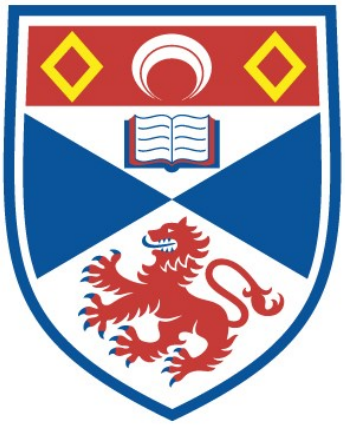
ASPECTS OF GROWTH AND METABOLISM IN THE MUSCULATURE OF ANTARCTIC FISHES : WITH PARTICULAR REFERENCE TO 'NOTOTHENIA NEGLECTA' NYBELIN

Neil Antony Fitch

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



1986

Aspects of growth and metabolism in the musculature of Antarctic fishes, with particular reference to Notothenia neglecta Nybelin.

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

by

Neil Antony Fitch

Department of Physiology

University of St.Andrews

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

CONTENTS

Declaration

I hereby declare that the research reported in this thesis was carried out by me and that the thesis is my own composition. No part of this work has been previously submitted for a higher degree.

The research was conducted in the Department of Physiology, United College of St.Salvatore and St.Leonard, University of St.Andrews, and on the British Antarctic Survey's biological station, Signy Island, Antarctica, under the direction of Dr. I.A.Johnston (St.Andrews) and M.G.White (B.A.S.).

Certificate

I hereby certify that Neil A. Fitch has fulfilled the conditions of Resolution of the University Court, No.1, 1967, and the Regulations, and that he is qualified to submit the accompanying thesis for the degree of Doctor of Philosophy.

ABSTRACT

1. Aspects of the physiology and biochemistry of Antarctic fish muscle have been examined, principally for the notothenid, Notothenia neglecta Nybelin.

2. Sampling was most successful during the late summer/autumn. The trammel net was the best method for catching fish above 200mm standard length. Smaller fish were best caught by SCUBA divers using hand-nets.

3. The condition factor for fish caught was highest during the summer (high food availability) and remained low during the winter months. Analysis of trunk muscle constituents (water, protein, lipid) indicated no evidence for marked depletion at any time of the year. Seasonal variation in relative gonad size and relative liver size were observed.

4. Metabolically the pectoral abductor muscle of N.neglecta had higher activities of anaerobic and aerobic enzymes than either the red or white trunk muscles. This is in line with the fact that this species normally swims using enlarged pectoral fins, while sprint swimming in short bursts is carried out by the white trunk muscle.

5. Differences in the metabolic profiles of the trunk muscles of 3 Antarctic species (N.neglecta, Notothenia gibberifrons and Trematomus newnesi) are related to their different lifestyles, particularily feeding behaviours.

6. Muscle growth in N.neglecta is probably both by hypertrophy, and recruitment of myosatellite cells. Both the fibres and capillaries in Antarctic species are much

larger in cross-sectional area than for temperate species.

7. Lactate dehydrogenase isozymes in the trunk and cardiac muscles of N.neglecta are electrophoretically and kinetically similar. This, along with it's high activities of anaerobic enzymes indicates that the cardiac muscle is adapted to utilise fuels anaerobically if necessary.

8. The majority of physiological and cellular adaptations can be explained on the basis of physiological/ecological constraints rather than cold temperature per se.

SUMMARY

Chapter 1.

In view of the fact that much of the research carried out for this thesis was under the auspice of the British Antarctic Survey (B.A.S.), a brief historical introduction is presented, outlining the discovery and exploration of the Antarctic, the initiation of scientific research, and the events leading up to a permanent British presence in Antarctica. This is followed by a short review of the characteristics of the South Orkney Islands, and Signy Island in particular, including a summary of the ichthyological research carried out at Signy up to 1980. Finally the physiology and biochemistry of fish muscle is reviewed, with a section on the particular adaptations found in the physiology and biochemistry of Antarctic fish species.

Chapter 2.

In this country specimens for research can be obtained at the expense of a phone call with no particular effort required by the researcher. In the Antarctic this is not the case, and no small amount of time and effort is spent in catching the specimens. The main sampling procedures are outlined, with notes on the different techniques required in summer and winter. Trammel nets proved to be the most effective method, both in terms of numbers of fish caught (about 7 fish per net on average) and the amount of time and effort required to deploy them. Long lines, baited traps and dip-nets were the most ineffective. The dominant

species overall was Notothenia neglecta, making up over 72% of the total catch. Most of the remainder was made up of Notothenia gibberifrons, Notothenia rossii and the Icefish Chaenocephalus aceratus. In terms of distribution N.neglecta and Trematomus newnesi were dominant nearshore, while further offshore C.aceratus and N.gibberifrons predominate.

Most fish undergo a natural seasonal starvation during the winter months. The muscle chemistry of N.neglecta was monitored throughout the year, and evidence is presented to show that the seasonal starvation in N.neglecta is not as severe as that found in temperate species. There is no evidence of a protein-water or lipid-water relationship in the muscle. Water content remains fairly constant throughout the year, while protein and lipid are high in the summer and low in winter. The main alteration in the condition of N.neglecta is associated with the spawning period. The relative gonad size (RGS) increases steadily during the spring and summer, while the condition factor (CF) remains steady. During May and June when spawning occurs, there is a dramatic fall both in the RGS (particularily in females) and the CF. During the winter months, RGS and CF remain low relative to the rest of the year, until the cycle starts again in the spring. The relative liver size also shows a cyclical variation, but this is not as pronounced as the RGS cycle. The feeding strategies of Antarctic fish are discussed; N.neglecta pursues different feeding habits during the winter months to maintain a constant supply of food, and thus avoids depletion of reserves in the trunk muscles and the liver.

Chapter 3.

Some key enzymes of energy metabolism were measured in the dominant nearshore species N.neglecta, a labriform swimming benthic species. The capacity for aerobic metabolism and utilisation of glucose and lipid was assessed by monitoring activities of hexokinase, cytochrome oxidase, malate dehydrogenase and 3-hydroxyacylCoA dehydrogenase. An assessment of maximum anaerobic capacity was achieved by measuring maximum activities of pyruvate kinase, phosphofructokinase and lactate dehydrogenase.

Enzymes were measured in the red and white trunk muscles, the red pectoral abductor muscle and cardiac muscle of mature N.neglecta. In terms of capacity for oxidative metabolism the ranking of the muscles was cardiac > red trunk >= pectoral > white trunk muscle. The ranking was not so clear cut for the anaerobic enzymes, although the cardiac muscle had the highest activities with the other three muscle types being approximately equal. The results are discussed with reference to the different functions of each muscle type.

The same enzymes were measured in the red and white trunk muscles of N.neglecta of increasing size (age). As fish size increases there appears to be a reduced capacity for oxidative metabolism and glucose utilisation. The latter is partially offset by an increased ability to use lipids as a fuel source, particularily in the red muscle. There is also a general reduction in activities of enzymes associated with anaerobic glycogenolysis. The results are discussed with reference to findings in pelagic species of

increasing size. Evidence is presented to show that size per se is not totally responsible for the changes observed. Other factors such as the change in lifestyle of larger (older) fish (they have different feeding strategies than smaller fish) may be of importance.

The same enzymes were also measured in three species of labriform swimming benthic species which inhabit different depths. Differences are most pronounced when comparing the shallow-living T.newnesi (0-20m) with the deeper-living N.gibberifrons (25-200m); N.gibberifrons has the lowest activities of enzymes associated with aerobic metabolism, but the highest activities of glycogenolytic enzymes. Evidence is provided to show that these inter-specific differences in enzyme activity are not due to depth of habitat (or size differences), but arise through the use of different feeding strategies.

Chapter 4.

Analysis of the muscle morphometry and capillarisation of the slow muscle of N.neglecta was carried out using stereological techniques. The results were compared with those for the haemoglobin-less icefish C.aceratus. An assessment of the changes in slow muscle morphometry and capillarisation with growth was carried out for N.neglecta.

It is immediately apparent that the slow fibre cross-sectional areas for Antarctic fish are much larger than those found in temperate species. Mean values were 6183 μm^2 for N.neglecta and 2663 μm^2 for C.aceratus. The reason for such large fibres is unknown, but may be related to energy-saving mechanisms; it takes less energy to

maintain electrochemical gradients across a small number of large fibres than across a large number of smaller fibres. The pattern of growth exhibited by the muscle fibres in N.neglecta is consistent with the theory of recruitment of myosatellite cells initially, followed by hypertrophy during later stages.

Associated with the large fibres are large bore capillaries, 3-4 times larger in cross-sectional area than capillaries from temperate species. Large bore capillaries help to maintain a low peripheral resistance as well as increasing the velocity of blood within the capillary. The latter provides an effective capillary-tissue oxygen gradient along the entire length of the capillary and a relatively high venous reserve.

Mitochondrial and myofibrillar volume densities were inversely correlated with size for N.neglecta, but there was no correlation between the suppliers of oxygen (the capillaries) and the oxygen utilisers (the mitochondria). Between species however, the higher capillary density found in the Icefish C.aceratus (almost twice that of N.neglecta) was paralleled by a higher mitochondrial volume density.

The results are discussed in terms of the adaptation of Antarctic species to the reduced oxygen carrying capacity of their blood; this condition reaches an extreme in members of the Icefish family, which have no haemoglobin or myoglobin, and no functional erythrocytes.

Chapter 5.

The LDH isozyme pattern was examined in the trunk and cardiac muscles of N.neglecta. The heart and muscle

isozymes were found to be elctrophoretically and kinetically similar. This finding is also seen in some flatfish species and is associated with the need to meet the energy requirements of the heart under conditions of reduced oxygen availability.

The effect of temperature on the Michaelis constant (Km) of LDH for pyruvate was monitored for the red and white trunk muscles and cardiac muscle of N.neglecta. The pH of the buffer was allowed to vary with temperature. At a temperature range experienced normally by N.neglecta, Km values were closely conserved, but above $4^{o}C$ rose sharply. The rise was less steep for white muscle LDH, perhaps because of the better buffering capacity of white muscle. The results are discussed in terms of the need to maintain essential regulatory responses over a range of microenvironmental conditions, and the fact that cardiac muscle of N.neglecta has a greater reliance on anaerobic provision of energy than in normal temperate species.

Chapter 6.

The results of all the work carried out for the thesis are discussed with reference to the adaptations of Antarctic species to overcome the problems of living at cold temperatures. Suggestions are made for future work that may help to elucidate the phenomenon of cold adaptation.

CHAPTER 1. General Introduction.

1.1.1. Antarctica - historical

The presence of a large land mass occupying the polar regions of the Southern hemisphere was first mooted by the ancient Greek philosophers, who called this land mass 'Antarktikos' ('opposite the bear'); they regarded the presence of Antarctica a necessity to balance out the land masses of the Northern hemisphere thus preserving the natural order of things. Polynesian records indicated voyages to the far south in AD 650 , while Phoenician mariners were recorded in the Southern hemisphere as far back as 1000 BC (although both groups did not necessarily reach Antarctica proper).

From the early 1500's, the presence of Terra Australis Incognita, rich in resources and suitable for human settlement, began to appear on maps, but it fell to Capt. James Cook, R.N. to prove the latter false. Cook's first voyage (1768 -1771) taking in Australia, New Zealand and Cape Horn proved that if Terra Incognita existed, it must all lay below 40^{o}S. This set the scene for the second voyage (1772 -1775), the first truly Antarctic voyage. In 1772 Cook set forth with HMS's 'Resolution' and 'Adventure', and circumnavigated the world between 50 and 60^{o}S. He first crossed the Antarctic circle, at 39^{o}E, on January 17th, 1772, and later on, twice more in the Pacific sector. The most southerly point was reached on January 30th, 1774, at

71°10´S, 106°54´W, where ice prevented any further movement to the south. Although the Antarctic continent itself remained undiscovered, Cook roughly charted the north coast of South Georgia, and discovered the South Sandwich Islands. Furthermore he proved that if a southern continent did exist, it must all lie below 60°S, and that it would be a land of snow and ice.

Cook´s reports of seals (and whales) in the vicinity of South Georgia and Cape Horn opened up the way for the American and British sealers, and prompted exploration further south as seal colonies were eradicated on the known islands. It wasn´t until 1820 that the Antarctic peninsula was sighted, by Capt. Edward Bransfield (GB), and the first documented landing occurred in the following year by Capt. John Davis (USA), in the vicinity of Hughes Bay on the Antarctic peninsula.

The first expedition solely concerned with Antarctica was that commanded by Sir James Ross between 1839-1843, in the ice-strengthened ships ´Erebus´ and ´Terror´, in which he discovered the Ross Sea and Ross Ice-shelf, and Mt. Erebus. The first parties to winter were in 1898; Adrian de Gerlache (Belgium) wintered in the Bellingshausen Sea, his ship the ´Belgica´ beset in the ice, while a party under C.Borchgrevink (GB) wintered at Cape Adare , on the continent itself. While most of the early expeditions centered on the Ross sea and it´s environs, the route via Australia/New Zealand being the most well known, one of the few expeditions to concentrate on the Weddell Sea was the Scottish Antarctic Expedition (1902-1904) led by W.S.Bruce in the ´Scotia´. He set up a wintering base on Laurie

Island in the South Orkney Islands in 1902. This base was handed over to the Argentine Meteorological Service in 1904 and has been occupied continuously ever since - the longest running base in Antarctica.

Once the presence of the Antarctic mainland had been established, it was inevitable that the race to the South Pole would begin. In 1909, Sir Ernest Shackleton and his party sledged to 88°23´S, a mere 97 miles from the pole. The goal was finally achieved in December 1911 by Roald Amundsen, only a month ahead of Capt. R.F.Scott´s ill-fated expedition. From the 1920´s onward, men no longer had to rely on their own strength (or that of dogs) as the mechanical age of exploration began. Less than twenty years later men had flown to the pole, in a minute fraction of the time taken by Amunsden and Scott.

Full time British participation in Antarctic research began with ´Operation Tabarin´ (1944-1947) under the auspice of the Royal Navy. Permanent bases were set up at Deception Island and Port Lockroy (Wienke Island), while the bases at Orcadas and Hope Bay were visited. After the end of the war, supervision was handed over to the newly-formed Falkland Islands Dependancies Survey, which in 1962 became the British Antarctic Survey, as it remains to this day. Other nations quickly followed suit in establishing permanent Antarctic stations, particularily Chile and Argentina.

As early as 1841, Mathew Murray of the U.S. Naval Observatory and Hydrographic Office had urged a pooling of international resources by all nations, in a concerted effort to gain a full knowledge of the Antarctic climate,

which he thought (rightly) had a profound effect on the world's climate. His dream became reality in 1957, with the inauguration of the International Geophysical Year (IGY). In an unprecedented show of scientific co-operation, 12 nations established over 60 bases in Antarctica, for continuous meteorological monitoring as well as glaciological, geological and physical experiments. Although IGY was due to end on December 31st,1958, such was it's success that the scientists urged it's continuation. Following from this, discussions of political status, logistics, etc. led to the signing of the Antarctic Treaty on December 1st 1959 (effective from June 21st,1961), whereby all territorial claims were suspended and the Antarctic (defined as all land masses lying below $60^{o}S$) was preserved for scientific research for 30 years, and protected against the ravages of industrial or commercial exploitation. Presently, over a dozen nations maintain permanent bases in Antarctica, and despite political troubles at international level, collaboration between nations in the Antarctic remains as good as ever.

1.1.2. Antarctica - geography and climate

Antarctica is the fifth largest continent, with a land mass of over 14 million km^2. It is overlain by a vast ice sheet, with an average thickness of 2100m,(containing over 90% of the world's ice), giving the Antarctic the highest elevation of any continent. The continent is split into East and West by the Trans-Antarctic mountains, and geological evidence seems to suggest that in ancient times, East and West

Antarctica were separate entities. The climate of the actual continent is considered 'dry'; the annual snowfall is only equivalent to about ten inches of rain, precipitation similar to that found over many hot deserts. The 'dryness' is accentuated by the low water vapour pressure, being only about 10% of that in tropical latitudes. In summer, the mean temperature is about $0^{o}C$ at the coast and -20 to $-30^{o}C$ inland, while in the winter, mean coastal temperatures vary between -20 and $-30^{o}C$, and inland the range is -40 to $-70^{o}C$; the lowest recorded temperature in Antarctica (and thus the world) was $-89^{o}C$ at Russia's 'Vostok' base.

The continent, in sharp contrast to the abundant oceanic life, is devoid of familiar vegetation and is virtually lifeless. The vegetation of lichens, bryophytes and algae occurs sparsely on a few exposed rocks, and rarely reaches above 5cm from ground level. The freshwater fauna is microscopic and there is no natural large land fauna - birds and seals are marine migrants who spend most of their lives at sea. The dominant land organisms are arthropods, mites and springtails.

1.1.3. Antarctica - oceanography

The Southern ocean covers an area of over 19 million km^2, exclusive of the ice shelves, to an average depth of 3700m. The northern limit of the Southern ocean is taken as the Antarctic Convergence, where cold Antarctic surface water sinks below the warmer, less dense south flowing sub-Antarctic waters. The position of the convergence

alters very little from year to year, and there is no seasonal fluctuation. It's presence is located by the sudden temperature change across it ; usually 1-3^{o}C in the winter, and 4-6^{o}C in the summer (King,1975). Topographically, there are three large basins in the Southern ocean, the Atlantic-Indian basin, South Indian basin and the South-East Pacific basin and there is a single marine trench, East of the South Sandwich Islands, which extends for 960km at an average depth of 7770m. As well, there is a submarine ridge, the Scotia ridge (arc) which connects a line through Tierra del Fuego - Falkland Islands - South Georgia - South Sandwich Islands - South Orkney Islands - South Shetland Islands - Antarctic Peninsula.

The main water transport between 40 and 60^{o}S is West to East (West wind drift) but closer to the continent the flow is reversed owing to the prevailing winds, which are Easterly in this region (East wind drift). Drifting matter circulates Eastwards around the continent at about 13km per day, a complete circuit taking up to four years (Deacon,1960). In the Atlantic sector, the Weddell drift imposes itself, with currents broadly following the direction of the prevailing winds (fig. 1.1).

The Antarctic surface water is a well defined low salinity-high oxygen layer, 100-250m thick. It lies over the warmer but more saline deep water layers, while Antarctic bottom water forms along the continental shelf, the Weddell sea being a major source. Antarctic bottom and intermediate layers extend far beyond the Equator in the Atlantic sector, but to a more limited extent in the Pacific and Indian sectors. A zone of divergence occurs between the

inshore Easterly winds and the offshore Westerly winds, on top of which the water transport at the surface has a northward component, which is compensated at depth by a southward flow. This vertical layering promotes nutrient mixing, especially at the Antarctic convergence, and accounts for the high productivity of the Southern ocean. The Antarctic marine environment is therefore a deep oceanic system with a very marked circulation. The light regime is seasonal, with abundant light for photosynthesis in the summer but very low illumination in the winter. Temperatures are consistently low throughout the region, often near the freezing point of seawater; Sea-ice is found all the year round in some areas (e.g. McMurdo Sound) while over most of the Antarctic the extent of the sea-ice varies according to the season. Peak sea-ice coverage is found in September with areas as far north as $55^{o}S$ being affected in the Atlantic sector. With tolerance of, or physiological adaptation to, the low temperatures, habitat conditions are favourable for life in the summer, but are very much less so in the winter, especially in the more Southern areas (Holdgate,1967).

1.2.1. Research in Antarctica - historical

During the 100 years up to 1914, biology (and indeed, all science) was usually a by-product of exploration; ships routes were chosen to enter unexplored areas, and science had to fit in as best it could. Even so, much material was amassed, the earliest biological investigations being recorded on Cook's voyages between 1772 and 1775. After

1918, there was less emphasis on privately sponsored expeditions, with more government interest and a great step forward occurred with the setting up of the Discovery Committee in 1925. This was an important step for two reasons: Firstly, on the Discovery cruises, biology took precedence over everything else and secondly, instead of a single expedition, the Committee maintained one or two research ships (RRS 'Discovery', 'Discovery II' or 'The William Scoresby'), continuously in Antarctic waters between 1926 and 1939. In fact so much material was accumulated by the Discovery cruises, that data is still being worked up today.

After the second World war, the modern phase in British Antarctic research was initiated by the Falkland Islands Dependancies Survey (now the British Antarctic Survey). There was less emphasis on ship-borne research, with the establishment of permanent land bases and research continuing the year round. The spirit of international scientific cooperation fostered by the International Geophysical Year (1957), continues even today, and currently over a dozen nations maintain overwintering bases in the Antarctic. The British Antarctic Survey currently have permanent research stations (fig. 1.2) at Bird Island, South Georgia ($54^{o}S, 38^{o}$ 03'W); Faraday, Argentine Islands ($65^{o}15'S, 64^{o}16'W$); Halley, Coats Land ($75^{o}31'S, 26^{o}56'W$); Rothera, Adelaide Island ($67^{o}34'S, 68^{o}08'W$) and Signy Island, South Orkney Islands ($60^{o}43'S, 45^{o}36'W$). The range of sciences studied includes marine , terrestrial and freshwater biology, glaciology, geology, meteorology, and atmospheric physics.

1.2.2. Ichthyological research in Antarctica

The first specimens of Antarctic fish viewed in this country were brought back from the voyages of HMS 'Erebus' and HMS 'Terror', 1839-1843. Four species were collected from the Kerguelen area, and described by J.Richardson (Norman,1938). Following expeditions all added new species to the list, notably Boulenger (1902), Lonnberg (1905) and Regan (1914). Although particular collections tended to be quite extensive for a specific region, they were never considered representative of the Antarctic as a whole. It was Norman (1938) who first collated material from early expeditions together with a large collection from the ships of the Discovery Committee. As a result he was able to divide the Antarctic region into distinct zoogeographical zones, a trend followed by Nybelin (1947) who described several new species and sub-species based on small taxonomic differences between collections of fish from several different zoogeographical zones. In the last 30 years shipborne research has recovered a few new species, but generally Norman's classifications have stood the test of time, with only minor revisions (DeWitt,1966; Andersen & Hureau,1979; Andersen,1984).

Using Norman's scheme, the South Orkney Islands are in the Glacial district (a sub group of the Antarctic zone, the other sub-group being the Kerguelen-Macquarrie district). Over 75% of the fish species in the Glacial district belong to the nototheniiformes, a division of the percoid fish of the order percomorphi (perch-like fish) which form the main

group of spiney-finned teleosts. The percomorph fish of the Antarctic, unlike percomorphs elsewhere, have flexible fin-rays, jugular pelvic fins and a single nostril, instead of two, on each side of the mouth (Marshall,1953). In the Glacial district only four out of the five families of the division nototheniiformes are present; the Nototheniidae, Harpagiferidae, Bathydraconidae and Channichthyidae. In the South Orkney Islands the Nototheniids are the most represented family (Table 1.1) and include the most dominant (in terms of biomass) nearshore species, Notothenia neglecta Nybelin. In deeper waters, N.gibberifrons Lonnberg and the Channichthyid, Chaenocephalus aceratus Lonnberg, become dominant.

Nybelin (1951) suggested several new species based on minor taxonomic variations between fish from different zoogeographical zones. Thus Notothenia rossii was split into N.rossii rossii, which inhabits East Antarctica, and N.rossii marmorata, the Scotia arc species. This separation still holds today, but less substantial is his claim for splitting N.neglecta into N.coriiceps coriiceps from the Kerguelen-Macquarie zone, and N.c.neglecta from the Glacial zone. Everson (1970b) studied over 1400 specimens of N.neglecta from the South Orkneys, as well as specimens from other localities, and specimens of N.c.coriiceps in the British Museum(Natural History), and concludes the distinction between the two not great enough to confer sub-species status on N.c.coriiceps, and more recent studies also take this view (Burchett et al,1983). DeWitt revised the genus Notothenia (family Nototheniidae) in 1966, while more recently Andersen and Hureau (1979) and Andersen (1984)

used the anatomy of the caudal skeleton and of the cephalic canals to define 3 genera of the sub-family Nototheniini - Notothenia, Nototheniops and Paranotothenia. Andersen (1984) suggested that N.c.neglecta and N.c.coriiceps were separate subspecies, and they were subsequently classified as N.neglecta and N.coriiceps, the former being the South Orkney species, and this is the currently accepted view.

Most of the initial research into Antarctic fish was confined to systematics, but recently more specialised studies have been undertaken, encompassing respiratory physiology (Wohlschlag,1960;1962;1964; Hemmingsen & Douglas,1972), growth and reproduction (Everson,1969;1970a; Burchett et al,1983), feeding behaviour and diet (Richardson,1975; Permitin & Tarverdieva,1978; Daniels,1982) and muscle physiology and biochemistry (Johnston & Walesby,1977,1979; McArdle & Johnston,1980; Walesby et al,1982; Fitch & Johnston,1983; Fitch et al,1984).

1.3.1. Signy Island - general

Signy Island ($60^{o}43'S, 45^{o}$ 36'W) is one of the smaller islands of the South Orkney Island group, lying on the Scotia ridge some 560km ENE of the northern tip of the Antarctic peninsula (fig. 1.3). It's discovery was as a direct result of the development of the southern sealing industry, and was first discovered by Powell in December 1821, and independantly, by Weddell in January 1822 (Marr,1935). The South Orkney Islands were 'opened up' by the arrival of the Scottish Antarctic Expedition headed by

W.S.Bruce in 1902. He established a land base on Laurie Island in 1903, and the island group has been occupied ever since, the present base on Signy Island being established in 1947. The island is roughly triangular in shape, with a total area of just under 20km^2 and a maximum elevation of only 279m (Tioga Hill). The island is made up of heavily metamorphosed sediments mainly garnetiferous quartz-mica-schists with some marbles and amphibolites (Mathews and Maling,1967). Rock outcrops show typical frost-shattering and glacial erosion features. An ice covered plateau covers approximately one-third of the island descending to sea level as the McLeod glacier to the South and the smaller Orwell glacier to the East (fig. 1.4). Signy is the most de-glaciated member of the South Orkneys, possibly due to it's low elevation and the strong North (easterly) winds which bring warm air whose moisture has already precipitated onto upland regions of adjacent Coronation Is. (maximum elev. 1266m, Mount Nivea), (Marr,1935). Coastal lowland is extensive on the East side but is discontinuous on the West coast; there are numerous fresh-water lakes scattered around the island.

Approximately 150 depressions each year pass from the Pacific to the Atlantic oceans. These either pass through the Drake passage from the Chilean coastal regions, or pass Northwards across the Antarctic peninsula towards the Indian sector of the Southern Ocean. In either case the first land encountered in the Scotia sea is the South Orkney Islands; this results in very changeable and very windy weather.(table 1.2). Equinoctal airflows cause heavy storms in March/April and September/October, but strong winds occur

every month. Holdgate (1964) has classified the South Orkney Islands as part of the Maritime Antarctic, with a fairly oceanic climate. In summer there is at least one month with a mean air temperature above zero at sea level and in winter the mean monthly temperature rarely falls below -10°C. Precipitation in the South Orkney Islands is frequent some falling as rain in the summer. The vegetation of Signy Island is also representative of the Maritime Antarctic; bryophytes, lichens and algae predominate with only two flowering plants present, Deschampsia antarctica Desv. and Colobanthus quitensis Hook (Lewis-Smith,1970). Various invertebrates inhabit the vegetation and screes, including springtails, tardigrades and nematodes.

During the short summer season the bluffs and cliffs all around the island are covered with nesting sea-birds, mainly petrels, while on the snow-free coastal areas three species of penguins breed in the South Orkneys - the Adelie (Pygoscelis adeliae Hombron & Jacquot), the Chinstrap (Pygoscelis antarctica Forster) and the less common Gentoo (Pygoscelis papua Forster). The Weddell seal (Leptonychotes weddelli Lesson) is the only seal commonly breeding on Signy Island, some two to three hundred pups being born each year (Mansfield,1958). Hundreds of juvenile Elephant seals (Mirounga leonina Linn.) mainly males, visit the South Orkneys to moult, with an occasional pup being born (Laws,1981). More recently the Southern Fur-seal (Arctocephalus gazella Peters) is returning in increasingly large numbers (Laws,1981); as yet no breeding colonies have been set up on Signy Island but, although rare, some pups have been born (Kightley & Caldwell,1982).

1.3.2. Signy island - marine research

When the station was first opened in 1947, meteorology was the major science studied, and biology had to compete alongside geology and surveying. The first biological research involved the breeding biology of the elephant seal (Laws,1953,1956a,1956b). Facilities were fairly basic in the early days, and much of the initial work was concerned with descriptive studies. As the base expanded, so the facilities improved, and the intricacy of the research became more involved. The building of a two storey fibre-glass hut, containing greatly improved laboratory (and living) accommodation was a great step forward, and with the advent of scuba-diving at Signy (1962/3) marine research could be extended from the land (seals and penguins) to the ocean. After a general biological survey of Factory cove and Borge bay work extended to the autecology of selected groups e.g. Amphipoda (Thurston,1972), Isopoda (White,1970) and fish (Everson,1968,1969,1970b). In 1970, a through-flow wet laboratory was set up, which greatly assisted marine research. After the general ecological and biological work had been carried out, then more detailed and specialised studies were undertaken.

Everson and Ralph (1968), Holeton (1970) and Twelves(1972) investigated the respiratory physiology of Antarctic fish, with particular emphasis on the haemoglobinless Icefish Chaenocephalus aceratus Lonnberg. Smith (1972a,b,c) studied freeze-resistance, principally in N.neglecta but also in species such as Trematomus

borchgrevinkii Boulenger which live quite comfortably just under the sea ice at temperatures of -1.8°C, while Richardson (1975) studied the diet of several local species.

1.4.1. Characterisation of fish muscle.

A number of fibre types can be identified in fish on the basis of their biochemical, histochemical or ultrastructural characterisitics. Classically fish trunk muscle types were identified by their colour, and classified as red or white accordingly, a system first used by Lorenzini in 1678. More recently workers have classified fish muscles as fast or slow, on the basis of their contractile properties (Johnston,1982a,1983); There is little published data on speeds of contraction of Antarctic fish muscles, however recent results suggest that the contractile properties of Antarctic fish fibres are comparable to those of temperate fish measured at their own environmental temperature (Gleeson et al,1983; Johnston & Harrison,1985). The nomenclature of red and white has been used here.

1.4.1.1. Red trunk muscle.

In most fish, myotomal muscle contains a discrete layer of red muscle usually forming a thin layer around the trunk (Bone,1966), or in the simplest case such as the brook trout (Salvelinus fontinalis Mitchell) red fibres form a thin wedge adjacent to the lateral line canal (Bone,1978; Johnston & Moon,1980a). With low ATP´ase activities and slow speeds of shortening red muscle has also been termed

slow muscle (Barany,1967). The proportion of red muscle in the trunk varies according to the activity of the species; active pelagic species (e.g. anchovy, Engraulis encrasicolus) possess a larger proportion of red muscle (29% in this case) than do sedentary demersal species such as the chimaerid Chimaera monstrosa (<1%).

The red fibres have a well developed capillary supply (Mosse,1978) and high densities of mitochondria, myoglobin (Matsuura & Hashimoto,1954), lipid droplets (George,1962; Bokdawala,1967) and high activities of citric acid cycle enzymes (Bostrom & Johansson,1972; Crabtree & Newsholme,1972) and respiratory chain enzymes (Crabtree & Newsholme,1972; Johnston et al 1977). Lipids are a major source of energy during swimming (Bilinski,1974; Driedzic & Hochachka,1978), with ketone bodies being the most important in elasmobranchs and fatty acids in teleosts (Zammit & Newsholme,1979).

Ultrastructurally red fibres have small diameters (10-50μm) and a mitochondrial volume density that is amongst the highest found in vertebrate striated muscle. Relative to other vertebrate red muscle, fish red muscle has a highly developed sarcoplasmic reticulum (SR) and T-tubule system, and even approaches quantities found in some twitch fibres (Johnston,1980).

Some pelagic fish have internalised red muscle e.g. Skipjack tuna (Katsuwonus pelamis) where an effective heat exchange mechanism maintains an elevated muscle temperature (Carey & Teal,1966). This muscle has a ten-fold increase in mitochondria and a more highly developed capillary supply than superficial red muscle (Bone,1978).

1.4.1.2. White trunk muscle.

The bulk of the myotome is made up of large diameter white fibres. They have a high myofibrillar ATP´ase activity and a fast maximal velocity of contraction (Bone,1978; Johnston,1980). White fibres are characterised by a poor capillary supply (Mosse,1978), although the supply is better developed in the more active pelagic species (Mosse,1979). The white fibres have a low myoglobin content, low lipid content, and on the whole rely on a predominantly glycolytic metabolism (George,1962; Bone,1966; Hamoir et al,1972). Sarcoplasmic reticulum and T-tubules are 2-3 times more numerous in white muscle compared to red, which aids faster relaxation-contraction cycling (Nag,1972; Johnston,1980). In addition, fish white muscle contains high concentrations of parvalbumins, low molecular weight calcium-binding proteins; parvalbumins may account for up to 15% of the soluble protein (Le Peuch et al,1978). Parvalbumins are also thought to play a role in rapid relaxation (Gerday & Gillis,1976; Gerday,1982) of white muscle, by quickly mopping up calcium ions released by the SR and allowing only transient activation of the crossbridges.

1.4.1.3. Innervation and recruitment.

The amount of power required for underwater locomotion is a function of body size and velocity^3 (Webb,1975) and reflects the way that drag forces are acting on the body as it´s size increases. Consequently burst speed swimming requires proportionally more effort than for sustained

swimming, which explains the development of a large muscle mass that can develop power rapidly and essentially independantly of the circulation. The possession of this large mass of muscle reserved solely for high speed swimming does not constitute a serious weight penalty as it would in terrestrial animals, since most fish preserve neutral (or near neutral) buoyancy. The differential power requirements of the red and white muscle are reflected in their respective metabolism; red muscle used for continuous sustained swimming uses aerobic oxidation of lipid and carbohydrate for it's energy supply, whereas the white muscle fibres rely on anaerobic glycogenolysis and glycolysis, and phosphagen-based stores to provide energy for muscle contraction.

All red fibres so far studied in fish are multiply innervated by small diameter fibres ending in 'en grappe' terminals, and are activated by junction potentials (Barets,1961; Bone,1964), although they may also be capable of propagating action potentials (Stanfield,1972). In white fibres two separate types of innervation are found. Elasmobranchs and more primitive fish groups (dipnoans, chondrosteans and holosteans) possess focally innervated white fibres with 'en grappe' terminals (Bone,1964; 1970). However among the majority of teleosts white fibres are multi-terminally and polyneuronally innervated, and can propagate spike action potentials (Bone,1964,1978; Hudson,1969), so that each fibre may recieve innervation from as many as 5 axons from each of 4 spinal nerves (Hudson,1969). Contraction velocities are commonly twice as fast in white fibres as they are in red fibres (Flitney &

Johnston,1979). In some species (e.g. the cottid Myoxocephalus scorpius) stimulation of the spinal nerve can give junction potentials leading to graded local contractions as well as spike potentials resulting in fast twitches (Hudson,1969). It is unclear as to whether both responses occur in vivo.

The recruitment of different fibre types during increasing speed of swimming has been studied in only a few of the 20,000 species of fish. However the results suggest a difference in the pattern of fibre recruitment according to the type of innervation. Focally innervated white fibres seem to be recruited only for burst activity (Bone,1966). For example, the Pacific herring (Clupea pallasii) recruits only red fibres at low swimming speeds that can be maintained for several hours. At speeds in excess of 5 body lengths/sec white fibres are recruited, and fatigue occurs in only a few minutes (Bone et al,1978). For species with multiply innervated white fibres the threshold speed for recruitment is lower (e.g. 2 lengths/sec for carp; Johnston, et al,1977), so that at sustainable swimming speeds both red and white fibres are active. A wide range of aerobic capacities is evident in white fibres from different species, and this appears to reflect the different degrees of involvement of this fibre type in sustained swimming (Johnston & Moon,1980b).

Histochemically, pink fibres have been demonstrated in some species occupying a position between the red and the white fibres. Relatively little work has been carried out with these fibres but in the mirror carp (Carassius carassius) they account for about 10% of the muscle mass

(Johnston et al,1974). They possess an aerobic capacity and miochondrial volume density that is intermediate between red and white fibres (Johnston & Maitland,1980). It is only in the carp that electromyographs have been taken from the pink fibres; the order of recruitment of the fibres during swimming at increasing speeds was red - pink - white.

1.4.1.4. Pectoral muscle.

Little work has been carried out on locomotory muscles other than those of the trunk. Labriform swimming (Breder,1926) whereby swimming is carried out by enlarged fan-shaped pectoral fins that beat synchronously in long wavelengths to form a characteristic sculling action (Webb,1973; Blake,1981) is widely developed amongst Antarctic species (Robilliard & Dayton,1969; DeWitt,1971; Twelves,1972; Walesby et al,1982). The mechanics of pectoral fin locomotion are complex, but it can be calculated that the propulsive efficiency is of the order of 0.2 (Blake,1981). The power stroke occurs as the adductor muscles pull the fins back towards the body. In the recovery stroke, the fin is 'feathered' and the forces acting upon it are only 1/20th of those during the power stroke (Blake,1981). The anatomy of the pectoral musculature has been poorly studied, but appears quite complex since the fin has to be able to rotate about the axis at it's point of attachment to the body, and the top half of the fin moves independantly of the lower half during the fin beat cycle. The characteristic metabolic profile of pectoral fibres in labriform swimmers is that they are

highly aerobic (Kryvi & Totland,1978; Walesby et al,1982), even more so than red myotomal fibres.

1.4.2. Physiological adaptations of Antarctic fish.

A physiological peculiarity of fish from high latitudes is that they have low levels of haemoglobin compared to fish from tropical waters (Tyler,1960; Everson & Ralph,1968). This reaches an extreme in the family Channichthyidae from Antarctic waters which totally lack both haemoglobin and myoglobin (Ruud,1954; Hureau,1966; Walesby et al,1982). The presence of blood pigments enhances the rate of diffusion of oxygen, even at low PO2's; this enhancement is therefore less effective in red-blooded Antarctic species such as N.neglecta, and will be totally absent in the channichthyids. Various respiratory and circulatory adaptations have therefore evolved to overcome this shortfall in oxygen supply.

1.4.2.1. Circulatory adjustments.

To overcome the increased blood viscosity caused by the constantly cold waters of the Southern ocean, Antarctic fish have a reduced number of circulating erythrocytes (Everson & Ralph,1968), which reaches an extreme in the channichthyids which have no functional erythrocytes, although they may be present in very small quantities (<40,000/ml; Martsinkevich,1958; Hureau,1966). The reduction in viscosity of the blood is therefore associated with a reduced oxgen carrying capacity; in the Icefish C.aceratus the oxygen carrying capacity of the blood is only 0.67 vols%

(Ruud,1954) compared to a figure of 12-17 vols% for temperate teleosts (Prosser,1973).

Due to the higher solubility of gases at colder temperatures, the waters of the Southern ocean contain almost twice as much dissolved oxygen as do temperate waters. Despite this, little anatomical modification has occurrred to the gills of Antarctic fish and they have a surface area available for exchange comparable to other fish species (Steen & Berg,1966; Hughes,1970). However the extraction efficiency of the gills seems high compared to temperate species (Steen & Berg,1966). Some of the most obvious changes occur in the circulation. The blood volume accounts for up to 10% of the body weight in C.aceratus (Hemmingsen et al,1972) and is higher in red-blooded Antarctic fish (Twelves,1972) than in temperate teleosts (Satchell,1971). This large blood volume is circulated by a large heart; in red-blooded Antarctic fish the relative heart size is similar to that of other teleosts, but that of channichthyids is larger, approaching values reported for tuna (among the most active of fish species) and small mammals (Johnston et al,1983). Antarctic fish have a low heart rate, and operate at low peripheral resistance with high stroke volumes (Holeton,1970; Hemmingsen & Douglas,1977). This low peripheral resistance is brought about by the presence of large bore capillaries rather than by increased capillary density (Fitch & Johnston,1983; Fitch et al,1984). The oxygen gradient from blood to the tissues is high in Antarctic fish, which maintain a high venous reserve compared to temperate species (Holeton,1970).

1.4.2.2. Respiratory adaptation.

The oxygen consumption of C.aceratus is comparable to that of red-blooded Antarctic species (Holeton,1970), but the oxygen consumption of Antarctic fish in general is low (Wohlschlag,1962; Holeton,1970) compared to temperate species, although at the lower end of their range (Prosser,1973). Low blood lactate levels at rest (2-6 mg/100ml) and after anoxic stress (24-36 mg/100ml) indicate no unusually high anaerobic metabolism (Hemmingsen & Douglas,1970; 1972). However, red-blooded Antarctic fish are better able to withstand hypoxia than icefish (Holeton,1970), with critical PO2's at 16-20mm Hg and 50mm Hg respectively. The aerobic scope for activity has been measured in only one instance, some twenty-five years ago. In the absence of corroborative data, the scope for activity (x5-6) is low compared to salmonids (x10-12), but is comparable to other less active temperate species (Wohlschlag,1960; Brett & Groves,1979).

1.4.2.3. Metabolic adjustments.

Without some form of compensation the effect of lowered cell temperature will be to reduce the rates of reaction of the various cell processes. To overcome this rate-depressing effect of low temperature an organism can respond in one (or more) of several ways. The microenvironment surrounding the enzyme(s) can be altered to maintain optimal enzyme activity, or more or better (i.e. functionally superior at the new temperature) enzyme can be produced.

The speeds of contraction of slow fibres of N.neglecta

are of similar magnitude to temperate species when measured at their respective environmental temperatures (Gleeson et al,1983; Johnston & Harrison,1985). In the case of Antarctic species, packing the cells with more protein (myofibrils) will not affect the speed of contraction, only the tension developed. Therefore the fish must make better protein, with or without changes in the microenvironment. In a detailed study of $Mg^{2+}Ca^{2+}$ -ATP´ases from the white trunk muscle of a number of species adapted to water temperatures from less than $0^{o}C$ to $37^{o}C$, the ATP´ases from the cold-adapted fish had much greater activity at lower temperatures than did those from warm water species (Johnston, Walesby et al,1977). This is brought about by having a reduced Gibbs free energy of activation (ΔG) as well as lowered free energies of entropy (ΔH) and enthalpy (ΔS). This increased activity of enzymes from cold-adapted fish is thought to arise from a changed tertiary structure of the molecule brought about by alteration in the primary structure (amino acid sequence). Associated with this are alterations in thermal stability; ATP´ases from Antarctic fish are more sensitive to thermal denaturation than those from warm water fish (Johnston & Walesby,1977; Cossins et al,1981). Other enzymes in Antarctic fish which have been shown to be more heat labile are aldolase and serum transferrin (Komatsu & Feeney,1970; Komatsu et al,1970).

The site of modification in evolutionary adapted fish is the catalytic part of the multi-protein actomyosin complex; the regulatory sub-units (tropomyosin and the troponins) are untouched (Johnston & Walesby,1979). In laboratory or seasonally acclimated fish modification of the troponins

occurs (Johnston,1979); because the turnover of troponins is much faster than that of myosin, modifications of these allow a much more rapid response to temperature variations experienced by the fish.

The physical state of membrane lipids has also been shown to affect the activity of membrane-bound enzymes (Cossins et al,1981). At lower temperatures there is no evidence that polar marine invertebrates store different types or amounts of membrane lipids when compared with temperate species (Clarke,1983). However, little is known of polar fish lipids apart from their utilisation for buoyancy (Eastman & DeVries,1981). It does seem that there is generally an increase in the unsaturation of phospholipid fatty acids within membranes at lower environmental temperatures, and that this is associated with maintaining membrane fluidity (Sinensky,1974). Some membranes, such as those from mitochondria seem more responsive to change than others, e.g. S.R. (Cossins,1981). The importance of the composition of the membrane environment for enzyme activity was shown by Hazel (1972a;b). Succinic dehydrogenase (SDH) isolated from the epaxial muscle of goldfish (C.auratus) acclimated to $5^{o}C$ had 1.3-2.2 times the activity of SDH from goldfish acclimated to $25^{o}C$. The SDH proteins were identical electrophoretically, and lipid extraction of crude SDH preparations reduced the activity by 75-80%. The enzyme activity could be partially restored by addition of purified mitochondrial lipids, and lipids isolated from $5^{o}C$ goldfish produced a higher V_{max} than those from $25^{o}C$ goldfish, regardless of whether the enzyme was purified from 5^{o} or 25^{o} goldfish.

1.4.2.4. Antifreeze proteins.

In some areas of the Antarctic such as McMurdo Sound the water temperature remains constantly at it's freezing point, and this may be as much as 1°C below the freezing point of the body fluids of the fish living there. Some polar fish avoid freezing by staying in deeper water away from any contact with the ice e.g. Rhigophilia dearbornii. A more common way of avoiding freezing in Antarctic (and Arctic) fish is by lowering the freezing point of the body fluids and blood; 40-70% of this is brought about by an increased NaCl content (Smith, 1972a,b,c). The remainder is brought about by the presence of macromolecular antifreezes, which may be either glycoproteins or peptides (DeVries,1969; DeVries et al,1970). The mode of action of these antifreezes appears to be that they bind to very small ice nuclei (through hydrogen-bonding) preventing them growing large enough to be stable (see DeVries,1980).

1.4.3.

The current study was undertaken to focus on seasonal and developmental aspects of the physiology and biochemistry of the trunk muscles of the major inshore species at Signy Island, Notothenia neglecta Nybelin. Various enzymes of energy metabolism were measured in fish of increasing length; the enzymes measured give an indication of maximum metabolic flux through their particular metabolic pathway. Muscle fibre cross-sectional areas and volume densities of the fibrillar components (e.g. mitochondria) and the morphometry of the vascular bed were measured to monitor

changes involved with growth. Seasonal changes in some aspects of gross muscle chemistry were monitored, followed by a four month starvation study to follow the survival strategy of N.neglecta in times of food deprivation. Due to the reduced haemoglobin levels characteristic of Antarctic fish (which reaches an extreme in the haemoglobinless condition of the Channichthyids) it is possible that some adaptation to increased anaerobic metabolism may have occurred. Kinetic parameters of Lactate dehydrogenase (LDH) have been measured in crude homegenates and purified extracts of red and white trunk muscle, and cardiac muscle. The particular isoenzymes of LDH found in N.neglecta have been separated and identified by polyacrylamide gel electrophoresis. This work makes a contribution to the understanding of the field of comparative physiology and the phenomenon of cold adaptation.

NOTOTHENIIDAE*	OCCURRENCE**
Notothenia neglecta	A
Notothenia rossii	C
Notothenia (Gobionotothen) gibberifrons	A
Nototheniops nudifrons	O
Nototheniops larseni	O
Trematomus newnesi	A
Trematomus eulepidotus	R
Pagothenia borchgrevinki	O
Pagothenia hansoni	O
Pagothenia bernacchii	O
HARPAGIFERIDAE	
Harpagifer bispinis	A
Pogonophyrne dolichobranchiata	R
CHANNICHTHYIDAE	
Chaenocephalus aceratus	A
Champsocephalus gunnari	O
BATHYDRACONIDAE	
Parachaenichthys charcoti	O
Prionodraco evansii	R
Cryodraco antarcticus	R

* Species named as in Andersen (1984).

** Based on data from Everson (1970), Richardson (1975), Permitin Tarverdieva (1980) and this study.
A - Abundant; C - Common; O - Occasional; R - Rare.

Table 1.1 List of fish species found at the South Orkney Islands

MINIMUM RECORDED AIR TEMPERATURE	-40.0°C
MAXIMUM RECORDED AIR TEMPERATURE	+19.8°C
MEAN ANNUAL AIR TEMPERATURE	- 3.7°C
MINIMUM SEA SURFACE TEMPERATURE	- 2.0°C (APPROX.)
MAXIMUM SEA SURFACE TEMPERATURE	+ 1.5°C (APPROX.)
MEAN ANNUAL WINDSPEED	13.7 KNOTS
MINIMUM DAY LENGTH	4-5 HOURS
MAXIMUM DAY LENGTH	19-20 HOURS
MINIMUM DURATION OF SEA-ICE	70 DAYS
MAXIMUM DURATION OF SEA-ICE	254 DAYS
MEAN DURATION OF SEA-ICE	148 DAYS

Table 1.2 Climatological information for Signy Island. (Data fr
unpublished research station records).

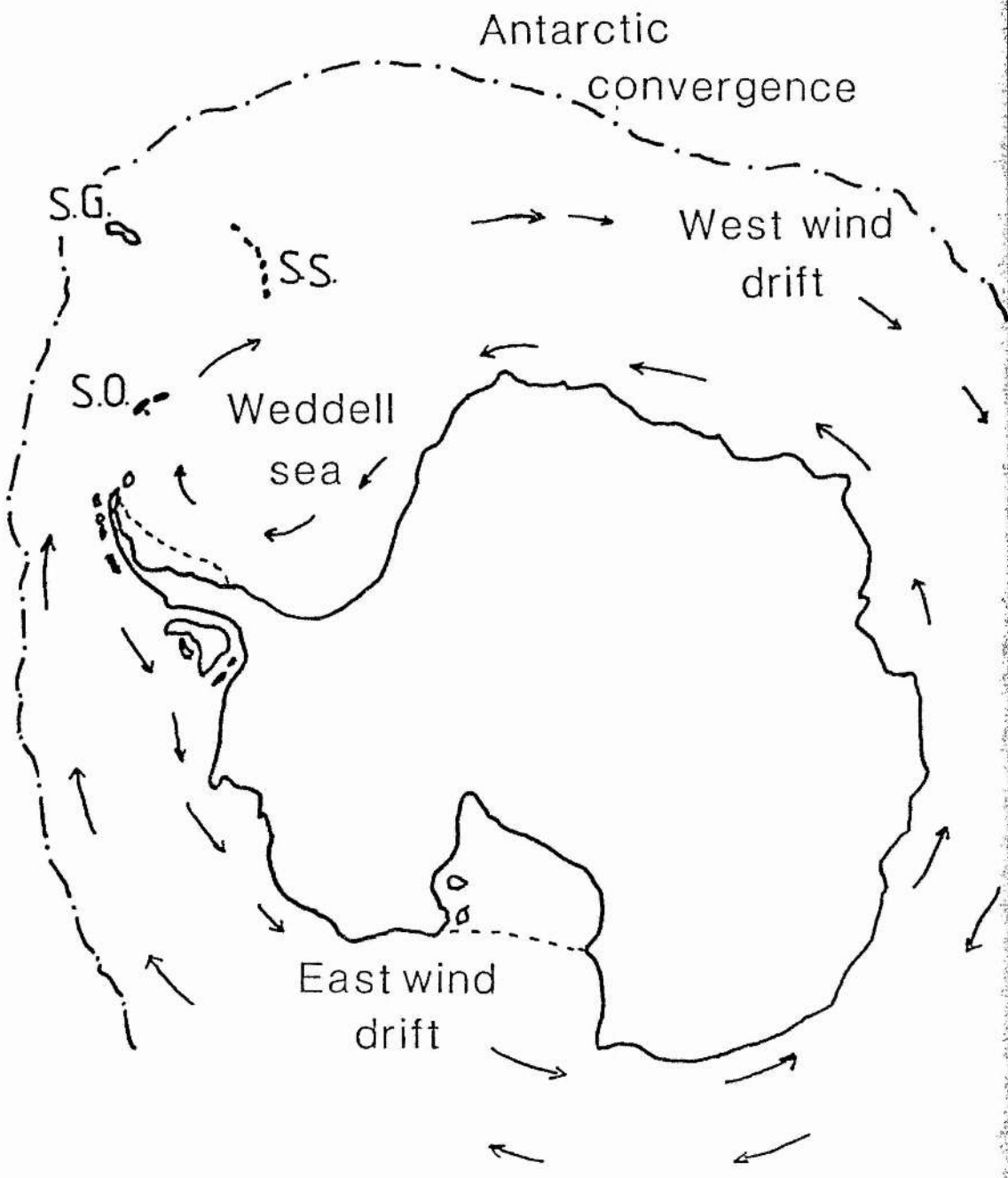


Fig. 1.1 Prevailing ocean currents around Antarctica
(S.G.-South Georgia, S.S.-South Sandwich Islands
S.O.-South Orkney Islands.)

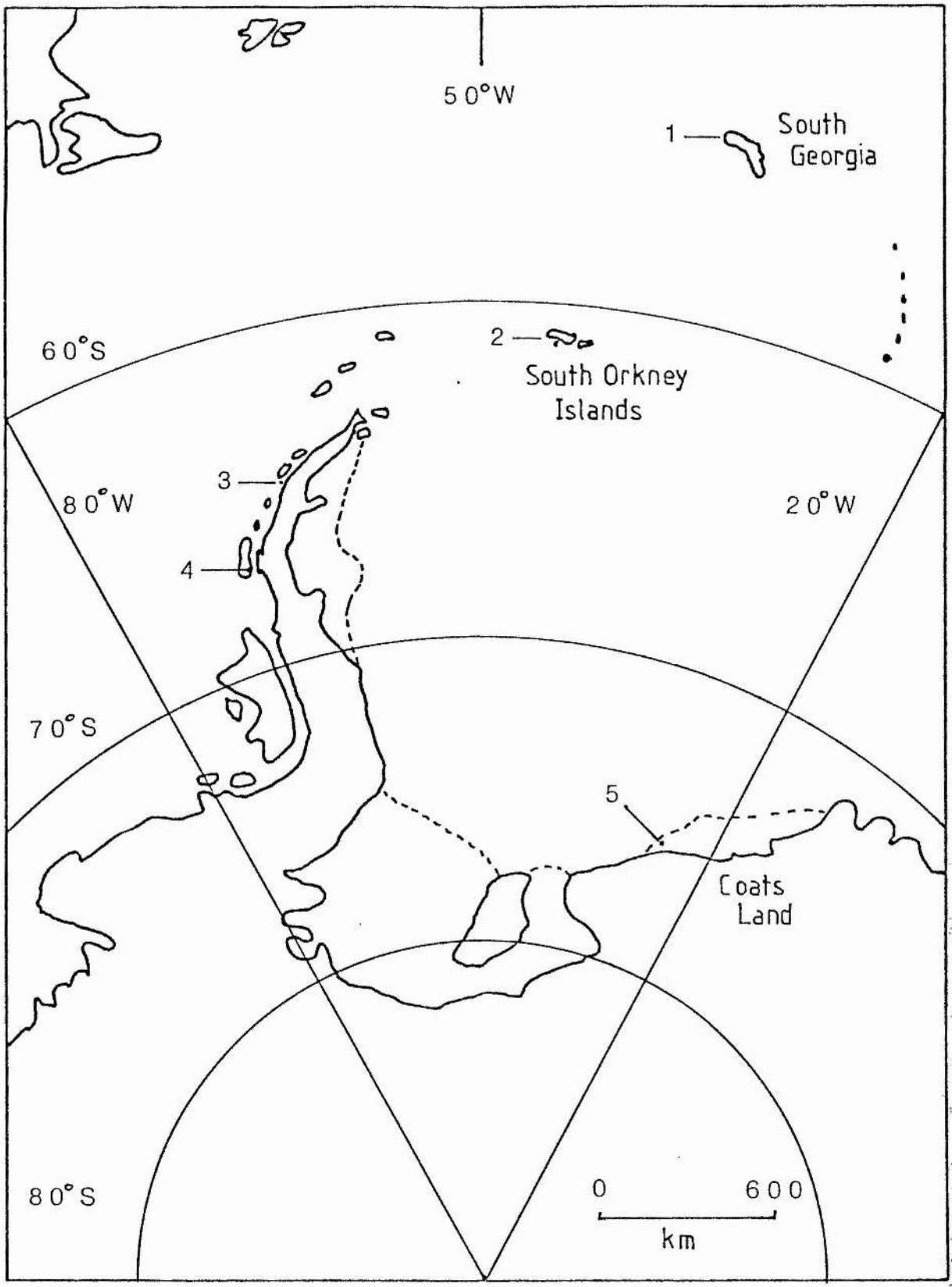


FIG 1.2 BRITISH BASES IN ANTARCTICA 1. BIRD ISLAND

2. SIGNY ISLAND 3. FARADAY 4. ROTHERA 5. HALLEY

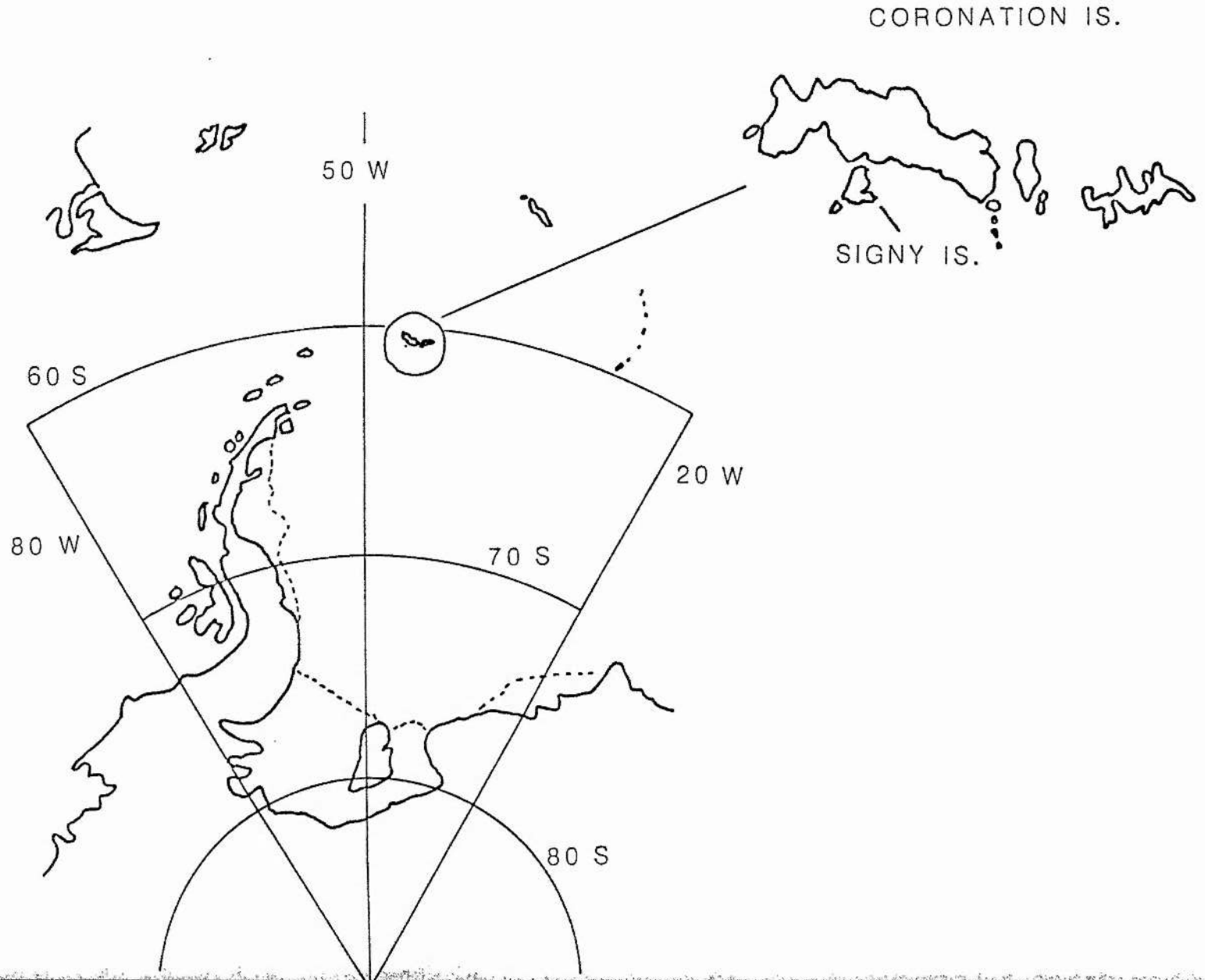


FIG 1.3 LOCATION OF THE SOUTH ORKNEY ISLANDS

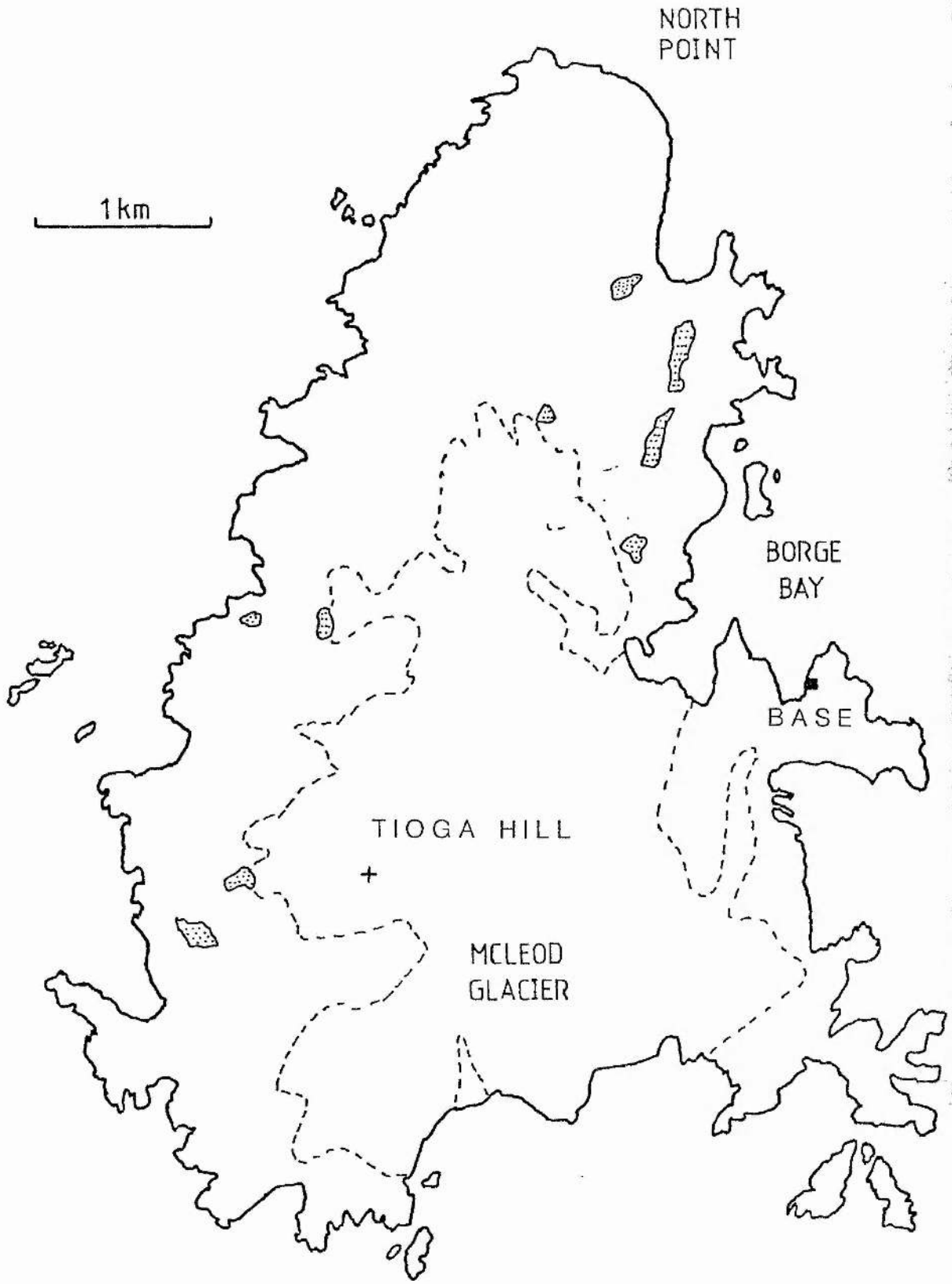


FIG 1.4 SIGNY ISLAND

Freshwater lakes

Limit of permanent icecap

CHAPTER 2. Seasonal variation in sampling and fish condition.

2.1 Introduction

Signy Island has a climate characteristic of the maritime Antarctic (Holdgate,1964), so that in the winter months the mean monthly air temperature rarely falls below $-10^{o}C$. Nonetheless, after the March equinoctal gales have blown themselves out the island group usually becomes surrounded by pack ice, and a cold calm spell leads to the formation of sea-ice. Thus the use of boats for open water fishing is only practical at Signy during the summer months (December to April), when the sea is clear of pack ice or brash ice (a loose collection of small pieces of ice, usually broken off from large floes or bergs). The duration of the pack ice and the water temperatures in Borge Bay are shown in figure 2.1. However, even during the summer months stray icebergs and loose pack ice are always liable to enter Borge bay without warning, with the attendant hazard of carrying off the sampling equipment.

Slight modifications of technique are required during the winter months (June-November) when fast ice is present, but generally fishing can carry on as usual. The period of maximum danger to fishing is during the formation, or break-up of the sea ice. During this time (May/June and November/December) fishing must wait until either the ice is thick enough to travel on, or it has disappeared and boating

is possible again.

The presence of up to 2m accumulation of ice and snow at the surface considerably reduces the amount of light penetrating the water column and this, allied with the reduced occurrence of sunlight in the winter months, is in part responsible for the highly seasonal patterns of primary productivity in the Southern Ocean. The area south of the Antarctic convergence represents a biological environment in which pronounced seasonal cycles occur at all trophic levels. The phytoplankton and small zooplankton represent a large standing crop, concentrated into roughly six months of the year, with a large increase occurring in the austral spring (Foxton,1956; El-Sayed,1968). During the austral winter however, primary productivity is very low and depletion will probably occur to some extent in most species. Many species of fish undergo natural periods of depletion each year of their life, either due to seasonal fluctuations or spawning migration (Love,1970; Shul´man,1974). The response to starvation differs between species but always brain and heart tissue are maintained at the expense of liver and muscle tissue (Love,1970). Commonly white muscle contractile proteins are broken down sparing red muscle fibres, and this has been seen in several species e.g. flounder,Platichthys flesus (Templeman & Andrews,1956), plaice Pleuronectes platessa (Patterson et al,1974; Johnston,1981) and saithe, Gadus virens (Beardall & Johnston,1983). Associated with this muscle breakdown there is generally a decrease in metabolic rate (as measured by oxygen consumption) and locomotory activity (Love,1970; Moon & Johnston,1980; Johnston,1981). The reduced oxygen

consumption is partly due to the lack of requirement for oxygen for food breakdown (Beamish,1964). There is some evidence that those fish with higher red muscle contents (e.g. saithe, 10% red muscle, Greer-Walker & Pull,1975) remain fairly active, even after some months of depletion (Beardall & Johnston,1983). There seems to be a tendency for N.neglecta to reduce it's food intake during spawning and also during the late winter months (Everson,1970a).

A number of methods were utilised for catching fish, and fishing sites of varied depth and habitat were tried (fig. 2.2). Routine biometrical measurements were taken for the majority of fish caught and seasonal trends in condition factor, relative gonad size, relative liver size etc. were monitored. An attempt was made to follow the monthly changes in the gross muscle chemistry of N.neglecta.

2.2 Materials and Methods

2.2.1 Sampling techniques

A. Trammel nets.

All the principle species under investigation were benthic, so fishing by means of trammel net was the preferred method, and it was also found to be the most efficient. During the summer months, nets were laid from the 7m launch 'Serolis'; they were set in the late afternoon and retrieved early the next day, since most inshore species are nocturnal (Burchett,1982). The nets were hauled in by hand, and coiled in large polypropylene buckets filled with sea water then returned to base. In the wet-lab. the fish were untangled from the net and placed in holding tanks,

with a through-flow water system. The water temperature in the wet-lab. tanks was never more than $\pm 1^{o}C$ different from the water temperature in Factory Cove. The time taken from hauling in the nets until sorting the fish on base was 20-30 minutes at the most, depending on which fishing site was used.

Three trammel nets were commonly used. Two nets measured 25m x 2m deep, with a triple mesh, and a polypropylene headline. The outer mesh of the nets was 450mm knot to knot while the inner mesh was 100mm knot to knot. These were anchored with old harpoon heads, and could be used at depths down to 300m. The other trammel net was the same size as the first two, but had polystyrene floats along the headline. This net couldn't be used at depths greater than 100m, because the polystyrene floats became waterlogged, and collapsed the net.

For smaller size fish, a fine mesh trammel net was used, with a mesh size of 350mm (outer diamond) and 35mm (inner). This net was only 8m long, by 2m deep, and was only employed in shallow water sites (A,B or C), where it could be laid from an RFD inflatable rather than having to use the launch. This net proved excellent for catching T.newnesi and small (105-200mm standard length) N.neglecta.

During the winter months a slightly different approach was required to sample through the sea ice. The use of Skidoo's and sledges greatly speeds up the operation, which was good for both fish and fishermen, considering the very cold temperatures prevalent during the winter ! Commonly it was possible to retrieve a net and be back on base within 15 minutes, even from the most distant fishing sites. After

the sea ice had thickened up, two holes about 1m square were cut in the ice using a chainsaw or hand axe, the distance between the holes was just greater than the length of the nets. A diver then positioned a line between the two holes; the line was weighted at it's centre to prevent it lying against the ice and freezing in (fig. 2.3a). The net was attached to one end of the line and pulled into the water from the other hole (fig. 2.3b). When this operation is completed, the net should be stretched out between the holes beneath the ice (fig. 2.3c). Both sides then lowered together until the net was on the sea floor, and the warps were fastened to a marker pole. Fishing during the winter was less weather-dependant because the skidoos can travel in conditions that would keep the boats moored. The nets were retrieved the next day, loaded into bins full of sea water to prevent the specimens freezing and returned to the wet-lab. for processing. The speed of the return journey was such that ice formation does not occur in the bins, and the fish survived in nearly every case. The small trammel net could also be set using this method, except that the access holes were closer together.

B. Long lines.

Long lines were made up of 50m of 6mm rope, hooks being attached to snood clips every 2m along the line. The ends of the line were weighted by old harpoon heads, and the warp and a marker buoy were attached to one end of the long line. The hooks were baited with fish or penguin meat, and the lines were set in much the same manner as the trammel nets. As with the nets, long lines were left out overnight, and

collected early the next day, weather permitting. Fish were removed from the hooks as soon as the line was retrieved, and placed in large bins of sea water for transportation back to the wet-lab.. The long lines could be set in the winter, using the same method as employed for the nets.

C. Baited traps.

A small number of traps were constructed on base using 'Dexion' framing, and small mesh chicken wire. The traps were rectangular and measured 40cm x 40cm x 60cm long. One end of the trap was hinged to allow placing of the bait and removal of the fish, while at the other end a cone-shaped entry point was constructed (155 or 75mm external diameter, 140 or 45mm internal diameter). The bait was generally fish carcasses, with the occasional penguin carcass; the bait attracts amphipods, which in turn attracts the fish. It was easy to use this method in the winter, simply by lowering the trap through a hole in the ice.

D. Agassiz trawl.

Since all the nearshore fish at Signy Island are benthic, bottom trawling was thought to be a useful method. A 2m Agassiz trawl, with a single net of mesh size 90mm was used. Trawling was usually carried out in the late afternoon/early evening, and only in reasonably shallow water - commonly the area between Bare Rock and Outer Island, and between Small Rock and Bare Rock.

E. Dip-netting.

Using a bright light suspended just above the water

surface and a dip-net had proved very successful at Grytviken, South Georgia for catching early juvenile stages, so it was decided to try this method at Signy. The light consisted of a 6V lamp encased in a glass sphere, and run off a 6V battery. Preliminary trials showed that large numbers of amphipods and serolids were attracted to the light, and it was hoped that this would attract the fish. This method was also tried during the winter months.

F. SCUBA-diving.

An alternative method for catching smaller fish was by SCUBA diving and using hand nets. Dives were carried out at night, so that the fish could be dazzled by the torch beam, and then easily netted. Dives were usually carried out at Bare Rock or in Factory Cove in front of the base, so that a quick return to base could be achieved by the divers, especially in winter.

G. Rod and line.

This method was used both in summer and in winter without any difficulty. Bait was usually fish or penguin meat, but even using a bare hook resulted in some success. Fishing was usually carried out only in shallow water, sometimes from the shore. In the winter, any convenient crack or hole in the ice could be used, or a hole was drilled using the 'Jiffy' drill.

2.2.2 Experimental techniques

A. Routine biometry.

The following parameters were generally measured:

Standard length (the length from the tip of the snout to the end of the caudal peduncle in mm); wet weight (g); gonad weight (g); liver weight (g); and sometimes heart weight (g); numbers of fin rays on dorsal, pectoral and anal fins; sex; date of capture/sacrifice. The following parameters were derived:

Condition factor (CF) = [wet wt.(g)/length(cm)^3] * 100

Relative gonad size (RGS) = [gonad wt.(g)/wet wt.(g)] * 100

Relative liver size (RLS) = [liver wt.(g)/wet wt.(g)] * 100

B. Experimental methods.

Water Content.

Samples of appproximately 100mg of muscle were dried to constant weight at 60^{o}C.

Ash content.

Samples of about 100mg weight were dried to constant weight at 60^{o}C and then ashed overnight at 600^{o}C.

Total Protein (soluble).

Muscle samples were homogenised in 5-10 volumes of teleost ringer, pH 7.4 at 4^{o}C, for 3 x 20 seconds using an MSE motorised homogeniser. This was followed by centrifugation at 600G for 10 minutes, the supernatant being retained. Total soluble protein was then assayed in the supernatant using a Boehringer test kit (ser.124281). In this analysis proteins form a coloured complex with cupric ions in alkaline solution, the intensity of the colour being proportional to the amount of protein present.

Total Lipid.

Lipid was extracted from the muscle using the method of Bligh and Dyer (1959) as modified by Hanson and Olley

(1965). The resulting extract in chloroform was assayed using a Boehringer test kit (ser.124303); lipids combine with sulphuric and phosphoric acids and vanillin to form a pink complex, the intensity of which is proportional to the amount of lipid present (Zollner & Kirsch,1962).

2.2.3 Fish.

For routine biometry all the specimens in a catch were analysed. For monthly estimation of gross muscle chemistry both adult and juvenile fish were used. After capture fish were returned to the wet-lab. and placed in through-flow aquaria (water temperature 0-2^{o}C) until feeding normally. This normally took 2-3 days by which time it was assumed that any changes in metabolism, behaviour etc. caused by the stress of capture would have reverted to normal. Food was withheld for 24 hours prior to sacrifice. Fish were sacrificed by stunning followed by spinal transection, then the relevant tissues were dissected out. Red myotomal muscle was taken from the region of the lateral line (fig. 2.4) care being taken to avoid contamination by the underlying white muscle mass. White muscle samples were taken from the deep epaxial muscle about two-thirds of the way along the body from the caudal end (fig. 2.4), while pectoral muscle samples were taken from the anterior deep abductor muscle (fig. 2.4a-c). Muscles I, II and IIb were superficially white with red fibres lying deeper within the muscle. Muscle I must be removed before II and IIb can be reached, and it was from here that pectoral samples were taken. These muscles make up the pectoral abductor muscles. If the fin is folded forward, then III and IV are revealed.

These are both primarily red muscles (Walesby et al,1982) and constitute the pectoral adductor muscles. Liver, heart and gonads were dissected out as necessary.

2.2.4. Statistics.

A pooled or two-sample t-test was used to determine the significance of the results.

2.3. Results

2.3.1. Different sites.

A number of different fishing sites were used (fig. 2.2) at varying depths and with different bottom substrates. The site(s) used on a particular day depended upon the species of fish required, and accessability. For instance, during ice formation or break-out, then it was unsafe to go to the distant sites (D-F), and only the inshore sites were sampled. At Porny Rock (A), weed beds and rock gullies made good hiding places for the smaller fish. Small N.neglecta (up to c.200mm standard length), and T.newnesi were the commonest species caught here. The Bare Rock-Outer Island site,(B), consisted of weed patches with stretches of sand, and reached depths of 10-20m. The 'weed bowl' (C) was a 20m depression in an area that was overall about 10m deep. The bowl was full of weed. The latter sites were very good for catching small to medium large N.neglecta, with usually very few other species represented. The largest sizes of N.neglecta, were caught at the deeper sites (D-F). Owen's Bank (D) was particularily good for catching specimens of

the Icefish, C.aceratus, while the majority of N.gibberifrons were caught off Powell Rock (E) and in the Orwell Bight (F). The few specimens of rarer species were caught in the nets laid in deeper waters, at sites E or F. These included two specimens of Parachaenichthys charcoti, two specimens of Prionodraco evansii and a solitary, even rarer, specimen of Pogonophryne dolichobranchiata. Overall, the commonest nearshore fish captured was N.neglecta, while in deeper waters, C.aceratus and N.gibberifrons become dominant.

2.3.2. Methods.

The trammel net was by far the most effective method, netting most of the larger fish species. Those fish too small to be captured by the large trammel nets were often caught by the smaller gill net. Catch rates in late winter - early spring (Aug-Nov) seemed to be lower than the rest of the year (table 2.1). The average catch per net was just under 7 fish , from a sample of 112 nets. N.neglecta made up over 72% of the catch, with C.aceratus, N.gibberifrons and N.rossii making up most of the remainder. Long lines were the next most effective method, often catching 2-3 fish per line. However, it was difficult to compare directly between the various methods, since the number of 'trials' was not the same for each method. The trammel nets were successful in meeting the requirements for experimental animals, and the other methods were usually tried ad lib to produce samples of a range of sizes and species.

One problem with the long lines was that the fish often swallowed the bait and the hook; removal of the hook led to

internal bleeding, and the fish caught on long lines rarely survived more than a day or two. Consequently specimens caught by this method were not used for enzyme studies, or muscle chemistry studies, but only for processing for electron microscopy. Long lines also proved to be less successful in the winter months, possibly because the fish do not feed so much during this period (Everson,1970a). Despite their reported success (Everson,1970a), our baited traps were not successful at all, either during summer or winter. The reason for this was unclear, and is not a design fault, since the traps were of the same construction as those used by Everson (1970a). The Agassiz trawl was successful in providing specimens of T.newnesi, and also a few post-larval fish (of a variety of species), along with masses of weed and stones from the bottom. One problem encountered was that even at it's lowest speed, 'Serolis' was still too fast for efficient trawling. As a result, the trawl bounces across the bottom, instead of being dragged smoothly, and this may account for it's less than optimum success rate. Like the traps, the dip net and light system proved ineffective, catching no fish at all, either in the summer, or the winter. Inspection by divers showed that some small fish were attracted, but tended not to get sufficiently close to the light source, remaining just above the level of the weed cover. The fish were mostly post-larval N.gibberifrons. SCUBA diving was successful for catching T.newnesi and small N.neglecta. The success of rod and line fishing depended upon fishing over weedy patches; in one instance a catch of 16 fish in half an hour (all N.neglecta) was recorded. If the line was located over

sandy areas, then no fish were usually caught, and after 30 minutes a new site was chosen.

In conclusion, trammel nets were the most successful means of sampling, both in winter and in summer. Fish of all the major species could be caught, down to a standard length of c.200mm. Smaller fish would just slip through the mesh of the large trammel nets but were successfully caught by the smaller mesh trammel net, or by SCUBA diving. The commonest species caught inshore were T.newnesi and juvenile N.neglecta, while further offshore, C.aceratus, N.gibberifrons and adult N.neglecta were predominant. On the whole, sampling was quicker and easier during the winter months, with fewer days lost due to bad weather.

2.3.3. Biometry.

Fish used for enzyme studies, muscle chemistry experiments and electron microscopy were processed for biometrical data. The weight- and length-frequency histograms for N.neglecta (fig. 2.5) show that the methods employed most often caught fish in the ranges 310-349mm standard length and 400-599g wet weight. This is at the size that N.neglecta reaches maturity (Everson,1970a). Monitoring the relative gonad size (RGS) with time is useful in determining the probable spawning period of a particular species. By far the largest changes were seen in female fish, when at spawning the gonads may account for over 16% of the wet weight (fig. 2.6). There was a gradual rise in RGS during the summer months, becoming more rapid as the summer progressed, peaking in May at a value of 17.0. Spawning occurs in May-June, so that by the end of June the

RGS was 5-6 times lower than it was before spawning. In mature males the pre-spawning increase in RGS was not so well defined (fig. 2.6), possibly because the gonads account for a much smaller proportion of the body weight. A rapid build-up occurred in early summer, so that by February the RGS was within 5% of it´s maximum value (7.8). The RGS was maintained until May, and thereafter declined rapidly, just as in female fish. During the winter months the RGS of both females and males remained fairly constant, and only starts to increase above basal levels (1-2 for males, 2-3 for females) with the onset of Spring. The RGS of juvenile fish also showed a peak in May, which was particularily noticeable in females. Even so, peak RGS values for juvenile fish were only about 10% of those for adult fish (fig. 2.6).

The condition factor (CF) of a fish is usually taken as an indication of the ´fitness´ of the fish. A high CF indicates that the fish is growing well, and by implication, food is plentiful. It can be seen that the CF was high, and remained high during the summer months, relative to the winter months (fig. 2.7), with a sharp and significant ($P<0.01$) fall in CF coinciding with the spawning period. Lowest values for the CF were found in November, i.e. the end of winter. There was a rapid rise in CF ($P<0.01$) between December and January as food availability increases.

The relative liver size (RLS) also showed a cyclical variation but this was not as pronounced as that for the RGS (fig. 2.8). The liver size in the female contributes a greater proportion of the wet weight, the RLS for females being about 30% larger than that of males.

2.3.4. Monthly variation in gross muscle chemistry.

Water content.

In adult fish the water content of the red and white muscle remained similar throughout the year, and was only significantly different in January ($P<0.02$) and April ($P<0.001$) (fig. 2.9). Thus the trends during the year were the same for male and female fish. October heralds the end of winter, and at this time the water content of the trunk muscle was at it's highest level. The water content fell and was then maintained at a lower level during the summer months, the overall reduction being significant by April ($P<0.001$). During autumn and up to spawning time the water content, although variable remained fairly low, possibly indicating that the fish are in good nutritional health (backed up by the fact that the CF's are at their highest during this period. Spawning occurs in May-June, and with this and the onset of winter, the water content of the trunk muscle increased steadily until October.

The water content in the trunk muscle of juvenile fish was much more variable (fig. 2.9), but as a rule trends were similar to those found in adult fish. There was no significant difference between the water content of the red and white muscle in juvenile fish except in April/May ($P<0.01$); the highest values were found in late winter, and the lowest values occurred in late summer. Overall the water content of adult muscles was higher than the corresponding muscle in juvenile fish. This was significant between the white muscles for every month except November/December, while it was only significant between the

red muscles during the summer and autumn months (December-June). On the whole, seasonal changes in the water content of the trunk muscles of adult and juvenile fish showed very little variation.

Total protein.

Juvenile values were higher than adult values (fig. 2.10) although the results are very variable. The levels of total protein between red and white muscle were not significantly different during the summer and early winter months, when presumably the fish are in good nutritional status (and positive nitrogen balance). The total protein content was generally at it's lowest during the winter, and highest during the spring or summer, but because of the large variation in standard errors, this trend was only significant for white muscle in juvenile fish ($P<0.05$).

Total lipid.

The seasonal trends for total lipid content of red and white muscles of N.neglecta were identical (fig. 2.11). Maximum levels of lipid in both red and white muscles were found in spring, and levels gradually fell throughout the year. During the winter levels were significantly lower ($P<0.01$) than in the summer in both muscle types.

The same patterns were seen in the trunk muscles of juvenile fish, red muscle lipid content again being 2-5 times higher than that of the white muscle. Again maximum levels of lipid occurred in spring and summer, and values were significantly reduced ($P<0.01$) by the winter. Overall there was no significant difference between similar muscle types in adult and juvenile fish, while differences between red and white muscles were most significant in spring and

summer (fig. 2.11).

2.4. Discussion

2.4.1. Sampling.

Everson (1970a) concluded that N.neglecta was the dominant nearshore species at Signy Island, and that further offshore N.rossii and C.aceratus are more prevalent. In deeper waters (>200m), it seems that N.gibberifrons becomes dominant (Targett,1981). The catch data from the period of this study are in agreement with these findings, although the proportion of N.rossii and C.aceratus in the total catch made up by was smaller in 1980-1982. During the early 1970's the fish stocks north and west of South Georgia were subject to intensive fishing, notably by the Soviet Union and other Eastern bloc countries (Poland and East Germany). This has reduced stocks of potential commercial species (including N.neglecta ,N.rossii and C.aceratus) to below a sustainable level (Everson,1978; Kock,1984). By the late 1970's the focus of attention had shifted to the South Orkneys (Everson,1978) and intensive fishing in this area may have similarily reduced stocks.

The ratio of male to female N.neglecta (1:1.3) was similar to that found by other workers (Burchett et al,1983). The reason for the slight disparity is that the female fish grow more quickly and live longer than males (Wohlschlag,1960; Everson,1970a); the males have a higher metabolic rate and a higher catabolic rate, so they are less efficient at converting food to growth (Wohlschlag,1960;

Everson,1969).

The methods used for sampling now and for the earlier study have changed very little during the intervening 20 years, but the results obtained using those methods show one or two differences. The major innovation has been the use of the trammel net, which although available in the United Kingdom, was not utilised in Antarctica in the 1960's. Trammel nets were first used at Signy Island in 1970 (Twelves,1970) and since then have become widely used both at Signy and South Georgia. They are the most productive method of catching benthic species, and require very little time or effort. The use of long lines and hand lines was not very successful, particularly when compared to the results of earlier workers who reported up to 10 fish per cast (Everson,1970a). The condition of fish caught by long lines was very poor since they tended to swallow the bait whole, thus getting the hook caught in the oesophagus or gut. N.gibberifrons and C.aceratus were most prone to this, and long lines rarely caught any other species. Since the emphasis of the research has become directed towards whole animal experiments or experiments where the condition of the fish is important then the use of long lines has become much less widespread in recent years.

Another method previously giving good results was the baited trap (Everson,1970a), but during the present study they failed to catch a single fish. The traps were of the same construction as used in the past so lack of success couldn't have been a design fault. Obviously correct placement of the trap is required otherwise there may be no fish close enough to be attracted by the bait. N.neglecta

is commonly found in weed patches in areas of sand and rock (Daniels,1982; Burchett et al,1983). At Signy Island traps were located in areas where good catches of fish had previously been obtained by trammel nets (sites B and C particularily). Inspection of the sites by diving showed the habitat to be of the sort favoured by N.neglecta , but still no fish were caught. Traps were also tried at Owen's Bank (site D) to attempt capture of Icefish (C.aceratus), but again without success. After numerous attempts the use of baited traps was abandoned. The traps were often left out overnight the same as for the trammel nets; it may be that traps ought to be left out for much longer periods to become effective, although the bait has usually been eaten after one night.

The post-larval and fingerling stages of N.neglecta are pelagic (Norman,1938; Everson,1968) and a number of methods were utilised to try and capture fish of this age range. Potentially large changes in the metabolic system of this species might occur as they switch from a pelagic to a benthic lifestyle during their second year, so it was considered important to obtain specimens at this stage in their development. Previous workers had found little difficulty in obtaining fingerlings either by trawling or using a light source and a dip net at night (Everson,1970a, at Signy; Morris & North,1984, at South Georgia) particularily between the months of November and February. Subsequently hand collection by SCUBA divers has been very effective. Both austral winters of 1980 and 1981 were quite severe and consequently the sea-ice and pack ice remained in and around the island for much longer than usual (until

January 1981 and December 1981 respectively; Fig. 2.1). This effectively reduced the optimal time for sampling down to 4-8 weeks. A number of trawls were carried out in the vicinity of Bare Rock and Outer Island and although early post-larval specimens of some species were caught, no fingerling stage N.neglecta were obtained; nor were any caught when trawling was extended through until March. Using a light and dip net was equally unsuccessful even after a number of sites were sampled. It is possible that the prolonged presence of sea/pack ice considerably delayed the start of the spring plankton bloom, causing a reduction in the available food supply for the newly-hatched fish and therefore high mortality. It is interesting to note that in the same season there was a >95% mortality rate for penguin chicks in the study colonies on Signy Island, and a similar high mortality among shag chicks (Shaw,1980; Lishman,1981).

Inspection by diving at the accessible fishing sites showed that the habitats frequented by the different species are similar at Signy Island to those found in other Antarctic localities e.g. South Georgia (Burchett et al,1983) and the Antarctic peninsula (Daniels,1982).

2.4.2. Condition

Most fish undergo seasonal depletions of relatively short duration, although they can survive long periods of total deprivation, the record being held by a male European eel at 1515 days (Love,1970). They are well adapted for mobilising their body constituents as fuel for survival; large quantities of catheptic enzymes are present in fish muscle and their activities are amongst the highest reported

for vertebrates (Siebert et al,1964; Love,1970). The white muscle provides a large reservoir of readily utilisable protein, and is generally preferentially degraded during starvation (Johnston & Goldspink,1973; Patterson et al,1974; Beardall & Johnston,1983). At lower temperatures fish are able to withstand longer periods of depletion, probably aided by their reduced basal metabolism (Wilkins,1967; Love et al,1968). A large proportion of the observations of seasonal variation are usually linked directly or indirectly with temperature changes, changes in food supply or sexual activity (Love,1980).

An overall estimate of the 'fitness' of a fish is given by it's condition factor. The variation of condition factor with month is shown (Fig. 2.7) for all specimens of N.neglecta caught at Signy Island from 1980 to 1982. The lowest condition factor was found in July (2.15), just after spawning, and in November (2.13) at the end of winter, the latter suggesting that Everson (1970a) was correct in stating that these fish do not feed as the seals are returning to the Islands. The condition factor was high and fairly stable throughout the summer months, falling in May and June as more body stores are directed to gonad development; presumably female fish will tend to be less 'fit' than males, since a larger proportion of the body mass is accounted for by the gonads. Comparison of (Figs. 2.6 & 2.7) shows that CF and RGS parallel each other. For instance, the relative gonad size in female N.neglecta rises during the summer months, peaking in May at a value of over 16%. In some species the weight of the genital products can be as high as 30% of the body weight

(Iles,1971). There was a rapid fall of RGS in June indicating spawning has occurred, then values remained steady during the winter rising again from November onwards. Immature female fish showed a similar peak in RGS in May (Fig. 2.6) although to a smaller extent than adult females.

A cyclical trend was also seen when comparing the RLS with month (Fig. 2.8). The RLS increased in both female and male fish from June to September, followed by a fall. This also supports the theory that N.neglecta ceases to feed in the late winter months and is utilising it's liver carbohydrate stores. Identical patterns of RLS varying with month were observed by Everson (1969). This partial-fast is coincedent with the return of weddell and elephant seals, both of whom consume fish in their diet (Dearborn,1965; Stonehouse,1972). The drop in RLS during the late winter might also be indicative of reduced food supplies, but Daniels (1982) has pointed out that the feeding strategy of N.neglecta is such that it can consume adequate prey items all the year round. It is possible that although the quantity of food ingested in the winter is still maintained, the quality of the food may be poorer in the winter leading to a less efficient conversion into energy supplies or storage materials. There is another fall in RLS just prior to the onset of spawning, that is most evident in the females (Fig. 2.8). Similar trends were seen in the cod (Gadus morhua), where the energy content of the liver was also measured. In this species the energy content of the liver fell just before spawning as the accumulation of gonadal material required more energy than could be provided by the diet (Eliassen & Vahl,1982). Since the female gonads

account for such a large proportion of the body weight in ripe fish, they would probably constitute a larger energy demand than the male gonads.

The gross muscle chemistry of N.neglecta was monitored on a monthly basis to see if there was any indication of changes occurring in parallel with those in condition factor. Generally it is very difficult to separate the effects due to the environment from those due to maturation, starvation etc. (Jacquot,1961). However, the marine environment of the Antarctic is very stable, with water temperatures remaining fairly constant throughout the year (Fig. 2.1), and despite the peak of primary production that occurs in the austral spring (Foxton,1956) it has already been stated that N.neglecta has evolved a feeding strategy to enable it to maintain it's food intake all the year round (Daniels,1982). Consequently the major seasonal stress on Antarctic fish would be that caused by gonadal growth and spawning. If the energy demand associated with reproduction exceeds the energy supplied by the food then the body reserves must be drawn upon. The seasonal changes in water, protein and lipid contents of the muscle (and liver) have therefore been related to growth of the gonads in several fish species (Love,1970; Shul'man,1974).

Inter-relationships of the main constituents of white muscle during depletion vary considerably in different species according to the location of stored lipid (Love,1970). 'Fatty' fish such as the mackerel (Scomber scombrus) and herring (Clupea harengus) have high concentrations of reserve lipid in the muscle tissue, and during times of depletion these are drawn upon first; even

after several months depletion there may still be very little protein breakdown (Inui & Ohshima,1966; Love,1970). There is therefore, a relationship between the muscle lipid and muscle water content; as the lipid levels fall during depletion, the water level rises (Brandes & Dietrich,1956). In 'non-fatty' fish such as the cod (Gadus morhua) and the plaice (Pleuronectes platessa) there are less extensive lipid depots in the muscle and the stored lipid is found mainly in the liver. During depletion these species utilise lipid from the liver first and then maintain carbohydrate at the expense of muscle proteins (Butler,1968; Renaud & Moon,1980); thus there is a relationship between muscle protein and muscle water. This relationship is seen better in the white muscle since red muscle proteins are conserved during starvation (Johnston & Goldspink,1973; Beardall & Johnston,1983). For example in the plaice, red muscle water content was not significantly different even after 14 weeks starvation although the water content of the white muscle had increased significantly (Johnston & Goldspink,1973).

N.neglecta is classified as 'non-fatty' (Crawford,1979), so that as protein levels fall in the muscle, the water content should rise. This relationship does not seem to hold for N.neglecta (Fig 2.9) even after 28 days starvation (Crawford,1979). Similarily, and as expected, there is no relationship between muscle lipid and muscle water (Fig. 2.9); rather the opposite seems to occur, with lipid trends following those of water. It has been noted in non-fatty fish that there is sometimes a considerable delay while body energy reserves are being utilised before the water content in the muscle starts to rise (Love,1970). It is possible

that N.neglecta can withstand a relatively short period of depletion by using it's liver stores, without the necessity to start utilising muscle energy stores, hence there will be no increase in muscle water content. During periods where the CF is relatively stable, then the increase in RLS is an indication of ample food supplies (Figs. 2.7 & 2.8). When the CF falls (May-July) there is a fall in RLS, but also a fall in water content of the trunk muscles (Figs. 2.7-2.9) instead of a rise, further evidence that the muscle energy stores are not being depleted.

Most studies showing lipid-water and protein-water relationships have been carried out using temperate species such as the cod or the plaice (Love,1970; Johnston & Goldspink,1973). However, both cod and herring have been shown to withstand depletion better when acclimated to lower temperatures (Wilkins,1967; Love et al,1968), so Antarctic fish are probably better able to withstand long periods of depletion when compared to temperate species, assisted by their lower rate of metabolism.

Therefore in the short term N.neglecta can mobilise liver stores which provide an adequate supply of fuels so that muscle stores are not called upon, and lipid-water and protein-water relationships do not occur in the muscle. Furthermore, females are probably better than males at withstanding depletion since they have a lower metabolic rate, and more importantly a lower catabolic rate than males (Wohlschlag,1960; Everson,1969). To demonstrate changes in the gross muscle chemistry of the trunk muscles of N.neglecta probably requires much longer periods of depletion than are normally used for temperate species.

(a)

MONTH	NETS	FISH	FISH/NET
JAN	15	72	4.80
FEB	9	61	6.78
MCH	25	245	9.80
APR	11	185	16.82
MAY	5	54	10.80
JUN	10	47	4.70
JUL	13	59	4.54
AUG	2	1	0.50
SEP	1	7	7.00
OCT	4	6	1.50
NOV	7	3	0.43
DEC	10	29	2.90

(b)

SPECIES	NUMBER	% OF TOTAL
Notothenia neglecta	554	72.04
Chaenocephalus aceratus	84	10.92
Notothenia rossii	66	8.58
Notothenia (Gobionotothen) gibberifrons	52	6.76
Pagothenia hansoni	7	0.91
Pagothenia bernacchii	3	0.39
Trematomus newnesi	1	0.13
Parachaenichthys charcoti	1	0.13
Pogonophryne dolichobranchiata	1	0.13

Table 2.1 TRAMMEL NET CATCH RATE (a) AND SPECIES COMPOSITION (b) DATA FROM MARCH 1980 TO MARCH 1982.

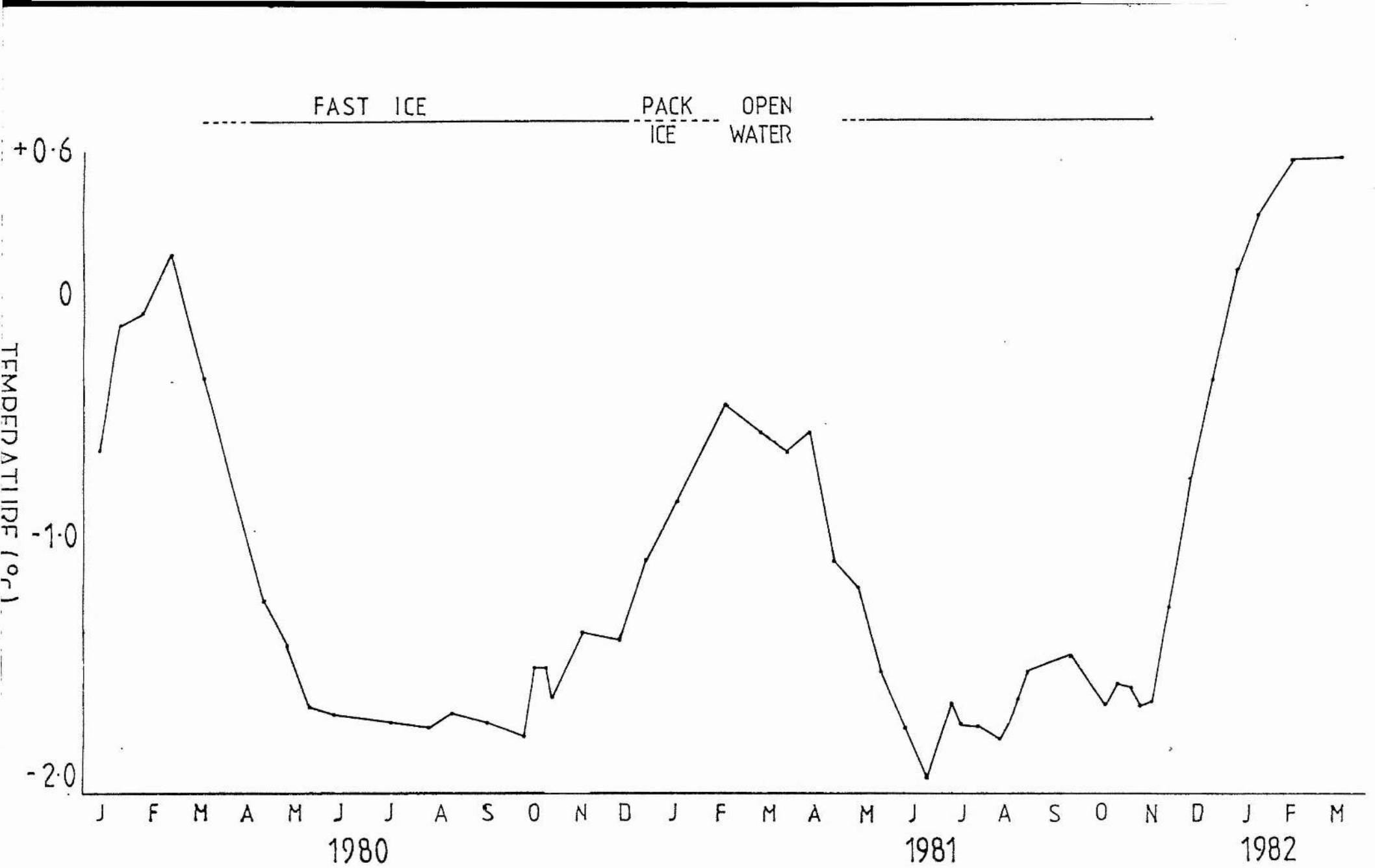


Fig. 2.1 Sea water temperature and sea-ice conditions, Signy island, 1980-1982

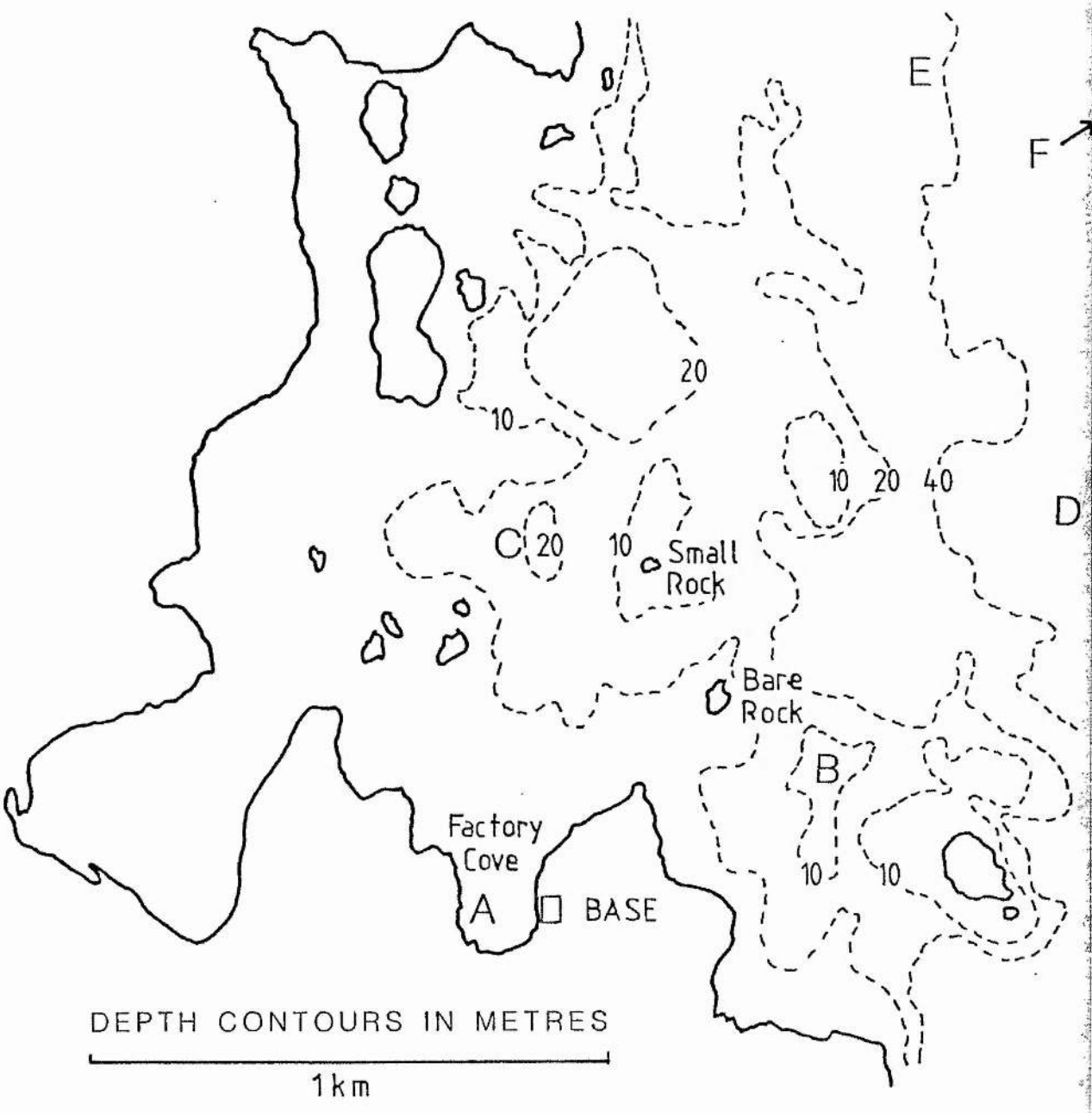


FIG 2.2 SAMPLE SITES IN BORGE BAY, SIGNY ISLAND

A. PORNY ROCK (3-4m)

B. BARE ROCK (10-15m)

C. WEED BOWL (20m)

D. OWEN'S BANK (60m)

E. POWELL ROCK (40m)

F. ORWELL BIGHT (200m+)

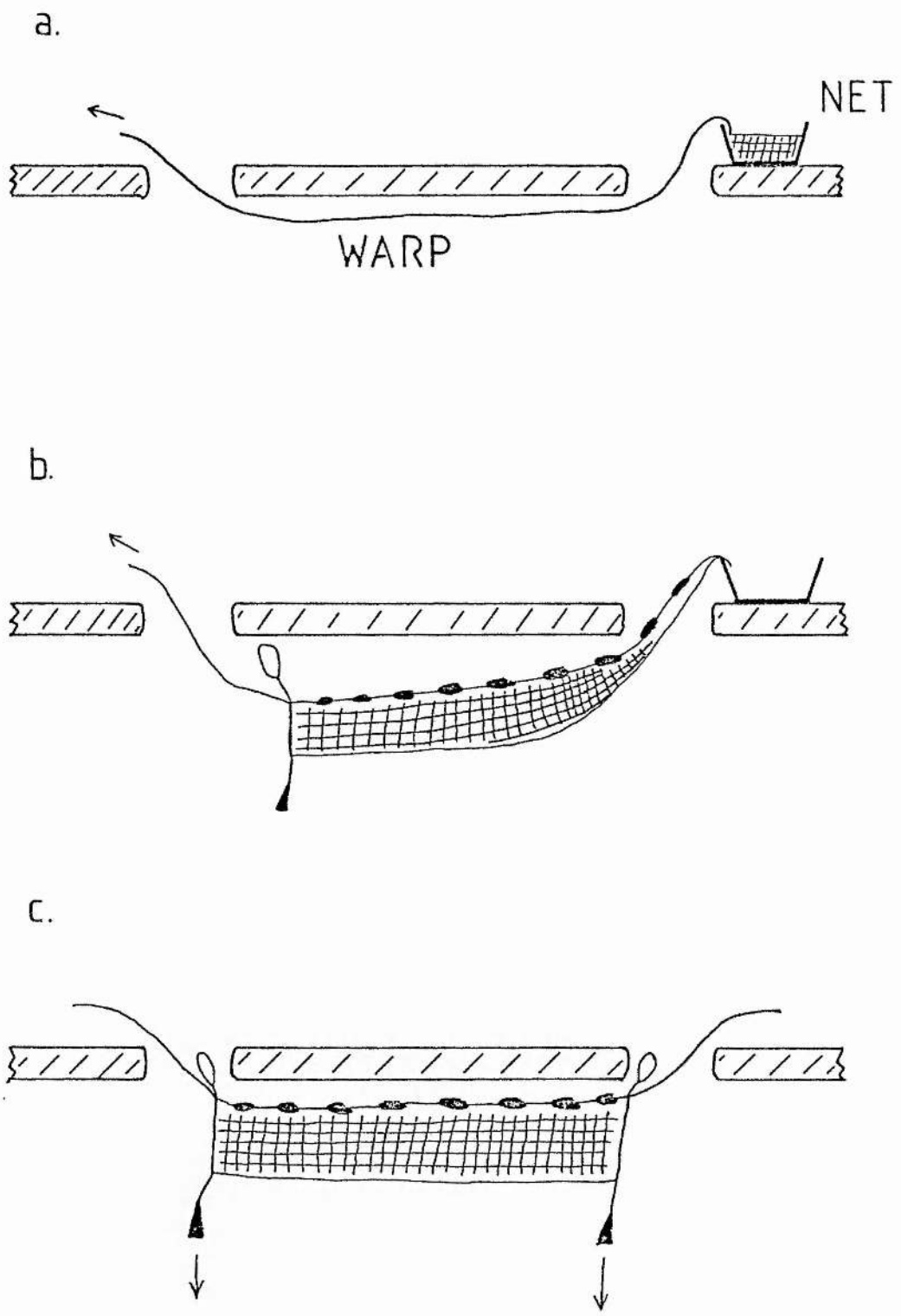


Fig. 2.3 Method for setting nets under ice

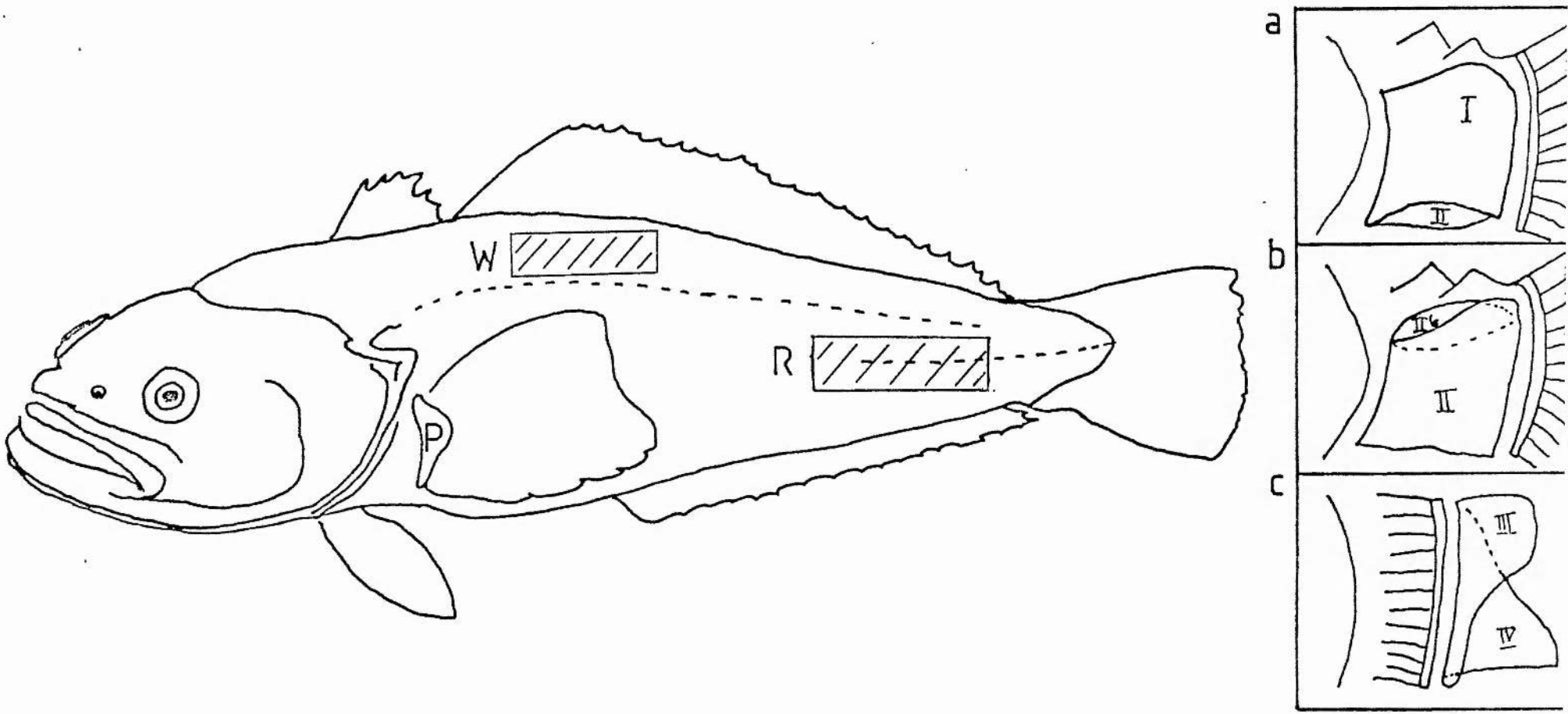


Fig 2.4 Sample sites for red (R), white (W) and pectoral (P) muscles.

a-c, pectoral abductor (I,II & IIb) and adductor (III & IV). See text for further details

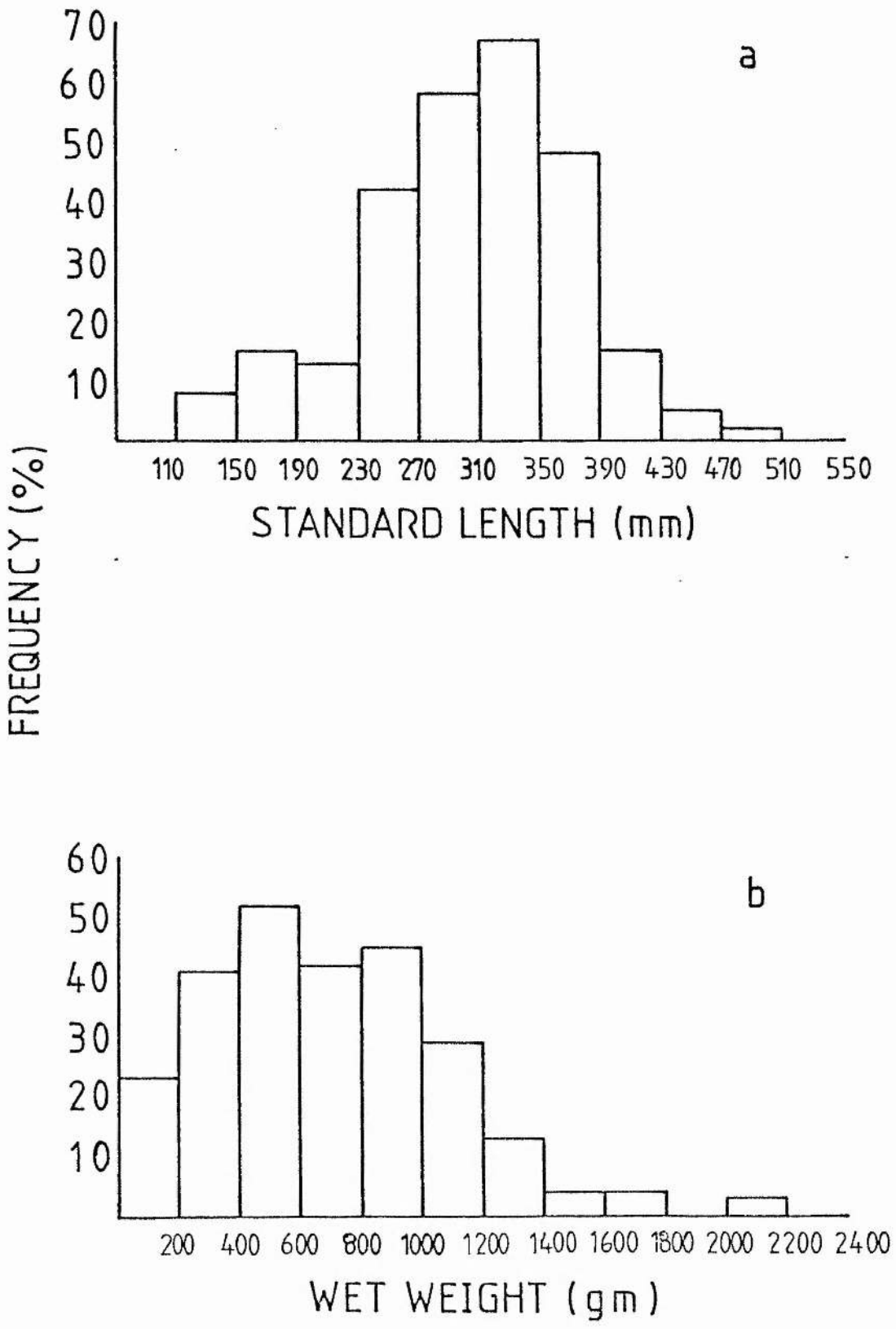


Fig 2.5 Distribution of standard length (a) and wet weight (b) frequencies for N. neglecta

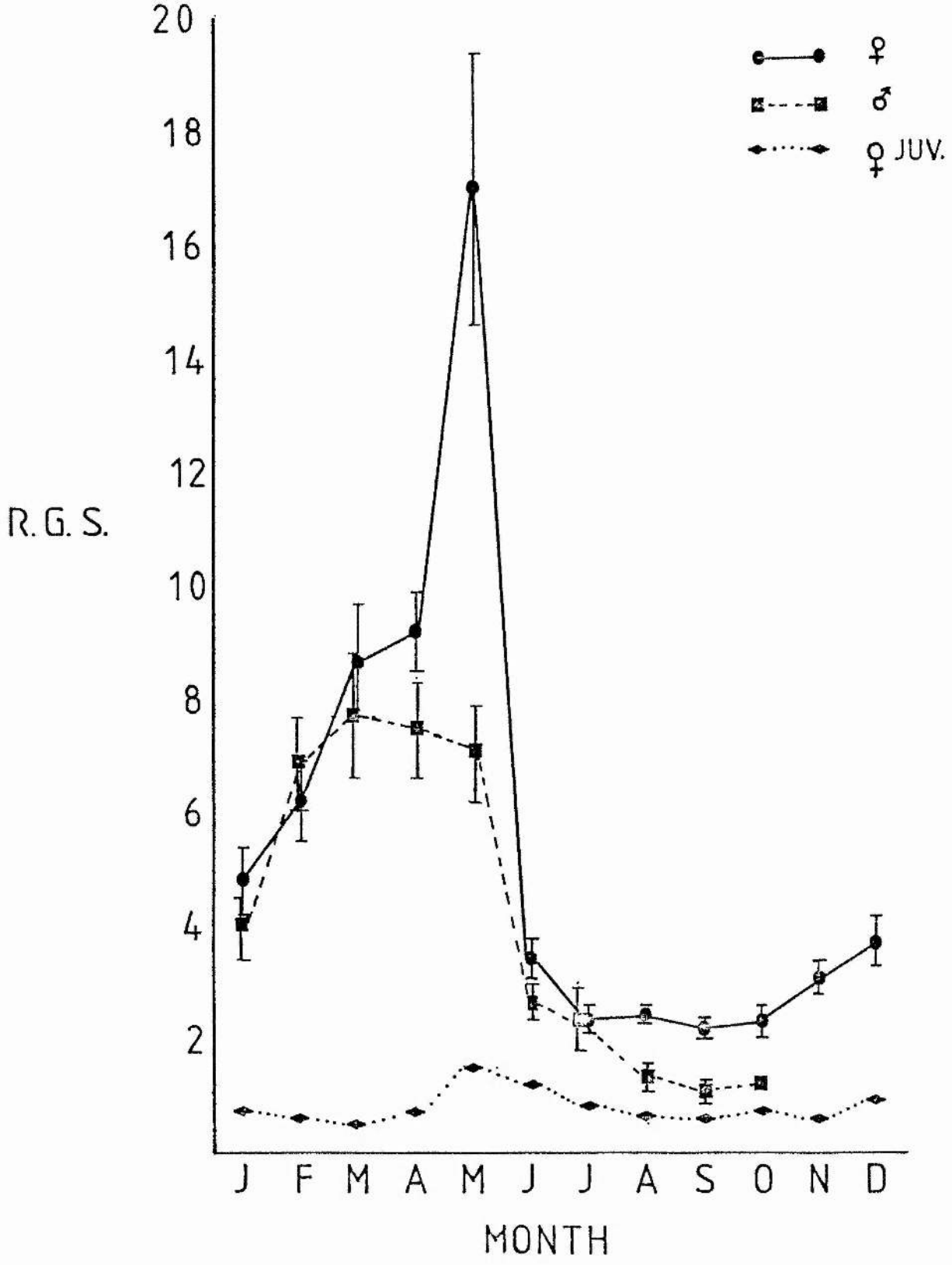


Fig. 2.6 Monthly variation of relative gonad size in N.neglecta (means ± sem, n at least 6 fish).

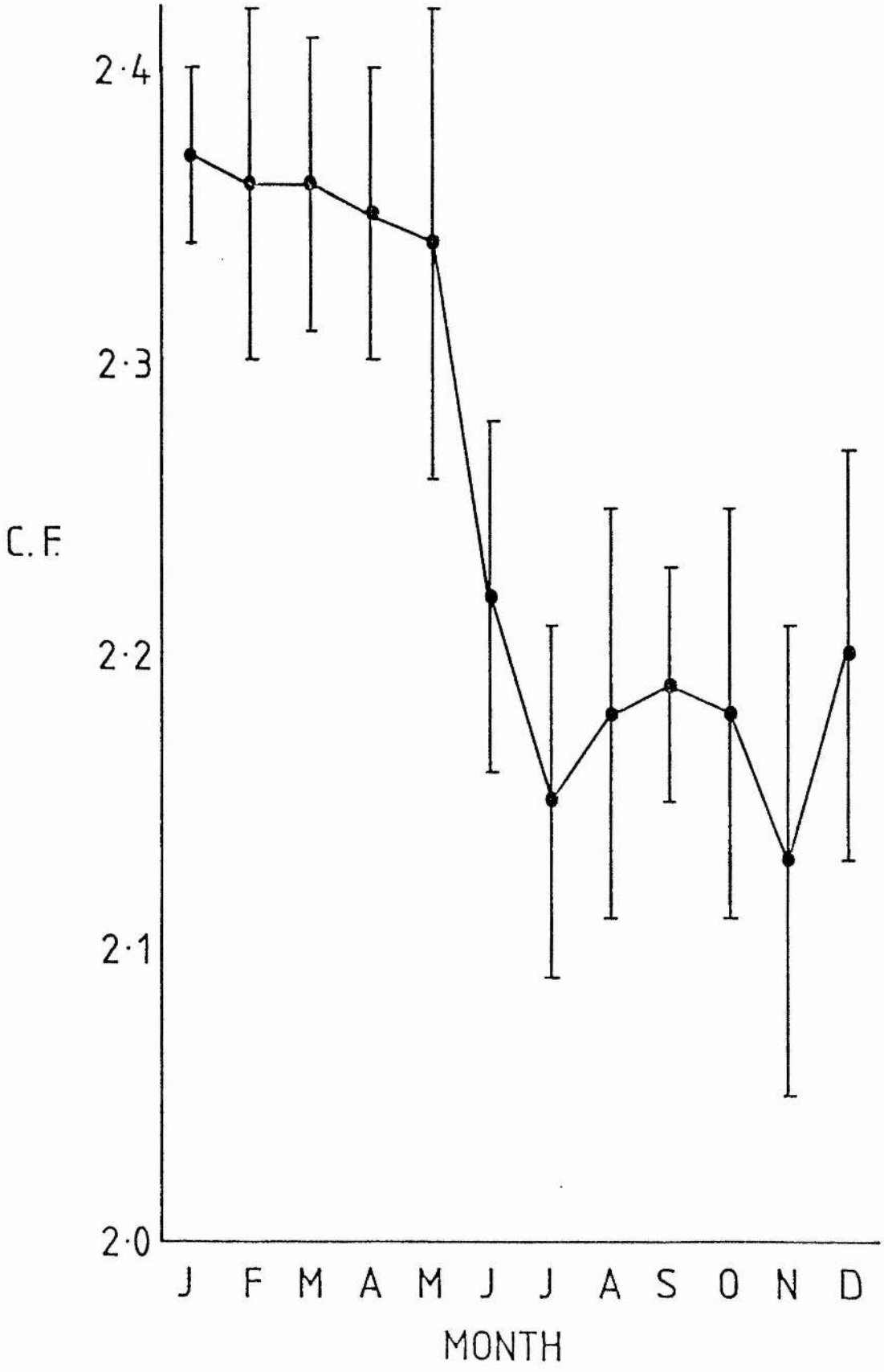


Fig 2.7. Monthly variation of condition factor for N.neglec

(means ± sem, n at least 6 fish)

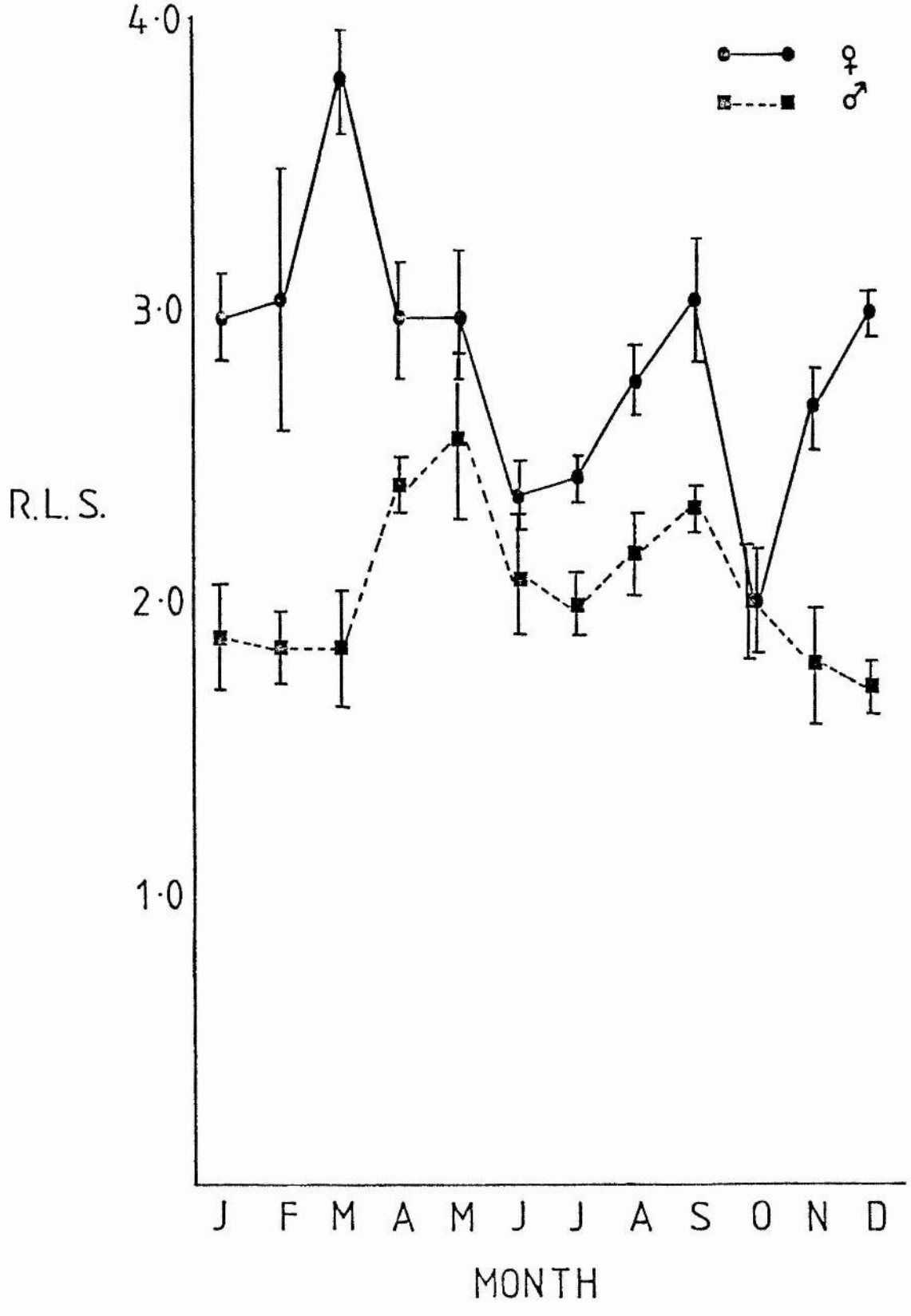


Fig 2.8 Monthly variation of relative liver size for N.neglecta (means ± sem, n at least 6 fish).

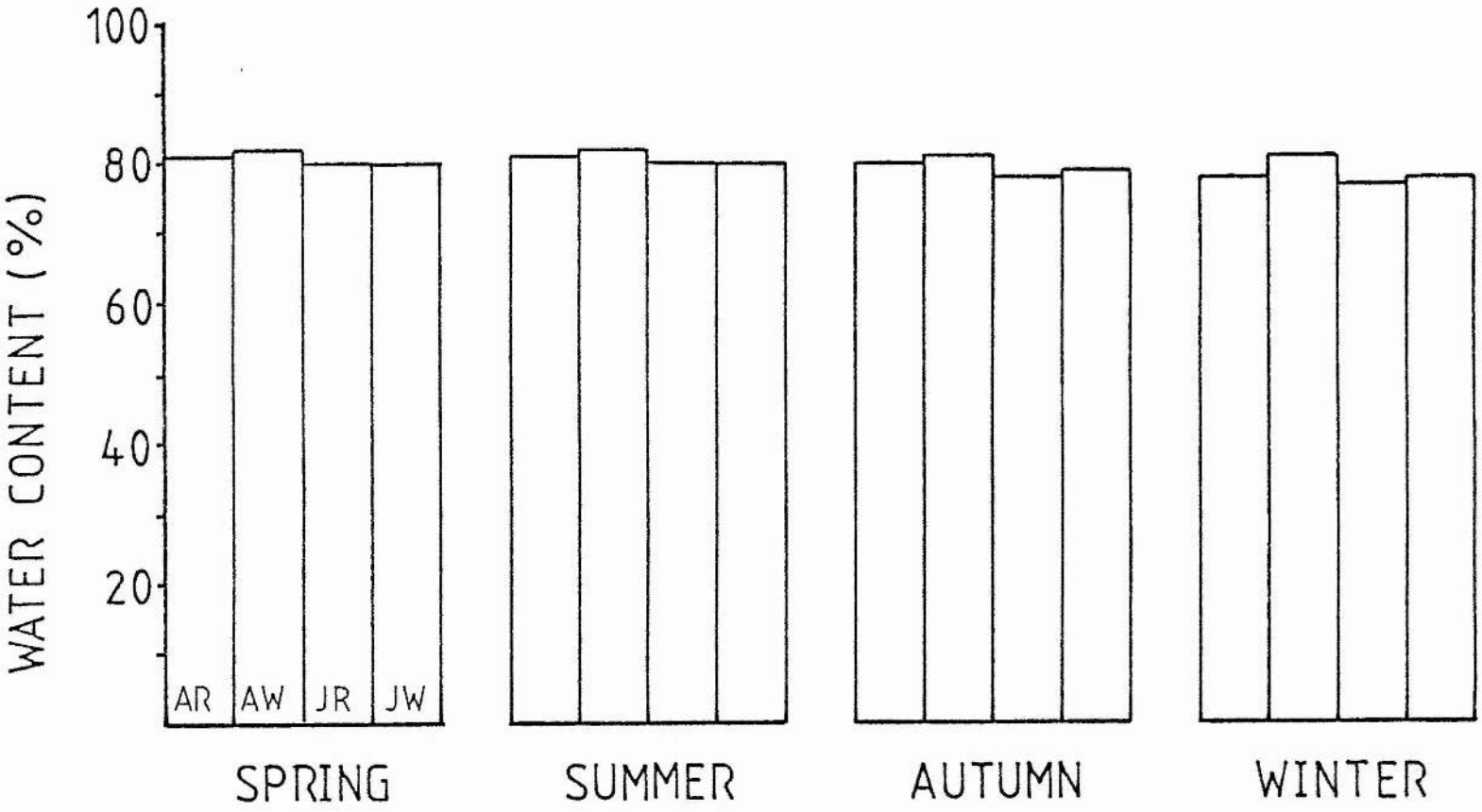


Fig. 2.9 .Seasonal variation in water content of the trunk muscles of N.neglecta. A-adult, J-juvenile, R-red, W-white.

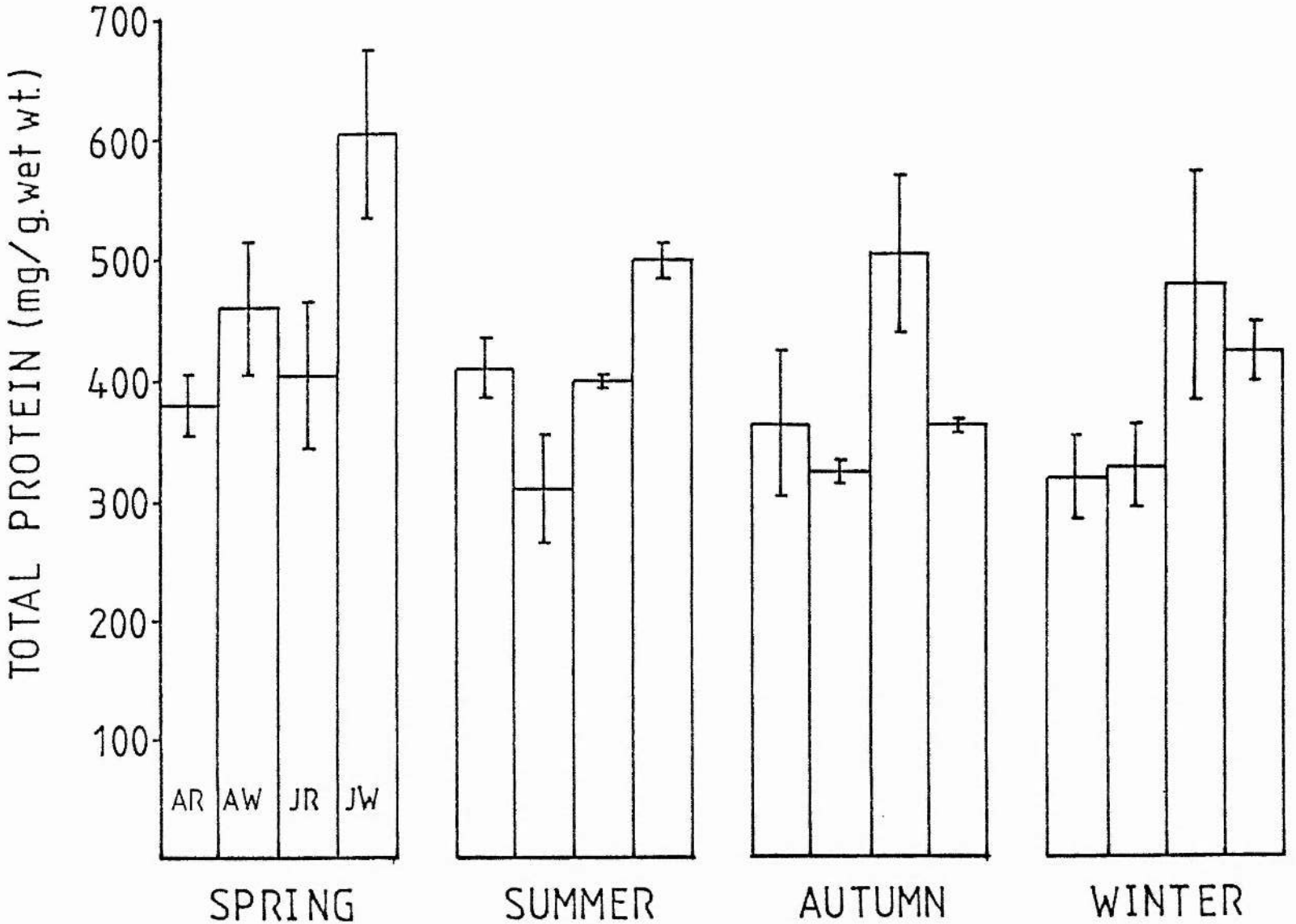


Fig. 2.10 Seasonal variation in total protein content of the trunk muscles of N.neglecta. (notation as fig. 2.9) Means ± sem n at least 6.

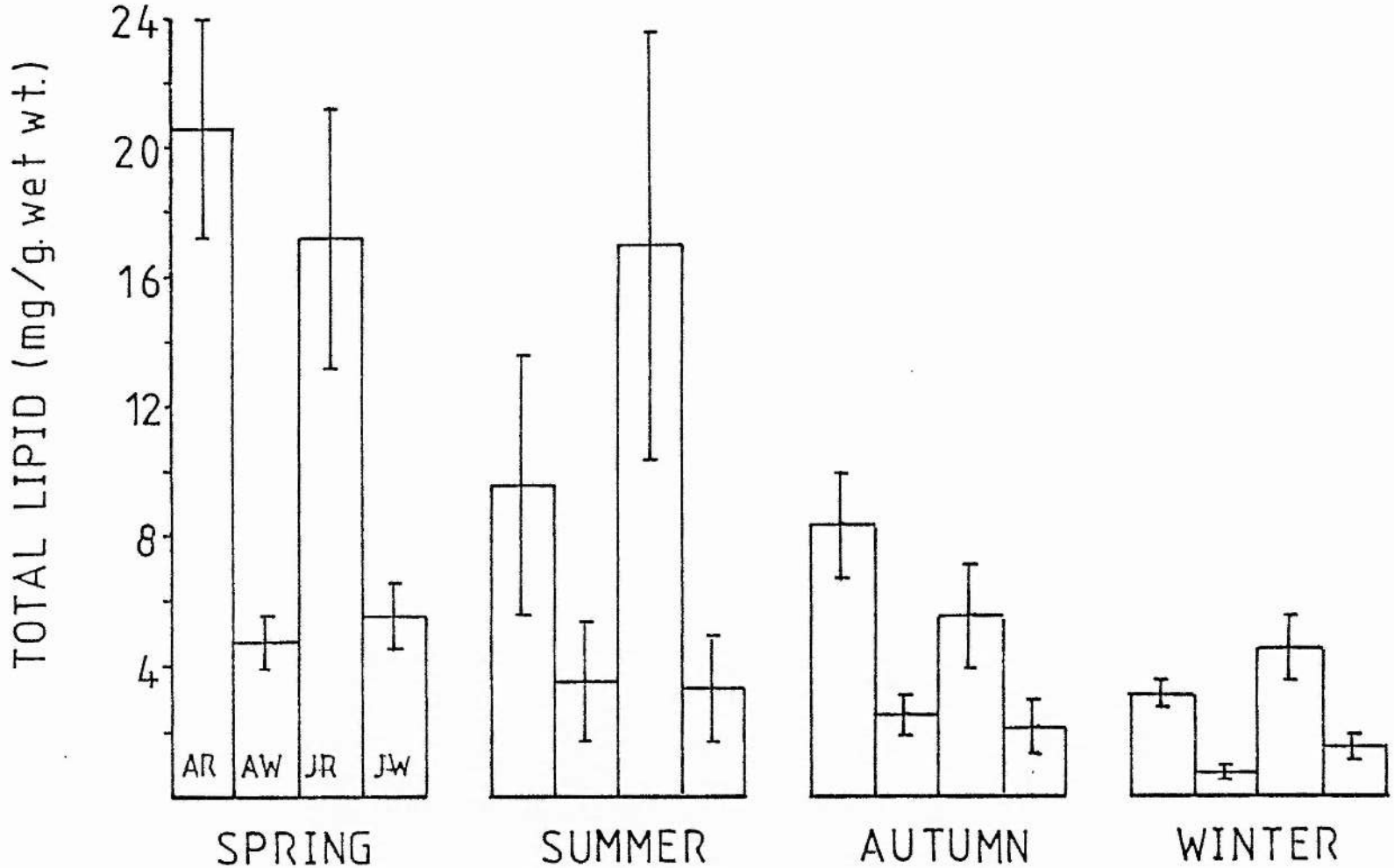


Fig. 2.11 Seasonal variation in total lipid content of the trunk muscles of N.neglecta. (notation as for fig. 2.9) . Means ± sem, n at least 6.

Chapter 3. Enzymes of energy metabolism in Antarctic fish species, with the emphasis on Notothenia neglecta.

3.1. Introduction

To assess the contribution of the various enzymes in a metabolic pathway usually involves determination of maximum *in vitro* activities. The particular enzymes chosen for study are those which catalyse the control steps in the pathway. Key enzymes generally catalyse reactions that are far from equilibrium and these 'non-equilibrium' steps in a pathway can be identified by one of two methods. The first is by comparison of equilibrium constants with mass action ratios. The equilibrium constant (K) of a reaction $A+B \rightleftharpoons C+D$ is calculated from the concentrations of the substrates (A,B) and the products (C,D) only, since the rate constants for the forward and back reactions cancel each other out. Thus;

$$K = [C][D] / [A][B]$$

Since an enzyme is a catalyst it cannot alter the position of the equilibrium, so that if an equilibrium (i.e. non-regulatory) reaction is being catalysed then the ratio of the concentrations of products/substrates (the mass action ratio, Γ) should approximate to K, which itself is usually determined from the thermodynamic properties (the free energy change $G = -RT \ln K$, where R is the gas constant and T is in degrees kelvin) of the reaction. However, if Γ is much smaller than K, it suggests that the reaction is displaced far from equilibrium, and that the enzyme is regulatory. The second approach is by measurement of maximum enzyme activities. A non-equilibrium reaction in a pathway arises because the enzyme catalysing

that step has not sufficient activity to bring the substrates and products to equilibrium. Subsequent and preceding enzymes have much higher reaction rates. So the enzymes in a metabolic pathway with comparatively low activities are possibly regulatory (Newsholme & Start,1973). However, a high activity measured in vitro may not necessarily reflect the situation in vivo where the enzyme will be affected by it's microenvironment e.g. pH, compartmentalisation etc. (Walesby & Johnston,1980).

To some extent all the enzymes in a metabolic pathway will be regulatory, since changes in flux through the pathway will alter the activities of all the enzymes (Kacser & Burns,1973). The difficulty lies in identifying the enzyme which responds to the original metabolic signal, and thereby initiates the subsequent changes in the activities of the remaining enzymes. Two methods can be used to identify this enzyme. Either the enzymes of a pathway can be studied in isolation under optimal conditions without consideration of pathway flux (Newsholme & Start,1973) or the pathway as a whole can be monitored using whole preparations or tissue slices, and the flux can be measured while perturbations of individual reactions are carried out. It can be argued that the latter is the most physiological method and is therefore the most valid (Kacser & Burns,1973). It can also be shown that a reaction far from equilibrium is not necessarily the one most sensitive to control; however, it is agreed that a reaction that is at equilibrium is the least sensitive (if not totally insensitive) to control, and so it cannot be regulatory (Kacser & Burns,1973; Newsholme & Start,1973). The

importance of assaying activities *in vitro* at *in vivo* concentrations where possible is shown by the work of Beitner et al (1979). In growing rats activities of phosphofructokinase (PFK) and hexokinase (HK) in hind leg muscles were regulated by tissue concentrations of glucose-1,6-diphosphate when the assays were carried out at *in vivo* concentrations of reactants. When assays were carried out under conditions for optimal activities, glucose-1,6-diphosphate had no regulatory effect.

The metabolic profiles of red and white muscles are not fixed at birth, but can be modified by development (Bostrom & Johansson,1972; Beitner et al,1979), starvation (Moon & Johnston,1980) and exercise (Johnston & Moon,1980a,b). Acclimation to cold temperatures can also lead to an increase in the potential for aerobic metabolism of both red and white muscles (Hazel & Prosser,1974; Johnston & Lucking,1978) and an alteration in the relative importance of particular substrates (Hochachka,1968; Stone & Sidell,1981). Adaptation to depth leads to a general decrease in respiratory activity (Childress,1971; Torres et al,1979) with a concomittant reduction in metabolism (Childress & Somero,1979).

Enzymes of carbohydrate and lipid metabolism (both regulatory and non-regulatory) in various muscles have been measured under optimal conditions for the wide-ranging benthic species, *N.neglecta*, and related to activities of the same enzymes found in the same muscles of the shallow-living (0-10m) benthic species, *T.newnesi* and the deeper living benthic species *N.gibberifrons*. Also size-related enzyme activity has been examined in the red and white trunk muscles of *N.neglecta*, in specimens of

125-490mm standard length.

3.2. Materials and Methods.

3.2.1. Fish.

Specimens of N.neglecta were caught by trammel net or by hand in Factory cove and Borge bay at all fishing sites (Fig.2.2). N.gibberifrons was also caught by trammel net, at sites D,E and F, while T.newnesi was caught either by hand-net while diving, or with the small trammel net at sites A and B. Fish were removed from the nets and maintained in aquaria with a through-flow water system, water temperature -1 to +1 ^{o}C. Fish were fed on a diet of chopped fish and amphipods, but no food was given during the 48 hours before being sacrificed. The range of standard lengths,SL,(mm) and weights (gm) used in the experiments were:

N.neglecta, 125-490 : 37-2100

N.gibberifrons, 270-350 : 270-580

T.newnesi, 80-96 : 6.0-10.5.

Specimens of N.gibberifrons and T.newnesi represented the adult stage and were captured between the months of December and February. For analysis of size-related enzyme activities specimens of N.neglecta were grouped as small (SL 125-240mm), intermediate (SL 245-335mm) and large (SL 340+ mm).

3.2.2. Preparation of muscle homogenates.

Fish were stunned by a blow to the head followed by transection of the spinal cord. Samples of the various muscles were dissected out, freed from connective tissue and cut into small pieces with fine scissors. Red myotomal

muscle was taken from the region of the lateral line care being taken to avoid contamination by the underlying white muscle mass. White muscle samples were taken from the deep epaxial muscle about two-thirds of the way along the body from the caudal end , while pectoral muscle samples were taken from the anterior deep abductor muscle (Fig. 2.4). The whole heart was removed, and cardiac muscle samples were taken from the ventricle. Muscle samples were immediately blotted to remove excess moisture, weighed and then homogenised at 0-4^{o}C in an MSE motorised homogeniser, for 3 x 20 seconds at maximum speed. The following extraction media were used for the individual enzymes:

Lactate dehydrogenase (LDH; Enzyme Commission number (EC),1.1.1.27), Malate dehydrogenase (MDH; EC.1.1.1.37), 3-Hydroxyacyl CoA dehydrogenase (3HACDH; EC.1.1.1.35) and Cytochrome oxidase (COX; EC.1.9.3.1), 5-15 volumes of 125mM sodium phosphate buffer, pH 7.6 at 4^{o}C.

Phosphofructokinase (PFK; EC.2.7.1.11), Hexokinase (HK; EC.2.7.1.1) and Pyruvate kinase (PK; EC.2.7.1.40), 3-10 volumes of 50mM tris-Hcl, 5mM EDTA, 2mM MgCl2, 1mM DTE, pH 7.5 at 4^{o}C.

Homogenates were centrifuged at 600G for 10 minutes in ice-jacketed centrifuge tubes and the supernatants were filtered through glass wool and retained for determination of enzyme activities.

3.2.3. Assay of enzyme activities.

Measurements of enzyme activity were carried out at 2-4^{o}C on a Pye-Unicam SP600UV spectrophotometer, at a wavelength of 340nm (except COX, 550nm), with appropriate enzyme and reagent blanks. Preliminary experiments were

carried out to determine optimal conditions of substrates and pH for the enzymes studied, and that the reaction rates increased linearly with increasing enzyme concentration. These preliminary experiments were repeated for each different species studied. Assay procedures for the individual enzymes for N.neglecta were as follows:

Lactate dehydrogenase

LDH was assayed in a medium containing 100mM sodium phosphate buffer, pH 7.8, 0.32mM NADH and saturating conditions of sodium pyruvate. Preliminary experiments showed that under the conditions employed, saturating conditions occurred at 5mM pyruvate for white fibres and 4mM pyruvate for red fibres.

Malate dehydrogenase

MDH was assayed in a medium containing 95mM sodium phosphate buffer, pH 7.8, 0.32mM NADH and 1.3mM cis-oxaloacetic acid.

3-Hydroxyacyl CoA dehydrogenase

3HACDH was assayed by the method of Storey and Bailey (1978). The reaction medium contained 100mM sodium phosphate buffer, pH 7.6, 0.16mM NADH and 5mM S-acetoacetyl-N-acetyl cysteamine. The latter was omitted in control reactions.

Cytochrome oxidase

COX was assayed by following the oxidation of reduced cytochrome C in 50mM sodium phosphate buffer, pH 7.6. Reduced cytochrome C was obtained by adding sodium dithionite to a solution of cytochrome C (giving an absorbance of 1.0) in 50 mM phosphate buffer, pH 7.6, then dialysing overnight against several volumes of the same buffer. Enzyme activity was calculated using $EmM_{(red-ox)} = 19.1$

Phosphofructokinase

PFK was assayed in a medium containing 50mM Tris-Hcl, pH 7.8, 50mM Kcl, 6mM $Mgcl_2$, 2.5mM disodium ATP, 0.16mM NADH, 1.5mM fructose-6-phosphate and excess alpha-glycerophosphate dehydrogenase, triosephosphate isomerase and aldolase. Normally the reaction rate speeds up with time, as fructose-1,6-diphosphate is formed, but the addition of 2mM AMP ensured maximal reaction rates from the start.

Hexokinase

HK was assayed using an ATP generating system. The reaction medium contained 80mM Tris-Hcl, pH 7.8, 0.8mM EDTA, 9.6mM $Mgcl_2$, 1.5mM Kcl, 1mM DTE, 10mM phosphocreatine, 0.65mM $NADP^+$, 4mM disodium ATP, 1mM glucose, 100ug creatine kinase and 100ug of glucose-6-phosphate dehydrogenase (Crabtree & Newsholme,1972).

Pyruvate kinase

PK was assayed in a medium containing 80mM sodium phosphate buffer, pH 7.8, 1.5mM phosphoenolpyruvate, 0.32mM NADH, 2mM ADP and excess LDH to remove the pyruvate formed, and so prevent feedback inhibition of PK.

All chemicals were from Sigma chemical company limited (Poole), or from Boehringer corporation (London).

3.2.4. Statistics.

The normality of the groups was tested to determine whether parametric or non-parametric tests should be used. A pooled or two-sample t-test, or Mann-Whitney test were used accordingly.

3.3. Results

3.3.1. Comparison of muscle types in mature N.neglecta.

Activites were determined for enzymes of aerobic energy metabolism: HK, which catalyses the phosphorylation of glucose; MDH, an enzyme of the citric acid cycle; 3HACDH, a marker for beta-oxidation of fatty acids and COX, a respiratory chain enzyme (Fig. 3.1). Activities of these enzymes were 2-5 times greater in the red myotomal muscle, than the corresponding enzyme in the white muscle ($P<0.001$). The pectoral abductor muscle had an aerobic capacity equivalent to that of the red trunk muscle, there being no significant differences in the activities of HK, MDH, or 3HACDH, although in each case the activity in red muscle was slightly higher. Cardiac muscle exhibited the highest aerobic capacity of any of the tissues studied; activities of MDH and 3HACDH were twice as high as the activities in the red trunk muscle ($P<0.001$, <0.01 respectively), while the activity of HK was about 6 times higher ($P<0.001$). Cardiac muscle also had the highest COX activity, being about four times higher than the red muscle enzyme ($P<0.01$). Overall therefore, the ranking of the muscles in terms of their aerobic capacity would appear to be cardiac muscle > red trunk muscle >= pectoral abductor muscle > white trunk muscle.

Activities of PFK and PK represent flux through their particular stage of the glycolytic pathway and LDH activity the capacity for lactate formation. Activities for PFK, a prime regulator of glycolysis were not significantly different between the two types of trunk

muscles (Fig. 3.2), indicating a similar potential maximum flux through this part of the glycolytic pathway. PFK activities in cardiac and pectoral abductor muscle were less than 50% of those in the trunk muscles ($P<0.05$). Red trunk muscle PK was twice as active as the PK of white muscle ($P<0.01$), while the LDH activities were comparable between the two muscle types (Fig. 3.2).

Cardiac and pectoral muscles, while having lower PFK activities than either red or white trunk muscles, had significantly higher PK activities ($P<0.001$). In terms of LDH activity cardiac muscle had the greatest activity, some 3-4 times higher than that found in the pectoral muscle ($P<0.001$). There was no significant difference between LDH activities of the pectoral muscle and either of the trunk muscles(Fig. 3.2). The ranking of the muscles in terms of overall anaerobic capacity was not so clear cut; pectoral abductor muscle and red and white trunk muscles were approximately equal, cardiac muscle being slightly higher.

3.3.2. Size-related enzyme activities in N.neglecta.

Malate dehydrogenase

The pattern of activity was the same in both muscle types, with the highest activities being found in the smallest (youngest) fish, and the lowest activities in the largest size fish. This reduction was significant in both the red and white trunk muscle ($P<0.01$). The red muscle had an activity about three times greater than the white trunk muscle at all sizes (Fig. 3.3).

3-Hydroxyacyl CoA dehydrogenase

Trends were quite different in the two muscle types. In

the white trunk muscle, although the activity was falling off in the larger fish, there were no significant differences between any of the size groups (Fig. 3.3). In the red trunk muscle however, the activity increased significantly ($P<0.05$) in the larger size fish.

Hexokinase

In the red muscle the highest activities were found in the intermediate size fish, with activities in larger and smaller fish being roughly the same (Fig. 3.3), and not significantly different from the intermediate group. In the white muscle activities in the small and intermediate groups were not significantly different, but there was a decline of about 40% in the larger fish ($P<0.05$).

Phosphofructokinase

Activities of PFK were relatively high in the smaller size fish, but showed a slight decline in both muscle types by the time the fish had matured (Fig. 3.4). In the white muscle PFK activity continued to decline, the overall fall in activity (small to large groups) being significant ($P<0.05$). PFK activity in the red muscle increased slightly as the fish were growing, but not significantly so.

Pyruvate kinase

In both the trunk muscles there was no significant change in PK activity as the size increased. The overall tendency was for activity in the red muscle to decline, while in the white muscle a slow increase was observed (Fig. 3.4).

Lactate dehydrogenase

In both red and white muscle the trends were the same (Fig. 3.4). There was a significant decline in activity

between the small and intermediate size groups ($P<0.005$,red; $P<0.001$,white), with a continued but insignificant decline as the size continued to increase.

3.3.3. Interspecific comparison of red and white myotomal muscle enzymes.

In the red trunk muscle the shallow living T.newnesi had the highest aerobic enzyme activities (MDH and COX), while the deeper living N.gibberifrons had the lowest activities (Table 3.1). 3HACDH activities were not significantly different in the red muscles of N.neglecta and T.newnesi. In terms of ability to utilise glucose, the red muscle of N.gibberifrons had the highest activity of HK, 6 times higher than N.neglecta. Red muscle LDH activities were broadly similar for all three species, as were PK activities (Table 3.1). However, PFK activity (the rate limiting step in glycolysis) varied 12-fold between T.newnesi and N.gibberifrons, the latter having the highest activity.

In the white trunk muscle trends were basically the same as they were for the red trunk muscle. T.newnesi had the highest MDH and COX activities; 3HACDH activities were not significantly different between T.newnesi and N.neglecta, and N.gibberifrons had the highest HK activity (Table 3.1). However, interspecific differences in anaerobic capacities were more highlighted in the white muscles of the various species. In each case (LDH, PK and PFK) the deeper living N.gibberifrons had the highest activity, with the shallow living T.newnesi having the lowest activities. Activities of anaerobic enzymes in the white muscle of N.neglecta were intermediate (Table 3.1).

3.4. Discussion

3.4.1. Enzyme activities in mature Notothenia neglecta.

3.4.1.1. Locomotory muscles.

Early studies on the musculature of Antarctic fish (Lin et al,1974) suggested that the myotomal musculature was composed entirely of white fibres, with red muscle fibres restricted to the pectoral muscles. However, more recent studies have shown that there is a wedge of aerobic red fibres adjacent to the lateral line (Walesby & Johnston,1980; Walesby et al,1982). These red myotomal fibres have aerobic enzyme activities of only about one quarter that of the red pectoral fibres, although they both have similar activities of enzymes associated with anaerobic metabolism (Walesby & Johnston,1980).

This study has shown that on the basis of enzyme activities, there are the same two characteristic fibre types in the trunk muscle of N.neglecta, as are found in other Antarctic fish. Activities of enzymes associated with aerobic metabolism (enzymes of the citric acid cycle and the respiratory chain) are 3-5 times greater in the red myotomal muscle compared to the white myotomal muscle (Fig. 3.1), a result characteristic of most fish species studied (Crabtree & Newsholme,1972; Johnston et al,1977; Johnston & Moon,1980a,b; Walesby & Johnston,1980; Johnston & Bernard,1982; Walesby et al,1982).

The activities of aerobic enzymes (MDH,COX and HK) in N.neglecta come within the lower limits of some temperate

species measured at their own environmental temperature, e.g. brook trout, Salvelinus fontinalis (Johnston & Moon,1980a). The low enzyme activities in Antarctic fish may be due in part to their low basal oxygen consumption (Wohlschlag,1964; Ralph & Everson,1968; Holeton,1970), and their possible low scope for activity (Wohlschlag,1960;1962).

The activities of PFK and PK were measured as an indication of glycolytic capacity. PFK and PK activities are usually 2-5 times higher in the white myotomal muscle compared to red myotomal muscle and this has been found in both temperate and Antarctic fish (Crabtree & Newsholme,1972; Johnston & Moon,1980a,b; Walesby & Johnston,1980; Johnston & Bernard,1982). While PFK is thought to be regulatory the situation is not so clear for PK. Although in mammals PK is a non-equilibrium enzyme there is little support for it being regulatory (Newsholme & Start,1973). However in some fish at least PK is under tight metabolic control (Johnston,1975), often (but not always) in cases where facultative anaerobiosis or tolerance of low oxygen concentrations is required (Johnston,1975; Storey & Hochachka,1974).

For N.neglecta the PFK activity is similar in both red and white trunk muscles, while the PK activity is twice as high in the red fibres. The same pattern of activities is seen in the yellow eel, A.anguilla (Bostrom & Johansson,1972) and with PK activity in the tench, Tinca tinca,(Johnston & Bernard,1982). Glycolytic enzymes in Antarctic species are lower in activity than comparable enzymes in temperate teleosts. The difference is more

pronounced when comparing white muscle enzymes and most species have an enzyme activity at least an order of magnitude higher than the Antarctic species. For example, PFK activity in the white muscle of N.neglecta is 1.22 μmol/min/gw, while the activity of the same enzyme in the brook trout is 13.4 μmol/min/gw (Johnston & Moon,1980a) and in the plaice, 29.0 μmol/min/gw (Johnston & Moon,1981). LDH is considered to catalyse the final step in glycolysis and again the activity is lower in Antarctic species (N.neglecta, 34.8 μmol/min/gw; crucian carp, 237 μmol/min/gw and brook trout, 330 μmol/min/gw: Johnston & Moon, 1980a;1981).

These low PFK and LDH activities when considered along with the low blood lactate found at rest and even after hypoxic stress in some Antarctic species (Hemmingsen & Douglas,1970) suggests a low capacity for anaerobic glycogenolysis. However the maximum mechanical power output of Antarctic fish fibres is similar to other fish (Gleeson et al,1983; Johnston & Harrison,1985). It has been suggested that the fuel for sprint activity in Notothenioid fish is phosphagen based, and that phosphocreatine stores in the white muscle are sufficient to support a large number of sprints of short duration (Johnston & Harrison,1985). Field and aquarium observations of swimming and feeding behaviour of N.neglecta and other Antarctic species are consistent with this theory (Twelves,1972; Daniels,1982; Montgomery & Macdonald,1984). Personal observation in the field and in aquaria shows N.neglecta to be a relatively sluggish species; it is a generalist feeder, feeding off sluggish prey, and is mainly active at night (Richardson,1975;

Permitin & Tarverdieva,1978; Burchett,1982; Daniels,1982).

The majority of Antarctic species are labriform swimmers, and such fibres in have been shown to be highly aerobic (Lin et al,1974; Kryvi & Totland,1978; Walesby & Johnston,1980).

The metabolic profiles of red fibres from the pectoral adductor muscle of other Antarctic species have been examined (Walesby & Johnston,1980; Walesby et al,1982; Johnston & Harrison,1985). The activity of aerobic enzymes in the pectoral fibres is greater than in the trunk muscles for both the red-blooded N.rossii and the haemoglobinless C.aceratus, but for N.neglecta the pectoral fibres and red trunk muscle fibres have similar aerobic enzyme activities (Fig. 3.1).

The activities of anaerobic enzymes in the pectoral fibres of N.neglecta are only about 40% of those in the white fibres (PFK activity), and are more similar to anaerobic enzyme activities in the red muscle. Some of the differences noted for the enzyme activities of the pectoral muscle of N.neglecta when compared to the other Antarctic species may be due to investigating different muscles. In N.rossii and C.aceratus, the pectoral adductor was examined, and it is this muscle that provides the main power stroke in labriform swimming (Webb,1973; Walesby & Johnston,1980; Blake,1981). However, the muscle studied in N.neglecta was the pectoral abductor muscle. This muscle pulls the fin away from the body, before the power stroke begins, and this movement requires only about 5% of the effort involved in the power stroke (Blake,1981). Consequently the activities of aerobic enzymes in the pectoral abductor muscle might be

expected to be less than those in the pectoral adductor.

If the propulsive effort for sustained swimming is provided by the pectoral fins, then the presence of aerobic red fibres in the trunk is something of a puzzle. In the pectoral-swimming holocephalan Chimaera monstrosa it is proposed that the trunk is used for rudder-like movements associated with fine adjustments in direction, and that the red fibres mediate these movements (Kryvi & Totland,1978). Similar rudder-like movements have been seen in Antarctic fish in the field (Robilliard & Dayton,1969; Twelves,1972; Montgomery & Macdonald,1984) and it is suggested that the function of the red myotomal fibres in Antarctic fish is the same as for C.monstrosa. Such adjustments of movement would be required both during high speed and low speed swimming, and could be the reason for the similar glycolytic capacity of the red and white myotomal fibres in N.neglecta.

3.4.1.2. Cardiac muscle.

Cardiac muscle requires a constant supply of ATP in order to meet the demands of constant contractile activity. In fish, cardiac muscle has a greater ability to utilise glucose for metabolism (high HK activity) than any other tissue, a high ability to oxidise fatty acids for fuel (Bilinski,1974), a higher aerobic capacity than either red or white trunk muscle, and a glycolytic capacity equal to or less than red muscle (Cowey & Walton,1982). During conditions of hypoxia or anoxia, the heart is able to use lactate and convert it to pyruvate for aerobic oxidation (Driedzic et al,1979). The data obtained from N.neglecta is in accordance with this scheme (Figs. 3.1 & 3.2), with high

cardiac HK, MDH and COX activities. Generally the hearts of ectothermic species such as fish have a greater dependance on a glucose based metabolism than endotherms (MacIntyre & Driedzic,1981) and the higher HK activity relative to 3HACDH activity in the cardiac muscle of N.neglecta supports this theory. Relative to other tissues the heart is best suited to utilise lipids as a fuel source (Fig. 3.1), and under anaerobic conditions the heart of N.neglecta also appears to have a good glycolytic capacity as shown by the high activities of PK and LDH (Fig. 3.2).

3.4.2. Size-related changes of enzyme activity in Notothenia neglecta.

There are relatively few studies on the effect of ageing in fish, and of these most are concerned with anatomical features rather than chemistry/biochemistry. Sometimes a striking loss of skeletal muscle mass occurs (Robertson,1961) and the fibre diameter decreases (Greer-Walker,1970). Degeneration of the gonads (Woodhead,1974a,b) and the thyroid (Woodhead & Ellett,1966) occurs, generally characterised by the laying down of increased amounts of connective tissue. There are even fewer reports concerning the effect upon enzyme activities of post-embryonic/postlarval growth (Bostrom & Johansson,1972; Beitner et al,1979; Somero & Childress,1980).

Although the size range studied for N.neglecta appears small, it does cover fish aged from 2-18+ years, and only

misses the postlarval fingerling stage. On the whole, changes of aerobic enzyme activity with size appear more marked in the red muscle (Fig. 3.3). In white muscle the changes in activity are smaller and usually not significant. Somero and Childress (1980) also found a decrease in citric acid cycle enzyme activities as fish size increases, and that the scaling of the decline was almost identical to that found for the decrease in maximum oxygen consumption (VO_2) with increasing size. A parallel decrease in the total mitochondrial content of the muscles with increasing size has also been noted (Mathieu et al,1981).

As well as a reduced aerobic capacity in older specimens of N.neglecta, there is a reduction in HK activity and therefore maximum rate of glucose utilisation, this reduction being significant in the white muscle ($P<0.05$). Lipids are an alternative fuel source, particularily for the red muscle (Jonas & Bilinski,1964; Zammit & Newsholme,1979), and as HK activity declines there is a rise in 3HACDH activity (Fig. 3.3). So as the ability to utilise glucose decreases there seems to be an increased reliance on fatty acids.

There is a general overall reduction in the activity of enzymes involved in anaerobic glycogenolysis (Fig. 3.4), particularily with regard to LDH activity. This finding is in contrast to that of Somero and Childress (1980) who found that in half of the species they studied, increasing size leads to a fall in VO_2 and aerobic enzyme activity but a rise in LDH activity. These results suggest that whereas MDH (and CS) activity, and therefore aerobic metabolism, appear to correlate well with respiratory function (VO_2),

something other than oxygen supply and demand is determining LDH activity in the muscle tissues. Also, the different trends shown by Antarctic and temperate fish for LDH activity with increasing size indicates that different regulatory mechanisms may be controlling LDH activity. Anaerobic enzyme activities in non-locomotory tissues, such as brain, or cardiac muscle, do not scale with increasing size or depth (Beitner et al,1979; Childress & Somero,1979; Somero & Childress,1980) and since the mode of locomotion of Antarctic species relies primarily on the pectoral fins, it may be that the enzyme activities in the pectoral fibres would scale better with increasing size or depth.

Factors other than increasing size *per se* may cause an alteration of the enzyme profile; migratory or spawning behaviour,which may involve a change in the lifestyle of the fish (e.g. the eel; Bostrom & Johansson,1972), or different feeding behaviour patterns between older and younger fish. Tagging experiments indicate that *N.neglecta* is neither migratory, nor does it move offshore to spawn (Everson,1969). However, there is some evidence that the feeding strategy of mature *N.neglecta* differs from that of the smaller and younger specimens (Daniels,1982). For fish below 231mm SL, isopods and amphipods make up 60-75% of the diet. In larger fish, euphausids and other fish make up most of the diet, with algae comprising about 20%. Amphipods and isopods account for less than 10% of the diet in the larger fish (258mm+ SL). Why this should alter the ability to utilise glucose and lipids is not clear. There is a broad tendency for polar zooplankton (and to some extent, polar marine invertebrates) to be rich in lipid

(Clarke,1983). So it is possible that the potentially less lipid-rich diet of the larger fish (due to the lower consumption of lipid-rich zooplankton) accounts for the reduction in 3HACDH activity in the white muscle (Fig. 3.3) while in the red muscle (where lipids are a more important fuel source) 3HACDH activity is maintained and increased to make better utilisation of the available lipid.

Finally, a reduction in enzyme activity with increasing size may just be part of an overall ageing process (Hocman,1979). Protein synthesis has been shown to decline as fish age (Tano & Shirihata,1975; Gomez-Jarabo et al,1976), so reduced enzyme activity may arise due to reduced synthesis of enzyme. However this ageing process doesn't appear to affect all enzymes equally, since overall there is no reduction in PK activity, and only slight reductions in other enzymes (Figs. 3.3 & 3.4).

In conclusion, size-related changes in enzyme activity appear to be of little overall significance for N.neglecta and those that do occur seem more related to utilisation of fuel sources rather than overall scope for activity. These changes may be related to the different feeding strategies and diets of small and large fish rather than any variation in size per se.

3.4.3. Interspecific comparison of the red and white trunk muscle of three species of Antarctic fish.

T.newnesi is a benthic teleost found only in shallow waters, rarely venturing any deeper than 20m, while adult N.gibberifrons can be considered a deep water species, found

at depths down to 350m (DeWitt,1971), and not recorded shallower than 25m (Everson,1970a). Like N.neglecta, both T.newnesi and N.gibberifrons are benthic and are labriform swimmers. From studies of pelagic species of similar behavioural ecotypes inhabiting different depths in the water column, respiratory rates decline exponentially with increasing depth (Childress,1971;1975; Torres et al,1979), while there is also a decrease in both aerobic and anaerobic enzyme activities particularily in the white trunk muscle (Childress & Somero,1979). There seems to be no increased reliance on anaerobic metabolism at depth, since the decline in PK activity occurs at the same rate as for MDH and CS (Childress & Somero,1979). If the same trends occur in benthic species as occurs in pelagic species, a reduction in enzyme activities might be expected in the progression T.newnesi >= N.neglecta > N.gibberifrons. However since N.neglecta is such a wide-ranging species (found at depths from 0-200m; DeWitt,1971), any differences will be more pronounced between T.newnesi and N.gibberifrons. Enzymes of aerobic metabolism in the three species are compared (Table 3.1). N.gibberifrons has the lowest activity of HK and respiratory chain enzymes (in both red and white muscle), while the highest activities are found in T.newnesi; this parallels the trends found by Childress and Somero(1979), despite the smaller depth range being used in this study. However, if glycolytic enzymes are compared (Table 3.1), the reverse trend is seen, so that in benthic species, unlike pelagic species, some form of compensation is taking place to make up for the reduced aerobic capacity at depth. The differences appear most marked in the white

muscles, and although the trends are the same in red muscles, the differences are generally not significant.

Size-related rather than depth-related differences can probably be discounted. In a study of a large number of pelagic species it was concluded that interspecific size effects explained none of the variation of enzyme activity either at a given depth or as a function of depth, and that the magnitude of depth-related changes in activity far outweighed any interspecific size-related changes (Childress & Somero,1979). It has already been shown that intra-specific size-related differences in enzyme activities for N.neglecta are only significant in a few cases (see section 3.4.2.). However, a more recent study by Somero and Childress (1980) indicated that glycolytic enzymes increased in activity in larger fish. Consequently the difference in activities of glycolytic enzymes between N.gibberifrons and T.newnesi maybe because of this interspecific scaling effect, N.gibberifrons being the larger of the two species.

One factor that might influence the musculature of the fish is their mode of life (Boddeke et al,1959), and possibly the different feeding habits of the three Antarctic species might account for their different enzyme profiles. The feeding strategies of Antarctic fish have been studied (Daniels,1982) and marked differences were observed between the three species. N.neglecta was characterised as an 'ambush' feeder, lurking among rocks or weeds until it's prey comes within lunging distance. This species would therefore require a high capacity for burst activity for lunging at it's prey. This is borne out by the high PFK

activity in this species compared to T.newnesi, and is further support for a phosphagen based metabolism for sprint swimming (Johnston & Harrison,1985). N.gibberifrons sometimes shows this type of behaviour but more often is a 'slurp' feeder. It swims along muddy bottoms sucking up and sifting through the mud, consuming any organisms it finds there. Pectoral fins are it's main mode of locomotion, and so this species has little requirement for a well developed active red myotomal musculature. However it has a higher glycolytic capacity than either of the other two species. This is probably more concerned with evading predators itself. Several species of seal consume fish (Dearborn,1965; Stonehouse,1972) and a muddy bottom is an exposed place; N.gibberifrons will need a high fast-burst capacity to reach the nearest cover as quickly as possible.

Of the three, only T.newnesi feeds in the water column, consuming motile invertebrates such as the krill Euphausia superba, and amphipods and copepods. Obviously T.newnesi will be the most active predator and would require the most active metabolism, especially if it has to maintain it's position in the water column without the aid of a swim-bladder; the high aerobic capacity found for T.newnesi supports this theory. Since euphausids and amphipods in the water column are relatively slow moving there would be no need to develop an extensive glycolytic metabolism for high speed swimming/cruising. Shoals of T.newnesi often hang over weed beds and dive into the cover of the weed bed when approached. Thus sprints will be of only a short duration so that like N.neglecta sprint swimming may be sustained by phosphagen based metabolism.

In conclusion, different enzyme profiles are found in the trunk muscles of three Antarctic species; N.neglecta, N.gibberifrons and T.newnesi. These species live at different habitat depths, have different feeding strategies, and are of different maximum sizes; it is probably the behavioural aspects that have influenced the development of the different enzyme patterns in these three species, rather than any other factors.

SPECIES	ENZYME ACTIVITY (μmol/min/g.wet wt.)	
	AEROBIC ENZYMES	
	CYTOCHROME CXIDASE	HEXOKINASE
Notothenia neglecta	10.86 / 1.69	0.294 / 0.124
Nctothenia gibberifrons	nd	1.980 / 2.360
Trematomus newnesi	17.93 / 3.81	0.091 / 0.032
	ANAEROBIC ENZYMES	
	PHOSPHOFRUCTO KINASE	PYRUVATE KINASE
N. neglecta	1.24 / 1.22	7.68 / 3.88
N.gibberifrons	3.67 / 3.87	12.2 / 10.3
T.newnesi	0.72 / 0.28	10.0 / 2.92

	LACTATE D´H´ASE
N. neglecta	42.6 / 34.8
N.gibberifrons	73.5 / 54.9
T.newnesi	69.7 / 28.3

Weights (gm) and Standard Lengths (mm), (Mean +/- SEM):

N.neglecta; 1072 +/- 48, 375 +/- 8

N.gibberifrons; 425 +/- 47, 281 +/- 17

T.newnesi; 8.25 +/- 0.55, 89 +/- 2

TABLE 3.1. Enzyme activities in the trunk muscles of three Antarctic species (red / white trunk muscle). nd - no data.

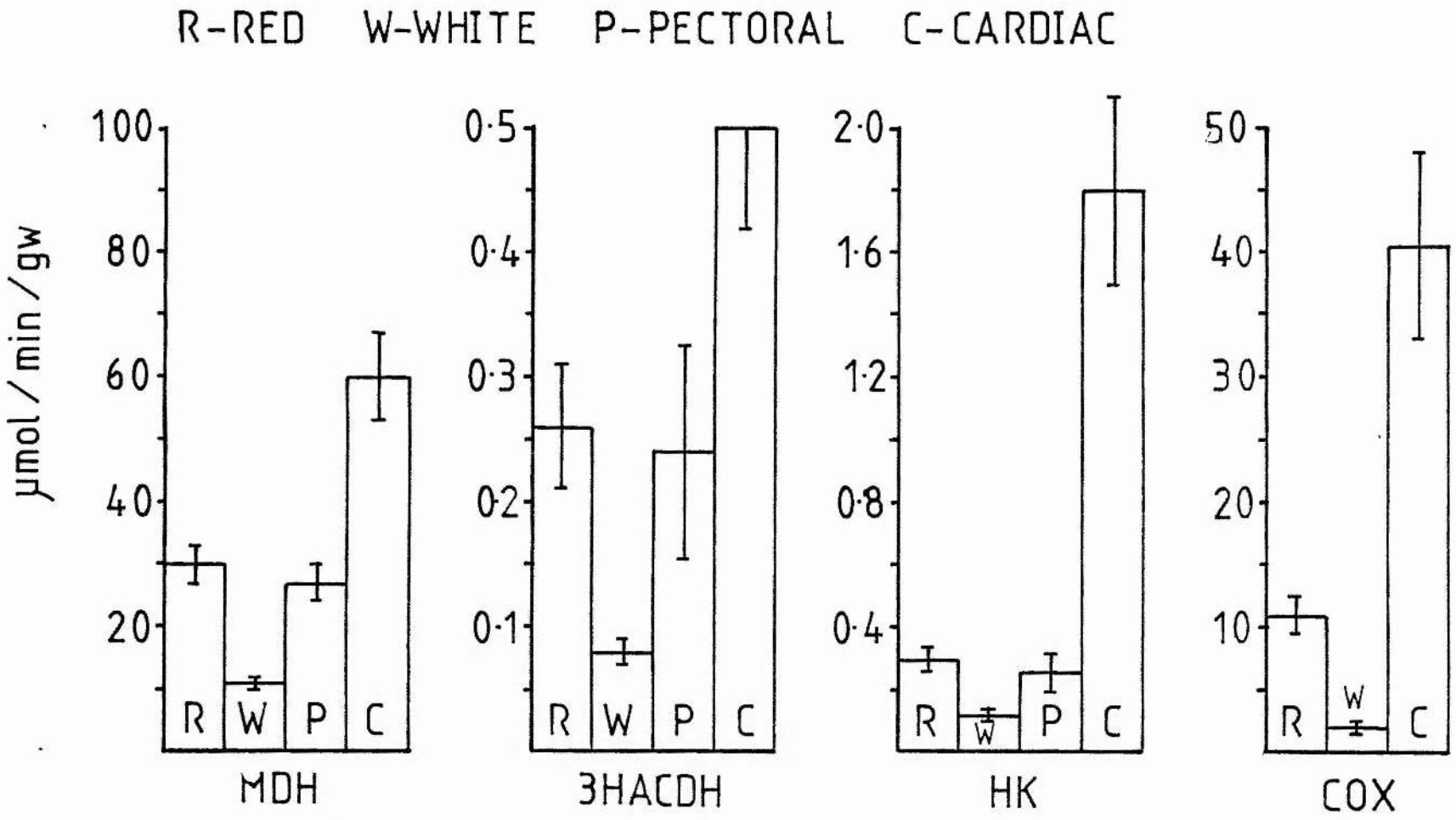


Fig. 3.1 Aerobic enzyme activities in various muscles of N.neglecta

(means ± sem. n at least 6 fish) MEAN WEIGHT (+/-SEM): 1001+/- 48 g

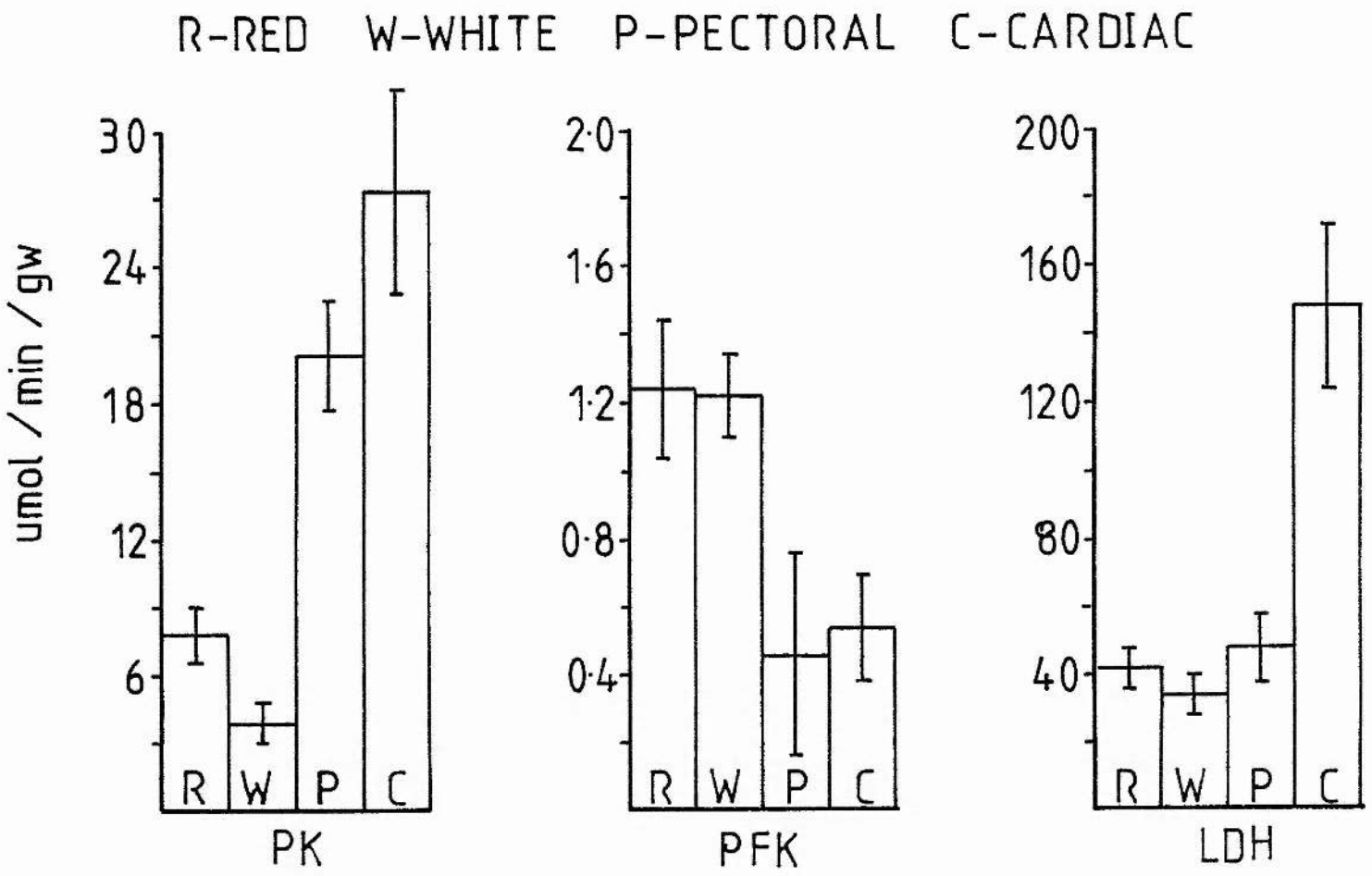


Fig.3.2 Anaerobic enzyme activities in various muscles of N.neglecta (means ± sem. n at least 6 fish) MEAN WEIGHT (+/- SEM): 1001+/- 48 g

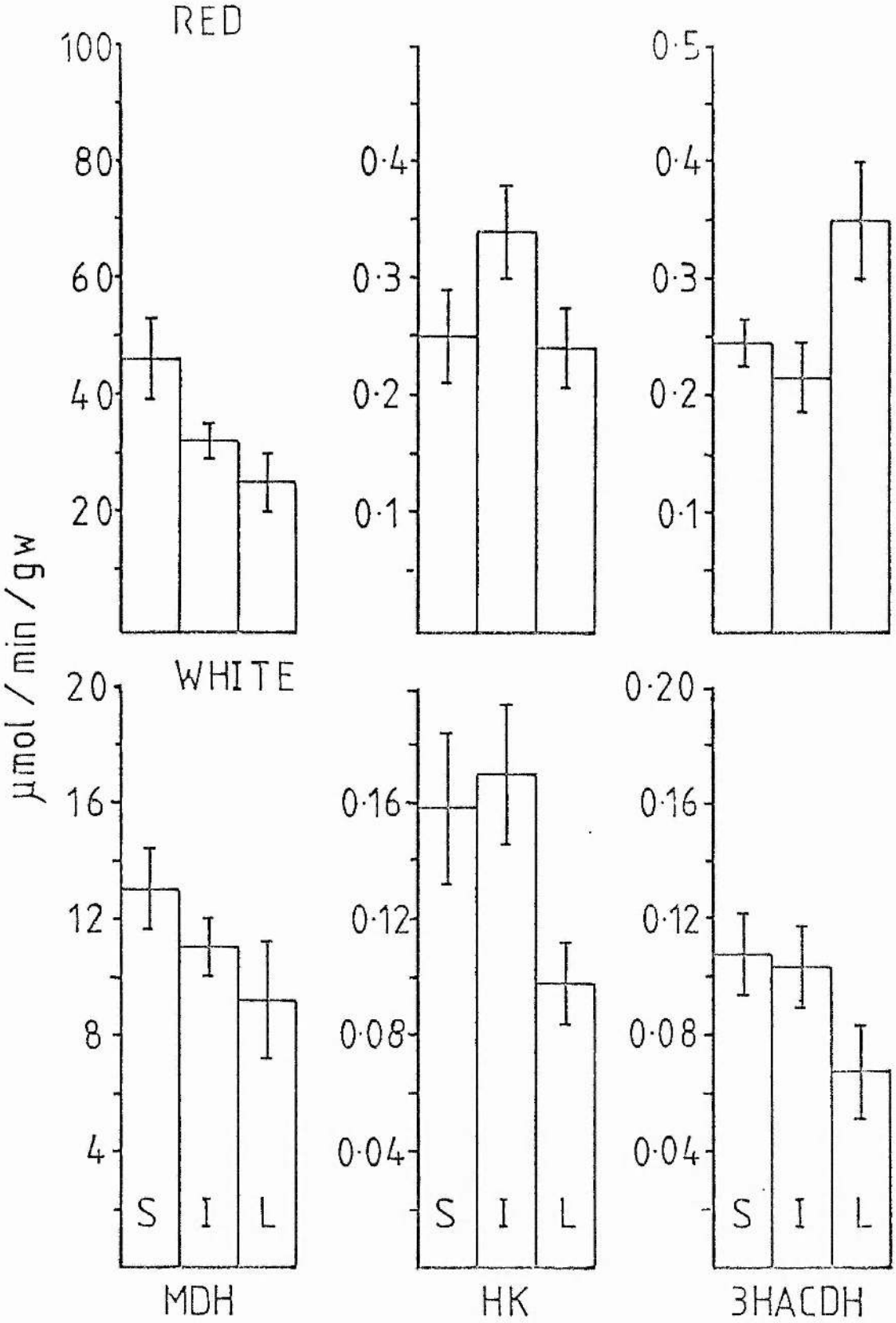


Fig. 3.3 Aerobic enzyme activities in trunk muscles of N.neglecta of increasing size. S-small: I-intermediate: L-large. (means sem, n at least 10 fish)

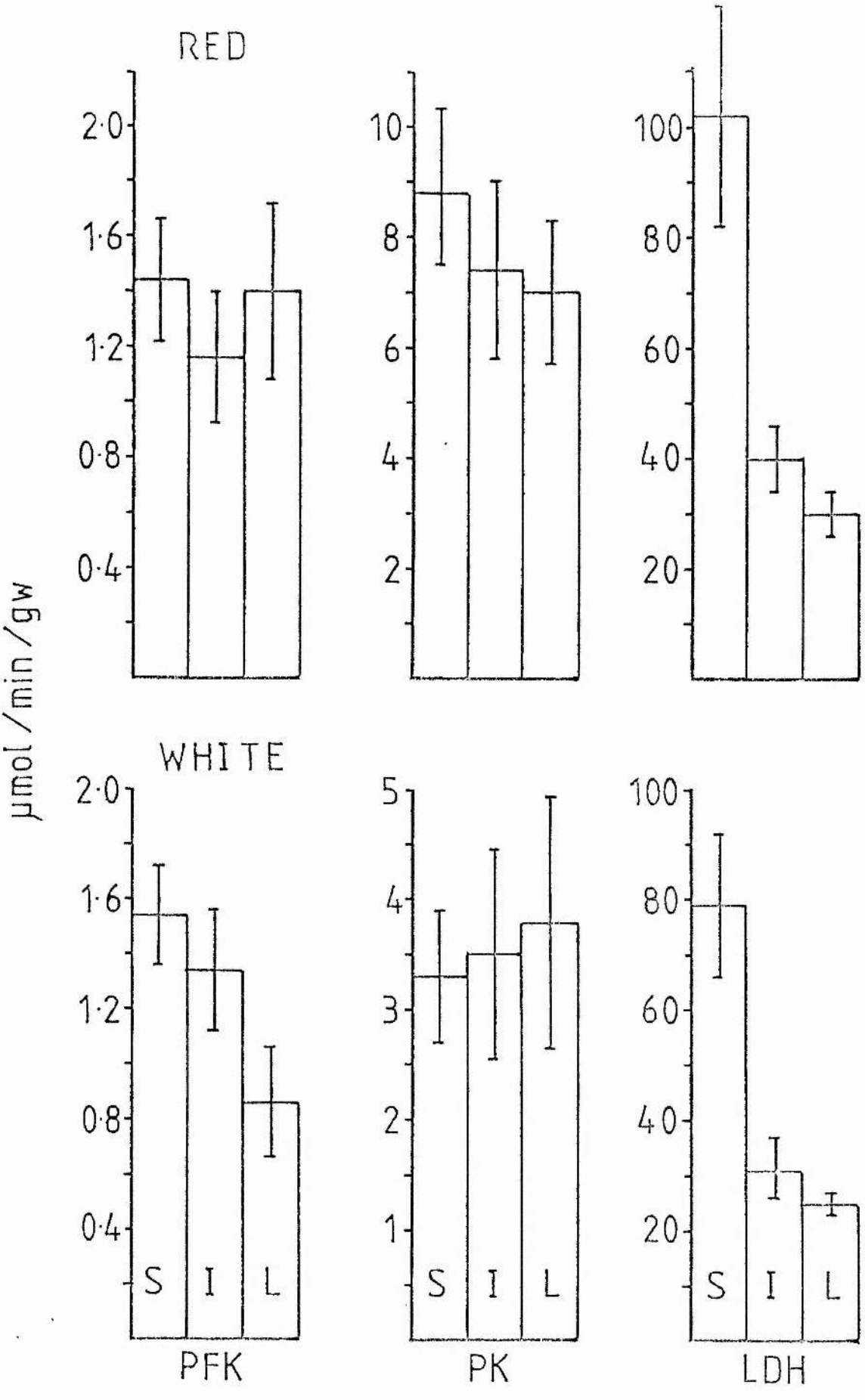


Fig. 3.4 · Anaerobic enzyme activities in trunk muscles of N.neglecta of increasing size. S-small: I-intermediate; L-large. (means± sem, n at least 10 fish

CHAPTER 4. Changes in muscle morphometry and capillarisation in NOTOTHENIA NEGLECTA and the Icefish CHAENOCEPHALUS ACERATUS.

4.1. Introduction

In most fish, growth is accompanied by an increase in the number of muscle fibres (Greer-Walker,1970; Strickland,1975; Weatherly & Gill,1985), as well as hypertrophy of existing fibres. This is unlike the situation in mammals where the number of fibres is fixed at birth and hypertrophy is the only means of growth, e.g. the mouse (Goldspink,1970). During early development myosatellite cells are found in the muscles of higher vertebrates and appear to be responsiblefor increasing the number of fibres (Nag & Nursall,1972). Their continued presence after birth may be to maintain muscle integrity or to replace damaged fibres (Goldspink,1970). Myosatellite cells are also present in fish muscle although precise evidence for their function is lacking. Hyperplastic growth could be responsible for the ´mosaic´ appearance of white muscle in some fish species. The small fibres are thought to be newly generated (Akster,1983), but whether from myosatellite cells or by splitting of existing fibres is uncertain (Scapolo et al,1984).

Hypertrophy does involve an increase in the number and size of myofibrils within the fibres (Greer-Walker,1970;

Goldspink,1970). It seems that new myofibrils may arise from longitudinal splitting of existing larger myofibrils (Goldspink,1970) and not through de novo synthesis. In the cod (Gadus morhua) the increase of fibre size with length is sigmoidal, the slope starting to decrease after the fish pass a standard length of 40-50cm, a point which coincides with the onset of spawning behaviour (Greer-Walker,1970).

Investigations of intrafibrillar components other than myofibrils have only recently been carried out. Mathieu et al (1981) have shown that the volume density of the mitochondria is inversely correlated with body size in a wide range of mammalian species, but quantification of this type of data with regard to fish (of increasing size) is scarce.

Measuring the density or surface area of the skeletal muscle capillaries provides information on the structural limitations for gas and/or metabolite exchange. The capillary supply to mammalian muscles has been correlated with metabolic fibre type (Romanul,1965), mitochondrial content of the muscle fibres (Hoppeler et al,1981), body size (Schmidt-Neilsen & Pennycuik,1961; Pietschmann et al,1982) and whole animal VO_2 (Weibel et al,1981). In fish species capillary supply to the trunk musculature has been linked with acclimation to lower temperatures (Johnston,1982) and hypoxia (Johnston et al,1983; Johnston & Bernard,1984). In mammals study of the capillary supply to the muscles is complicated by the fact that a single capillary may supply three or more metabolically distinct fibre types. The situation in fish is simplified by the arrangement of the different fibre types into anatomically

discrete layers, and this provides a useful model for determining quantitative relationships between fibre type and capillary supply.

Undoubtedly the advent of simplified stereological methods has made the quantification of capillary density and intrafibrillar components more precise and simpler to perform. Stereology is a mathematical means of relating 3-dimensional parameters defining a structure to 2-dimensional measurements obtainable on sections of that structure (Weibel,1979). The capillary supply to the trunk muscles of adult N.neglecta and the intrafibrillar components have been quantified and compared to the same parameters in a range of fish of increasing size to see if any structural/ultrastructural changes have occurred with growth. A comparison has also been made with the haemoglobinless Icefish C.aceratus.

4.2. Materials and Methods

4.2.1. Fish.

Specimens of N.neglecta were caught by trammel net in Borge bay (sites C, E & F, fig.2.2) and returned live to the wet-lab., where they were maintained in through-flow aquaria (water temperature 0-2^{o}C). Fish were fed on a diet of chopped fish and amphipods until 24-48 hours before use. Commonly the fish were used for other experiments, and samples of red and white muscle fixed for electron microscopy as a by-product. Adult fish (320-385mm SL) were investigated and compared with fish of increasing size from 117-300mm SL. In some examples the fish of increasing size

were grouped as 117-200mm SL, 200-250mm SL and 250-300mm SL. Adult specimens of C.aceratus were also caught by trammel net, off Owen's Bank (site D, fig.2.2). Mean weight and standard length (+/- SEM) were 1040g (+/- 190g) and 476mm (+/- 29mm) respectively. Fish were killed by stunning followed with transection of the spinal cord. Red muscle samples were dissected out from the region adjacent to the lateral line canal, and white muscle was taken from the deep epaxial muscle mass (i.e. same regions as used for the enzyme studies -see Fig. 2.4).

4.2.2. Tissue processing.

Samples were fixed overnight in 3% glutaraldehyde in 0.15M sodium phosphate buffer, pH 7.4 at 4°C. The following day samples were cut into small tissue blocks of dimension 5 x 1 x 1mm, and further fixed in the same fixative for 2 hours to allow complete penetration. This was followed by two washes in the phosphate buffer and post-fixation for 1 hour in 1% osmium tetroxide in 0.15M sodium phosphate buffer, pH 7.4 at 4°C. Samples were then washed twice in distilled water and dehydrated through a series of alcohols to absolute, at room temperature. Clearing was carried out with epoxypropane (3 x 20 minutes) and the tissue was left overnight in a 1:1 epoxypropane/araldite mixture. Infiltration was carried out with freshly prepared araldite CY212 for 4 hours, followed by embedding in fresh araldite. The embedding trays were left in an oven at 60°C for 48 hours for hardening. At least 6 transverse (TS) blocks were prepared for each muscle type in each fish, as well as 3-6 longitudinal (LS) blocks. Blocks from each fish were selected at random for sectioning.

4.2.3. Measurement of fibre cross-sectional area and capillary density.

Semi-thin (0.5-1.0um) sections were cut on a Reichert OM-U2 ultramicrotome, stained with toluidine blue and photographed using a Zeiss optical microscope. An optical graticule was also photographed at the same magnification for accurate scaling. Negatives were projected onto plain white paper (final mag. x180) and fibres were drawn around and capillaries marked in. Fibre cross-sectional areas were measured using a digital planimeter interfaced to an Olivetti P6060 microcomputer. Reproducability was to within 2%. Capillary numbers were counted directly. Total area of the section was digitised and capillary density calculated.

4.2.4. Capillary anisotropy, surface and volume densities.

Capillaries were identified using criteria established by Bruns and Palade (1968), as thin-walled exchange vessels with well developed pericytes. The orientation of the capillaries in the vascular bed can be calculated using TS and LS sections. The length density of a component is related to it's numerical density such that Jv is proportional to QA(theta), where QA (theta) is the numerical density derived from a section whose normal (a projection at 90^{o} to the section) forms an angle (theta) with the axis of anisotropy. For TS sections theta = 0, and for LS sections theta = pi/2, and so;

for perfect anisotropy, Jv = QA(0)

for perfect isotropy, Jv ~ 2QA(pi/2) (Weibel,1980)

As the muscle capillary bed has a high (but unknown) degree of anisotropy, and as it is technically very

difficult to measure the angle theta, it is necessary to evaluate a concentration parameter k. So, Jv = c(k,theta) x QA(theta). Assuming the distribution of capillary orientations approximates to a spherical normal distribution it can be shown that (Cruz-Orive,1982),
k = QA(0)/QA(pi/2) = c(k,pi/2)/c(k,0). QA(0) and QA(pi/2) are the capillary densities in TS and LS sections respectively, and c(k,0) is found from a table (Mathieu et al,1985, table 1). Once this factor is known the capillary length density can be calculated from any TS section thus; Jv(c,f) = c(k,0) x QA(0) (= NA(c,f), capillary density). This then allows calculation of capillary volume and surface densities, Vv(c,f) and Sv(c,f) respectively;

Vv(c,f) = a(c) x Jv(c,f)

Sv(c,f) = b(c) x Jv(c,f).

a(c) and b(c) are the mean capillary cross-sectional area and perimeter length respectively. The capillary volume density is an estimate of the volume of blood within the capillary network, and the capillary surface density is an estimate of the total capillary surface area available for gas exchange.

The concentration parameter was calculated from ten LS and ten TS blocks.

4.2.5. Volume densities of intrafibrillar organelles.

Ultra-thin (0.06-0.07um) sections were cut on the Reichert ultramicrotome and those sections showing silver or gold interference patterns were picked up on pyroxyline coated 150 mesh copper grids. Sections were double stained in 2% uranyl acetate (40-60 mins) and Reynold's (1963) lead citrate (4-6 mins). Sections were viewed and photographed

on a Phillips 301B transmission electron microscope at 40kV.

The stereological analysis of organelle volumes requires the use of theoretical models proposed by Weibel (1973,1979), using a simple coherent quadratic test system (Weibel,1980). All stereological measurements are ratios of at least two joint measurements; one relating to the component under study (e.g. the mitochondria), and the other to the structure as a whole (e.g. the muscle fibre). Stereology also comes with it's own notation; the ratios are expressed as a double symbol of two capital letters, the first defining the parameter under study, the second the parameter of the reference system. For example, the volume density of the mitochondria in a muscle fibre is a ratio of the volume density of the mitochondria to the volume of the fibre, and is expressed as; Vv(mit,f). Electronmicrographs (x2500-9100) were projected onto a 1cm square lattice counting grid at varying projector magnifications (x2.5-3.5) such that the grid spacing was between 1.0 and 1.5 times the average diameter of the component being studied. Volume densities were determined by point counting at line intercepts such that;

$Vv = Pi/Pt$ Vv - volume density; Pi-number of points falling on a component; Pt-total number of points falling on a profile (fibre).

With components of different sizes, a different grid spacing is optimal so that for maximum accuracy multiple grid sizes are required (Weibel,1973). However for components of roughly similar sizes this error is small compared to experimental error. Point counting methods are to an extent insensitive to the angle of sectioning, or the

degree of anisotropy of the component (Weibel,1980) so that this method can be used even if the sections are slightly out of true. Orientation of the counting grid with respect to the fibre seems not to be of great importance (Egginton,1982).

4.2.6. Statistics.

Student's t-test and linear regressions were used where appropriate.

4.3 Results

4.3.1. Gross morphology.

4.3.1.1. The slow muscle of N.neglecta of increasing size.

The fibres in the slow muscle of N.neglecta are large and exhibit a wide variation in cross-sectional area (plate 4.1a) and overall shape. Smaller fibres are usually circular or roughly triangular in shape, while the larger fibres are either square or rectangular. The distribution of slow fibre cross-sectional areas in fish of increasing size groups are shown in (Fig. 4.1). The distribution of areas in the smallest size range shows that over half the fibres have an area of less than 1999μm^2. As fish size increases, a bimodal pattern is seen with peaks at 5000-5999μm^2 and 1000-1999μm^2. As further increases in fish size occur, the distribution becomes unimodal again, the peak value increasing to 5000-5999μm^2 in the adult fish (Fig. 4.1). Over three-quarters of the cross-sectional areas in adult fish lie between 3000 and 7999μm^2. Myosatellite cells were observed in both large and small N.neglecta, but were much more common in the smaller

specimens.

Capillaries were identified as thin-walled exchange vessels with pericytes (plate 4.3). Capillaries were often situated at the junctions of muscle fibres, although not exclusively so (plate 4.1b). The mean capillary area and perimeter in mature (>320mm SL) N.neglecta (n=30) were 55± 5 um^2 and 35.2 ± 1.5 um^2 (means ± SEM) respectively. The orientation of the capillary bed appeared to be highly anisotropic with less than 3% of capillary profiles appearing on longitudinal sections. Accordingly a value of 1.015 was calculated for the concentration parameter (k). A mean value for other functional parameters are given in table 4.1.

The capillary density for mature specimens was 237 ± 16 capillaries/mm^2 (mean ± SEM, n=6 fish); although the capillary density appeared higher in smaller fish, there was no correlation (fig. 4.3). Whether or not a decreasing capillary density in larger fish is offset by increasing capillary cross-sectional area is difficult to ascertain, because of the fewer measurements of capillary cross-sectional area in smaller specimens.

4.3.1.2. Comparison between N.neglecta and the Icefish C.aceratus.

The slow fibres of the Icefish are smaller in cross-sectional area than for N.neglecta of equivalent standard length, and show a much smaller variation in cross-sectional area (plate 4.1c). The distribution of slow fibre cross-sectional areas for mature N.neglecta and C.aceratus is compared in figure 4.2. The distribution is more spread out for N.neglecta which has the higher mean cross-sectional area (6183um^2). Over 75% of the

cross-sectional areas for C.aceratus lie between 1000 and 3000μm^2, (mean 2663μm^2).

Slow fibres of C.aceratus appear to show a greater region of subsarcolemmal mitochondria (compare plates 4.1a,b with c,d, and 4.3a,b with 4.3c,d).

Capillaries were slightly larger in the Icefish (plate 4.3c,d), the mean cross-sectional area and perimeter (±SEM) being 64± 7μm^2 and 33 ± 3 μm respectively. This difference was not significant (t-test). As for N.neglecta the capillaries were usually located at the junction of three or more fibres. The capillary density was significantly higher ($P<0.001$) in the slow muscle of C.aceratus with a value of 544 ± 38 capillaries/mm^2 (n=6 fish). Other functional parameters of the capillary bed for the Icefish are shown in table 4.1, which compares the data obtained for Antarctic species with that obtained from temperate species.

4.3.2. Organelles.

4.3.2.1. Slow muscle fibres of N.neglecta of increasing size.

The volume densities of mitochondria and myofibrils were monitored for a number of specimens of N.neglecta over a size range of 117-380mm SL. Myofibrillar volume density was inversely correlated with fish size as was mitochondrial volume density (Fig. 4.4). The difference in mitochondrial volume density between juvenile and adult specimens is shown by plate 4.4a,b. Since the mitochondria are the actual utilisers of the oxygen supplied, some relationship between mitochondrial volume density and capillary density might be expected. There was no direct correlation between these two

parameters for N.neglecta, although smaller fish appeared to have higher densities of capillaries and mitochondria than larger fish (Figs. 4.3 & 4.4).

Myofibrillar splitting has been proposed as a means of hypertrophy in fish muscle, and regions of myofibrillar splitting were observed in juvenile N.neglecta (plate 4.2c).

In determining the mitochondrial volume density no distinction was made between intermyofibrillar (IF) or subsarcolemmal (SS) mitochondria, but from a small random sample (n=18) intermyofibrillar mitochondria accounted for about 65% of the total. There appeared to be no correlation of either IF or SS mitochondria with fish size. Morphologically there appeared to be no difference in the structures of either IF or SS mitochondria in both small and large specimens of N.neglecta(plates 4.5a,b).

4.3.2.2. Comparison between mature N.neglecta and C.aceratus.

The volume density of mitochondria in the 'red' muscle of C.aceratus was nearly twice that of the red-blooded N.neglecta(plates 4.4b,c); the same relationship as found for capillary density when comparing the two species. So trends between species are for a higher capillary density to be associated with a higher mitochondrial volume density.

As for N.neglecta there appeared to be no difference in the morphology of IF and SS mitochondria in the Icefish (plates 4.5c,d). A few samples of fast muscle from the Icefish were examined. The large size of the fibres (often

greater than 25000 μm^2 in cross-sectional area) prevented proper stereological analysis because only a small portion of the fibre was in the field of view of the microscope at any one time. However it was noticed that the morphology of IF mitochondria in the fast muscle was different to that of the analagous mitochondrial population in the slow muscle (plates 4.5e,f). The cristae in fast muscle mitochondria was much less extensive than for slow muscle mitochondria

4.4 Discussion

A major characteristic in the physiology of Antarctic fish is their reduced levels of haemoglobin when compared to temperate species (Everson & Ralph,1970). This condition reaches an extreme in the Channichthyidae ('Icefishes') which totally lack the respiratory pigments haemoglobin and myoglobin (Ruud,1954; Hureau,1966; Walesby et al,1982). The presence of blood pigments greatly enhances the rate of oxygen diffusion, even at low PO_2's (Wittenberg,1963), so the reduced levels of haemoglobin in N.neglecta implies a reduced delivery of oxygen to the tissues. In mammals adapted to low PO_2's (e.g. animals living at altitude) alterations in the uptake efficiency of oxygen occur (increased alveolar capacity, increased diffusion capacity across the lungs)(Weibel et al,1981). In Antarctic fish there seems to be no adaptation at the level of the oxygen uptake apparatus; the gills are similar in morphology and size to those of temperate species and are not well adapted for increased efficiency of oxygen extraction (Hughes,1966;

Steen & Berg,1966; Westermann et al,1985). There is some debate as to whether or not cutaneous respiration has a role to play in channichthyids (Hemmingsen & Douglas, 1970). So any adaptation to overcome the reduced oxygen carrying capacity of the blood must occur in the transport process or in oxygen uptake by the tissues. The principle of symmorphosis states that the structural design of a system is matched to it´s functional requirements through regulated morphogenesis (Taylor & Weibel,1981). How does the respiratory physiology of Antarctic fish, and in particular N.neglecta, bear this out ?

At the physiological level several adaptations have taken place to overcome this supply problem. The blood volume is larger in Antarctic fish than in temperate teleosts, and in the icefish C.aceratus accounts for over 9% of the total body weight (Everson & Ralph,1970; Hemmingsen & Douglas,1970). There is a significant increase in cardiac output (Holeton,1970; Hemmingsen et al,1972) delivered at a high stroke volume and low heart rate by a heart that is much larger than temperate teleosts (Hemmingsen et al,1972; Twelves,1972; Johnston, Fitch et al,1983). The systemic blood pressure is low (Hemmingsen & Douglas,1970;1972) and this is brought about by enlarged capillaries (Fitch & Johnston,1983). The overall respiratory capacity may be low as inferred by a low basal metabolic rate (Everson & Ralph,1970; Holeton,1970).

What is happening at the cellular level ?

It is immediately apparent that the mean cross-sectional area for the slow fibres of N.neglecta and C.aceratus are much larger than fibre cross-sectional areas found in

temperate teleosts (Table 4.2). It appears that there is a general trend for Antarctic fish to have larger fibres than temperate species (Smialowska & Kilarski,1981). Why this should be so is unclear. One advantage of having few large fibres rather than many smaller fibres is that the resulting lower surface area/volume ratio means that less energy is required to maintain the electrochemical gradient of the cell membranes (Smialowska & Kilarski,1981). With the low basal metabolism characteristic of Antarctic fish (Ralph & Everson,1968; Hemmingsen et al,1969; Holeton,1970; Hemmingsen & Douglas,1972) then any energy-saving mechanism would be favoured.

As fish size increases, both hypertrophy and hyperplasia occur (Greer-Walker,1970; Strickland,1975), unlike the situation in mammals where hypertrophy alone accounts for increases in muscle size. In the cod, G.morhua the total number of muscle fibres in the trunk rose steeply after hatching until a total length of about 40cm was achieved. Thereafter the rise in fibre numbers was only about half as steep (Greer-Walker,1970). For N.neglecta no direct estimate of fibre number was obtained because total muscle weights are not known. The pattern of distribution of fibre areas in growing N.neglecta is similar to that seen in some temperate species (Weatherly & Gill,1985). In species where growth is by hypertrophy alone e.g. bluntnose minnow (Pimephales notatus) then as the fish grow the number of smaller fibres decreases, eventually reaching zero. In other species hyperplasia also occurs e.g. rainbow trout (Salmo gairdneri), so that as fish size increases a bimodal pattern of fibre distributions occurs as myosatellite cells

are recruited. After a certain point the supply of myosatellite cells is depleted, and further growth is by hypertrophy alone. At this point the bimodal pattern disappears and a unimodal pattern is seen again (Weatherly & Gill,1985). From figure 4.1 it appears that growth in N.neglecta is by both recruitment of myosatellite cells and then by hypertrophy. Such myosatellite cells are found in smaller N.neglecta In large cod there is a decrease in fibre cross-sectional areas with increasing size fish (Greer-Walker,1970). The decrease in fibre size in older cod is explained as a mechanical constraint caused by movement at speed through the water column. Such constraints do not seem to come into play for the labriform swimming N.neglecta, possibly because it does not use such fast swimming speeds as the cod.

Muscle fibres are generally surrounded by capillaries in proportion to their aerobic metabolic properties (Romanul,1965; Gray & Renkin,1978), and their oxygen requirement (Krogh,1919; Weibel,1979). In fish, slow muscle fibres are usually found at densities of about 1:1 with capillaries, if not higher (Mosse,1979). With such large fibres it might be expected that the capillary density of the muscle would have to be high in order to supply sufficient oxygen to the central portions of the fibres. In N.neglecta there is no correlation between either fish standard length or wet weight and capillary density, although the line of best fit has a slightly negative slope. Hoppeler et al (1981b) have shown a negative correlation between capillary density and size, in a large weight range of African mammals. Fibre cross-sectional area or number is not a

good indicator of the oxygen requirement of the muscle since it is not the fibres themselves, but the mitochondria which are the actual utilisers of the oxygen supplied. The mitochondrial complement of muscle fibres has been correlated with maximum aerobic capacity (Hoppeler et al,1973; Bylund et al,1977).

If the capillary density is matched with the mitochondrial volume density there is a good agreement in mammals (Hoppeler et al,1981a) and for fish species although the Antarctic species and the conger eel appear not to fit this trend (Fig. 4.5). N.neglecta and C.aceratus therefore have low capillary densities when compared with species of similar mitochondrial volume density. However, the capillary surface and volume densities for the Antarctic species (Table 4.1) are comparable with temperate fish species which have higher haematocrits and haemoglobin concentrations (Egginton & Johnston,1982a). This means that despite the lower capillary density, the volume of blood in the capillary bed, and the surface area of capillaries available for gas/metabolite exchange are similar in Antarctic and temperate fish. This is brought about in the Antarctic species through the existence of large bore capillaries (Fitch & Johnston,1983; Fitch et al,1984), which are much greater in cross-sectional area than in temperate fish of similar size (Table 4.1). So the capillary density appears not be a good descriptor of the exchange properties of the vascular bed, at least in Antarctic species. Quantification of surface and volume densities of the capillaries might be a better indicator of the vascular bed (Egginton & Johnston,1982a). It must also be remembered that

capillary density does not relate exclusively to aerobic metabolism; capillarisation of the white fibres is relatively too abundant when comparing oxidative capacities with red muscle (Gray & Renkin,1978). The involvement of capillaries with substrate supply, lactate removal and heat removal must all be accounted for (Hoppeler et al,1981).

If the mitochondrial volume density is plotted against size (Fig. 4.4) there is a negative correlation ($r= -0.863$; $P<0.01$). The same situation occurs in mammals although with a large variability (Mathieu et al,1981), and quantitative histochemical observations seem to support a decrease in the mitochondrial content of fibres with increasing muscle fibre cross-sectional area (Gauthier & Padykula,1966). The situation is further complicated by the presence of two mitochondrial populations; subsarcolemmal mitochondria (SS) found at the periphery of the fibres and intermyofibrillar mitochondria (IF) distributed among the myofibrils. It has been suggested that the former support the transport of substances across the sarcolemma, while only the latter are directly involved with muscle contraction (James & Meek,1979; Salamonski & Johnston,1982).

In mammals the internal structure of the mitochondria (cristae) appears unrelated to either mitochondrial type (IF or SS) or to fibre type, and the distribution of IF and SS mitochondria is roughly equal (Hoppeler et al,1981a). In fish this is not the case; there are differences in IF mitochondrial packing between fast and slow fibres (Johnston,1982b; Egginton & Johnston,1982b; Fitch et al,1984: plate4.5e,f), and there does not seem to be the same sort of grouping of SS mitochondria adjacent to the

capillaries as seen in mammals (Hoppeler et al,1981a: plate 4.2).

It may be more functionally relevant to match total mitochondrial volume rather than mitochondrial volume density to the oxygen consumption of a muscle, but absolute muscle masses and proportions of red to white muscle are unknown for Antarctic species, so it is difficult to estimate absolute volumes of mitochondria.

As N.neglecta grows there is an increase in the total oxygen requirement possibly related to an increase in the total volume of mitochondria (a similar ocurrence is seen in mammals; Mathieu et al,1981), although weight-specific oxygen consumption will decrease with size (Prosser,1973b). There is no correlation between increasing body size and capillary density for N.neglecta (Fig. 4.3) so this increased total oxygen requirement may be met by increasing the total capillary numbers. At the same time the mitochondrial density is decreasing (Fig. 4.4). On the whole, Antarctic fish seem well equipped to overcome any handicap caused by the reduced haemoglobin levels; in fact, far from being sluggish and torpid, some species (including representatives of the icefish family, e.g. Champsocephalus gunnari) are active pelagic predators (Permitin & Tarverdieva,1978).

It must be concluded that the design of the respiratory system of Antarctic fish is ably matched to their functional requirements.

SPECIES	AREA (um^2)	DENSITY (mm^{-2})	Sv(c,f) (cm^{-1})	Vv(c,f)
Eel (1) (*Anguilla anguilla*)	14	2364	379	0.036
Crucian carp (2) (*Carassius carassius*)	20	1639	312	0.034
African catfish (3) (*Clarius mossambicus*)	20	1899	398	0.043
Tench (3) (*Tinca tinca*)	22	5092	1106	0.120
Icefish (4) (*Chaenocephalus aceratus*)	64	544	198	0.035
Notothenia neglecta (Mature)	55	237	79	0.013

Weights(gm), Means ± SEM. Eel: 0.14 +/- 0.1; Carp: 16.1 +/- 1.2; Tench: N.D. ; Icefish: 1040 +/- 175; *N.neglecta*: 900 +/- 50.

Abbreviations: Sv(c,f) - Surface density of capillaries per unit fibre volume;
Vv(c,f) - Volume density of capillaries per unit fibre volume.

References: (1); Egginton & Johnston,1982. (2); Johnston & Bernard,1984. (3); Johnston & Bernard (unpublished). (4); Fitch & Johnston,1983.

Table 4.1. Capillarisation data for the red trunk muscle of various teleost species.

SPECIES	CROSS-SECTIONAL AREA (um^2)	MEAN WEIGHT ± SEM (gm)	REF
Notothenia neglecta	6029 ± 268	900 ± 50	
Chaenocephalus aceratus	2721 ± 141	1040 ± 175	
Conger eel (Conger conger)	981 ± 105	2444 ± 945	1.
Crucian carp (Carassius carassius)	752 ± 37[a]	16.1 ± 1.2	2.
	705 ± 40[b]	15.9 ± 0.9	
African catfish (Clarias mossambicus)	660 ± 30[c]	50 ± 5.0	3.
	581 ± 33[d]	48 ± 5.2	
European anchovy (Encrasiolus engraulis)	1115 ± 52	(120-140mm SL)	4.
Cod (Gadus morhua)	7855	(1001 ± 50mm SL)	5.

a; 2°C acclimated. b; 28°C acclimated. c; aerated water. d; hypoxic water SL; Standard Length.

References

1. Egginton & Johnston,1983; 2. Johnston & Bernard,1984; 3. Johnston, Bernard & Maloiy,1983; 4. Johnston,1982c; 5. Greer-Walker,1970.

Table 4.2. Slow fibre cross-sectional areas for Antarctic and temperate species. (Means ±SEM.)

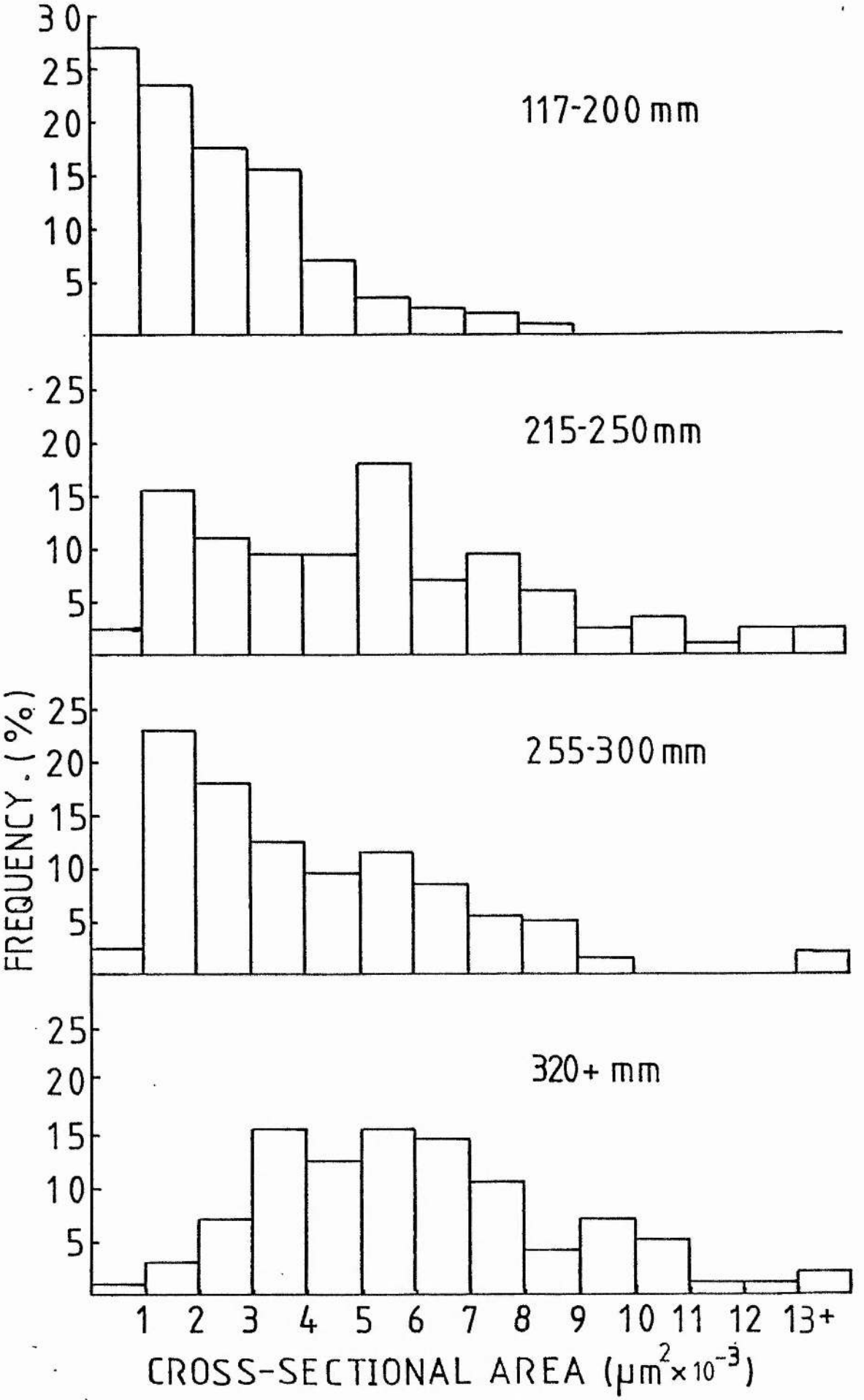


Fig. 4.1 Distribution of fibre cross-sectional area for N.neglect of increasing size

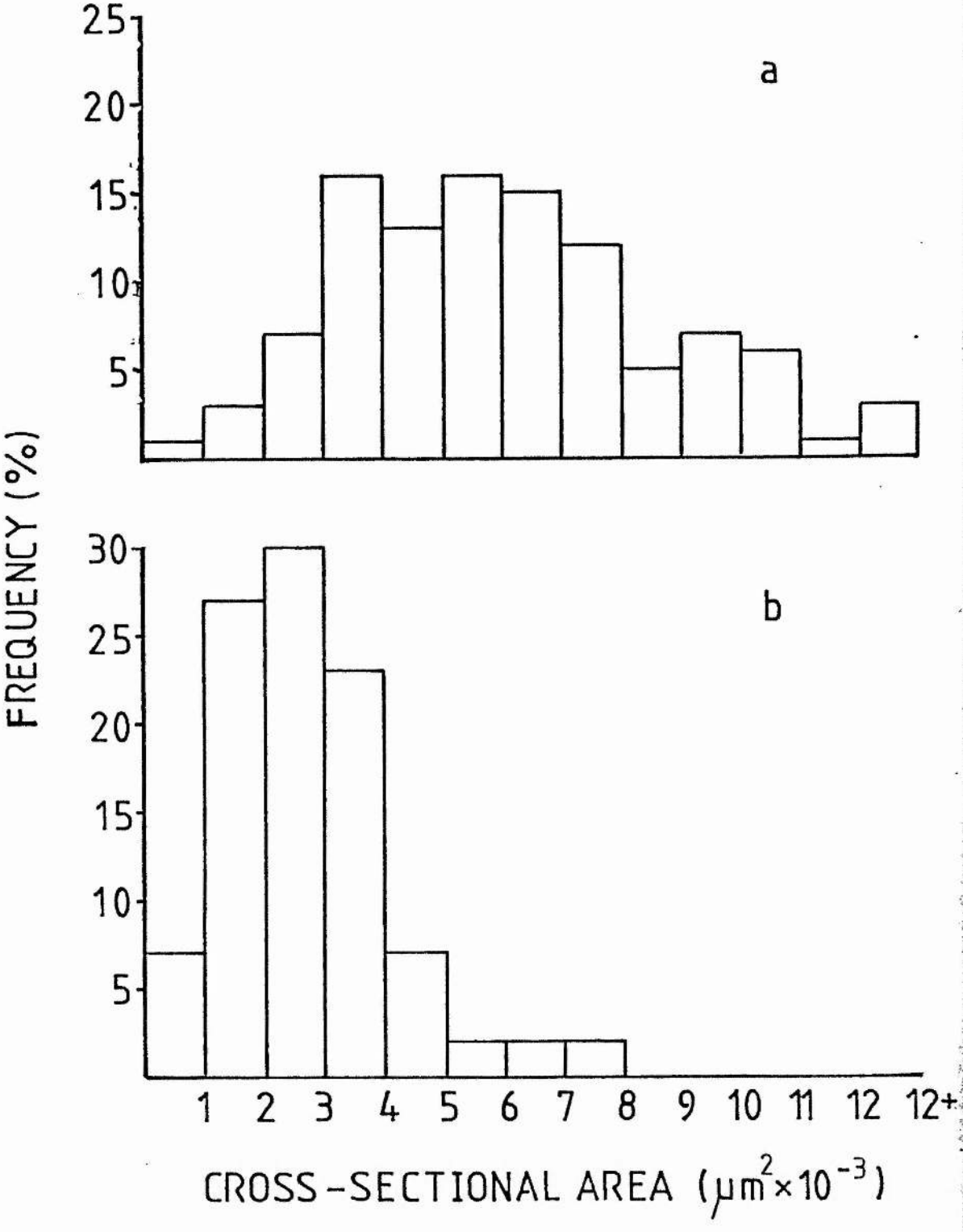


Fig. 4.2 Distribution of slow fibre cross-sectional areas i
adult N.neglecta (a) and the icefish C.aceratus (b)

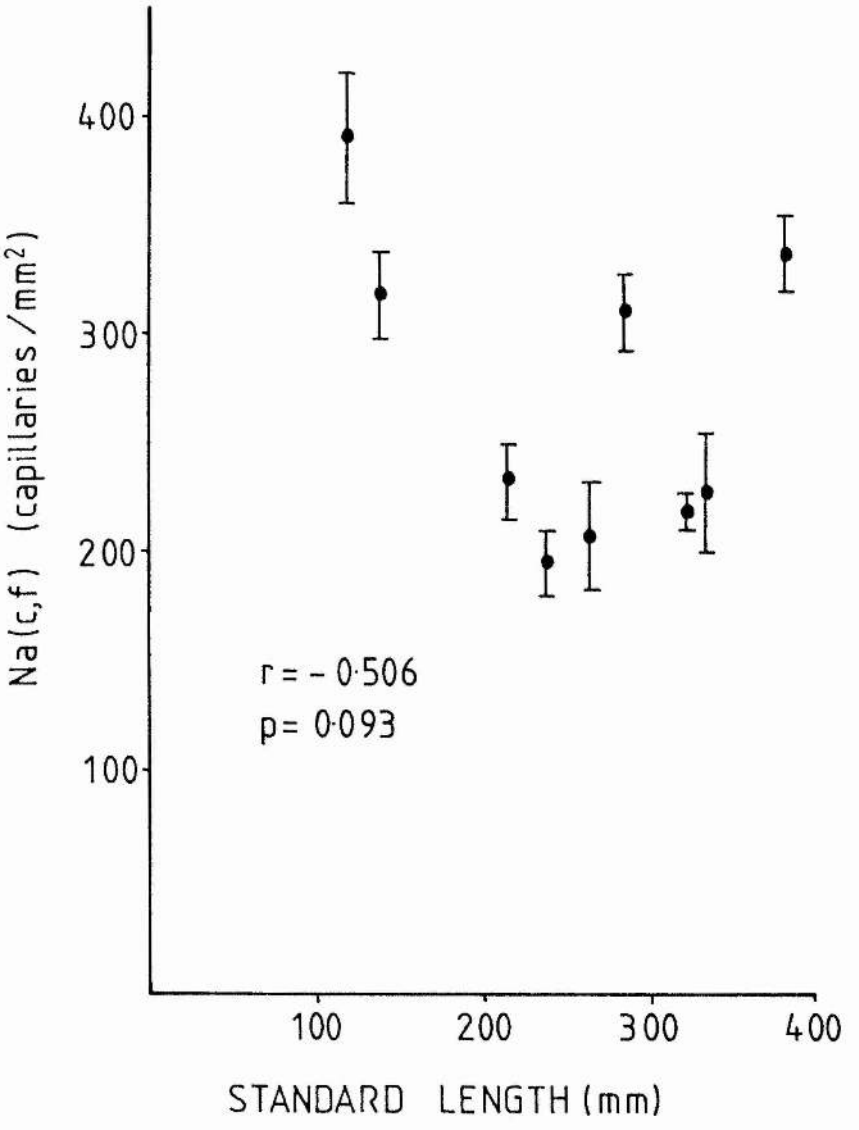


Fig. 4.3 Variation of capillary density Na(c,f) with standard length for N.neglecta.
(means ± sem, n at least 4 fish)

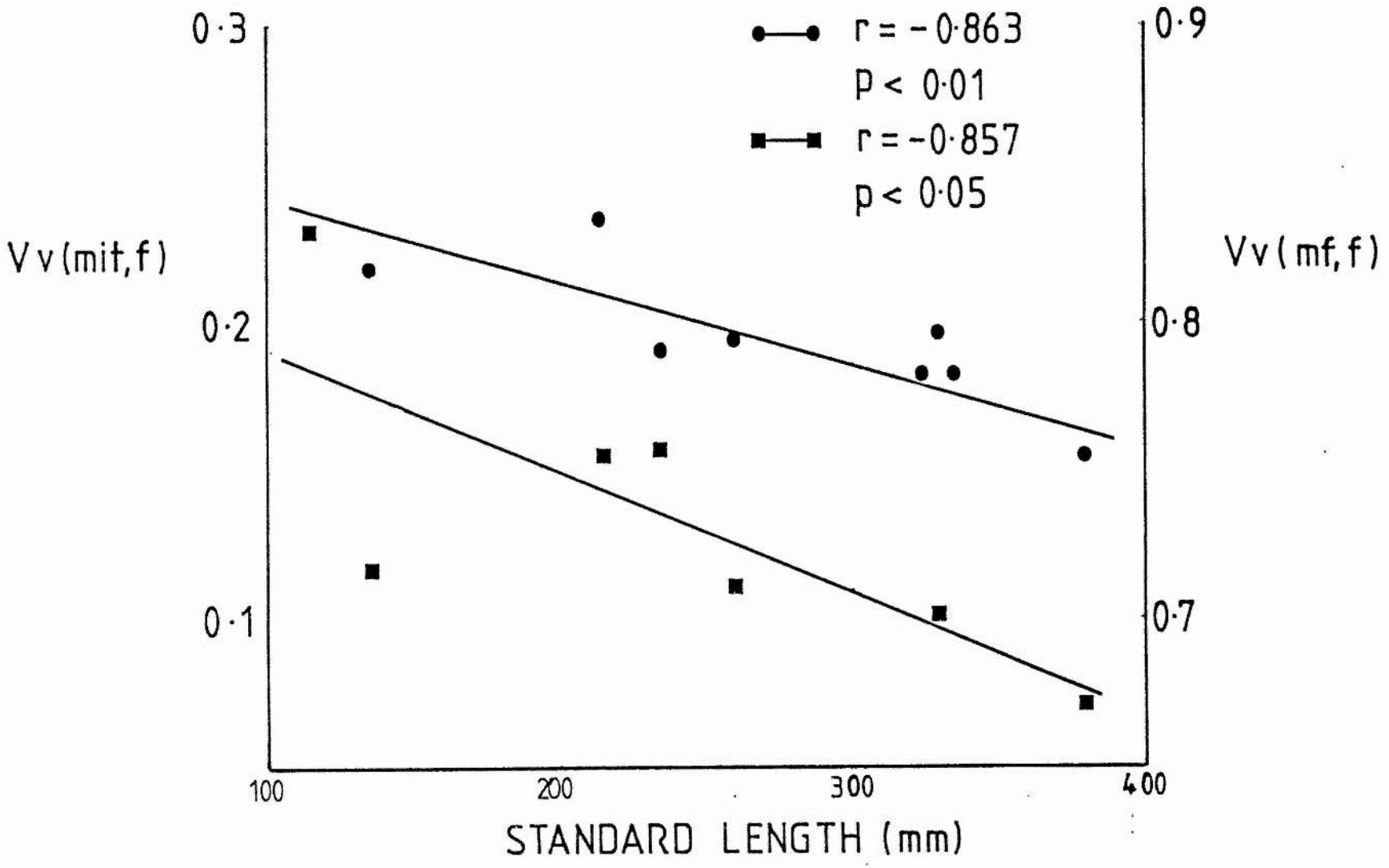


Fig. 4.4 Variation of mitochondrial (●) and myofibrillar (■) volume density with increasing standard length

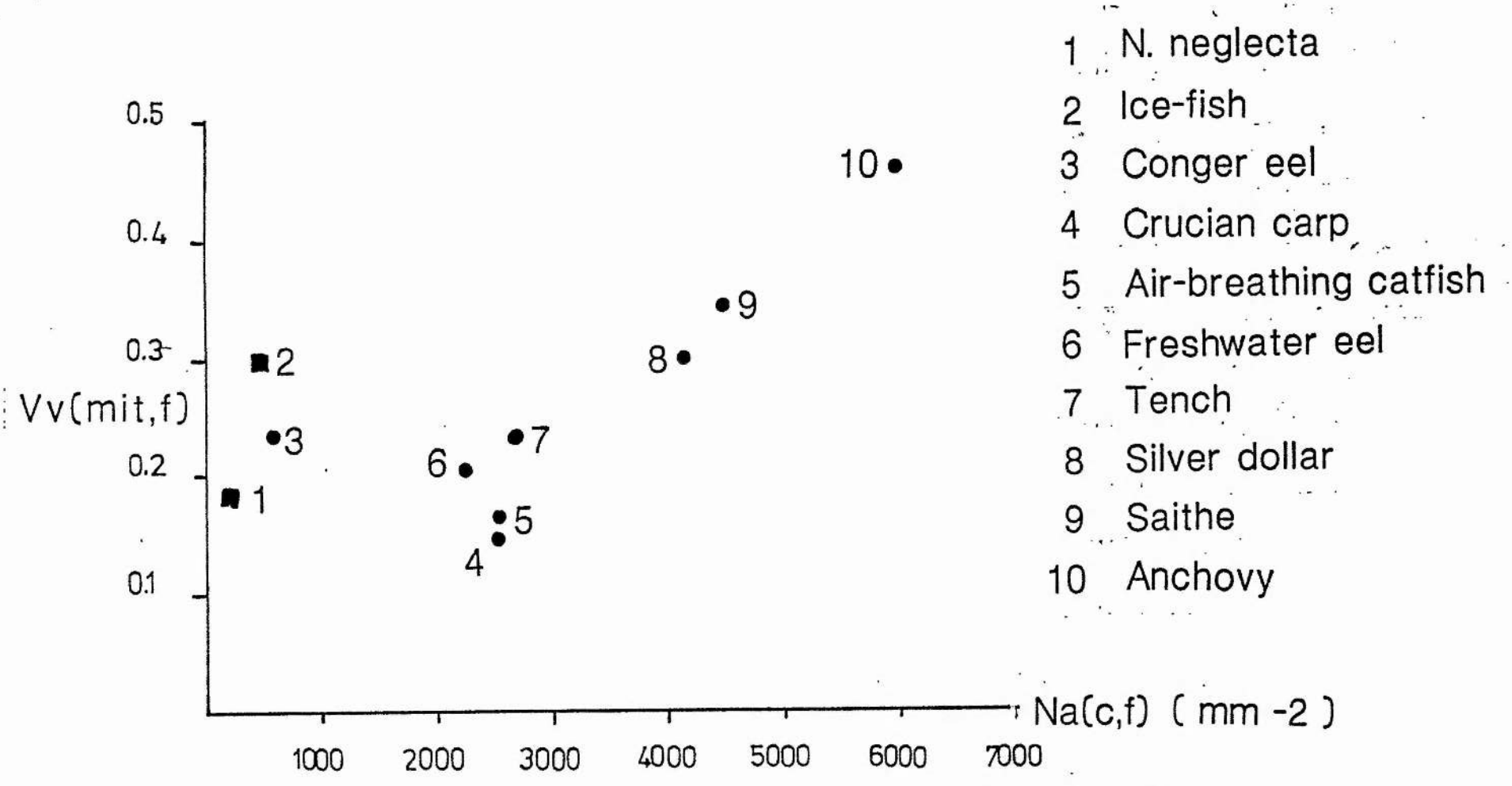
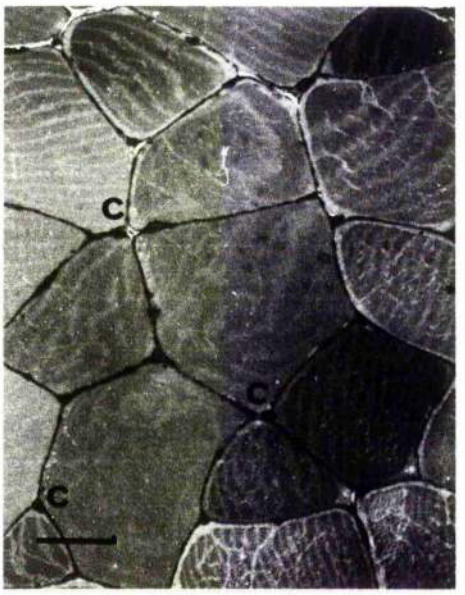


Fig. 4.5 Variation of mitochondrial volume density, Vv(mit,f), with capillary density, Na(c

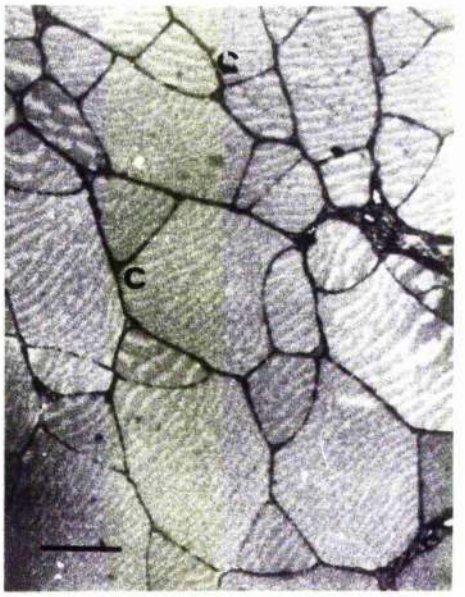
for various species

PLATE 4.1. Transverse micrographs showing the variation in slow muscle fibre cross-sectional areas for Notothenia neglecta (a,b) and Chaenocephalus aceratus (c,d). Note the location of capillaries (C) mainly at fibre junctions. Scale bars: a-d, 50μm. Magnification: a-d, 200x.

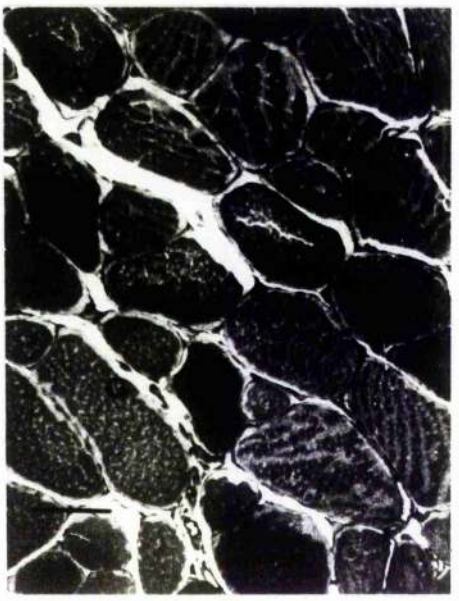
a



b



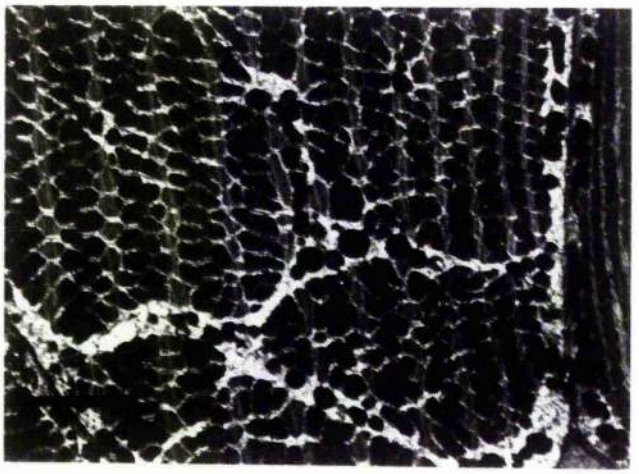
c



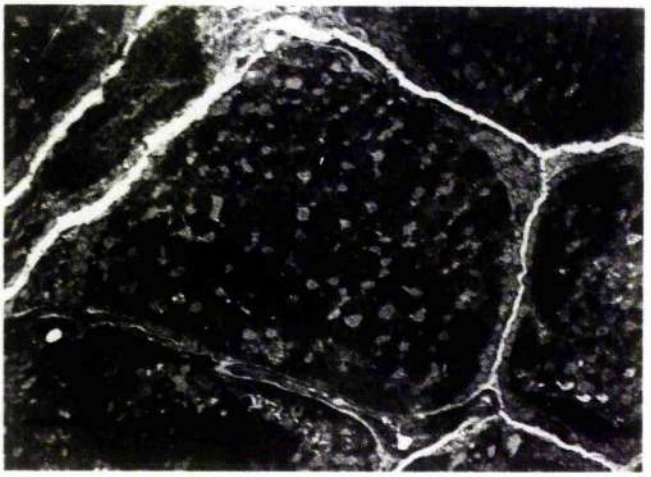
d

PLATE 4.2. Low power transverse electronmicrographs showing the distribution of subsarcolemmal mitochondria in Notothenia neglecta (a) and Chaenocephalus aceratus (b). 4.2c shows regions of myofibrillar splitting in small Notothenia neglecta. Scale bars: a, 5μm; b, 10μm; c, 1μm. Magnification: a, 2900x; b, 1020x; c, 18780x.

a



b

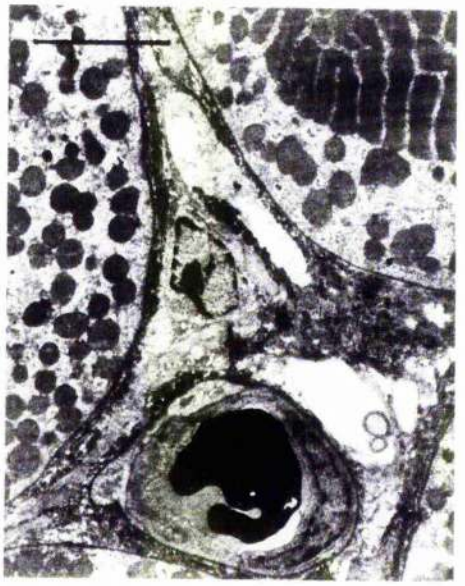


c

PLATE 4.3. Low power transverse electronmicrographs showing the structure of slow muscle capillaries in Notothenia neglecta (a,b) and Chaenocephalus aceratus (c,d). Scale bars: a-d, 5μm. Magnification: a, 2450x; b, 3990x; c, 1660x; d, 2620x.

a

b

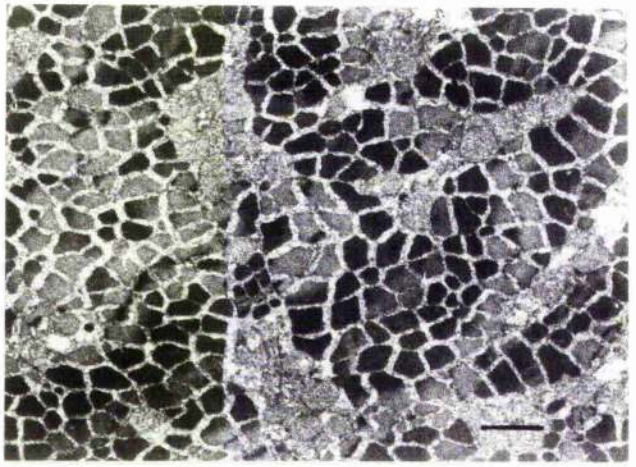


c

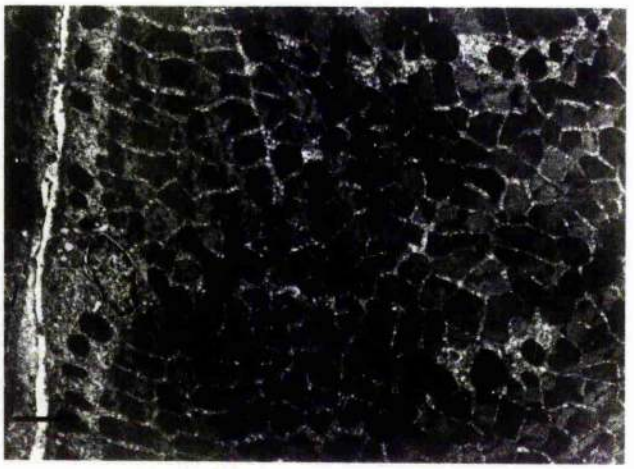
d

PLATE 4.4. Low power transverse electronmicrographs comparing the mitochondrial volume density in the slow muscle fibres of juvenile (a) and adult (b) Notothenia neglecta and mature Chaenocephalus aceratus (c). Scale bars: a,b, 2.5μm; c, 5μm. Magnification: a, 4385x; b, 5180x; c, 1910x.

a

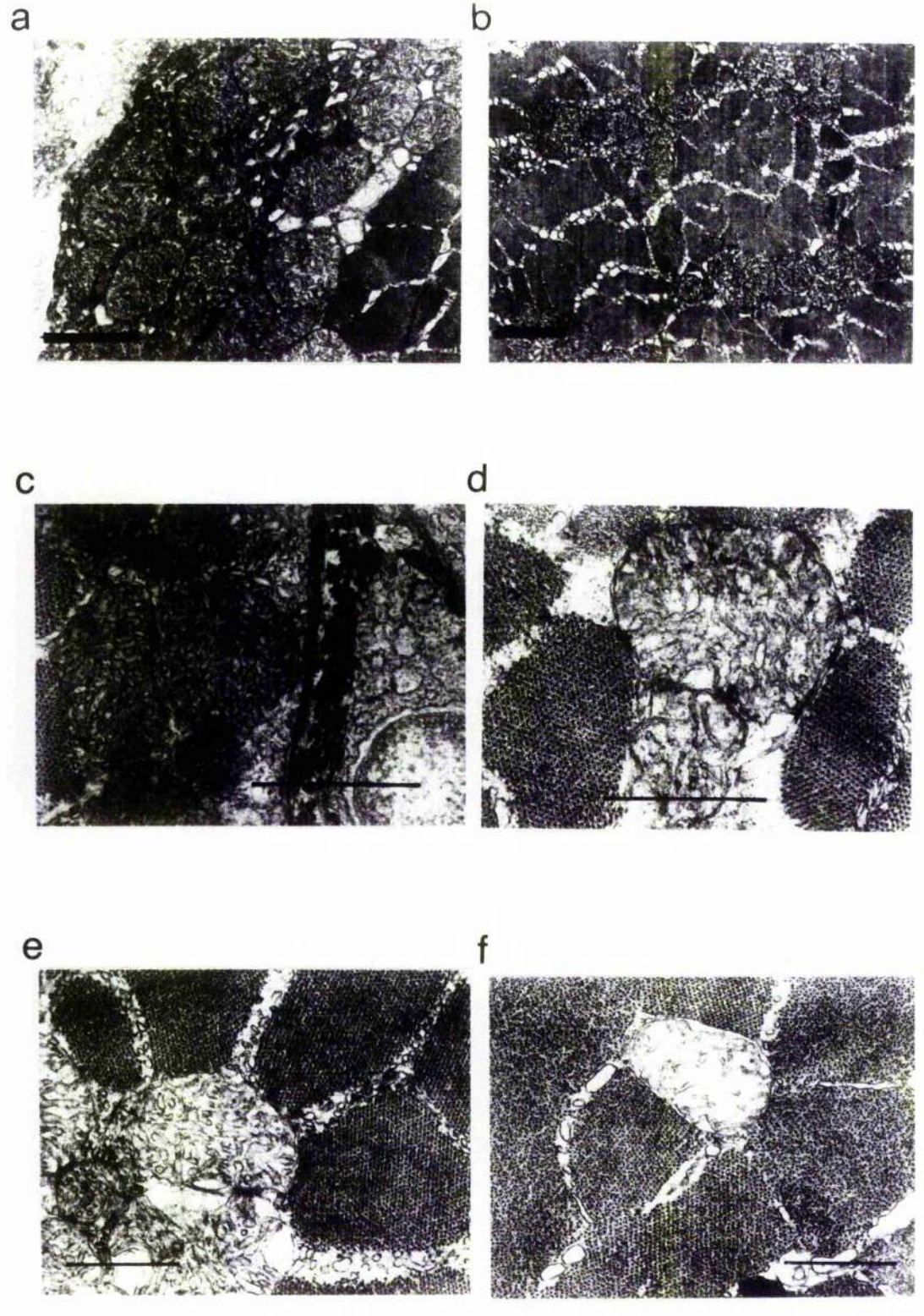


b



c

PLATE 4.5. High power transverse electronmicrographs showing the morphology of intrafibrillar (a,c) and subsarcolemmal (b,d) mitochondria in Notothenia neglecta (a,b) and Chaenocephalus aceratus (c,d), and the difference between intrafibrillar mitochondria in slow (e) and fast (f) muscle. Scale bars: a,c-f, 1μm; b, 1.5μm. Magnification: a, 12240x; b, 6430x; c,d, 28990x; e,f, 21260x.


a
b
c
d
e
f

CHAPTER 5. Characterisation and purification of Lactate Dehydrogenases in the trunk and cardiac muscles of NOTOTHENIA NEGLECTA.

5.1. Introduction

Most vertebrates have two dominant forms of the tetrameric lactate dehydrogenase (LDH). The M4 (A4) type is the primary form in the skeletal muscles; it has a moderately high Michaelis constant (Km) for pyruvate, and continues to function well even at relatively high pyruvate concentrations. The H4 (B4) type is dominant in the heart and other aerobically respiring tissues; it has a low Km and is generally severely inhibited at pyruvate concentrations at the upper end of the physiological range (Kaplan et al,1960; Dawson et al,1964; Everse & Kaplan,1973). It is postulated that the H-type isozyme may function as a lactate dehydrogenase in the heart, while the M-type isozyme functions as a pyruvate reductase in the muscles (Everse et al,1970).

Fish lower down the evolutionary scale such as the lampreys have only the M4 type LDH (Coppes de Achaval,1984) while the closely related but more 'modern' hagfish (e.g. Myxine glutinosa) have a H4 type LDH, but it's properties are more like those of the M4 type isozyme than the H4 variant in higher orders of fish i.e. a high Km for pyruvate, with relatively low inhibition at high pyruvate concentrations (Sidell & Beland,1980). Consequently, ancestral LDH may have been an M4 type enzyme, with the H4

enzyme arising by duplication of the ancestral gene (Markert et al,1975). Theoretically the heteropolymers M3H, M2H2 and MH3 could be formed, although in advanced teleosts only the M2H2 form is found, and that only in some species (Coppes de Achaval,1984). This absence of heteropolymers is believed to be due to genetically specified intrinsic properties of the sub-units (Whitt,1970).

The relative distribution of H and M isozymes may be determined by the oxygen availability to the tissues (Gesser & Poupa,1973). Support for this theory has been found in birds (Wilson et al,1963), amphibians (Salthe,1965) and mammals (Anderson & Bullard,1971). In fish however, where the heart receives it's blood supply direct from the venous circulation and so is constantly being perfused with blood containing a high lactate and low oxygen concentration (relative to the rest of the body), the lactate input to the heart may be the determining factor for heart LDH isozyme patterns (Gesser & Poupa,1973). Also, since the red and white muscles of fish are metabolically quite distinct, their respective LDH isozyme patterns may differ.

Cold acclimation leads to changes in some isoenzyme patterns, the physiological reason for the change being explained on the basis of conservation of important enzyme characteristics such as the Michaelis constant, Km (Hochachka & Somero,1973; Somero,1978). Isoenzyme patterns of LDH have often been studied (Hochachka,1965; Shaklee et al,1977; Yamawaki & Tsukuda,1979); the Km value is usually found to be minimum at the environmental temperature of the species and cold acclimation produces a Km value that is minimum at the acclimated temperature (Hochachka &

Somero,1968). Above the environmental temperature the Km for pyruvate increases with increasing temperature (Somero & Hochachka,1969; Hochachka & Lewis,1971) while it appears that the Km decreases with decreasing temperature, if pH and enzyme concentration are kept constant (Hochachka & Lewis,1971; Walsh & Somero,1981). The LDH system in the heart and trunk muscles of N.neglecta has been investigated; muscle extracts have been purified using a two stage affinity chromatography process, and the kinetics of the crude homogenate and purified extracts have been determined. The effect of temperature on the Km for pyruvate has been investigated and isoenzyme patterns have been analysed using polyacrylamide gel electrophoresis.

5.2. Materials and Methods

5.2.1. Fish.

Fish were caught by trammel net in Borge Bay or gill net in Factory Cove, and maintained in through-flow aquaria, water temperature 0-1.5^{o}C. Fish were killed by stunning followed by spinal transection. Mean weights and lengths ($\pm$ SEM) were 819 $\pm$ 144g and 313 $\pm$ 20mm respectively. Red and white muscle samples were taken from the same sites used for enzyme assays (Fig. 2.4). The heart was dissected out and samples were taken from the apex of the ventricle. Assays were carried out immediately, or the samples were frozen and stored at -40^{o}C until required.

5.2.2. Preparation of Homogenates.

Muscle samples were freed from connective tissue, cut into small pieces with scissors and weighed. Tissue was

then homogenised at 0-4°C with an MSE motorised homogeniser for 3x20s at below maximum speed, in 5-10 volumes of 125mM sodium phosphate buffer, pH 7.6 at 4°C. Homogenates were centrifuged at 600g for 10 minutes in ice-jacketed centrifuge tubes, and the supernatants were filtered through glass wool and retained.

5.2.3. Purification.

Purification of the LDH extract was carried out by a two-stage affinity chromatography procedure. All procedures were carried out in a refrigerated cabinet at 4°C. The first column was packed with blue dextran-sepharose (Cibachron Blue - Biorad) with a dimension of 1 x 7.5cm, and a bed volume of 4ml. The column was equilibrated with 50mM sodium phosphate buffer, pH 7.4 at 4°C. Cibachron Blue (CB) complexes with a wide number of enzymes all of which contain a dinucleotide fold (Thompson et al,1975); it is this fold that forms the NAD binding site in lactate dehydrogenase. As the extract passes down the column, LDH (and other NAD-linked dehydrogenases) bind to the CB. The LDH is washed off the column by eluting with low concentrations of the nucleotide cofactor (NADH) in the buffer.

The extract (2ml) was applied to the column with 3-4 bed volumes of buffer, and the eluant was collected and assayed for LDH activity (see below). If no activity was found, then the enzyme was washed off the column with 50mM sodium phosphate buffer, pH 7.4 at 4°C, containing 0.15mM NADH. Aliquots of 1ml were collected and assayed for LDH activity. Fractions containing the majority of the activity were pooled, and the volume was reduced to 2ml using Millipore

MX-10 ultrafiltration filters. The pooled extract was then carried forward to the next column.

The second column was more specific for LDH; an oxamate-sepharose column was used (O´Carra & Barry,1972). The column dimensions were 1 x 4cm with a bed volume of 2.5ml, and it was equilibrated with 0.5M sodium chloride in 0.05M sodium phosphate buffer, pH 6.8 at 4^{o}C, and primed with buffer plus 0.15mM NADH before the sample was applied.

Oxamate is a structural analogue of pyruvate (Fig. 5.1) and is linked to the sepharose matrix by a short hydrocarbon chain. Lactate dehydrogenase has a compulsory ordered mechanism of action in which the nucleotide cofactor binds first (Cleland,1963). This causes the conformational change in the structure of the enzyme enabling it to then bind pyruvate (or oxamate). As long as NADH is present the enzyme will stay bound to it´s substrate (O´Carra & Barry,1972). If NADH is omitted from the eluting buffer, LDH washes off the column almost one bed volume later.

Once the oxamate column had been primed, the sample was added and washed on with 4-5 bed volumes of buffer plus NADH, and the eluant tested for LDH activity (see below). If no activity was found the column was eluted with buffer minus NADH, and 1 ml aliquots were collected. The aliquots containing most of the activity were pooled; one portion was kept for testing the kinetics of the LDH reaction while the remaining part was stored at -20^{o}C until required for polyacrylamide gel electrophoresis.

Protein estimations were carried out by the method of Lowry et al (1951)

5.2.4. Assay of LDH Activity.

LDH activity was assayed spectrophotometrically by monitoring the change in absorbance at 340nm. The assay medium contained 100mM sodium phosphate buffer, pH 7.6 at 4^{o} 0.32mM NADH and the relevant concentration of pyruvate. Normal assays for maximal activities used 4-5mM pyruvate for all muscle types. In kinetic experiments a range of pyruvate concentrations from 0.1 to 60mM were used. The experiments involving determination of Km were carried out at -1^{o}, 2^{o}, 4^{o}, 8^{o} and 12^{o}C. Extracts were prepared as above, and the pH of the buffer was allowed to vary with temperature as this was assumed to be more physiological than maintaining constant pH (Reeves,1977; Somero,1983).

5.2.5. Polyacrylamide Gel Electrophoresis (PAGE).

A 5.5% polyacrylamide gel was prepared according to the method of Dietz & Lubrano (1967) using plate gels instead of disc gels. Various amounts of sample were loaded, the optimum amount being 20ul. The electrophoresis run was carried out at constant voltage with a current of 30mA, and generally took about 4 hours. The gels were stained using the staining solution of van der Helm (1961) as modified by Dietz & Lubrano (1967). Commercially available preparations (Sigma) of H- and M-type enzymes were run alongside the test samples.

5.2.6. Statistics.

Normality was tested and then either a t-test or Mann-Whitney test was employed to test for significance.

5.3. Results

5.3.1. Purification.

A typical elution profile through the CB column is shown in (Fig. 5.2). The aliquots containing 80-90% of the activity were pooled , their volume reduced to 2ml, and the pooled extract was then applied to the oxamate column. A typical elution profile through the latter column is shown in (Fig. 5.3). Almost 100% binding was achieved by both columns since the eluants rarely showed the presence of LDH activity.

When eluting the enzyme off the CB column it was impossible to gain a sharp peak, either by changing the flow rate or the NADH concentration. The pooled aliquots were tested for LDH activity and compared to the crude homogenate to assess the yield. This was in the range of 66-129%; a better than 100% recovery is possible if the purification procedure removes compounds that are normally inhibitory to the enzyme. Eluting off from the oxamate column provided a much sharper peak most of the activity coming off in 2-3 aliquots, one bed volume after removing the NADH from the buffer. Around 90% of the original activity was recovered on passing through the oxamate column, but in terms of specific activity generally a 6-13 fold purification was obtained.

5.3.2. Kinetics.

Graphs of LDH activity with increasing pyruvate concentration are shown in (Fig. 5.4). The shape of the curve was the same for all three muscle types with maximal

activities at 4-5mM pyruvate and the inhibition less than 50% even at pyruvate concentrations exceeding 60mM. The heart enzyme was only about 20% inhibited at this point. The kinetics of the purified extract were qualitatively no different from those of the crude homogenate.

The Michaelis constant (Km) for pyruvate was determined by a Lineweaver-Burke double reciprocal plot (Lehninger,1975). There was no significant difference between Km values for any of the muscles (Table 5.1)

5.3.3. Polyacrylamide Gel Electrophoresis.

Electrophoresis of muscle and cardiac extracts from N.neglecta showed the two enzymes to be similar. Thus it appears that N.neglecta has a heart isozyme that is structurally and kinetically similar to the muscle isozyme.

5.3.4. Temperature-dependance of Km.

The effect of temperature on the Km for pyruvate for LDH in the red and white trunk muscles, and the cardiac muscle is shown in Fig. 5.5. Between the physiological temperature range experienced by N.neglecta at Signy island (-1.8 to +1.5^{o}C) the Km values were affected similarily in all the tissues, rising between 2.3 -3.2 times. Increasing the temperature above 2^{o}C had a much greater effect on the red muscle enzyme than the LDH in the other tissues. Further increases in temperature caused a sharp rise in the Km for the cardiac muscle enzyme, but the white muscle LDH maintained a steady rise throughout the temperature range studied (Fig. 5.5).

5.4. Discussion

The general potential for affinity chromatography in enzyme isolation and purification is well known (Cuatrecasas & Anfinsen,1971; Dean & Watson,1978). The recovery (yield) of LDH using CB columns is usually in the order of 70-80% (Mosbach et al,1972; Dean & Watson,1978); For N.neglecta LDH's the yield of the CB column were between 66 and 129% so the columns were working at a similar efficiency to those used by other workers. Oxamate columns have greater specificity due to the compulsory ordered mechanism of the LDH reaction (Cleland,1963). A sharp elution profile is given by the oxamate column for N.neglecta LDH, similar to the profiles observed for other species and yields were around 92-93% which also compares well with those found elsewhere (O'Carra & Barry,1972). The specificity of this column is increased not only because the initial presence of NADH promotes efficient separation of LDH from other proteins with no affinity for oxamate, but also the elution of LDH by discontinuing NADH should leave behind any other adsorbed proteins as long as they do not have the same ordered reaction mechanism as LDH (O'Carra & Barry,1972).

It was considered that the efficiency of the oxamate column made the CB column redundant in some ways, particularily in view of the dispersed elution profile of the latter. The kinetics of pooled extracts from both columns used in isolation were no different so that for the later experiments in the series a single step process using the oxamate column was used. This achieved savings in time

with no loss of quality.

The 7-13 fold purification achieved was an order of magnitude lower than that found in similar studies. For example, a 300-fold purification was obtained for LDH isozymes in the Atlantic hagfish (Sidell & Beland,1980), and isolation of Glutamate dehydrogenase by a similar process gave a 245-fold purification (Dean & Watson,1978).

The effect of increasing pyruvate concentration on the rate of reaction of LDH is shown in figure 5.4. The same pattern is shown by all muscle types and unlike the situation in most species, the heart isozyme is less inhibited at high concentrations than the skeletal muscle enzyme (Everse & Kaplan,1973; Sidell & Beland,1980). The Km values are not significantly different for the LDH from the three muscle types, but the values for N.neglecta are higher than that found for M-type isozymes in other teleost species (Table 5.1). This means that the LDH isozymes in Antarctic fish are not as effective at converting pyruvate to lactate as the same isozymes in temperate species. In fact the low capacity of Antarctic fish to produce lactate under conditions of anoxic stress has already been demonstrated (Hemmingsen & Douglas,1970;1972).

Comparative studies of LDH's from vertebrates adapted to a wide range of environmental temperatures show up major conservative trends in protein evolution and adaptation. For instance the Km for pyruvate for M-LDH is widely conserved at normal body temperatures (Somero,1983). In most species the Km of LDH for pyruvate is held at a value close to that of the pyruvate concentration of resting muscle (Somero,1983). This ensures a reserve capacity so

that in times of activity, increasing pyruvate concentration can cause at least a two-fold increase in LDH activity. Information is required about tissue pyruvate concentrations in N.neglecta to assess whether they are also higher than found in temperate species, but recent studies suggest this is not the case (Dunn & Johnston, in preparation). In vivo, pyruvate concentrations in Gillichthys mirabilis have been shown to vary in parallel with Km as the temperature changes so that the ratio of pyruvate to Km is maintained (Walsh & Somero,1981). This may be important for maintaining regulatory ability in the enzyme, so that given responses to modulators can occur at all temperatures. As long as the pyruvate concentration is held below Km, increased production of pyruvate during elevated glycolytic rate can be met with concomittant increases in LDH activity (Walsh & Somero,1981). This conservation of substrate concentration and substrate binding capacity (as approximated by Km) is an evolutionary trend noted in all enzyme systems so far studied (Somero,1978).

To maintain key protein functions within optimal ranges usually requires one (or more) of three responses (Somero,1983). The first involves changes in the primary structure of the protein which become transmitted through the other orders of structure to cause inherent changes in the properties of the enzyme. Secondly, there may be an alteration in the concentration of the enzyme, and finally the type and amount of protein may remain unchanged, but the microenvironment surrounding the enzyme may be altered. It seems that the first strategy is responsible for conservation of Km, and a temperature difference of only

5-8^{o}C can lead to expression of different LDH proteins (Graves & Somero,1982; Somero,1983).

Only recently has it become realised that the microenvironment, particularily pH, may play an important role in determining catalytic mechanisms in enzymes (see review by Nuccitelli & Heiple,1982), so that investigation of temperature induced changes in enzyme function should also account for the temperature change on the pH of the system. For a rise in temperature of one degree centigrade the pH of the body fluids falls by 0.015-0.020 pH units (Reeves,1977). The net charge on most proteins can be held relatively constant during intracellular pH changes, either by maintaining an approximately constant $[OH^-]/[H^+]$ ratio relative to that of water (Howell et al,1973) or by maintaining a constant fraction of dissociated histidine imidazole residues on the protein (alphastat regulation; Reeves,1972), so that protein properties dependant upon it's charged state are conserved. Close conservation of Km values only occurs when using biologically realistic pH regimes so that ideally the pH of the buffer should vary with temperature in the same manner as the body fluids (Somero,1983).

The effect of temperature (at variable pH) on the Km for pyruvate of M-LDH in various muscles is shown in figure 5.5. Values for Km are well conserved over the physiological temperature range of this species (-1.8 to +1.5^{o}C), with the Km for the heart muscle enzyme being slightly higher than that of the red and white trunk muscle enzymes. The rise in Km of between 2-3 fold over the physiological range is greater than that experienced by temperate species

(Hochachka & Lewis,1971). At higher temperatures the Km for pyruvate of the white muscle is least affected, possibly because white muscle has a larger buffering capacity than the other muscle types (Damm Hansen & Gesser,1980; Castellini & Somero,1981), and can deal better with the larger amount of dissociated protons at the higher temperatures.

It has been demonstrated in the flounder (Platichthys flesus) that LDH from heart and skeletal muscles are identical using electrophoretic and kinetic criteria (Jorgensen & Mustafa,1980a), and that the isozyme is the M-type variant, showing little inhibition at high pyruvate concentrations and with a relatively high Km for pyruvate. The Atlantic hagfish (Myxine glutinosa) has both M- and H-type LDH, but the kinetics of the H-type enzyme are relatively similar to the M-type enzyme (Sidell & Beland,1980). The hearts of both these species, and indeed flatfish in general may have to function in conditions of severe hypoxia related to the mode of life of these species (Jorgensen & Mustafa,1980a; Sidell & Beland,1980). Also the cardiac cells of fish are always under low pO2's since they receive blood direct from the oxygen utilisation sites (skeletal muscle) en route to the oxygenisation site (gills) and have no coronary circulation of their own (Gesser & Poupa,1973). It would be advantageous therefore for these species to have M-type isozymes of LDH in their hearts since in times of hypoxia glycogen and glucose can be broken down by the glycolytic pathway without the resulting build-up of pyruvate causing inhibition of the enzyme. It is interesting to note that the flounder also has a high

activity of hexokinase and pyruvate kinase in the heart muscle (Jorgensen & Mustafa,1980a) and high concentrations of glycogen and glucose (Jorgensen & Mustafa,1980b) all indicative of a high glycolytic potential. Similarly N.neglecta has high activities of hexokinase and pyruvate kinase in it's cardiac muscle (Chapter 3). So the presence of M-type LDH in the cardiac muscle of N.neglecta may be a further adaptation in Antarctic species to the lower oxygen carrying capacity of the blood caused by the reduced haemoglobin levels (Tyler,1960; Everson & Ralph,1968).

It has already been noted that due to the circulatory characteristics of fishes, the heart is under the direct influence of lactate formed in the skeletal muscles. The lactate concentration in the blood can reach high concentrations under many conditions (Black et al,1962); perhaps there is some correlation between the capacity of the skeletal muscles to produce lactate and the LDH isozyme in the heart ? However Nototheniid species have a generally low activities of anaerobic enzymes (see chapter 3; Johnston & Harrison,1985) so that it is probable that like the Icefish (C.aceratus)they do not produce large amounts of lactate (Hemmingsen & Douglas,1972; Johnston & Harrison,1985). Based on a number of considerations Gesser and Poupa (1973) used the ratio of LDH activity to COX activity as an estimate of the capacity of the muscles to produce lactate and they found that generally a positive correlation existed between LDH/COX and the heart isozyme pattern, except for flatfish which showed no correlation. The units used in their paper for the activities of COX and LDH were not in μmol/min/gw and so are not directly

comparable with the result for N.neglecta. If the LDH/COX ratio is calculated for the same species using values from Johnston and Moon (1981) the sets of results do become comparable (Fig. 5.6). The capacity of Antarctic fish skeletal muscle to produce lactate is 3-5 fold lower than flatfish, which corroborates direct measurements of lactate concentrations in Antarctic fish before and after anoxic stress (Hemmingsen & Douglas,1970). The H/M pyruvate ratio is also lower in Antarctic species than would be predicted by Gesser and Poupa (1973).

At the cellular level we find that N.neglecta (like the flatfish) has an M-type LDH isozyme with the M-type kinetics in it's heart muscle. If the cardiac muscle of N.neglecta had the isozyme with H-type characteristics, anaerobic breakdown of carbohydrates would lead to a build up of pyruvate; this would inhibit LDH and halt the re-equilibration of the redox state necessary to maintain glycolysis.

Thus the Antarctic fish heart is well adapted for anaerobic breakdown of glycogen, in much the same manner as the flounder (Jorgensen & Mustafa,1980b) and has a high capacity for oxidative metabolism (chapter 3) and so is capable of functioning adequately under all conditions that may prevail in the Antarctic marine environment.

(A)

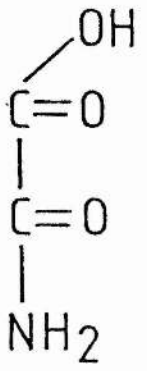
MUSCLE TYPE	Km
RED,TRUNK	0.69 +/- 0.15 (10)
WHITE,TRUNK	0.85 +/- 0.14 (16)
CARDIAC	0.87 +/- 0.15 (16)

(B)

SPECIES	Km
Atlantic hagfish	0.53 (M4)
	0.45 (H4)
Atlantic salmon	0.40 (M4)
	0.05 (H4)
Plaice	0.56 (M4)
	0.08 (H4)

TABLE 5.1. Km for pyruvate of lactate dehydrogenase in various muscles of Notothenia neglecta (A), and in skeletal (M4) and cardiac (H4) muscles of temperate species (B) - data from Sidell & Beland,1980. Values in (A) are means +/- SEM (n).

(a)



(b)

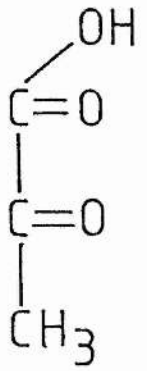


FIG. 5.1 STRUCTURAL SIMILARITY BETWEEN OXAMATE (a) AND PYRUVATE (b).

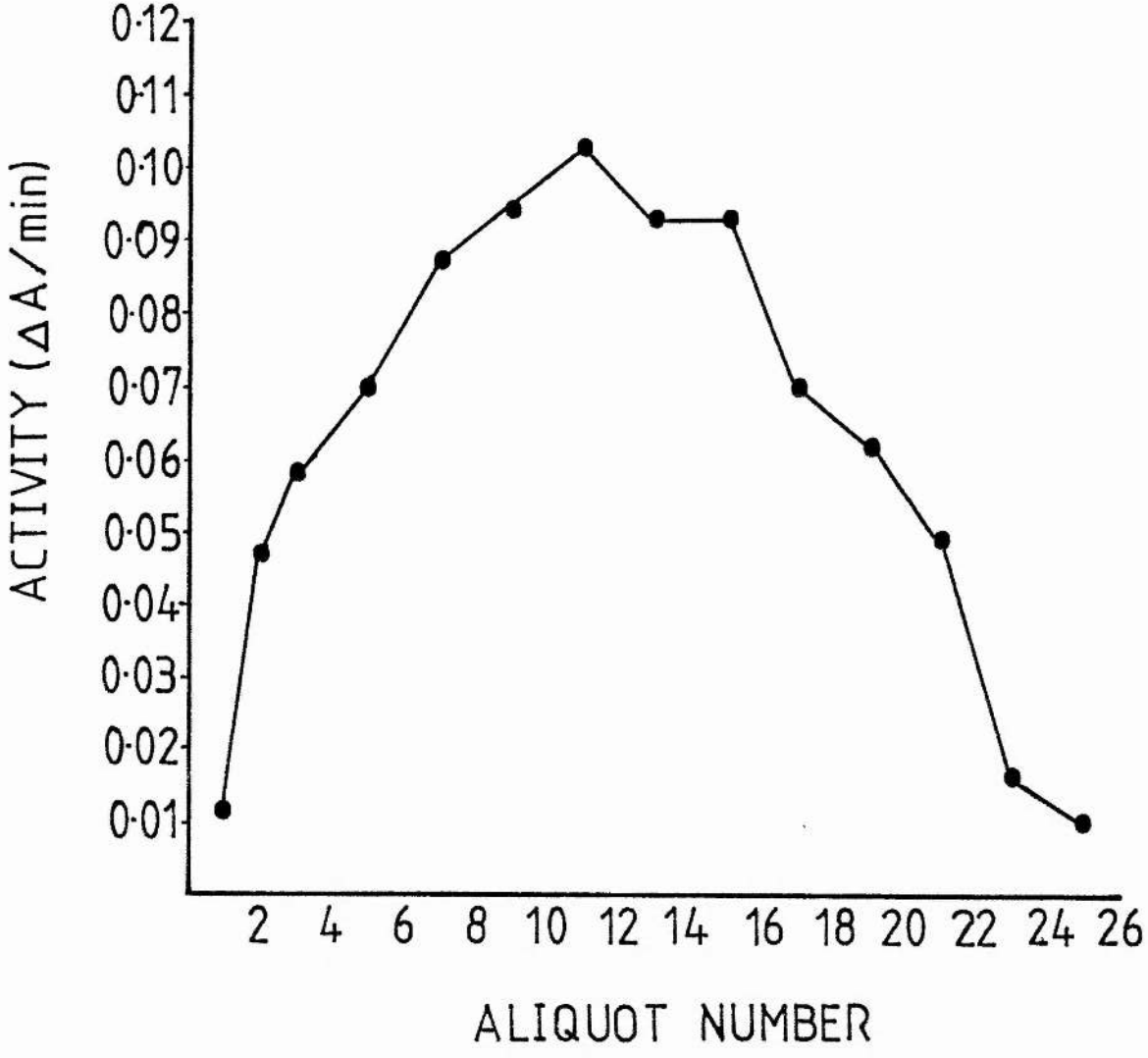


Fig. 5.2 Typical elution profile for the cibachron blue column

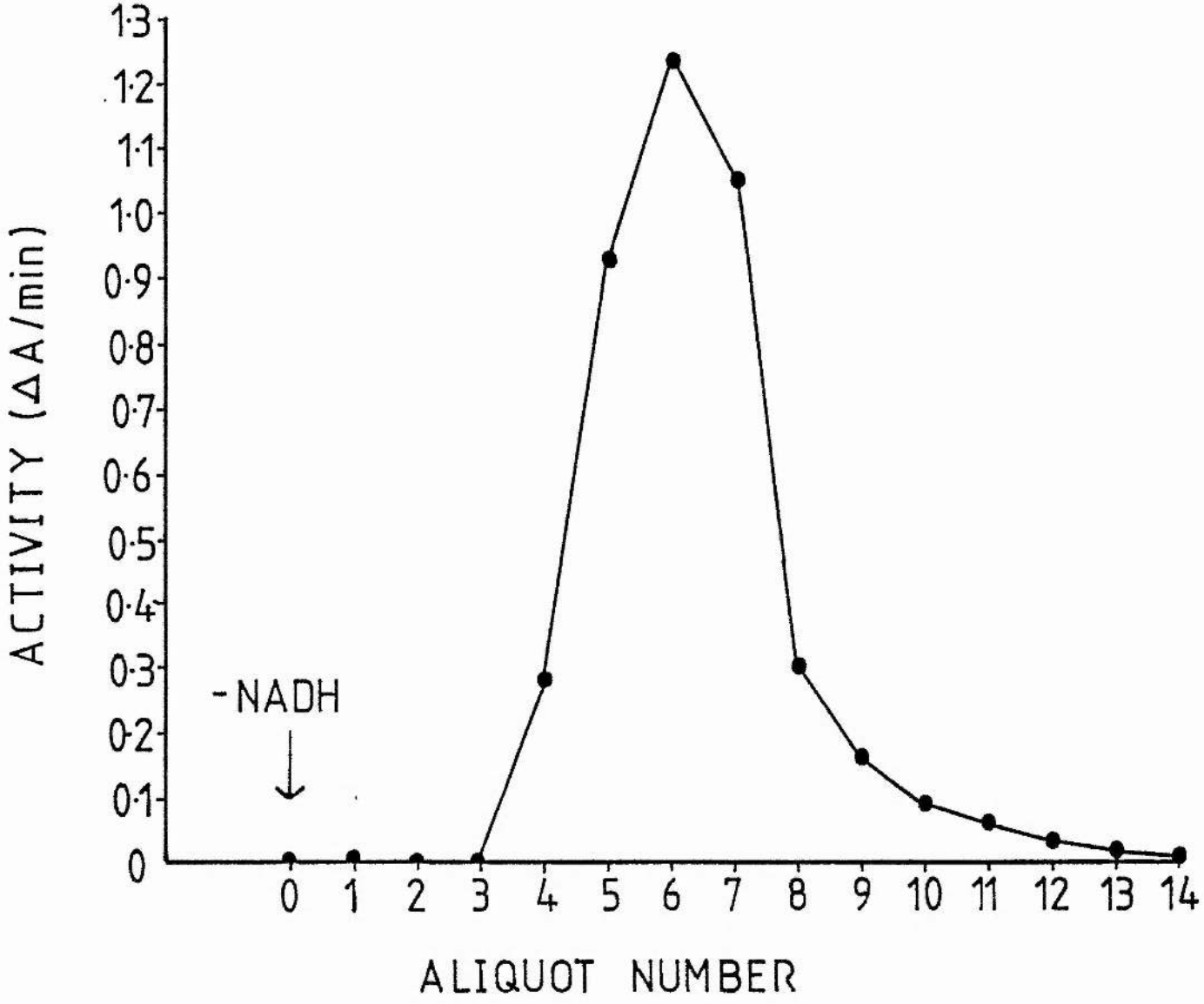


Fig. 5.3 Typical elution profile for the oxamate column

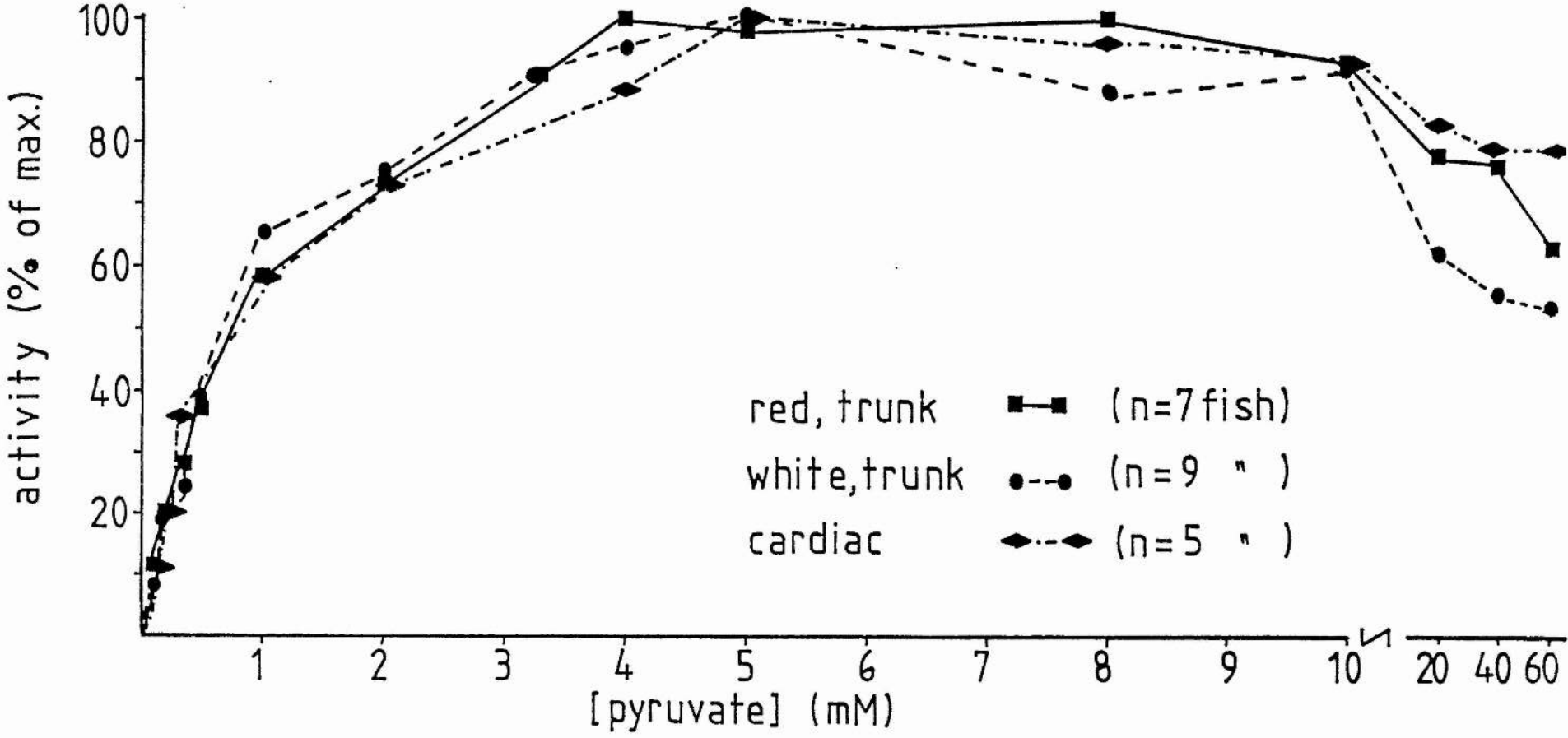


Fig. 5.4

Effect of increasing pyruvate concentration on LDH activity in various muscles of N.neglecta. Error bars omitted for clarity.

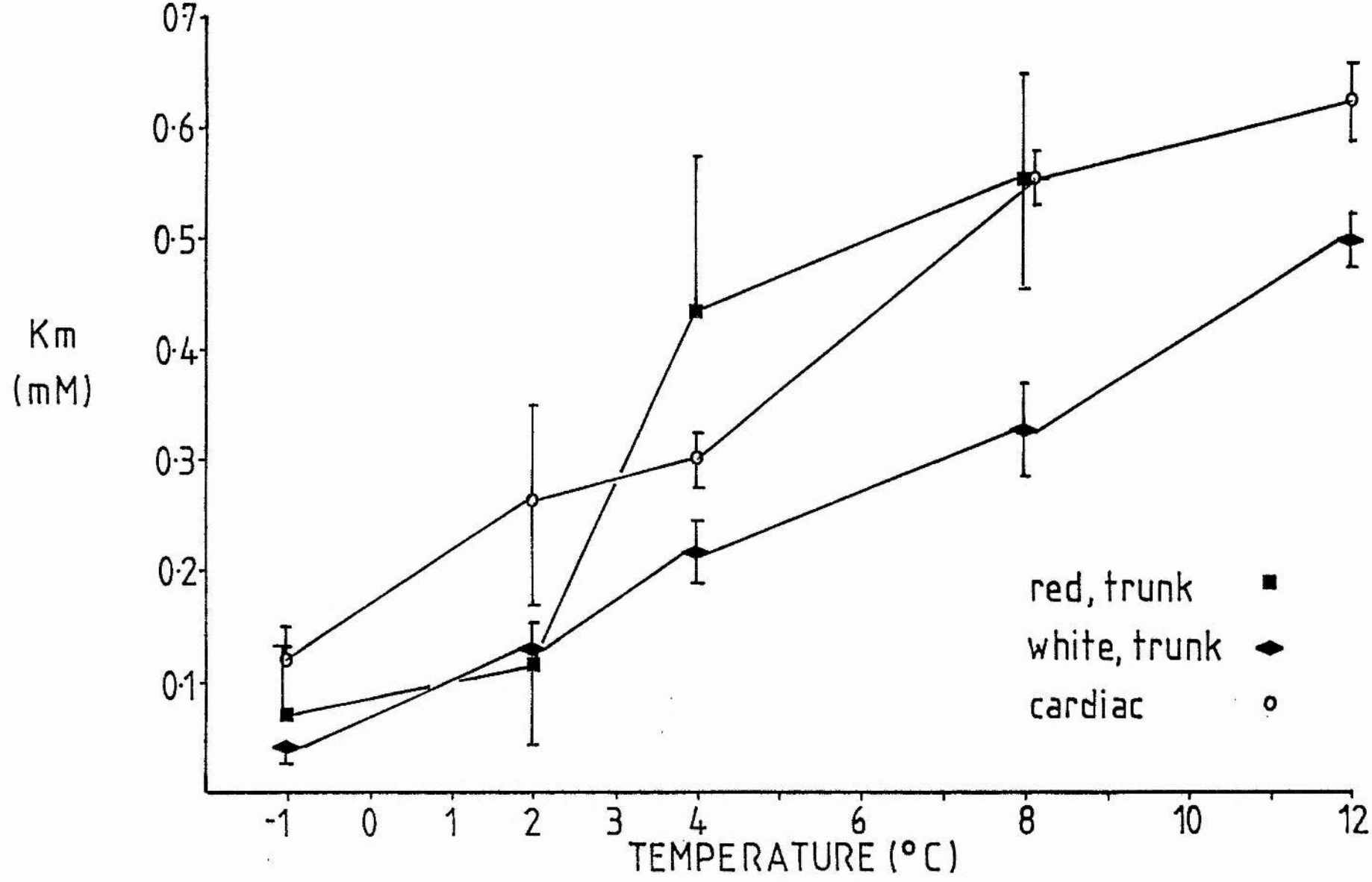


Fig. 5.5 Effect of temperature on Km (pyruvate) at variable pH (n at least 4 fish) means ± sem

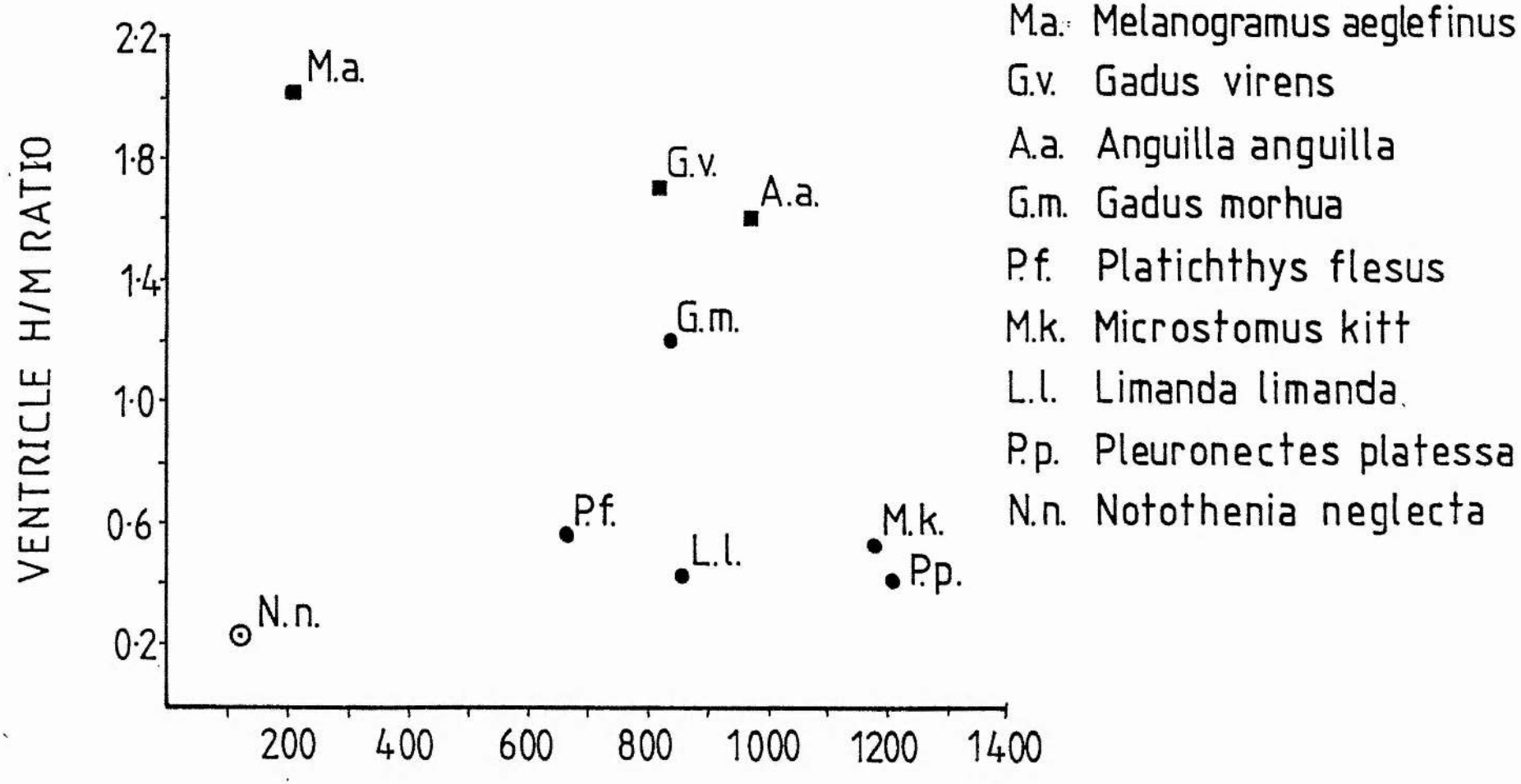
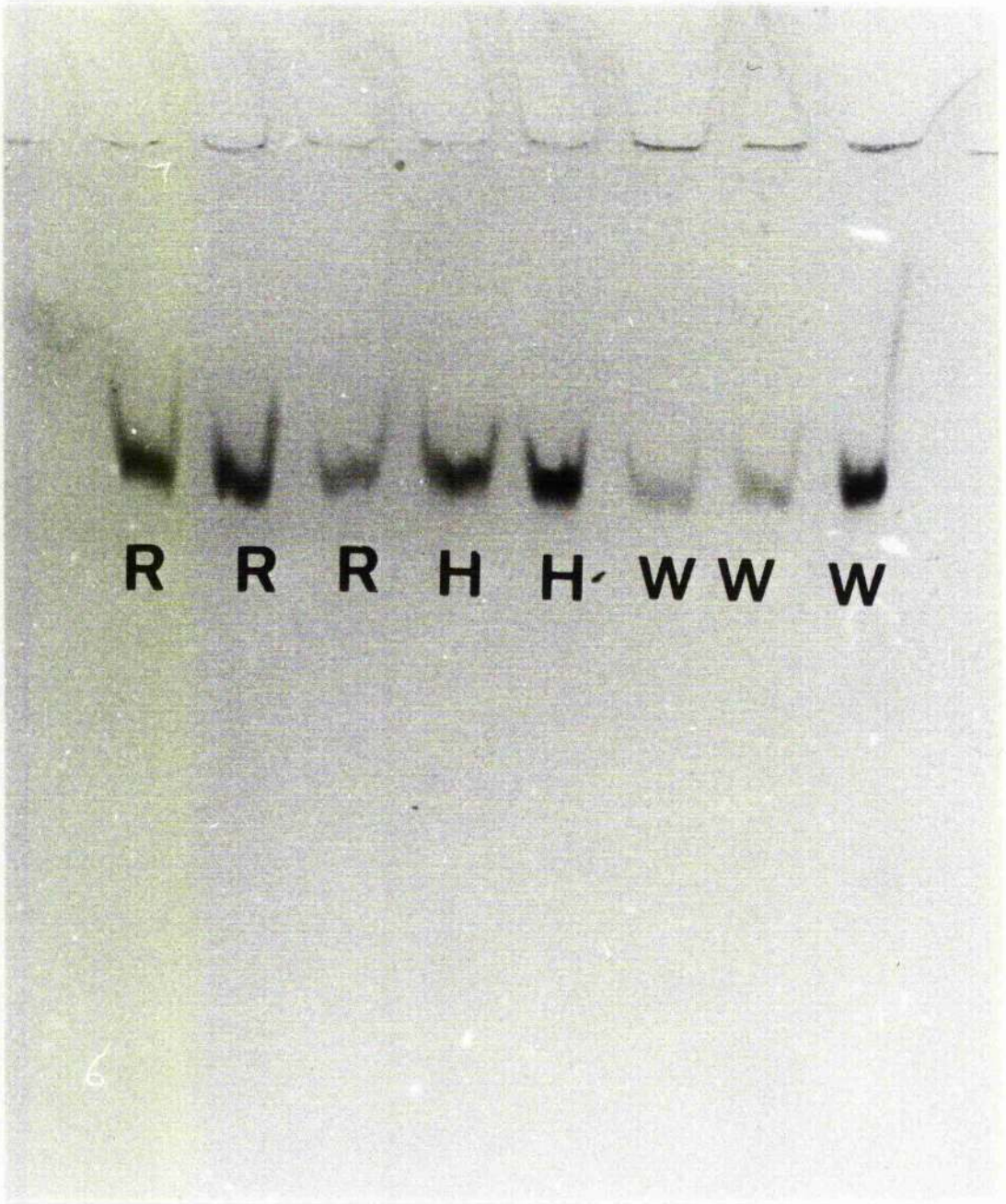


Fig. 5.6 Activity of cardiac LDH at low (0.32mM) divided by high (10mM) pyruvate concentration correlated with capacity for lactate formation. (COX activity/LDH activity).

• data from Gesser & Poupa (1973), ■ Gesser & Fenge (1978), Johnston & Moon(1981)

PLATE 5.1. Polyacrylamide gel showing the similar staining reaction for lactate dehydrogenase in muscle extracts from red (R), white (W) and cardiac (H) muscles of Notothenia neglecta.


R R R H H W W W

Chapter 6. General Discussion.

The largest proportion of work in the field of fish physiology (and indeed, marine biology as a whole) has been carried out with animals from temperate, shallow waters, and to a lesser extent, tropical areas. This prediliction with temperate species seems to have produced the opinion that animals living at high latitudes (and in deep seas), where water temperatures are generally 4°C or less, are living under some sort of handicap. In fact if we define cold water to be water equal to or lower than 5°C, then for marine life cold water is the norm rather than the exception, and the polar oceans are rich in marine life.

There is a limited amount of data to suggest that the scope for activity of Antarctic species is similar to those with similar behavioural ecologies from temperate waters. The scope for activity is the difference between the basal metabolic rate and the active metabolic rate and to date only Wohlschlag (1960) has investigated oxygen consumption during active swimming in an Antarctic species. The maximum active oxygen consumption for T.bernacchii was 140-180 mg/kg/hr; a typical basal oxygen consumption for Antarctic species is 30 mg/kg/hr (Holeton,1970), so the scope for activity is x5-6. This is low in comparison to salmonid species where the scope for activity can reach x10-12 (Fry,1971), but is of similar magnitude to those values found for less active temperate species (Brett & Groves,1979). The finding that the swimming performance of the whole animal is similar in Antarctic and some temperate

species (thus showing that the cold temperature per se is not a limiting factor) is further corroborated at the molecular level if the mechanical performance of individual skinned muscle fibres is examined. When measured at their own environmental temperatures the maximum power output of white fibres in Antarctic species is about 60% of that reported for tropical species (Johnston,1985; Johnston & Altringham,1985). However, maximum isometric tension at $0^{o}C$ is 5-8 times higher for muscle fibres from Antarctic species compared with tropical species, and there appears to little evidence for compensatory increases in maximum contraction velocities at low temperatures for Antarctic species (Johnston,1985; Johnston & Harrison,1985). Evolutionary temperature compensation of power output may involve differences in the ability of cross-bridges in the muscle to generate force (Johnston & Altringham,1985).

So Antarctic species have overcome the first potential problem of living in cold waters; that of the rate-depressing effect of the cold temperature per se on living systems. It has already been mentioned that Antarctic fish produce proteins at lower temperatures with reduced thermodynamic activation parameters (see chapter 1) which makes them functionally superior to 'warm' proteins made to perform at lower temperatures.

The enzymes of energy metabolism measured in this study in the trunk muscles of N.neglecta have low activities when compared with the corresponding enzymes in temperate species (chapter 3). This may be due to a combination of factors:

1. The majority of Antarctic species are labriform swimmers (Lin et al,1974; Walesby & Johnston,1980; Walesby

et al,1982). Thus the pectoral muscles provide the main locomotory power, and the role of the trunk muscle maybe just to function as a rudder (Kryvi & Totland,1978).

2. Sprint swimming in Antarctic species is probably mediated primarily by high energy phosphocreatine stores (Johnston & Harrison,1985; Dunn & Johnston, in preparation).

3. Antarctic species have a low resting rate of oxygen consumption although there is some overlap with the lower end of the scale for resting oxygen consumption of temperate species (Wohlschlag,1964; Ralph & Everson,1968; Holeton,1970).

So the reduced dependance on the trunk musculature in turn leads to a reduced requirement for fuels by the enzymes in the normal metabolic pathways. The greater involvement of the pectoral muscles in the locomotion of Antarctic species is shown by the high (relative to red muscle) activities of enzymes associated with aerobic metabolism (chapter 3) a finding common to both Antarctic (Walesby et al,1982) and temperate (Kryvi & Totland,1978) labriform swimmers.

Another problem encountered by Antarctic species that is directly attributable to the low temperatures is the increased viscosity of the blood. At $0^{o}C$ the viscosity of the blood of C.aceratus is 25% greater than at $10^{o}C$ (Hemmingsen & Douglas,1972). If Antarctic species maintained erythrocyte numbers at the same level as temperate species the increase in viscosity would be even more pronounced and without some form of adaptation to the circulatory system the energy cost of transporting the more viscous solution would be too great for the fish to cope

with. In fact there is a general trend for Antarctic fish to have reduced haematocrits and haemoglobin concentrations (Tyler,1960; Everson & Ralph,1968; Wells et al,1980), which maintains the fluidity of the blood, but at the expense of a reduced oxygen carrying capacity. The latter has been overcome by various circulatory and respiratory adjustments (see chapter 1) at the physiological level, and also it appears, at the cellular level. There seems to be a slightly greater reliance on anaerobic metabolism by cardiac muscle in particular; it has an LDH isozyme kinetically (and structurally) similar to the muscle isozyme (chapter 5) and a high glycolytic capacity compared to temperate species (chapter 3). Thus a high concentration of pyruvate caused by an increase in the glycolytic rate will not inhibit the cardiac LDH isozyme, and so glycolysis can continue. A similar occurrence of muscle type LDH in the heart, or very similar heart and muscle enzymes is found in temperate species whose lifestyles incorporate some degree of oxygen shortage as a matter of course e.g. flatfish (Gesser & Poupa,1973; Jorgensen & Mustafa,1980a,b), and in fish further down the evolutionary scale such as the hagfish and lampreys (Sidell & Beland,1980; Coppes de Achaval,1984). There is no evidence to suggest that Antarctic fish experience an oxygen shortage under normal conditions.

Fish acclimated to cold temperatures in the laboratory respond to the reduced temperature by increasing the capillary density in both fast and slow fibres and increasing the mitochondrial volume density (but not the myofibrillar density). This acts to increase the supply of oxygen to the tissues and to decrease the diffusion path

length within the tissues (Johnston & Maitland,1980; Sidell,1980; Johnston,1982b; Sidell,1983). Antarctic fish have however adopted a different strategy. They maintain a low capillary density when compared with temperate fish of comparable mitochondrial volume density (chapter 4), but their capillary cross-sectional areas are three times larger (chapter 4). Consequently parameters defining volume and surface area of the capillary bed available for gas and metabolite exchange ($Vv_{(c,f)}$ and $Sv_{(c,f)}$ respectively) are similar in Antarctic and temperate species (chapter 4).

Beside the cold temperature there is another phenomenon characteristic of the Southern ocean; the highly seasonal pattern of primary production and consequent changes of standing stock of phytoplankton, upon which the rest of the food chain depends. This 'bloom' is usually fairly predictable; at Signy Island it commences during the first two weeks of December and terminates during the final two weeks of February (Whittaker,1982). As a consequence it might be expected that food availabilty is much reduced in the winter months, and that seasonal depletion would occur in Antarctic species, in the same manner as occurs for temperate species (Love,1970). Although the condition factor of N.neglecta does fall during the winter months (chapter 2), analysis of muscle constituents does not point to any severe depletion taking place (chapter 2). It is suggested by Daniels (1982) that N.neglecta changes it's feeding strategy during the winter months to make the most of the available prey. Only at the end of the winter does the condition of the fish fall. This could be due to either cessation of feeding with the return of foraging seals,

primarily as an avoidance measure (Everson,1970a) since most seals consume fish as part of their diet (Dearborn,1965; Stonehouse,1972), or because the quality of the prey ingested in the winter is not as good as it is in the summer because the prey items themselves are in poor condition.

The cold waters of the Southern ocean have not proved to be a hindrance to survival; far from 'just managing to get by', Antarctic fish stocks are quite large and several species are commercially fished by Eastern bloc countries (Everson,1978; Kock,1985). However, while the biomass is large, the rate of production of Antarctic fish species is low, so that overfishing can soon lead to non-sustainable levels, as has occurred for N.rossii at South Georgia (Kock,1985). Physiological and cellular adjustments have occurred in Antarctic species but the majority of examples seen can be explained on the basis of ecological constraints other than the low temperature per se.

Suggestions for future work.

1. Measurement of capillary dimensions in smaller fish to see the scaling with growth.
2. Measure the capillary anisotropy in smaller fish to see if it is the same as for mature fish.
3. Measure whole animal VO_2's at rest and in relation to excercise.
4. Measure the effects of increasing lactate concentration on the Km for pyruvate of lactate dehydrogenase.
5. Measure enzymes of energy metabolism in fingerling N.neglecta.

BIBLIOGRAPHY

Akster,H.A. (1983). A comparative study of fibre type characteristics and terminal innervation in head and axial muscles of the carp (Cyprinus carpio): a histochemical and electron microscopal study. Neth. J. Zool. 33: 164-188.

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

Andersen,N.C. & Hureau,J.C. (1979). Proposition pour une nouvelle classification des Notothenilnae (Pisces, Perciformes, Notothenidae). Cybiem 3^{e} serie, 1979(6): 47-53.

Anderson,G. & Bullard,R.W. (1971). Effect of high altitude on lactic dehydrogenase isozymes and anoxic tolerance of the rat myocardium. Proc. Soc. exp. Biol. Med. 138: 441-443.

Barany,M. (1967). ATP´ase activity of myosin correlated with speed of muscle shortening. J. Gen. Physiol. 51: 197-216.

Barets,A. (1961). Contribution a l´etude des systemes moteur lent et rapide du muscle lateral des teleosteans. Archs. Anat. Morph. exp. 50: 91-187.

Bass,A., Brdiczka,D., Eyer,P., Hofer,S. & Pette,D. (1969). Metabolic differentiation of distinct muscle types at the level of enzymatic organisation. Eur. J. Biochem. 10: 198-206.

Beamish,F.W.H. (1964). Respiration of fishes with special

emphasis on standard oxygen consumption. II. Influence of weight and temperature on respiration of several species. Can. J. Zool. 42: 177-188.

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

Beitner,R., Nordenberg,J. & Cohen,T.J. (1979). Correlation between the levels of glucose 1,6 diphosphate and the activities of phosphofructokinase, phosphoglucomutase and hexokinase in skeletal and heart muscles from rats of different ages. Int. J. Biochem. 10: 603-608.

Bertin,A. (1956). Eels. A biological study. Clever Hulme Press, London.

Bilinski,E. (1974). Biochemical aspects of fish swimming. In 'Biochemical and Biophysical Perspectives in Marine Biology', Malins,D.C. & Sargent,J.R. (eds). Vol.1, 247-288. Academic Press, London & New York.

Black,E.C., Connor,A.R., Lam,K-C & Chiu,W.G. (1962). Changes in glycogen, pyruvate and lactate in rainbow trout (S.gairdneri) during and following muscular activity. J. Fish. Res. Bd. Can. 19: 409-436.

Blake,R.W. (1981). Mechanics of drag-based mechanisms of propulsion in aquatic vertebrates. Symp. zool. Soc. Lond. 48: 29-52.

Bligh,E.G. & Dyer,W.J. (1959). A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37: 911-917.

Boddeke,R., Slijper,E.J. & van der Stelt,A. (1959). Histological characteristics of the body musculature of fishes in connection with their mode of life. Proc.

Roy. Acad. Sci. Amsterdam, 62(c): 576-588.

Bokdawala,F.D. (1967). Histochemical study of fat in red and white fibres of fish skeletal muscle. J. Anim. Morph. Physiol. 14: 230-241.

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

Bone,Q. (1966). On the function of the two types of myotomal muscle fibre in elasmobranch fish. J. mar. biol. Ass. U.K. 46: 321-350.

Bone,Q. (1970). Muscular innervation and fish classification. Simp. 1st. Zoofil. Univ. Salamanca. I: 369-377.

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

Bone,Q., Kiceniuk,J. & Jones,D.R. (1978). On the role of the different fibre types in fish myotomes at intermediate swimming speeds. Fish. Bull. 76: 691-699.

Bostrom,S-L. & Johansson,R.G. (1972). Enzyme activity patterns in white and red muscle of the eel (A.anguilla) at different developmental stages. Comp. Biochem. Physiol. 42B: 533-542.

Boulenger,G.A. (1902). Pisces. In 'Report on the Collections of Natural History made in the Antarctic Regions during the Voyage of the "Southern Cross"', 174-189. British museum (N.H.), London.

Brandes,C.H. & Dietrich,R. (1956). Fat and water content in redfish. Fette Seifen Anstr. Mittel. 58: 433-439.

Breder,C.M. (1926). The locomotion of fishes. Zoologica (NY) 4: 159-297.

Brett,J.R. & Groves,T.D.D. (1979). Physiological energetics. In 'Fish Physiology', Hoar,W.S., Randall,D.J. and Brett,J.R. (eds), Vol 8, 279-352. Academic Press, New York.

Bruns,R.R. & Palade,G.E. (1968). Studies on blood capillaries. I. General organisation of blood capillaries in muscle. J. Cell Biol. 37: 244-276.

Burchett,M. (1982). The ecology of some coastal fish populations at South Georgia. Prog. Underwater Sci. 7: 15-20.

Burchett,M., Sayers,P.J., North,A.W. & White,M.G. (1983). Some biological aspects of the nearshore fish populations at South Georgia. Br. Antarct. Surv. Bull. 59:63-74.

Butler,D.G. (1968). Hormonal control of gluconeogenesis in the North American eel (Anguilla rostrata). Gen. Comp. Endocr. 10: 85-91.

Bylund,A.C., Bjuroe,T., Cederblad,G., Holm,J., Lundholm,K., Sjoestroem,M., Aengquist,K.A. & Schersten,T. (1977). Physical training in man. Eur. J. Appl. Physiol. 36: 151-169.

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

Carroll,N.V.R., Longley,W. & Roe,J.H. (1956). The determination of glycogen in liver and muscle by use of the anthrone reagent. J. biol. Chem. 220: 583-593.

Castellini,M.A. & Somero,G.N. (1981). Buffering capacity of vertebrate muscle: correlations with potentials for anaerobic function. J. Comp. Physiol. 143: 191-198.

Crawford,R.E. (1979). Effect of starvation and experimental feeding on the proximate composition and caloric content of an Antarctic teleost N.coriiceps neglecta. Comp. Biochem. Physiol. 62A: 321-326.

Childress,J.J. (1971). Respiratory rate and depth of occurrence of midwater animals. Limnol. Oceanogr. 16: 104-106.

Childress,J.J. (1975). The respiratory rates of mid-water crustacea as a function of depth of occurrence and relation to the oxygen minimum layer off Southern California. Comp. Biochem. Physiol. 50B: 787-799.

Childress,J.J. & Somero,G.N. (1979). Depth related enzyme activities in muscle brain and heart of deep-living pelagic marine teleosts. Mar.Biol. 52: 273-283.

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

Cleland,W.W. (1963). The kinetics of enzyme catalysed reactions with two or more substrates or products. I. Nomenclature and rate equations. Biochim. Biophys. Acta 67: 104-137.

Coppes de Achaval,Z. (1984). Isozymes of lactate dehydrogenase in fishes of the superorder Acanthopterygii - An update. Comp. Biochem. Physiol. 79B: 1-8.

Cossins,A.R. (1981). The adaptation of membrane dynamic structure to temperature. In 'Effects of Low Temperature on Biological Membranes', Morris,G.J. and Clarke,A. (eds), 82-106. Academic Press, London & New York.

Cossins,A,R., Bowler,K. & Prosser,C.L. (1981). Homeoviscous adaptation and it´s effects upon membrane-bound enzymes. J. therm. Biol. 6: 183-187.

Cowey,C.B. & Walton,M.J. (1982). Aspects of intermediary metabolism of salmonid fish. Comp. Biochem. Physiol. 73B: 59-79.

Crabtree, B. & Newsholme,E.A. (1972). The activities of phosphorylase, hexokinase, phosphofructokinase, lactate dehydrogenase and the glycerol-3-phosphate dehydrogenases in muscles from vertebrates and invertebrates. Biochem. J. 126: 49-58.

Cruz-Orive,L.M. (1982). Modelling structural anisotropy. Acta Stereol.(Sheffield) 1982.

Cuatrecasas,P. & Anfinsen,C.B. (1971). Affinity Chromatography. Ann. Rev. Biochem. 40: 259-278.

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

Dawson,D.M., Goodfriend,T.L. & Kaplan,N.O. (1964). Lactic dehydrogenase; function of the two types. Rates of synthesis of the two forms can be correlated with metabolic differentiation. Science 143: 929-933.

Deacon,G.E.R. (1937). The hydrology of the Southern Ocean. Dis. Rep. XV: 1-124.

Deacon,G.E.R. (1960). The southern cold temperate zone. Proc. Roy. Soc. B152: 441-447.

Dean,P.D.G. & Watson,D.H. (1978). Affinity chromatography of enzymes. In ´Affinity Chromatography´, Hoffman-Ostenhof,O. et al (eds), 25-38. Pergamon Press, Oxford.

Dearborn,J.H. (1965). Food of Weddell seals at McMurdo

Sound, Antarctica. J. Mammal. 46: 37-43.

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

DeWitt,H.H. (1966). A revision of the Antarctic and southern genus Notothenia (Pisces, Nototheniidae). Stanford University, Ph.D. Thesis.

Dewitt,H.H. (1971). Coastal and deep-water benthic fishes of the Antarctic. Am. Geogr. Soc. Antarct. Map Folio Ser. 15: 1-10.

Dietz,A.A. & Lubrano,T. (1967). Separation and quantitation of Lactic Dehydrogenase isoenzymes by disc electrophoresis. Anal. Biochem. 20: 246-257.

Driedzic, W.R. & Hochachka,P.W. (1978). Metabolism in fish during excercise. In 'Fish Physiology', Randall,D.J. & Hoar,W.S. (eds). Vol.7, 503-543. Academic Press, London & New York.

Driedzic,W.R., Macintyre,A.B. & McMorran,L.E. (1979). Lactate - the preferred anaerobic fuel of the metabolism of the fish heart. Abstracts, 1st Conference Eur. Soc. Comp. Physiol. Biochem. (Liege), 67-68.

Eastman,J.J. & DeVries,A.L. (1981). Buoyancy adaptations in a swimbladder-less Antarctic fish. J. Morphol. 167: 91-102.

Egginton,S. (1982). Structural and Functional Differentiation of Teleost Skeletal Muscle. Ph.D. thesis, University of St.Andrews.

Egginton,S. & Johnston,I.A. (1982a). Muscle fibre differentiation and vascularisation in the juvenile

European eel (Anguilla anguilla L.). Cell Tiss. Res. 222: 563-577.

Egginton,S. & Johnston,I.A. (1982b). A morphometric analysis of regional differences in myotomal muscle ultrastructure in the juvenile eel A.anguilla. Cell Tiss. Res. 222: 579-596.

Egginton,S. & Johnston,I.A. (1983). An estimate of capillary anisotropy and determination of surface and volume densities of capillaries in skeletal muscles of the conger eel (Conger conger L.). Quart. J. Exp. Physiol. 68: 603-617.

Eliassen,J-E. & Vahl,O. (1982). Seasonal variations in biochemical composition and energy content of liver, gonad and muscle of mature and immature cod G.morhua. J. Fish Biol. 20: 707-716.

El-Sayed,S.Z. (1968). Primary productivity. Am. Geogr. Soc. Antarctic Map Folio Ser. 10: 1-6.

Everse,J. & Kaplan,N.O. (1973). Lactate dehydrogenase: structure and function. Adv. Enzym. 37: 61-133.

Everse,J., Berger,R.L. & Kaplan,N.O. (1970). Physiological concentrations of lactate dehydrogenases and substrate inhibition. Science 168: 1236-1238.

Everson,I. (1968). Larval stages of certain Antarctic fishes. Br. Antarct. Surv. Bull. 16: 65-70.

Everson,I. (1969). The population dynamics and energy budget of Notothenia neglecta Nybelin at Signy Island, South Orkney Islands. Br. Antarct. Surv. Bull. 23: 25-50.

Everson,I. (1970a). The ecology of the inshore fish of Signy Island (South Orkneys). University of London

(Queen Mary College), Ph.D. Thesis.

Everson,I. (1970b). Reproduction in Notothenia neglecta Nybelin. Br. Antarct. Surv. Bull. 23: 81-92.

Everson,I. (1978). Antarctic fisheries. Polar Record 19: 233-251.

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

Everson,I. & Ralph,R. (1970). Respiratory metabolism in Chaenocephalus aceratus. In 'Antarctic Ecology', Holdgate,M.W. (ed), Vol.1, 313-319. Academic Press, London & New York.

Fitch,N.A. & Johnston,I.A. (1983). Muscle capillary supply in an Antarctic fish (Chaenocephalus aceratus) that lacks respiratory pigments. J. Physiol. (London) 340: 65P.

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

Flitney,F.W. & Johnston,I.A. (1979). Mechanical properties of isolated fish red and white muscle fibres. J. Physiol. (London) 295: 49-50P.

Foxton,P. (1956). The distribution of the standing crop of zooplankton in the Southern Ocean. Dis. Rep. 28: 191-236.

Fry,F.E.J. (1971). The effect of environmental factors on the physiology of fish. In 'Fish Physiology', Hoar,W.S. and Randall,D.J. (eds), Vol.6, 1-98. Academic Press, New York.

Gauthier,G.F. & Padykula,H.A. (1966). Cytological studies

of fibre types in skeletal muscle. A comparative study of the mammalian diaphragm. J. Cell Biol. 28: 333-354.

George,J.C. (1962). A histophysiological study of the red and white muscles of the mackerel. Am. Midl. Nat. 68: 487-494.

Gerday,Ch. (1982). Soluble calcium-binding proteins from fish and invertebrate muscle. Mol. Physiol. 2: 63-87.

Gerday,Ch. & Gillis,J.M. (1976). The possible role of parvalbumins in the control of contraction. J. Physiol. (Lond.) 258: 96P.

Gesser,H. & Fange,R. (1971). Lactate dehydrogenase and cytochrome oxidase in the swimbladder of fish. Int. J. Biochem. 2: 163-166.

Gesser,H. & Poupa,O. (1973). The lactate dehydrogenase system in the heart and skeletal muscle of fish: A comparative study. Comp. Biochem. Physiol. 46B: 683-690.

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

Goldspink,G. (1970). The proliferation of myofibrils during muscle growth. J. Cell Sci. 6: 593-603.

Gomez-Jarabo,G., Mataix,F., Illera,M. & Varela,G. (1976). The influence of age on the nutritive utilisation of protein in trout (Salmo gairdneri). Investigacion pesq. 40: 561-569.

Graves,J.E. & Somero,G.N. (1982). Electrophoretic and functional enzymic evolution in four species of Eastern Pacific barracudas from different thermal environments.

Evolution 36: 97-106.

Gray,S.D. & Renkin,E.M. (1978). Microvascular supply in relation to fiber metabolic type in mixed skeletal muscles of rabbits. Microvasc. Res. 16: 406-425.

Greer-Walker,M. (1970). Growth and development of the skeletal muscle fibres of the cod (G.morhua). J. Cons. perm. int. Explor. Mer. 33: 228-244.

Greer-Walker,M. & Pull,G.A. (1975). A survey of red and white muscle in marine fish. J. Fish Biol. 7: 295-300.

Guppy,M., Hulbert,W.C. & Hochachka,P.W. (1979). Metabolic sources of heat and power in tuna muscles. II. Enzyme and metabolite profiles. J. Exp. Biol. 82: 303-320.

Hamoir,G., Focant,B. & Disteche,M. (1972). Proteinic criteria of differentiation of white, cardiac and various red muscles in carp. Comp. Biochem. Physiol. 41B: 665-674.

Hansen,H.D. & Gesser,H. (1980). Relation between nonbicarbonate buffer value and the tolerance to cellular acidosis: A comparative study of myocardial tissue. J. Exp. Biol. 84: 161-167.

Hanson,S.W.F. & Olley,J. (1965). Application of the Bligh and Dyer method of lipid extraction to tissue homogenates. Biochem. J. 89: 101-102.

Hazel,J.R. (1972a). The effect of temperature acclimation upon succinic dehydrogenase activity from the epaxial muscle of the common goldfish (Carassius auratus)-I. Properties of the enzyme and the effect of lipid extraction. Comp. Biochem. Physiol. 43B: 837-861.

Hazel,J.R. (1972b). The effect of temperature acclimation upon succinic dehydrogenase activity from the epaxial

muscle of the common goldfish (Carassius auratus)-II. Lipid reactivation of the soluble enzyme. Comp. Biochem. Physiol. 43B: 863-882.

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

van der Helm,H.J. (1961). Simple method of demonstrating lactic acid dehydrogenase isoenzymes. Lancet 1961(2): 108-109.

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

Hemmingsen,E.A. & Douglas,E.L. (1972). Respiratory and circulatory responses in a haemoglobin-free 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 to the absence of haemoglobin in Channichthyid fishes. In 'Adaptations in the Antarctic Ecosystem', Llano,G.A. (ed), 479-487. Gulf Publishing Co., Texas.

Hemmingsen,E.A., Douglas,E.L. & Grigg,G.C. (1969). Oxygen consumption in an Antarctic haemoglobin-free fish Pagetopsis macropterus and in three species of Notothenia. Comp. Biochem. Physiol. 29: 467-470.

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

Hochachka,P.W. (1965). Isoenzymes in metabolic adaptation of a poikilotherm: sub-unit relationships in lactic dehydrogenase of a goldfish. Archs. Biochem. Biophys. 111: 96-103.

Hochachka,P.W. (1968). Action of temperature on branch points in glucose and acetate metabolism. Comp. Biochem. Physiol. 25: 107-118.

Hochachka,P.W. & Lewis,J.K. (1971). Interacting effects of pH and temperature on the Km values for fish tissue lactate dehydrogenases. Comp. Biochem. Physiol. 39B: 925-933.

Hochachka,P.W. & Somero,G.N. (1968). The adaptation of enzymes to temperature. Comp. Biochem. Physiol. 27: 659-668.

Hochachka,P.W. & Somero,G.N. (1973). Strategies of Biochemical Adaptation, 358pp. W.Saunders, Philadelphia.

Hocman,G. (1979). Biochemistry of Ageing. Int. J. Biochem. 10: 867-876.

Holdgate,M.W. (1964). Terrestrial ecology in the maritime Antarctic. In 'Biologie Antarctique', Carrick,R., Holdgate,M. & Prevost,J. (eds), 181-194. Herman, Paris.

Holdgate,M.W. (1967). The Antarctic ecosystem. Phil. Trans. R. Soc. 252: 363-389.

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. (1976). Respiratory morphometrics of white and red blooded Antarctic fish. Comp. Biochem. Physiol.

54A: 215-220.

Hoppeler,H., Luthi,P., Claassen,H., Weibel,E.R. & Howald,H. (1973). The ultrastructure of the normal human skeletal muscle: a morphometric analysis on untrained men, women and well-trained orienteers. Pflugers Arch. 344: 217-232.

Hoppeler,H., Mathieu,O., Krauer,R., Claassen,H., Armstrong,R.B. & Weibel,E.R. (1981a). Design of the mammalian respiratory system. VI. Distribution of mitochondria and capillaries in various muscles. Resp. Physiol. 44: 87-111.

Hoppeler,H., Mathieu,O., Weibel,E.R., Krauer,R., Lindstedt,S.L. & Taylor,C.R. (1981b). Design of the mammalian respiratory system. VIII. Capillaries in skeletal muscles. Resp. Physiol.44: 129-150.

Howell,B.J., Rahn,H., Goodfellow,D. & Herreid,C. (1973). Acid-base regulation and temperature in selected invertebrates as a function of temperature. Am. Zool. 13: 557-563.

Hudson,R.C.L. (1969). Polyneuronal innervation of the fast muscles of the marine teleost Cottus scorpius L. J. Exp. Biol. 50: 47-67.

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

Hughes,G.M. (1970). A comparative approach to fish respiration. Experientia 26: 113-122.

Hughes,G.M. (1972). Morphometrics of fish gills. Resp. Physiol. 14: 1-25.

Hureau,J.C. (1966). Biologie de Chaenichthys rhinoceratus Richardson, et probleme du sang incolore des

chaenichthyidae, poissons des mers Australes. Bull. de la Soc. Zool. de France 91: 735-751.

Iles,T.D. (1971). Ecological aspects of growth in African cichlid fishes. J. Cons. perm. int. Explor. Mer. 33: 363-385.

Inui,Y. & Ohshima,Y. (1966). Effect of starvation on metabolism and chemical composition of eels. Bull. Jap. Soc. scient. Fish. 32: 492-501.

Jacquot,R. (1961). Organic constituents of fish and other aquatic animal foods. In "Fish as Food", Borgstrom,G. (ed), Vol.1, 145-209. Academic Press, London & New York.

James,N.T. & Meek,G.A. (1979). Stereological analyses of the structure of mitochondria in pigeon skeletal muscle. Cell Tiss. Res. 202: 493-503.

Johnston,I.A. (1975). Pyruvate kinase from the red skeletal musculature of the carp (Carassius carassius L.). Biochem. Biophys. Res. Comm. 63: 115-120.

Johnston,I.A. (1979). Calcium regulatory proteins and temperature acclimation of actomyosin ATP´ase from a eurythermal teleost (Carassius auratus L.). J. Comp. Physiol. 129: 163-167.

Johnston,I.A. (1980). Contractile properties of fish fast muscle fibres. Mar. Biol. Lett. 1: 323-328.

Johnston,I.A. (1981). Quantitative analysis of muscle breakdown during starvation in the marine flatfish Pleuronectes platessa. Cell Tiss. Res. 214: 369-386.

Johnston,I.A. (1982a). Biochemistry of myosins and contractile properties of fish skeletal muscle. Mol. Physiol. 2: 15-29.

Johnston,I.A. (1982b). Capillarisation, oxygen diffusion distances and mitochondrial content of carp muscles following acclimation to summer and winter temperatures. Cell Tiss. Res. 222: 325-337.

Johnston,I.A. (1982c). Quantitative analyses of ultrastructure and vascularisation of the slow muscle fibres of the anchovy. Tissue & Cell 14: 319-328.

Johnston,I.A. (1983). Comparative studies of contractile proteins from the skeletal and cardiac muscles of lower vertebrates. Comp. Biochem. Physiol. 76A: 439-446.

Johnston,I.A. (1985). Temperature, muscle energetics and locomotion in inshore Antarctic fish. Oceanis 11: 125-142.

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. Pflugers Arch. 405: 136-140.

Johnston,I.A. & Bernard,L.M. (1982). Ultrastructure and metabolism of skeletal muscle fibres in the tench: Effects of long term acclimation to hypoxia. Cell Tiss. Res. 227: 179-199.

Johnston,I.A. & Bernard,L.M. (1984). Quantitative study of capillary supply to the skeletal muscles of crucian carp Carassius carassius L.: Effects of hypoxic acclimation. Physiol. Zool. 57: 9-18.

Johnston,I.A. & Goldspink,G. (1973). Some effects of prolonged starvation on the metabolism of the red and white myotomal muscles of the plaice (Pleuronectes platessa). Mar. Biol. 19: 348-353.

Johnston,I.A. & Harrison,P. (1985). Contractile and metabolic characteristics of muscle fibres from Antarctic fish. J. Exp. Biol. (IN PRESS).

Johnston,I.A. & Lucking,M. (1978). Temperature induced variation in the distribution of different types of muscle fibre in the goldfish (Carassius auratus). J. Comp. Physiol. 124: 111-116.

Johnston,I.A. & Maitland,B. (1980). Temperature acclimation in crucian carp, Carassius carassius L. Morphometric analyses of muscle fibre ultrastructure. J. Fish Biol. 17: 113-125.

Johnston,I.A. & Moon,T.W. (1980a). Excercise training in the skeletal muscles of brook trout (Salvelinus fontinalis). J. Exp. Biol. 87: 177-195.

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

Johnston,I.A. & Moon,T.W. (1981). Fine structure and metabolism of multiply innervated fast muscle fibres in teleost fish. Cell Tiss. Res. 219: 93-109.

Johnston,I.A. & Walesby,N.J. (1977). Molecular mechanisms of temperature compensation in fish myofibrillar ATP´ases. J. Comp. Physiol. 119: 195-206.

Johnston,I.A. & Walesby,N.J. (1979). Evolutionary temperature adaptation and the calcium regulation of fish myosin ATP´ases. J. Comp. Physiol. 129: 169-177.

Johnston,I.A., Bernard,L.M. & Maloiy,G.M. (1983). Aquatic and aerial respiration rates, muscle capillary supply and mitochondrial volume density in the air breathing catfish (Clarius mossambicus) acclimated to either

aerated or hypoxic water. J. Exp. Biol. 105: 317-338.

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

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

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

Johnston,I.A., Fitch,N.A., Zummo,G., Wood,R., 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.

Jonas,R.E. & Bilinski,E. (1964). Utilisation of lipids by fish III. Fatty acid oxidation by various tissues from sockeye salmon. J. Fish Res. Bd. Can. 21: 653-656.

Jorgensen,J.B. & Mustafa,T. (1980a). The effect of hypoxia on carbohydrate metabolism in the flounder (Platichthys flesus). I. Utilisation of glycogen and the accumulation of glycolytic end-products in various tissues. Comp. Biochem. Physiol. 67B: 243-248.

Jorgensen,J.B. & Mustafa,T. (1980b). The effect of hypoxia on carbohydrate metabolism in the flounder (Platichthys flesus). II. High energy phosphate compounds and the role of glycolytic and gluconeogenetic enzymes. Comp. Biochem Physiol. 67B: 249-256.

Kacser,H. & Burns,J.A. (1973). The control of flux. S.E.B.

symp. Ser. XXVII: 65-104.

Kaplan,N.O., Ciotti,M.M., Hamolsky,M. & Beiber,R.E. (1960). Molecular heterogeneity and evolution of enzymes. Science 131: 392-397.

Kightley,S.P.J. & Caldwell,J.R. (1982). The first record of a fur seal birth on Signy Island, South Orkney Islands. Br. Antarct. Surv. Bull. 51: 287-289.

King,C.A.M. (1975). Introduction to Physical and Biological Oceanography. 372pp. Edward Arnold, London.

Kock,K-H. (1985). The state of exploited Antarctic fish stocks around South Georgia (Antarctic). Arch. Fischwiss. 36: 155-183.

Komatsu,S.K. & Feeney,R.E. (1970). A heat labile fructosediphosphate aldolase from cold adapted Antarctic fish. Biochim. Biophys. Acta 206: 305-315.

Komatsu,S.K., Miller,H.T., DeVries,A.L., Osuga,D.T. & Feeney,R.E. (1970). Blood plasma proteins of cold-adapted Antarctic fishes. Comp. Biochem. Physiol. 32: 519-527.

Krogh,A. (1919). The number and distribution of capillaries in muscles with calculations of the oxygen pressure head necessary for supplying the tissue. J. Physiol (Lond.) 52: 409-415.

Kryvi,H. & Totland,G.K. (1978). Fibre types in locomotory muscles of the cartilaginous fish Chimera monstrosa. J. Fish Biol. 12: 257-265.

Laws,R.M. (1953). The Elephant seal (Mirounga leonina). I. Growth and age. F.I.D.S. Sci. Rep. No.8, 62pp.

Laws,R.M. (1956a). The Elephant seal (Mirounga leonina). II. General, social and reproductive behaviour.

F.I.D.S. Sci. Rep. No.13, 88pp.

Laws,R.M. (1956b). The Elephant seal (Mirounga leonina). III. The physiology of reproduction. F.I.D.S. Sci. Rep. No.15, 64pp.

Laws,R.M. (1981). Seal surveys, South Orkney Islands, 1971 and 1974. Br. Antarct. Surv. Bull. 54: 136-139.

Lehninger,A.L. (1975). Biochemistry. The Molecular Basis of Cell Structure and Function. 2nd Edn. Worth, New York.

Le Peuch,C.J., Demaille,J. & Pechere,J.F.(1978). Radioelectrophoresis: a specific microassay for parvalbumins, application to muscle biopsies from man and other vertebrates. Biochim. Biophys. Acta 537: 153-159.

Lewis-Smith,R.I. (1970). Vegetation of the South Orkney Islands with particular reference to Signy Island. B.A.S. Sci. Rep. No.68, 124pp.

Lin,Y., Dobbs,G.H. & DeVries,A.L. (1974). Oxygen consumption and lipid content in red and white muscles of Antarctic fish. J. Exp. Zool. 189: 379-386.

Lishman,G.L. (1981). The comparative feeding ecology, breeding biology and bioenergetics of Pygoscelid penguins on Signy Island. B.A.S. Report Q2/1981/H (unpublished).

Lonnberg,A.J.E. (1905). The fishes of the Swedish South Polar expedition. Wissensch. Ergebn. Schwed. Sudpol-Exped. No.5, 69pp.

Love,R.M. (1970). The Chemical Biology of Fishes. Vol.1. Academic Press, London & New York.

Love,R.M. (1980). The Chemical Biology of Fishes. Vol.2.

Academic Press, London & New York.

Love,R.M., Robertson,I. & Strachan,I. (1968). Studies on the North sea cod. VI. Effects of starvation. 4. sodium and potassium. J. Sci. Fd. Agric. 19: 415-422.

Lowry,O.H., Rosebrough,N.J., Farr,A.L. & Randall,R.J. (1951). Protein measurement with the Folin phenol reagent. J. biol. Chem. 190: 265-275.

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

MacIntyre,A.B. & Driedzic,W.R. (1981). Activities of enzymes in cardiac energy metabolism. Can. J. Zool. 59: 325-328.

Mansfield,A.W. (1958). The breeding behaviour and reproductive cycle of the Weddell seal (Leptonychotes weddelli Lesson). F.I.D.S. Sci. Rep. No.18, 41pp.

Markert,C.L., Shaklee,J.B. & Whitt,G.S. (1975). Evolution of genes. Science 189: 102-114.

Marr,J.W.S. (1935). The South Orkney Islands. Dis. Rep. X: 283-382.

Marshall,N.B. (1953). Egg size in Arctic, Antarctic and deep-sea fishes. Evolution 7: 328-341.

Marshall,N.B. (1964). Some convergence between the benthic fishes of polar seas. In 'Biologie Antarctique', Carrick,R., Holdgate,M. & Prevost,J. (eds), 273-278. Hermann, Paris.

Martsinkevich,L.D. (1958). Cellular composition of blood in white-blooded fishes (Chaenichthyidae) of the Antarctic. Inform. Byul. Sov. Antarkt. Eksp. 3: 67-68.

Mathews,D.H. & Maling,D.H. (1967). The geology of the South

Orkney Islands: I. Signy Island. F.I.D.S. Sci. Rep. 25: 32pp.

Mathieu,O., Cruz-Orive,L.M., Hoppeler,H. & Weibel,E.R. (1985). Estimating length density and quantifying anisotropy in skeletal muscle capillaries. J. Microsc. (In Press).

Mathieu,O., Krauer,R., Hoppeler,H., Gehr,P., Lindstedt,S.L., Alexander,R.McN., Taylor,C.R. & Weibel,E.W. (1981). Design of the mammalian respiratory system. VII. Scaling mitochondrial volume in skeletal muscle to body mass. Resp. Physiol. 44: 113-128.

Matsuura,F. & Hashimoto,K. (1954). Chemical studies on the red muscles of fishes. II. Determinations of the content of haemoglobin, myoglobin and cytochrome c in the muscles of fishes. Bull. Jap. Soc. Scient. Fish. 20: 308-312.

Mendez,D.J. & Keys,A. (1960). Density and composition of mammalian muscle. Metabolism 9: 184-188.

Montgomery,J.C. & Macdonald,J.A. (1984). Performance of motor systems in Antarctic fishes. J. Comp. Physiol. 154: 241-248.

Moon,T.W. & Johnston,I.A. (1980). Starvation and the activities of glycolytic and gluconeogenic enzymes in skeletal muscle and liver of the plaice (P.platessa). J. Comp. Physiol. 136: 31-38.

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.

Mosbach,K., Guilford,H., Ohlsson,R. & Scott,M. (1972). General ligands in affinity chromatography. Biochem. J.

127: 625-631.

Mosse,P.R.L. (1978). The distribution of capillaries in the somatic musculature of two vertebrate types with particular reference to teleost fish. Cell Tiss. Res. 187: 281-303.

Mosse,P.R.L. (1979). Capillary distribution and metabolic histochemistry of the lateral propulsive musculature of pelagic teleost fish. Cell Tiss. Res. 203: 141-160.

Nag,A.C. (1972). Ultrastructure and ATP´ase activity of red and white muscle fibres of the caudal region of a fish, Salmo gairdneri. J. Cell Biol. 55: 42-57.

Nag,A.C. & Nursall,J.R. (1972). Histogenesis of white and red muscle fibres of trunk muscles of a fish Salmo gairdneri. Cytobios 6: 227-246.

Newsholme,E.A. & Start,C. (1973). Regulation in Metabolism, 349pp. Wiley & Sons, London & New York.

Newsholme,E.A., Zammit,V. & Crabtree,B. (1978). The role of glucose and glycogen as fuels for muscle. Biochem. Soc. Trans. 6: 512-520.

Norman,J.R. (1938). Coast fishes, III: The Antarctic zone. Dis. Rep. 18: 1-104.

North,A.W. & White,M.G. (1982). Key to fish post-larvae from the Scotia seas, Antarctica. Cybium, 6: 13-32.

Nuccitelli,R. & Heiple,J.M. (1982). Summary of the evidence and discussion concerning the involvement of pH in the control of cellular functions. In ´Intracellular pH: Its Measurement, Regulation and Utilisation in Cellular Function´, Nuccitelli,R. & Deamer,D.W. (eds.), 567-586. Allan R. Liss, New York.

Nybelin,O. (1947). Antarctic Fishes. In ´Scientific

Results of the Norwegian Antarctic Expeditions, 1927-1928', Holtedahl,O. (ed). Vol II, 390-466. Jacob Dybwad, Oslo.

Nybelin,O. (1951). Sub-Antarctic and Antarctic fishes. Scientific Results of the Bratteg Expedition, 1947-1948. No.2, 1-32.

O'Carra,P. & Barry,S. (1972). Affinity chromatography of lactate dehydrogenase. FEBS Lett. 21: 281-285.

Patterson,S., Johnston,I.A. & Goldspink,G. (1974). The effects of starvation on the chemical composition of red and white muscles in the plaice (Pleuronectes platessa). Experientia 30: 892-894.

Permitin,Yu.E. & Tarverdieva,I.M. (1978). Feeding of the fishes of the families Nototheniidae and Channichthyidae in the South Orkney Islands,Antarctica. Sov. J. Mar. Biol. 4: 619-622.

Pietschmann,M., Bartels,H. & Fons,R. (1982). Capillary supply of heart and skeletal muscle of small bats and non-flying mammals. Resp. Physiol. 50: 267-282.

Prosser,C.L. (1973a). Respiratory functions of the blood. In 'Comparative Animal Physiology', Prosser,C.L. (ed), Vol 1, 317-361. W.B. Saunders Co., Philadelphia, London & Toronto.

Prosser,C.L. (1973b). Oxygen: Repiration and metabolism. In 'Comparative Animal Physiology', Prosser,C.L. (ed), Vol 1, 165-211. W.B. Saunders Co., Philadelphia, London & Toronto.

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

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

Reeves,R.B. (1977). The interaction of body temperature and acid-base balance in ectothermic vertebrates. A. Rev. Physiol. 39: 559-586.

Regan,C.T. (1914). Fishes. In 'British Antarctic ('Terra Nova') Expedition,1910.' Zool. 1: 1-54. Oxford University Press, London.

Renaud,J.M. & Moon,T.W. (1980). Characterisation of gluconeogenesis in hepatocytes isolated from the American eel Anguilla rostrata Leseur. J. Comp. Physiol. 135: 115-125.

Reynolds,E.S. (1963). The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J. Biophys. Biochem. Cytol. 10: 308-312.

Richardson,M.G. (1975). The dietary composition of some Antarctic fish. Br. Antarct. Surv. Bull. 41 & 42: 113-120.

Robillard,G.S. & Dayton,P.K. (1969). Notes on the biology of the chaenichthyid fish Pagetopsis macropterus. Antarct. J. U.S. 4: 304-306.

Robertson,O.H. (1961). Prolongation of the life span of kokanee salmon (Oncorhynchus nerka kennerlyi) by castration before beginning of gonad development. Proc. natn. Acad. Sci. USA. 47: 609-621.

Romanul,F.C.A. (1965). Capillary supply and metabolism of muscle fibers. Arch. Neurol. 12: 497-509.

Ruud,J.T. (1954). Vertebrates without erythrocytes and

blood pigment. Nature (London) 173: 848-850.

Salamonski,J.H. & Johnston,I.A. (1982). Capillary supply and mitochondrial volume density in the axial muscles of the mesopelagic teleost Argyropelecus hemigymnus. Mar. Biol. 69: 1-5.

Salthe,S.N. (1965). Comparative catalytic studies of lactic dehydrogenases in the amphibia: environmental and physiological correlations. Comp. Biochem. Physiol. 16: 393-408.

Satchell,G.H. (1971). 'Circulation in Fishes'. Cambridge University Press, Cambridge.

Scapolo,P.A., Veggetti,A., Rowlerson,A., Mascarello,F. & Carpere,E. (1984). Do the small new fibres of grey mullet white muscle arise by fibre splitting ? J. Muscle Res. Cell Mot. 5: 214-226

Schmidt-Neilsen,K. & Pennycuik,P. (1961). Capillary density in mammals in relation to body size and oxygen consumption. Am. J. Physiol. 200: 746-750.

Shaklee,J.B., Christiansen,J.A., Sidell,B.D., Prosser,C.L. & Whitt,G.S. (1977). Molecular aspects of temperature acclimation in fish: Contributions of changes in enzyme activities and isozyme patterns to metabolic reorganisation in the green sunfish. J. Exp. Zool. 201: 1-20.

Shaw,P. (1980). Age-related breeding biology of the blue-eyed shag. B.A.S. Report Q2/1980/H (Unpublished).

Shul'man,G.E. (1974). Life Cycles of Fish: physiology and biochemistry. John Wiley, New York.

Sidell,B.D. (1980). Responses of goldfish (Carassius auratus) muscle to acclimation temperature: alterations

in biochemistry and proportions of different fibre types. Physiol. Zool. 53: 98-107.

Sidell,B.D. (1983). Cardiac metabolism in the Myxinidae: physiological and phylogenetic considerations. Comp. Biochem. Physiol. 76A: 495-506.

Sidell,B.D. & Beland,K.F. (1980). Lactate dehydrogenases of Atlantic hagfish: Physiological and evolutionary implications of a primitive heart isozyme. Science 207: 769-770.

Siebert,G., Schmitt,A. & Bottke,I. (1964). Enzymes of the amino acid metabolism in cod musculature. Arch. Fisch. Wiss. 15: 233-244.

Siefter,S., Dayton,S., Novic,B. & Muntwyler,E. (1949). The estimation of glycogen with the anthrone reagent. Archs. Biochem. 25: 191-200.

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

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

Smith,R.N. (1972a). The freezing resistance of Antarctic fish: I. Serum composition and it's relation to freezing resistance. Br. Antarct. Surv. Bull. 28: 1-10.

Smith,R.N. (1972b). " II. The freezing points of body fluids. Br. Antarct. Surv. Bull. 29: 91-102.

Smith,R.N. (1972c). " III.

An experimental study of the death of supercooled fish resulting from contact with ice. Br. Antarct. Surv. Bull. 30: 81-94.

Somero,G.N. (1978). Temperature adaptation of enzymes: Biological optimisation 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: 621-634.

Somero,G.N. & Childress,J.J. (1980). A violation of the metabolism-size paradigm. Activities of glycolytic enzymes in muscle increase in larger size fish. Physiol. Zool. 53: 322-337.

Somero,G.N. & Hochachka,P.W. (1969). Isoenzymes and short-term temperature compensation in poikilotherms: activation of lactate dehydrogenase isoenzymes by temperature decreases. Nature (Lond.) 223: 194-195.

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

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

Stone,B.J. & Sidell,B.D. (1981). Metabolic responses of striped bass (Morone saxatilis) to temperature acclimation. 1. Alterations in carbon sources for hepatic energy metabolism. J. Exp. Zool. 218: 371-379.

Stonehouse,B. (1972). Animals of the Antarctic: The Ecology

of the far South. 171pp. Holt,Rinehart & Winston, New York.

Storey,K.B. & Bailey,E. (1978). Intracellular distribution of enzymes associated with lipogenesis and gluconeogenesis in the fat body of the adult cockroach, Periplaneta. Insect Biochem. 8: 125-131.

Storey,K.B. & Hochachka,P.W. (1974). Enzymes of energy metabolism in a vertebrate facultative anaerobe, Pseudemys scripta. J. biol. Chem. 249: 1423-1427.

Strickland,N.C. (1975). Relationship between size of muscle fibres and body dimensions in a number of teleosts. Experientia 31: 1279-1280.

Tano,S. & Shirihata,S. (1975). Effects of age and growth on macromolecular biosynthesis in the salmon brain. Radioisotopes 24: 93-96.

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

Taylor,C.R. & Weibel,E.R. (1981). Design of the mammalian respiratory system. I. Problem and strategy. Resp. Physiol. 44: 1-10.

Templeman,W. & Andrews,G.L. (1956). Jellied condition in the American plaice (Hippoglossoides platessoides). J. Fish. Res. Bd. Can. 13: 147-182.

Thompson,S.T., Cass,K.H. & Stellwagen,E. (1975). Blue-dextran sepharose: An affinity column for the dinucleotide fold in proteins. Proc. Natl. Acad. Sci. U.S.A. 72: 669-672.

Thurston,M.H. (1972). The crustacea amphipoda of Signy

Island, South Orkney Islands. B.A.S. Sci. Rep. No.71, 127pp.

Torres,J.J., Belman,B.W. & Childress,J.J. (1979). Oxygen consumption rates of midwater fishes of California. Deep Sea Res. 26A: 185-197.

Twelves,E.L. (1970). Study of the ecology of Chaenocephalus aceratus and aspects of the physiology of C.aceratus and Notothenia neglecta. B.A.S. Report N2/1970/H (unpublished).

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

Tyler,J.C. (1960). Erythrocyte counts and haemoglobin determinations for two Antarctic Nototheniid fishes. Stanford Ichthyol. Bull. 7: 199-201.

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

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

Walsh,P.J. & Somero,G.N. (1982). Interactions among pyruvate concentration, pH and Km of pyruvate in determining *in vivo* Q_{10} values of the lactate dehydrogenase system. Can. J. Zool. 60: 1293-1299.

Weatherly,A.H. & Gill,H.S. (1985). Dynamics of increase in muscle fibers in fishes in relation to size and growth. Experientia 41: 353-354.

Webb,P.W. (1973). Kinematics of pectoral fin propulsion in

Cymatogaster aggregata. J. Exp. Biol. 59: 697-710.

Webb,P.W. (1975). Hydrodynamics and energetics of fish propulsion. Bull. Fish. Res. Bd. Can. 190: 1-159.

Weibel,E.R. (1973). Stereological techniques for electron microscopic morphometry. In 'Principles and Techniques of Electron Microscopy', Mayat,A. (ed.). Vol.3, 237-296. Van Nostrand-Rinhold, Amsterdam.

Weibel,E.R. (1979). Stereological Methods. Vol.1: Practical Methods For Biological Morphometry. Academic Press, London, New York & Toronto.

Weibel,E.R. (1980). Stereological Methods. Vol.2: Theoretical Foundations. Academic Press, London, New York & Toronto.

Weibel,E.R., Gehr,P.G., Cruz-Orive,L.M., Muller,A.E., Kwangi,D.K. & Haussener,V. (1981a). Design of the mammalian respiratory system. IV. Morphometric estimation of pulmonary diffusing capacity: critical evaluation of a new sampling method. Resp. Physiol. 44: 39-59.

Weibel,E.R., Taylor,C.R., Gehr,P., Hoppeler,H., Mathieu,O. and Maloiy, G.M. (1981b). Design of the mammalain respiratory system. IX. Functional and structural limits for oxygen flow. Resp. Physiol. 44: 151-164.

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

Westermann,J.E.M., Barber,D.L. & Malo,L.G. (1984). Observations on gills of pelagic and demersal juvenile Notothenia rossii. Br. Antarct. Surv. Bull. 65: 81-89.

White,M.G. (1970). Aspects of the breeding biology of Glyptonotus antarcticus at Signy Island, South Orkney Islands. In 'Antarctic Ecology', Holdgate,M.W. (ed.), 279-285. Academic Press, London.

White,M.G. (1972). Biomass estimates from Borge bay, Signy Island, South Orkney Islands. Br. Antarct. Surv. Bull. 31: 45-50.

Whitt,G.S. (1970). Directed assembly of polypeptides of the isozymes of lactate dehydrogenase. Archs. Biochem. Biophys. 138: 353-354.

Whittaker,T. (1982). Primary production of phytoplankton off Signy Island, South Orkney Islands, the Antarctic. Proc. R. Soc. Lond. 214B: 169-189.

Wilkins,N.P. (1967). Starvation of the herring, Clupea harengus L.: survival and some gross biochemical changes. Comp. Biochem. Physiol. 23: 503-518.

Wilson,A.C., Cahn,R.D. & Kaplan,N.O. (1963). Functions of the two forms of lactic dehydrogenase in the breast muscles of birds. Nature (Lond.) 197: 331-334.

Wittenberg,J.B. (1963). Facilitated diffusion of oxygen through haemerythrin solutions. Nature (Lond.) 199: 816-817.

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

Wohlschlag,D.E. (1962). Metabolic requirements for the swimming activity of three Antarctic fishes. Science 137: 1050-1051.

Wohlschlag,D.E. (1964). Respiratory metabolism and growth of some Antarctic fishes. In 'Biologie Antarctique', Carrick,R., Holdgate,M.W. & Prevost,J. (eds.), 489-502.

Hermann, Paris.

Woodhead,A.D. (1974a). Ageing changes in the Siamese fighting fish, Betta splendeus - I. The testis. Exp. Gerontol. 9: 75-81.

Woodhead,A.D. (1974b). Ageing changes in the Siamese fighting fish, Betta splendeus - II. The ovary. Exp. Gerontol. 9: 131-139.

Woodhead,A.D. & Ellett,S. (1966). Endocrine aspects of ageing in the guppy Lebistes reticulatus - I. The thyroid gland. Exp. Gerontol. 1: 315-330.

Yamawaki,H. & Tsukuda,H. (1979). Significance in the variation in isozymes of liver lactate dehydrogenase with thermal acclimation in the goldfish. I: thermostability and temperature dependency. Comp. Biochem. Physiol. 62B: 89-93.

Zammit,V.A & Newsholme,E.A. (1979). Activities of enzymes of fat and ketone-body metabolism and effects of starvation on blood concentration of glucose and fat fuels in teleost and elasmobranch fish. Biochem. J. 184: 313-322.

Zollner,N. & Kirsch,K. (1962). The quantitative determination of lipids by means of the sulfophosphovanillin reaction common to many natural lipids (all known plasma lipids). Zeit. ges. exptl. Med. 135: 545-561.

Acknowledgements

It is impossible to carry out a research programme in the Antarctic without the help and consideration of all base members. I would like to thank all personnel who were on Signy Island between February 1980 and April 1982 for helping to make my stay such a pleasant and enjoyable one. I would particularily like to thank Boatmen Mark Redpath and Adrian Overbury; Marine Assistants Rick Price and Bob Forster; and Diving Officers Doug Allan and Damien Sanders. My grateful thanks are also due to the Director of B.A.S., Dr. R.M.Laws,FRS, for giving me the opportunity of working in Antarctica, and for permission to publish this thesis.

In St. Andrews thanks for advice and help are due to Tony Crockford and Murray Coutts in particular, and all other members of the Fish Lab., most of whom have been of assistance at one time or another. Mr. C.D. Sinclair of the Department of Statistics was very helpful in discussions about the statistics employed in this thesis.

Finally my sincere thanks go to my supervisors, Dr. I.A.Johnston, and M.G.White for their tolerance, understanding, enthusiasm and support (and patience) over a sustained period.

I would like to dedicate this thesis to my wife Stephanie.

Place-names. The following names used locally at Signy Island are not official place-names: Porny Rock; Weed Bowl; Owen's Bank; Powell Rock.

Tel. ST. ANDREWS 76161 GA

PROFESSOR XMSXLAVERACK, BSc, RXD, FRSBIOLXFRSE
Director: Professor Ian A. Johnston

Dr. Dominic Houlihan,
Department of Physiology,
University of Aberdeen,
ABERDEEN.

Dear Dominic,

In order to speed things up, I am sending a copy of
directly since College Gate invariably takes a while to
Thanks very much for agreeing to be the External, and I
seeing you in mid November or after Ireturn from the Ant

Best wishes

Ian

Professor Ian A. Johnston