Ecological and Behavioural Correlates of Intracellular Buffering Capacity in the Muscles of Antarctic Fishes

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Summary. Five species of antarctic fishes can be arranged in order of increasing anaerobic capacity of the white muscles for burst swimming: *Rhigophila dearborni* (Zoarcidae), icefish (Channichthyidae), *Dissostichus mawsoni, Trematomus centronotus,* and *Pagothenia borchgrevinki* (Nototheniidae). This order reflects increasing dependence on anaerobic work done during short bursts of speed during prey capture or predator avoidance. Buffer capacity (β) for white muscle was lower than that of behaviourally equivalent fish from lower latitudes and β is itself temperature-dependent.

Introduction

Temperature is an environmental factor which has profound effects on both aerobic and anaerobic activities in poikolothermic animals. Nowhere is this better illustrated than in the freezing waters of Antarctica where the metabolic rates of the fish living at -1.8 ° C are extremely low (Macdonald et al. 1987).

During aerobic activity, hydrogen ions from acidic metabolites are consumed by oxidative phosphorylation and thus tissue pH remains in balance (Hochachka and Mommsen 1983). When anaerobic bursts of activity occur, this balance no longer exists and hydrogen ions must be buffered to prevent an excessive fall in pH which might otherwise inhibit normal physiological processes such as muscular contraction and solute transport. These two patterns of energy expenditure are broadly associated with red and white muscle types, respectively.

Intracellular buffering capacity therefore becomes critical in muscles which function under conditions where perfusion limits acid transport (Castellini and Somero 1981). (The system is efficient however, because anaerobic metabolites are subsequently recycled by oxidative phosphorylation.)

Clearly, acid-base balance depends, for the most part, on the buffering capacity of muscle tissue in relation to metabolic rate and external gas exchange, which are in turn influenced by environmental factors such as temperature. Temperature alters the protonation state of many compounds and thus a key feature of intracellular buffering requires that protonation should be preserved over a range of temperatures (Hazel et al. 1978). In this respect, Abe et al. (1985) proposed that imidazole-based histidine compounds play a crucial role in buffering fish muscle over a wide range of operating temperatures.

Antarctic fish provide a special opportunity to test this thesis at an extreme low temperature. Further, Antarctic fish are predominantly endemic and have radiated into a diverse assemblage of forms ranging from sedentary benthic species to those adapted for a cryopelagic mode of living (Eastman and DeVries 1985). The present study focuses on the differences in buffering capacities in relation to work performed at very low temperature for the species, ranked in decreasing order of scope for activity: *Pagothenia borchgrevinki, Dissostichus mawsoni, Trematomus centronotus* (Nototheniidae), *Pagetopsis macropterus* (Channichthyidae), and *Rhigophila dearborni* (Zoarcidae).

Materials and Methods

Collection of Fishes

This study was conducted during the southern summer of 1986-1987 near New Zealand's Scott Base (77°49'S, 166°40'E) on Ross Island in McMurdo Sound. Our principal fishing site was located 1500 m south of Scott Base on the fast ice over a depth of 420m water. A 1m diameter hole drilled through the ice provided access to water for fishing and for maintaining aquaria inside a heated hut.

The cryopelagic notothenid *Pagothenia borchgrevinki* was taken on hand lines using shiny lures. Giant antarctic toothfish (erroneously called "cod", *Dissostichus mawsoni,* were removed from a seal during its visits to our fishing hole. Capture times of the cod were in the order of a few minutes and the fish were in better condition than could be obtained from setlines. The stomach of one cod revealed a live icefish which had apparently only just been swallowed. The opportunity was taken to sample tissues from this single, channichthyid *(Pagetopsis macropterus).* (The term Channichthyidae, as recognized by DeWitt (1971), takes precedence over Chaenichthyidae which mutated through a spelling error in older literature and tends to be perpetuated.) Other species were collected from various localities by hand line, SCUBA, or bottom-set traps. These included the notothenid, *Trematomus centronotus,* and the zoarcid, *Rhigophila dearborni.* Several specimens were kindly given to us by Professor A. L. DeVries.

Selection of Tissues

With the exceptions of *D. mawsoni* and the single icefish specimen, all fish were rested, tank-acclimated specimens. White skeletal muscle was excised from the trunk of freshly pithed fish dorsal to, and just posterior to the anus. Red muscle was identified by its pinkish colour and by its position just anterior to the insertion of the pectoral fins. Sections of ventricular muscle were taken from the hearts, and blood was taken from large veins into heparinised syringes.

The tissues were rinsed briefly in cold saline and blotted dry on filter paper. Samples which could not be analysed immediately were frozen for measurement within 48 h.

Blood hemoglobin was determined optically as the cyanmethemoglobin derivative (Dacie and Lewis 1975).

Measurement of Buffering Capacity

In vitro muscle buffering capacity (β) was estimated according to the methods of Castellini and Somero (1981). Approximately 0.5g fresh or frozen tissue was ground in a glass homogenizer with 5 ml ice-cold 0.85% NaCl. The homogenate was made up to 10ml with cold saline and placed in a reaction vial thermostatted to 25 °C, fitted with a stirrer and a semi-micro combination Ingold pH electrode coupled to an Orion pH meter. The effect of measurement temperature was examined by titrations at 0°, 15°, and 37 °C. The electrode was calibrated at the measurement temperature using NBS buffer standards. When necessary, the pH of the homogenate was adjusted down to pH 6.00 with 0.2 M HCI. The buffer line was then determined by titrating 20-µl aliquots of 0.2 M NaOH and recording stable pH. Titration was continued to >pH 8.00. Buffering capacity (β) was measured in Slykes, where 1 Slyke is the number of µmol base required to titrate the pH of 1 g wet weight of tissue by one pH unit over the pH range 6.5 to 7.5.

Results are reported as means with standard errors, and Student's-t-test was used in the conventional way to discriminate means. Data are recorded as replicates from two *D. mawsoni,* and one icefish. Data for heart muscle is also replicated from pooled samples. Statistical comparisons are therefore valid only for skeletal muscles from other species for which *n* represents separate measurements on individual fish.

Results

The variation in β observed among individuals of the same species was generally smaller than the extremes in β for interspecific comparisons (Figs. 1 and 2). Buffer values for white trunk muscle showed significant pH-dependence as evident from the upward curving plots in Fig. 1. For the purpose of comparing values of buffer capacity (β) with published data, linearity was assumed between pH 6.5 and 7.5 where the buffer capacity of fish muscle is maximal (Abe et al. 1985). The shape of the titration curves in Fig. 1 show no such zone of maximal buffering and hence it must be recognised that buffering capacity itself is a function of pH. R was calculated in this range for each titration and the mean and standard deviations calculated. A summary of this analysis for muscles and blood from five species of Antarctic fish is given in Fig. 2.

White muscle has an appreciably higher value of β than values from other tissues ($P<0.01$), with the exception of *T. centronotus* where white vs. pink muscle titrated similarly ($P>0.2$). White muscle from the nototheniids *D. mawsoni, P. borchgrevinki,* and *T. centronotus* had significantly higher ($P<0.01$) buffer capacities than those of the channichthyid and the zoarcid. Values of (3 from the former group were, in fact, higher in all tissues than those of the latter (Fig. 2). Pink muscle could not be located in the region of pectoral fin insertion in *R. dearborni.*

The buffer capacity of blood was highest in fish with high hemoglobin (Hb) concentration (Table 1). *R. dearborni* was exceptional in having a low value of R for blood ($R = 3.5\pm0.3$ slykes) and comparatively high Hb (36.6 ± 3.1).

The buffer capacity of white muscle from P. *borchgrevinki* was temperature dependent with (3 significantly lower at 0 ° C ($P<0.01$) than at any higher temperature (Fig. 3). Since tissue pH shifts with in vivo temperature, then it is obvious that so might buffering capacity via the temperature dependence of pH.

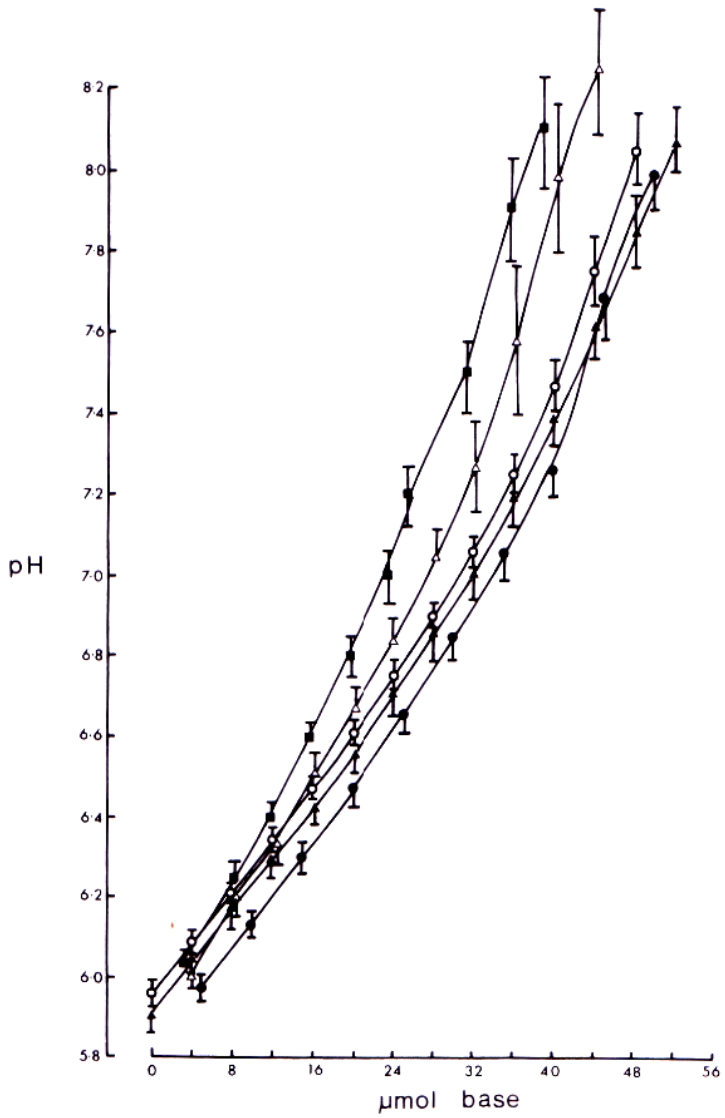

Fig. 1. Buffer titration curves for white trunk muscles of antarctic fishes. Values are mean ± SD for *P. borchgrevinki* (o - - - - o), *D. mawsoni* (▲ - - - -▲), *T. centronotus* (● - - - - ●), *P. macropterus* (Δ ----Δ), and *R. dearborni* (■----■)

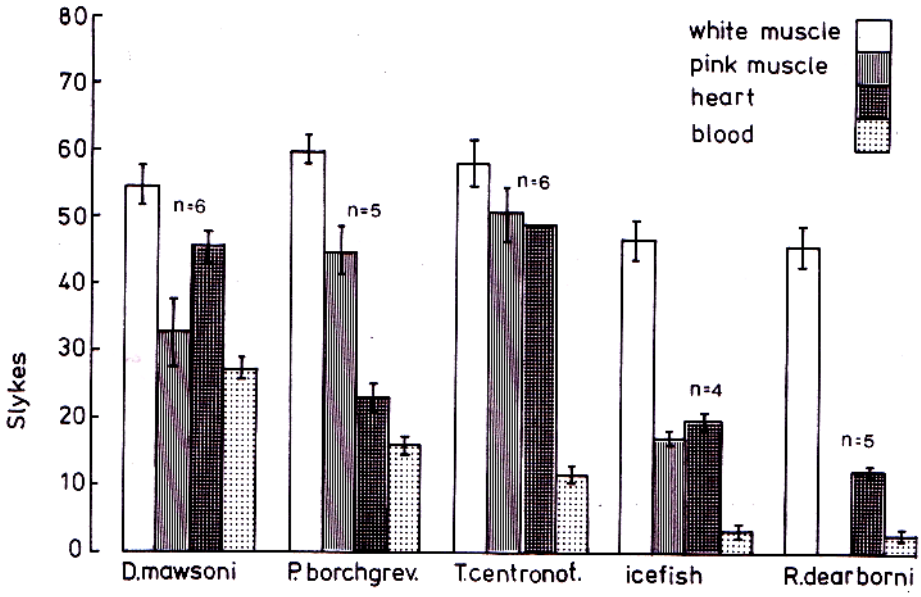

Fig. 2. Buffer capacities (β)±SD measured in Slykes, for various tissues from antarctic fishes

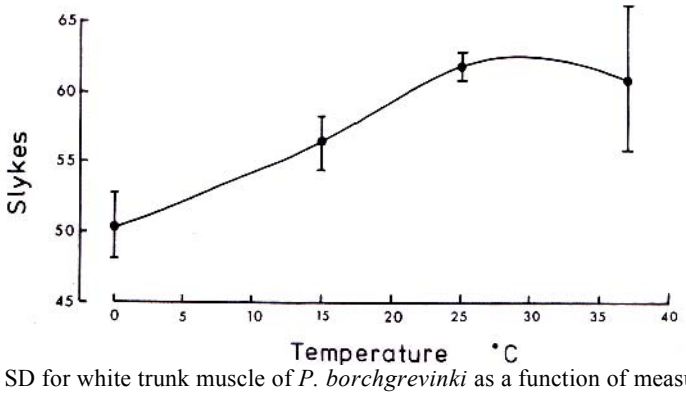

Fig. 3. Buffer capacity ± SD for white trunk muscle of *P. borchgrevinki* as a function of measurement temperature

Table 1. Relationship between hemoglobin concentration (Hb) and blood buffering capacity (0)

Species	(n)	(Hb) g l^{-1}	β Slykes
Dissostichus mawsoni	(6)	*41.2±2.4*	*27.6± 1.47*
Pagothenia borchgrevinki	(5)	*39.6± 1.8*	*16.1 ± 1.3*
Trematomus centronotus	(6)	*32.2±1.1*	*12.5±0.9*
Pagetopsis macropterus	(4)	*0*	*3.4±0.2*
Rhigophila dearborni	(5)	*36.3±3.1*	*3.5±0.3*

Discussion

Evaluation of the Method

Pilot experiments were carried out to assess potential sources of error. Firstly, buffering by fresh muscle could not be distinguished from that of tissue frozen for up to 48 h at -20 °C. Secondly, the change in pH was negligible in relation to the time required to complete a titration. Thirdly, it was expected that dilution of tissues by saline would decrease the apparent pK values of titratable groups and thus the true buffer capacity was not estimated. Fourthly, destruction of tissue and cell compartments promotes hydrolysis of ATP and creatine phosphate to liberate free phosphate which may be expected to increase the buffer value, particularly at higher pH. An attempt to overcome the latter problem using 0.16 M KF, or 5 mM iodoacetic acid as recommended by Dobson et al. (1986) did not materially affect β and was not subsequently employed.

While recognising these potential and real sources of error, we adhered to the original method with measurement temperature at 25°C, because the aim of the study was broadly comparative and enabled us to compare our results with those from other recent studies using warmer water species.

White Muscle Buffering

Buffer capacity is often used as an indicator of the anaerobic potential of muscle tissue. For example, Baldwin et al. (1984) correlated values of β in penguin swimming muscles with interspecific breatholding abilities.

Castellini and Somero (1981) classified fish into three groups - warm bodied, active pelagic, and deep sea fishes which corresponded with decreasing values of β for white muscle, thus recognising their differing potential for anaerobic function. Our values for the active pelagic antarctic species, *P. borchgrevinki* and the mid-water predator *D. mawsoni* were 60.6±2.0 and 55.0±3.2 slykes, respectively, and are lower than values of β for the active pelagic group, but higher than 0 in the deep sea fish reported. On the other hand, values for β from the deep sea icequab *R. dearborni* (β = 45.2±3.3 slykes) which has an extremely low metabolic rate (Wells 1986), and the "sit-and-wait" predator, the icefish (β = 47.3 ± 3.0 slykes), corresponded with the value of 46±3 slykes given by Castellini and Somero (1981) for behaviourally equivalent fishes living at lower latitudes. Thus our observations confirm that the highest values of β in antarctic fish occur in white skeletal muscle suited for intense burst activity. Further, the pH-dependence of the buffer curves in poorly buffered fish muscle (Castellini and Somero 1981) was also a feature of buffer curves for antarctic fish (Fig. 1). In the extreme case of highly active muscle in a warm bodied fish, Dobson et al. (1986) recorded a value of >100 slykes for white muscle, a

figure nearly twice that of the most active antarctic fish, yet values for red muscle and blood were not dissimilar to those from active antarctic fish.

Temperature as a Determinant of β

Body and environmental temperature appeared not to be determinants of white muscle buffer capacity in the study of Castellini and Somero (1981) who found R to be insensitive to temperature between 10 and 37 °C, yet our results suggest that environmental temperature is a significant determinant of buffer capacity at the extreme low temperature in the case of an active antarctic species (Fig. 3). The value of β= 50.1 ± 3.0 slykes at 0 ° C in *P. borchgrevinki* white muscle was significantly lower than that of 60.8±6.3 slykes at 37ºC ($P<0.01$). The unexpectedly lower values of β for active antarctic fish may be explained if the pK values of the principal intracellular buffering species do not encompass efficient proton buffering at extreme low temperature. At -1.8°C, the apparent pK of imidazole rises to ~7.4 and thus its effective buffer range is 6.4±8.4. In severely exercise-stressed antarctic fish Wells et al. (1984) recorded extracellular pH down to 7.2 with lactate concentration of 5 mM. Although intracellular pH was not measured, it seems unlikely that in this extreme situation pH will fall below the buffer range of imidazole groups. A more plausible explanation is that at the higher measurement temperatures, hydrolysis of organic phosphates and changes in protein conformation result in additional buffering capacity from free phosphate. This consideration implies that the true buffer capacities of antarctic fish are lower than those measured here, thus strengthening our contention that the active species are closely aligned with deep sea species from lower latitudes.

Muscle Structure and Biochemistry

Deep water or mesopelagic fish without swim bladders have soft bodies with high water and low protein contents (Blaxter et al. 1971). Antarctic fish also have soft bodies high in unsaturated lipid as adaptations for buoyancy and mobility in extreme cold (Eastman and DeVries 1982; Clarke et al. 1984) and may provide a structural explanation for low muscle buffering capacities.

The glycolytic capacity of white muscle is greatly reduced in antarctic fish and indicates reduced anaerobic capacity (Johnston 1985), an observation indirectly supported by the inability of stressed fish to accumulate high lactate loads (Wells et al. 1984). Biochemical constraints place constraints on burst swimming in the antarctic fish *Notothenia neglecta* (Dunn and Johnston 1986) which is physiologically supported by the observation that twitch contraction times in *P. borchgrevinki* match closely with maximum swimming speed (Montgomery and Macdonald 1984).

The zoarcid, *Rhigophila dearborni* has an extremely low metabolic rate with little or no scope for burst activity or labriform swimming (Wells 1986) and moves with anguilliform locomotion. Davison and Macdonald (1985) demonstrated that the muscle types of this species could not be divided up into classical red and white muscle types. These observations explain why we were unable to isolate "pink" muscle (Fig. 2) and the low values of P for other tissues.

Red Muscle Buffering

Red and white muscle types are less well defined and not easily distinguished in other antarctic fish. Red muscle appears pinkish and is found in appreciable amounts in nototheniids only in the pectoral region (Lin et al. 1974) where it is employed in the peculiar sculling action of the pectoral fins in the so-called labriform mode of swimming used to sustain low level aerobic activity (Montgomery and Macdonald 1984). The oxygen consumption of red muscle in *P. borchgrevinki is* approximately 3 times higher than that of white (Lin et al. 1974) and because of the higher capillary density allowing removal of acid metabolites (Davison and Macdonald 1985), the requirement of buffering capacity is expected to be less in the pectoral muscles. This was confirmed in the consistently lower values for R in red than in white muscle.

Myoglobin is reportedly absent in pectoral muscles from icefish (Walesby et al. 1982) though we noted slight colouration. The recent analysis of very small amounts of myoglobin in icefish by Douglas et al. (1985) give grounds for believing that it may also be present in very low concentrations in pectoral muscles. In any case, a functional distinction in buffer capacity was noted (Fig. 2).

Blood Buffering

As noted in the results, blood buffer capacity is broadly correlated with hemoglobin content, though the plasma and red cell organic phophates also contribute greatly to buffering (Lykkeboe and Johansen 1975). High plasma buffering may account for the unexpectedly high value of β in the hemoglobinless icefish.

Relatively high blood buffering capacity may have an interesting functional consequence. Dobson et al. (1986) pointed out that in the marlin a high Bohr factor may be needed to overcome the high blood buffer capacity if proton transport is to be efficient. The blood of nototheniids shows relatively large Bohr factors

which indicates appreciable proton binding (Tetens et al. 1984). We must await futher data on correlations of β with the Bohr factor before an association is established for antarctic fishes. The contribution of blood to buffering tissue effluxes of protons from fixed acids such as lactate is strictly limited, however, because the blood comprises only a small fraction (ca. 6%) of total body mass.

In summary, the five species of fish examined in this study can be arranged as follows in order of increasing anaerobic capacity of the muscles for burst swimming: *Rhigophila dearborni,* icefish, *Dissostichus mawsoni, Trematomus centronotus,* and *Pagothenia borchgrevinki.* This order reflects increasing dependence on anaerobic muscle work during short bursts of speed during prey capture or avoidance, and is matched by an increasing order of resting metabolic rate (Wells 1987). Those with the highest values of β for white muscle have the highest resting rates of metabolism (e.g. P. *borchgrevinki),* and are more likely to operate under oxygen-limiting conditions. There is no known explanation as to why antarctic fish have lower values of β than behaviourally equivalent fish from lower latitudes. It may be that there are subtle specific differences in metabolic pathways. For example, pH-dependent hydrolysis of creatine phosphate, or decarboxylation of lipids as alternatives to lactate metabolism may be important metabolic strategies in antarctic fishes.

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