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Phosphoadenylate concentrations and energy charge in two freshwater crustaceans: Responses to physical and chemical stressors

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With 6 figures and 5 tables in the text

Introduction

In the past, the effects of chemical and physical alteration of the environment have been monitored by observing changes at the population or community levels of biotic organization (Gaufin 1973; Lugo 1979; Ward et al. 1979). This type of monitoring often only chronicles environmental disasters. While responses to stressors are observed at the ecosystem, community and population levels of organization, the effects of environmental stressors are manifested at the organismal level by causing death or impairing organismal function. Thus, monitoring and predicting environmental stressors at the organismal level of organization has been investigated. This has been done by toxicity tests (Eaton 1973; Sprague 1973), behavior modifications (Warner et al. 1966; Klein & Lincer 1974) and physiological changes (Dawson et al. 1977). Laboratory determined toxicity limits may not represent the actual intensity of a single stressor on an organism in its natural environment due to additive and synergistic effects of other stressors and environmental conditions.

Presently, biochemical measures appear to be the most successful in yielding recognizable indications of chronic, sublethal stress in organisms. Sublethal changes in enzyme systems in response to chronic exposure to toxicants, have been investigated (Weiss 1961; Jackim et al. 1970; Matsumura & Narahashi 1971; Yap et al. 1971; Christensen 1972; Hodson 1976; Heitz et al. 1974; Jackim 1974; Towle et al. 1976). Other potential biochemical indicators of stress include amino acid content (Jeffries 1972), hormone concentrations (Donaldson & Dye 1975; Shreck & Lory 1978) and various metabolic products (Statham et al. 1976; Christensen et al. 1977).

While biochemical measures are very sensitive to chronic exposure to many stressors, their major disadvantage is that they are often very specfiic for particular stressors. Thus, if one has not chosen the appropriate biochemical system, the probability of observing a response to a chronic stressor may be quite small. For this reason, a more general measure of stress in organisms is desirable.

Atkinson (1977) describes the adenylate energy charge [AEC = (ATP + $^1/_2$ ADP)/(ATP + ADP + AMP)] as a key ratio involved in the regulation of energy transfer through catabolic and anabolic enzyme systems. This ratio has a theoretical range of 0 to 1 but is generally between 0.8 and 0.9 in healthy organisms in a non-limiting environment (Ivanovici 1980). Values close to 1 indicate a high metabolic energy potential, while lower values reflect lower energy potential. This ratio represents the internal energy status of the cell, from which the condition of the organism can be estimated (Chapman et al. 1971; Ivanovici 1979; Ivanovici & Wiebe 1980). Since any one of the many enzyme systems associated with cellular energy transfer or increased energy costs to organisms could affect AEC the specificity of other biochemical measure is avoided. Also, the phosphoadenylate system is an integral part of the energy metabolism of all organisms from bacteria to mammals and adenosine-5'-triphosphate is the major energy source for all metabolic activities in organisms (Strumpf 1953). This study investigated the effects of cadmium on the AEC and phosphoadenylate concentrations in the freshwater crayfish *(Procambarus*

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pubescens) and the glass shrimp *(Palaeomonetes paludosis)*. Effects of sampling, acclimation and tissue type as well as relationships between adenylates were investigated in *P. plaudosis*.

Methods

Collection

Crayfish and glass shrimp were collected with dip nets from Upper Three Runs and Meyers Branch Creeks, respectively. Both of these streams were located on the U. S. Department of Energy's Savannah River Plant, near Aiken, South Carolina.

Acclimation and maintenance

Crayfish were acclimated in stream water for 7 d prior to use in experiments. Shrimp were acclimated for 3 d before use in experiments. All animals were maintained in covered 7 l, opaque, polethylene boxes (25.5 × 30.0 × 8.5 cm) which contained water from which the organisms had been captured (over approximately 2 cm of washed builders sand). Water was aerated and changed every 24 hr in shrimp experiments. Crayfish were exposed to Cd in a continuous-flow bioassay system. Crayfish were maintained in individual boxes, while several shrimp were maintained per box. Rocks were placed in pans, containing shrimp, as refugia. All animals were maintained at 20 ± 3 °C under a 12—12 hr photoperiod. Crayfish were fed mealworms and alfalfa pellets ad libitum daily. Shrimp were fed tetramin® fish food ad libitum daily.

The effects of physical exertion on the adenylate pools in shrimp were studied by chasing shrimp with a dipnet until they no longer responded to provocation with the net (approximately 5 min), after which they were sampled immediately. Absolute and relative adenylate pools in these shrimp were compared to unexerted shrimp and "killed controls" (shrimp, which had been pithed and maintained on ice for 30 min). The sample size for each treatment was five shrimp.

Exposure and sampling

Crayfish were exposed to 0, 10 and 30 μg Cd · l^{-1}, added as $CdCl_2$. Replicate organisms were sampled after 2, 5, 7 and 14 d. Shrimp were exposed to 0.0, 0.4, 10.0 and 30.0 μg Cd · l^{-1}, added as $CdCl_2$. Replicate organisms were sampled after 2, 5, 7 and 14 days. Crayfish were exposed in a continuous flow system. Shrimp were exposed in static systems with water changed daily.

Tissue for adenylate analyses was removed from the dorsal portion of the tail muscle of crayfish. Whole tails (including exoskeletons) were used in the shrimp studies of Cd exposure, physical exertion, temporal variation in the field and laboratory acclimation. In an additional study, adenylates were measured to determine relative variability in whole animals, whole tails and dissected tail muscle tissue taken above the dorsal abdominal artery. Crayfish and shrimp were placed in plastic buckets and allowed to acclimate for approximately 30 min (Dickson 1980). Samples were placed in labeled polyethylene bags and quickly frozen by flattening the tissue between two blocks of aluminum at —196 °C. The entire process of dissection and enzymatic inactivation by freezing was completed in approximately 20 sec for whole tails and 50—60 sec for tail muscle tissue. Frozen samples were kept in liquid nitrogen until the adenylates were extracted.

Adenylate extraction and assay

Adenylates were extracted by the method of Giesy et al. (1981). Between 5 and 25 mg, dry weight of tissue was ground to a fine powder in steel grinders at —196 °C with 1 ml 6 % (V/V) perchloric acid to inactivate enzymatic activity. Samples were thawed and centrifuged and the supernatant neutralized to pH 6.0—6.4 with K_2CO_3 and diluted volumetrically with 0.02 M Tris-HCl (pH = 7.4). This extraction procedure resulted in recoveries of 95 % or better.

ADP and AMP were converted to ATP by pyruvate kinase and pyruvate kinase and myokinase respectively in the presence of phosphoenolpyruvate and $MgSO_4$. ATP concen-

trations were measured by a modification of he luciferin-luciferase bioluminescence method (GIESY et al. 1981). All samples were assayed with a SAI model-2000 ATP photometer which was equipped with a kinetics injection attachment. Individual adenylate concentrations were calculated by difference. 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 injection. This procedure results in a RSD for assay of approximately 4 %. Sample adenylate concentrations were compared to standards and reported as $\mu M \cdot g^{-1}$, dry weight.

All statistical analyses were done with the SAS software package (BARR et al. 1979). Overall treatment and time effects were examined with ANOVA. Differences among individual means were tested for by either TUKEY's HSD test (STOLINGE 1976) or SCHEFFE's test (KIRK 1968) where appropriate. The relationships among adenylates were examined by generalized linear PEARSON correlations and profile analysis with WILKS' criterion (MORRISON 1967). 96-hr LC_{50} values were calculated by the method of LITCHFIELD & WILCOXON (1949). A two-way ANOVA of the response of shrimp adenylate concentrations and AEC is not appropriate because time of exposure is confounded by the fact that the Cd exposure was terminated after 16 d. Thus, comparisons among samplings within treatment were made with a one-way ANOVA and the appropriate multiple range test.

Results

Glass shrimp

Tissue types

There were no significant differences in the AEC or any of the adenylate concentrations between the whole body and whole tail in shrimp (Table 1). However, the AMP concentration was significantly greater and AEC significantly less in tail muscle, which had been dissected from the exoskeleton, than in the other two tissues (profile analysis and SCHEFFE's S-test). The decrease in ATP resulted in an increase in AMP concentration and concomitant decrease in AEC. The ATP concentrations in the dissected tail muscle tissue were not only lower but also more variable.

Table 1. Adenylate concentrations and adenylate energy charge in whole body, whole tail and dissected tail muscle tissue of *P. paludosis* $\bar{X} \pm 95\%$ CI.

	Whole body (n = 8)	Whole tail (n = 6)	Tail muscle (n = 7)
ATP[1]	6.8 ±1.8[a]	7.8 ±3.8[a]	5.1 ±6.1[a]
ADP[1]	3.2 ±1.3[a]	3.1 ±2.8[a]	2.3 ±1.4[a]
AMP[1]	0.6 ±0.5[a]	0.4 ±0.4[a]	3.5 ±2.5
Total[2]	10.6 ±1.8[a]	11.2 ±6.1[a]	10.9 ±6.8[a]
AEC[3]	0.79 ±0.07[a]	0.85 ±0.06[a]	0.45 ±0.20

[1] $\mu M \cdot g^{-1}$, dry weight

[2] Total = (ATP + ADP + AMP)

[3] $AEC = \frac{(ATP + \frac{1}{2} ADP)}{(ATP + ADP + ADP)}$

[a] Means within the same adenylate group with this superscript are not significantly different from one another (SCHEFFE's S-test, $\alpha = 0.05$).

Field variation and laboratory acclimation

In general the laboratory acclimated shrimp had lower adenylate concentrations than shrimp sampled in the field (Table 2). Both the ATP and total adenylate con-

Table 2. Adenylate concentrations and AEC in whole tails of *P. paludosis* sampled in the field and acclimated to laboratory conditions for three days. $\bar{X} \pm 90\%$ CI.

	Field collected		Laboratory acclimated
	1 Feb., 1978 (n = 8)	6 March, 1979 (n = 10)	(n = 6)
ATP[1]	23.4 ± 9.8	12.2 ± 1.9[a]	7.8 ± 3.1[a]
ADP[1]	5.4 ± 4.5[a]	5.2 ± 1.3[a]	3.1 ± 2.4[a]
AMP[1]	0.9 ± 1.7[a]	1.5 ± 0.9[a]	0.4 ± 0.3[a]
Total[1,2]	29.6 ± 11.9	18.9 ± 2.0[a]	11.2 ± 5.1[a]
AEC[3]	0.84 ± 0.10[a]	0.79 ± 0.05[a]	0.85 ± 0.05[a]

[1] $\mu M \cdot g^{-1}$, dry weight
[2] Total = (ATP + ADP + AMP)
[3] $AEC = \frac{(ATP + \frac{1}{2}ADP)}{(ATP + ADP + AMP)}$
[a] Means within the same adenylate with this superscript are not significantly different from one another, SCHEFFE's S-test, $\alpha = 0.10$.

centrations were significantly lower in laboratory acclimated shrimp. The ATP concentrations in shrimp tails collected on 6 March 1978 were significantly lower than the ATP concentrations in tails collected from animals on 1 February 1978. Coefficients of variation were also lower at the March sampling. The 7 March sample was collected the day after an intense storm event which drastically decreased the samplable population at the collection site. The AEC was not significantly different among the laboratory acclimated or field collected animals.

Physical exertion and death

When shrimp were physically exerted until they did not respond to tactile stimulation, the concentrations of ATP and total adenylate decreased significantly relative to laboratory acclimated shrimp (Table 3). The ADP concentration decreased by a factor of two but this decrease was not significant. The AEC also decreased in the tails of physically exerted animals but not significantly so. Contrary to the dis-

Table 3. Adenylate concentrations and AEC in whole tails of *P. paludosis* sampled after being laboratory acclimated, physically exerted and killed and held on ice. $\bar{X} \pm 90\%$ CI.

	Laboratory acclimated (n = 6)	Physically exerted (n = 5)	Killed controls (n = 5)
ATP[1]	7.8 ± 3.1	3.0 ± 1.0[a]	3.0 ± 0.7[a]
ADP[1]	3.1 ± 2.4[a]	1.4 ± 0.5[a]	0.5 ± 0.3[a]
AMP[1]	0.4 ± 0.3[a]	0.4 ± 0.3[a]	1.1 ± 0.2
Total[1,2]	11.2 ± 5.1	4.7 ± 1.3[a]	4.4 ± 1.0[a]
AEC[3]	0.9 ± 0.05[a]	0.8 ± 0.08[ab]	0.7 ± 0.04[b]

[1] $\mu M \cdot g^{-1}$, dry weight
[2] Total = (ATP + ADP + AMP)
[3] AEC = (ATP + ½ADP)/(ATP + ADP + AMP).
[a] Means within the same adenylate with the same superscript (a, b) are not significantly different from one another, SCHEFFE's S-test, $\alpha = 0.10$.

section of tail tissue, physical exertion and killing by pithing resulted in decreases in ATP concentrations without concomitant increases in AMP.

When shrimp were killed and placed on ice for 0.5 hr, the ATP concentration was significantly lower than in laboratory acclimated shrimp and the AMP concentration was significantly greater. However, after the animals had been dead for 0.5 hr the AEC, while significantly lower than in laboratory acclimated shrimp, decreased only to 0.7 (Table 3). The concentrations of ATP, ADP, total adenylates and the AEC were not significantly different between the physically exerted and killed shrimp.

Cadmium effects on shrimp

The 96-hr LC_{50} for *P. paludosis* in Meyers Branch stream water at 21 °C was 68 ± 10.4 μg Cd · l^{-1} (± 95 % CI) with a slope parameter of 2.06 ± 0.96.

Placing the shrimp in the experimental containers caused great fluctuations in the adenylate concentrations in the tail (Figs. 1—5). The general trend was an immediate decrease in ATP concentration (Fig. 1) and AEC (Fig. 5) and concomitant

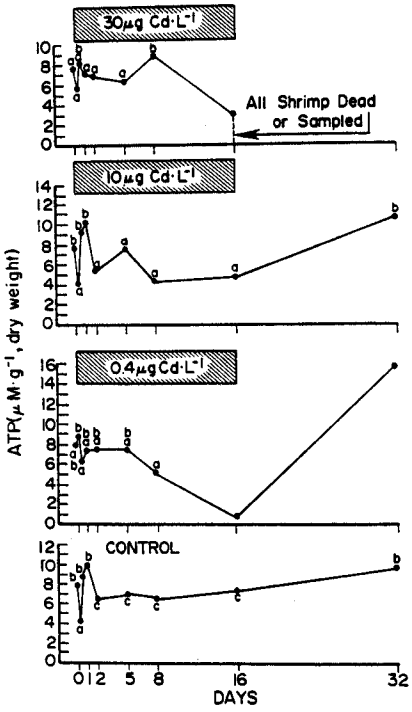

Fig. 1. ATP concentration in *P. paludosis* whole tail. Hatched area represents period of Cd exposure and Cd concentration. Each point represents the mean of 2 replications. Points denoted by the same letter, within treatment, are not significantly different from one another, TUKEY's HSD-Test, $\alpha = 0.05$.

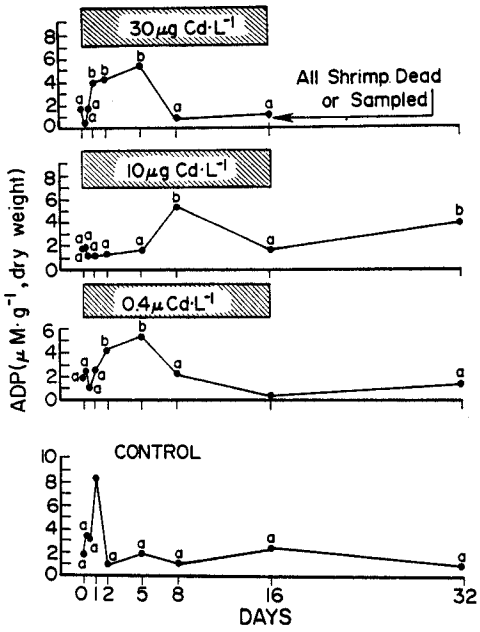

Fig. 2. ADP concentrations in *P. Paludosis* whole tail. Hatched area represents period of Cd exposure and Cd concentration. Each point represents the mean of two replications. Points denoted by the same letter within treatment are not significantly different from one another, TUKEY's HSD-Test, $\alpha = 0.05$.

increase in AMP concentration (Fig. 3). During the first two days the adenylate concentration and AEC fluctuated in the tails of controls animals, as well as those exposed to Cd. Between days 2 and 16 the ATP concentration was significantly lower than the initial concentrations in acclimated shrimp. By day 32 the ATP concentrations were not significantly different from those in laboratory-acclimated shrimp. After initial fluctuations, the concentrations of ADP, AMP, AEC and total adenylate concentration remained constant throughout the experiment in the shrimp, which were not exposed to Cd (Figs. 2—5). Exposure to Cd caused significant decreases in ATP and total adenylate concentrations during the period of Cd

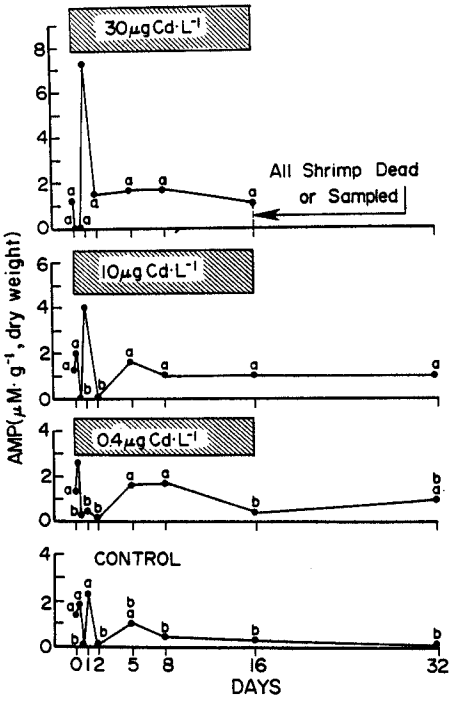

Fig. 3. AMP concentrations on *P. paludosis* whole tail. Hatched area represents period of Cd exposure and Cd concentration. Each point represents the mean of two replications. Points denoted by the same letter within treatment are not significantly different from one another, TUKEY's HSD-Test, $\alpha = 0.05$.

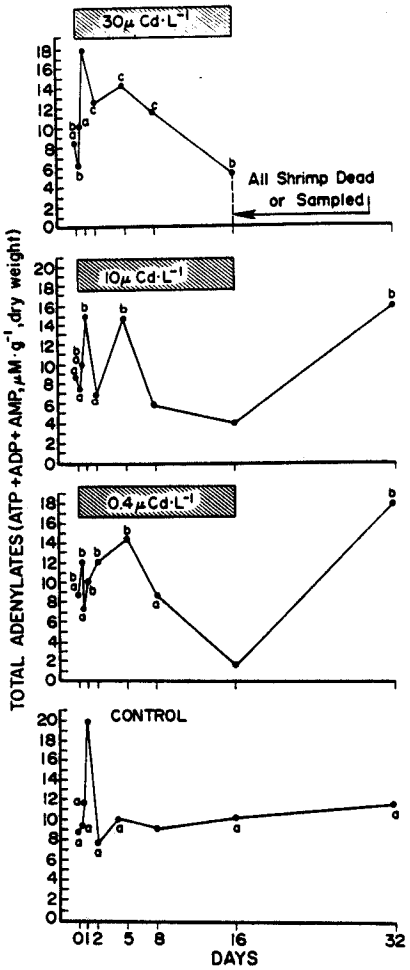

Fig. 4. Total adenylate concentration in *P. paludosis* whole tail. Hatched area represents period of Cd exposure and Cd concentration. Each point represents the mean of two replications. Points denoted by the same letter within treatment are not significantly different from one another, TUKY's HSD-Test, $\alpha = 0.05$.

exposure (Figs. 1 and 4). In all cases, these decreases were preceded by an initial increase in adenylate concentration. The AEC was also significantly lower in shrimp exposed to Cd. However, the amount of decrease was small (Fig. 5).

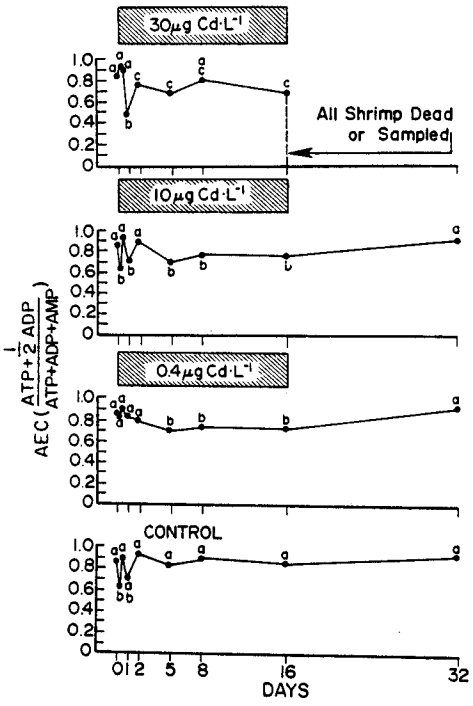

Fig. 5. Adenylate energy charge (AEC) in *P. paludosis* whole tail. Hatched area represents period of Cd exposure and Cd concentration. Each point represents the mean of two replications. Points denoted by the same letter, within treatment, are not significantly different from one another, TUKEY's HSD-Test, $\alpha = 0.05$.

Cadmium exposures were terminated on day 16 of the experiment to see if the effects observed during exposure to Cd were reversible. The concentrations of ATP, ADP and the total adenylate pool increased significantly between day 16, when exposure to 0.4 and 10 μg Cd · l^{-1} was terminated and day 32 when the experiment was completed. The AEC also increased so that there was no significant difference between shrimp sampled at day 32 and the laboratory acclimated animals at the beginning of the experiment or control shrimp which had been held in experimental chambers for 32 d.

When exposed to 30 μg Cd · l^{-1}, which is approximately half of the 96-hr LC_{50}, all of the shrimp were dead or sampled after 16 d. Death at 16 d was preceded by a significant increase in ATP and slight increase in AEC in shrimp sampled on day 8. While Cd exposure caused significant changes in ATP concentrations, the concentrations of ADP and AMP varied less. ADP concentrations were significantly decreased by exposure to Cd but were more variable than ATP concentrations. Most of the decrease in total adenylate concentrations was caused by decreases in ATP concentrations. Decreases in ATP and ADP concentrations did not result in concomitant increases in AMP concentrations. Thus, ATP and ADP were not simply degraded to AMP. When shrimp were exposed to Cd, ATP exhibited significant positive correlations with ADP, and total adenylate concentrations as well as AEC

but ATP was not significantly correlated with AMP concentration (Table 4). The total adenylate concentration was positively and significantly correlated with all three individual adenylates. AEC was positively correlated with ATP, negatively correlated with ADP and AMP and uncorrelated with the total adenylate concentration.

Table 4. PEARSON pair-wise correlations between adenylate concentrations and adenylate energy charge in *P. paludosis* cadmium exposure experiment (n = 60, R with significance, P <, in parentheses).

	ADP	AMP	Total adenylates	Energy charge
ATP	0.24 (*)	0.11 (NS)	0.85 (****)	0.29 (*)
ADP		0.32 (**)	0.65 (****)	−0.42 (***)
AMP			0.49 (****)	−0.75 (***)
Total adenylates				−0.16 (NS)

P<0.0001 = ****; P<0.001 = ***; P<0.01 = **; P<0.05 = *; P>0.05 = NS.

Crayfish

Field adenylate concentrations

The adenylate concentrations and adenylate energy charge of *Procambarus pubescens* measured in the field are reported in Table 5. When acclimated to laboratory conditions the AEC remained at 0.82. However, when crayfish were transfered to the treatment containers, the AEC of the control crayfish increased to over 0.90 on day 2, before returning to 0.82, where it remained for the duration of the experiment (Fig. 6).

Table 5. Adenylate concentrations and adenylate energy charge of the crayfish *Procambarus pubescens* tail muscle tissue determined in the field ($\bar{X} \pm 95\%$ CI, n = 6, adenylate concentrations as $\mu M \cdot g^{-1}$).

ATP	ADP	AMP	Total	AEC
74.4 ± 3.0	21.0 ± 5.4	8.5 ± 4.0	103.4 ± 18.5	0.82 ± 0.07

Cadmium effects

When freshwater crayfish *(Procambarus pubescens)* were exposed to 10 and 30 μg Cd · l^{-1} the AEC was significantly lower, relative to control organisms after 2, 7 and 14 d exposure but not after 5 d (Fig. 6). There were no significant differences between the AEC of animals exposed to the two Cd concentrations after 7 or 14 d.

Discussion

Organisms existing in their environments are subjected to a variety of natural fluctuations as well as human caused factors. Many natural phenomena can be

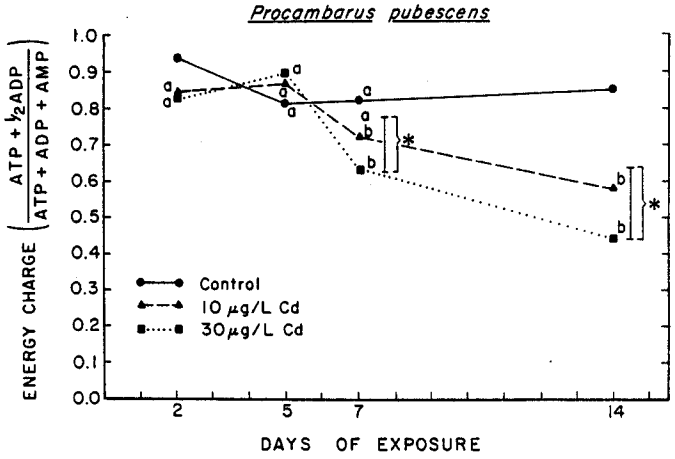

Fig. 6. Adenylate energy charge (AEC) in dorsal tail muscle of the freshwater crayfish *(Procambarus pubescens)* exposed to Cd. Each point represents the mean of two replications. The length of the lines (✱) represents the values of the TUKEY's HSD critical value ($\alpha = 0.05$) on days 7 and 14. Points denoted by the same letter, within sampling are not significantly different from one another, TUKEY's HSD-test, $\alpha = 0.05$.

stressful to aquatic organisms and these organisms are often exposed to a number of chemical and physical stressors concurrently. Through biological and medical research, it has been found that various forms of stress (e. g., disease, injury or exposure to pollutants) act in a similar manner by eliciting physiological defense reponses (SELYE 1976). Because of the metabolic activity associated with these defenses, increased energy utilization occurs when an organism is subjected to a stressor. One would expect a decrease in ATP (adenosine triphosphate), which is the primary source of biochemical energy, during this period of increased energy utilization. Such a decrease in ATP concentration occurs in the cladoceran *Daphnia pulex* (BUIKEMA et al. 1979) and the copepod *Calanus finmarchicus* (BALCH 1972) when these organisms are subjected to a stressor. However, examination of only ATP concentrations to evaluate stressor effects is complicated by the extensive natural variability of ATP concentrations associated with season, life stage and activity level (SKJOLDAL & BAMSTEDT 1976; BAMSTEDT & SKJOLDAL 1976; GIESY 1980).

The adenylate energy charge [AEC = (ATP + $^1/_2$ ADP)/(ATP + ADP + AMP); ATP = adenosine triphosphate, ADP = adenosine diphosphate, AMP = adenosine monophosphate], which represents the amount of available energy that is stored in the adenylate system, has been proposed as a biochemical measure of environmental stress (BALL & ATKINSON 1975; WIEBE & BANCROFT 1975; IVANOVICI 1979). The numerical value of the energy charge ranges, theoretically, from zero to one. Values close to one indicate a high metabolic energy potential, while lower values indicate decreased energy potential. The AEC possesses four significant properties which allow the detection of sublethal stress in organisms, as well as indicating its severity.

1) *General reaction to stressors:* Because the AEC represents available biochemical energy, any stressor has the potential of causing a recognizable shift of this ratio due to increased energy utilization associated with physiological defense me-

chanisms. As indicated by previous studies (CHAPMAN et al. 1971; IVANOVICI 1979), the point at which this adenylate ratio deflection occurs can vary depending on the organism tested and the type and severity of the stressor.

2) *Universality:* The ATP-phosphoadenylate system is an integral part of the energy metabolism of all living organisms, from bacteria to mammals. These molecules can be isolated from both prokaryotic and eukaryotic cells (CHAPMAN et al. 1971).
3) *Integrated view of metabolism:* AEC is kinetically important in the enzyme regulation of catabolic, amphibolic and anabolic sequences (ATKINSON 1968, 1969, 1971, 1977; EBBERINK et al. 1976; COFFEE & SOLANO 1977). AEC values close to one cause increased anabolism, while lower ratios induce increased catabolism. It has been suggested that the AEC represents a highly integrated view of the energy processes of metabolism (ATKINSON 1977).
4) *Values associated with physiological state:* Under optimal conditions the AEC of actively metabolizing cells and tissues of organisms from bacteria to mammals range between 0.75 and 0.99 (CHAPMAN et al. 1971; ATKINSON 1977). This stabilized upper range is associated with normal metabolic homeostasis (ATKINSON 1971). while lower values, in the range of 0.55 to 0.75, indicate stress conditions, and below 0.50 death normally occurs (CHAPMAN et al. 1971; MONTAGUE & DAWES 1974; BEIS & NEWSHOLME 1975; HOLMSEN & ROBKIN 1977; WALKER-SIMMONS & ATKINSON 1977). Inactive but viable life stages such as seeds and spores, however, exhibit lower energy charges than those of active systems (CHING & CHING 1972; BALL & ATKINSON 1975).

The important advantage of energy charge measurements is that they consistently appear to correspond well to biochemical condition in many organisms studied (WALKER-SIMMONS & ATKINSON 1977; CHING & CHING 1972; MONTAGUE & DAWES 1974; WIEBE & BANCROFT 1975; CHAPMAN et al., 1971). For example, energy charge values of *E. coli* cells subjected to nutritional stress can be maintained at 0.80 indefinitely, provided there is continuous slow addition of glucose. Once the culture is starved for glucose, there is a 60 % drop in energy charge accompanied by decreases in functional capacities and finally by cell death (WALKER-SIMMONS & ATKINSON 1977). FALKOWSKI (1977) observed a decrease in the energy charge of the marine diatom *Skeletonema costatum* (GREV.) CLEVE, due to starvation by phosphorus limitation. By extending the relationship of energy charge and condition in organisms further, one may hypothesize that organisms in any phase of a stressed condition have energy charge values different than those of animals in an unstressed condition. While energy charge may be influenced by a large number of interconnected enzymatic reactions and may not have a theoretical basis as a measure of stress for any particular enzyme system, we were seeking an operational measure which is easily quantified and could be related to stressors.

Investigations specifically designed to evaluate the AEC as a sublethal stress indicator in macroorganisms have only recently been initiated. In addition to studies of the effect of natural stressors and environmental conditions reviewed by IVANOVICI (1980) and GIESY et al. (1980), very recent work has been conducted on stressors associated with man's activities, such as cadmium (DICKSON 1980; GIESY et al. 1980), hydrocarbons (IVANOVICI 1977), toluene (SKJOLDAL & BAKKE 1978), and rapid temperature change (KUNNEMANN & BASHAMOHIDEEN 1976).

(ENGLE et al. (1975) found that osmotic stress also caused a decrease in the ATP content of blue crab gills. The results of these studies indicate that adenylate concentrations and the adenylate energy charge has the potential to indicate stress in metazoans.

BEIS & NEWSHOLME (1975) reported adenylate concentrations for abdominal muscle of the lobster *Homarus vulgaris*. When their values were corrected to a dry weight basis with a conversion factor (2.5 dry to fresh tissue weight ratio) the adenylate concentrations were as follows: ATP = 17.4; ADP = 1.4; AMP = 0.05 $\mu M \cdot g^{-1}$, dry weight. This results in an AEC value of 0.96. This AEC is much greater than any values calculated in our study. The total adenylate concentration reported for *H. vulgaris* is much lower than in the shrimp studied in the field but similar to those in laboratory acclimated shrimp.

Energy charge can remain constant while ATP concentrations decrease greatly. This is thought to be due to an energy charge regulated reduction of the total adenylate pool by degradation of AMP. SKJOLDAL & BAKKE (1978) found that stressor induced decreases in ATP concentrations resulted in an eventual decrease in energy charge in the marine isopod *Cirolana borealis* and that energy charge stabilization through the reduction of the total adenylate concentration as ATP decreased was of little significance for the survival of the isopods. In our studies of shrimp we found that chronic exposure of Cd caused a decrease in ATP and total adenylate concentrations without a concomitant increase in AMP concentration. However, the short-term physical exertion experiments resulted in an increase in the AMP concentration in tail muscle associated with a decrease in ATP, these results indicate that ATP is utilized rapidly but that AMP is not rapidly decomposed to maintain a constant AEC as is the case in the chronic Cd exposure experiment. ATKINSON (1977) states that cellular metabolism and catabolism will be adjusted to maintain a constant AEC. The fact that AMP is not rapidly degraded during the rapid physical exertion, which is a situation which is within the normal scope of activity of *P. paludosis*, indicates that AMP may be accumulated to form a pool from which ADP and ATP can be resynthesized.

Muscle tissue was selected for phosphoadenylate analyses because of the (1) relatively homogeneous tissue composition in crustacean tail muscle (PILGRIM & WIERSMA 1967), (2) lack of major pools of ATP-degrading enzymes commonly associated with liver or digestive tissue (ROUCHE & BETS 1956; WIJSMAN 1976), and (3) evidence from earlier studies (IVANOVICI 1977; GIESY et al. 1980) which indicate that adenylate energy charge values of this tissue type can be affected by various external stressors. The major disadvantage of utilizing muscle tissue for an assay is the possibility of rapid changes in phosphoadenylate pools caused by muscular contraction. Earlier work indicates that muscular contractions can induce major changes in tissue phosphoadenylate concentrations, with large decreases in ATP and increases in AMP (REY 1956; SAITO et al. 1958; CARLSON & SIGER 1960; WOJCIECHOWSKA et al. 1975).

Shrimp are popular bioassay organisms (KHORRAM & KNIGHT 1977). However, VERNBERG et al. (1977) found grass shrimp *(Palaemonetes pugio)* to be too resistant a species to be a useful bioassay organism. We used the closely related *Palaemonetes paludosis* and observed significant changes in ATP concentrations and AEC at a Cd concentration as low as 0.4 μg Cd $\cdot l^{-1}$, even though the 96-hr LC_{50} value of 68 μg

$Cd \cdot l^{-1}$ indicates that this crustacean is rather resistant to acute exposures to Cd, as measured by mortality (GIESY et al. 1977). The fact that the shrimp exposed to 30 μg $Cd \cdot l^{-1}$ died supports the contention of EISLER & HENNEKEY (1977) that a 168-hr LC_{50} is not sufficient to evaluate the toxicological properties of Cd to biota.

In a study of chronic exposure of the crayfish *Cambarus latimanus* to Cd, THORP et al. (1979) observed mortalities of approximately 5 and 18 % after chronic exposures for 5 mo to 5 and 10 μg $Cd \cdot l^{-1}$, respectively. While these chronic exposures resulted in mortalities, which could not be measured by standard bioassay techniques, a mortality of 18 % could have drastic effects on population and community structures (GIESY et al. 1979). In our study we observed significant decreases in AEC due to 10 and 30 μg $Cd \cdot l^{-1}$ after only 7 d. Thus, energy charge indicated long-term chronic effects in a relatively short time, with a small number of replications. Further work is required to calibrate the relationship between decreses in AEC and chronic mortality or organismal disfunction.

Future studies should investigate the value of adenylate energy charge as a general measure of stress induced changes in cellular energy balance. The response of a variety of organisms to a variety of physical and chemical stressors, singly or in concert, must be evaluated and energy charge responses compared to more traditional measures responses to stressors, such as changes in fecundity, respiration, electrolyte balance, behavior and specific enzymatic activities and substrate concentrations.

Summary

Concentrations of ATP, ADP and AMP were measured in tissues of the freshwater crayfish *Procambarus pubescens* (FAXON) (Cambarinae, Decapoda) and glass shrimp *Palaeomonetes paludosis* (GIBBS) (Palaemonidae, Decapoda) by the luciferin-luciferase bioluminescence technique. Total adenylate concentration $\mu M \cdot g^{-1}$, dry weight and adenylate energy charge [AEC = (ATP + $^1/_2$ ADP)/(ATP + ADP + AMP)] were calculated. Effects of dissection on tissue adenylate concentrations were observed in shrimp tissue. The 96-hr LC_{50} for *P. paludosis* exposure to cadmium was 68 μg $Cd \cdot l^{-1}$ (95 % CI = 10.4). Cadmium concentrations of 0.4, 10 and 30 μg $Cd \cdot l^{-1}$ caused significant decreases in ATP and total adenylate concentrations as well as AEC in two weeks. When cadmium exposures of 0.4 and 10 μg $Cd \cdot l^{-1}$ were terminated, adenylate concentrations and AEC values returned to near normal values. All shrimp exposed to 30 μg $Cd \cdot l^{-1}$ died in 16 days. Physically exerting shrimp until they would no longer respond caused significant decreases in ATP and total adenylate concentrations but did not affect the AEC. Field monitoring of *P. paludosis* revealed a significant decrease in AEC after an intense storm event, which eliminated much of the population. The AEC of *P. pubescens* was reduced significantly after 7 days exposure to 10 and 30 μg $Cd \cdot l^{-1}$, with greater decreases observed when exposed to 30 μg $Cd \cdot l^{-1}$.

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