High-Energy Phosphate Metabolism during Exercise and Recovery in Temperate and Antarctic Scallops: An In Vivo ^{31}P-NMR Study

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ABSTRACT

In vivo ^{31}P-nuclear magnetic resonance (NMR) spectroscopy was used to measure the levels of ATP, phospho-L-arginine (PLA), and inorganic phosphate in the adductor muscle of the Antarctic scallop *Adamussium colbecki* and two temperate species, *Aequipecten opercularis* and *Pecten maximus*. Graded exercise regimes from light (one to two contractions) to exhausting (failing to respond to further stimulation) were imposed on animals of each species at its habitat temperature (0° vs. 12°C, respectively). NMR spectroscopy allowed noninvasive measurement of metabolite levels and intracellular pH at high time resolution (30–120-s intervals) during exercise and throughout the recovery period. Significant differences were shown between the magnitude and form of the metabolic response with increasing levels of exercise in each species. After exhaustion, short-term (first 15 min) muscle alkalosis was followed by acidosis of up to 0.2 pH units during the recovery process. *Aequipecten opercularis* had similar resting muscle PLA levels compared with either *P. maximus* or *A. colbecki* but used a fivefold greater proportion of this store per contraction and was able to perform only half as many claps (maximum of 24) as the other species before exhaustion. All species regenerated their PLA store at a similar rate despite different environmental temperatures. These findings argue for some cold compensation of muscular performance and recovery capacities in the Antarctic scallop, albeit at levels of performance similar to scallops with low activity lifestyles from temperate latitudes.

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Introduction

The ability to swim is of great short-term importance to the survival and success of individual scallops (Barbeau and Scheibling 1994*b*, 1994*c*). In the longer term, repeated swimming affects resource partitioning, which results in greater muscle growth at the expense of the gonad (Kleinman et al. 1996). Avoiding unnecessary escape responses by predator recognition (Thomas and Gruffydd 1971), minimising the cost of swimming in terms of energy use per effective escape, and recovering as quickly and efficiently as possible are likely to be important in maximising both survival and fecundity.

Several descriptions exist of the metabolic processes used by scallop fast muscle to power contractions and the processes involved in recovery using techniques including muscle sampling and in vivo measurements of blood chemistry, pulse rate, and oxygen consumption. Thompson et al. (1980) showed that during the period immediately after swimming blood oxygen fell and carbon dioxide rose while other metabolites showed little change. Oxygen uptake from the water was 0 for at least 1 h after exercise. This left a substantial "black box" of muscle-based metabolism, which, until now, has only been accessible by sampling muscle for the biochemical analyses on which the existing literature is based (Baldwin and Opie 1977; Gäde et al. 1978; Grieshaber 1978; de Zwaan et al. 1980; Livingstone et al. 1981). In scallops, the ATP used during muscular activity is initially regenerated by the breakdown of phospho-L-arginine (PLA) followed by anaerobically and aerobically supported glycolytic ATP production (Grieshaber 1978; Livingstone et al. 1981). In anaerobic metabolism, octopine is formed from pyruvate, arginine, and NADH, releasing NAD. This replaces the pyruvate to lactate pathway of vertebrates (Gäde et al. 1978).

The need for tissue samples to measure the levels of muscle metabolites constrains the number of experimental conditions possible and the time resolution with which recovery could be followed. ^{31}P-nuclear magnetic resonance (NMR) spectroscopy provides a sensitive and noninvasive tool for the online measurement of changes in high-energy phosphate levels and tissue pH in vivo in the intact animal. This allows continuous measurements to be carried out on each animal (Hitzig et al. 1987), which is usually resting; however, the focus of this study was

the observation of graded exercise from light (one to three muscle contractions) to exhausting as well as the time course of subsequent recovery. Numerous studies using NMR to observe changes in metabolite levels during and after muscular activity focus on a single muscle in vivo or dissected muscle preparations. See Argov et al. (1987) and Curtin et al. (1997) for work on dogfish and rat, respectively, or extensive ^{31}P-NMR work on human volunteers (Vestergaard-Poulsen et al. 1995; Cooke et al. 1997; Mercier et al. 1998; Combs et al. 1999; Haseler et al. 1999; Mole et al. 1999). NMR experiments that comprise the whole locomotory system are rare. One example among marine animals is the work by Thébault et al. (Thébault et al. 1987, 1994, 1997; Thébault and Raffin 1991) on the tail musculature of prawns. In the scallop, the adductor muscle is very prominent, which allows such analyses to be undertaken. Existing studies have shown that metabolite transport to and from the fast adductor is limited (de Zwaan et al. 1980). Focusing on the fast adductor, therefore, allows the fate of all relevant phosphate metabolites to be observed. Few NMR studies about scallop (or other marine invertebrate) physiology exist. NMR spectroscopy has been successfully used on scallops to assess seasonal changes in metabolic state (Jackson et al. 1994); the kinetic properties of scallop arginine phosphokinase have been examined in vitro (Graham et al. 1986), and resting and stimulated muscle have been compared (Meyer et al. 2001).

The ability of cold-water ectotherms to perform work during locomotion has been studied in the Antarctic scallop *Adamussium colbecki* Smith 1902 (Ansell et al. 1998; Bailey 2001) and a variety of Antarctic fish (Franklin and Johnston 1997; Franklin 1998; Wakeling and Johnston 1998). Studies in Antarctic fish have shown compensation for force production without any corresponding maintenance of ATP use rates, which makes isometric force maintenance more efficient at low temperatures (Altringham and Johnston 1986). These effects are associated with changes to both the myosin heavy and light chains that increase ATPase activity at low temperature (Johnston et al. 1975; Johnston and Walesby 1978). However, when compared with a range of tropical and temperate fish species with similar morphologies and lifestyles, aerobic factorial scope for activity in Antarctic fish reaches similar levels despite low temperature. Conversely, little temperature compensation is seen for muscle power output during fast start performance (Wakeling and Johnston 1998)

Although muscle performance in Antarctic marine fish is relatively well described, their ability to withstand fatigue (Altringham and Johnston 1985; Lowe and Wells 1997; Van Dijk et al. 1998) and recover from exercise (Hardewig et al. 1998) is less well known. The increased mitochondrial density observed in Antarctic fish red muscle has been proposed as an adaptation to facilitate aerobic metabolism by compensating for reduced diffusion rates (Tyler and Sidell 1984) and mass-specific aerobic capacities of mitochondria (Johnston et al. 1998) at low temperatures. Accordingly, increased mitochondrial densities may also facilitate recovery from exercise. It is certain that Antarctic eelpout (*Pachycara brachycephalum*) were found to recover faster than temperate relatives (*Zoarces viviparus*) at the same temperature (Hardewig et al. 1998). These Antarctic fish also maintained high glycolytic capacities while the dominant Antarctic group, the Notothenioids, did not (Dunn and Johnston 1986; Dunn 1988; Van Dijk et al. 1998). Clearly, there are interesting differences both between fish from different environments and between different species in the same environment. This study represents a first investigation of metabolism during exercise and recovery in a polar invertebrate and two related, temperate species of scallop for comparison. The two temperate species differ in growth rate (Allison 1994), in activity level and swimming behaviour (Baird 1957; Moore and Trueman 1971; Stephens and Boyle 1978; DeMont 1988), and in phylogenetic distance to *A. colbecki* (Canapa et al. 2000).

Material and Methods

Animals

Adamussium colbecki were collected using SCUBA at Rothera Research Station in March 1999 and maintained at 0°C in through-flow aquaria at Rothera and in aerated, recirculating seawater as they were shipped to Bremerhaven, Germany, by way of Cambridge. Two temperate species, *Aequipecten opercularis* (Linnaeus 1758) and *Pecten maximus* (Linnaeus 1758), were obtained from the marine biological station in Roscoff, Brittany, and maintained at 12°C at the Alfred Wegener Institute for Polar and Marine Research in Bremerhaven. Experiments on *P. maximus* were undertaken in February 1999 and on *A. opercularis* and *A. colbecki* in June 1999. Temperate animals were used within 2 wk of collection. *Adamussium colbecki* specimens had been in captivity for a longer period but were highly responsive to light and handling, indicating reasonable physical condition.

Twenty-four hours before experimentation, a 2-cm^2 piece of Velcro was glued to the lower valve of each animal, which was also intubated (through the rear jet aperture) with two pieces of narrow-bore plastic tubing. These tubes were held in place by dental wax; one allowed the injection of saturated saline into the mantle cavity to act as a stimulus to swim. Monitoring of swimming activity was achieved with a pressure transducer (UFI, Motro, Calif.) fitted to the end of the other tube and connected to a bridge amplifier of a MacLab system (AD-instruments).

Experimental animals were attached to the bottom of a 1.5-L insulated plastic chamber containing approximately 1.2 L of seawater using Velcro glued to the base of the chamber. The chamber was then placed within the magnet of the NMR. Two thermocirculators were used to maintain the low temperatures in the chamber required for experiments in *A. colbecki* without freezing the seawater. The first controlled the temperature of

the seawater to be pumped through the chamber and returned it to a 50-L reservoir where it was aerated. The second pumped cooled antifreeze through a coil around the water tubes. Temperature was measured inside the chamber using a fluoroptic thermometer (Luxtron, Polytec). Temperatures remained within 2°C of the animal's maintenance temperature throughout and were within 1°C during experimentation. Temperature and mantle pressure readings were collected continuously throughout experimentation and captured by a Macintosh computer running MacLab software.

^{31}P-NMR Spectroscopy

^{31}P-NMR spectra were obtained from live scallops after placing the chamber containing the experimental animal within the magnet (Bruker 47/40 Biospec DBX system). The scallop's adductor muscle was centred over a triple tunable surface coil (^{31}P, ^{13}C, ^{1}H; 5 cm diameter). After adjusting the coil circuit and optimising the field homogeneity to the experimental set up, scout magnetic resonance images were collected using a gradient echo sequence in all three directions to position the animal. For the calculation of the adductor muscle diameter and to monitor the ventilatory activity after exhaustion, anatomical gradient, or spin echo magnetic resonance images, were collected similar to Bock et al. (2001*b*). In vivo ^{31}P-NMR spectra were acquired from the adductor muscle using a 200-μs-bp pulse and a repetition delay of 0.5 s for *P. maximus* and a 100-μs-bp pulse with a repetition delay of 0.26 s for the other species; acquisition size was 4 k. Depending on the size of adductor muscle and the physiological condition of the animal, 64–256 scans were collected for *P. maximus* (resulting in scan times from 32 s to 2.10 min for each spectrum), and 466–932 scans were collected, resulting in measurement times from 7 to 12 min for the smaller species. Spectra were collected consecutively using an automation routine and postprocessed as described in Bock et al. (2001*a*).

The peaks of interest (inorganic phosphate [Pi], PLA, and ATP) were identified from the spectra, and their exact resonance frequency, height, width, and area were calculated (XWIN NMR, Bruker). Peak area correlates with the amount of the substance within the detection area of the coil. Intracellular muscle pH (pH_i) was calculated from the chemical shift of the Pi signal relative to PLA using a calibration curve provided by Pörtner et al. (2000). Free energy of ATP hydrolysis was calculated from pH_i and ATP, PLA, and Pi concentration (Pörtner et al. 1996). For this calculation, the summed levels of arginine containing metabolites and of L-arginine were determined from published ratios of PLA over L-arginine. The ratio of Pi to PLA was used as a measure of muscle energetic status appropriate to the metabolism of scallops (Jackson et al. 1994). Time codes in the temperature and pressure (activity) data collected using the MacLab program allowed cross correlation with the ^{31}P-NMR data.

Preexercise "resting" levels of PLA and total ATP were measured where possible and compared between species using GLM Univariate ANOVA with Tukey's post hoc test. Estimates of changes in absolute concentrations of metabolites have been made on the basis of published data for *A. opercularis* from the same tissue (Grieshaber 1978). Resting pH_i cannot be measured directly because the calculation requires a Pi peak to be present. Resting pH_i was estimated by back-calculation of the claps (muscle contractions) versus minimum pH_i relationship and the relationships between Pi and pH_i. At low Pi levels, pH calculations become increasingly unreliable (Figs. 2*B*, 3*A*). Pi versus pH data were ranked by increasing Pi concentration and a 10-point running mean; a standard error calculation was applied to the data. The average standard error of the data increased with reducing Pi levels from 0.6% of the mean pH at 2–2.9 μmol Pi g^{-1} to 1.6% at 1–1.9 μmol Pi g^{-1}. pH data were not used for Pi values of <2 μmol Pi g^{-1}.

Graded Exercise

Each animal was allowed to rest for a minimum of 1 h after placement within the chamber before experimentation was begun. Manipulation of the animal was minimised during transfer to the chamber; however, initial spectra typically showed elevated Pi levels because of muscular activity during handling. No stimulation was given until an appropriate control spectrum (no detectable Pi) was obtained. Measurements were begun before exercise and continued until full recovery in most cases.

Saturated saline was prepared using natural seawater and Instant Ocean salt. This saline was introduced into the mantle cavity until a response was measured on the pressure transducer, which indicated activity by the animal. An injection typically contained 2–5 cm^3 saline, injected at 1 cm^3 s^{-1}. Further injections were made until the desired number of "claps" (one to 20) had been achieved. In addition to these, exhaustive exercise was imposed. This was defined as the animal becoming refractory to further stimulation. After the experimentation, all animals were returned to the holding aquarium and were still alive and responding normally at least 3 mo later.

Workloads in Each Exercise Regime

It was not possible to measure the power output of the adductor from the pressure trace without a synchronised measure of flow through the jets of the animal (Marsh et al. 1992). This was not possible in the current equipment configuration but will be incorporated into future designs. Muscle cross-sectional, area-specific, peak-force production (F, kNm^{-2}) was estimated from the peak pressure recorded within the mantle (P, Pa), the shell area (S, m^2), and the muscle cross-sectional area (M, m^2)

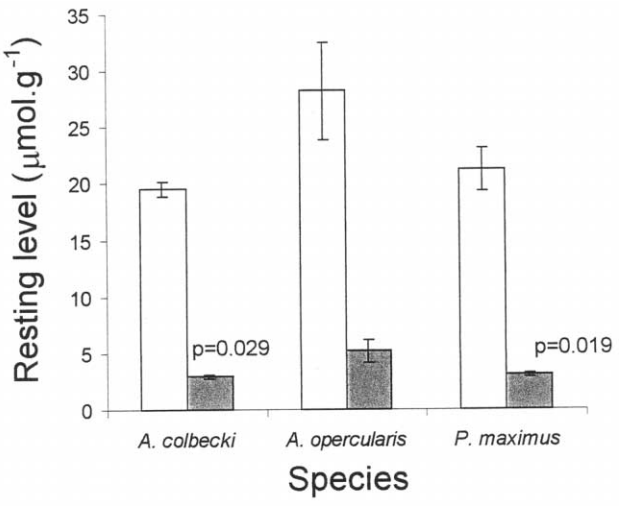

Figure 1. Phospho-L-arginine (PLA; *white bars*) and ATP levels (*shaded bars*) in resting scallops, that is, from control spectra where no inorganic phosphate could be detected before each experiment. Absolute values for phosphagen levels have been calculated on the basis of those measured by Grieshaber (1978) in *Aequipecten opercularis* from the same sampling site. ATP levels were significantly higher in *A. opercularis* than in the other two species (GLM Univariate ANOVA with Tukey's post hoc, $P = 0.029$ and $P = 0.019$, respectively; df = 9); there was no significant difference in PLA level between species. Data points are the means and standard errors; $n = 3$ for *A. opercularis* and *Adamussium colbecki*; $n = 6$ for *Pecten maximus*.

determined from coronal magnetic resonance images obtained for each individual using the formula

$$F = \left[\frac{(P \times S)}{M}\right]1{,}000.$$

Technical Considerations

During NMR spectroscopy, the sample must be fixed relative to the surface coil. This is a constraint when investigating locomotion in animals. The hydrodynamics of "swimming" while in a fixed position are different from those experienced while moving. A moving animal "levers" its way past the water rather than forcing water backward while remaining stationary. This effect is minimised by using scallops as a model animal. The biomechanics of swimming in *A. colbecki* (Ansell et al. 1998; Bailey 2001) and in *P. maximus* (D. M. Bailey, personal observation) show that the animals come close to a standstill during each clap cycle as the valves open to refill the mantle cavity. During swimming, 99% of the work is used to form the propulsive jets (Cheng et al. 1996) with only a small amount required to accelerate the shells themselves and the associated external mass of water. The work of jet formation and therefore the proportion of the animal's energy store used will therefore be similar in a fixed, stationary scallop as opposed to a swimming scallop.

Differences in reproductive state between species might possibly confound the results of muscle metabolism experiments such as those described here (Brokordt et al. 2000). Spawning is possible in *A. opercularis* at the time of the experiment (Allison 1994) though in our animals none was observed. Spawning was less likely in the other species because they were both studied during their respective winters.

Results

Resting Metabolite Levels

ATP and PLA levels were measured in each animal before stimulation while Pi levels were undetectable. Peak areas for these metabolites were converted to absolute values using the measured ATP level in *Aequipecten opercularis* (5.18 μmol g^{-1} wet weight) obtained by Grieshaber (1978). The animals used by Grieshaber (1978) were of similar size and from the same location as the animals used in this study. ATP level changes less during handling than the other metabolites, so that it is the most reliable metabolite with which to scale the others.

At rest, muscle ATP concentration was significantly higher in *A. opercularis* than in either *Adamussium colbecki* or *Pecten maximus* (GLM Univariate ANOVA with Tukey's post hoc, $P = 0.029$ and $P = 0.019$, respectively; df = 9). There was no significant difference between ATP levels in *A. colbecki* and *P. maximus*. There were also no significant differences in PLA content between the different species (Fig. 1).

Because of differences in body size, the time resolution of the measurements varied from 90 or 91 s in *P. maximus* to 7–12 min in *A. colbecki* and *A. opercularis*. The data for *P. maximus* allowed the observation of extremely short-term changes in metabolite levels and therefore permitted the study of all grades of exercise. The majority of the data presented is for this species. Although the data from the smaller-bodied *A. colbecki* and *A. opercularis* were of reduced temporal quality, the data allowed the comparison of similar numbers of muscle contractions between species.

Muscle Metabolism during Exercise

A range of exercise regimes were imposed on the animal, from one to two claps to exhausting. The number of claps required ranged from a mean of 13 in *A. opercularis* to 36.8 in *P. maximus*. The greatest number of claps performed by one animal in a single bout of activity was 57 by *P. maximus* (Table 1).

Exercise in all species was associated with decreases in PLA concentration and with rises in Pi. These changes were progressively greater with increasing workload as shown for *P. maximus* (Fig. 2*A*, 2*B*). PLA and Pi concentrations immediately following exercise were significantly related to the number of

Table 1: Interspecific differences in the number of claps (muscle contractions) performed before exhaustion

	Number of Claps to Exhaustion		Interspecific Differences	
Species	Maximum	Mean	*Aequipecten opercularis*	*Pecten maximus*
Aequipecten opercularis	24	13		
Pecten maximus	57	36.8	df = 7, t = 2.64, $\boldsymbol{P = .033}$	
Adamussium colbecki	48	30.8	df = 6, t = 2.48, $\boldsymbol{P = .048}$	df = 7, t = .61, P = .56

Note. Exhaustion was defined as the animal becoming unresponsive to further stimulation. *Aequipecten opercularis* performed significantly fewer claps than the other two species (by two to three times) before exhaustion, with *P. maximus* performing the most. Significant differences are indicated in boldface.

claps performed by the animal (PLA: $y = -0.261x + 20.356$, $r^2 = 0.41$, $P = 0.01$, df = 13; Pi: $y = 0.119x + 2.737$, $r^2 = 0.44$, $P = 0.01$, df = 13). Changes in ATP concentration were small and not significantly related to workload (Fig. 2*C*). For each spectrum, the ratio of Pi to PLA was calculated. At rest, with little free Pi, Pi/PLA was close to zero. Pi/PLA increased with increasing numbers of contractions (Figs. 3, 4).

At higher workloads (Fig. 2; $t = 130$ min to end of experiment), the effects of exercise on muscle metabolism of *P. maximus* differed in form and was of greater magnitude. As at lower levels of work, PLA level fell and Pi level rose following exercise. However, immediately following exercise, PLA concentration increased rapidly (Fig. 2*B*). There was no concurrent fall in Pi level at this stage, with Pi (Fig. 2*A*) and PLA levels decoupling briefly. There was an apparent drop in ATP, though this is within the range of the noise in the data (Fig. 2*C*). The ATP free energy change of hydrolysis fell with increasing workload from a resting level of -57 kJ mol^{-1} to -47 kJ mol^{-1} at exhausting levels of exercise (Fig. 3*B*).

Following exhaustive work, Pi levels continued to increase after the end of exercise (Fig. 3; $t > 120$ min). Magnetic resonance images during this period showed that this increase was associated with the animal remaining closed. Pi levels only fell after the animal opened its shell and began to ventilate its gills. Water flows within the mantle were clearly visible in magnetic resonance images. The double minima in the PLA concentration was typical of exhausted animals (Fig. 2*B*). PLA level fell during exercise and then displayed an initial and rapid increase. This turned into a slight progressive drop for approximately 5–7 min, throughout the period of valve closure. This progressive fall in PLA and subsequent PLA recovery mirrored changes in Pi. Other than the period immediately following heavy exercise total Pi + PLA remained approximately constant.

Figure 4 summarises the effect of workload on peak Pi/PLA and time to subsequent recovery in *P. maximus*. Increased workload increases both the metabolic perturbation and the time required to recover.

pH_i

Exercise was followed by a short period of muscle alkalosis (rise of up to 0.5 pH units), followed by more prolonged acidosis (Fig. 3). At high to exhausting levels of exercise, the relationship between pH and the levels of the measured metabolites was not simple and differed according to whether Pi and PLA levels were rising or falling. Falling pH_i during recovery correlated significantly with a decrease in Pi concentration and an increase in PLA concentration in all experiments with *P. maximus* (Pearson's coefficient > 0.8, $P < 0.001$, $n > 100$). Back-calculation to Pi = 0 μmol g^{-1} resulted in a mean resting pH_i of 7.37 (SE $\pm$ 0.02) for *P. maximus*.

Interspecific Differences in Muscle Metabolism during Exercise

As before, the number of claps was used as a proxy for workload. Peak-force production was estimated for *P. maximus* on the basis of peak intramantle pressures during 20 claps from two animals. Peak-force production for *P. maximus* averaged 98 $\pm$ 12 kN m^{-2} (mean $\pm$ range). The pressure traces for the other two species were not of sufficiently high resolution to allow accurate measurement of the intramantle pressures. Estimates of force production exist for both *A. opercularis* (109 kN m^{-2} at 10°C) and *A. colbecki* (100 kN m^{-2} at 0°C) from kinematic studies of scallops acclimated to, and swum at, the same temperatures as in our NMR experiments (Bailey 2001).

The relationship between the number of muscle contractions and metabolic change was investigated. The slope of the relationship between number of claps and Pi/PLA (linear regression) differed between species with *A. opercularis* showing a greater change in Pi/PLA than either of the other species (Fig. 5). There was no significant difference between *A. colbecki* and *P. maximus*. There was no detectable relationship between number of claps and the time to peak Pi/PLA at different exercise levels. This is probably because the Pi increase was very rapid compared with the time resolution of the measurements, probably occurring within the first 1–2 min from the beginning of stimulation.

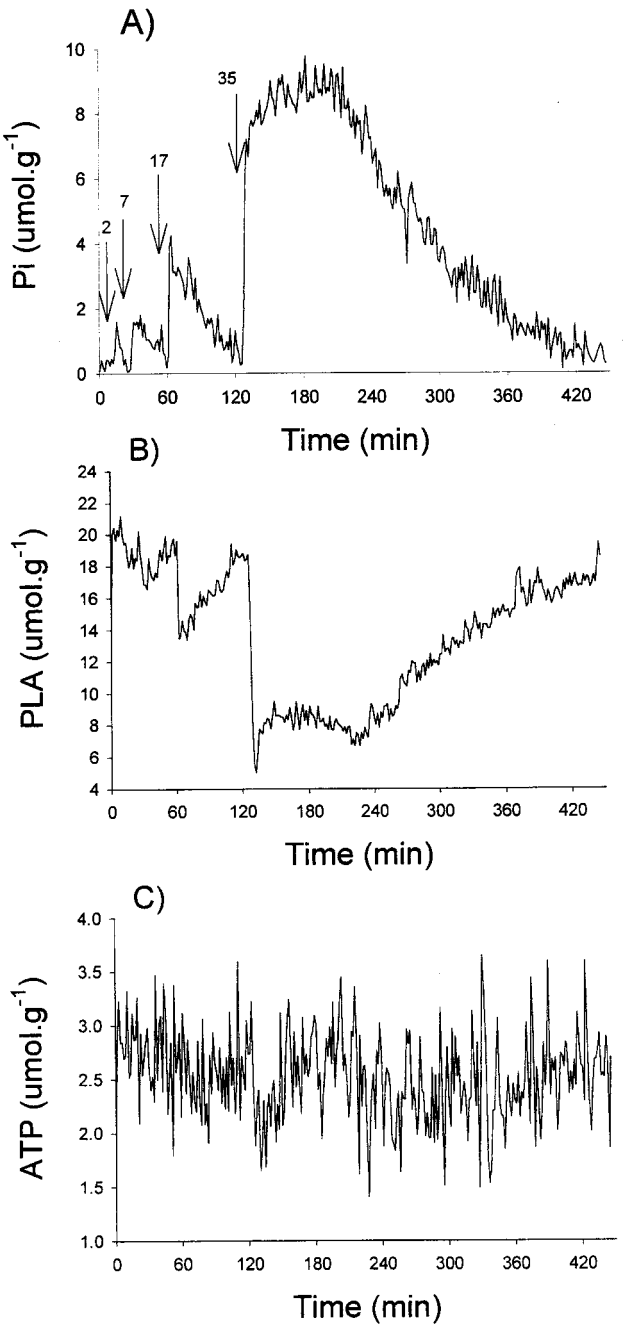

Figure 2. Typical traces of inorganic phosphate (Pi), phospho-L-arginine (PLA), and ATP levels in *Pecten maximus* with increasing exercise. The arrows in *A* indicate the timing of the onset of exercise and the number of claps imposed (two, seven, 17, and 35). Units are scaled relative to the Pi trace of the first experiment. Pi level rises following exercise (*A*), while PLA level falls (*B*). Note the double minima in the PLA trace typical at high workloads in all species. No consistent pattern of ATP change could be detected because any transient changes (*C*; time = 125 min) were within the range of the noise in the measurements.

The free energy change of ATP hydrolysis fell with increasing workload in each species, from resting levels of −55.0 to −58.0 kJ mol^{-1} to −46.0 to −50.2 kJ mol^{-1} at exhaustion. There was no significant difference between either resting or exhaustion levels of free energy change between species.

Time to 50% recovery, that is, the time taken for Pi/PLA to fall to half of its maximum level, was used to compare recovery rates between species. This parameter was used because it was seldom possible to measure accurately the time needed to return to preexercise levels because of spontaneous activity by the animals in the later stages of recovery. There were significant relationships between maximum Pi/PLA and the time required for 50% recovery in Pi/PLA ratios in both *A. colbecki* and *P. maximus* (Fig. 6), with 50% recovery time increasing by 1.1 and 4.2 h, respectively, for each 0.5 unit change in Pi/PLA ratio. This relationship was weaker in *A. colbecki* than in *P. maximus* ($r^2 = 0.63$, $P < 0.05$, df = 4 and $r^2 = 0.56$, $P < 0.001$, df = 18, respectively). There was no significant difference between the slopes or intercepts of the lines for *A. colbecki* and *P. maximus*, partly because numbers were limited.

pH_i

The alkalosis (maximum pH) reached was not significantly related to the number of muscle contractions in any species. The minimum measurable pH_i (Pi > 2 μmol g^{-1}) following exercise was significantly negatively related to the number of muscle contractions in *A. colbecki* and *P. maximus* (see Fig. 7 for details) and was significantly more alkaline in *A. colbecki* than *P. maximus* (GLM Univariate ANOVA with clap number as a covariate). The intercepts of the pH_i versus clap number lines at zero claps gave resting pH_i values of 7.75 for *A. colbecki* and 7.65 for *P. maximus*, respectively. There was insufficient data to compare *A. opercularis*, but the three points for this species were all significantly lower than the data for *P. maximus* and *A. colbecki* because they fall outside the 95% confidence intervals of the latter two. The difference in pH between the Antarctic (*A. colbecki*) and temperate (*A. opercularis* and *P. maximus*) species was less than would be expected on the basis of maintenance of intracellular neutrality.

The net muscle acidification (maximum-minimum pH) increased significantly with increasing clap number in *A. colbecki* ($r^2 = 0.869$, $P = 0.021$, df = 3) but not in *P. maximus*, despite the parallel pH decrement. There were insufficient data to compare *A. opercularis*. The relationships between peak Pi/PLA and maximum pH shift were significant in both *A. colbecki* and *P. maximus* (linear regressions, $r^2 = 0.805$, $P = 0.03$ and $r^2 = 0.767$, $P < 0.001$, respectively).

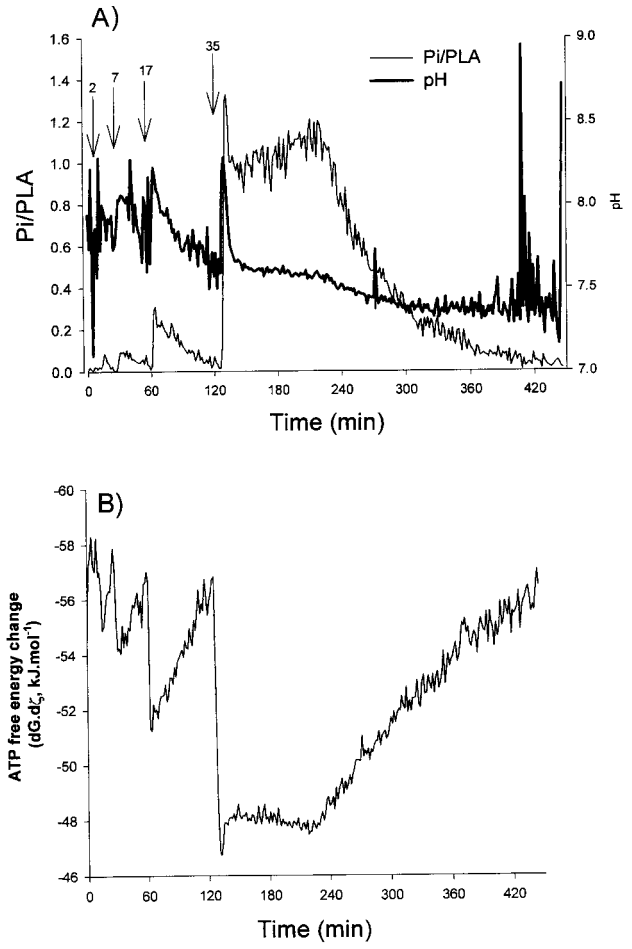

Figure 3. Calculated changes in intracellular environment during graded exercise. *A*, Inorganic phosphate/phospho-L-arginine (Pi/PLA) and muscle pH during graded exercise in *Pecten maximus*. This is the same experiment as Figure 4. As before, arrows indicate the timing of onset of exercise and the number of claps. The adductor muscle experienced a period of alkalosis (raised pH) followed by acidosis. pH was calculated from the chemical shifts of the Pi signal relative to PLA (Pörtner et al. 2000); the noise at the beginning and end of the experiment was caused by the disappearance of the Pi peak. At high workloads, Pi level exceeded PLA level. *B*, ATP free energy change calculated from ATP, PLA, and Pi levels, and intracellular pH fell with increasing workload from −57 to −47 kJ mol^{-1}.

Discussion

Metabolism during Exercise and Recovery

PLA level fell on initial work because the ATP used was regenerated from PLA, keeping ATP level stable. At lower workloads, the rapid onset of recovery indicated that aerobic metabolism began shortly following work (Livingstone et al. 1981). ADP and Pi were removed by oxidative phosphorylation, which produced ATP and allowed the regeneration of PLA by arginine phosphokinase. The contribution of octopine formation was probably minimal, and the pH_i perturbation was small.

By considering the findings in earlier literature (see "Introduction"), the following scenario arises for higher workloads. Here, anaerobic metabolism was prolonged and was often extended further by the animal remaining closed (Grieshaber 1978). Pi and PLA changes were decoupled immediately after the peak in Pi levels because of a rapid rise and then gradual fall in PLA levels. This suggests that the increase in PLA was derived from transphosphorylation of ATP by arginine phosphokinase at the very low PLA and high L-arginine levels following heavy exercise. PLA reformation was probably arrested by arginine use in the octopine dehydrogenase pathway. ATP formation by anaerobic glycolysis would therefore be supplemented by increased ATP availability from the phosphagen as arginine removal increases the favourability of ATP regeneration from PLA.

The theory that octopine formation in anaerobic metabolism may increase the ATP available from PLA has been previously rejected on the basis that PLA use and octopine formation occurred over different timescales (Gäde et al. 1978) and that arginine removal was exclusively due to PLA regeneration (Livingstone et al. 1981). These considerations neglected the role of free ADP in also determining the net use of PLA (Pörtner et al. 1996). The high resolution of this study demonstrates that further PLA use may occur throughout the first half of the recovery process when octopine is probably being formed (Gäde et al. 1978; Fig. 2*B*). The transient recovery and subsequent fall in PLA levels indicates the activation of octopine dehydrogenase by pyruvate production and accumulation of free ADP. The sequestration of arginine by the octopine pathway under anaerobic conditions may be adaptive due to the

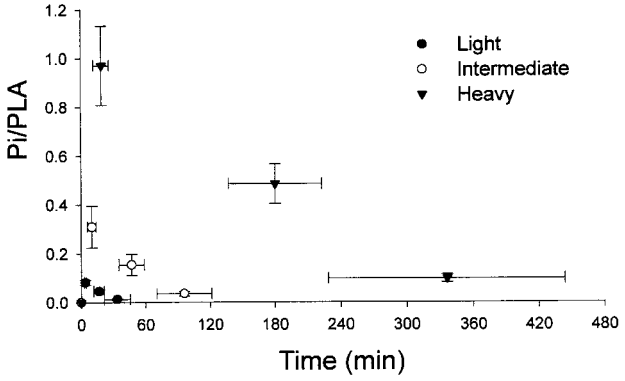

Figure 4. Summary of the effects of workload on the peak inorganic phosphate/phospho-L-arginine (Pi/PLA) ratio observed and the time taken to return Pi/PLA to resting levels in *Pecten maximus*. The three workloads presented are light (*solid circles*; <11 claps), intermediate (*open circles*; 11–21 claps), and heavy (*solid triangles*; >21 claps). Error bars are 1 SE. At higher workloads, higher Pi/PLA changes occur and recovery takes longer.

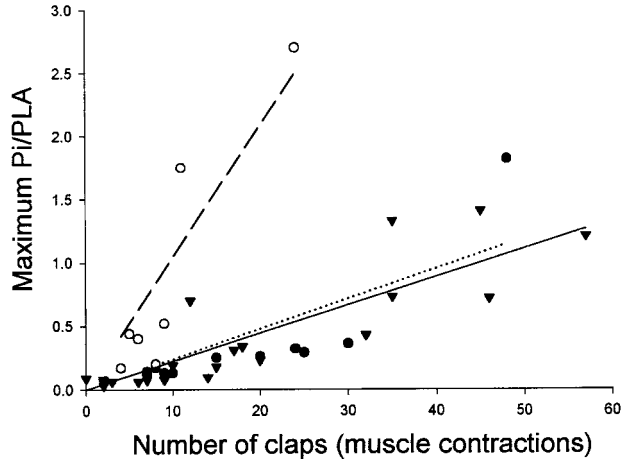

Figure 5. Maximum inorganic phosphate/phospho-L-arginine (Pi/PLA) following exercise with increasing number of claps. Increases in the number of muscle contractions resulted in higher levels of Pi release and PLA breakdown, indicated by changes in the Pi/PLA ratio. Data points are the maximum Pi/PLA recorded following exercise in *Adamussium colbecki* (*solid circles*), *Aequipecten opercularis* (*open circles*), and *Pecten maximus* (*solid triangles*). Regression lines were driven through the origin, the resting Pi/PLA value. *Adamussium colbecki* (*dotted line*): $y = 0.024x$, $r^2 = 0.787$, $P < 0.001$, df = 9. *Aequipecten opercularis* (*dashed line*): $y = 0.104x$, $r^2 = 0.901$, $P < 0.001$, df = 7. *Pecten maximus* (*solid line*): $y = 0.0222x$, $r^2 = 0.863$, $P < 0.001$, df = 25. The slope of the relationship between number of claps and peak Pi/PLA was significantly steeper in *A. opercularis* than in *A. colbecki* (*t*-test, $P < 0.001$, df = 7) or *P. maximus* ($P < 0.001$, df = 25).

relatively small difference in free energy of hydrolysis between PLA and ATP that would otherwise result in low ATP levels as PLA was depleted. The double peak in Pi/PLA following heavy exercise indicates the lag between high work levels and the commencement of octopine synthesis.

Another suggestion was that octopine accumulation allows the animal to survive a further attack by closing its shell (Grieshaber 1978). In predator-prey interaction experiments, scallops would almost always be consumed on capture; closing the shell did not increase survival (Barbeau and Scheibling 1994*a*). In accordance with observations in squid (Pörtner et al. 1996), it appears that octopine metabolism enables the maximum use of the PLA store and produces additional ATP by glycolysis, recovering the adenylate energy charge.

Recovery rates in this study were all obtained at realistic natural temperatures for the animals concerned and therefore indicate performances at the temperatures for which they are adapted. The rates recorded were in reasonable agreement with PLA depletions and their reversal during exercise in *Pecten jacobaeus* (Grieshaber and Gäde 1977) and *Placopecten magellanicus* (Livingstone et al. 1981). Recovery took substantially longer in *Placopecten*, with complete return of PLA levels not occurring for up to 24 h compared with a maximum of 17 h in the *P. maximus* in this study. In comparison, the razor shell (*Ensis directus*) recovers from total exhaustion within 3 h (Schiedek and Zebe 1987). However, compared with scallops in this and previous studies (Grieshaber 1978; de Zwaan et al. 1980; Schiedek and Zebe 1987), *E. directus* retained a higher proportion of its PLA store when exhausted.

Scallops appear to be adapted to aerobic recovery during intracellular acidosis; mitochondrial metabolism was elevated at pH 6.4 compared with pH 7.0 in the tropical scallop *Euvola ziczac* at 22°–28°C and indicated adaptation for aerobic recovery during acidosis (Boadas et al. 1997). The maximum pH shift recorded in this study (around 0.4 units) was similar to that recorded by Curtin et al. (1997) for exercise in dogfish muscle and relates in a similar way to the proportion of phosphagen used. Acidosis following exercise in molluscs has been attributed to respiratory acidosis (Thompson et al. 1980) on the basis that octopine is less acidic than lactate and at low levels in the blood. However, it became clear later that octopine formation during anaerobic glycolysis causes the same extent of proton production as lactic acid formation (Hochachka and Mommsen 1983; Pörtner et al. 1984; Pörtner 1987), evidenced by a severe acidosis found during octopine formation in squid muscle (Pörtner et al. 1991, 1996).

Causes of Fatigue

Exhaustion in the animals was defined as failure to respond to further stimulation. In wild animals, reaching such a level of

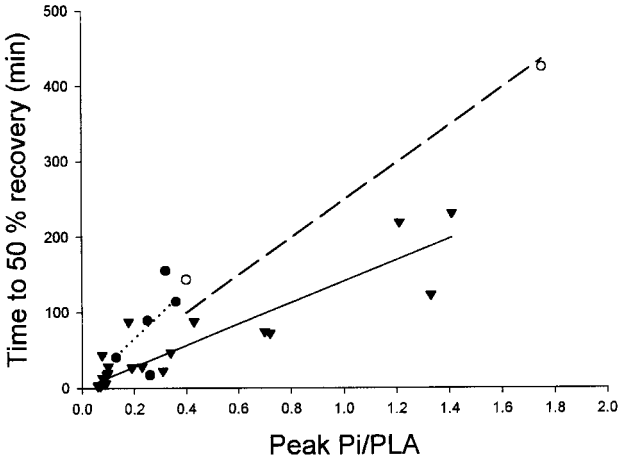

Figure 6. Time to 50% recovery from increasing levels of inorganic phosphate/phospho-L-arginine (Pi/PLA) perturbation. Full recovery was defined as the return of Pi/PLA to resting levels. Data points are the time at which Pi/PLA reached 50% of the maximum Pi/PLA recorded following exercise. The time to 50% recovery increased with increasing Pi/PLA (see text for statistics). *Adamussium colbecki* (*solid circles, dotted line*): $y = 19{,}443x$, $r^2 = 0.63$, $P < 0.05$, df = 4. *Pecten maximus* (*solid triangles, solid line*): $y = 8{,}455.7$, $r^2 = 0.56$, $P < 0.001$, df = 18. Data were insufficient to produce a regression for *Aequipecten opercularis* (*open circles, dashed line*).

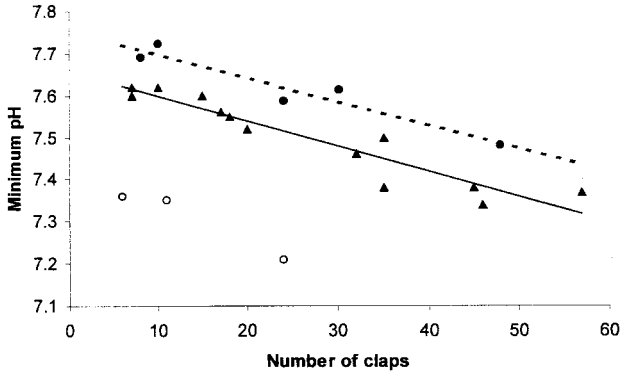

Figure 7. The effect of workload (number of claps) on minimum muscle pH recorded after the completion of the experiment. The drop in muscle pH following exercise increased significantly with increasing workload in both *Pecten maximus* and *Adamussium colbecki*. *Pecten maximus* (*solid triangles, solid line*): $y = -0.006x + 7.658$, $r^2 = 0.889$, $P < 0.001$, df = 11. *Adamussium colbecki*: $y = -0.006x + 7.754$, $r^2 = 0.923$, $P = 0.009$, df = 3 (*solid circles, dotted line*). Minimum pH was significantly lower in *Aequipecten opercularis* (*open circles, no line*) than in *P. maximus* (ANCOVA, $P < 0.001$, df = 13) and significantly higher in *A. colbecki* than in *P. maximus* (ANCOVA, $P < 0.001$, df = 5).

fatigue after escaping from a predator would likely result in capture if attacked again. The physiological mechanisms of fatigue in scallops remain unclear. As pH_i is elevated following exercise owing to the early consumption of PLA (Pörtner 1987), proton levels are not likely to be the cause. What is more likely is a relaxation effect by accumulating Pi at high pH (Pörtner et al. 1996). *Aequipecten opercularis* tolerated Pi levels almost double those of the other species before becoming exhausted, probably by virtue of its lower postexercise pH_i. The relationships between number of claps, Pi/PLA, Pi, or PLA did not change as the animal approached exhaustion without an obvious threshold being crossed. There was a great deal of variability in the number of claps achieved (and therefore the metabolic changes) within species. Because no consistent ATP depletion was detected during exercise or recovery, this is not implicated as a cause of fatigue. Calculations of ATP free energy change showed that for *Pecten maximus* exhaustion occurs at a mean free energy of -49 kJ mol ATP^{-1} with the lowest value reached for this species being -45 kJ mol^{-1}. These values were very similar to those found for fatigue in exercising squid, and certainly of the order where suppression of ATPase activity is possible. The resting free energy change of *P. maximus* was -57 kJ mol^{-1}, the same as for the squid *Lolliguncula brevis* (Pörtner et al. 1996). Values for ATP free energy change were similar in all three scallop species when exhaustion occurred, implicating loss of ATPase activity as the eventual cause of fatigue in these animals.

Interspecific Differences in Muscle Metabolism

Individual *A. opercularis* were able to perform around half as many claps to exhaustion as either *Adamussium colbecki* or *P. maximus* despite having the highest resting free ATP levels and resting levels of PLA similar to those of the other two species. *Pecten maximus* clapped at a very low rate (typically 0.6–0.7 Hz), which was found previously in response to stimulation by starfish homogenate (Gäde et al. 1978). Higher numbers of claps have been recorded in *A. opercularis* when the scallops are stimulated with a starfish leg (Grieshaber 1978) than were observed here. *Aequipecten opercularis* demonstrates clap frequencies of 2.4–3.2 Hz at the test temperatures (Bailey 2001). In contrast, *A. colbecki* has a clap frequency of 1.7–2.1 Hz at the test temperature (Bailey 2001). Ansell et al. (1998) recorded a maximum number of 18 claps from a single stimulation in *A. colbecki* in the wild.

The number of claps was significantly related to the muscle Pi/PLA ratio following exercise in all species, though the slopes of the relationships differed. The maximum Pi/PLA ratios attained by *A. opercularis* were significantly higher (two to three times) than either of the other two species for the same number of claps.

Previous studies have shown that peak muscle power output was significantly higher in *A. opercularis* (mean 169 W Kg^{-1}) than in *A. colbecki* (100 W Kg^{-1}) at the temperatures used in this study (Bailey 2001). No comparable power output data exist for *P. maximus*. Muscle cross-sectional area-specific force production (as calculated from the pressure trace) in *P. maximus* was similar to that produced by both the other species. Rates of Pi/PLA recovery were similar in *A. colbecki* and *P. maximus* with the available data for *A. opercularis* falling in between. Following heavy exercise (>22 claps), 50% recovery of PLA levels in *P. maximus* took an average of 3 h, with 90% recovery taking an average of 5.6 h (Fig. 7). The increased PLA depletion observed in *A. opercularis* indicated a greater use of ATP, which would initiate anaerobic glycolysis earlier. In each species, the eventual cause of fatigue appeared to be the reductions in the free energy change of ATP hydrolysis, with exhaustion being reached at a similar value of free energy change despite the differences in cellular pH and metabolite use. The high swimming performance of *A. opercularis* is demonstrated by the much-reduced mortality this species suffers from trawls compared with less motile species such as *Pecten jacobaeus* (Hall-Spencer et al. 1999). Although *A. opercularis* used its energy stores the most quickly, its rate of PLA regeneration did not appear to be higher than those of the other two species.

Conclusions

There was more difference between the two temperate species than there was between *P. maximus* and *A. colbecki*. While recent

studies found no evidence of cold adaptation in terms of the muscle performance of *A. colbecki* (Bailey 2001), these new NMR data demonstrated that this Antarctic species recovered from exercise at a similar rate at 0°C to *P. maximus* at 10°C. Because *P. maximus* is the more closely related (Canapa et al. 2000) of the two temperate species to *A. colbecki*, these findings indicate some cold compensation of muscular recovery capacities in the Antarctic scallop. Differences in muscle metabolism between the two temperate species probably reflect differences in their natural activity levels and muscle performance requirements and when compared with the Antarctic species were at least as influential in determining muscle physiology and recovery rates as adaptations to different temperatures. Along the same lines, the mode of life of *A. colbecki* resembles more closely that of *A. opercularis*, however, at significantly lower levels of energy expenditure despite the 20 million years of selection for this lifestyle in the Antarctic. These observations may reflect the general limitation to high-activity modes of life linked to general cellular design constraints in permanently cold conditions (Pörtner 2002).

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