Comp. Biochem. Physiol. Vol. 70A, pp. 421 to 425, 1981
Printed in Great Britain.

0300-9629/81/011421-05$02.00/0

VARIATION OF PHOSPHOADENYLATES AND ADENYLATE ENERGY CHARGE IN CRAYFISH (DECAPODA: ASTACIDAE) TAIL MUSCLE DUE TO HABITAT DIFFERENCES

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(*Received* 9 *March* 1981)

Abstract—1. Phosphoadenylate concentrations (ATP, ADP and AMP) were measured in dorsal tail muscle of five crayfish species collected in four aquatic habitats (pond, artesian spring, stream and cave).

2. Phosphoadenylates and adenylate energy charge (AEC = (ATP + ½ADP)/(ATP + ADP + AMP)) were not significantly different ($P > 0.05$) in crayfish tail muscle from species collected in the different aquatic habitats.

3. Concentrations of tail muscle ATP appear to be associated with behavioral activity. The most active species examined, *Cambarus tenebrosus*, contained significantly greater amounts of tail muscle ATP ($P < 0.05$) than did the least active form, *Orconectes australis australis*.

INTRODUCTION

The adaptation of organisms to various habitats can involve adjustments in behavior, physiology and biochemistry (Hochachka & Somero, 1973). The array and intensity of environmental factors present within a particular habitat will influence the type and importance of these adaptations.

Freshwater crayfish (Decapoda: Astacidae) represent an invertebrate group which exhibits adaptation to a variety of distinct habitats. Over 284 species of crayfish in the United States reside in aquatic habitats which include rivers, streams, lakes, ponds, swamps and subterranean waters (Hobbs, 1972).

Physiological adaptations of crayfish associated with environmental conditions are numerous and range from major changes in gill chamber volume (Hobbs, 1975) to subtle adjustments in tissue metabolic rates (Dickson and Franz, 1980). Biochemical adaptations can include differences in tissue protein structures (Brown, 1981) and hemolymph amino acid content (Rogala *et al.*, 1978).

Nothing is presently known about the effects of habitat adaptation on phosphoadenylate concentrations of crayfish muscle tissue. Studies related to this subject involve an examination of ATP concentrations in marine mollusks from a number of different habitats (Wijsman, 1976; Ansell, 1977). Knowledge of phosphoadenylate variability due to habitat adaptations is important in defining the extent of possible uses of the adenylate energy charge (AEC = (ATP + ½ADP)/(ATP + ADP + AMP)) as a biochemical stress indicator. In addition to utilization under controlled laboratory conditions, the AEC has the potential for use in assessing the physiological condition of organisms in their natural environment (Dickson, 1980; Giesy *et al.*, 1981). Studies such as these could yield information on the overall physiological effects of single or multiple pollutant stressors in combination with stress associated with normal environmental fluctuations and seasonal physiological state.

To allow correct interpretation of changes in phosphoadenylate concentrations of *in situ* populations, the variability of phosphoadenylates and AEC were determined for crayfish living in different habitats. In this study, phosphoadenylate concentrations of dorsal tail muscle were determined in crayfish collected from the following four habitats; stream, pond, artesian spring and subterranean waters.

MATERIALS AND METHODS

During July and August 1978, collections of five crayfish species were made from three different aquatic habitats on the Savannah River Plant, South Carolina and a stream in Merrybranch Cave, White Co., Tennessee (Table 1). Because of the potential fragility of cave ecosystems, the specific cave habitat was chosen because of the presence of a relatively large population of crayfish, minimizing potential population disruption.

Adult, intermolt crayfish were collected in each habitat through use of baited minnow traps. In addition to collecting crayfish, dissolved oxygen, pH, temperature and current velocity were recorded.

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

Adenylates were extracted by a method described by Giesy *et al.* (1981). Between 25 and 75 mg (dry wt) of muscle tissue was ground to a fine powder in steel grinders

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Table 1. Sampling locations and environmental conditions at the time of collection for crayfish species utilized in habitat comparisons of phosphoadenylates and energy charge

Species	Habitat	Location	Dissolved[1] Oxygen (mg/l)	pH	Temperature (°C)	Current[2] Velocity (cm/sec)
Orconectes australis[3] *australis* (Rhoades)	Cave Stream	Merrybranch Cave, White Co, TN	9.3	7.7	12.0	0
Cambarus tenebrosus[4] Hay						
Cambarus latimanus (Le Conte)	Pond	Adjacent to Four Mile Creek, SRP[5]	5.7	6.2	22.0	0
Procambarus raneyi Hobbs	Stream	Tributary of Upper Three Runs, SRP	8.1	5.9	21.5	35.0
Procambarus acutus acutus (Girard)	Artesian Spring	Brinkley Well, SRP	3.3	4.3	27.0	0

[1] Dissolved oxygen determined by Winkler titration.
[2] Measured by Pygmy Water Meter.
[3] Troglobite-obligatory cave species.
[4] Troglophile-facultative cave species.
[5] Savannah River Plant, SC.

at −196°C, with 1 ml 6% (v/v) perchloric acid to inactivate enzymatic activity. Samples were thawed, centrifuged and the supernatant neutralized to pH 7.4 with K_2CO_3. Samples were then centrifuged at 40,000 *g* at 5°C. This extraction procedure resulted in recoveries of total adenylates of 95% or better.

ADP and AMP were converted to ATP by pyruvate kinase (ADP → ATP) and pyruvate kinase and myokinase (AMP → ATP) respectively, in the presence of phosphoenolpyruvate and $MgSO_4$. A modification of the luciferin–luciferase bioluminescence method (Giesy *et al.*, 1981) was used to measure ATP concentrations. Samples were assayed with a SAI model-2000 ATP photometer which was equipped with an enzyme kinetics injection attachment. Five or six replicate injections were made for each sample incubation and means of replicate injections used for calculations. Six second integrations of bioluminescence were made from the time of injections. Sample adenylate concentrations were compared to standards and reported as nM/mg, dry wt. Individual adenylate concentrations were calculated by difference. Statistical analyses of data were conducted as previously described in Giesy & Dickson (1981).

RESULTS

Ambient temperature, pH, dissolved oxygen and current velocity were quite different in each of the four habitats at the time of collection (Table 1). Although these hydrologic parameters vary considerably within each habitat, they indicate some of the inherent environmental differences between habitats.

Significant differences (ANOVA; $P < 0.01$) in ATP and total adenylate concentrations were only observed between the two species inhabiting the cave stream, *Orconectes australis australis* and *Cambarus tenebrosus* (Table 2, Fig. 1). No other significant differences in phosphoadenylate concentrations were

Table 2. Phosphoadenylate concentrations and adenylate energy charge in dorsal tail muscle of five species of crayfish collected from four different habitats. Means that do not have the same letter in their superscripts are significantly different ($\alpha = 0.05$)

Species	Habitat	Mean dry weight concentration (SD) ATP nM/mg	ADP nM/mg	AMP nM/mg	Total[1] nM/mg	AEC[2]
O. a. australis[3] $n = 13$	Cave Stream	11.11^{a} (4.37)	1.46^{a} (0.97)	0.99^{a} (1.52)	13.56^{a} (4.90)	0.87^{a} (0.12)
C. tenebrosus[4] $n = 7$		20.51^{b} (10.69)	2.74^{a} (2.43)	0.81^{a} (0.83)	24.06^{b} (11.38)	0.91^{a} (0.07)
C. latimanus $n = 11$	Pond	16.10^{ab} (5.67)	1.70^{a} (2.28)	1.25^{a} (1.24)	18.98^{ab} (5.96)	0.89^{a} (0.08)
P. raneyi $n = 12$	Stream	14.59^{ab} (5.27)	1.63^{a} (2.10)	2.50^{a} (2.71)	18.57^{ab} (5.21)	0.83^{a} (0.16)
P. a. acutus $n = 15$	Artesian Spring	14.96^{ab} (5.23)	1.66^{a} (1.82)	2.23^{a} (1.59)	18.13^{ab} (6.50)	0.85^{a} (0.05)

[1] Total = ATP + ADP + AMP.
[2] AEC = (ATP + ½ADP)/(ATP + ADP + AMP).
[3] Troglobite-obligatory cave species.
[4] Troglophile-facultative cave species.

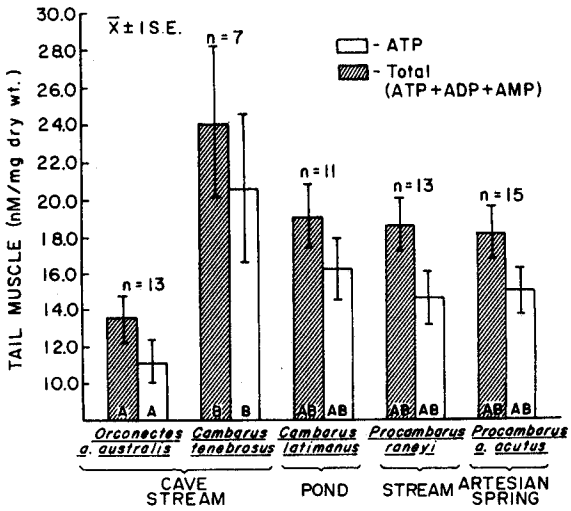

Fig. 1. Mean ATP and total adenylate concentrations of dorsal tail muscle tissue in five species of crayfish. For comparisons of ATP and total adenylate values among the five species, bars which do not have the same letter in their base are significantly different ($\alpha = 0.05$).

found among the five crayfish species. AEC ranged between 0.83 and 0.91 and did not differ significantly among any of the species examined (Table 2). No significant differences (ANOVA; $P > 0.05$) in adenylate concentrations or AEC were found between sexes in the five crayfish species examined. No significant correlations (Spearman rank, $P > 0.05$) were found between carapace length and phosphoadenylate concentrations.

DISCUSSION

Differences in physical, chemical and biological parameters associated with the four types of sampled habitats (Poulson and White, 1969; Hynes, 1970; Cole, 1975; Table 1) suggest the potential for adaptations in behavior, physiology and biochemistry of the crayfish fauna. Some of the associations between environmental conditions and these adaptations in crustaceans are reviewed by Florkin (1960), Vernberg & Vernberg (1970) and Prosser (1973).

Of the four habitats from which crayfish were collected in this study, the cave stream possessed the most unusual environmental conditions. The subterranean environment is characterized by relatively stable physical and chemical parameters. In addition to climatic constancy, caves are considered to be food-poor systems because of the lack of autotrophic production and sporadic allochthonous input. Caves can be considered to be one of the most extreme environments inhabited by crayfish (Hobbs *et al.*, 1977).

The environmental factors studied here did not appear to affect the tail muscle phosphoadenylates (ATP, ADP, AMP and total adenylates) as concentrations were similar in crayfish collected from all four areas (Table 2). The only significant differences in ATP and total adenylate concentrations were found between the two cave species (Table 2, Fig. 1). The presence of low ATP concentrations in muscle tissue of *O. a. australis*, and high values in *C. tenebrosus* are thought to result from divergences in the manner in which these species cope with the ecological properties of the subterranean environment.

Troglobitic (obligatory cave-dwelling) crayfish, such as *O. a. australis*, have evolved a number of unique adaptations which allow them to successfully exploit the cave environment. These include the loss of eye structures (Mellon, 1977) and body pigmentation (Wolfe & Cornwell, 1964), reduced metabolic rates (Jegla, 1964; Weingartner, 1977; Caine, 1978; Dickson & Franz, 1980), and the presence of K-selected life history traits (Hobbs, 1973; Cooper, 1975; Franz, 1978). In contrast, troglophilic (facultative cave-dwelling) forms, such as *C. tenebrosus* are less specialized than troglobitic species and are considered to represent more recent invasions of cave habitats. They are able to exploit the subterranean environment primarily through adaptations in behavior and life history adjustments (Weingartner, 1977).

These dissimilarities in habitat adaptations cause distinct differences in energy flow through the two species of cave-dwelling crayfish examined. In the highly specialized troglobite, the combination of lower metabolic rates and increased sensory enhancement (Cooper, 1969) enables the crayfish to expend small amounts of energy in foraging activities. This is supported by studies of two species of troglobitic crayfish (Hobb, 1973; Weingartner, 1977), which indicated that these cave forms have much smaller foraging ranges than those reported for surface species (Camougis & Hichar, 1959; Black, 1963; Hazlett *et al.*, 1974). In contrast, troglophilic crayfish do not possess major changes from surface species in sensory enhancement mechanisms or metabolic rates (Weingartner, 1977). The troglophilic form must, because of this lack of specialization, increase its foraging range and activity to secure adequate food in the depauperate cave habitat. Weingartner (1977) determined that the foraging range of the troglophilic crayfish, *Cambarus laevis*, was significantly larger in a

cave population than in individuals of the same species living in a nearby surface stream.

The direct association between the level of behavioral activity (i.e., walking, prey capture and agonistic actions) and muscle ATP concentrations apparently results from the utilization of tail musculature in these activities. Anatomical examination (Pilgrim & Wiersma, 1967) indicates that muscle groupings originating in the crayfish tail are intimately associated with the musculature of the periopods (walking legs) and pleopods (swimming legs). The researchers also determined that contraction of tail muscles was necessary in tail-body positioning during walking. In addition, greater activity should increase the number of encounters with other organisms or the same species, resulting in more frequent escape responses. Through selection pressures, ATP concentrations would be expected to be greater in more active species. Ansell (1977) found a similar situation in marine molluscs, in which the most active forms contained higher concentrations of ATP in muscle tissue than less active species. The association between behavioral (muscular) activity and crayfish tail muscle ATP is also supported by evidence that the peak breeding month (i.e., greatest agonistic behavior) corresponded to the highest level of tail muscle ATP during the year examined (Dickson & Giesy, 1981).

The AEC values, ranging from 0.83 to 0.91, were quite similar in the five species of crayfish analyzed (Table 2). These values are comparable to the normal ranges found in a variety of organisms, including bacteria, algae, macrophytes, invertebrates and vertebrates (Chapman *et al.*, 1971; Ivanovici, 1980). The lack of significant variation in crayfish muscle AEC associated with habitat adaptations should allow a more accurate evaluation of the effects of pollutants on *in situ* populations.

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

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