Comp. Biochem. Physiol. Vol. 72A, No. 2, pp. 295 to 299, 1982
Printed in Great Britain.

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0300-9629 82 020295-05$03.00 0
Pergamon Press Ltd

SEASONAL VARIATION OF PHOSPHOADENYLATE CONCENTRATIONS AND ADENYLATE ENERGY CHARGE IN DORSAL TAIL MUSCLE OF THE CRAYFISH, *PROCAMBARUS ACUTUS ACUTUS* (DECAPODA: ASTACIDAE)

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(*Received* 16 *October* 1981)

Abstract—1. Phosphoadenylate concentrations (ATP, ADP, and AMP) in dorsal tail muscle of the crayfish, *Procambarus acutus acutus*, were recorded monthly for one year.

2. Phosphoadenylates and the adenylate energy charge (AEC = (ATP + ½ADP)/(ATP + ADP + AMP)) varied significantly over the twelve month period. This variability was not associated directly with environmental conditions (temperature, dissolved oxygen, or pH of the habitat at time of collection), sex, breeding condition of males or limb regeneration.

3. Seasonal peaks of tail muscle ATP concentrations coincided with the breeding period of the crayfish population examined. This pattern could reflect greater energy production associated with increased muscular activity involved in mating and agonistic encounters.

INTRODUCTION

Environmental fluctuations associated with seasonal climatic changes are of major importance in triggering adjustments in the physiology and behaviour of aquatic organisms. Cyclic variations in parameters such as temperature, dissolved oxygen, pH and food availability can induce biological changes at both the molecular and organismal levels (Hochachka & Somero, 1973; Prosser, 1973).

Freshwater crayfish (Decapoda:Astacidae) exhibit seasonal fluctuations in protein synthesis (McWinnie & Mohrherr, 1969), blood glucose (Telford, 1974), lipid content (Collatz, 1969), carbohydrate types (Speck, 1969) and urine production (Ono & Kamemoto, 1969). Studies involving crayfish on an organismal level indicate the presence of differences in agonistic behavior (Thorp, 1978) and reproductive activities (see review in Jegla, 1964) associated with seasonal periods.

Although not previously examined in crayfish, seasonal differences in phosphoadenylate (ATP, ADP and AMP) levels and AEC have been noted in frogs (L'vova, 1978), in two crustaceans (Bamstedt & Skjoldal 1976; Skjoldal & Bamstedt, 1976) and in two freshwater clams (Giesy & Dickson, 1981). In an evaluation of the adenylate energy charge (AEC = (ATP + ½ADP)/(ATP + ADP + AMP)) as a general stress indicator, as proposed by Dickson (1980), Ivanovici (1980) and Giesy *et al.* (In press), it is necessary to define possible seasonal variation of phosphoadenylate concentrations. The present study was conducted to determine the seasonal variability of phosphoadenylate and AEC in crayfish dorsal tail muscle.

METHODS AND MATERIALS

Beginning in July 1978, monthly collections of the crayfish *Procambarus acutus acutus* (Girard) were made from a ditch associated with Brinkley Well, an artesian system located on the Savannah River Plant, South Carolina. Adult, intermolt crayfish ($n = 15$) were collected for a period of 12 months with baited minnow traps. This habitat was selected for the seasonal study because of the large population of *P. a. acutus* and the almost complete absence of any other crayfish species. Dissolved oxygen, temperature and pH of the aquatic habitat were recorded in the morning during the monthly crayfish collections.

Crayfish dorsal tail muscle was rapidly dissected at the collection site using standardized procedures (Dickson, 1980). Tissue samples were placed in labelled polyethylene bags and quickly frozen by flattening the tissue between two aluminium blocks immersed in liquid nitrogen (−196°C). The entire process of dissection and enzymatic inactivation was completed in approximately 10 sec. Samples were stored in liquid nitrogen and transported to the laboratory for extraction of phosphoadenylates.

Adenylate extraction was conducted by a method described by Giesy *et al.* (In press). Between 25 and 75 mg (dry weight) of muscle tissue was ground to a fine powder in steel grinders at −196°C, with 1 ml 6% perchloric acid to inactivate enzymatic activity. Samples were thawed, centrifuged and the supernatant neutralized with K_2CO_3. Samples were then centrifuged at 40,000 *g* at 5°C. This extraction protocol resulted in total adenylate recoveries of 95% or greater.

ADP and AMP were converted to ATP by pyruvate kinase (ADP → AMP) and pyruvate kinase and myokinase (AMP → ATP) respectively, in the presence of $MgSO_4$.

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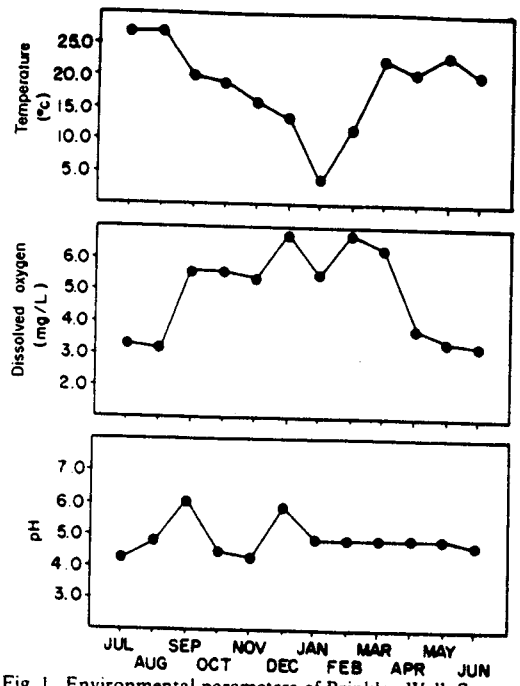

Fig. 1. Environmental parameters of Brinkley Well, Savannah River Plant, SC, recorded at the time of monthly collections of *P. a. acutus*.

ATP concentrations were measured by a modification of the luciferin-luciferase bioluminescence assay (Giesy *et al.*, In press). Samples were assayed by a SAI model-2000 ATP photometer which was equipped with an enzyme kinetics injection attachment. ADP and AMP concentrations were calculated by subtraction. Five or six replicate injections were made for each sample incubation and means of replicate injections used for calculations. Six second intergrations of bioluminescence were made from the time of sample injections. Sample adenylate concentrations were compared to standards and reported as nM/mg dry weight. Statistical analyses of data were conducted as previously described in Giesy & Dickson (1981).

RESULTS

Temperature and dissolved oxygen of the artesian spring exhibited major changes during the study (Fig. 1). Temperatures ranged from 4 to 27°C, while dissolved oxygen concentrations ranged from 3.3 to 7.2 mg/l.

Phosphoadenylate concentrations (ATP, ADP, AMP and total phosphoadenylates) exhibited significant (ANOVA, $P < 0.05$) seasonal variation (Table 1). The highest ATP concentrations were observed in late summer with lower values in winter and spring (Fig. 2). The highest monthly ATP concentrations ($\bar{X}$ = 48.91 nM/mg) were recorded in September, and the lowest ($\bar{X}$ = 3.58 nM/mg) were observed in animals collected in December. The AEC of tail muscle also exhibited significant variation ($P < 0.01$) during the twelve months (Table 1). Values ranged from 0.93 in March and April to 0.80 in May , with no distinct seasonal pattern.

Phosphoadenylate concentrations and AEC were not correlated with temperature, dissolved oxygen or pH of the habitat (Spearman rank, $P > 0.05$). In addition, phosphoadenylate concentrations were not

Table 1. Adenylate concentrations and adenylate energy charge in dorsal tail muscle of *P. a. acutus* for monthly collections over one year. Means that do not have the same letter in their superscripts are significantly different ($\alpha = 0.05$)

Month	Mean (standard deviation) ATP nM/mg	ADP	AMP dry tissue wt	Total*	AEC†
July	14.96^{a}	1.66^{ab}	2.33^{a}	18.13^{a}	0.85^{abc}
n = 15	(5.23)	(1.82)	(1.59)	(6.50)	(0.05)
August	20.99^{b}	2.18^{b}	1.25^{abc}	24.14^{b}	0.91^{a}
n = 15	(4.64)	(1.95)	(1.66)	(4.48)	(0.07)
September	48.91^{c}	5.33^{c}	2.47^{a}	56.70^{c}	0.91^{a}
n = 15	(8.18)	(4.58)	(3.33)	(8.56)	(0.08)
October	22.17^{d}	2.12^{b}	1.95^{ab}	26.17^{b}	0.89^{abc}
n = 15	(3.32)	(1.60)	(1.40)	(2.60)	(0.06)
November	34.23^{e}	4.54^{c}	2.40^{a}	41.18^{b}	0.89^{abc}
n = 14	(7.36)	(1.75)	(2.61)	(10.00)	(0.04)
December	3.58^{f}	0.33^{d}	0.15^{c}	4.06^{e}	0.92^{a}
n = 13	(0.71)	(0.22)	(0.20)	(0.64)	(0.08)
January	3.65^{f}	0.76^{d}	0.69^{cd}	5.09^{e}	0.81^{bc}
n = 15	(0.69)	(0.58)	(0.80)	(0.95)	(0.14)
February	8.35^{g}	1.05^{de}	0.28^{c}	9.68^{e}	0.93^{a}
n = 15	(1.52)	(0.91)	(0.77)	(2.13)	(0.07)
March	4.45^{fg}	0.41^{d}	0.14^{c}	5.00^{e}	0.93^{a}
n = 15	(0.74)	(0.28)	(0.28)	(0.70)	(0.05)
April	4.31^{fg}	0.35^{d}	0.19^{c}	4.84^{e}	0.93^{a}
n = 15	(0.65)	(0.32)	(0.22)	(0.73)	(0.06)
May	5.28^{fg}	0.63^{d}	1.30^{abc}	7.21^{e}	0.80^{c}
n = 14	(1.90)	(0.72)	(1.51)	(2.97)	(0.17)
June	6.75^{fg}	0.74^{d}	0.18^{c}	7.67^{e}	0.93^{a}
n = 15	(1.40)	(0.38)	(0.24)	(1.38)	(0.04)

* Total = ATP + ADP + AMP.
† AEC = (ATP + ½ADP)/(ATP + ADP + AMP).

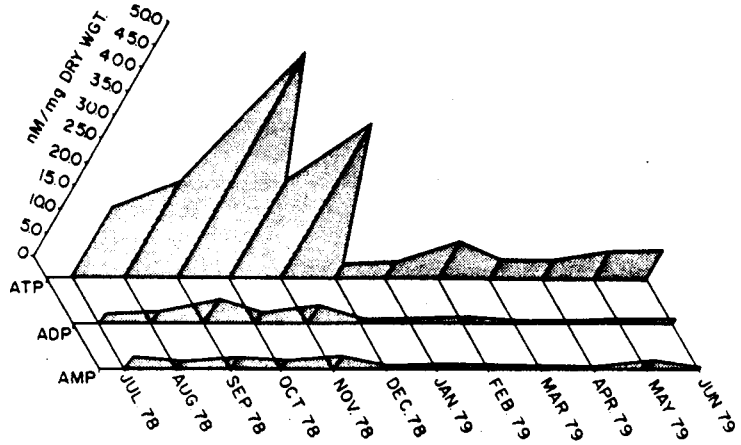

Fig. 2. Mean adenylate concentrations of *P. a. acutus* dorsal tail muscle during monthly collection.

correlated with crayfish carapace length (Spearman rank, $P > 0.05$). Although a significant positive linear regression ($P < 0.01$) was found between carapace length and AEC over the 12 month period it explained little of the variability present ($r^2 = 0.08$). This regression did not differ between males and females (Wilks slope analysis; $P > 0.05$).

No significant differences (ANOVA; $P > 0.05$) in phosphoadenylate concentrations or AEC were found in the following comparisons during the twelve months; (1) males vs. females, (2) Form I (breeding) and Form (II) (non-breeding) males and (3) injured vs. uninjured individuals (Table 2).

DISCUSSION

Seasonal differences in ATP, ADP, AMP and total phosphoadenylate concentrations are present in dorsal tail muscle of the crayfish species examined (Table 1). A seasonal peak is most apparent in ATP concentration (Fig. 2). Seasonal variation in physical and chemical conditions did not appear to affect phosphoadenylate concentrations directly. Instead, these environmental changes are thought to influence the energy status of muscle indirectly, through yearly hormonal cycles.

Similar seasonal patterns in phosphoadenylate concentrations have been reported in marine crustaceans and a freshwater mollusk. The euphausid, *Meganyctiphanes norvegica* exhibited one seasonal peak (Skjoldal & Bamstedt, 1976), and two peaks were found in populations of the copepod, *Euchaeta norvegica* (Bamstedt & Skjoldal, 1976). Based on evaluations of body size, lipid content and nucleic acid (RNA and DNA) concentrations, these researchers hypothesized that the highest seasonal concentrations of phosphoadenylates were directly associated with peak reproductive periods of the respective species. Giesy & Dickson (1981) observed a seasonal peak in phosphoadenylate concentrations of foot muscle in

Table 2. Adenylate concentrations and adenylate energy charge in dorsal tail muscle of selected groups of *P. a. acutus* based on twelve monthly collections

Classification	Mean (Standard deviation) ATP	ADP	AMP nM/mg dry tissue wt	Total*	AEC†
Males	14.18	1.36	0.91	16.31	0.90
$n = 110$	(14.40)	(1.68)	(1.37)	(15.84)	(0.09)
Females	16.12	2.24	1.41	19.77	0.88
$n = 67$	(14.39)	(3.02)	(2.22)	(18.14)	(0.09)
Form I males	15.25	1.31	0.89	17.28	0.90
$n = 61$	(15.64)	(1.56)	(1.47)	(16.96)	(0.08)
Form II males	12.85	1.41	0.93	15.09	0.89
$n = 49$	(12.70)	(1.84)	(1.26)	(14.38)	(0.11)
Non-injured	14.75	1.63	1.08	17.40	0.89
$n = 175$	(14.36)	(2.26)	(1.70)	(16.71)	(0.09)
Injured	16.20	2.14	1.26	19.34	0.89
$n = 20$	(14.92)	(2.74)	(2.17)	(17.66)	(0.08)

* Total = ATP + ADP + AMP.
† AEC = (ATP + ½ADP)/(ATP + ADP + AMP).

Anodonta imbecillis, associated with egg presence in the marsupial pouch. From observational data on the relative abundance of Form I (breeding condition) males, it appears that the major breeding period of the population of *P. a. acutus* sampled is in the fall, rather than the more typical spring period. The corresponding seasonal peak in ATP levels in the fall could indicate an association between these factors in this crayfish species.

Before possibilities for the association of breeding period and phosphoadenylate peaks are hypothesized, it must be realized that the particular tissue or tissues assayed may exhibit seasonal peaks because of different physiological actions. The adenylate analyses of the two marine crustaceans (Bamstedt & Skjoldal, 1976: Skjoldal & Bamstedt, 1976) were conducted with whole animal preparations, rather than just muscle tissue. They would represent a cumulative energy status of the whole organism and the associated metabolic changes (increased basal metabolism, lipid catabolism, protein synthesis and production of reproductive products) commonly associated with reproductive periods. The increase of muscle tissue adenylates corresponding to the crayfish breeding period may result from a different physiological origin.

As in an earlier study of habitat influence on phosphoadenylate concentrations (Dickson & Giesy, 1981), the origin of high ATP levels in crayfish tail muscle during breeding periods could be associated with increased activity. Thorp (1978) found evidence of increased behavioral activity during the reproductive period of another fall breeding crayfish, *Cambarus latimanus*. A seasonal cycle of agonistic behavior could then account for a similar pattern in the energy status of dorsal tail muscle. The origin of this ATP increase during the breeding period is not known, but probably involves a tissue response to hormonal cycles associated with reproduction. A further study of seasonal fluctuations in phosphoarginine (secondary high energy molecule in crustacean muscle) could help determine the extent of increased phosphate metabolism.

The AEC of tail muscle also exhibited significant differences during the twelve months examined (Table 1), although levels ranged within limits found in most unstressed organisms (Chapman *et al.*, 1971; Atkinson, 1977). In a recent study, Giesy & Dickson (1981) also found significant seasonal changes in AEC of muscle tissue in two freshwater pelecypods, *Corbicula fluminea* and *Anodonta imbecillis*. It was hypothesized that factors influencing this adenylate ratio could include normal aging of the population, reproductive periods and environmental fluctuations. In additon to the lack of influence due to measured environmental parameters, the AEC of crayfish tail muscle did not exhibit any distinct seasonal pattern (Table 1). The importance of differences in seasonal changes in AEC between pelecypods and crayfish is not understood at this time. These differences could result from specific developmental and physiological systems found in these two invertebrate groups.

Developmental stage and sex were associated with ATP concentrations in the copepod, *E. norvegica* (Bamstedt & Skjoldal, 1976). In contrast, evidence from the present study indicates that these biological differences did not affect ATP concentrations (Table 2) in the crayfish examined. These dissimilar results could again be attributed to the assay of whole animals utilized in the copepod study, and analyses of muscle tissue in the present study. It appears that any behavioral differences between sexes or breeding conditions of males is not sufficient to alter tail muscle ATP concentrations significantly in the crayfish species analyzed.

No major differences were found in phosphoadenylate concentrations between crayfish with missing or regenerating appendages and those whith normal limb development (Table 2). This would suggest that increased energy metabolism associated with regeneration is localized or not of sufficient magnitude to influence phosphoadenylate concentrations in dorsal tail muscle.

Acknowledgements—This study was supported by contract DE-AC09-76SR00819 between the U.S. Department of Energy and the University of Georgia. M. Allred and L. Briese helped with sample collection and provided valuable technical assistance.

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