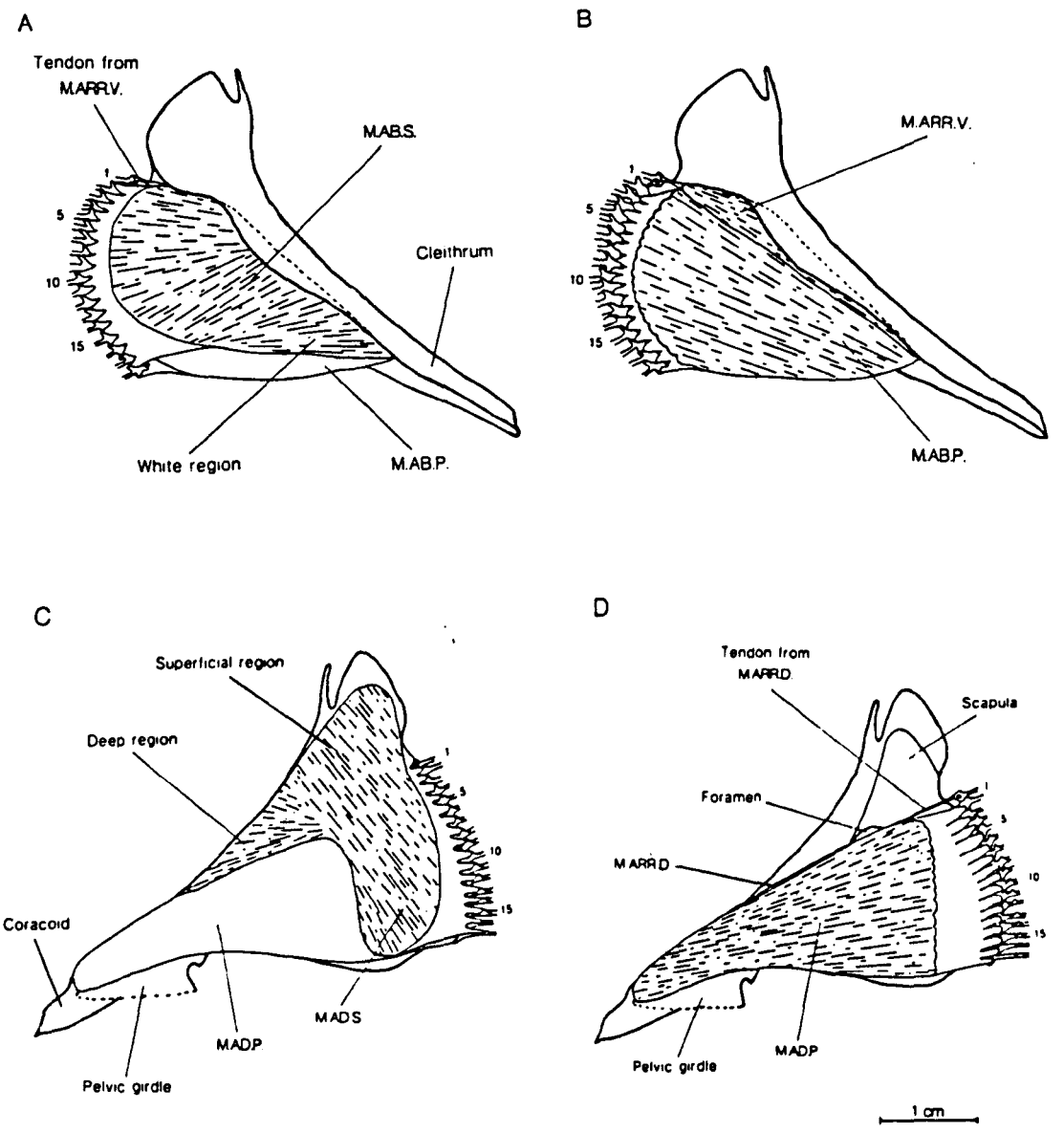

A
Tendon from M.ARR.V.
M.AB.S.
Cleithrum
White region
M.AB.P.
B
M.ARR.V.
M.AB.P.
C
Superficial region
Deep region
Coracoid
M.AD.P
M.AD.S
Pelvic girdle
D
Tendon from M.ARR.D.
Scapula
Foramen
M.ARR.D
M.AD.P
Pelvic girdle
1 cm

Fig.4:6. Transverse sections through the junctional region between the large diameter superficial fibres and the small diameter deeper fibres of the M. abductor profundis of Notothenia neglecta. **a:** Stained for succinic dehydrogenase activity. **b:** Stained for myosin ATPase, incubated at pH 9.5. **c:** stained for myosin ATPase, incubated at pH4.8.

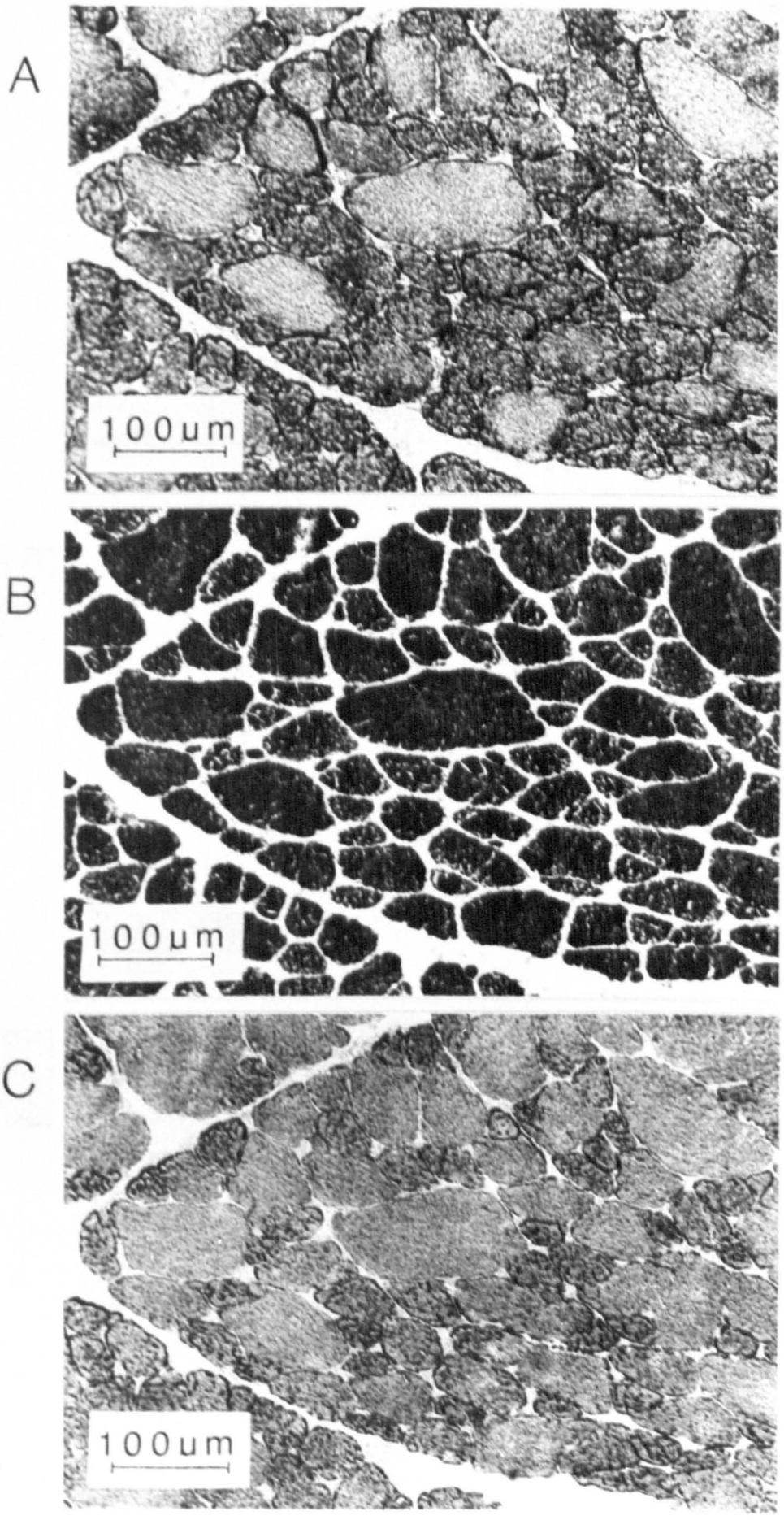
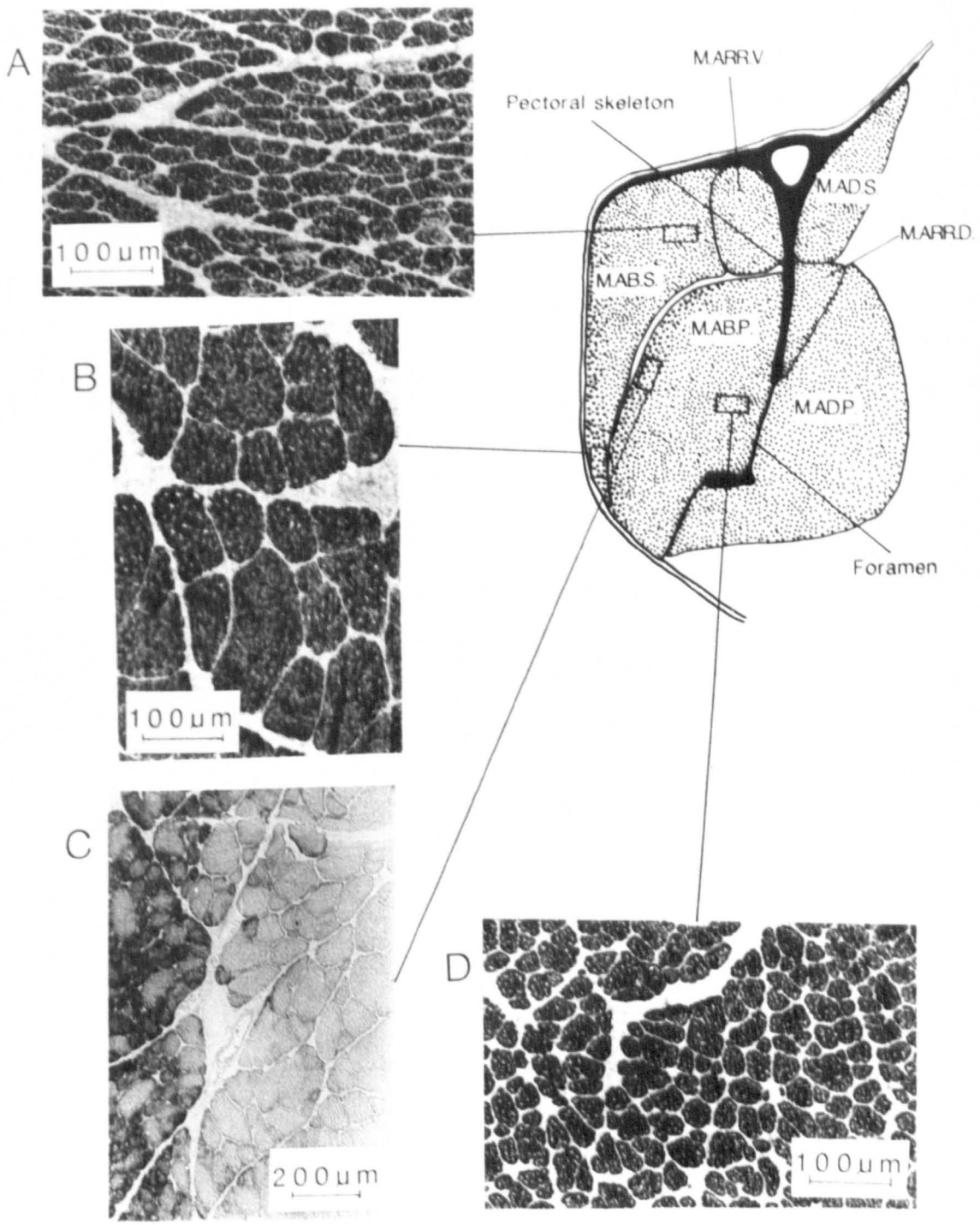

A
100um
B
100um
C
100um

Fig.4:7. Cross-section through the pectoral muscle mass of Notothenia neglecta at the level of the scapular foramen showing positions of the muscles. Plates show details of regions of the muscles. **a** and **d:** Core of small diameter fibres in the abductor muscles stained for myosin ATPase (incubated at pH 9.5). **b:** Superficial band of large diameter fibres of M.AB.S. stained for myosin ATPase (incubated at pH 9.5). **c:** Junctional region between lightly stained large diameter fibres and core of darker, small diameter fibres in M.AB.P., stained for succinic dehydrogenase activity.


A
100 µm
M.ARR.V
Pectoral skeleton
M.AD.S
M.ARR.D.
M.AB.S.
M.AB.P.
M.AD.P.
Foramen
B
100 µm
C
200 µm
D
100 µm

CHAPTER 5

The Force-Velocity Characteristics of Demembranated White and Red Pectoral Fibres from the South Polar Teleost *Notothenia neglecta*: Strategies of Adaptation to Low Temperatures.

INTRODUCTION

Fish muscles have evolved to function over a wide range of average temperatures: for example, high latitude antarctic species live in perennially freezing sea-water at -1.9°C, whereas some African cichlids occur in hot springs at more than 40°C.

Demembranated fibre preparations have been used extensively to investigate the functional properties of contractile proteins in the locomotory muscles of fish adapted to different temperatures. Resistance adaptations are apparent in comparisons between tropical and cold-adapted species: fibres from south polar notothenioids fail to relax completely following activations between 10 and 15°C (Gleeson et al., 1983; Johnston and Brill, 1984; Johnston and Altringham, 1985) whilst those of tropical species maintain contractile function up to at least 30°C (Johnston and Brill, 1984). These studies also indicate that partial temperature compensation of power output has occurred in cold-adapted species. For example, maximum power output for white fibres from the channichthyid Chaenocephalus aceratus at -2°C is 17Wkg^{-1} compared to 44 and 2Wkg^{-1} in homologous fibres from the tropical marlin, Makaira nigricans, at 20 and 0°C respectively (Johnston and Altringham, 1985). This is achieved largely by capacity adaptations in tension generation: contraction velocities are not temperature compensated in polar species. In addition, Johnston and Altringham (1985) have identified

adaptive differences in the shapes of the force-velocity (P-V) curves of white fibres which serve to enhance power output in cold-adapted species at low temperatures. They showed that, in teleosts from different thermal environments, the P-V relationships became less curved (represented by increases in Hill's (1938) constant a/Po) with decreasing experimental temperature such that, at the preferred body temperature of each species, the curvature decreased in the order tropical>temperate>south polar. Less curved P-V relationships are advantageous to cold-adapted species in that they are associated with higher contraction velocities, and therefore higher power outputs, at given loads.

Most of the studies concerning temperature compensation of mechanical properties have concentrated on white fibres. There are however, reasons to believe that the mechanisms involved in cold-adaptation of red fibres may be different from these. One may assume that during periods in which any particular muscle is generating maximum power output all of the constituent white fibres will be recruited. Under these conditions, increases in power output can only be achieved through modifications in the intrinsic properties of these fibres. In contrast, during sustained activity which is powered mainly by red fibres, increased power output may be generated by recruitment of some portion of the white musculature. This strategy is apparent in the carp, Cyprinus carpio, in which acute exposure to low temperatures results in a reduction in the threshold speed at which white fibres are recruited (Rome et al., 1984).

The principal aim of the present study was to extend the observations on the contractile properties of notothenioid fibres at low temperatures to demembranated preparations of white and red pectoral fibres from Notothenia neglecta. The inclusion of red fibres is of particular interest in view of the possibility of alternative strategies of temperature compensation in this muscle type. Data is also presented concerning the effects of temperature on the contractile properties of the pectoral fibres.

MATERIALS AND METHODS

Fish collection and maintenance.

Thirty-four specimens of Notothenia neglecta Nybelin (mean body weight ± S.E.M. = 0.83 ± 0.04kg) were used in these experiments. The fish were caught with trammel nets in Borge Bay and by SCUBA divers in Factory Cove, off Signy Island, South Orkney Islands, British Antarctic Territory. All of the experiments were carried out at the British Antarctic Survey's research station on Signy Island. Live fish were maintained in tanks in a flow-through sea-water aquarium at 0 to +2°C and fed every other day on fish and amphipods. Experimental animals were killed with a blow to the head followed by spinal cord transection.

Force-velocity (P-V) characteristics of demembranated muscle fibres.

The P-V characteristics of single, demembranated white and red pectoral muscle fibres were determined at a range of temperatures between -5 and +10°C. The fibres were isolated from regions of the M.abductor superficialis in which pure fibre populations had been identified in an earlier study (Chapter 4). The experimental technique used to determine the P-V characteristics of these fibres was different in two respects from that described in Chapter 4. Firstly, the pH of the experimental solutions was set to 7.56 at 0°C and allowed to vary with temperature according to the dissociation constant of the imidazole buffer, such that the pH varied from pH 7.65 at -5°C to pH 7.38 at 10°C. pH changes over this range have been shown to have no significant effect on the contractile properties of teleost fibres (Johnston and Altringham, 1985; Mutungi, 1988). The second major departure from the technique employed in Chapter 4 was the inclusion of 7% (by volume) glycerol in the solutions used at -5°C to prevent freezing. Preliminary experiments at 0°C with these solutions indicated that this addition did not affect the contractile properties.

P-V data from releases up to 0.6Po were fitted to a linear form of Hill's (1938) equation, as described in Chapter 4, in order to determine Vmax and Hill's constants a and b.

Temperature dependencies.

The temperature dependencies of Po and Vmax were

expressed by the terms R_{10} and Q_{10} respectively (Bennett, 1984). These were obtained using the following equation:

$$R_{10} \text{ or } Q_{10} = (R_2/R_1)^{10/(T_2-T_1)}$$

where R_1 and R_2 are either tensions (R_{10}) or velocity (Q_{10}) at temperatures T_1 and T_2 respectively, T_2 being greater than T_1.

Statistics.

Differences in Po, Vmax and a/Po between white and red fibres at each temperature were compared using Student's t-test.

RESULTS

Contractile properties of white and red fibres at low temperatures (+1°C).

Table 5:1 shows the P-V characteristics of white and red fibres from N.neglecta at +1°C and these results, together with comparative data from the literature, are shown in Figs.5:1 and 5:2. Values of Po for both fibre types in notothenioids demonstrate a high degree of temperature compensation compared to warmer-water species (Fig.5:1a and b). In contrast, Vmax does not appear to be temperature compensated: values of Vmax are considerably higher in warm than cold-adapted species at their preferred body temperatures (Figs.5:2a and b).

Effects of temperature on P-V characteristics.

Effects of temperature on a/Po: a/Po for both white and red pectoral fibres from N.neglecta decreased with increasing temperature (Fig.5:3). This trend is illustrated in Fig.5:4 which shows increasing curvature of the P-V relationship at higher temperatures (Fig.5:4).

Red fibres had consistently higher values of a/Po at all of the experimental temperatures employed. However, the differences were only statistically significant ($P<0.05$) at +1 and +10°C.

Effects of temperature on maximum isometric tension (Po):The effects of temperature on maximum tensions are shown in Fig.5:5 and the temperature dependencies of Po over two temperature ranges are shown in Table 5:2. White fibres generated significantly higher tensions than red fibres at each experimental temperature ($P<0.01$). Po was relatively independent of temperature both over the physiological temperature range ($R_{10(-2 \text{ to } +1°C)} = 1.2$ to 1.3) and at higher temperatures ($R_{10(+1 \text{ to } +10°C)} = 1.2$).

Both fibre types relaxed completely following activations at up to 10°C (Fig.5:6a). Above this temperature they developed marked post-contraction resting tensions. In red fibres, resting tension was approximately 55% of the maximum isometric tension at 15°C (Fig.5:6b).

Effects of temperature on maximum unloaded contraction velocity (Vmax):The results are shown in Fig.5:7 and Table

5:2. In common with Po, Vmax was significantly higher in white than in red fibres at each experimental temperature ($P<0.01$). The temperature dependencies of Vmax were similar for both fibre types. Over the physiological temperature range Q_{10} for Vmax was 1.8, increasing to 2.1 and 2.4 for white and red fibres respectively between +1 and +10°C.

DISCUSSION

Adaptation to low environmental temperature.

Altringham and Johnston (1985b) have reported values of Po and Vmax for red pectoral fibres in N.neglecta at 0°C (approximately $100kNm^{-2}$ and $0.58Ls^{-1}$) which are substantially higher than those obtained in the present study at +1°C ($58kNm^{-2}$ and $0.24Ls^{-1}$). The disparity between these results may be related to differences in the condition of the experimental animals. The present study was carried out using freshly caught fish over an austral winter, whereas the animals used by Altringham and Johnston (1985b) had been maintained in an aquarium under constant lighting conditions and fed daily for periods of up to two years prior to experimentation. The possibility of seasonal deterioration in muscle function is supported by data from Fitch (1986) which show significant changes in the gross muscle chemistry of freshly caught specimens of N.neglecta at different times of the year.

The comparative data presented in Fig.5:1a and b indicate that, in spite of the apparently low values

obtained in this study, Po for both white and red pectoral fibres from N.neglecta demonstrate a high degree of temperature compensation. In contrast, there is no evidence that Vmax is compensated for temperature in notothenioids: the values of Vmax at low temperatures for both fibre types in these fish are between 6 and 8 times less than the highest values reported for tropical species at their preferred body temperatures (Fig.5:2a and b).

a/Po for white pectoral fibres at +1°C (Table 5:1) was within the range reported in other studies for notothenioid white trunk fibres at low temperatures (Johnston and Altringham, 1985; Johnston and Harrison, 1985). This represents a secondary adaptive mechanism, as described in the Introduction, which serves to enhance power output at low temperatures. There are relatively few published values of a/Po for red fibres in other species. However, the decrease in a/Po with increasing experimental temperature for this fibre type in the present study parallels that seen in white fibres (Fig.5:3) and is similar to the trend described for white trunk fibres in tropical and temperate species (Johnston and Altringham, 1985). On this basis it is likely that modifications in curvature of the P-V relationship are also important in cold-adaptation of red fibres. In general, demembranated fibres generate lower tensions than live fibres and have different P-V relations (Curtin and Woledge, 1987; Altringham and Johnston, 1988a). Thus the contribution of a/Po to increases in power output cannot be accurately assessed with data from demembranated fibres. Recently, Langfeld et al., (1988) have estimated

that in live white fibres from the temperate teleost Myoxocephalus scorpius, the change in a/Po on reducing the temperature from 8 to 1°C increases relative power output by only some 15%. Since the pattern of change in a/Po with experimental temperature is similar in fish from different thermal environments (Johnston and Altringham, 1985), this suggests that, in comparison with changes in Po (Fig.5:1), interspecific differences in a/Po play a relatively minor role in temperature adaptation of fibre power output.

The results obtained in this study indicate that the mechanisms involved in temperature adaptation of red fibres in notothenioids are similar to those of white fibres. This does not exclude the possibility that differences in recruitment patterns may also play some part in compensating the power output of red fibres in cold-adapted species in vivo. This must be investigated in further studies using electromyographic techniques.

Effect of temperature on Po and Vmax.

Macdonald and Montgomery (1982) have investigated the influence of temperature on whole extraocular muscle preparations isolated from the nototheniid Pagothenia borchgrevinki. On the basis of their results they suggested that the mechanical properties of the contractile proteins in this species were not impaired at temperatures in excess of 30°C. Data obtained from demembranated fibres are more easily interpreted than those from whole muscles since they allow the properties of the contractile proteins to be investigated in isolation from nerve and membrane effects

(see Chapter 1). The results obtained with demembranated fibres in the present study indicate that contractile failure occurs at temperatures substantially lower than 30°C: in common with white trunk fibres from other notothenioids (Gleeson et al., 1983; Johnston and Brill, 1984; Johnston, 1985; Johnston and Altringham, 1985) the pectoral fibres from N.neglecta failed to relax completely following activations above 10°C. The development of residual tension at these temperatures is associated with an increase in fibre stiffness and is probably the result of the formation of abnormal crossbridges (Johnston and Altringham, 1985). These observations indicate that adaptive modifications in the structure of the cold-adapted contractile proteins, which confer high levels of performance at low temperatures, result in limitations in the thermal range over which they can function.

At temperatures up to 10°C Po and Vmax for the pectoral fibres in N.neglecta increased with experimental temperature. Po for both fibre types had a lower thermal dependence than Vmax (Table 5:2). This is common to all of the teleost species studied to date (Johnston and Brill, 1984; Johnston and Salamonski, 1984; Johnston and Altringham, 1985; Johnston and Wokoma, 1986) and is thought to reflect differences in the kinetics of the crossbridge reactions responsible for generating isometric tension and active shortening (Johnston and Altringham, 1985).

South polar notothenioids are probably unique amongst vertebrate ectotherms in that they experience only very

slight seasonal variations in environmental temperature. One might expect this to be reflected in higher thermal dependencies of Po and Vmax compared to warmer-water species. This appears to be the case in the muscles of endotherms in which rate processes have higher temperatue coefficients than those of ectotherms (see Bennett, 1984). However, the influence of temperature on Po and Vmax in the pectoral muscles of N.neglecta (Table 5:2) is very similar to that described in warmer-water teleosts (Johnston and Brill, 1984; Johnston and Salamonski, 1984; Johnston and Altringham, 1985) suggesting that the stenothermal nature of their environment does not influence the thermal dependencies of their contractile properties.

Table 5:1. Force-velocity (P-V) characteristics of demembranated white and red pectoral fibres from Notothenia neglecta at +1°C.

MUSCLE	MAXIMUM TENSION (KNm^{-2})	Vmax (MUSCLE LENGTHS sec^{-1})	a/Po	b
WHITE FIBRES	122.8±5.9	0.43±0.04	0.28±0.03	0.12±0.02
RED FIBRES	58.0±6.7	0.24±0.02	0.36±0.04	0.08±0.01

Table 5:2. Temperature dependencies of maximum isometric tension (Po) and maximum unloaded contraction velocity (Vmax) of demembranated white and red pectoral fibres from Notothenia neglecta.

	R_{10} for Po		Q_{10} for Vmax	
	-2 to +1°C	+1 to +10°C	-2 to +1°C	+1 to +10°C
White Fibres	1.20	1.20	1.80	2.45
Red Fibres	1.33	1.21	1.84	2.11

Fig.5:1. Comparison of maximum isometric tension of demembranated white (a) and red (b) muscle fibres from a range of teleosts measured at their normal body temperatures.

1. Notothenia neglecta pectoral fibres (present study).
2. Trematomus hansonii red pectoral, white trunk fibres (Johnston and Harrison, 1985).
3. Notothenia rossii red pectoral, white trunk fibres (Johnston and Harrison, 1985).
4. Chaenocephalus aceratus red pectoral, white trunk fibres (Johnston and Harrison, 1985).
5. Notothenia neglecta white trunk fibres (Johnston and Altringham, 1985).
6. Gadus morhua trunk fibres (Altringham and Johnston, 1982).
7. Myoxocephalus scorpius white trunk fibres (Johnston and Sidell, 1984).
8. Morone saxatilis trunk fibres (Moerland and Sidell, 1984).
9. Coryphaena hippurus trunk fibres (Johnston and Brill, 1984).
10. Mugil cephalus trunk fibres (Johnston and Brill, 1984).
11. Katsuwonus pelamis deep red trunk, white trunk (Johnston and Brill, 1984).
12. Carangus melampygus trunk fibres (Johnston and Brill, 1984).
13. Makaira nigricans white trunk fibres (Johnston and Altringham, 1985).
14. Makaira nigricans red trunk fibres (Johnston and Salamonski).

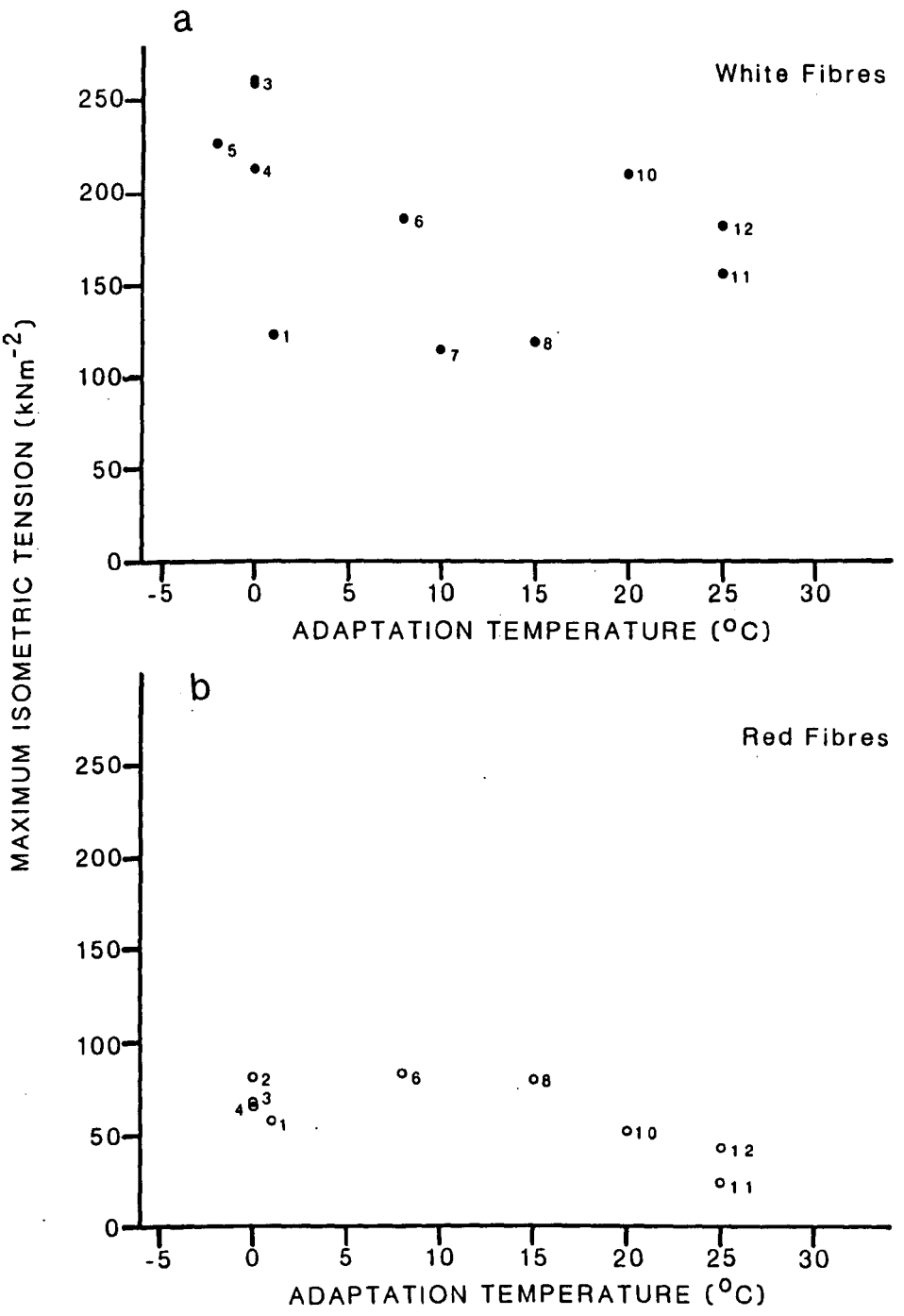

a
White Fibres
MAXIMUM ISOMETRIC TENSION (kNm^{-2})
250
200
150
100
50
0
-5
0
5
10
15
20
25
30
ADAPTATION TEMPERATURE (^{o}C)
b
Red Fibres
ADAPTATION TEMPERATURE (^{o}C)

Fig.5:2. Comparison of maximum unloaded shortening velocities of demembranated white (a) and red (b) muscle fibres from a range of teleosts measured at their normal body temperatures.

1. Notothenia neglecta pectoral fibres (present study).
2. Trematomus hansonii red pectoral, white trunk fibres (Johnston and Harrison, 1985).
3. Notothenia rossii red pectoral, white trunk fibres (Johnston and Harrison, 1985).
4. Chaenocephalus aceratus red pectoral, white trunk fibres (Johnston and Harrison, 1985).
5. Notothenia neglecta white trunk fibres (Johnston and Altringham, 1985).
6. Gadus morhua trunk fibres (Altringham and Johnston, 1982).
7. Myoxocephalus scorpius white trunk fibres (Johnston and Sidell, 1984).
8. Morone saxatilis trunk fibres (Moerland and Sidell, 1984).
9. Coryphaena hippurus trunk fibres (Johnston and Brill, 1984).
10. Mugil cephalus trunk fibres (Johnston and Brill, 1984).
11. Katsuwonus pelamis deep red trunk, white trunk (Johnston and Brill, 1984).
12. Carangus melampygus trunk fibres (Johnston and Brill, 1984).
13. Makaira nigricans white trunk fibres (Johnston and Altringham, 1985).
14. Makaira nigricans red trunk fibres (Johnston and Salamonski).

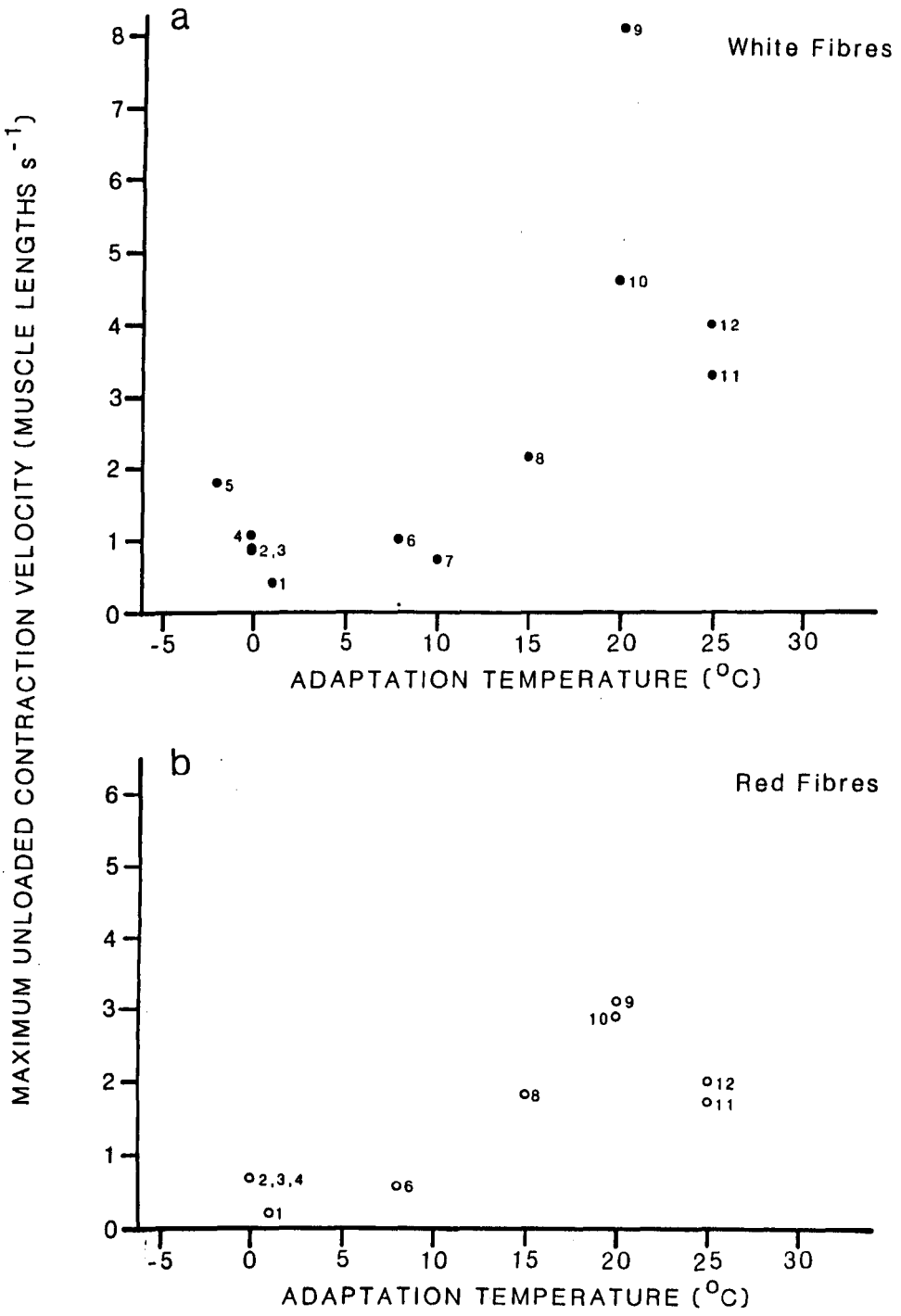

a
White Fibres
MAXIMUM UNLOADED CONTRACTION VELOCITY (MUSCLE LENGTHS s^{-1})
ADAPTATION TEMPERATURE (°C)
b
Red Fibres
ADAPTATION TEMPERATURE (°C)

Fig.5:3. Effect of temperature on Hill's (1938) constant a/Po for white (●) and red (○) pectoral fibres from Notothenia neglecta. Asterix denotes level of significance (*$P<0.05$) from Student's t-test. Error bars represent ± S.E.M.

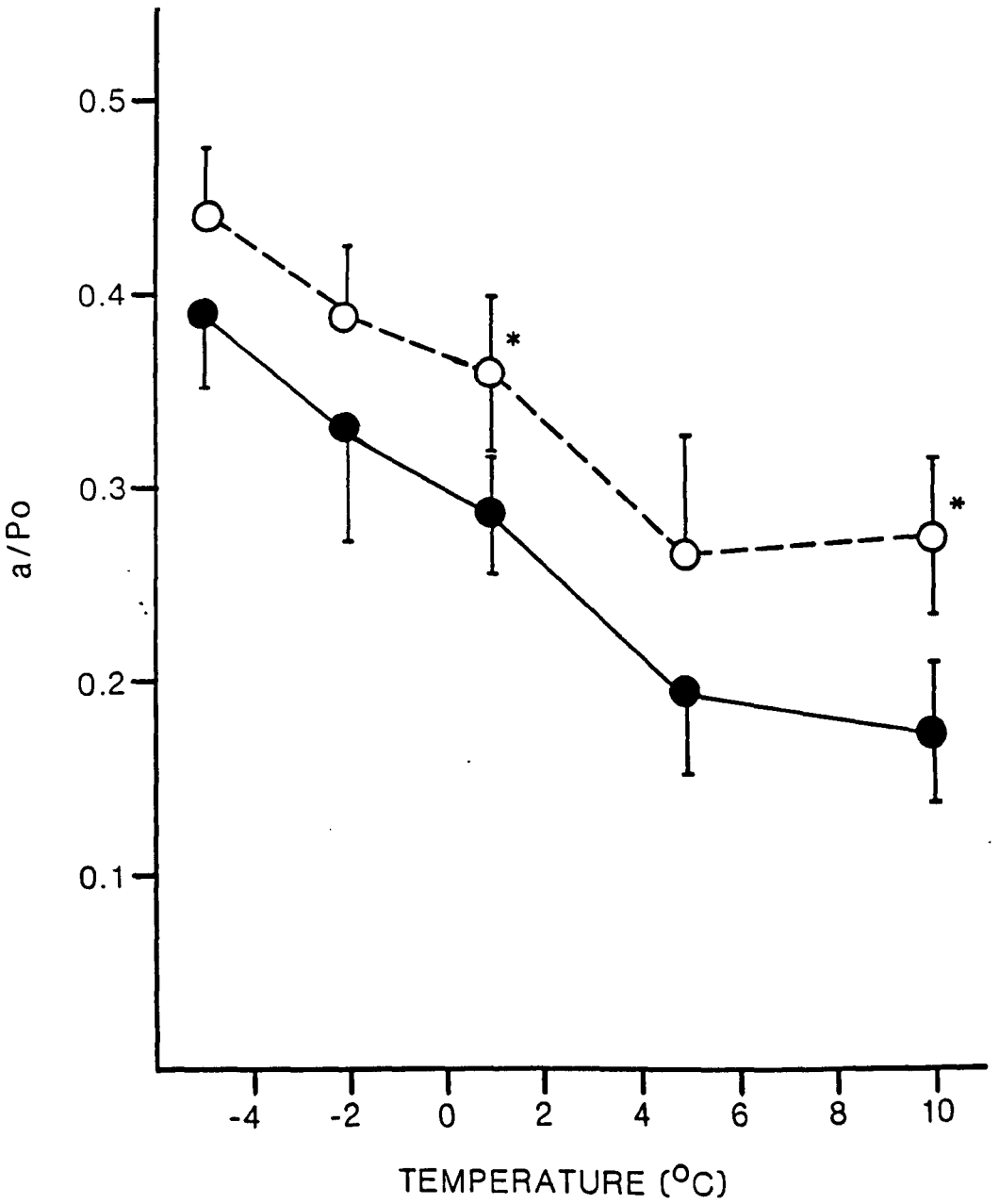

0.5
0.4
0.3
0.2
0.1
a/Po
*
*
-4
-2
0
2
4
6
8
10
TEMPERATURE (°C)

Fig.5:4. Effect of temperature on the force-velocity relationship for white (**a**) and red (**b**) fibres from Notothenia neglecta. Note the increasing curvature with increasing experimental temperature.

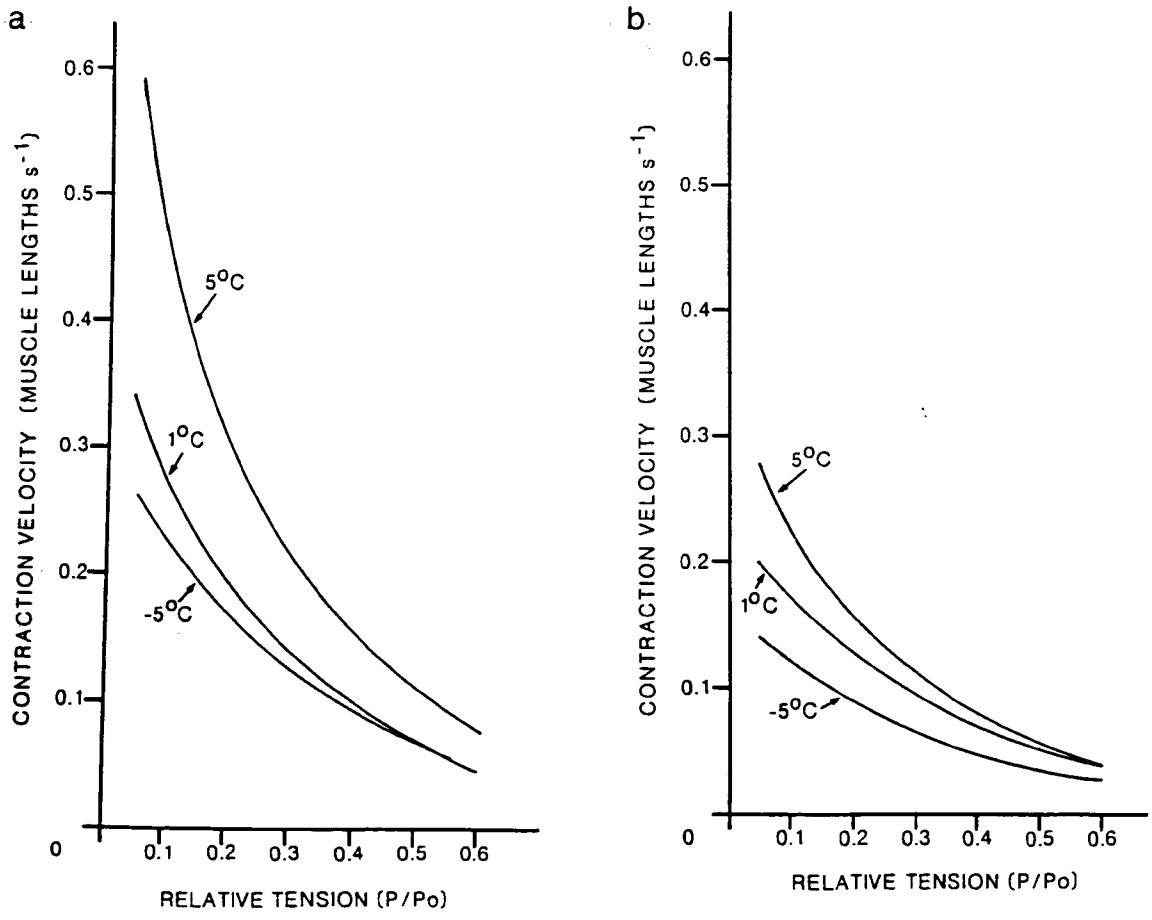

a
b
CONTRACTION VELOCITY (MUSCLE LENGTHS s-1)
RELATIVE TENSION (P/Po)
0
0.1
0.2
0.3
0.4
0.5
0.6
5°C
1°C
-5°C

Fig.5:5. Effect of temperature on maximum isometric tension for white (●) and red (O) pectoral fibres from Notothenia neglecta. Asterix denotes level of significance (** $P<0.01$) from Student's t-test. Error bars represent ± S.E.M.

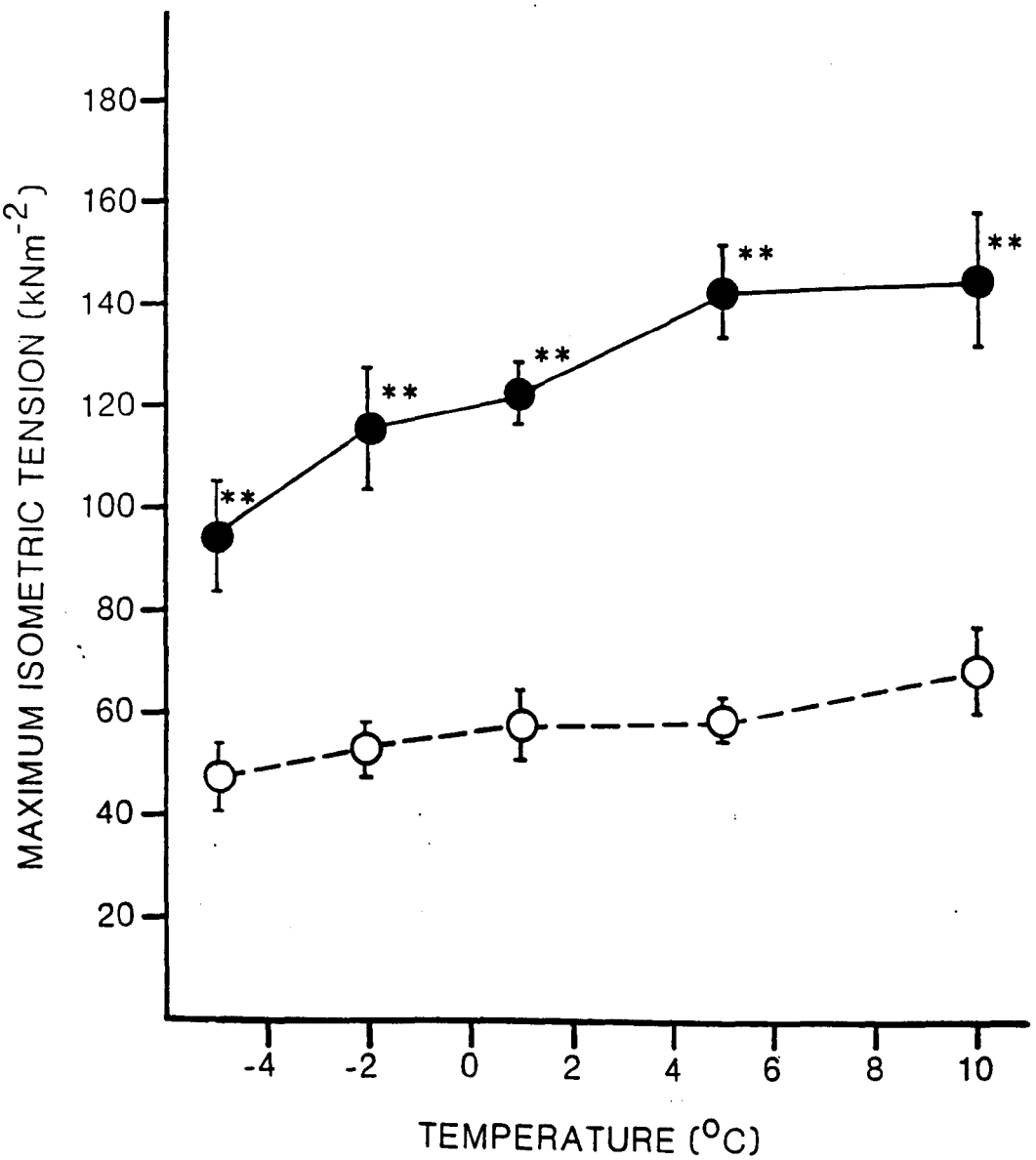

MAXIMUM ISOMETRIC TENSION (kNm^{-2})
180
160
140
120
100
80
60
40
20
**
**
**
**
**
-4
-2
0
2
4
6
8
10
TEMPERATURE (^{o}C)

Fig.5:6. Isometric tension records for red pectoral fibres from Notothenia neglecta at 5°C (**a**) at 15°C (**b**). Note the post contraction resting tension at the higher temperature.

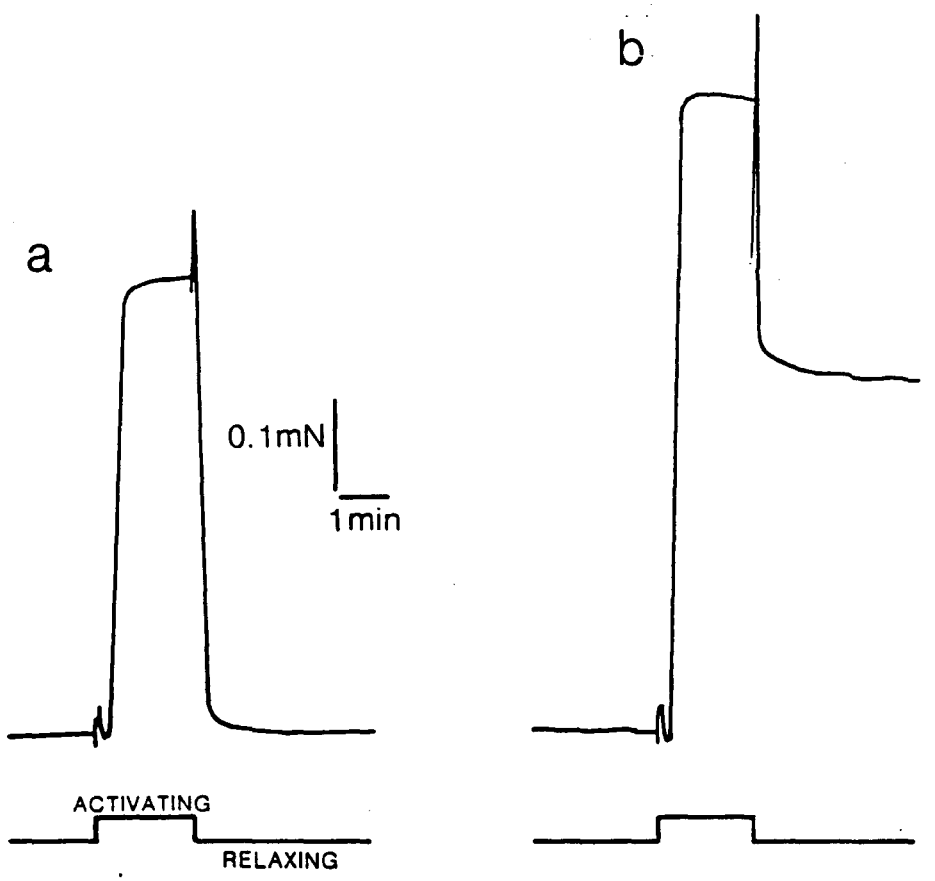
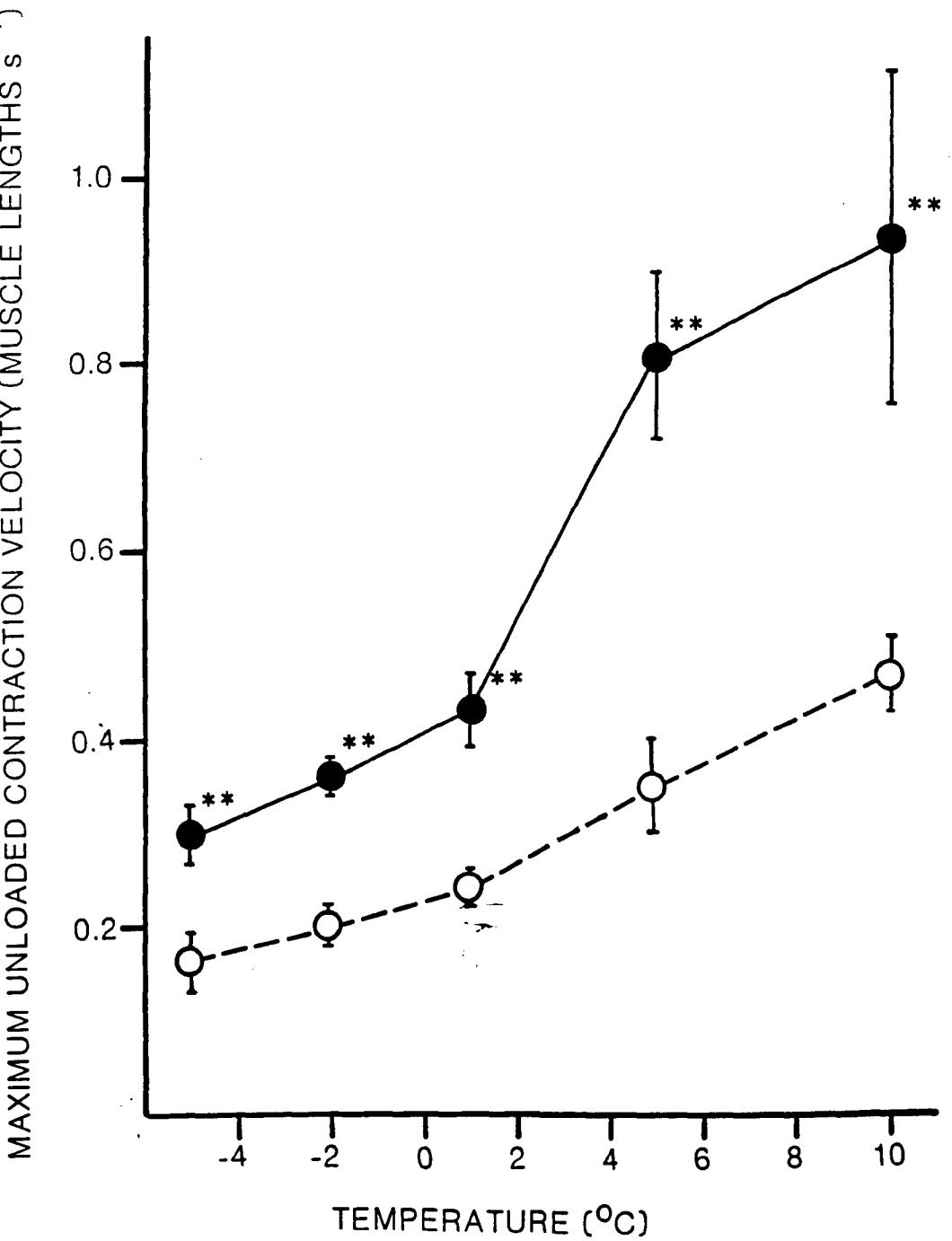

a
0.1mN
1min
ACTIVATING
RELAXING
b

Fig.5:7. Effect of temperature on maximum unloaded shortening velocity for white (●) and red (○) pectoral fibres from Notothenia neglecta. Asterix denotes level of significance (** $P<0.01$) for Student's t-test. Error bars represent ± S.E.M.


MAXIMUM UNLOADED CONTRACTION VELOCITY (MUSCLE LENGTHS s^{-1})
1.0
0.8
0.6
0.4
0.2
**
**
**
**
**
-4
-2
0
2
4
6
8
10
TEMPERATURE (^{o}C)

CHAPTER 6

A Re-examination of Isometric Myofibrillar ATPase Activity in Demembranated Teleost Fibres Using a Novel Technique.

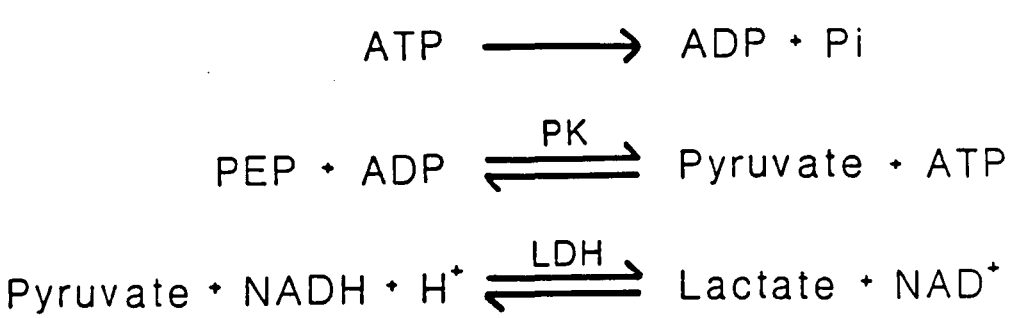
INTRODUCTION

In a recent study, Altringham and Johnston (1986b) compared the isometric myofibrillar ATPase activities of demembranated white trunk fibres isolated from teleosts adapted to south polar, temperate and tropical environments. The results obtained for fibres from each species were rather similar with values rangeing from approximately $0.5 ATPS^{-1}s^{-1}$ at -5°C to approximately $6 ATPS^{-1}s^{-1}$ at 25°C. However, in the south polar species, Notothenia neglecta, low ATPase activities at low temperatures were associated with the generation of high isometric tensions. This combination resulted in higher economies of contraction (tension developed/ATP turnover rates) for N.neglecta than for the warmer—water species at their preferred body temperatures.

The experimental method used in this study was first described by Levy et al. (1976). Single, demembranated fibres were incubated in small volumes of activating solution and the ATP hydrolysed during the course of the contractions was quantified by measuring the increment in the concentration of ADP in the chamber. Reliance on changes in [ADP] precludes the incorporation of an ATP regenerating system in the activating solution. Such regenerating systems are commonly employed in mechanical studies with demembranated preparations to ensure substrate saturation within the fibres. In their absence, ATP supply to the myofibrills is dependent on diffusion from the activating solution and ATP depletion may occur at the core of the fibres. This may influence measured rates of ATP hydrolysis:

for example, extremely low ATP concentrations are associated with the development of abnormal actomyosin crossbridges which generate tension without concomitant ATPase activity (Ferenczi et al., 1984). It is not clear whether this affected the results obtained by Altringham and Johnston (1986b) for N.neglecta, however, it should be noted that diffusion limitations are more likely to occur in notothenioid fibres since these have larger diameters than fibres from other teleost groups (Smialowska and Kilarski, 1981).

The aim of the present study was to repeat the experiments performed by Altringham and Johnston (1986b) using a method which overcame the potential problems associated with substrate supply to the fibres, i.e. one which included an ATP regenerating system in the activating solutions. One such technique is already available (Loxdale, 1976; Takashi and Putnam, 1979; Griffiths et al., 1980). It uses phosphoenolpyruvate (PEP) and pyruvate kinase (PK) to regenerate ATP according to the following reaction sequence:



ATP hydrolysis is measured indirectly by monitoring the

change in [NADH]. Unfortunately, there is evidence that the PEP/PK system does not adequately buffer [ATP] at low temperatures or under conditions in which the rate of ATP hydrolysis is high (Glyn and Sleep, 1985). It was therefore considered unsuitable for the purposes required in this study. Instead, a novel method was developed in which the ADP product of crossbridge cycling was rephosphoylated from phosphocreatine (PCr) by the Lohmann reaction, mediated by the enzyme creatine phosphokinase (CPK):

$$ADP + PCr \overset{CPK}{\rightleftharpoons} ATP + Cr$$

ATP hydrolysis was determined indirectly by measuring the accumulation of free creatine [Cr]. This technique was used to measure the economies of contraction in white trunk fibres from four teleost species, two of which were common to the earlier study by Altringham and Johnston (1986b) allowing a direct assessment of the validity of their results.

MATERIALS AND METHODS

Fish Collection and Maintenance

Four species of fish from three thermal environments were used in the present study: south polar, Notothenia

neglecta Nybelin; temperate, Myoxocephalus scorpius L. (cottus); tropical, Oreochromis niloticus L. and Oreochromis andersonii Castelnou.

All the experiments described were carried out in St. Andrews. N.neglecta (6 fish, mean total weight and mean standard length ± S.E.M. = 276±47g and 243±18mm) were caught using trammel nets in Borge Bay and Factory Cove, off Signy Island, British Antarctic Territory and returned to the U.K. in refrigerated sea water tanks on board the British Antarctic Survey's ship, R.R.S. Bransfield. In St. Andrews the fish were maintained in filtered, recirculated sea water at 0°C and fed three times weekly on chopped squid and krill. Cottus (12 fish, 367±45g and 282±14mm) were caught in traps in St. Andrews Bay off the east coast of Scotland. They were kept in tanks with a filtered, flow-through sea-water supply just above ambient North Sea temperatures (approximately 10°C) and used very soon after capture. O.niloticus (8 fish, 329±44g and 240±7mm) and O.andersonii (3 fish, 770g and 370mm) were obtained from the Department of Aquaculture, University of Stirling. Both species were kept at 28°C in large tanks with a filtered, recirculated fresh-water supply and fed daily on proprietory fish pellets.

Mechanical Apparatus

The mechanical apparatus used in these studies was designed to measure the force generated by single, demembranated muscle fibres incubated in small volumes of activating solution. The fibres were attached to the

apparatus via two stainless steel hooks. One of these hooks was held in a fixed position; the other was glued to the silicon beam of an A.M.E. 801 tension transducer (A.M.E., Horton, Norway) (sensitivity = $0.5mNV^{-1}$). The transducer was attached to a micromanipulator which allowed it to be moved relative to the fixed hook.

Experimental solutions were contained in three perspex baths (two with volumes of 2ml and one of 0.175ml) suspended on a rail beneath the hooks. The bath assembly was linked to an electric motor which was used to vibrate the baths and effect mixing of the solutions bathing the fibres. Experiments were performed at temperatures between 0 and 20°C. The temperature of the solutions was controlled to within $\pm$0.1°C with a recirculating solution of 20% ethylene glycol in water.

Solutions

Relaxing solution had the following composition: 65mM PIPES (piperazine-N,N'-bis (2-ethane-sulphonic acid)); 6.5mM $MgCl_2$; 15mM EGTA (ethyleneglycol-bis-(B-aminoethyl ether)-N,N,N'N'-tetraacetic acid); 6mM ATP. The pH of the solutions was set to 7.4 at 0°C and allowed to vary freely with temperature. The resulting pH range was pH 7.4 at 0°C to pH7.2 at 20°C. Changes in pH over this range have been shown to have no significant effect on the mechanical properties of teleost muscle fibres (Johnston and Altringham, 1985; Mutungi, 1988). Activating solution was made by adding $CaCl_2$ to a final pCa of 4.69. Preliminary experiments indicated that this calcium concentration was

sufficient to produce maximum tensions in fibres from the four species at each of the temperatures. The concentration of PIPES in the solutions was varied to give a pMg between 3.01 and 3.05, a pMgATP of 2.27 to 2.35 and an ionic strength of 0.175 to 0.180mM. Free ion concentrations were calculated using an iterative computer programme incorporating corrections for pH and temperature (Nicol, 1985). Skinning solution was made by adding 1% brij 58 (polyoxyethylene-20-cetyl-ether) to the relaxing solution.

Preliminary experiments were conducted at 0°C (the temperature at which the activity of CPK may be expected to be least) and at 20°C (the temperature at which ATPase activity was maximal) to determine the concentration of CPK which had to be added to the solutions to ensure that [ATP] was buffered adequately. These indicated that at CPK concentrations in excess of approximately $20Uml^{-1}$ the rates of ATP hydrolysis were independent of [CPK]. In practice, $40Uml^{-1}$ of crystaline CPK was added to the solutions just before experimentation.

Fibre preparation and activation.

Experiments were performed on white fibres isolated from deep regions of the anterior myotomes of the experimental animals. Thin strips of tissue, several myotomes in length, were dissected from the fish and stored in relaxing solution on ice. Single fibres were isolated from these strips as described previously (see Chapter 4) and transferred to the two metal hooks attached to the apparatus. The fibres were held in place with plexiglass in

acetone glue; care was taken to cover the regions of the fibres wrapped around the hooks to prevent them contributing to metabolite turnover in the baths.

The fibres were demembranated by immersion in skinning solution for twenty minutes and subsequently transferred to relaxing solution where they were allowed to equilibrate for five minutes. The sarcomere lengths of the fibres were measured by laser diffraction and set to 2.3μm using the micromanipulator attached to the tension transducer. At this stage the lengths and diameters of the fibres were measured using an eyepiece graticule in a binocular microscope mounted above the hooks.

The fibres were activated in 175μl of activating solution in the smallest bath. The resulting tension development was monitored on a chart recorder. Contractions were maintained for periods between 1 and 8 minutes and terminated by returning the fibres to relaxing solution. During the course of some activations the tension showed a tendency to decline. Activations in which this decline was more than 5% of the maximum recorded tension were abandoned and the post-contraction [Cr] in the activating solution was not assayed.

Following each contraction, fibres were re-equilibrated in relaxing solution for five to twenty minute intervals before reactivation.

Determination of ATP hydrolysed.

The utilization of ATP by the fibres during activation was determined indirectly by measuring the increase in free creatine concentration in the post-contraction activating solution compared to that in the pre-contraction activating solution. In aqueous solution creatine exists in equilibrium with creatinine. It was also necessary therefore to measure changes in creatinine concentrations in the solutions. At neutral pH however the equilibrium between creatine and creatinine lies towards creatine (Edgar and Shiver, 1925), and, under the conditions used in the present study, changes in creatinine were not significant.

Creatine and creatinine were separated from the other constituents of the test solutions using a Gilson high pressure liquid chromatography system and measured spectrophotometrically at 215nm. The column was of stainless steel (150mm long x 4.6mm internal diameter) prepacked with Lichrosorb RP18 (mean particle diameter 10um). Samples for assay were injected into a 100µl loop above the column.

Two mobile phase buffers were used: buffer A was 0.3M sodium dihydrogen orthophosphate and $0.25gl^{-1}$ sodium dodecyl sulphate with pH adjusted to 2.75 with orthophosphoric acid; buffer B had the same composition as buffer A except that the pH was set to pH6.5 with 10M NaOH. All the reagents were analytical or HPLC grade. Both solutions were degassed under vacuum prior to use. The gradient profile, superimposed on a typical chromatogram, is shown in Fig.6:1. An initial ramp, from 35 to 80% B, was used over the first seven minutes, 80%

B was held up to ten minutes followed by a return to 35% B between ten and twelve minutes post injection. The flow rate was $1ml min^{-1}$ throughout. The retention time of creatine was approximately 9.6 minutes and that of creatinine approximately 9.2 minutes. The other solutes, most of which also absorbed at 215μm, produced a large, composite peak with a short retention time.

The column was calibrated with standard solutions of creatine in 65mM PIPES. The spectrophotometric response of creatine was linear up to a concentration of 0.12mM.

It was important to establish that the CPK reaction was in equilibrium in the pre-contraction activating solution to ensure that the ADP product of cross-bridge cycling was rapidly converted to ATP. This was achieved by measuring the concentration of creatine in the pre-activating solution at intervals after the addition of CPK. When consecutive analyses had identical [Cr] it was assumed that equilibrium had been reached.

Post-contraction activating solutions were analsyed immediately after each contraction. This avoided problems associated with the breakdown of PCr, which proved unstable in storage, and with the conversion of creatine into creatinine. One consequence of the instability of PCr was that the concentration of creatine in the pre-contraction activating solutions had to be measured at regular intervals.

Control experiments were carried out to assess non-actomyosin ATPase activity: demembranated fibres from all four species were incubated in relaxing solution for 20 minutes at a range of temperatures. Free creatine concentrations were measured before and after incubation. In common with Altringham and Johnston (1986a) changes in [Cr] following this procedure proved negligible and non-actomyosin ATPase activity was considered to be zero.

No attempt was made to inhibit aerobic pathways of ATP synthesis or adenylate kinase since these do not contribute significantly to adenylate metabolism in demembranated teleost white fibres (Altringham and Johnston, 1985a, b).

Calculation of ATP hydrolysed.

Utilization of ATP was calculated from the post-contraction increment in [Cr] assuming a 1:1 stoichiometry between ATP split and free creatine liberated according to the Lohmann reaction (see Introduction). The rate of hydrolysis was expressed as ATP split per myosin S_1 head per second ($ATPS_1^{-1}s^{-1}$). The calculation, detailed below, was based on that described by Altringham and Johnston (1986a).

According to Bendall, (1969), myosin accounts for 8% of the wet weight of fibres composed of 80% myofibrils. Wet weights were calculated from fibre volumes, assuming a specific gravity of 1.1. It was also assumed that the myofibrillar volume density in cottus and the Oreochromis

species were 80% but a correction was made to the calculation of ATP splitting for N.neglecta to account for the slightly higher myofibrillar packing in these fibres (86.3%) (Johnston and Camm, 1987).

The maximum isometric tension (Po) was determined from the transducer output on the chart recorder. A series of activation records and corresponding chromatograms is shown in Fig.6:2. The economy of contraction was calculated by dividing the tension generated by the ATP hydrolysed.

Statistics.

Student's t-test was used to compare Po values for O.andersonii and O.niloticus and interspecific differences in the economies of contraction between N.neglecta and the other species at 0 and 5°C.

RESULTS

Resolution of the creatine assay.

Analysis of standard solutions of creatine, between 0 and 0.12mM, indicated that 0.03nM differences in [Cr] could be resolved to within 95% accuracy. Outside of this range absorbtion at 215nm was not linearly related to [Cr] and the resolution was considerably less. Creatine concentrations measured during the course of the experiments never exceeded 0.12mM.

Establishment of equilibrium between Cr in the fibres and bathing medium.

Fig.6:3 shows that the relationship between free creatine concentration and incubation times was linear between 1 and 8 minutes. This suggests that the diffusion of Cr from the fibres into the bathing medium did not limit the accuracy of the technique in activations which lasted more than one minute.

Interspecific comparisons of tension and ATPase activity.

Maximum isometric tensions (Po) and ATPase activities for each of the four species investigated in the present study are shown in Figs.6:4a and b. Both parameters increased with temperature. Interspecific differences in Po were closely related to normal ranges of body temperature (Fig.6:4a): for example, at 0°C, fibres isolated from N.neglecta developed approximately 1.6 times the tension generated by those of cottus and 3.2 times those of the two Oreochromis species. Compared at the physiological temperature range for each species however, the values obtained for Po were quite similar. Maximum isometric tensions generated by fibres from the two tropical congeneric species O.niloticus and O.andersonii were not significantly different at any of the experimental temperatures employed in the present study (Fig.6:4a).

In contrast to Po, ATPase activities for all four species were similar throughout the experimental temperature range (Fig. 6:4b). Between 0 and 2°C ATPase activities for all four species were between 1.0 and 1.4 ATP $S_l^{-1}s^{-1}$ rising

to approximately 4.8 ATP $S_1^{-1}s^{-1}$ for 0.niloticus and 0.andersonii at 20°C.

The relationship between economy of contraction and experimental temperature is shown in Fig.6:5. Three of the species, cottus, 0.andersonii and 0.niloticus, had similar economies, whereas fibres from N.neglecta achieved high levels of tension with relatively low ATPase activities. At 0 and 5°C the economies of contraction for N.neglecta were approximately 50% higher than for the other species ($P<0.05$).

DISCUSSION

Changes in the concentrations of creatine and phosphocreatine in whole muscle preparations following periods of work have been measured previously (Mommaerts et al., 1962; Marechal and Mommaerts, 1963; Gilbert et al., 1971; Crow and Kushmerick, 1982). However, to the author's knowledge, the technique described here is the first in which an increase in [Cr] has been used to determine ATP hydrolysis in single demembranated muscle fibres. It proved superior to an alternative method based on PEP and PK in that PCr/CPK regenerating system buffered [ATP] both at low temperatures and at relatively high rates of ATPase activity (up to approximately 4.5ATP$S_1^{-1}s^{-1}$ (Fig.6:4b).

In the present study, the indirect creatine technique was used to determine the actomyosin ATPase activities of

white trunk fibres from teleosts adapted to a range of thermal environments. The general pattern of results (Fig. 6:4b) was comparable to that obtained by Altringham and Johnston (1986b) in a similar study in which ATP hydrolysis was measured directly by monitoring changes in [ADP]: all of the species had similar ATPase activities at each experimental temperature. Values ranged from approximately $1ATPS_l^{-1}s^{-1}$ at 0°C to approximately $4.5ATPS_l^{-1}s^{-1}$ at 20°C (Fig.6:4). Two species, N.neglecta and O.niloticus, were common to both studies allowing direct comparison of the results (Fig.6:6). Data obtained using the two techniques differed only slightly: for example, at 5°C, values for N.neglecta were 2.0 (present study) and 2.1 $ATPS_l^{-1}$ s^{-1} (Altringham and Johnston, 1986b) and, at 10°C, values for O.niloticus were 3.3 and 3.1 $ATPS_l^{-1}s^{-1}$ respectively. This suggests that the lack of an ATP regenerating system in the solutions used in the earlier study did not, in fact, limit substrate delivery to the fibres and that the measured ATPase rates were therefore accurate.

In N.neglecta, low myofibrillar ATPase activities at low temperatures (Fig.6:4b) were associated with the generation of high isometric tensions (Fig.6:4a). This combination resulted in significantly higher economies of contraction for this species than for the warmer water species at their preferred body temperatures (Fig.6:5). Again, this confirms the results obtained by Altringham and Johnston (1986b). These authors considered the mechanisms which could lead to this phenomenon. They suggested two possible explanations: either force generation becomes

uncoupled from ATP hydrolysis in warmer water species at low temperatures or there are genuine increases in force generated per ATP hydrolysed in the cold adapted species. Further work is required to distinquish between these possibilities. In addition, the functional consequences of the high economies of contraction in notothenioid fibres are important areas for future research; for example, the reliance of the white fibres in N.neglecta on phosphagens as the primary sources of immediate energy supply (Dunn and Johnston, 1986) may be related to their more economical use of ATP.

Fig.6:1. Typical chromatogram (solid line) showing positions of the creatinine (retention time = 9.2 minutes) and creatine (retention time = 9.6 minutes) peaks. The large peak at approximately 2 minutes is a composite of the other solutes in the experimental solutions. The dashed line shows the change in buffer B through the column on the same time scale.

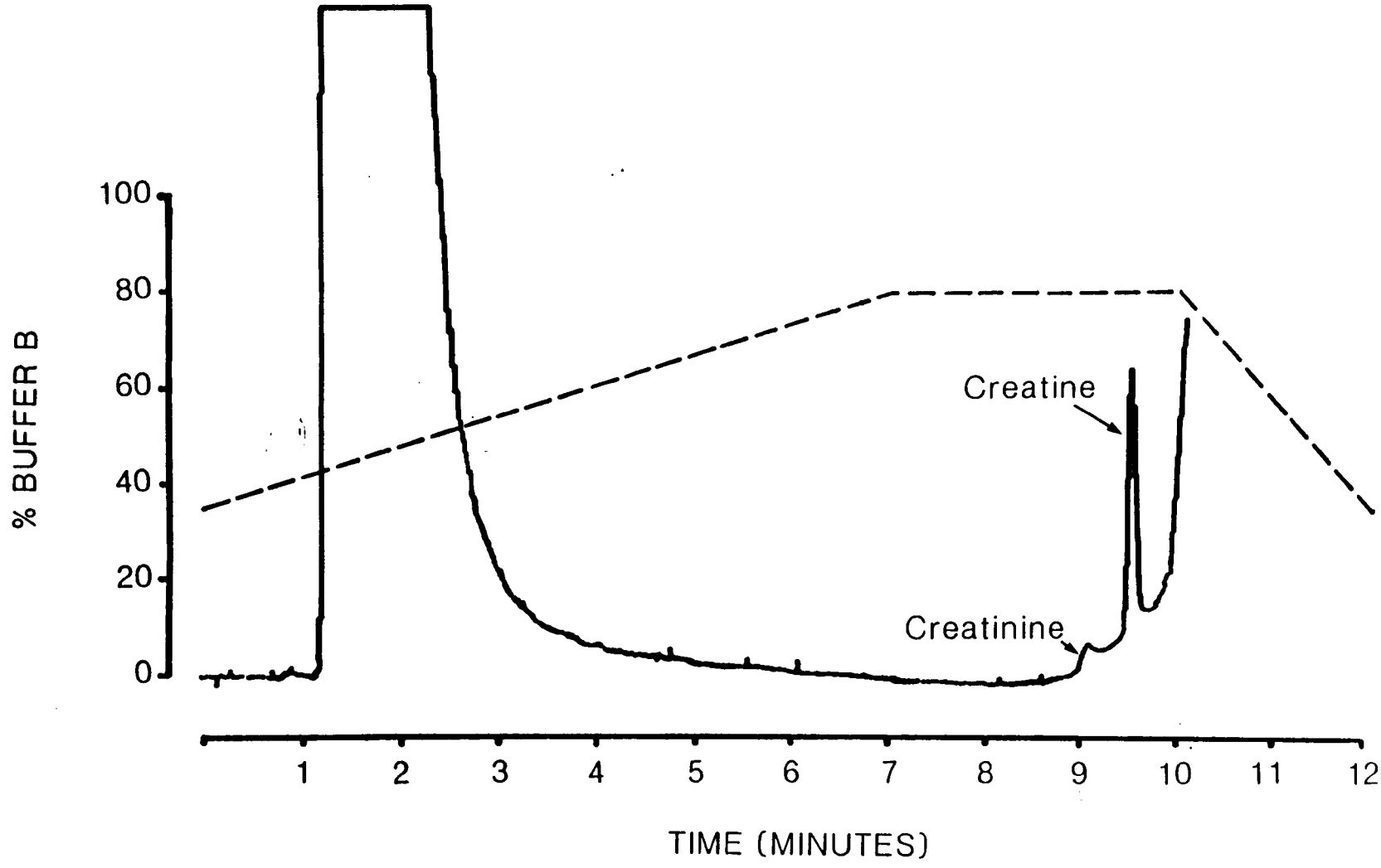
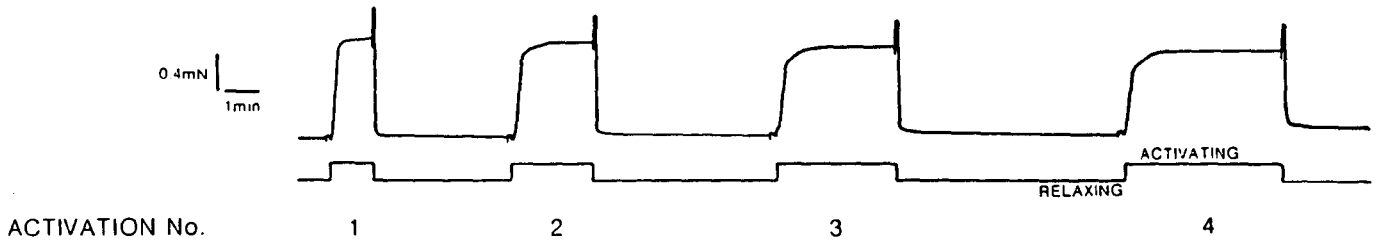

100
80
60
40
20
0
% BUFFER B
Creatine
Creatinine
1
2
3
4
5
6
7
8
9
10
11
12
TIME (MINUTES)

Fig.6:2.a: Isometric tension records for a single white fibre from Myoxocephalus scorpius at 10°C. **b:** Chromatograms showing control and post-activation creatine concentrations associated with the four activations in (a).

a


0.4mN
1min
ACTIVATING
RELAXING
ACTIVATION No.
1
2
3
4

b

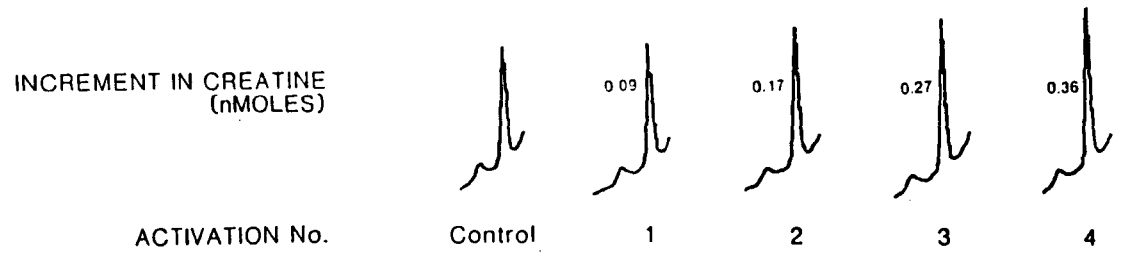

INCREMENT IN CREATINE
(nMOLES)
0.09
0.17
0.27
0.36
ACTIVATION No.
Control
1
2
3
4

Fig.6:3. Plot of creatine content of activating solutions against fibre incubation time for white fibres from Myoxocephalus scorpius at 10 and 15°C. The points for 10°C are derived from the data in Fig.6:2.

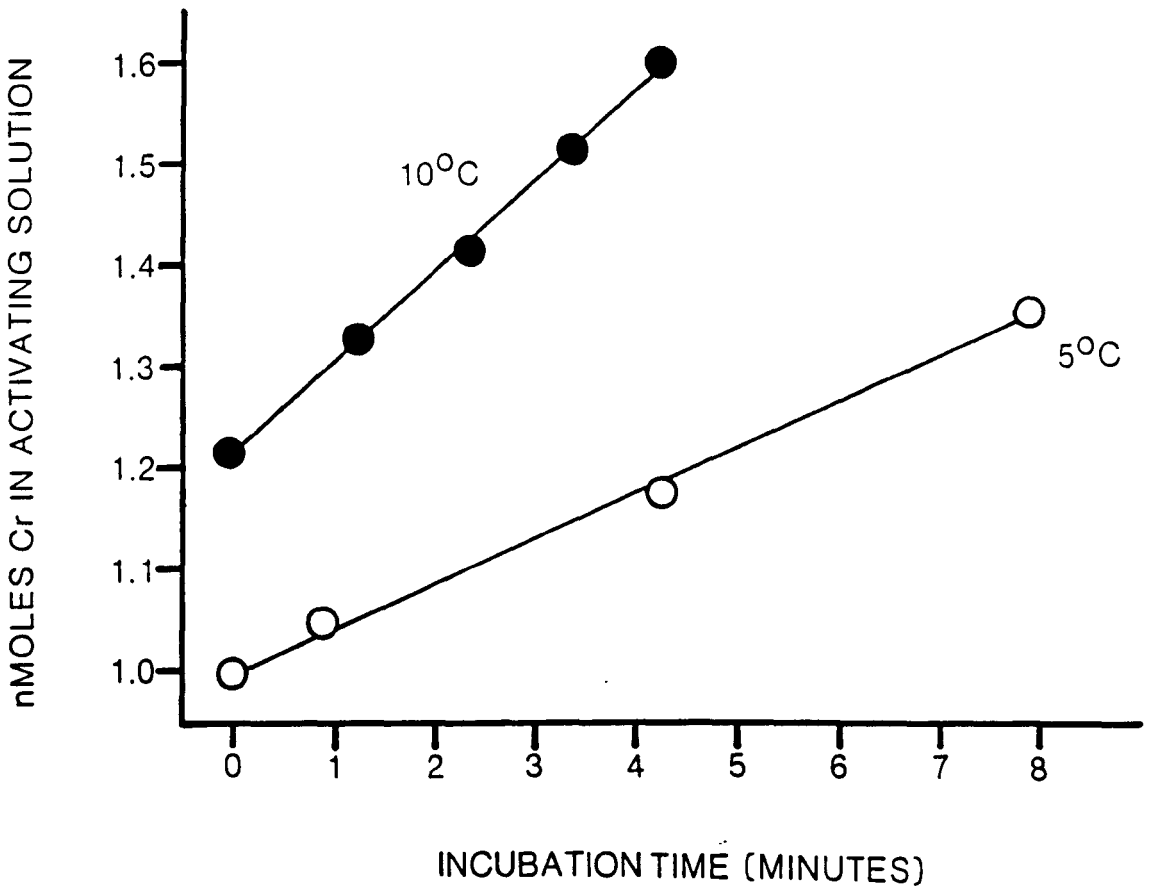
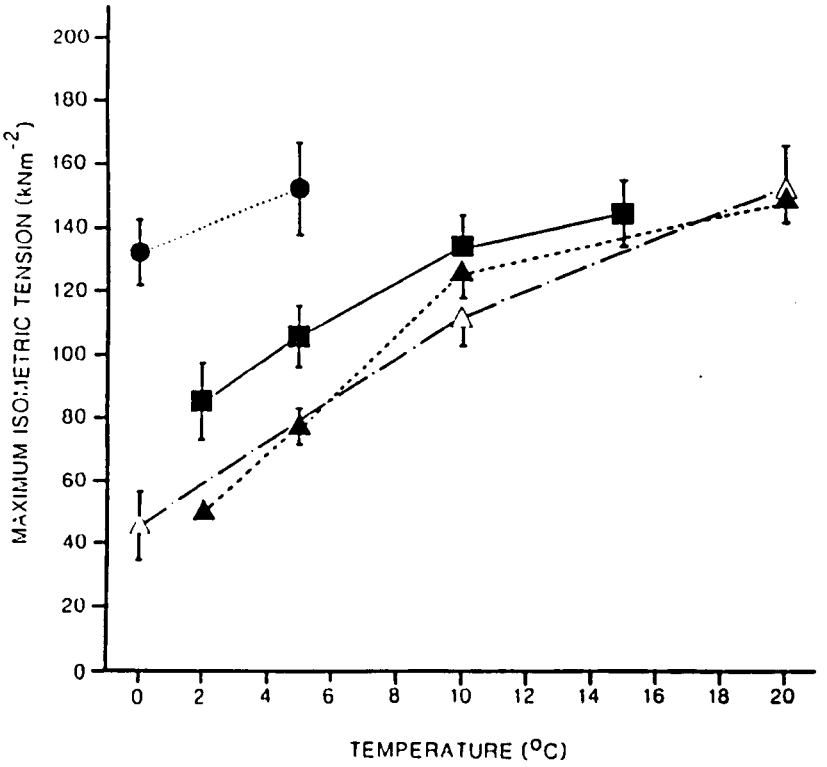

nMOLES Cr IN ACTIVATING SOLUTION
1.6
1.5
1.4
1.3
1.2
1.1
1.0
10°C
5°C
0
1
2
3
4
5
6
7
8
INCUBATION TIME (MINUTES)

Fig.6:4. Maximum isometric tension (a) and ATPase activity (b) plotted against temperature. The species are: Notothenia neglecta (●); Myoxocephalus scorpius (■); Oreochromis andersonii (Δ); Oreochromis niloticus (▲). Error bars represent mean ± S.E.M..

a


MAXIMUM ISOMETRIC TENSION (kNm^{-2})
200
180
160
140
120
100
80
60
40
20
0
0
2
4
6
8
10
12
14
16
18
20
TEMPERATURE (^{o}C)

b

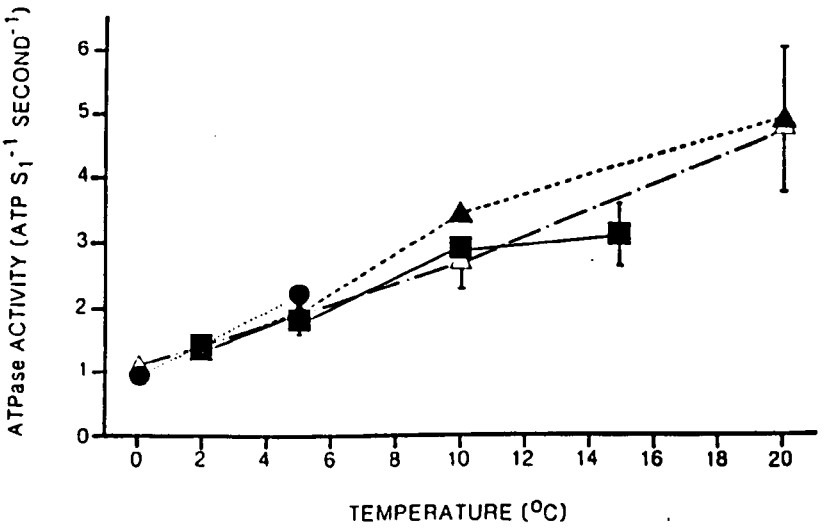

ATPase ACTIVITY (ATP S_1^{-1} SECOND^{-1})
6
5
4
3
2
1
0
0
2
4
6
8
10
12
14
16
18
20
TEMPERATURE (^{o}C)

Fig.6:5. Economy of contraction (tension/ATP turnover rates) against temperature. The species are: Notothenia neglecta (●); Myoxocephalus scorpius (■); Oreochromis andersonii (Δ); Oreochromis niloticus (▲). Error bars represent mean ± S.E.M..

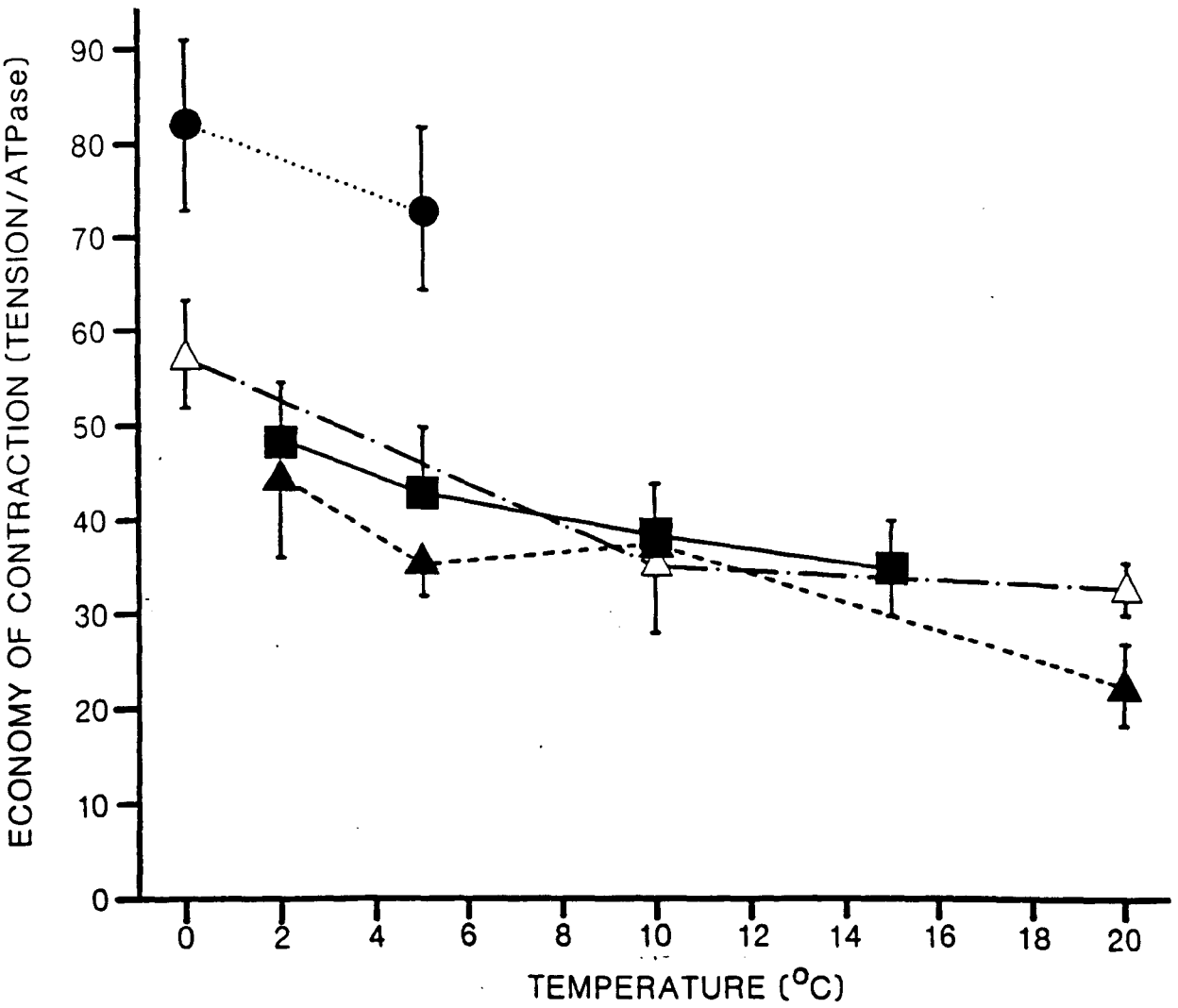
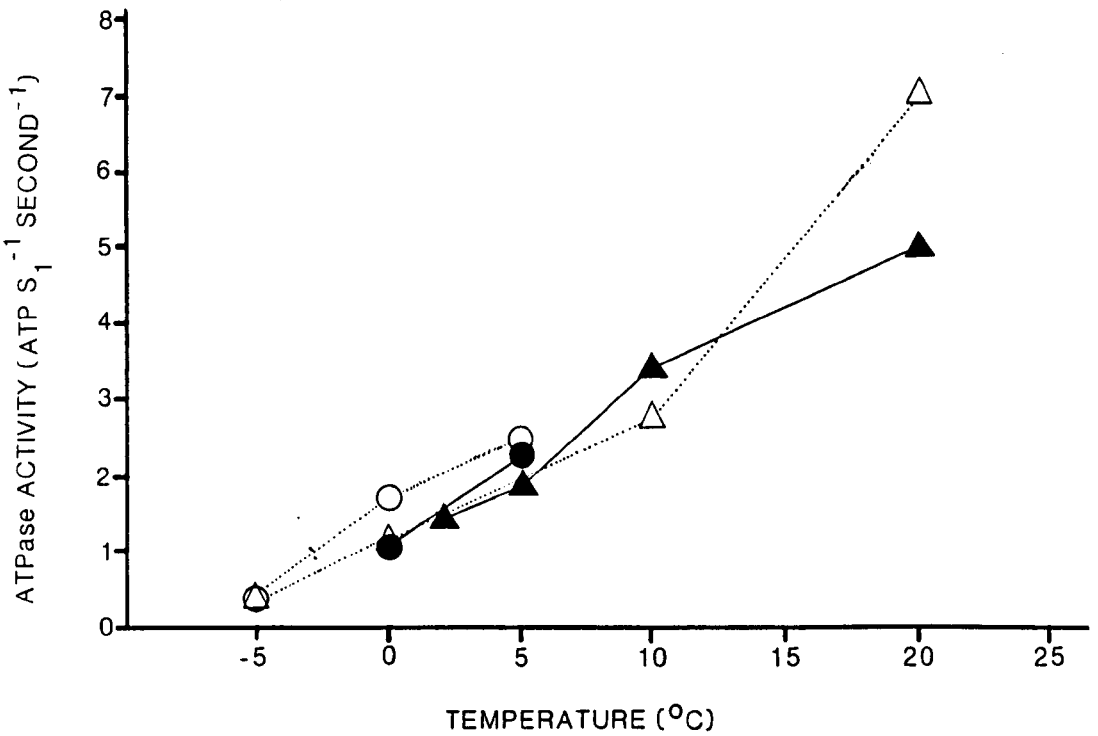

ECONOMY OF CONTRACTION (TENSION/ATPase)
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
16
18
20
TEMPERATURE (°C)

Fig.6:6. Comparison of myofibrillar ATPase activities measured using the direct nucleotide technique (Altringham and Johnston, 1986b) and the indirect creatine method (present study). The species are: Notothenia neglecta (O) (Altringham and Johnston, 1986b); Oreochromis niloticus (Δ) (Altringham and Johnston, 1986b); Notothenia neglecta (●) (present study); Oreochromis niloticus (▲) (present study).


ATPase ACTIVITY (ATP S_1^{-1} SECOND^{-1})
0
1
2
3
4
5
6
7
8
-5
0
5
10
15
20
25
TEMPERATURE (^{o}C)

CHAPTER 7

General Discussion.

The original experimental work described in this thesis can be related to two broad aspects of the physiology of the Notothenioidei: the lack of respiratory pigments in the family Channichthyidae and adaptations in the suborder as a whole associated with low environmental temperatures. These are discussed below with final suggestions for future research.

Is there a compromise between the maintenance of aerobic capacity and tension generation in channichthyid oxidative muscle tissue?

Mitochondrial volume densities in aerobic skeletal muscle fibres (0.50; Johnston, 1987) and ventricular myocytes (0.43; Chapter 3) from the channichthyid Chaenocephalus aceratus are considerably higher than in other teleosts with similar activity patterns. The proliferation of these organelles is thought to compensate for the rate depressing effects of low temperatures and low myoglobin concentrations on oxidative metabolism (see Chapter 3). In both cell types the high mitochondrial volume densities are achieved at the expense of contractile elements: myofibrillar volume densities in skeletal fibres (0.35; Johnston, 1987b) and myocytes (0.31; Chapter 3) are only slightly more than half those measured in sedentary temperate species.

The capacity of contractile cells to generate tension depends on myofibrillar packing; therefore, one might expect the low myofibrillar volume density in oxidative muscle tissue in C.aceratus to be reflected in low tensions.

However, maximum isometric tensions for C.aceratus red skeletal fibres are similar to those reported for myoglobin-rich aerobic fibres in other south polar and warmer-water teleosts (Table 7:1). Clearly, tension generation in C.aceratus fibres are compensated for the effects of low temperature and reduced myofibrillar packing. Compensation for the latter is illustrated in Table 7:1 which shows that the force generated per unit area of myofibrills in C.aceratus is considerably higher than in other species for which tensions and myofibrillar volume densities are available. Maintenance of aerobic capacity in C.aceratus red skeletal fibres therefore is not associated with a reduction in force generation. The low power outputs reported for these fibres (Johnston and Altringham, 1985) and myocardial tissue (Chapter 2) in this channichthyid are probably the result of limitations in contraction velocity (see Chapter 5) which is independent of myofibrillar packing.

The selective advantage of the haemoglobinless condition in channichthyids.

Compensation for the haemoglobinless condition in channichthyids is achieved largely by increases in blood volume and cardiac output (see Chapter 1). These necessitate extensive cardiovascular remodelling so that high cardiac outputs can be achieved at relatively low ventricular power outputs (Chapter 2). In view of the extraordinary compensatory adaptations described in these fish it is clear that the selection pressures which influenced the evolution of the haemoglobinless must have been very strong.

Several authors have suggested that the lack of haemoglobin in channichthyids represent the extreme in the general trend towards low haematocrits in fish from arctic (Graham and Fletcher, 1985) and antarctic (Hureau et al., 1977) latitudes. The haematocrits of antarctic nototheniids for example, are all lower than those of confamilial species which occur outside of the Antarctic Region (Tetens et al., 1984). This trend is thought to compensate for the increase in blood viscosity and concomitant increase in circulatory work load which occurs with decreasing environmental temperature (Everson and Twelves, 1977). Comparative studies have shown that channichthyid blood is indeed considerably less viscous than that of nototheniids (Twelves, 1972). However, the sympatric distribution of these two families in many parts of the Southern Ocean, and, in particular, the dominance of 'red-blooded' nototheniids at extreme high southern latitudes, suggests that ascribing the evolution of the haemoglobinless condition directly to reductions in blood viscosities may be overly simplistic. Unfortunately, the general acceptance of this interpretation (for example, see Andriashev, 1987) appears to have forestalled further investigation of the adaptive significance of this remarkable phenomenon.

Temperature compensation.

Many adaptations have been described in notothenicids which serve to offset the effects of low temperature (see Chapter 1). However, many physiological processes in these fish demonstrate little or no temperature compensation. Such

observations are often considered to indicate limitations in evolutionary capacity to overcome the rate effects of temperature. Recently, Clarke (1980, 1983) challenged this view. He maintained that polar ectotherms have achieved complete physiological adjustment to their thermal environment and related differences between these and warmer-water organisms to ecological constraints and to evolutionary limitations other than the direct effects of temperature. Two characteristics of the Notothenioidei, their slow growth rates and benthic habits, serve to illustrate Clarke's arguments. He suggested that their growth rates are a result of the marked seasonality of primary production in the Southern Ocean (see Chapter 1) and that their limited diversification into the water column reflects the fact that ancestral forms lacked a swim-bladder.

Whilst it is true that the influence of factors other than low temperature on the biology of notothenioids have been understressed, Clarke's contention, that complete thermal adaptation has been achieved in these animals, is probably an overstatement. For example, data presented in Chapter 5 indicate that one contractile parameter in notothenioid muscle fibres, unloaded contraction velocity (Vmax), demonstrates no temperature compensation. Despite compensation in tension generation and adaptive modifications in curvature of the force-velocity relationship (Chapter 5), the low values of Vmax in notothenioid fibres limit power output to values which are generally lower than in warm-water species at their

preferred body temperatures (Johnston and Altringham, 1988).

Energetic advantages of life at low temperatures.

In support of his arguments against metabolic cold adaptation, Holeton (1974) emphasised the energetic advantages of low resting metabolic rates (see Chapter 1). This has been elaborated by Clarke (1980) who proposed that reductions in basal metabolism would allow a greater proportion of an organism's energy budget to be directed towards reproduction, growth and activity.

Available evidence suggests that polar invertebrates do have lower resting metabolic rates than warmer-water species (Clarke, 1980). They also have energy budgets which are similar to those predicted by Clarke (1980) (see Clarke, 1983). However, low resting metabolic rates remain to be demonstrated in polar fish (see Chapter 1) and there is therefore no evidence that low temperatures confer an energetic advantage on south polar notothenioids. However, recent studies (Altringham and Johnston (1986b) and Chapter 6) have shown that axial muscle fibres from notothenioids generate more than twice as much isometric tension per ATP hydrolysed than homologous fibres from warmer-water species at their preferred body temperatures (Fig.6:5). At any experimental temperature, myofibrillar ATPase activities for species from different thermal environments are broadly similar (Fig.6:4b): thus the high economies of contraction for nototheniods may be related directly to the influence of low temperatures. It is not clear how these observations translate to working muscle *in vivo* since axial muscles do

not operate isometrically. However, these results are important in that they represent the first direct demonstrations of an energetic advantage to notothenioids of adaptation to low temperatures.

Suggestions for future research.

The present state of knowledge concerning cardiac function in channichthyids is rather elementary. In the immediate future more information must be collected concerning the gross anatomy and ultrastructural morphometry of the ventricles in channichthyids with a range of activity patterns. One might expect the increased circulatory load imposed by sustained swimming in pelagic species to be reflected in these characteristics. Driedzic (1983) has developed a preparation which allows the mechanical characteristics and rates of oxygen consumption to be measured simultaneously in isolated teleost hearts. Application of techniques such as this to the hearts of channichthyids would allow accurate determinations of their dynamic characteristics and resistance to hypoxia.

In the case of skeletal muscles, demembranated fibres will continue to be used to investigate metabolite turnover during contractions as in Chapter 6. However, their applications in mechanical studies will soon be superseded by the use of single or small bundles of live fibres. Indeed such preparations have already been developed (Curtin and Woledge, 1987; Johnston, 1987b; Altringham and Johnston, 1988a). These represent a significant improvement over demembranated fibres in that their contractile properties

are similar to isolated whole muscles: they generate approximately twice the tensions and have twice the contraction velocities of demembranated fibres (Altringham and Johnston, 1988a; Curtin and Woledge, 1988). In addition, they are superior to whole muscle preparations because they do not suffer from hypoxia and all of the constituent fibres can be activated with a single electrical stimulus. In view of the differences between the mechanical properties of live and demembranated fibres it would be worthwhile to repeat the experiments performed with demembranated notothenioid fibres using live fibres in order to validate the mechanisms of cold adaptation.

Of particular importance for future work is the potential use of live preparations to investigate the power output of teleost fibres under the constraints imposed during swimming. In vivo, fish axial muscle fibres are subjected to sinusoidal length changes as they first contract and then are stretched by the activity of surrounding fibres (Hess and Videler, 1984). These changes in length have a profound effect on the mechanical properties of the fibres. For example, Altringham and Johnston (1988a) have shown that a 5% stretch increases force in live teleost fibres by some 30%. Techniques have been described which allow the power output of muscles during oscillatory length changes to be measured. These have been applied to insect (Josephson, 1985a, b) and amphibian (Stevens, 1988) muscles. Determination of the power output of teleost live fibres under conditions which simulate in vivo length changes, together with available data concerning

fibre trajectories (Alexander, 1969), hydrodynamics (Webb, 1971) and energetics (Hess and Videler, 1984) will allow modelling of the biomechanics of undulatory swimming in these animals - a major goal in fish locomotory physiology.

Table 7:1. Maximum isometric tensions generated by red skeletal muscle fibres in fish.

	TEMP. (°C)	Vv(mf,f)	MAXIMUM ISOMETRIC TENSION EXPRESSED PER UNIT FIBRE CROSSSECTIONAL AREA (kNm^{-2})	MAXIMUM ISOMETRIC TENSION EXPRESSED PER UNIT MYOFIBRILLAR CROSSSECTIONAL AREA (kNm^{-2})
Chaenocephalus aceratus	0	0.35[a]	66[b]	189
Notothenia neglecta	0	0.54[c]	81[d]	150
Carp (*Cyprinus carpio*)	23	0.57[e]	64[f]	112
Dogfish (*Scyliorhinus canicula*)	8	0.62[g]	82[h]	132

Abbreviation: Vv(my,f) = myofibrillar volume density

References:

a. Johnston (1987b).

b. Johnston and Harrison (1985).

c. Johnston and Camm (1987).

d. Altringham and Johnston (1986a).

e. Akster (1985).

f. Johnston *et al.* (1985).

g. Bone *et al.* (1985).

h. Altringham and Johnston (1982).

REFERENCES

Ahlgren J.A., Cheng, C-H.C., Schrag, J.D., DeVries, A.L. (1988). Freezing avoidance and the distribution of antifreeze glycopeptides in body fluids and tissues of antarctic fish. J. Exp. Biol., 137:549-563.

Akster, H.A. (1985). Morphometry of muscle fibre types in the carp (Cyprinus carpio L.): Relationships between structural and contractile characteristics. Cell Tiss. Res., 241:193-201.

Alexander, R. McN. (1969). The orientation of muscle fibres in the myomeres of fishes. J. Mar. Biol. Assoc., U.K., 49:263-290.

Altringham, J.D., Johnston, I.A. (1982). The pCa-tension and force velocity characteristics of skinned fibres isolated from fish fast and slow muscles. J. Physiol., 333:421-449.

Altringham, J.D., Johnston, I.A. (1985a). Changes in tension generation and ATPase activity in skinned muscle fibres of the carp following temperature acclimation. Pflugers Arch., 403:449-451.

Altringham, J.D., Johnston, I.A. (1985b). Effects of phosphate on the contractile properties of fast and slow muscle fibres from an antarctic fish. J. Physiol., 368:491-500.

Altringham, J.D., Johnston, I.A. (1986a). Energy cost of contraction in fast and slow muscle fibres isolated from an elasmobranch and an antarctic teleost fish. J. Exp. Biol., 121:239-250.

Altringham, J.D., Johnston, I.A. (1986b). Evolutionary adaptation to temperature in fish muscle cross bridge mechanisms: tension and ATP turnover. Comp. Physiol., 156B:819-821.

Altringham, J.D., Johnston, I.A. (1988a). Activation of multiply innervated, fast and slow myotomal muscle fibres of the teleost Myoxocephalus scorpius. J. Exp. Biol. In press.

Altringham, J.D., Johnston, I.A. (1988b). The mechanical properties of polyneuronally innervated, myotomal muscle fibres isolated from a teleost fish (Myoxocephalus scorpius). Pflugers Arch.. In press

Andersen, N.C. (1984). Genera and subfamilies of the family Nototheniidae (Pisces, Perciformes) from the antarctic and Subantarctic. Steenstrupia, 10:1-34.

Andriashev, A.P. (1965). A general review of the antarctic fish fauna. In: "Biogeography and Ecology in Antarctica", van Mieghem, J. and van Oye, P. (eds.), 491-550. Dr.W.Junk Publishers, The Hague,

Andriashev, A.P. (1987). A general review of the antarctic bottom fish fauna. In: "Proc. V Congr. europ. Ichthyol. Stockholm 1985", Kullander, S.O. and Fernhom, B. (eds.), 357-372.

Archer, S.D., Johnston, I.A. (1988). Kinematics of labriform and subcarangiform swimming in the antarctic fish, Notothenia coriiceps neglecta. J. Exp. Biol. Submitted.

Axelsson, M., Nilsson, S. (1986). Blood pressure control during exercise in the antarctic cod, Gadus morhua. J. Exp. Biol., 126:225-236

Bailey, J.R., Driedzic, W.R. (1986). Function of myoglobin in oxygen consumption by isolated perfused fish hearts. Am. J. Physiol., 251:R1144-R1150.

Baldwin, J. (1971). Adaptation of enzymes to temperature: acetylcholinesterases in the central nervous system of fishes. Comp. Biochem. Physiol., 40B:181-187.

Bancroft, J.D., Stevens, A. (1972). "Theory and practice of histological techniques". Churchill, Edinburgh.

Barany, M. (1967). The ATPase activity of myosin correlated with speed of muscle shortening. J. Gen. Physiol., 50:197-208.

Barker, P.F., Burrell, J. (1977). The opening of the Drake Passage. Mar. Geol., 25:15-34.

Beardall, C.H., Johnston, I.A. (1983). Muscle atrophy during starvation in a marine teleost. Eur. J. Cell Biol., 29:209-217.

Bendall, J.R. (1969). "Muscles, Molecules and Movement". Heinemann, London.

Bennett, A.F. (1984). Thermal dependence of muscle function. Am. J. Physiol., 247:R217-R229.

Bentley C.R., Ostenso N.A. (1961). Glacial and subglacial topography of West Antarctica. J. Glaciol., 3:882-911.

Blake, R.W. (1981). Mechanics of drag-based mechanisms of propulsion in aquatic vertebrates. In:"Vertebrate Locomotion", Day, M.H. (ed.). Sym. Zool. Soc. Lond., 48:29-52.

Blake, R.W. (1982). Median and paired fin propulsion. In: "Fish Biomechanics", Webb, P.W. and Weiss, D. (eds.), 214-247. New York.

Bone, Q. (1964). Patterns of muscular innervation in the lower chordates. Internat. Rev. Neurobiol., 6:99-147.

Bone, Q. (1966). On the function of the two types of myotomal muscle fibres in elasmobranch fish. J. Mar. Biol. Assoc. U.K., 46:321-349.

Bone, Q. (1978). Locomotor muscle. In: "Fish Physiology", Hoar, W.S. and Randall, D.J. (eds.), 7:361-424. Academic Press, New York and London.

Bone, Q., Johnston, I.A., Pulsford, A., Ryan, K.P. (1986). Contractile properties and ultrastructure of three types of muscle fibre in the dogfish myotome. J. Muscle Res. Cell Motility, 7:47-56.

Bone, Q., Ono, R.D. (1982). Systematic implications of innervation patterns in teleost myotomes. Brevoria, 470:1-23.

Brandt, P.W., Cox, R.N., Kawai, M., Robinson, T. (1982). Regulation of tension in skinned muscle fibres. Effect of cross bridge kinetics on apparent Ca^{2+} sensitivity. J. Gen. Physiol., 79:997-1016.

Breisch, E.A., White, F., Jones, H.M., Laurs, R.M. (1983). Ultrastructural morphometry of the myocardium of Thunnus alalunga. Cell Tiss. Res. 233:427-438.

Canty, A.A., Driedzic, W.R. (1987). Evidence that myoglobin does not support heart performance at maximal levels of oxygen demand. J. Exp. Biol., 128:469-473.

Carey, F.G., Teal, J.M. (1966). Heat conservation in tuna fish muscle. Proc. U.S. Nat. Acad. Sci., 56:1464-1469.

Carmack, E.C., (1977). Water characteristics of the Southern Ocean south of the polar front. In: "A Voyage of Discovery", Angel, M. (ed.), 15-41. Pergamon Press, Oxford.

Carmack, E.C., Foster, T.D. (1975). On the flow of water out of the Weddle Sea. Deep-Sea Res., 22:711-724.

Cimini, V., Maresca, A., Tajana, G., Tota, B. (1977). On the heterogenity of the fish heart ventricle 1. Preliminary morphological observations. Boll. Soc. Ital. Biol. Sper., 53:543-548.

Clark, A.G. (1927). Comparative Physiology of the Heart. Cambridge University Press, Cambridge.

Clarke, A. (1980). A reappraisal of the concept of metabolic cold adaptation in polar marine invertebrates. In: "Ecology in the Antarctic", Bonner, W.N. and Berry, R.J. (eds.), 77-92. Academic Press, London.

Clarke, A. (1983). Life in cold water: the physiological ecology of polar marine ectotherms. Oceanogr. Mar. Biol. Ann. Rev., 21: 341-453.

Clarke, A., Doherty, N., DeVries, A.L., Eastman, J.T. (1984). Lipid content and composition of three species of antarctic fish in relation to buoyancy. Polar Biol. 3:77-83.

Connell, J.J. (1961). The relative stabilities of the skeletal muscle myosins of some animals. Biochem. J., 80:503-538.

Cossins, A.R. (1977). Adaptations of biological membranes to temperature. The effect of temperature acclimation of goldfish upon the viscosity of synaptosomal membranes. Biochim. Biophys. Acta, 470:395-4.

Cossins, A.R., Prosser, C.L. (1978). Evolutionary adaptation of membranes to temperature. Proc. Nat. Acad. Sci. U.S.A., 75:2040-2043.

Craddock, C. (1982). Antarctica and Gondwanaland. In "Antarctic Geoscience", Craddock, C. (ed.). The University of Wisconsin Press, Madison, Wisconsin.

Crow, M.T., Kushmerick, M.J. (1982). Chemical energetics of slow- and fast-twitch muscles of the mouse. J. Gen. Physiol., 79:147-166.

Curtin, N.A., Woledge, R.C. (1987). Force velocity relation of living fibres from dogfish white muscle. J. Physiol., 377:92P.

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.

Davison, W., Macdonald, J.A. (1985). A histochemical study of the swimming musculature of antarctic fish. New Zealand J. Zool., 12:473-483.

Denton G.H., Armstrong, R.L., Stuiver M. (1971). The late Cenozoic glacial history of Antarctica. In: "The late Cenozoic glacial Ages", Turekian, K.K. (ed.), 267-306. Yale University Press, New Haven.

DeVries, A.L., (1974). Survival at freezing temperatures. In: "Biochemical and Biophysical Perspectives in Marine Biology", Malins, D.C. and Sargent, J.R. (eds.), 289-330. Academic Press, London.

DeVries, A.L., (1978). The physiology and biochemistry of low temperature adaptations in polar marine ectotherms. In: "Polar Research. To the Present, and the Future", McWhinnie, M.A. (ed.), 175-202. AAAS Selected Symposium, Washington, D.C.

DeVries, A.L., Eastman, J.T. (1978). Lipid sacs as a buoyancy adaptation in an antarctic fish. Nature 271:352-353.

DeVries, A.L., Eastman, J.T. (1981). Physiology and ecology of notothenioid fishes of the Ross Sea. J. Roy. Soc. N.Z., 11:329-340.

DeVries, A.L., Lin, Y. (1977). The role of glycoprotein antifreezes in the survival of antarctic fishes. In: "Adaptations within Antarctic Ecosystems", Llano, G. (ed.), 703-712. Gulf Publishing, Houston.

DeVries, A.L., Komatsu, S.K., Feeney, R.E. (1970). Chemical and physical properties of freezing point depressing glycoproteins from antarctic fishes. J. Biol. Chem., 245:2901-2908.

DeWitt, H.H. (1971). Coastal and Deep-water benthic fishes of the Antarctic. Antarctic map folio series, Folio 15:1-10. Bushnell, V.C. (ed.) Am. Geog. Soc., New York.

DeWitt, H.H., Hopkins, T.L. (1977). Aspects of the diet of the antarctic silverfish, Pleuragramma antarcticum, . In: "Adaptations within Antarctic Ecosystems", Llano, G.A. (ed.),
557-567. Gulf Publishing, Houston.

Douglas, E.L., Chapman, D.J., Hemmingsen, E.A. (1973). Absence of porphyrin respiratory pigments in the blood in the antarctic icefish Chaenocephalus aceratus. Cryobiol., 10:260-261.

Douglas, E.L., Peterson, K.S., Gysi, J.R., Chapman, D.J. (1985). Myoglobin in the heart tissue of fishes lacking hemoglobin. Comp. Biochem. Physiol., 81A:885-888.

Driedzic, W.R. (1983). The fish heart as a model system for the study of myoglobin. Comp. Biochem. Physiol. 76A:487-493.

Driedzic, W.R., Stewart, J.M. (1982). Myoglobin content and the activities of enzymes of energy metabolism in red and white fish hearts. J. Comp. Physiol., 149:67-73.

Driedzic, W.R., Stewart, J.M., Scott, D.L. (1982). The protective effect of myoglobin during hypoxic perfusion of isolated fish hearts. J. Mol. Cell. Cardiol., 14:673-677.

Dunn, J.F., (1988). Muscle metabolism in antarctic fish. Comp. Biochem. Physiol., 90B:539-545.

Dunn, J.F., Archer, S.D., Johnston, I.A. (1988). Muscle fibre types and metabolism in post-larval and adult stages of fish from South Georgia (Antarctica). Pol. Biol. Submitted.

Dunn, J.F., Johnston, I.A. (1986). Metabolic constraints on burst-swimming in the antarctic teleost (Notothenia neglecta). Mar. Biol., 91:433-440.

Du Toit, A.L. (1937). "Our Wandering Continents". Oliver and Boyd, Edinburgh.

Eastman, J.T., DeVries, A.L. (1981a). Buoyancy adaptations in a swimbladderless antarctic fish. J. Morphol. 167:91-102.

Eastman, J.T., DeVries, A.L. (1981b). Hepatic ultrastructural specialization in antarctic fishes. Cell Tiss. Res. 219:489-496.

Eastman, J.T., DeVries, A.L. (1982). Buoyancy studies of notothenioid fishes in McMurdo Sound, Antarctica. Copeia 1982:385-393.

Eastman, J.T., DeVries, A.L. (1985). Adaptations for cryopelagic life in the antarctic nototheniid fish Pagothenia borchgrevinki. Pol. Biol.,4:45-52.

Eastman, J.T., DeVries, A.L. (1986). Antarctic fishes. Sci. Am., 255 (No.3):96-105.

Edgar, G., Shiver, H.E. (1925). The equilibrium between creatine and creatinine, in aqueous solution. The effect of hydrogen ion. J. Am. Chem. Soc., 47:1179-1188.

El-Sayed, S.Z. (1971). Observations on phytoplankton bloom in the Weddell Sea. In: "Biology of the Antarctic Sea IV", Llano, G.A. and Wallen, I.E. (eds.), 301-312. Am. Geophys. Union, Washington, D.C.

El-Sayed, S.Z.(1985). Plankton of the Antarctic Seas. In: "Antarctica", Bonner, W.N. and Walton, D.W.H. (eds.), 135-153. Pergamon Press, Oxford.

Emery, S.H., Mangano, C., Randazzo, V. (1985). Ventricle morphology in pelagic elasmobranch fishes. Comp. Biochem. Physiol., 82A:635-643.

Everson, I. (1977). Antarctic marine secondary production and the phenomenon of cold adaptation. Phil. Trans. Roy. Soc. Lond., 279B:55-66.

Everson, I., Ralph, R. (1970). Respiratory metabolism of Chaenocephalus aceratus. In: "Antarctic Ecology, Volume 1", Holdgate, M.W. (ed.), 315-319. Academic Press, London.

Everson, I, Twelves, E.L. (1977). The evolution of the haemoglobinless condition in the antarctic Channichthyidae. In: "Adaptations within Antarctic Ecosystems", Llano, G.A., (ed.), 1231. Gulf Publishing, Houston.

Farrell, A.P. (1985). Cardiovascular and hemodynamics energetics of fishes. In: "Circulation, Respiration and Metabolism", Gilles, R. (ed.), 377-385. Springer-Verlag, Berlin

Feeney, R.E., Osuga, D.T. (1976). Comparative biochemistry of antarctic proteins. Comp. Biochem. Physiol., 54A:281-286.

Feller, G. (1987). Les channichthyidae: poissons de l'antarctique adaptations metaboliques et structurales a l'absence d'hemoglobine et de myoglobine. Doctoral thesis, University of Liege.

Feller, G., Bassleer, R., Goessens, G., Hamoir, G. (1983). Relative size and myocardial structure of the heart of an antarctic fish devoid of haemoglobin and myoglobin Channichthys rhinoceratus. J. Zool. (Lond.), 199:51-57.

Feller, G., Goessens, G., Gerday, Ch., Bassleer, R. (1985). Heart structure and ventricular ultrastructure of hemoglobin- and myoglobin-free icefish Channichthys rhinoceratus. Cell Tiss. Res., 242:669-676.

Ferenczi, M.A., Goldman, Y.E., Simmons, R.M. (1984). The dependence of force and shortening velocity on substrate concentration in skinned muscle fibres from Rana temporaria. J. Physiol., 350:519-543.

Fitch, N.A., Johnston, I.A. and Wood, R.E. (1984). Skeletal muscle capillary supply in a fish that lacks respiratory pigments. Resp. Physiol.,57: 201-211.

Fitch, N.A. (1986). Aspects of the physiology and biochemistry of antarctic fish muscle with emphasis on Notothenia neglecta. Ph.D thesis, University of St.Andrews.

Foster, T.D. (1978). Temperature and salinity fields under the Ross Ice Shelf. Ant. J. U.S. 13:81-82.

Forster, M.E., Franklin, C.E., Taylor, H.H., Davison, W. (1987). The aerobic scope of an antarctic fish, Pagothenia borchgrevinki and its significance for metabolic cold adaptation. Pol. Biol., 8:155-159.

Gilbert, C., Kretzschmar, K.M., Wilkie, D.R., Woledge, R.C. (1971). Chemical change and energy output during muscular contraction. J. Physiol., 218:163-193.

Giovane, A., Greco, G., Maresca, A., Tota, B. (1980). Myoglobin in the heart ventricle of tuna and other fishes. Experientia, 36:219-220.

Gleeson, T.T., Johnston, I.A., Sidell, B.D., Stephens, W.G.S. (1983). Temperature dependence of contraction velocity in some ectotherm fast muscles. J. Physiol. 346:65.

Glyn, H., Sleep, J. (1985). Dependence of adenosine triphosphatase activity of rabbit psoas muscle fibres and myofibrils on substrate concentration. J. Physiol., 365:259-276.

Graham, M.S., Fletcher, G.L. (1985). On the low viscosity blood of two cold water, marine sculpins. J. Comp. Physiol., 155B:455-459.

Grande, L., Eastman, J.T. (1986). A review of antarctic ichthyofaunas in the light of new fossil discoveries. Palaeontology, 29:113-137.

Grant, R.T., Regnier, M. (1926). The comparative anatomy of the cardiac coronary vessels. Heart, 13:285-317.

Greco, G., Lipari, D., Tota, B., Zummo, G. (1980). Preliminary observations on the heart of the haemoglobinless antarctic fish Champsocephalus gunnari, Loennberg. Boll. Soc. It. Biol. Sper., LVI:1807-1813.

Greene, F.C., Feeney, R.E. (1970). Properties of muscle glyceraldehyde-3-phosphate dehydrogenase from the cold-adapted antarctic fish Dissostichus mawsoni. Biochim. et Biophys. Acta, 220:430-442.

Greer-Walker, M. (1971). The effects of starvation and exercise on the skeletal muscle fibres of the cod (Gadus morhua L.) and coalfish (Gadus virens L.) respectively. J. Cons. Int. Explor. Mer., 33:421-427.

Griffiths, P.J., Guth, K., Kuhn, H.J., Ruegg, J.C. (1980). ATPase activity in rapidly activated skinned muscle fibres. Pflugers Arch., 387:167-173.

Guth, L., Samaha, F.J. (1969). Qualitative differences between actomyosin ATPase of slow and fast mammalian muscle. Exp. Neurol., 25:138-152.

Guth, L., Samaha, F.J. (1972). Erroneous interpretations which may result from applications of the "myofibrillar ATPase" histochemical procedure to developing muscle. Exp. Neurol., 34:465-475.

Hamoir, G., Gerardin-Otthiers, N. (1980). Differentiation of the sarcoplasmic proteins of white, yellowish and cardiac muscles of an antarctic hemoglobin-free fish, Champsocephalus gunnari. Comp. Biochem. Physiol., 65B:199-206.

Harrison, P., Nicol, J.M., Johnston, I.A. (1987). Gross morphology, fibre composition and mechanical properties of pectoral fin muscles in the antarctic teleost, Notothenia neglecta Nybelin. In: "Proc. V. Congr. europ. Ichthyol. Stockholm 1985", Kullander, S.O. and Fernholm, B. (eds.):459-465.

Hardman, K.D., Eylar, E.H., Ray, D.K., Banaszak, L.J., Gurd, F.R.N. (1966). Purification of sperm whale myoglobin by low temperature fractionation with ethanol and metallic ions. J. Biol. Chem., 241:432-442.

Haschemeyer, A.E.V. (1976). Kinetics of protein synthesis in higher organisms in vivo. Trends Biochem. Sci., 1:133-136.

Haschemeyer, A.E.V. (1985). Cold adaptation and genome organization in an antarctic fish species. Comp. Biochem. Physiol., 81B:523-530.

Haschemeyer, A.E.V., Persell, R., Smith, M.A.K. (1979). Effect of temperature on protein synthesis in fish of the Galapagos and perlas Islands. Comp. Biochem. Physiol., 64B:91-95.

Haschemeyer, A.E.V., Smith, M.A.K. (1979). Protein synthesis in liver, muscle and gill of mullet (Mugil cephalus L.) in vivo. Biol. Bull., 156:93-102.

Hazel, J.R., Garlick, W.S., Sellner, P.A. (1978). The effects of assay temperature upon the pH optima of enzymes from poikilotherms: a test of the imidazole alphastat hypothesis. J. Comp. Physiol., 123:97-104.

Hazel, J., Prosser, C.L. (1974). Molecular mechanisms of temperature compensation in poikilotherms. Physiol. Rev., 54:620-677.

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

Hemmingsen, E.A., Douglas, E.L. (1972). Respiratory and circulatory responses in a hemoglobin-less fish, Chaenocephalus aceratus, to changes in temperature and oxygen tension. Comp. Biochem. Physiol., 43A:1031-1043.

Hemmingsen, E.A., Douglas, E.L. (1977). Respiratory and circulatory adaptations in the absence of haemoglobin in Chaenichthyid fishes. In: "Adaptations within Antarctic Ecosystems", Llano, G.A., (ed.), 479-487. Gulf Publishing, Houston.

Hemmingsen, E.A., Douglas, E.L., Johansen, K., Millard, R.W. (1972). Aortic blood flow and cardiac output in the hemoglobin-free fish Chaenocephalus aceratus. Comp. Biochem. Physiol., 43A:1045-1051.

Herdman, H.F.P., Wiseman, J.D.H., Ovey, C.D. (1956). Proposed names for features on the deep-sea floor: 3. Southern or Antarctic Ocean. Deep-sea Res., 3:253-261.

Hess, F., Videler, J.J. (1984). Fast continuous swimming of saithe (Pollachius virens): a dynamic analysis of bending moments and muscle power. J. Exp. Biol., 109:229-251.

Hill, A.V. (1938). The heat of shortening and the dynamic constants of muscle. Proc. Roy. Soc. B,126:136-195.

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

Holeton, G.F. (1973). Respiration of arctic char (Salvelinus alpinus) from a high arctic lake. J. Fish. Res. Board Canada, 30:717-723.

Holeton, G.F. (1974). Metabolic cold adaptation of polar fish: fact or artefact? Physiol. Zool., 47:137-152.

Holeton, G.F. (1975). Respiration and morphometrics of hemoglobinless antarctic icefish. In: "Respiration of Marine Organisms", Cech, Jr., J.J. Bridges, D.W. and Horton, D.B. (eds.). Res. Inst. Gulf of Maine, South Portland, Maine.

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

Hudson, R.C.L. (1967). Histological and electrophysiological investigations of the fast muscle of the teleost Cottus bubalis. Ph.D. thesis, University of St. Andrews.

Hudson, R.C.L. (1973). On the function of the white muscles in teleosts at intermediate swimming speeds. J.Exp. Biol., 58:509-522.

Hudson, J.D. (1977). Oxygen isotope studies on Cenozoic temperatures, oceans and ice accumulation. Scott. J. Geol., 13:313-23.

Hughes, G.M. (1966). The dimensions of fish gills in relation to their function. J. Exp. Biol., 45:177-195.

Hume, W.F. (1948). "Terrestrial Theories". Government Press, Cairo.

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

Jakubowski, M., Byczkowska-Smyk, K. (1970). Respiratory surfaces of the white-blooded fish Chaenichthys rugosus, Regan (Perciformes). Polish Arch. Hydrobiol., 17:273-281.

Johnston, I.A., (1987a). Muscle function in antarctic fish. In: "Proc. V. Congr. europ. Ichthyol., Stockholm 1985", Kullander, S.O. and Fernhom, B. (eds.), 455-458.

Johnston, I.A., (1987b). Respiratory characteristics of muscle fibres in a fish (Chaenocephalus aceratus) that lacks haem pigments. J. Exp. Biol., 133:415-428.

Johnston, I.A., Altringham, J.D. (1985). Evolutionary adaptation of muscle power output to environmental temperature: force-velocity characteristics of skinned fibres isolated from antarctic, temperate and tropical marine fish. Pfluegers Arch., 405:136-140.

Johnston, I.A., Brill, R. (1984). Thermal dependence of contractile properties of single skinned muscle fibres isolated from antarctic and various Pacific marine fishes including Skipjack Tuna (Katsuwonus pelamis) and Kawakawa (Euthynnus affinis). J. Comp. Physiol., 155B:63-70.

Johnston, I.A., Camm, J.-P. (1987). Muscle structure and differentiation in pelagic and demersal stages of the antarctic teleost Notothenia neglecta. Mar. Biol., 94:183-190.

Johnston, I.A., Davison, W., Goldspink, G. (1977a). Energy metabolism of carp swimming muscles. J. Comp. Physiol., 114B:203-216.

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 haemoglobin-less icefish Chaenocephalus aceratus. Comp. Biochem. Physiol., 76A:475-480.

Johnston, I.A., Goldspink, G. (1973a). A study of glycogen and lactate in the myotomal muscles and liver of the coalfish (Gadus virensL.) during sustained swimming. J. Mar. Biol. Ass. U.K., 53:17-26.

Johnston, I.A., Goldspink, G. (1973b). A study of the swimming performance of the Crucian carp (Carassius carassius L.) in relation to the effects of exercise and recovery on biochemical changes in the myotomal muscles and liver. J. Fish Biol., 5:249-260.

Johnston, I.A., Goldspink, G. (1975). Thermodynamic activation parameters of fish myofibrillar ATPase enzyme and evolutionary adaptations to temperature. Nature, 257, No.5527:620-622.

Johnston, I.A., Harrison, P. (1985). Contractile and metabolic characteristics of muscle fibres from antarctic fish. J. Exp. Biol., 116:223-236.

Johnston, I.A., Harrison, P. (1987). Morphometrics and ultrastructure of myocardial tissue in notothenioid fishes. Fish Physiol. Biochem., 3:1-6.

Johnston, I.A., Maitland, B. (1980). Temperature acclimation in crucian carp: A morphometric analysis of muscle fibre ultrastructure. J. Fish Biol. 17:113-125.

Johnston, I.A., Moon, T.W. (1980a). Exercise training in skeletal muscle of brook trout (Salvelinus fontinalis). J. Exp. Biol. 87:177-194.

Johnston, I.A., Moon, T.W. (1980b). Endurance exercise training in the fast and slow muscles of a teleost fish (Pollachius virens). J. Comp. Physiol., 135B:147-156.

Johnston, I.A., Patterson, S., Ward, P., Goldspink, G. (1974). The histochemical demonstration of myofibrillar adenosine triphosphatase activity in fish muscle. Can. J. Zool., 52:871-877.

Johnston, I.A., Salamonski, J. (1984). Power output and force-velocity relationship of red and white muscle fibres from the pacific blue marlin (Makaira nigricans). J. Exp. Biol., 111:171-177.

Johnston, I.A., Sidell, B.D. (1984). Differences in temperature dependence of muscle contractile properties and myofibrillar ATPase activity in a cold-temperate fish. J. Exp. Biol., 111:179-189.

Johnston, I.A., Sidell, B.D., Driedzic, W.R. (1985). Force-velocity characteristics and metabolism of carp muscle fibres following temperature acclimation. J. Exp. Biol., 119:239-249.

Johnston, I.A., Walesby, N.J., (1977). Molecular mechanisms of temperature adaptation in fish myofibrillar adenosine triphosphatases. J. Comp. Physiol., 119B:195-206.

Johnston, I.A., Walesby, N.J., (1979). Evolutionary temperature adaptation and the calcium regulation of fish actomyosin ATPases. J. Comp. Physiol., 129:169-177.

Johnston, I.A., Walesby, N.J., Davison, W., Goldspink, G. (1975a). Temperature adaptation in myosin of antarctic fish. Nature. 254:74-75.

Johnston, I.A., Walesby, N.J., Davison, W., Goldspink, G. (1977b). Further studies on the adaptations of fish myofibrillar ATPases to different cell temperatures. Pflugers Arch., 371:257-262.

Johnston, I.A., Ward, P., Goldspink, G. (1975b). Studies on the swimming musculature of the rainbow trout I: Fibre types. J. Fish Biol., 7: 451-458.

Johnston, I.A., Wokoma, A. (1986). Effects of temperature and thermal acclimation on contractile properties and metabolism of skeletal muscle in the flounder (Platichthys flesus L.). J. Exp. Biol., 120:119-130.

Josephson, R.K. (1985a). Mechanical power output from striated muscle during cyclic contraction. J. Exp. Biol.,114:493-512.

Josephson, R.K. (1985b). The mechanical power output of a tettigonid wing muscle during singing and flight. J. Exp. Biol.,117:357-368.

Kennett, J.P. (1977). Cenozoic evolution of Antarctic glaciation, the circum-Antarctic Ocean and their impact on global paleoceanography. J. Geophys. Res., 82:3843-60.

Kiceniuk, J.W., Jones, D.R. (1977). The oxygen transport system in trout (Salmo gairdneri) during sustained exercise. J. Exp. Biol., 69:247-260.

Kilarski, W., Kozlowski, M. (1983). Ultrastructural characteristics of the teleostean muscle fibres and their nerve endings. The stickleback (Gasterosteus aculeatus L.). Z. microsk. -anat. Forsch., 97:1022-1036.

Kreuzer, F., Hoofd, L.J.C. (1972). Factors influencing facilitated diffusion of oxygen in the presence of haemoglobin and myoglobin. Resp. Physiol., 15:104-124.

Krogh, A. (1914). The quantitative relation between temperature and standard standard metabolism in animals. Int. Zeitschr. fur Physikal.-Chem. Biol., 1:491-508.

Krogh, A. (1916). "The respiratory exchange of animals and man". Longmans, London.

Kyle, P.R., Elliot, D.H., Sutter, J.F. (1981). Jurassic Ferrar Super-group tholeiites from the Transantarctic Mountains, Antarctica, and their relationship to the initial fragmentation of Gondwana. In: "Gondwana V", Cresswell, M.M. and Vella, P. (eds.), 283-7. A.A. Balkema, Rotterdam.

Langfeld, K.S., Altringham, J.D., Johnston, I.A. (1988). The effects of temperature on the force-velocity relation of live, fast teleost muscle fibres. J. Exp. Biol., submitted.

Lemanski, L.F., Fitts, E.P., Marx, B.S. (1975). Fine structure of the heart in the Japanese medaka, Oryzias latipes. J. Ultrastruct. Res., 53:37-65.

Levy, R.M., Umazume, Y., Kushmerick, M.J. (1976). Ca^{2+} dependence of tension and ADP production in segments of chemically skinned muscle fibers. Biochim. et Biophys. Acta, 430:352-365.

Lin, Y., Dobbs, G.H., DeVries, A.L. (1974). Oxygen consumption and lipid content in red and white muscles of antarctic fishes. J. Exp. Zool., 189:379-385.

Littlepage, J.L. (1965). Oceanographic investigations in McMurdo Sound, Antarctica. Ant. Res. Ser. 5:1-37.

Llano, G.A. (1978). Polar research: A synthesis with special reference to biology. In: "Polar research: To the present, and the future", McWhinnie, M.A. (ed.). AAAS Selected Symp. Ser. No. 7:27-61. Westview Press, Boulder, Colarado.

Loxdale, H.D. (1976). A method for the continuous assay of picomole quantities of ADP released from glycerol-extracted skeletal muscle fibers on MgATP activation. J. Physiol., 260:4P-5P.

McArdle, H.J., Johnston, I.A. (1980a). Evolutionary temperature adaptation of fish sarcoplasmic reticulum. J. Comp. Physiol.,135:157-164.

McArdle, H.J., Johnston, I.A. (1980b). Isolation and properties of sarcoplasmic reticulum from the fast muscles of plaice (Pleuronectes platessa). J. Fish Biol., 18:209-221.

Macdonald, J.A., Montgomery, J.C. (1982). Thermal limits of neuromuscular function in an antarctic fish. J. Comp. Physiol., 147:237-250.

Macdonald, J.A., Montgomery, J.C. (1986). Rate-compensated synaptic events in antarctic fish: consequences of homeoviscous cold-adaptation. Experientia, 42:806-808.

Macdonald, J.A., Montgomery, J.C., Wells, R.M.G. (1987). Comparative physiology of antarctic fishes. Adv. Mar. Biol., 24:321-388.

Macdonald, J.A., Wells, R.M. (1978). Physiological adaptation in antarctic fishes. Ant. Record (N.Z.) 1(1):17-23.

McElhinny, M.W. (1973). "Palaeomagnetism and Plate Tectonics". Cambridge University Press, Cambridge.

Mackintosh, N.A. (1946). The Antarctic convergence and the distribution of surface temperatures in Antarctic waters. Discovery Repts., 22:177-210.

McNurlan, M.A., Tomkins, A.M., Garlick, P.J. (1979). The effect of starvation on the rate of protein synthesis in rat liver and small intestine. Biochem. J., 178:373-379.

Marechal, G., Mommaerts, W.F.H.M. (1963). The metabolism of phosphocreatine during an isometric tetanus of frog sartorius muscle. Biochim. Biophys. Acta, 70:53-67.

Marshall, N.B. (1964). Some convergences between the benthic fishes of polar seas. In: "Biologie antarctique", Carrick, R., Holdgate, M. and Prevost, J. (eds.), 273-278. Hermann, Paris..

Mathews, R.W., Haschemeyer, A.E.V. (1978). Temperature dependency of protein synthesis in toadfish liver in vivo. Comp. Biochem. Physiol., 61B:479-484.

Matthews, R.K., Poore, R.Z. (1980). Tertiary - O record and glacio-eustatic sea-level fluctuations. Geology, 8:500-14.

Mazzocchi, G., Robba, C., Gottardo, G., Meneghelli, V., Nussdorfer, G. (1982). Ultrastructure of aldosterone-secreting adrenal adenomata. J. Submicrosc. Cytol., 14:179-185.

Mercer, J.H. (1973). Cainozoic temperature trends in the southern hemisphere: Antarctic and Andean glacial evidence. In: "Palaeoecology Africa", van Zinderen Bakker, E.M. (ed.), 8:85-114. Balkema: Capetown.

Mercer, J.H. (1983). Cenozoic glaciation in the Southern Hemisphere. Ann. Rev. Earth Planet Sci, 11:99-132.

Meyer-Rochow, V.B., Pyle, C.A. (1980). Fatty acid analysis of lens and retina Orchomene plebs. Comp. Biochem. Physiol., 65B:395-398

Miller, R.G. (1987). Origins and pathways possible for the fishes of the Antarctic Ocean. In: "Proc. V. Congr. europ. Ichthyol., Stockholm 1985", Kullander, S.O. and Fernhom, B. (eds.), 373-380.

Moerland, T.S., Sidell, B.D. (1986). Biochemical responses to temperature in the contractile protein complex of striped bass Morone saxatilis. J. Exp. Zool., 238:287-295.

Molnar, P., Atwater, T., Mammerickx, J., Smith, S.W. (1975). Magnetic anomalies, bathymetry and the tectonic evolution of the South Pacific since the late Cretaceous. Geophys. J., 40:383-420.

Mommaerts, W.F.H.M., Seraydarian, K., Wallner, A. (1962). emonstration of phosphocreatine splitting as an early reaction in contacting frog sataius muscle. Biochim. Biophys. Acta, 63:75-81.

Montgomery, J.C. and MacDonald, J.A. (1984). Performance of motor systems in antarctic fishes.J. Comp. Physiol. A., 154: 241-248.

Morgan, D.L., Proske, U. (1984). Vertebrate slow muscle: its structure,pattern of innervation and mechanical properties. Physiol. Rev., 64:103-169.

Morris, D.J., North, A.W. (1984). Oxygen consumption of five species of fish from South Georgia. J. Exp. Mar. Biol. Ecol., 78:75-86.

Morris, R.W., Schneider, M.J. (1969). Brain fatty acids of an antarctic fish, Trematomus bernacchii. Comp. Biochem. Physiol., 28:1461-1465.

Mutungi, G.M. (1988). The interaction of pH and temperature on the contractile properties of skinned muscle fibres isolated from various ectotherms. Ph.D. thesis. University of St. Andrews.

Nachlas, M.M. Tsou, K.C., De Sonza, E., Cheng, C.S., Seligman, A.M. (1957). Cytochemical demonstration of succinic dehydrogenase by the use of a new p-nitrophenyl substituted dittrazele. J. Histochem. Cytochem. 5:420-436.

Norman, J.R. (1938). Coast fishes. Part III. The Antarctic zone. Discovery Rept., 18:1-104.

Norton I.O. and Sclater J.G. (1979). A model for the evolution of the Indian Ocean and the breakup of

Gondwanaland. J. Geophys. Res. 84:6803-6830.

Nybelin, O. (1947). Antarctic fishes. Sci. Results Norweg. Antarct. Exped. 1927-1928. 26:1-76.

Ocean Drilling Program, Leg 114 shipboard scientific party. (1987). Palaeoceanography of the subantarctic South Atlantic. Nature, 328:671-672.

O'Neill, J.G. (1987). Temperature adaptation of the immune response in the antarctic teleost Notothenia rossii Richardson. In: "Proc. V. Congr. europ. Ichthyol., Stockholm 1985", Kullander, S.O. and Fernhom, B (eds.), 443-451.

Ostadal, B., Rychter, Z., Poupa, O. (1970). Comparative aspects of the development of the terminal vascular bed in the myocardium. Physiol. Bohemslov., 19:1-7.

Ostadal, B., Shiebler, T.H. (1971). Uber die terminale Strombahn in Fischherzen. Z. Anat. Entwicklungsgesch., 134:101-110.

Ostadal, B, Shiebler, T.H. (1972). Blood supply of the heart in different species of fish. Physio. Bohemoslov, 21:103.

Patton, J.S. (1975). The effect of pressure and temperature on phospholipid and triglyceride fatty acids of fish white muscle: a comparison of deepwater and surface marine species. Comp. Biochem. Physiol., 52B:105-110.

Permitin, Yu E., Tarverdieva, M.J. (1978). Feeding of fishes of the families Notothenidae and Chaenichthyidae in the South Orkney Islands. Biologiya Marya, 3:75-81. (Transl. from the Russian).

Peter, J.B., Barnard, R.J., Edgerton, V.R., Gillespie, C.A., Stempel, K.E. (1972). Metabolic profiles of three fiber types of skeletal muscle in guinea pigs and rabbits. Biochem., 11:2627-2633.

Pittock, A.B., Frakes, L.A., Jenssen, D., Peterson, J.A., Zillman, J.W. (1978). "Climatic change and Variability: a southern Perspective". Cambridge University Press, Cambridge.

Pockett, S, Macdonald, J.A. (1986). Temperature dependence of neurotransmitter release in the antarctic fish Pagothenia borchgrevinki. Experientia, 42:414-415.

Poupa, O., Rakusan, K., Ostadal, B. (1970). The effect of physical activity upon the heart of vertebrates. In "Physical Activity and Aging. Medicine and Sport", Brunner, D. and Jokl, E. (eds.), 4:202-233. Karger, Basel.

Poupa, O., Lindstrom, L., Maresca, A, Tota, B. (1981). Cardiac growth, myoglobin proteins and and DNA in developing tuna (Thunnus thynnus thynnus L.) Comp. Biochem. Physiol., 70A:217-222.

Poupa, O., Ostadal, B. (1969). Experimental cardiomegalies and "cardiomegalis" in free living animals. Am. N.Y. Acad. Sci., 156: 445-467.

Precht, H. (1958). Concepts of the temperature adaptation of unchanging reaction systems of cold-blooded animals. In: "Physiological Adaptation", Prosser, C.L. (ed.), 50-78. American Physiological Society, Washington, D.C..

Pritchard, A.W., Hunter, J.R., Lasker, R. (1971). The relation between exercise and biochemical changes in red and white muscles and liver in the jack mackeral Trachurus symmetricus. U.S. Fish Wildlife Service Fish. Bull., 69:379-386.

Prosser, C.L. (1973). "Comparative Animal Physiology". W.B. Saunders, Philadelphia.

Qvist, J., Weber, R.E., DeVries, A.L., Zapol, W.M. (1977). pH and haemoglobin oxygen affinity in blood from the antarctic cod Dissostichus mawsoni. J. Exp. Biol., 67:77-88.

Ralph, R., Everson, I. (1968). The respiratory metabolism of some antarctic fish. Comp. Biochem. Physiol., 27:299-307.

Raymond, J.A. DeVries, A.L. (1977). Absorption inhibition as a mechanism of freezing resistance in polar fishes. Proc. Nat. Acad. Sci., U.S.A., 74:2589-2593.

Reeves, R.B. (1972). An imidazole alphastat hypothesis for vertebrate acid-base regulation: tissue carbon dioxide content and body temperature in bullfrogs. Respir. Physiol., 14:219-236.

Regan, C.T. (1912). The antarctic fishes of the Scottish Antarctic Expedition. Trans. Royal Soc. Edinburgh, 49:229-292.

Regan, C.T. (1914). Fishes. Brit. Antarct. ("Terra Nova") Exped., 1910, Nat. Hist. Rept. Zool., 1:1-54.

Robilliard, G.A., Dayton, P.K. (1969). Notes on the biology of the chaenichthyid fish Pagetopsis macropterus from McMurdo Sound, Antarctica. Ant. J. U.S., 4:304-306.

Rome, L.C., Loughna, P.T., Goldspink, G. (1984). Muscle fiber activity in carp as a function of swimming speed and muscle temperature. Am. J. Physiol., 247:R272-R279.

Ruegg, J.C., Schadler, M., Steiger, G.J., Muller, G. (1971). Effects of phosphate on the contractile mechanism. Pflugers Archiv 325:359-364.

Ruud, J.T. (1954). Vertebrates without erthrocytes and blood pigment. Nature, Lond., 173:848-850.

Saetersdal, T.S., Justesen, N-P, Krohnstad, A.W. (1974). Ultrastructure and innervation of the teleostean atrium.

J. Mol. Cell Cardiol., 6:415-437.

Santer, R.M. (1976). The distribution of collagen bundles and an epicardial coronary vasculature in the plaice (Pleuronectes platessa L.) heart ventricle at different ages. J. Mar. Biol. U.K., 56:241-246.

Santer, R.M. (1984). Morphology and innervation of the fish heart. Adv. Anat. Embryol. Cell Biol., 89.

Santer, R.M., Cobb, J.L.S. (1972). The fine structure of the heart of the teleost Pleuronectes platessa L. Z. Zellforsch. Mikrosk. Anat., 131:1-14.

Santer, R.M., Greer-Walker, M. (1980). Morphological studies on the ventricle of teleost and elasmobranch hearts. J. Zool. (Lond.), 190:259-272.

Santer, R.M., Greer-Walker, M., Emerson, L., Witthames, P.R. (1983). On the morphology of the heart ventricle in marine teleost fish (Teleostei). Comp. Biochem. Physiol., 76A:453-459.

Satchell, G.H. (1971). "Circulation in Fishes". Cambridge monographs in Experimental Biology, no.18. Cambridge University Press, Cambridge.

Savin, S.M. (1977). The history of the Earth's surface temperature during the past 100 million years. Ann. Rev. Earth Planet. Sci. 5:319-55.

Schlesinger, M.J., Ashbuner, M., Tissieres, A. (1982). "Heat shock from bacteria to man". Cold Spring Harbour Laboratory, New York.

Scholander, P.F., Flagg, W., Walters, V., Irving, L. (1953). Climatic adaptation in arctic and tropical poikilotherms. Physiol. Zool., 26:67-92.

Scholander, P.F., van Dam, L., Kanwisher, J.W., Hammel, H.T., Gordon, M.S. (1957). Supercooling and osmoregulation in arctic fish. J. Cell. Comp. Physiol., 49:5-24.

Sclater, J.G., Fisher, R.L. (1974). Evolution of the east central Indian Ocean with emphasis on the tectonic setting of the Ninety East Ridge. Geol. Soc. Am. Bull., 85:683-702.

Shackleton, N.J., Kennett, J.P. (1975). Paleotemperature of the Cenozoic and the initiation of Antarctic glaciation: oxygen and carbon isotope analyses in DSDP sites 277,279, and 281. Init. Repts. Deep Sea Drill. Proj., 29: 743-56.

Shann, E.W. (1920). The comparative myology of the shoulder girdle and pectoral fin of fishes. Trans. R. Soc. Edinburgh,52:531-570.

Shriver, J.W. (1984). Energy transduction in myosin. Trends Biochem. Sci., 9:322-327.

Sidell, B.D. (1983). Cellular acclimatisation to environmental change by quantitative alterations in enzymes and organelles. In: "Cellular Acclimatization to Environmental Change", Cossins, A.R., and Sheterline, P. (eds.), Soc. exp. Biol. Seminar Ser., 17:103-120. Cambridge University Press, Cambridge, New York.

Sinensky, M. (1974). Homeoviscous adaptation - a homeostatic process that regulates the viscosity of membrane lipids in Escerichia coli. Proc. Natl. Acad. Sci., 71:522-525.

Smialowska, E., Kilarski, W. (1981). Histological analysis of fibres in myotomes of antarctic fish (Admiralty Bay, King George Island, South Shetland Islands). I. Comparative analysis of muscle fibre size. Pol. Polar Res., 2:109-129.

Smith, M.A.K., Haschemeyer, A.E.V. (1980). Protein metabolism and cold adaptation in antarctic fishes. Physiol. Zool., 53:373-382.

Smith-Woodward, A. (1908). On fossil remains from Snow Hill and Seymour Islands. Wiss. Erg. Schwed. Sudpol. Exp. 1901-1903 3(4):1-37.

Snedecor, G.W., Cochran, W.G. (1972). "Statistical Methods". 6th. ed. Iowa State University Press, Iowa.

Somero, G.N. (1969). Enzymic mechanisms of temperature compensation: immediate and evolutionary effects of temperature on enzymes of aquatic poikilotherms. Amer. Naturalist, 103:517-530.

Somero, G.N. (1978). Temperature adaptation of enzymes. Biological optimization through structure-function compromises. A. Rev. Ecol. Syst., 9:1-29.

Somero, G.N. (1983). Environmental adaptation of proteins: strategies for the conservation of critical functional and structural traits. Comp. Biochem. Physiol., 76A:621-633.

Somero, G.N., DeVries, A.L. (1967). Temperature tolerance of some antarctic fishes. Science, 156:257-258.

Somero, G.N.and Hochachka, P.W. (1968). The effect of temperature on catalytic and regulatory functions of pyruvate kinases of the rainbow trout and the antarctic fish Trematomus bernacchii. Biochem. J., 110:395-400.

Stanfield, P.R. (1972). Electrical properties of white and red muscle fibres of the elasmobranch fish (Scyliorhinus canicula). J. Physiol., 222:161-186.

Starks, E.C. (1930). The primary shoulder of the bony fishes. Stanford Univ. Publ. Ser. Biol. Sci., 6:147-239.

Steen, J.B., Berg, T. (1966). The gills of two species of haemoglobin-free species compared to those of other teleosts with a note on severe anaemia in an eel. Comp. Biochem. Physiol., 18:517-526.

Stevens, E.D. (1972) Some aspects of gas exchange in tuna. J. Exp. Biol., 56:809-823.

Stevens, E.D. (1988). Effect of pH and stimulus phase on work done by isolated frog sartorius muscle during cyclical contraction. J. Musc. Res. Cell Motility, 9:329-333.

Stevens, E.D., Randall, D.J. (1967). Changes in blood pressure, heart rate and breathing rate during moderate swimming activity in rainbow trout. J. Exp. Biol., 46:307-315.

Sverdrup, H.U., Johnson, M.W., Fleming, R.H. (1942). "The Oceans". Prentice-Hall, New York.

Takashi, R., Putnam, S. (1979). A fluorimetric method for continuously assaying ATPase: application for small specimens of glycerol-extracted muscle fibers. Analyt. Biochem., 92:375-382.

Targett, T.E. (1981). Trophic ecology and structure of coastal antarctic fish communities. Mar. Ecol. Prog. Ser., 4:243-263.

Taylor, D.J., Matthews, P.M., Radda, G.K. (1986). Myoglobin-dependent oxidative metabolism in the hypoxic rat heart. Respir. Physiol., 63:275-283.

Tetens, V., Wells, R.M.G., DeVries, A.L. (1984). Antarctic fish blood: respiratory properties and the effects of thermal acclimation. J. Exp. Biol., 109:265-279.

Tota, B. (1978). Functional cardiac morphology and biochemistry in Atlantic bluefin tuna. In: "The Physiological Ecology of Tunas", Sharp, G.D. and Dizon, A.E. (eds.), 89-112. Academic Press, New York.

Tota, B. (1983). Vascular and metabolic zonation in the ventricular myocardium of mammals and fishes. Comp. Biochem. Physiol., 76A:423-437.

Tota, B, Cimini, V., Salvatore, G., Zummo, G. (1983). Comparative study of the arterial and lacunary systems of the ventricular myocardium of elasmobranch and teleost fishes. Am. J. Anat., 167:15-32.

Travis, D.F., Travis, A.(1972). Ultrastructural changes in the left ventricular rat myocardial cells with age. J. Ultrastruct. Res., 39:124-148.

Twelves, E.L. (1972). Blood volume of two antarctic fishes. Br. Antarct. Surv. Bull., 31:85-92.

Tyler, S., Sidell, B.D. (1984). Changes in mitochondrial distribution and diffusion distances in muscle of goldfish upon acclimation to warm and cold temperatures. J. Exp. Zool., 232:1-9.

Veevers, J.J., McElhinny, M.W. (1976). The separation of Australia from other continents. Earth Sci. Rev., 12:139-159.

Walesby, N.J. and Johnston, I.A. (1980). Fibre types in the locomotory muscles of an antarctic teleost, Notothenia rossii. A histochemical, ultrastructural and biochemical study. Cell. Tissue Res., 208:143-164.

Walesby, N.J., Nicol, C.J.M. and Johnston, I.A. (1982). Metabolic differentiation of muscle fibres from a haemoglobinless (Champsocephalus gunnari Lonnberg) and a red blooded (Notothenia rossii Fischer) antarctic fish. Br. Ant. Surv. Bull., 51: 201-214.

Walvig, F. (1960). The integument of the icefish Chaenocephalus aceratus (Loennberg). Nytt Magasin for Zoologi, 9:31-36.

Webb, P.W. (1971). The swimming energetics of trout. I. Thrust and power output at cruising speeds. J. Exp. Biol., 55:489-520.

Webb, P.W. (1975a). Efficiency of pectoral-fin propulsion of cymatogaster aggregata. In: "Swimming and flying in nature", 2, Proc. second half of the Symp. on swimming and flying in nature, Wu, T.Y.T., Brozaw, C.J., Brennen, C. (eds.), 573-584. Plenum Press, N.Y., London.

Webb, P.W. (1975b). Hydrodynamics and energetics of fish propulsion. Bull. Fish Res. Board of Canada., 190:1-158.

Wegener, A. (1912). Die Entstehung der Kontinente. Petermanns Mittelungen 1912, 185-195.

Wegener, A. (1929). "The origin of continents and oceans". Translated by John Biram (1966) from the 6th. (1929) German edition. Dover, New York.

Weibel, E.R. (1985). Design and performance of muscular systems: an overview. J. Exp. Biol., 115:405-412.

Wells, R.M.G. (1987). Respiration of antarctic fishes from McMurdo Sound. Comp. Biochem. Physiol., 88A:417-424.

Wheeler, A. (1978). "Key to the fishes of Northern Europe". Warne, London.

Winterbottom, R. (1974). A descriptive synonymy of the striated muscles of the teleostei.Proc. Acad. Nat. Sci. Philadelphia, 125:225-317.

Wohlschlag, D.E. (1960). Metabolism of an antarctic fish and the phenomenon of cold adaptation. Ecology, 41:287-292.

Wohlschlag, D.E. (1963). An antarctic fish with unusually low metabolism. Ecology 44:557-564.