

## Seasonal changes in adenylate energy metabolism in the muscle and liver of the redear sunfish, *Lepomis microlophus*

Paula F. Dehn

Department of Biology, Canisius College,  
Buffalo, N.Y. 14208, U.S.A.

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### Abstract

Levels of muscle and liver adenylates, phosphocreatine (PCr), total adenylates (TA) and the adenylate energy charge (AEC) exhibited significant seasonal changes. Fish size and condition factors were significantly different throughout this study; however there were no correlations between fish size or condition factor and metabolite levels. Muscle adenosine triphosphate (ATP), TA and AEC were lowest in winter and positively correlated with temperature; while adenosine diphosphate (ADP) and adenosine monophosphate (AMP) levels were high in winter and negatively correlated with temperature. Muscle AEC's dropped into the stressed range (0.5-0.75) during winter months. ATP/ADP ratios were lowest while PCr/ATP ratios were highest in winter months; indicating that a capacity for phosphate buffering existed and could not account for these low muscle AEC's. Liver ATP, TA, PCr, AEC, and adenylate ratios were lowest in the spring and were not correlated with temperature, but rather coincided with reproduction. Liver AEC's dropped into the lethal range (<0.5) in April; although no fish mortalities were observed. Liver AEC's were lower than muscle AEC's throughout the study. The AEC appears to have some validity as a long-term indicator of physiological status and may have application in monitoring/predictive programs due to its predictable response to seasonal change. However, cautious use is suggested until temperature effects on adenylate metabolism and seasonal effects on metabolic recovery following capture stress can be established.

**Keywords:** Adenylate energy charge, metabolism, seasonal responses, *Lepomis microlophus*.

*Changements saisonniers du métabolisme des adénylates du muscle et du foie du poisson Lepomis microlophus.*

### Résumé

Les adénylates du foie et du muscle, la phosphocréatine (PCr), les adénylates totaux (TA) et la charge énergétique adénylique (AEC) varient de façon significative en fonction de la saison. Les tailles des poissons et les facteurs de condition sont significativement différents tout au long de cette étude; cependant il n'y avait pas de corrélation entre la taille du poisson ou le facteur de condition et la valeur des métabolites. L'adénosine triphosphate du muscle (ATP), TA et AEC étaient les plus bas en hiver et corrélés positivement avec la température; tandis que les valeurs de l'adénosine diphosphate (ADP) et de l'adénosine monophosphate (AMP) étaient élevées en hiver et corrélées négativement avec la température. L'AEC du muscle tombe dans une gamme limite (0,50-0,75) durant les mois d'hiver. Les rapports ATP/ADP étaient les plus bas tandis que les taux PCr/ATP étaient les plus élevés en hiver, indiquant qu'une capacité de tampon-phosphate existait et n'expliquait pas les faibles valeurs de l'AEC. Les ATP, TA et PCr du foie et les taux en adénylates étaient les plus faibles au printemps et n'étaient pas corrélés avec la température mais coïncidaient plutôt avec la reproduction. En avril, les AEC du foie atteignaient une zone létale (<0,5), bien qu'aucune mortalité de poissons n'ait été observée. Les AEC du foie étaient toujours inférieurs à ceux du muscle tout au long de cette étude. L'AEC semble avoir une certaine validité comme indicateur de l'état physiologique et peut

s'appliquer éventuellement dans des programmes de surveillance ou de prévision, grâce à sa réponse prédictible à un changement de saison. Cependant, une utilisation prudente est suggérée tant que les effets de la température sur le métabolisme adénylique et les effets de la saison sur le métabolisme de récupération suivant le stress de capture ne peuvent être confirmés.

**Mots-clés :** Charge énergétique adénylique, métabolisme, saison, *Lepomis microlophus*.

## INTRODUCTION

Environmental fluctuations associated with seasonal climatic changes are important triggers for adjusting the physiology and behavior of aquatic organisms (Hochachka and Somero, 1984). Cyclical variations in temperature, food availability, dissolved oxygen, and pH can induce changes at both the molecular and organismal levels (Reid and Wood, 1976). Food deprivation, temperature extremes and acute physical exertion have been associated with the release of catecholamines and/or corticotropin and the alteration of energy metabolism at the blood and/or tissue level in fishes (Mazeaud and Mazeaud, 1981; Leatherland and Sonstegard, 1984).

The adenylate energy charge (AEC) has been proposed as an indicator of the physiological status of an organism (Haya and Waiwood, 1983; Ivanovici, 1980). The AEC [ $AEC = (ATP + 0.5 ADP) / (ATP + ADP + AMP)$ ] is a measure of the amount of energy available from the adenylate pool and, theoretically, is a prime factor that regulates the flux of energy in catabolic and anabolic processes in the cell (Atkinson, 1971). Although the AEC theory of cellular energy regulation has been disproved (Erecinska and Wilson, 1978; 1982), the AEC does appear to be a reasonably good indicator of both pollution stress (Ivanovici, 1980) and physiological well-being (Haya and Waiwood, 1983).

Seasonal variations in the levels of adenylates and/or the AEC have been reported for a variety of invertebrate species (Bamstedt and Skjoldal, 1976; Cantelmo-Cristini *et al.*, 1985; Dickson and Giesy, 1982; Giesy and Dickson, 1981; Moal *et al.*, 1987, 1991; Picado *et al.*, 1988), but only cyclostomes (Savina and Derkachev, 1983) and a single teleost (Reinert and Hohreiter, 1984) fish have been examined for seasonal changes in adenylate metabolism. In some organisms the AEC compensated and exhibited seasonal acclimatization, even though levels of adenylates or energy precursors (phosphagens, glucose, glycogen) were altered (Cantelmo-Cristini *et al.*, 1985; Savina and Derkachev, 1983).

The AEC also has been suggested as a potentially useful diagnostic tool in evaluating stress responses in aquatic organisms. One application being examined is that of incorporating the AEC into routine, long-term ecological monitoring/predictive programs, and as a early warning signal of toxic contamination

(Haya and Waiwood, 1983). However, before the applicability of the AEC as a bioassay tool can be fully assessed, examination of how the AEC responds to conditions normally encountered in the environment, e.g. seasonality and the myriad of factors affected by seasonal changes, need to be examined; as many of these same factors are known to affect toxicity. Additionally these factors would comprise the normal background within which any such long-term program would be conducted.

The purpose of this study was to examine seasonal changes in adenylate energy metabolism and the AEC in the muscle and liver of the redear sunfish, *Lepomis microlophus*. A secondary goal was to examine the suitability of utilizing the AEC as a long-term indicator of physiological status for application in monitoring/predictive programs. The muscle and liver were chosen as they reflect the energy condition of the whole animal and are sites of both regulation of metabolism and detoxification, respectively. The sunfish was chosen as: 1) members of this family (Centrarchidae) are widely distributed throughout the United States (*L. microlophus* is distributed from Missouri to southern Indiana and south to Florida and Texas, and is present throughout California); 2) members of this family are important game and/or pan fishes (Eddy and Underhill, 1978); and 3) one member of the family (bluegill, *L. macrochirus*) is a recommended species for aquatic toxicity testing.

## MATERIALS AND METHODS

### Collection and sampling

Redear sunfish, *Lepomis microlophus*, were seined monthly from Blue Wing Lake in South Bexar County, Texas, using a beach seine in shallow water. No attempt was made to separate or identify age cohorts; although, when possible, fish of similar weights (8-15 g) and lengths (80-100 mm) were used for monthly samples so as to avoid any size effects on adenylate metabolism. This sampling methodology did not allow a truly representative estimate of the sizes of fishes within the population to be obtained. At the time of collection lake temperatures were recorded, while dissolved oxygen measurements were made only in the spring and summer months (D.O. = 7.4 - 11.6 ppm) as the lake was equipped with

acrators. Fish were returned to the laboratory and maintained overnight (24 hrs) in holding tanks to allow for recovery from capture stress. Fish were weighed, the length measured to the base of the caudal peduncle, and decapitated. A sample of the white epaxial muscle just below the dorsal fin and the liver were excised in the cold (4°C) within 60 sec of decapitation, which has been shown to have no effect on adenylate levels (Reinert and Hohreiter, 1984). Tissues were freeze-clamped in liquid nitrogen and stored at -80°C prior to extraction (Hess and Brand, 1974).

### Preparation and analysis of samples

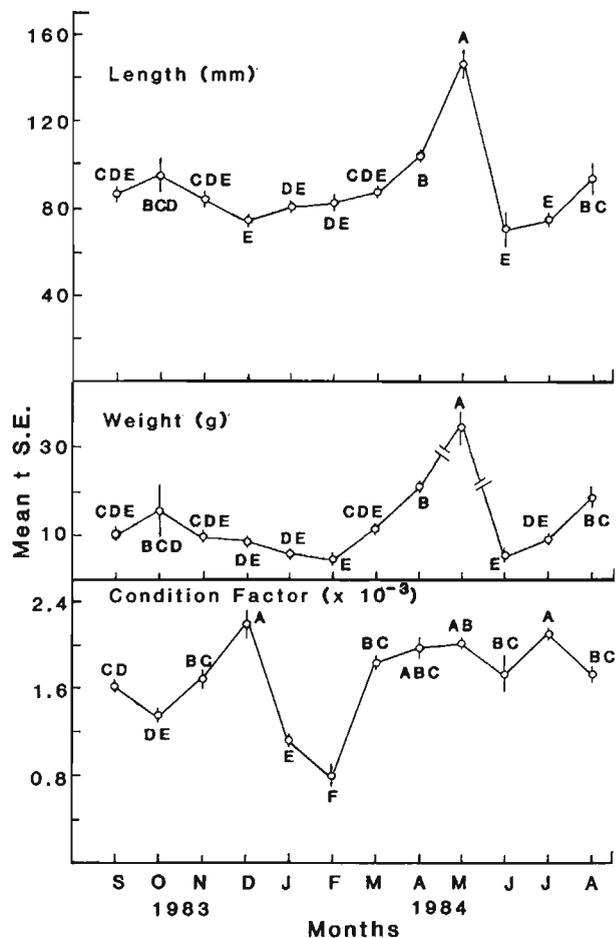
The frozen muscle was ground in liquid nitrogen and then an approximately 0.5 g sample, accurately weighed, was extracted in 2 ml of perchloric acid, while the whole liver (approximately 0.2 g) was extracted in 1 ml of perchloric acid (Dehn *et al.*, 1985). The concentrations of ATP, ADP, AMP and phosphocreatine (PCr) in the neutralized extract were determined spectrophotometrically using modifications of the enzymatic methods of Lamprecht and Trautschold (1974), Lamprecht *et al.*, (1974), and Jaworek *et al.*, (1974). Tris buffer (pH 7.6, 50 mM) was substituted for trichloroamine buffer and the amount of extract used was maximized for each tissue examined.

### Calculations and statistical analyses

Determination of the levels of adenylates and calculation of the adenylate energy charge (AEC) were performed according to Dehn and Wene (1985). The calculation of the condition factor ( $CF = \text{weight}/\text{length}^3 \times 100$ ), adenylate ratios (ATP/ADP, PCr/ATP) and all statistical analyses were performed using SAS Subprograms. An ANOVA (alpha 0.05) and Duncan's new multiple range test (NMRT) were used to determine monthly differences. Correlation coefficients were calculated to determine if any relationships existed between fish size (length, weight), condition factor or temperature and levels of adenylates, phosphagen and AEC.

## RESULTS

Fish lengths, weights and condition factors were significantly different throughout this study. Fish lengths ranged from a monthly average of 71 mm to 147 mm, while weights ranged from a monthly average of 5.7 g to 33 g (*fig. 1*). Fish were significantly larger in May compared to all other months. The condition factors ranged from 0.8 to 2.25 and was significantly lower than all other months in February



**Figure 1.** — Changes in mean  $\pm$  SE length, weight, and condition factor ( $\text{weight}/\text{length}^3 \times 100$ ) of redear sunfish captured during a seasonal cycle. Letters represent the results of the Duncan's NMRT (New multiple range test). The same letter indicates no significant difference. Sample size ( $n$ ) is indicated with the muscle data on table 1.

(*fig. 1*). No correlations were observed between fish size or condition factor and metabolite levels or the AEC.

Muscle tissue exhibited significant seasonal differences in the levels of all of the adenylates, phosphocreatine (PCr), total adenylates (TA), and the AEC (*fig. 2*). Levels of ATP were significantly lower in winter months (Jan., Feb.) and ranged from 0.48 to 4.61  $\mu\text{mol/g}$  wet weight. Levels of ADP ranged from 0.35 to 0.83  $\mu\text{mol/g}$  wet weight, while levels of AMP ranged from 0.06 to 0.53  $\mu\text{mol/g}$  wet weight. Highest AMP levels were observed in winter months. Both TA and AEC exhibited the same basic pattern of change as levels of ATP, being lowest in winter months. Levels of the TA ranged from 1.57 to 5.09  $\mu\text{mol/g}$  wet weight, while AEC ranged from 0.494 to 0.951. Levels of PCr also were significantly lower in winter and spring months, and values ranged from 0.31 to 6.55  $\mu\text{mol/g}$  wet weight. Levels of ATP, TA and the AEC were positively correlated with

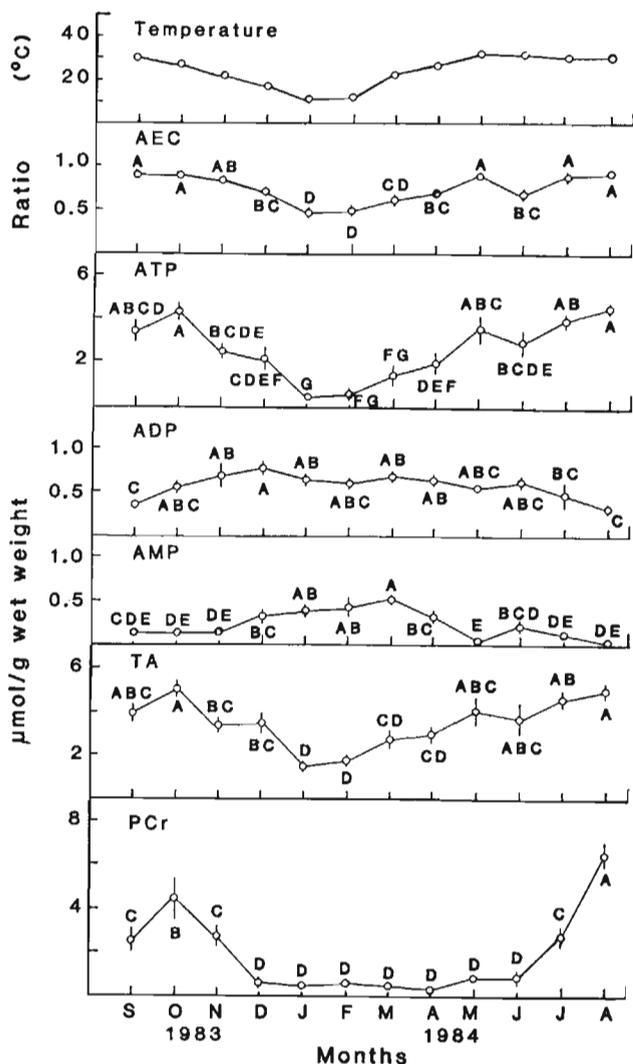


Figure 2. — Seasonal changes in temperature, mean  $\pm$  SE levels of ATP, ADP, AMP, total adenylates (TA), phosphocreatine (PCr), and the calculated AEC in white epaxial muscle of redear sunfish. Letters represent the results of the Duncan's New multiple range test. The same letter indicates no significant difference. No standard error bar indicates errors were smaller than the symbol size. Sample size ( $n$ ) is indicated with the muscle data on table 1.

temperature ( $r=0.815$ ,  $F=0.0012$ ;  $r=0.796$ ,  $F=0.0019$ ;  $r=0.787$ ,  $F=0.0024$ , respectively), while levels of ADP and AMP were negatively correlated with temperature ( $r=-0.648$ ,  $F=0.023$ ;  $r=-0.710$ ,  $F=0.0097$ , respectively). No correlation was observed between levels of PCr and temperature.

The muscle tissue exhibited significant seasonal differences in the PCr/ATP and ATP/ADP ratios (table 1). The PCr/ATP ratios were highest in the winter months (Jan., Feb.) and in August. The ATP/ADP ratio in July was significantly higher than all other months. The ATP/ADP ratio was positively correlated with temperature ( $r=0.747$ ,  $F=0.0053$ ). No correlation was observed between muscle PCr/ATP ratio and temperature.

The liver tissue exhibited significant seasonal differences in the levels of all the adenylates, PCr, TA and AEC (fig. 3). Levels of ATP were lowest in the spring (Apr.), and ranged from 0.14 to 2.51  $\mu\text{mol/g}$  wet weight. Levels of ADP ranged from 0.32 to 2.17  $\mu\text{mol/g}$  wet weight, while levels of AMP ranged from 0.36 to 2.43  $\mu\text{mol/g}$  wet weight. Highest levels of AMP were observed in June. Both TA and AEC exhibited the same basic pattern of change as levels of ATP, being lowest in spring. Levels of the TA ranged from 1.71 to 7.86  $\mu\text{mol/g}$  wet weight, while AEC ranged from 0.173 to 0.747. Levels of PCr also were significantly lower in spring, and values ranged from 0.21 to 8.87  $\mu\text{mol/g}$  wet weight. No correlations were observed between any of the levels of metabolites or the AEC and temperature.

The liver ATP/ADP ratio exhibited significant seasonal differences, while the PCr/ATP ratio exhibited no significant seasonal alterations (table 1). The ATP/ADP ratio was lowest in the late spring (Apr., May) and early fall (Sept.). No correlations were observed between any of the liver adenylate ratios and temperature.

## DISCUSSION

Centrarchids move into shallow, near shore waters during the reproductive season and the males establish a territory and build nests (Keenleyside, 1979). The reproductive season for the warm water redear sunfish is late spring, and fish could only be sexed in April and May (pers. obs.). In the present study, the largest animals seined were in May, which undoubtedly reflects: 1) the behavioral changes that cause larger fish to move inshore for reproductive activities displacing the younger, smaller fish, and 2) the sampling methodology employed that results in only fish in shallow near shore waters to be captured. Even though size, as measured by length and weight, of captured fish underwent large seasonal fluctuations, the condition factor remained somewhat stable throughout the year except in late winter (Feb.) when it dropped to a low of 0.8, indicating an overall decrease in the condition of these late winter individuals. Wilber (1969) found that the condition factor of *L. macrochirus* populations in Florida increased with length and were highest in the spring (Mar. & Apr.) (cited in Carlander, 1977). In the present study, this length relationship did not occur as the longest animals were captured in May, yet the greatest condition factor was observed in December (length=147 vs 75 mm, respectively). In the present study the overall condition of these sunfish was greatest in December, April, May and June, and worst in January and February.

The mean levels of adenine nucleotides, phosphocreatine (PCr), and the AEC in the muscle were similar to those reported for white muscle in a variety

**Table 1.** — Seasonal changes levels of the PCr/ATP and ATP/ADP ratios (mean  $\pm$  SE) for white epaxial muscle and liver of redear sunfish. Letters represent the results of the Duncan's new multiple range test (NMRT). The same letter indicates no significant difference.

Month	Muscle			Liver		
	n	PCr/ATP	ATP/ADP	n	PCr/ATP	ATP/ADP
September	10	0.8 + 0.2BC	9.5 + 0.9B	10	4.1 + 1.9A	0.7 + 0.1C
October	11	1.0 + 0.2BC	8.3 + 0.9B	10	1.6 + 0.2A	2.2 + 0.5ABC
November	10	1.3 + 0.3BC	4.1 + 0.8B	9	2.0 + 0.4A	1.9 + 0.5ABC
December	10	0.4 + 0.1BC	2.6 + 0.5B	9	3.4 + 1.4A	3.0 + 1.4AB
January	10	2.6 + 1.1A	0.7 + 0.1B	10	4.7 + 1.3A	1.3 + 0.2BC
February	11	1.8 + 5.1AB	1.1 + 0.4B	10	2.8 + 1.3A	1.7 + 0.5ABC
March	10	0.5 + 0.1BC	1.9 + 0.5B	10	3.0 + 0.7A	1.5 + 0.4ABC
April	10	0.2 + 0.04C	3.4 + 1.3B	10	3.9 + 0.7A	0.5 + 0.1C
May	3	0.2 + 0.01C	6.6 + 1.1B	3	0.3 + 0.1A	0.8 + 0.1C
June	10	1.1 + 0.5BC	3.9 + 0.1B	10	3.6 + 0.5A	3.4 + 0.9A
July	10	0.2 + 0.1BC	154 + 62A	10	8.1 + 3.3A	1.6 + 0.2ABC
August	8	1.5 + 0.2ABC	39 + 26B	8	0.9 + 0.1A	2.3 + 0.3ABC

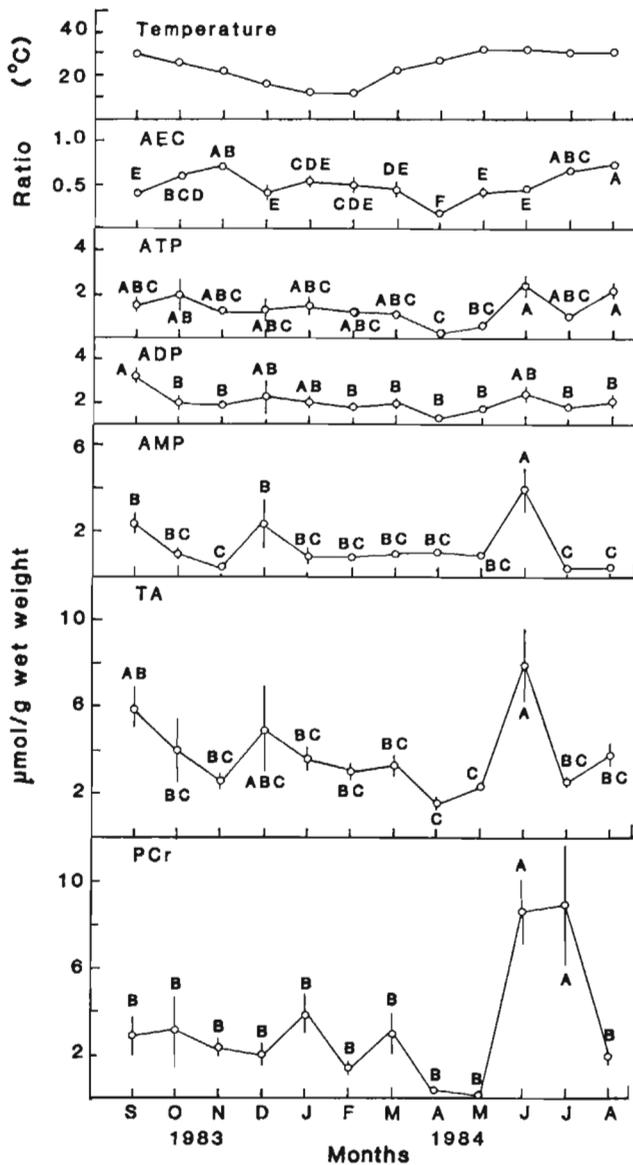
of non-stressed freshwater fish (Dehn and Schirf, 1986), except for the late fall-winter months when ATP, PCr and the AEC were considerably lower. The mean levels of adenine nucleotides and the AEC in the liver were similar to those reported for liver in a variety of non-stressed fish (Dehn, unpubl.; Haya *et al.*, 1985; Heath, 1984; Jorgensen and Mustafa, 1980; Savina and Derkachev, 1983), except for the late spring when ATP and the AEC were lower. PCr concentrations in the liver generally were above those previously observed for juvenile salmon (Haya *et al.*, 1985) and largemouth bass (Dehn, unpubl.). The mean levels of ATP were consistently lower in the liver than in the muscle while ADP and AMP were similar or higher. As a results liver AEC's were lower than muscle. The mean levels of PCr were similar in the muscle and liver.

Seasonal differences have been observed in the adenylate concentrations and the AEC of the boreal marine zooplankton *Euchaeta norwegica* (Bamstedt and Skjoldal, 1976); bivalves *Corbicula fluminea* (Cantelmo-Cristini *et al.*, 1985; Giesy and Dickson, 1981) *Crassostrea gigas* (Moal *et al.*, 1991), *Cardium* sp. (Picad *et al.*, 1988) and *Mytilus edulis* (Skjoldal and Barkati, 1982); and the crayfish *Procambarus acutus acutus* (Dickson and Giesy, 1982). For the crayfish, lowest levels of muscle ATP, total adenylates and AEC occurred in the winter and late spring (Dickson and Giesy, 1982). No discernable correlations existed between energy metabolism and environmental conditions (temperature, pH, D.O.), but it was suggested that the AEC varied with reproductive season. Recently a study by Moal *et al.* (1991) indicates that seasonal differences in AEC in the oyster, *Crassostrea gigas*, may be correlated with nutritional status and/or maturation. In an *in situ* study the AEC of the foot muscle of *Corbicula fluminea* remained constant within a season (July-November), but varied between seasons (1981 vs 1982) (Cantelmo-Cristini, *et al.*, 1985).

Little is know of the seasonal variations of the adenylates or AEC of aquatic vertebrates. The European eel, *Anguilla anguilla* exhibited seasonal variations in ATP content when acclimatized under natural conditions (Anderson *et al.*, 1985). The concentration of ATP in eel erythrocytes was greatest during the summer and lowest in April. The muscle of the lamprey, *Lampetra fluviatilis*, showed seasonal (November-May) variations, but the AEC remained constant (Savina and Derkachev, 1983). Rainbow trout muscle exhibited seasonal changes in AEC with lowest values observed in winter and highest values associated with summer months (Reinert and Hohreiter, 1984). In the present study differences in the levels of muscle adenylates, total adenylates (TA) and AEC occurred. The highest ATP, TA and AEC were observed in late summer (Aug) with lowest values in winter (Jan).

The availability of phosphagens may influence the AEC. The accepted role of phosphocreatine (PCr) is to buffer changes in ATP concentrations (Beis and Newsholme, 1975) and it has been suggested that the stability of muscle energy status is associated with this buffering effect (Jorgensen and Mustafa, 1980). When phosphocreatine levels decrease, a reduction occurs in the buffering capacity and hence leads to a reduction in the AEC. In the present study it appears that the occurrence of low muscle AEC's in winter months might be explained by low levels of PCr. However when the PCr/ATP ratios are examined it is apparent that PCr/ATP is extremely high when ATP/ADP is low, indicating that a high potential exists for buffering.

Metabolic and/or behavioral responses to low temperatures may better explain low muscle AEC's observed during winter as levels of adenylates and the AEC were correlated with temperature. In the present study lake temperatures fell to lows of 12.5°C. Walesby and Johnston (1980) found that ATP, total adenylates and AEC of white muscle of cold-accli-



**Figure 3.** — Seasonal changes in temperature, mean  $\pm$  SE levels of ATP, ADP, AMP, total adenylates (TA), phosphocreatine (PCr), and the calculated AEC in the liver of redear sunfish. Letters represent the results of the Duncan's New multiple range test. The same letter indicates no significant difference. No standard error bar indicates errors were smaller than the symbol size. Sample size (n) is indicated with the liver data on table 1.

mated (4°C) brook trout, *Salvelinus fontinalis* were lower than those of warm-acclimated (24°C) fish. When an organism encounters changes in temperature it may acclimatize by altering its behavior and/or biochemical composition (Reynolds and Casterlin, 1980). Decreased swimming activity has been observed to occur in response to reduced temperature (Love, 1970) and both reduced temperature and starvation (Lemons and Crawshaw, 1985; Turner, 1986). Centrarchids have been observed to undergo both reduced activity (Turner, 1986) and cessation of

feeding when environmental temperatures decrease (Carlander, 1977). When feeding cessation occurs, endogenous food sources are mobilized, resulting in a depletion of muscle constituents (Love, 1970). Elevated muscle ATP levels and AEC have been associated with reductions in swimming activity (Villarreal and Dehn, 1984). In the present study higher PCr/ATP ratios occurred during cold winter months suggesting that reductions in activity might have occurred. Whether feeding cessation occurred during winter months in Blue Wing Lake is not known, but decreases of over 83% and 45% in the level of muscle ATP and AEC, respectively, were seen during this period. It should be noted that muscle AEC values did fall below levels associated with the irreversible loss of viability (AEC < 0.5). However, no fish kills were reported in the lake at this time. Additionally these low concentrations of adenylates, phosphagens and the AEC's in winter may reflect, in part, differences in the recovery potential of the animal following capture stress. The lowest water temperature and poorest condition of the fish were observed in the late winter, and these factors are known to effect recovery from capture stress (Jones and Murray, 1960). However, the high PCr/ATP ratios indicate that the phosphate buffering capacity is unaffected by low concentrations of these individual metabolites. Also, previous studies have shown that muscle adenylates phosphocreatine, and the AEC recover from exhaustive muscle activity within 1 hour (Breed and Dehn, 1985). Further laboratory studies are underway to determine any possible temperature cause-effect relationships for these observed muscle metabolic changes and to examine possible seasonal differences in the recovery times for muscle metabolites following capture stress.

Liver energy metabolism of the lamprey, *Lampetra fluviatilis*, has been shown to undergo seasonal variations (Savina and Derkachev, 1983). Lowest levels of ATP, TA and AEC were associated with spring (Apr.). Thermal adaptation was shown to have an effect on adenylate concentrations, *i.e.* increasing the temperature from 4.5°C to 17-18°C resulted in an approximate 1000% and 434% increase in ATP and AEC, respectively (Savina and Derkachev, 1983). In the present study differences in the levels of liver adenylates, TA, and AEC occurred. The highest ATP, TA and AEC's were observed in summer.

Temperature does not appear to play a direct role in lowering levels of ATP and AEC in the liver in the present study; as relatively high levels of ATP and AEC were found in winter when lake temperatures reached lows of 12.5°C, while lowest ATP levels and AEC occurred in spring (Apr.) when lake temperatures ranged from 23-28°C. Instead these low levels of liver energy metabolites coincided with the reproductive season. Temperature may be indirectly responsible for these lows as it is known to trigger feeding cessation which is associated with the repro-

ductive patterns of many organisms. The liver of many fish is known to be exceedingly rich in unsaturated lipids (Love, 1970), which become the main energy substrate during periods of prolonged starvation. Utilization of these compounds results in an increase in acyl CoA derivatives which have been suggested to inhibit the adenylate translocase system hence interfering with the transfer of mitochondrial ATP to cytoplasmic pools (Savina and Derkachev, 1983). Temperature is not the only factor which induces metabolic alterations. Hypoxia has been shown to have an effect on the liver energy metabolism of flatfishes (Jorgensen and Mustafa, 1980). A decrease in oxygen supply also is known to result in the accumulation of acyl CoA derivatives, which leads to the inhibition of the translocase system (Savina and Derkachev, 1983). In the present study however, hypoxia did not appear to be involved in these lowered levels of ATP and AEC in spring, as lake dissolved oxygen levels were relatively high (10-11 ppm). The underlying cause of these seasonal lows are not known and remain areas of future investigation.

## CONCLUSION

Seasonal fluctuations in adenylate energy metabolism do occur in the muscle and liver of redear sunfish. Seasonal changes in muscle adenylate metabolism are correlated with temperature, while seasonal changes in liver adenylate metabolism coincide with reproductive activity. In the context of utilizing the AEC as a biochemical indicator of stress for long-term monitoring/predictive work, it appears from this study that predictable seasonal changes in the AEC do occur in redear white muscle and perhaps to a lesser extent in liver; indicating that this system may be suitable for biological monitoring. However until any temperature cause-effect relationships for seasonal changes in muscle metabolism and any seasonal effects on metabolite recovery following capture stress can be established, the use of the AEC in biological monitoring should be cautioned. In addition, in light of the work involving invertebrate species, which indicates differences occurring between seasons, further cautions in utilizing the AEC in monitoring work are advised.

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