^{31}P NMR studies of the metabolic changes in the prawns *Palaemon serratus* and *P. elegans* during exercise

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ABSTRACT: *In vivo* ^{31}P NMR (Nuclear Magnetic Resonance Spectroscopy) was used to examine the changes in phosphometabolites in the abdominal muscle of 2 closely related prawns, *Palaemon serratus* and *P. elegans*, after exercise. Two types of exercise were produced by electrical stimulation: brief maximal exercise and exercise to exhaustion. ATP was extracted and analyzed by HPLC to calculate the absolute concentrations of metabolites measured by NMR. The main findings were: (1) At rest, *P. serratus* had lower muscle concentrations of phosphoarginine, arginine and ATP than *P. elegans*. (2) During exercise, the ATP concentration in *P. serratus* muscle decreased by 23 % from that at the start of the exercise, while that of *P. elegans* remained unchanged. (3) The patterns of post-exercise phosphometabolite recovery in the 2 species were similar. Thus, *P. elegans*, which is more tolerant of environmental anaerobiosis, is also more able to maintain its cellular energy state during bursts of muscular activity.

KEY WORDS: Exercise · ^{31}P NMR · Crustaceans · Phosphometabolites

INTRODUCTION

The glass prawn *Palaemon elegans* inhabits rock pools in the upper littoral zone while its close relative *P. serratus* is essentially a sublittoral species. Mature *P. serratus* migrate towards deeper water in winter to find more stable conditions, but most young specimens (a few months old) stay in shore pools where the environmental conditions are more extreme. The reasons why young *P. serratus* occupy different habitats from adults are likely to be complex. Although young specimens of *P. serratus* tolerate low temperature poorly and high temperature well (Richard 1978), their spatial distribution cannot be attributed to differences in their tolerance of any one environmental factor. Recent studies have shown that there are major differences in the way the 2 species tolerate environmental hypoxia and anoxia (Taylor & Spicer 1987): *P. elegans* tolerates severe hypoxia better than *P. serratus*.

Animals generally use more oxygen during intense muscular activity than is available in the blood (Gäde 1983). In the same way, the blood O_2 tension drops rapidly when these animals are subjected to environmental anoxia. Zebe et al. (1981) defined environmental anaerobiosis as exposure of the whole organism to hypoxic or anoxic conditions in the natural environment, and functional anaerobiosis as hypoxic or anoxic conditions in specific tissues initiated by vigorous muscular activity. In molluscs, aspartate is consumed in response to environmental anoxia (Zurburg & Ebberink 1981), but not during functional hypoxia, when energy is derived from arginine phosphate and glycolysis. Aspartate is not involved in environmental or functional hypoxia in crustaceans; arginine phosphate and glycogen are the main substrates of anaerobic metabolism (Gäde 1983, Taylor & Spicer 1987; see also Albert & Ellington 1985).

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Thus, a tolerance of environmental hypoxia should be correlated with tolerance of functional hypoxia.

All the comparisons of the metabolic activities of species that have been made to date have relied on *in vitro* biochemical methods. This study uses ^{31}P NMR (Nuclear Magnetic Resonance Spectroscopy) spectroscopic and biochemical extraction techniques to compare the characteristics of *Palaemon serratus* and *P. elegans* metabolism *in vivo* at rest, after exercise and during recovery. The data are used to establish whether there is any difference between the metabolic responses of these species to functional anaerobiosis and in the time they take to recover.

MATERIALS AND METHODS

Specimens and exercise protocols. Young common prawns *Palaemon serratus* and glass prawns *P. elegans* (2 to 3 cm long) were collected in Concarneau Bay, France, kept in aerated seawater and fed regularly on mussels. The muscular performances of the 2 species were compared by subjecting individual prawns to electrical stimulation in small jars either for 10 ± 2.5 s, or until they were exhausted. The electrical stimulation was 10 ms rectangular pulses, 8 V at 2 Hz. Prawns performed vigorous tail flips for about 15 s (short-term maximal exercise). Thereafter, the contractions became progressively less powerful until the prawns were exhausted (maximal exercise). Immediately after electrical stimulation, the prawns were placed in the NMR probe and allowed to recover over a period of 7.5 min. A total of 16 prawns were used. In addition to the exercise protocol, another set of NMR measurements was acquired before, or at least 1 h following, the exercise bout.

NMR measurements. Individual prawns were placed in the NMR probe so that the abdominal part of the prawn was oriented in the transceiver coil. The temperature was maintained at $13 \pm 1 °C$, the seawater temperature during the experiments. The prawn was kept at the bottom of the tube in filtered seawater during the NMR measurements. The seawater was circulated (3 ml min^{-1}) using a peristaltic pump and the water level was maintained about 1 cm above the head of the prawn. The tubes were cone-shaped in order to increase the signal-to-noise ratio. Phosphorus NMR spectra were generated at 121.47 MHz in a pulsed Fourier transform mode on a Bruker AC300 spectrometer. The probe diameter was 10 mm. A deuterium lock was used for field frequency stabilization; a $^2H_2O/80\% H_3PO_4$-filled capillary was placed inside the 10 mm (o.d.) NMR tube.

In resting conditions, each spectrum consisted of 32 data acquisitions accumulated with a delay of 2.0 s

(interpulse delay: 3.0 s), a tip angle of 72° (pulse width: 16 µs) and a sweep width of 8064 Hz. The line-broadening was 5 Hz. After the electrical stimulation, each recovery experiment consisted of 4 series of 8 data acquisitions followed by 3 series of 32 data acquisitions, accumulated with a delay of 2.0 s (interpulse delay: 3.0 s). Saturation factors, described by Dawson et al. (1977), were obtained as previously described (Thébault & Raffin 1991). The saturation factor was 1.0 for phosphoarginine and ATP and 1.31 for inorganic phosphate. The relative concentration, expressed as ^{31}P magnetization units (MU) of inorganic phosphate, phosphoarginine, ATP and phosphomonoesters, was determined by integrating the peak under the appropriate resonance. The intracellular pH (pH_i) was estimated from the chemical shifts of the inorganic phosphate and arginine phosphate (Thébault & Raffin 1991).

Metabolite assays and HPLC analysis. Whole prawns were rapidly frozen in liquid nitrogen. Those showing spontaneous escape behaviour were excluded from analysis. The frozen muscle was dissected free of cuticle and divided into 2 parts, one heated at 60 °C for 2 d to determine dry weight, the other extracted with perchloric acid (Thébault & Raffin 1991). The samples were stored at –80 °C until HPLC analysis. ATP and ADP were quantified on a Whatman Partisphere SAX (12.5 cm × 4.6 mm i.d., particle size 5 µm) column as previously described (Thébault & Raffin 1991). Arginine was quantified after precolumn derivatization with phenylisothiocyanate (Heinrikson & Meredith 1984) by HPLC on a Waters Picotag (15 cm × 3.9 mm i.d.) column. The mobile phase was: TEA-sodium acetate (kit from Millipore Co., Milford, MA, USA) containing 6% acetonitrile (buffer A), and 60% acetonitrile (solution B).

Calculation of absolute metabolite concentrations. The absolute levels of phosphoarginine, inorganic phosphate and phosphomonoesters were estimated from the integrals of the NMR spectra, assuming that ATP measured biochemically is equivalent to the βATP peak (Dawson et al. 1977).

Statistics. Reported values are means ± SD. One-way ANOVA was used to compare the metabolite concentrations in the 2 species. When the ANOVA resulted in a significant F-value ($p \leq 0.05$), the differences between the means were located by the Newman-Keuls test.

RESULTS

Muscle metabolism at rest

The NMR spectra for *Palaemon serratus* and *P. elegans* abdominal muscle at rest (Fig 1) are very similar.
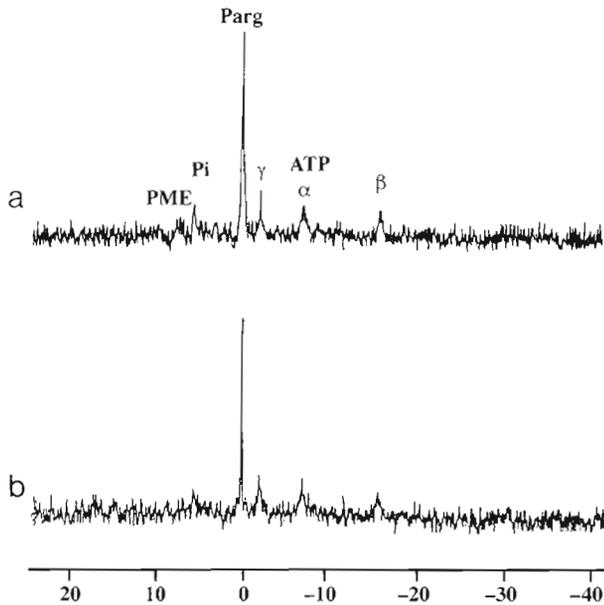
Fig. 1 Typical ^{31}P NMR spectra of (a) *Palaemon serratus* and (b) *P. elegans* abdominal muscle. Spectra were acquired under the following conditions: 32 acquisitions, pulse angle 72°, interpulse delay 3 s. Zero ppm reference: phosphoarginine. MPE: monophosphate esters; Pi: inorganic phosphate; Parg: phosphoarginine; γ, α, β: phosphorus atoms of ATP

The only difference was a substantially larger phosphomonoester peak in *P. serratus*.

The metabolite concentrations and pH$_i$ at rest are shown in Table 1. The pH$_i$ was similar in the muscle of the 2 species. The phosphomonoester and inorganic phosphate levels were not significantly different, but the phosphoarginine, arginine and ATP concentrations were significantly higher in *Palaemon elegans*. The arginine concentration was 6.51 ± 1.26 µmol ml^{-1} tissue water in *P. serratus* and was 12.23 ± 5.02 µmol ml^{-1} tissue water in *P. elegans* (p $\leq$ 0.05). However, the

Arg/Parg ratios in the 2 species were similar. Some of the biochemical parameters measured by *in vivo* ^{31}P NMR spectroscopy reflect the muscle metabolism. The ATP+Parg/ATP+Parg+Pi ratio (Lavanchy et al. 1985) was significantly higher in *P. elegans*.

Muscle metabolism during contraction

The changes in muscle metabolite concentrations after stimulation for 10 s are shown in Table 1. The phosphoarginine concentration changed significantly. The phosphomonoester concentration in *Palaemon elegans* was 1.6-fold higher than in *P. serratus*, and the ATP concentration was 2-fold higher. However, the inorganic phosphate concentrations in the muscle of the 2 species were similar. The ATP+Parg/ATP+Parg+Pi ratio was significantly higher in *P. elegans* than in *P. serratus*, but the Pi/ATP ratio was lower. The post-exercise pH$_i$ values were similar to the resting values in the muscle of the 2 species.

The changes in muscle metabolite concentrations after exercise to exhaustion are shown in Table 1. The phosphoarginine and the ATP were about 2-fold higher in *Palaemon elegans* than in *P. serratus*.

The ATP+Parg/ATP+Parg+Pi ratios, the Arg/Parg ratios and the pH$_i$ in the 2 species were not significantly different.

Muscle metabolism during recovery

NMR spectra were collected every 1.5 min for a total of 7.5 min starting at the end of exercise. The changes in metabolite and biochemical parameters after short-term maximal exercise are shown in Fig. 2. The relative concentrations of phosphomonoesters and ATP in the 2 species were not significantly different. The rela-

Table 1. *Palaemon serratus* and *P. elegans*. Biochemical characteristics of prawn tail muscle at rest, after short-term maximal exercise (STME) and after maximal exercise (ME). Absolute levels of phosphomonoesters (PME), phosphoarginine (Parg), total ATP and inorganic phosphate (Pi) were calculated from ^{31}P NMR and biochemical data, and are expressed as µmol ml^{-1} tissue water. Values are means ± SD (*P. serratus*: n = 9; *P. elegans*: n = 7). Significant differences between species (*p $\leq$ 0.05 and **p $\leq$ 0.01) were calculated by 1-way ANOVA as described in the 'Methods'

|	Control		STME		ME	
	P. serratus	*P. elegans*	*P. serratus*	*P. elegans*	*P. serratus*	*P. elegans*
PME	1.39 ± 1.05	1.84 ± 1.22	1.66 ± 1.38	2.73 ± 1.51	2.47 ± 0.88	3.29 ± 1.63
Pi	3.19 ± 1.85	3.75 ± 2.04	9.31 ± 2.52	9.07 ± 2.32	9.21 ± 1.52	11.96 ± 3.37
Parg	$6.38 \pm 3.37**$	13.11 ± 4.43	$4.09 \pm 1.71*$	6.60 ± 1.90	$2.53 \pm 1.08**$	5.09 ± 1.99
ATP	$2.77 \pm 0.60*$	3.76 ± 0.72	$2.14 \pm 1.24**$	4.26 ± 0.89	$2.11 \pm 0.43**$	3.61 ± 0.82
Parg+ATP/Parg+ATP+Pi	$0.67 \pm 0.15*$	0.81 ± 0.11	$0.41 \pm 0.08**$	0.55 ± 0.11	0.34 ± 0.09	0.43 ± 0.13
Pi/ATP	2.15 ± 1.46	1.22 ± 0.67	$4.26 \pm 2.02*$	2.25 ± 0.82	4.52 ± 1.11	3.69 ± 2.05
pH$_i$	7.08 ± 0.11	7.11 ± 0.20	7.10 ± 0.09	7.06 ± 0.11	7.03 ± 0.07	6.99 ± 0.09
Arg/Parg	1.02 ± 0.54	1.07 ± 0.36	2.15 ± 0.90	2.84 ± 0.82	4.10 ± 1.75	3.98 ± 1.56
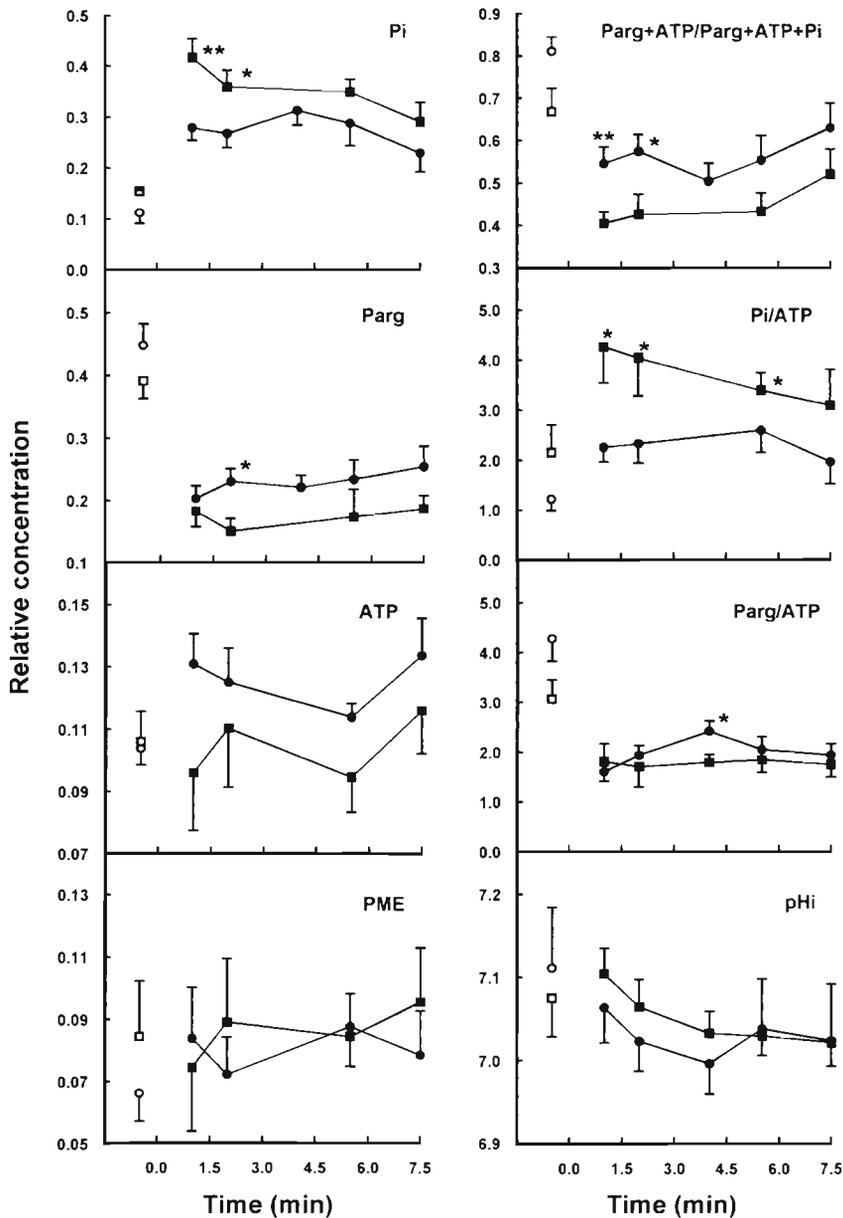
Fig. 2. *Palaemon serratus* and *P. elegans*. Recovery of phosphometabolites (relative concentrations) and biochemical parameters after short-term exercise in prawn abdominal muscle. Spectra were acquired under the following conditions: 4 series of 8 data acquisitions followed by 3 series of 32 data acquisitions, accumulated with a delay time of 2.0 s; pulse angle 72°, interpulse delay 3 s. Values are means ± SD (n = 9). Significant differences between species ($^{*}p \leq 0.05$; $^{**}p \leq 0.01$) were calculated by 1-way ANOVA as described in the 'Methods' (■) *P. serratus*; (●) *P. elegans*; open symbols: control values at rest

tive amount of phosphoarginine in *Palaemon elegans* at 2 min recovery was significantly higher than in *P. serratus*. But, the relative concentrations of inorganic phosphate at 1 and 2 min recovery were significantly higher in *P. serratus* than in *P. elegans*. The kinetics of recovery of the metabolites were similar. The Parg+ATP/Parg+ATP+Pi ratio, at the start of the recovery period, was significantly higher in *P. elegans* than in *P. serratus*, while the Pi/ATP ratio was significantly lower throughout the 7.5 min recovery. The Parg/ATP ratios in the 2 species were similar, as were the kinetics of recovery of the energetic parameters. The pH_i in the muscle of the 2 species declined similarly during the first minutes of recovery. The pH_i in *P. elegans*,

which was lower than in *P. serratus*, returned to its usual level more rapidly.

The recovery of the metabolite and the biochemical parameters after maximal exercise is shown in Fig. 3. The relative level of inorganic phosphate in *Palaemon serratus* was significantly lower than in *P. elegans* until 4 min of recovery. The patterns of ATP and Parg recovery were similar in the 2 species. Phosphomonoesters during recovery tended to be higher in *P. elegans*, and were significantly higher than in *P. serratus* at 7.5 min. The kinetics of recovery in the 2 species were similar. During the recovery period, the Parg+ATP/Parg+ATP+Pi ratio was slightly higher in *P. elegans*, with a significant difference at 4 min. The Pi/ATP ratio
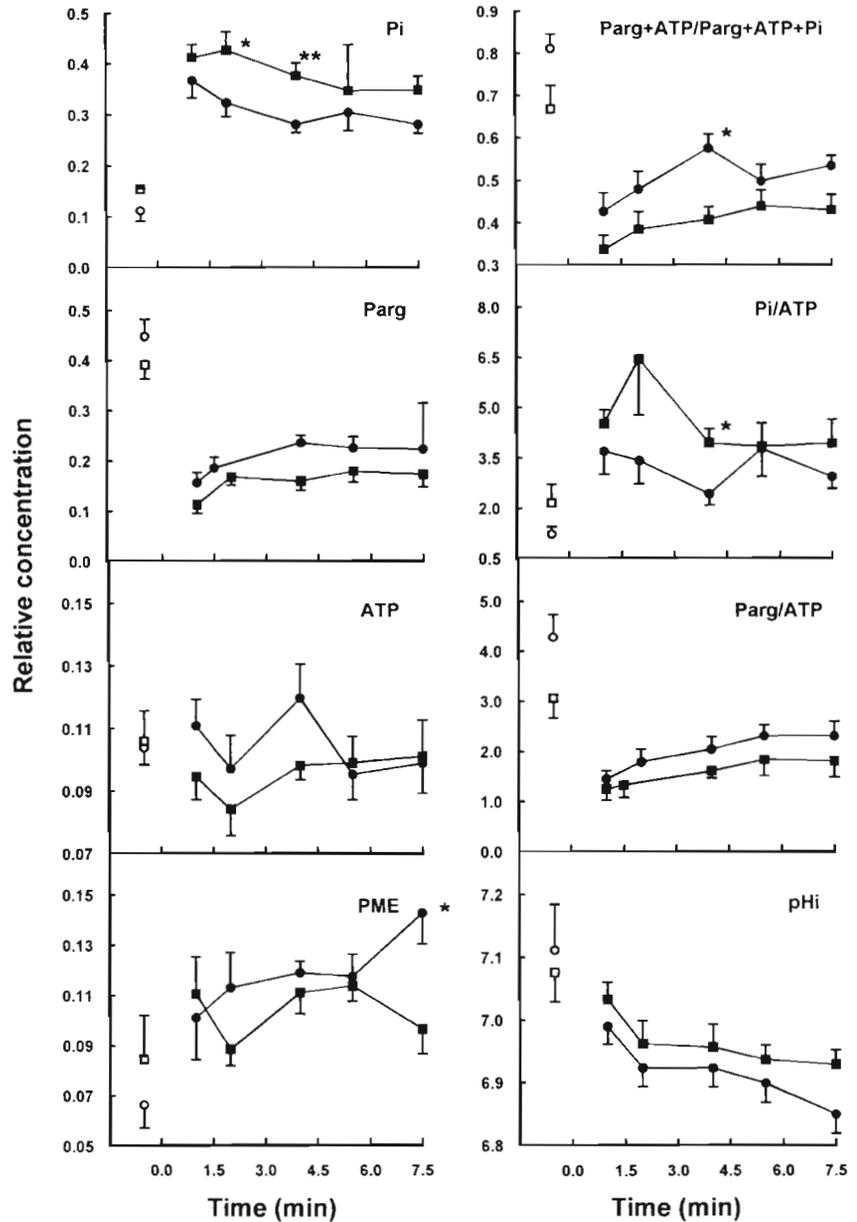
Fig. 3. *Palaemon serratus* and *P. elegans*. Recovery of phosphometabolites (relative concentrations) and biochemical parameters at exhaustion in prawn abdominal muscle. Spectra were acquired under the following conditions: 4 series of 8 data acquisitions followed by 3 series of 32 data acquisitions, accumulated with a delay time of 2.0 s; pulse angle 72°, interpulse delay 3 s. Values are means ± SD (n = 9). Significant differences between species (*$p \leq 0.05$; **$p \leq 0.01$) were calculated by 1-way ANOVA as described in the 'Methods' (■) *P. serratus*; (●) *P. elegans*; open symbols: control values at rest

tended to be lower in *P. elegans*, with a significant difference at 4 min. The recoveries of pH$_i$ and Parg/ATP in the 2 species were similar.

DISCUSSION

Biochemical characteristics of *Palaemon serratus* and *P. elegans* tail muscle

Palaemon elegans tail muscle contained higher concentrations of ATP, phosphoarginine and arginine than did *P. serratus* muscle. Thus, *P. elegans* has a higher phosphagen content, providing greater ATP-

buffering capacity for burst contractions (Kushmerick 1985). The amount of inorganic phosphate was low in the 2 species, showing that the animals were in good condition.

Effect of exercise

Most of the energy demand of exercise is met by the breakdown of arginine phosphate (Gäde 1983, Onnen & Zebe 1983, Thébault et al. 1987, Raffin et al. 1988), and the rest by glycolysis. The phosphomonoesters (corresponding mostly to increases in sugar phosphates) were higher in *Palaemon elegans* during exer-

cise than in *P. serratus*, indicating that glycolysis is very active in *P. elegans* muscle. The phosphoarginine decreased by 50 % in *P. elegans* and by 35 % in *P. serratus*, showing that *P. elegans* used more of the high energy stores at the onset of exercise than did *P. serratus*. The drop in arginine phosphate after maximal exercise, relative to control values, was identical (60 %) in the 2 species. The ATP decreased by 23 % at the start of exercise in *P. serratus* muscle, and then stabilized. In contrast, the muscle ATP content in *P. elegans* did not drop throughout the exercise protocol. Thus, *P. serratus* and *P. elegans* abdominal muscles form ATP at different rates by transphosphorylation of arginine phosphate (shown by the 2-fold difference in the total arginine pool) and by substrate phosphorylations in glycolysis.

Phosphometabolite levels during recovery

Post-exercise recovery occurs in 2 phases, with the greatest changes in metabolite concentration occurring during the initial nonlinear period (Challiss et al. 1989). This study examined only this immediate post-exercise period. During this period, the replenishment of arginine phosphate stores is balanced by the disappearance of inorganic phosphate. The abdominal muscles of both *Palaemon serratus* and *P. elegans* contain large amounts of glycogen (Taylor & Spicer 1987). Glycogen is broken down during functional anaerobiosis in marine invertebrates (Gäde 1983, Albert & Ellington 1985, de Zwaan & van den Thillart 1985). Kamp (1989) showed that glycogen is degraded in the abdominal muscle of shrimp *Crangon crangon* during recovery from work. The resynthesis of ATP depends on glycogen phosphorylase activity, which is restricted by the cytoplasmic concentration of inorganic phosphate (Kamp 1989).

The kinetics of restoration of the phosphometabolites were similar in the 2 species, while the phosphometabolite levels during recovery and at exhaustion were different. As a result, the *Palaemon serratus* abdominal muscle took longer to recover. Since recovery from exercise involves aerobic metabolism, the metabolic differences between the 2 species probably involve anaerobic metabolism.

In conclusion, our results show that *Palaemon elegans*, which is more tolerant of hypoxia and anoxia, also has a greater capacity for burst contractile activity without incurring severe energy deficit. This is probably due to its greater Arg+Parg stores, as Arg/Parg ratios in the 2 species are similar. The differences in the way the 2 species tolerate environmental and functional hypoxia may be correlated with their different spatial distribution.

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