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Variation in Adenylate Energy Charge and Phosphoadenylate Pool Size in Estuarine Organisms After an Oil Spill

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Adenylate energy charge (AEC), defined by Atkinson and Walton (1967) as:

$$AEC = \frac{[ATP] + \frac{1}{2}[ADP]}{[ATP] + [ADP] + [AMP]} \quad ,$$

is the proportion of the total phosphoadenylate pool charged with "high-energy" bonds. AEC values vary between zero and one by definition. Low values were originally correlated with substrate depletion and loss of growth potential in bacteria (Chapman et al. 1971; Wiebe and Bancroft 1975). Since AEC can be measured in any organism, decreases might be a universal measure of sublethal environmental stress (Ivanovici 1980a; Giesy et al. 1981; Din and Brooks 1986). In many cases AEC does decrease during unnatural stress. Examples include Euglena exposed to heavy metals (De Filippis et al. 1981), molluscs exposed to low salinity or high temperature (Ivanovici 1980b), and fish exposed to low pH (MacFarlane 1981). However, several cases where AEC is conserved during stress argue against the universality of this indicator (Phelps et al. 1981; Fitzwater et al. 1983; Turner and Wellburn 1985; Verschraegen et al. 1985). In some organisms which maintain high AEC while withstanding natural or anthropogenic stress, the absolute concentration of ATP and the total phosphoadenylate pool (TPP) decrease proportionally. Bakke and Skjoldal (1979) have suggested than TPP or simply ATP pool size may be a more universal measure of sublethal stress that AEC. However, in certain organisms the TPP shows dramatic natural fluctuations unrelated to pollution or stress (Giesy and Dickson 1981; McKee and Mendelssohn 1984). The variety of previous results indicates the confusion which has resulted from narrowly focused studies.

On 28 June 1983, a tanker spilled approximately 42,000 gallons of #6 diesel oil in the Cape Fear River, North Carolina, USA. Oil covered the tidal marshes on the east side of the river and provided an opportunity to determine if either the AEC or TPP in a variety of organisms would respond to this stress. Five test species were examined as long as one year after the spill. We compared AEC and TPP values of the organisms between contaminated and uncontaminated sites at all seasons. This is the first investigation to monitor AEC in a number of taxonomically distinct estuarine species during an extended period after an oil spill.

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MATERIALS AND METHODS

The principle study site was a tidal marsh dominated by Juncus roemerianus near the mouth of Mott's Creek (16 km south of Wilmington, NC; 77°56' W, 34°07' N). This site was covered with a heavy coating of oil mostly within 4 m of the marsh edge. Samples were collected from within 2 m of the edge. Polymesoda caroliniana, a clam, was not abundant at this site and so was collected from a site 0.5 km east, which was exposed only to lighter oil fractions transported by incoming tides. Uncontaminated control sites were located 2 km south of the spill site.

Collections began on 7 July 1983 and continued weekly during July and monthly thereafter through May 1984. The five species collected from July through November were P. caroliniana, Uca minax (a fiddler crab), J. roemerianus (needlerush), Spartina alterniflora (smooth cordgrass), and S. cynosuroides (giant cordgrass). From November 1983 through February 1984, only clams and needlerush were collected. New growth S. alterniflora was sampled along with P. caroliniana and J. roemerianus in spring 1984. S. cynosuroides did not reappear in the oiled area. Tissues analyzed were muscular foot of P. caroliniana, muscle from the large claw of male U. minax, base of the leaves of J. roemerianus and base of the stem of S. alterniflora and S. cynosuroides. Replicate samples (3-5) of each species were taken from oiled and control sites on each collection. (7 July and 14 July data are combined.)

Tissues were dissected in the field and frozen in liquid nitrogen within 60 sec. Samples were stored in liquid nitrogen until analyzed. Lyophilization, extraction in boiling ethylenediamine tetraacetic acid (EDTA), enzymatic conversion of ADP and/or AMP to ATP, quantification of ATP by the bioluminescence of luciferin, and calculation of AEC followed Mendelssohn and McKee (1981). There is evidence that for some animal tissues extraction in cold perchloric acid (PCA) is more efficient than the hot EDTA method (Sklar and McKee 1984). However, when the PCA method was used to extract muscle tissue of P. caroliniana, ATP averaged only 74% of the values reported here for the same months (data not shown).

AEC and TPP data were subjected to two-way analysis of variance with date and site as the main effects. In addition, Student's t-tests determined significant differences between control and oiled sites for each species on each collection date.

RESULTS AND DISCUSSION

The oil spill had no significant effect on phosphoadenylate pools of Polymesoda caroliniana (Figure 1). Previous studies with mollusc species and other types of pollution varied. Cadmium at 5 mg/L caused a significant reduction in AEC in Corbicula, a genus closely related to Polymesoda (Giesy et al. 1978). Conversely, AEC was not affected by a range of pollutants in two more distantly related molluscs (Chowdary et al. 1979; Phelps et al. 1981).

TPP and AEC varied significantly among collection dates (Figure 1). Variability was not obviously related to season. The increase in TPP

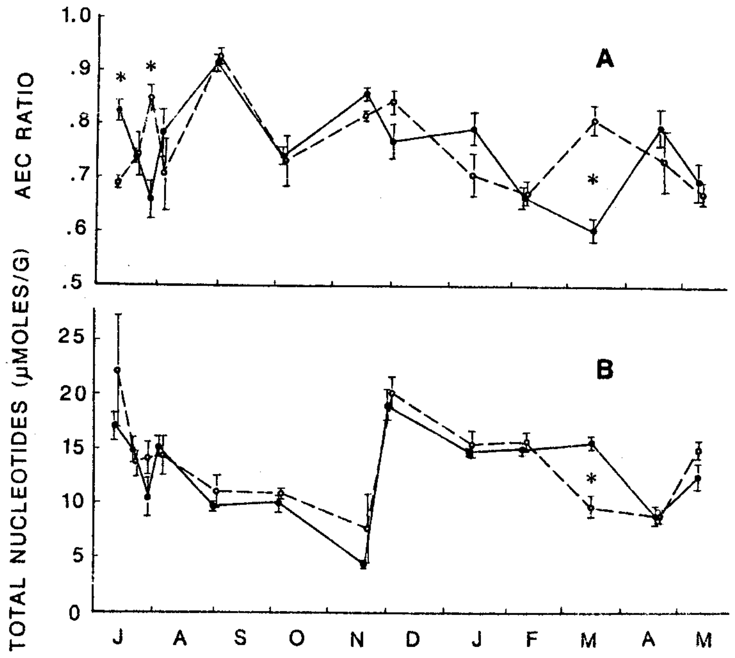

Figure 1. Changes in adenylate energy charge (A) and total phospho-adenylates [ATP + ADP + AMP] (B) expressed on a dry weight basis in the Carolina marsh clam, Polymesoda caroliniana, after the oil spill. Closed circles represent controls and open circles oiled groups. Vertical bars represent ± standard error. * = significant differences (P=.05) between control and oiled means for that date.

during late November (Figure 1B) corresponds to the end of the clams' reproductive season (Hackney 1983). Low AEC for February and March in controls and for February in the oiled clams (Figure 1A) are in the range often characteristic of limiting conditions (Ivanovici 1980a) and may reflect cold stress (Hackney 1985) in these populations which are near the northern range limit. Record cold temperature for the area occurred in January.

The large between-collection variability in AEC and TPP in *P. caroliniana* makes the measurement of these quantities of little value in assessing the long-term effects of pollutants (Figure 1). Phelps et al. (1981) reached a similar conclusion for the blue mussel, *Mytilus edulis*. Furthermore, a year-long study of bivalves in South Carolina showed between-date variability in AEC and TPP of the same wide magnitude that we report (Giesy and Dickson 1981).

In *Uca minax*, the AEC was extremely low immediately following the oil spill (Figure 2A). The AEC value at the control site was also low in the 21 July collection. It is generally assumed that organisms with AEC below 0.5 have been exposed to severe stress and may not recover (Ivanovici 1980a). It is possible that AEC in *U. minax* is extremely sensitive to dissolved hydrocarbons and that tidal mixing had carried

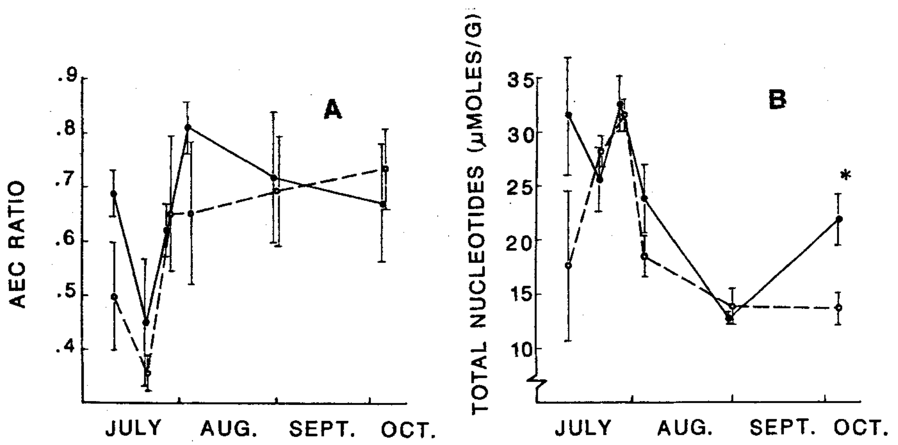

Figure 2. Changes in adenylate energy charge (A) and total phosphoadenylates [ATP + ADP + AMP] (B) expressed on a dry weight basis in the fiddler crab, Uca minax, after the oil spill. Closed circles represent controls and open circles oiled groups. Vertical bars represent ± standard error. * = significant differences (P=.05) between control and oiled means for that date.

contaminated water to the presumably pristine site by 21 July. However, we have no other evidence of pollution at this site. If the low AEC at either site is an effect of the oil, then recovery was complete within weeks of the spill and no long-term effects occurred (Figure 2A).

A comparison of AEC values for U. minax (Figure 2A) and for other test species shows that fiddler crabs have the most variability between samples within collections. Lack of molt synchrony is one possible explanation. However, we believe that crabs sampled at the oiled site represent a mixed group of animals which had migrated in after the spill. We observed U. minax in various unhealthy states during early July. Some individuals, although alive, had limited mobility and were coated with oil. Others from the same site reacted normally and were not covered. Fiddler crab mortality was difficult to determine in the field since dead animals quickly decompose or are eaten. However, a laboratory mortality study showed a mean lethal time of 3-4 days for U. minax coated with #6 diesel oil (unpublished data). Krebs and Burns (1977) also report lethal effects of oil on Uca. If most crabs at the oiled site had been killed outright by the initial oil coating, then animals sampled were less contaminated males which had migrated into the vacated territory. Variable AEC measurements could reflect different amounts of time immigrants had been exposed to residual hydrocarbons in the sediment.

The TPP in U. minax differed significantly for both site and date (Figure 2B). The control site had a higher overall mean. However, within-collection variability was great and the site difference inconsistent from date to date, making it difficult to use TPP to monitor pollution stress. Thus, neither AEC or TPP is a consistent, reliable indicator of oil-produced stress in U. minax. Bakke and Skjoldal (1979) also found that TPP changes had little predictive value in assessing hydrocarbon-induced stress in crustacea. They reported that sublethal amounts of toluene, a common component of petroleum mixtures, had no effect on ATP level in a marine isopod. Moderate but eventually lethal concentrations depressed the ATP content just before death. Highly toxic concentrations were lethal before the ATP levels responded.

There was no seasonal (between-date) effect of AEC for Juncus roemerianus (Figure 3A). There was also no significant difference between the AEC at the control and oiled sites, even though the black needlerush was heavily coated with oil for up to 3 months after the spill. Furthermore, plant biomass studies at the oiled site did not show severe alterations of the normal seasonal pattern for the species (data not shown). Another study had indicated that single, acute exposures to crude oil do not affect productivity in J. roemerianus (de la Cruz et al. 1981).

Two conclusions are apparent from changes in TPP in Juncus (Figure 3B). First, the pool size was lowest in August through November and increased significantly in the winter and spring. Second, the oiled site was not significantly different from the control in months immediately following the spill but had a significantly higher TPP after late autumn. Pamatmat and Skjoldal (1979) found that ATP content expressed on a dry weight basis correlated well with respiratory activity in belowground biomass in this species while AEC did not. It is thus possible that larger TPP indicates more active metabolism. If so, autumn was a time of relatively low metabolic acitivity and when metabolism resumed it was actually stimulated by the oil spilled the previous summer. An alternative explanation for these data has to do with the fact that the portion of J. roemerianus sampled included the basal leaf meristem. Plants with less meristematic activity would have fewer young cells in the collected material. We reason that in such samples more of the dry weight would be contributed by cell wall and therefore the apparent concentration of cytoplasmic nucleotides would be lower. If this is true, then our data suggest that autumn is a time of relatively slow growth and that the winter and spring growth period was more pronounced at the oiled site that the control. Regardless, Figure 3B does not indicate detectable stress for J. roemerianus as a result of the environmental insult.

The oil spill caused no visible damage to either Spartina species during summer or autumn 1983. Plants grew, flowered and underwent normal autumn senescence. However, the emergence and growth of new shoots the following spring was adversely affected by the oil spill. The stand of S. alterniflora at the oiled site exhibited slow growth and was much less dense than before the spill. S. cynosuroides did not even reappear at the oiled site in the spring. New growth at the control was normal for both species in 1984. The oil may have interfered with either belowground storage of photosynthate during late summer of 1983 or with the mobilization of stored energy from oil-soaked rhizomes in the spring.

AEC in S. alterniflora was not significantly affected by the oil spill in 1983, but site differences were obvious in spring 1984 (Figure 4A). While the AEC of healthy plants at the control increased, a decrease occurred at the oiled site. By May, the mean AEC for new growth at the oiled site was below 0.6, an apparent reflection of metabolic disfunction. The seasonal changes in AEC at the control site (but not the oiled site)

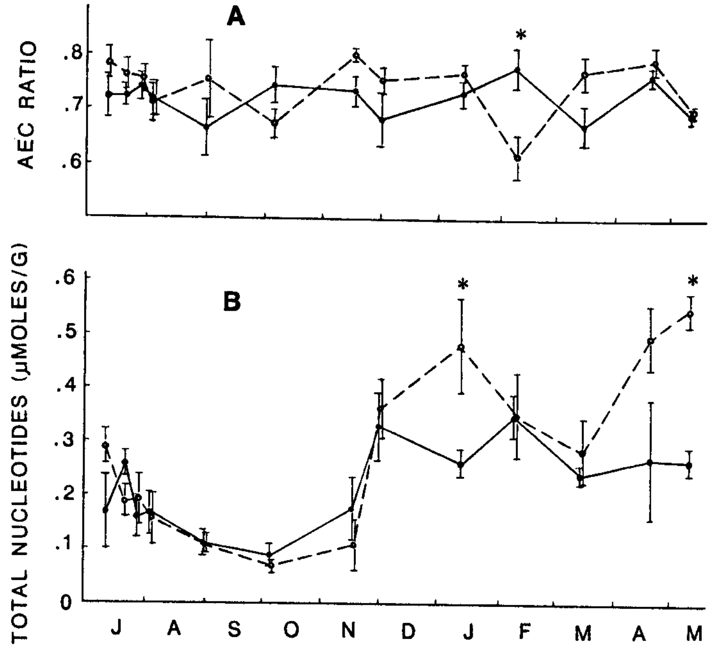

Figure 3. Changes in adenylate energy charge (A) and total phosphoadenylates [ATP + ADP + AMP] (B) expressed on a dry weight basis in the black needlerush, Juncus roemerianus, after the oil spill. Closed circles represent controls and open circles oiled groups. Vertical bars represent ± standard error. * = significant differences (P=.05) between control and oiled means for that date.

agree with a previous report for field-collected S. alterniflora from Louisiana (McKee and Mendelssohn 1984), where AEC increased from March to May and the decreased from July to November in unstressed plants.

For S. cynosuroides, the mean AEC at the oiled site decreased to 0.52 in the autumn of 1983, though the small sample size and large variability within collections kept the two sites from being statistically different (Figure 4C).

Our findings that a decrease in AEC in Spartina can be related to hydrocarbon-induced damage agree with the preliminary investigations of I. A. Mendelssohn, Wetland Resources, Louisana State University (unpublished). He exposed S. alterniflora to a range of concentrations of crude oil and found that the AEC measured after two days of exposure decreased with increasing amounts of oil. More importantly, this change in AEC roughly predicted a decrease in the ratio of living to dead biomass measured a month later.

The TPP for S. alterniflora and S. cynosuroides was the smallest for both species at both sites during the autumn (Figure 4B and 4D). This result was similar to Juncus. It also agrees with seasonal data from the same two species collected from populations in Louisiana (McKee and

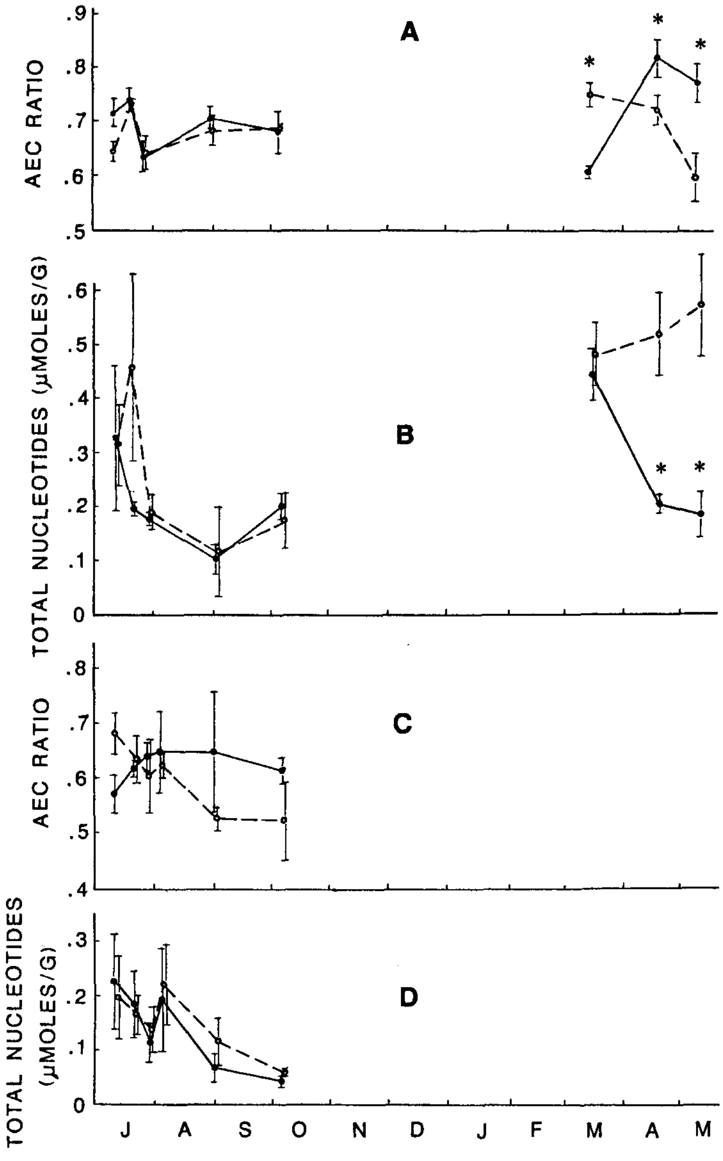

Figure 4. Changes in adenylate energy charge (A and C) and total phosphoadenylates [ATP + ADP + AMP] (B and D) expressed on a dry wieght basis in smooth cordgrass, Spartina alterniflora, (A and B) and giant cordgrass, S. cynosuroides, (C and D). Collections were not possible during winter as above-ground portions of plants were dead. S. cynosuroides did not regenerate in oiled areas the following spring. Closed circles represent controls and open circles oiled groups. Vertical bars represent ± standard error. * = significant differences (P=.05) between control and oiled means for that date.

Mendelssohn 1984). Site-related differences in TPP existed for S. alterniflora in the spring 1984 (Figure 4B). The pool size in young emergent plants in early March was high at both sites. It decreased to typical summer levels in April and May at the control but remained high at the oiled site, leading to the significant difference.

Three general conclusions can be made. First, long-term changes in AEC may result from sublethal stress due to oil pollution in some tidal marsh plants and animals but not in others. Second, TPP is not a more sensitive nor a more universal monitor of stress than AEC itself. Finally, seasonal variation in the phosphoadenylate system in estuarine organisms can be greater that the effects of an environmental insult and thus must be fully understood for the species and locality in question before AEC or ATP measurements can provide any useful information about a particular pollution incident.

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