Oecologia (Berl) (1981) 49:1–7

723

Oecologia

The Effect of Season and Location on Phosphoadenylate Concentrations and Adenylate Energy Charge in Two Species of Freshwater Clams

John P. Giesy[1] and Gary W. Dickson[2]
Savannah River Ecology Laboratory, University of Georgia, P.O. Drawer E, Aiken, South Carolina 29801, USA

Summary. Concentrations of phosphoadenylate nucleotides and the adenylate energy charge ((ATP+1/2ADP)/(ATP+ADP+AMP)) have been suggested as sensitive integrating measures of the energy state of organisms. This synoptic study investigated the seasonal and spatial variation of phosphoadenylate concentrations and AEC in two freshwater bivalve molluscs, the papershell clam, *Anodonta imbecillis* and the asian clam, *Corbicula fluminea*. Concentrations of all three adenylates, as well as the total adenylate concentration and adenylate energy charge of both species varied seasonally. These fluctuations were closely related to reproductive periods in both species. Total adenylate concentrations and ATP concentrations were slightly negatively correlated with shell length in *A. imbecillis* but the ADP and AMP concentrations and AEC were not significantly correlated with shell length. In *C. fluminea* the AEC was negatively correlated with shell length, while all of the adenylate concentrations were positively correlated with shell length. Neither species exhibited significant differences in AEC between two collection locations. When *C. fluminea* collected from the Savannah River were acclimated and fed in the laboratory their AEC increased significantly.

Introduction

The adenylate energy charge (AEC=(ATP+½ADP)/(ATP+ADP+AMP)) is a measure of the metabolic energy available to an organism (Atkinson 1977) and has been shown to vary in response to natural and man-made environmental changes (Ivanovici 1979). 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). 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 and Wiebe 1980). Values close to 1 indicate a high metabolic energy potential, while lower values reflect lower energy potential. Many natural phenomena can be stressful to aquatic organisms and 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 responses (Selye 1976). Because of the metabolic activity associated with these defenses, increased energy utilization occurs when an organism is subjected to a stress. One would expect a decrease in ATP (adenosine triphosphate), which is the primary source of biochemical energy, during this period of increased energy utilization (Giesy et al. 1981b). 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. The adenylate concentrations and AEC values have been catalogued for a number of organisms and the significance of the values and their relationship to environmental conditions discussed (Ivanovici 1979, 1980; Ivanovici and Wiebe 1980; Giesy et al. 1981b). Rainer et al. (1979) observed significant decreases in energy charge due to reduced salinity in three estuarine molluscs. 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. Giesy et al. (1981a, 1981b) have observed changes in adenylate concentrations and adenylate energy charge due to chronic exposures of crustaceans and mollusks to cadmium under laboratory conditions.

Little is known about the seasonal variation in adenylate concentrations or AEC. Skjoldal and Bamstedt (1976) observed seasonal differences in the adenylate concentrations of *Meganyctiphanes norvegica* and Bamstedt and Skjoldal (1976) found that season, sex and developmental stage influenced adenylate concentrations in the marine zooplankton (*Euchaeta norvegica*). Concentrations of ATP, ADP, AMP and total adenylate concentrations in dorsal tail muscle of the freshwater crayfish (*Procambarus acutus acutus* (Girard)) exhibited significant seasonal variation in adenylate concentrations or AEC in *P. acutus acutus* (Girard) but these differences could not be attributed to environmental conditions (temperature, dissolved oxygen or pH), sex or limb regeneration (Dickson 1980). Seasonal peaks of ATP concentrations in dorsal tail muscle coincide with the breeding period of these crayfish and could reflect greater energy production associated with increased activity involved in mating and agonistic encounters.

A number of factors may or may not affect adenylate concentrations or AEC. Thus, these parameters must be considered as the natural background that any monitoring program must be conducted within. If the adenylate system is to be a useful tool for monitoring multiple chronic stressors an understanding of seasonal variation is essential. This study investigated the

1 Present address: Pesticide Research Center and Department of Fisheries and Wildlife, Michigan State University, E. Lansing Michigan 48824, USA
2 Present address: Office of Toxic Substances Health and Environmental Review Division, U.S. Environmental Protection Agency TS-792, M-Street, S.W., Washington, D.C., 20460, USA

0029-8549/81/0049/0001/$01.40

seasonal and size related variability of ATP, ADP, AMP and total adenylate concentrations as well as that of AEC in the papershell clam (*Anodonta imbecillis*) and Asian clam (*Corbicula fluminea*).

Materials and Method

Papershell clams (*Anodonta imbecillis* Say, Unioninae) were collected, by SCUBA, from the thermally enriched portion of Par Pond, an impoundment on the U. S. Department of Energy's Savannah River Plant, near Aiken, South Carolina. Asian clams (*Corbicula fluminea;* Müller (previously *C. manilensis*, Philippi), Corbiculidae) (Britton and Morton 1979), were collected by SCUBA from the Savannah River, adjacent to the Savannah River Plant. Samples of both species represented all size classes so that size effects could be evaluated and segregated statistically.

C. fluminea collected in the Savannah River in October when the Savannah River was 15° C were held in aerated tanks, containing river water over washed sand. The tanks were kept at 21° C under a 12–12 h light-dark cycle and fed Gordon's clam food formula (Innes 1966) to determine the effect of holding clams where they were not exposed to potential toxicants and were supplied with optimum food under constant environmental conditions.

Clams were opened at the collection site and a sample of foot muscle tissue removed. Samples were placed in labeled polyethylene bags and quickly frozen by flattening the tissue between two blocks of aluminum at liquid nitrogen temperature (−196° C). The entire process of dissection and enzymatic inactivation by freezing was completed in approximately 10 sec. Samples were stored in liquid nitrogen until they were extracted.

Adenylates were extracted by a method described by Giesy et al. (1981b). Between 5 and 25 mg, dry weight, of tissue was ground to a fine power in steel grinders at −196° C, with 1 ml 6% (V/V) perchloric acid to inactivate enzymatic activity. Samples were thawed, centrifuged and the supernatent neutralized to pH 7.4 with K_2CO_3. Samples were 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 → ADP) respectively in the presence of phosphoenolpyruvate and $MgSO_4$. ATP concentrations were measured by a modification of the luciferin-luciferase bioluminescence method (Giesy et al. 1981b). 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 bioluminescense were made from the time of injection. Sample adenylate concentrations were compared to standards and reported as $\mu M \cdot g^{-1}$, dry weight (Giesy et al. 1981b).

Table 1. Mean shell length of *A. imbecillis* used for adenylate analyses

Month	*n*	$\bar{X}$	S.D.
September[a]	11	6.45	0.42
November	9	6.41	0.53
December	10	6.07	0.21
January[b]	8	6.45	0.20
March	12	6.70	0.24
April	12	6.83	0.19
May	10	7.15	0.35
June	12	6.91	0.47
July	12	6.90	0.36
August	12	6.51	0.24
September	12	7.18	0.30
October	12	6.54	1.31

[a] 1977
[b] 1978

All statistical analyses were done with the SAS software package (Barr et al. 1979). Overall seasonal effects were examined with a one-way ANOVA with time as the independent variable. Tukeys' HSD test (Stolinge 1978) was used to test for significant differences among individual means. The relationships among adenylate concentrations and between adenylates and size were examined by analyses of covariance, generalized linear correlations and Spearman rank-correlation analysis. The relationships between adenylate concentrations, AEC and shell length were examined with the Spearman rank correlation analysis because this procedure is less sensitive to singularities than Pearson correlations. Because the AEC is a ratio, values were transformed by taking the arcsine before correlations were calculated, however, there was no difference in correlations or ANOVA's calculated with transformed and non-transformed data. The relationship among individual adenylate concentrations and total adenylate concentrations was examined by profile analysis with Wilks' criterion (Morrison 1967).

Results

A. imbecillis

The size range of *A. imbecillis* collected from Par Pond (Table 1) did not change during the study, which indicates that most of the organisms were not rapidly growing and that there were no massive mortalities or recruitments to the population. The clams, which we collected, had visible marsupia (developing eggs in gills) during the months of April, May and July (Fig. 1).

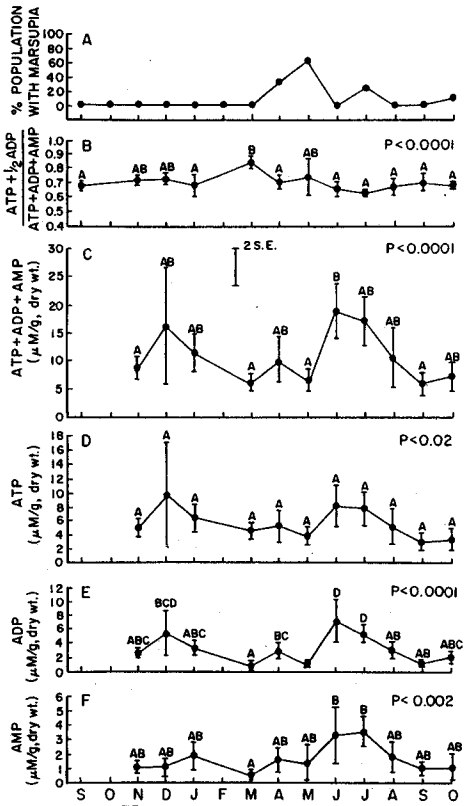

Fig. 1. *A. imbecillis*: **A)** percent of sampled population with visible marsupia, **B)** adenylate energy charge ((ATP+½ADP)/(ATP+ADP+AMP)), **C)** total adenylate concentration, **D)** ATP concentration, **E)** ADP concentration, **F)** AMP concentration. Each point represents the mean of 12 individuals. Confidence intervals are ±2 S.E. The probabilities of the F statistic for the one-way ANOVA test among months are given for each variable. Means which are not significantly different from one another (Tukey's HSD test, Stolinge 1978) are denoted by the same letter. Concentrations are expressed as $\mu M \cdot g^{-1}$, dry weight

Table 2. Spearman rank correlations (R) between adenylate concentrations, energy charge and shell length of the papershell clam *A. imbecillis*. $n=121$

	Adenylate energy charge	Total adenylates	AMP	ADP	ATP
Shell length	0.019; NS	−0.16[e]	−0.056; NS	−0.14; NS	−0.17[d]
ATP	0.082; NS	0.93[a]	0.46[a]	0.71[a]	
ADP	−0.33[b]	0.88[a]	0.48[a]		
AMP	−0.70[a]	0.633[a]			
Total adenylates	−0.22[c]				

[a] $p<0.0001$
[b] $p<0.001$
[c] $p<0.01$
[d] $p<0.05$
[e] $p<0.10$
NS = $P>0.10$, not significant

Table 3. Mean shell length of *C. fluminea* used for adenylate analyses

Month	n	$\bar{X}$	S.D.
December[a]	14	2.39	0.30
January[b]	15	2.15	0.28
February	24	2.01	0.44
March	24	2.01	0.40
April	24	1.95	0.46
May	23	2.39	0.24
June	24	2.23	0.45
July	24	2.27	0.44
August	24	2.34	0.34
September	24	2.20	0.44
October	24	2.32	0.46

[a] 1977
[b] 1978

The maximum percentage of collected organisms with marsupia was 70%, which occurred in May.

The one-way ANOVA of among month variation was significant for all parameters: ATP, ADP, AMP, total adenylate concentrations and adenylate energy charge (AEC) (Fig. 1). Within and among month variability was less for AEC than for ATP, ADP, AMP or total adenylate concentrations. The seasonal trends for ATP, ADP, AMP and total adenylates were all similar with maxima in December–January and June–July. Minimum concentrations of ATP, ADP, and AMP as well as total adenylate concentrations were observed in May when the highest proportion of the population had marsupia present. The concentrations of all four adenylates increased between the May and June samplings during which time the proportion of the population with marsupia decreased. After reaching a maximum in the June sample, concentrations of all three adenylates decreased to the minimum values observed in September. The relative standard deviation (RSD) was greatest at the times of maximum concentration and at times of maximum change.

The adenylate energy charge was much less variable than were the adenylate concentrations (Fig. 1). The maximum energy charge value of 0.83 was observed in March, just prior to the onset of marsupia formation (Fig. 1). The energy charge then decreased during the reproductive period of April and May but did not decrease below values observed earlier in the year. Concentrations of ATP as well as total adenylate concentrations in *A. imbecillis* foot muscle tissue exhibited slight negative correlations with shell lenght (Table 2). ATP was not correlated with the AEC but positively correlated with ADP, AMP and the total adenylate concentrations. ADP, AMP and total adenylate concentrations were all negatively correlated with the AEC.

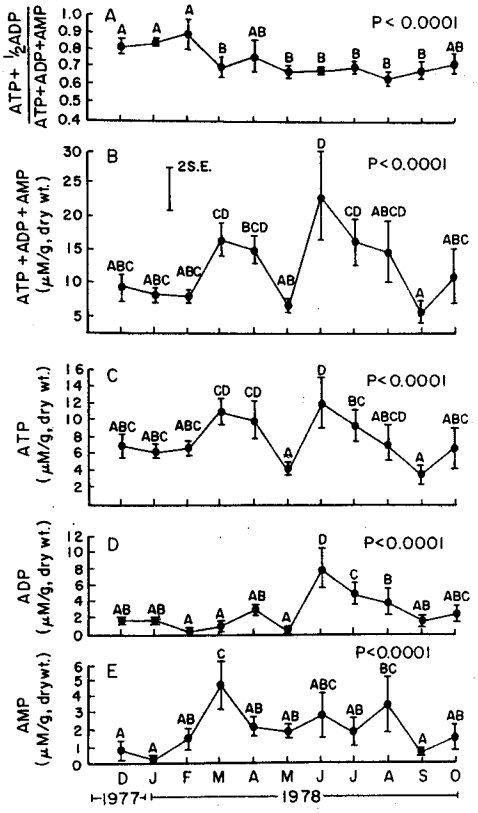

Fig. 2. *C. fluminea* adenylates: **A)** adenylate energy charge ((ATP+½ADP)/(ATP+ADP+AMP)), **B)** total adenylate concentration, **C)** ATP concentration, **D)** ADP concentration, **E)** AMP concentration. Each point represents the mean of 24 individuals. Confidence intervals are ±2 S.E. The probabilities of the **F** statistic for the one-way ANOVA months are given for each variable. Means, which are not significantly different from one another (Tukey's HSD test, Stolinge 1978) are denoted by the same letter. Concentrations are reported as $\mu M \cdot g^{-1}$, dry weight

C. fluminea

There was no significant seasonal trend in the mean size of *C. fluminea*, which were used for adenylate analyses (Table 3). However, small clams were more numerous in spring, while more large clams were observed in late summer.

Concentrations of ATP, ADP and AMP, as well as the total adenylate concentrations increased significantly between February and March then decreased until May when the concentrations increased again (Fig. 2). Maximum concentrations of ATP and ADP as well as the maximum concentrations of the total adenylates were observed in June, after which time concentrations decreased until September. The above trends for ATP, ADP and total adenylates were not significantly different from one another (profile analysis, $\alpha=0.05$). The trends for AMP concentrations were similar to those of the other adenylates but more variable and significantly lower (profile analysis, $\alpha=0.05$) from the other adenylate concentrations.

Table 4. Spearman rank correlations (R) between adenylate concentrations, energy charge and shell length of the Asian clam, *C. fluminea*. $n=244$

	Adenylate energy charge	Total adenylates	AMP	ADP	ATP
Shell length	-0.137^{c}	0.174^{b}	0.133^{c}	0.320^{a}	0.114^{d}
ATP	0.030; NS	0.950^{a}	0.478^{a}	0.434^{a}	
ADP	-0.135^{c}	0.567^{a}	0.097; NS		
AMP	-0.712^{a}	0.570^{a}			
Total adenylates	-0.198^{a}				

[a] $p<0.0001$
[b] $p<0.01$
[c] $p<0.05$
[d] $p<0.10$
NS = $P>0.1$, not significant

Table 5. Analysis of covariance of adenylate concentrations and adenylate energy charge and shell length in *C. fluminea*. DF, Source = 1, error = 242

Dependent variable	F	Prob > F	R^2
ATP	1.54	0.2100	0.006
ADP	8.79	0.0030	0.035
AMP	0.28	0.6000	0.001
Total adenylates	3.71	0.0550	0.015
Adenylate energy charge	14.47	0.0002	0.056

Table 6. Partial Pearson correlations (R) between *C. fluminea* adenylate concentrations and energy charge with effect of length covariate removed. $n=244$

	Adenylate energy charge	Total adenylates	AMP	ADP
ATP	0.505^{b}	0.930^{a}	0.505^{a}	0.630^{a}
ADP	-0.142^{b}	0.804^{a}	0.306^{a}	
AMP	-0.533^{a}	0.671^{a}		
Total adenylates	-0.124^{c}			

[a] $p<0.0001$
[b] $p<0.01$
[c] $p<0.05$

Table 7. Spearman rank correlations (R) between *C. fluminea* shell length and adenylate energy charge by month

Month	*n*	R	Prob > R
October	9	−0.829	0.0001
November	10	−0.950	0.0001
December	14	−0.546	0.0430
January	15	−0.168	0.5500
February	24	−0.740	0.0001
March	24	+0.465	0.0220
April	24	−0.434	0.0340
May	23	−0.090	0.6850
June	24	−0.114	0.5960
July	24	+0.195	0.3620
August	24	−0.186	0.3840
September	24	−0.056	0.7930

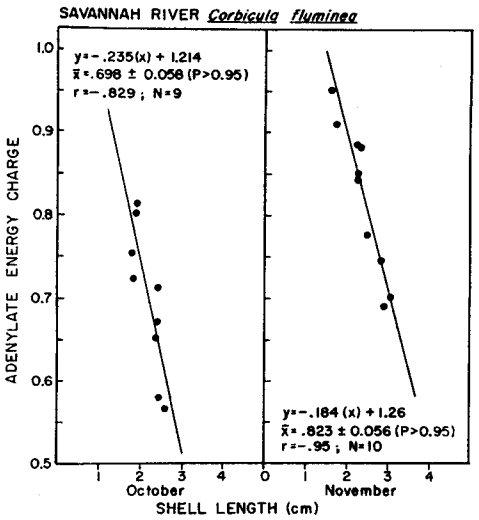

Fig. 3. Regression of AEC on shell length of *C. fluminea* from the Savannah River during October and November 1977

The adenylate energy charge was much less variable, both within and among months (Fig. 2). The greatest energy charge values were observed in the winter months of December, January and February. There was no significant difference (Tukeys' HSD, $\alpha=0.05$) among the energy charge values for March through October (Fig. 2).

Shell length was positively correlated with all adenylate concentrations but negatively correlated with AEC (Table 4). ATP concentration was not correlated with energy charge, while ADP, AMP and total adenylate concentrations were all negatively correlated with energy charge. Total adenylate concentrations were positively correlated with each of the individual adenylates as well as shell length. ADP and AMP concentrations were also positively correlated with ATP concentrations.

The results of an analysis of covariance with shell length as the independent variable, are given in Table 5. Because all of the dependent variables were significantly related to shell length, partial Pearson pairwise correlations were calculated with the effects of shell length (age) removed (Table 6). This causes two changes in the correlations observed. After removing the effects of shell length ADP and AMP concentrations are positively correlated; in the Spearman rank correlation there was no significant relationship between the two concentrations. Also, after removing the effects of shell length, ATP concentration is positively correlated with AEC, as expected.

The relationship between AEC and shell length, while highly significant, explained a relatively small amount of the variability observed, when all samples were considered together. Thus, the correlations between shell length and AEC were examined on a monthly basis (Table 7). The correlation between shell length and AEC was negative and significant in October–December and February through April (Table 7 and Fig. 3). A notable exception to this relationship was in January. There was no significant correlation between shell length and AEC during the period May through September.

The AEC of *A. imbecillis* collected from Par Pond and Clark Hill Reservoir (on the Savannah River above Augusta, Georgia) were not significantly different from one another (Table 8). Similarly, *C. fluminea* collected from the Savannah River near the Savannah River Plant and an overbank area of Clark Hill Reservoir were not significantly different (Table 8).

Table 8. Comparison of AEC of *A. imbecillis* from Par Pond Reservoir and Clark Hill Reservoir and *C. fluminea* from the Savannah River at the Savannah River Plant and Clark Hill Reservoir overbank area in October, 1977; $\bar{X} \pm 95\%$ CI, n for each sample is indicated in parenthesis

A. imbecillis[a]	
Par Pond	Clark Hill Reservoir
0.68 ± 0.03 (11)	0.72 ± 0.04 (12)
C. fluminea[a]	
Savannah River	Clark Hill Reservoir
0.76 ± 0.05 (5)	0.76 ± 0.04 (5)

[a] The AEC of individuals collected from the two locations were not significantly different from one another (Tukey's HSD, $\alpha = 0.05$)

When *C. fluminea* collected from the Savannah River in October, 1977 were taken to the laboratory and fed and maintained in aerated river water the AEC increased from 0.76 ± 0.06 to 0.95 ± 0.01 ($\bar{X} \pm 95\%$ CI, $n = 8$) after only three days acclimation in the laboratory.

Discussion

The concentrations of all adenylates as well as AEC values were similar for *A. imbecillis* and *C. fluminea*. Concentrations of all adenylates were greater than those reported for the Pacific oyster (*Crassostrea gigas*) (Wylie and Smith 1964), after correcting for the fact that their values were reported on a wet weight basis. The energy charge calculated from the data of Wylie and Smith (1964) was 0.19, which is much lower than energy charge values observed for the two bivalves studied here. This is probably due to differences in techniques since the samples of Wylie and Smith (1964) were not frozen or extracted by methods which would allow complete inactivation of enzymes which can degrade phosphoadenylates. Thus, the values reported by Wylie and Smith (1964) are probably underestimates.

The range of ATP concentrations observed in the two species studied here are in the middle of the range of values reported by Ansell (1977) for a large number of marine bivalves from different locations. The adenylate concentrations measured in our study were generally greater than those reported for the marine bivalve (*Mytilus edulis*) (Beis and Newsholme 1975). The energy charge calculated from the mean adenylate concentrations reported by Beis and Newsholme (1975) was 0.77, which is within the range of values observed for both species studied here.

Decreased energy charge at certain seasons of the year may reflect less active growth or senescence. Ansell (1977) observed no effect of size on ATP concentrations in marine bivalves. Thus, the relationship between energy charge and length in *C. fluminea* may be more a function of age than size, since these two parameters are confounded together.

We observed a bimodal frequency distribution of *C. fluminea* shell lengths similar to that of Britton et al. (1979), which indicates two age classes. If we postulate two reproductive periods per year, a population containing two year classes would be expected (Britton et al. 1979). The clams apparently live to be only 2 years old, as determined by frequency histograms and annuli. When the clams reach a shell length of approximately 2.5 cm they die. Most clams greater than 2.0 cm in length had eroded umbos and most of the relic shells had holes eroded through the umbo. In the period October–December, age class II *C. fluminea* died and the significant negative correlation between size and AEC in this period may have been due to the physiological changes concomitant with eminent death. In January, following the loss of senescent individuals from the population and prior to a new growth period, there was no significant correlation between shell length and AEC.

Because the growth rate of bivalve molluscs decreases as individuals become larger and older, one might expect to find metabolic correlations with size and age (Wilbur and Owen 1964). Specifically, the activity of carbonic anhydrase, the enzyme responsible for shell formation in bivalves is inversely proportional to size (Wilbur and Anderson 1950; Kawai 1955). The metabolic rates of the marine bivalves *Mytilus* (Zeuthen 1947), and *Teredo pedicellata* (Lane and Tierney 1951) decrease with increasing size. This trend has been reported for a number of organisms (Zeuthen 1947) and may be due to the fact that the feeding efficiency of bivalves decreases as individuals become larger (Wilbur and Owen 1964). The filtration rates and oxygen consumption of the marine softshell clam (*Mya arenaria* L.) and the blue mussel (*Mytilus edulis* L.) are inversely proportional to size (dry weight of soft tissue) (Capuzzo et al. 1977). These results both indicate a decreased metabolic activity in larger bivalve molluscs.

Corbicula apparently spawn in two major peaks, in the spring and in the fall. Heinsham (1958, as cited in Britton on Morton 1979) found a major peak in May and June. Sickel (1979) also reported two reproductive peaks in the Altamaha River, Georgia. However, the maximum density of larvae was in August. The data of Caldiron (1975, as cited in Britton and Morton 1979) showed little reproductive activity in the midsummer and midwinter months.

We observed the greatest AEC values during the midwinter period, while there was no significant difference between the spring and fall periods when reproduction could be expected. Individual adenylate concentrations, as well as total adenylate concentrations, exhibited minimum values in May and September, which could be expected to be periods of maximum reproduction.

Anodonta, as opposed to *Corbicula*, are long-term breeders (Pennak 1978). Eggs are fertilized in midsummer and carried until the next spring or summer. Energy charge increased just prior to the period of greatest egg development in April and May and decreased during the period of reproductive activity. In June, the total adenylate concentration in the clams rebounded from the minimum reached during maximum egg growth, immediately after the disappearance of visible eggs from the gills. A similar trend was observed in the individual adenylates as well.

The large increase in adenylates in *C. fluminea* between May and June was not accompanied by any change in AEC and both the within and among month variability of the AEC was much less than that of adenylate concentrations. The relative standard deviation (S.D./$\bar{X} \cdot 100\%$) was approximately 10% for the AEC, while RSD values for ATP, ADP and AMP concentrations were approximately 30, 60% and 100%, respectively.

There are several possible factors, which may contribute to the lower variability of the AEC, relative to adenylate concentrations. Atkinson (1977) argues that the AEC has a central regulatory function in cellular metabolic relationships. Furthermore, the AEC is not only regulatory but regulated and that organisms will adjust adenylate concentrations and rates of metabolism and catabolism to maintain a constant AEC. Agnew et al. (1973) reported that the AEC and total adenylate pool in *E. coli* varied in the same direction, however, the AEC was more tightly controlled. In the adenine requiring mutant P CO294 of *E. coli*,

however, when the adenylate pool dropped by 50%, the AEC dropped only from 0.88 to 0.80. Agnew et al. (1973) interpret this to mean that AEC and total adenylate pool are relatively independent.

In addition to the biochemical rationale for less variability in the AEC, there are several more practical reasons for the AEC to be less variable. First, the AEC is a pure number, not normalized to tissue weight, protein or Kjeldahl nitrogen, as are adenylate concentrations. We have observed tissue weight determinations to be much more variable than the adenylate assays. Second, because the AEC is a ratio, variation would be damped, relative to individual adenylate concentrations (Sokal and Rohlf 1969) and the fact that ATP and ADP concentrations are included in both the numerator and denominator causes further damping of the variability.

The variability was greatest for AMP concentrations. This may have biochemical significance but is probably more variable because the AMP concentrations are much lower than ATP or ADP and more incubation and assay steps are necessary to measure AMP concentrations. Thus, variability of the AMP concentrations contains that from three incubations and 3 assays. This is further supported by the fact that the RSD for ADP is twice that of ATP and that of AMP is three times as great as that of ATP. Ansell (1977) reported wide variability within tissue from a single organism as well as in the same tissue between organisms. The RSD calculated from data given by Ansell (1977) ranged from 5 to 30% for sample sizes of between 2 and 8 individuals, which is similar to the variability observed in this study.

In an extensive comparison of adenylate concentrations in tissues from a variety of organisms, Beis and Nesholme (1975) found that molluscs had relatively low tissue ATP concentrations and indicated that ATP/AMP ratios were lower in tissues where energy expenditure values were small. Tissues such as crustacean abdominal muscle which are capable of rapid energy expenditure have very high ATP/AMP ratios. We have observed that adenylate concentrations and AEC values are much more variable in crustaceans than molluscs. This is probably due to the fact that tissue can be dissected from bivalves more quickly and with less trauma to the tissue than can that of crustaceans since the potential to respond is less in molluscs. For this reason we suggest that molluscan muscle tissue will be more useful as indicator organisms of chronic environmental stressors. The Asian clam has already been suggested as a standard invertebrate for toxicity testing (Burress and Chandler 1976).

The fact that increases and decreases of individual adenylate concentrations are accompanied by similar fluctuations in total adenylate concentrations indicates that there is not simply interconversion of adenylates but synthesis and degradation of adenylates. ATP is degraded to ADP then AMP immediately upon death of the scallop (*Placopecten magellanicus*) (Hiltz and Dyer 1970). In rat cerebral cortex slices, periods of high respiration, decrease the ATP concentration without increasing ADP or AMP concentrations, which results in decreases in the total adenylate pool (Bull and O'Neill 1975). In this study AEC was not correlated with ATP in *A. imbecillis* but was positively correlated with AEC in *C. fluminea* when the effect of shell length was removed. All adenylates, including the total adenylate pool, varied together.

ADP and AMP were both negatively correlated with AEC in both species. However, the correlation between AEC and ADP concentration was less strong. The reason for the lack of correlation between AEC and ATP concentration is probably more mathematical than biochemical, since ATP appears in both the numerator and denominator of the AEC equation. The same problem applies to ADP concentration but since only half of the ADP concentration occurs in the numerator the effect is less severe.

Most of the variability in total adenylate concentration was explained by ATP variation, which indicates that for monitoring purposes ATP can be measured and reflect the same changes as the total adenylate pool. Seasonal changes in AEC as well as adenylate concentrations are probably related to reproductive state and must be considered when monitoring a population of molluscs. Size or age may also be important covariates, especially in populations with age or size mediated senescence.

We observed a significant increase in AEC after three days of acclimation in the laboratory at 21° C with aeration and feeding. These results indicate that *C. fluminea* in the Savannah River were living under suboptimal conditions. Since the clams were held in river water, which was changed every day, the increase in AEC was probably not due to release from exposure to a chemical stressor in the river water. Contaminants from the Augusta, Georgia Metropolitan area probably did not cause the difference between the AEC of clams collected in the field and acclimated in the laboratory because there was no significant difference above and below this area. The percent saturation of oxygen in the water was not different between the river and laboratory environments, thus this was probably not a factor in the increased AEC observed in the laboratory. The most probable factors influencing the AEC in *C. fluminea* were the increased temperature (from 15° C to 21° C and increased food availability. Other factors such as current and non-nutritional suspended solids may also have been factors. However, current velocity was probably not a significant variable between the laboratory and river environments, since the AEC of *C. fluminea* collected from the Savannah River, in an area with a rapid current, and those collected from an overbank area of Clark Hill Reservoir, which had essentially no current, were not significantly different. The most probable reason for the increase in AEC was nutritional augmentation.

Acknowledgments. L.A. Briese, K. Hahn and J. Inhat assisted with sample collection and analysis. J. Coleman drew the figures and Sheri Belew and Tonya Willingham typed the manuscript. An earlier version of the manuscript was reviewed by E. Liu, G.J. Leversee and J.W. Bowling. This research was supported under an interagency agreement between the U.S. Department of Energy and the University of Georgia (No. DE-AC09-76SR00819).

References

Agnew JS, Sedo RJ, Atkinson DE (1973) Relative independence of adenylate energy charge and adenylate pool level in *E. coli*. Abstract Fed Proc 32:575

Ansell AD (1977) The adenosene triphosphate content of some marine bivalve molluscs. J Exp Mar Biol Ecol 28:269–283

Atkinson DE (1977) Cellular Energy Metabolism and its Regulation. Academic Press, New York p 293

Balch N (1972) ATP content of *Calanus finmarchicus*. Limnol Oceanogr 17:906–908

Bamstedt V, Skjoldal HR (1976) Studies on the deepwater pelagic community of Korstjarden, western Norway. Adenosine phosphates and nucleic acids in *Euchaeta norwegica* (Copepod) in relation to its life cycle. Sarsia 60:63–80

Barr AJ, Goodnight JH, Sall JP (1979) SAS User's Guide. Raleigh, North Carolina: SAS Institute, Inc p 294

Beis I, Newsholme EA (1975) The contents of adenine nucleotides, phosphagens and some glycolytic intermediates in resting muscles from vertebrates and invertebrates. Biochem J 152:23–32

Britton JC, Morton B (1977) *Corbicula* in North America. The evidence reviewed and evaluated. In: JC Britton, JS Mattice, CE Murphy,

and LW Newland (eds), Proceedings, First international *Corbicula* Symposium, Texas Christian University, Fort Worth, TX, October 13–15, 1977. Texas Christian University Research Foundation. Forth Worth, TX p 249–287

Britton JC, Coldiron DR, Evans Jr, Golightly C, O'Kane DD, Teneyck JR (1979) Reevaluation of the growth pattern in *Corbicula fluminea* (Muller). In: Proceedings, First International Corbicula Symposium, Texas Christian University, Forth Worth, TX, October 13–15, 1977, JC Britton, CE Mattice, CE Murphy, and LW Newland (eds), Texas Christian University Research Foundation. Forth Worth, TX p 177–192

Buikema AL, Sherberger SR, Knauer GW, Newbern LA, Reading JT, Cairns J (1979) Effects of simulated entrainment on the biology of a freshwater cladoceran. In: JH Thorp and JW Gibbons (eds), Energy and Environmental Stress in Aquatic Systems, DOE Symposium Series 48, CONF-771114 p 809–825

Bull RJ, O'Neill JJ (1975) Spectral changes in the respiratory chain of cerebral cortex slices. Correlation with the energy status of the tissue. Psychopharmacology Communications 1:109–115

Burress RM, Chandler JH Jr (1976) Use of the Asiatic Clam, *Corbicula leana* Prime, in Toxicity Tests. Prog Fish Cult 38:10

Capuzzo JM, Sasner JJ (1977) The effect of chromium on filtration rates and metabolic activity of *Mytilis edulis* L. and *Mya arenaria* L. In Physiological Responses of Marine Biota to Pollutants. FJ Vernberg, A Calabrese, FP Thurberg and WB Vernberg (eds), Academic Press, New York pp 225–237

Chapman AG, Fall L, Atkinson DE (1971) Adenylate energy charge in *Escherichia coli* during growth and starvation. J Bacteriol 108:1072–1086

Dickson GW (1980) Phosphoadenylate concentrations and adenylate energy charge in freshwater crayfish (Decapoda: Astacidae). Natural and stress related variation. PhD Dissertation, University of Georgia, Athens, Ga p 112

Giesy JP, Denzer SR, Duke CS, Dickson GW (1981a) Phosphoadenylate concentrations and energy charge in two fresh-water crustaceans: Response to physical and chemical stressors. Verh Internat Verein Limnol 21:173–188

Giesy JP, Duke CS, Bingham RD, Dickson GW, Leversee GJ (1981b) Changes in phosphoadenylate concentrations and energy charge in the Asian Clam (*Corbicula fluminea*) due to starvation or chronic cadmium exposure. Society of Environmental Toxicology and Chemistry (in press)

Hiltz DF, Dyer WJ (1970) Principal acid-soluble nucleotides in adductor muscle of the scallop (*Placopecten magellanicus* and their degradation during postmortem storage on ice. J Fish Res Bd Canada 27:83–92

Innes WT (1966) Exotic Aquarium Fishes. Maywood New Jersey: Metaframe Co

Ivanovici AM Adenylate energy charge (1979) Potential value as a tool for rapid determination of toxicity effects. p 241–255 In Proc 5th Ann Aquatic Toxicity Workshop, Hamilton Ontario, November 7–9, 1978. Fish Mar Serv Tech Rep p 862

Ivanovici AM (1980) The adenylate energy charge in the estuarine mollusc, *Pyruzus ebeninus*. I: Laboratory studies of responses to salinity and temperature. Comp Biochem Physiol 66A:43–55

Ivanovici AM, Wiebe WJ (1980) For a working definition of "stress": a review and critique In: GW Barrett and R Rosenberg (eds), Stress and Natural Ecosystems. Wiley and Sons, New York (in press)

Kawai DK (1953) Carbonic anhydrase in pearl oyster. Changes of the enzyme activity in relation to growth and seasons. Publis Seto Marine Biol Lab Kyoto Japan: Kyoto Univ 5:89–94

Lane CE, Tierney JQ (1951) Hydrodynamics and respiration in Teredo. Bull Marine Sci Gulf and Caribbean 1:104–110

Morrison DF (1967) Multivariate Statistical Methods. McGraw-Hill Book Co., New York, NY p 338

Pennak RW (1978) Fresh-water Invertebrates of the United States, second edition. John Wiley and Sons, New York p 803

Rainer SF, Ivanovici AM, Wadley VA (1979) Effect of reduced salinity on adenylate energy charge in three estuarine molluscs. Mari Biol 54:91–99

Selye H (1976) Stress in Health and Disease. Butterworth, Inc Boston, Ma, p 334

Sickel JB (1979) Population dynamics of *Corbicula* in the Altamaha River, Georgia. In Proceedings, First International *Corbicula* Symposium, Texas Christian University, Fort Worth, TX, October 13–15, 1977. JC Britton, JS Mattice, CE Murphy, and LW Newland (eds), Texas Christian University Research Foundation Fort Worth, TX p 69–80

Skjoldal HR, Bamstedt U (1976) Studies of the deepwater pelagic community of Korstjorden, western Norway. Adenosine phosphates and nucleic acids in *Meganyctiphanes norvegica* (Euphausiacea) in relation to the life cycle. Sarsia 61:1–14

Sokal RR, Rohlf FJ (1969) Biometry, the principles and practice of statistics in biological research. WH Freeman and Co, San Francisco p 386–387

Stolinge MR (1978) Tables of the studentized augmented range and applications to problems of multiple comparisons. J Amer Stat Assoc 73:656–660

Wilbur KM, Anderson NG (1950) Carbonic anhydrase and growth in the oyster and busycon. Biol Bull 98:19–24

Wilbur KM, Owen G (1964) Growth. In Physiology of Mollusca Vol 1, KW Wilbur and CM Yonge (eds), Academic Press New York p 211–242

Wylie V, Smith M (1964) Nucleotides of the Pacific oyster *Crassostrea gigas* (Thunberg). Can J Biochem 42:1347–1351

Zeuthen E (1947) Body size and metabolic rate in the animal kingdom with special regard to the marine micro-fauna. Compt Rend Trav Lab Carlsberg Ser Chim 26:17–161

Received November 25, 1980