Otsuka, Cary Mineo

NUTRIENT STORAGE AND ADENYLATE ENERGY CHARGE IN LITTORINA LITTOREA (L.)

City University of New York PH.D. 1986

NUTRIENT STORAGE AND ADENYLATE ENERGY CHARGE IN *LITTORINA LITTOREA* (L.)

by

CARY M. OTSUKA

A dissertation submitted to the Graduate Faculty in Biology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

1986

This manuscript has been read and accepted for the Doctoral Faculty in Biology in satisfaction of the dissertation requirement for the degree of Doctor of Philisophy.

5/16/86
date

Linda K. Mantel
Chairman of Examining Committee

June 17, 1986
date

Executive Officer

John B. Pearce
Dr. John B. Pearce, National Marine Fisheries Service

David R. Franz
Professor David R. Franz, Brooklyn College

Norman M. Saks
Professor Norman M. Saks, City College

John H. Tietjen
Professor John H. Tietjen, City College

Supervisory Committee

The City University of New York

Abstract

NUTRIENT STORAGE AND ADENYLATE ENERGY CHARGE IN LITTORINA LITTOREA (L.)

by

Cary M. Otsuka

Adviser: Professor Linda H. Mantel

Seasonal changes in stored nutrients (lipids, carbohydrates, and proteins), the adenylate pool (ATP, ADP, AMP), and adenylate energy charge (AEC) were examined in the marine gastropod Littorina littorea (L.). Populations from two locations, Orchard Beach, a rocky intertidal site, and Breezy Point, a sand flat, were compared because the latter population exhibited a disrupted reproductive cycle for the two consecutive years prior to 1984. During 1984, animals from both populations released eggs in the laboratory. Although differences existed in population size and composition, no significant differences existed in the measured biochemical components during the reproductive phase. The disrupted reproductive cycle of the previous years had no apparent effect on the ability of the Breezy Point population to reproduce during 1984.

The Orchard Beach population was followed for two consecutive reproductive cycles. Reductions in lipid and carbohydrate composition coincided with the events of gametogenesis, fertilization, and egg release. Monthly fluctuations in the ADP and AMP concentrations were not directly related to any seasonal pattern. Reduced ATP/ADP

ratios coincided with the reproductive phase, indicating the high ATP demand during this period. This is supported by the significant positive correlation coefficients that existed between the visceral ATP-lipid composition and between the visceral ATP-carbohydrate composition. The calculated AEC (a ratio based on ATP, ADP, and AMP concentrations used to indicate whether an organism is in an optimal or stressed condition) appeared to be independent of season and reproduction. Low AEC values did not consistently coincide with the reproductive period indicating that reproduction was not a stressful event in terms of AEC.

Littorina littorea was exposed to sublethal concentrations of benzene (1 ppm) in the laboratory to examine the effect of this hydrocarbon on the AEC, adenylates, and stored nutrients. After a three week dosing period, no significant differences existed in AEC between control and experimental animals. Adenylate concentrations, lipid, and carbohydrate compositions were slightly greater in experimental animals. Protein composition was greater in control L. littorea. L. littorea is a very resistant organism that is able to maintain its levels of stored nutrients and adenylates at relatively constant levels and release viable eggs even when exposed to short term hydrocarbon perturbation.

Acknowledgements

I thank the members of my committee, Drs. David Franz, Linda Mantel, John Pearce, Norman Saks, and John Tietjen, for their many helpful comments on all phases of my research. My major professor, Linda Mantel, provided continuous support, enthusiasm, and guidance. Mr. Gerard Cannella and his staff provided the liquid nitrogen which was a precious commodity in short supply. I thank Jack Downey and the other members of the City College Biology Department technical staff for all of their help during the past several years. Dr. Charlotte Russell and Dr. Mike Sommer helped me remedy two different problems I encounterd in the preliminary stages of my research. Tom Stokes of the NYC DEP provided me with the 1983 and 1984 NY Harbor Water Quality Survey reports and the National Ocean Service, NOAA, supplied me with temperature and salinity data. The Lerner Gray Fund for Marine Research and the City College Biology Department provided the funds which made this research possible.

Table of Contents

List of Tables

List of Figures

Introduction

The common periwinkle, Littorina littorea (L.) is a marine prosobranch gastropod that most often inhabits rocky coasts of the northern Atlantic. Due to its abundance, relatively large size, and ease of care in the laboratory, L. littorea has been the subject of extensive study for the past century (Fretter and Graham 1962; Grahame 1973; Hayes 1927; Lebour 1937; Newell 1958; Tattersall 1920; Thorson 1946; Williams 1964, 1970). Several recent studies have examined the ecology of New England populations of L. littorea (Bertness 1984; Brenchley and Carlton 1983; Hunter and Russell-Hunter 1983; Lubchenco 1978, 1982, 1983; Lubchenco and Menge 1978; Petraitis 1982, 1983). Littorina littorea is the most abundant and important herbivore in the mid and low intertidal zones of New England rocky shores (Lubchenco 1978). This species is common at both Orchard Beach and Breezy Point, two sites in the metropolitan New York City area. To examine the relationship between patterns of nutrient storage and reproduction in L. littorea, I monitored both populations for egg production and seasonal changes in biochemical composition for the past three breeding seasons. The two populations differ in number of animals, size of animals, and in the frequency of the reproductive cycle. Although determination of the exact nature of these differences is not feasible, it provides a unique opportunity to investigate

two apparently physiologically different populations under natural conditions.

Hypotheses of the study

To examine the seasonal changes in stored nutrients (lipids, carbohydrates, proteins), adenylate concentrations (ATP, ADP, AMP), and AEC in Littorina littorea, I sampled animals from the field at monthly intervals between November 1983 and June 1985 and assayed the snails for the above biochemical components. I hypothesized that:

1. reproduction, i.e., gametogenesis, fertilization, and egg release, would represent a physiologically expensive period, which would be reflected in changes in these biochemical components.
   a. the known seasonal fluctuation in gross biochemical components would be positively correlated with ATP and negatively correlated with ADP and AMP.
   b. the monthly adenylate energy charge (AEC) values would decrease with the onset of the reproductive phase and would only increase following this period.
2. overall AEC values of Littorina littorea from Orchard Beach would be greater than those of the snails from Breezy Point reflecting the disrupted reproductive cycle of the latter population. The lipid composition and carbohydrate composition of the snails from Breezy Point would not exhibit reductions during the reproductive cycle as would the L. littorea collected from Orchard Beach.

The potential for short term or chronic hydrocarbon perturbation is very real for both Littorina littorea populations because each is located along waterways regularly used by power boats. To examine the effects of such a physiological stress on the gross biochemical components and adenylates of L. littorea, I exposed animals in the

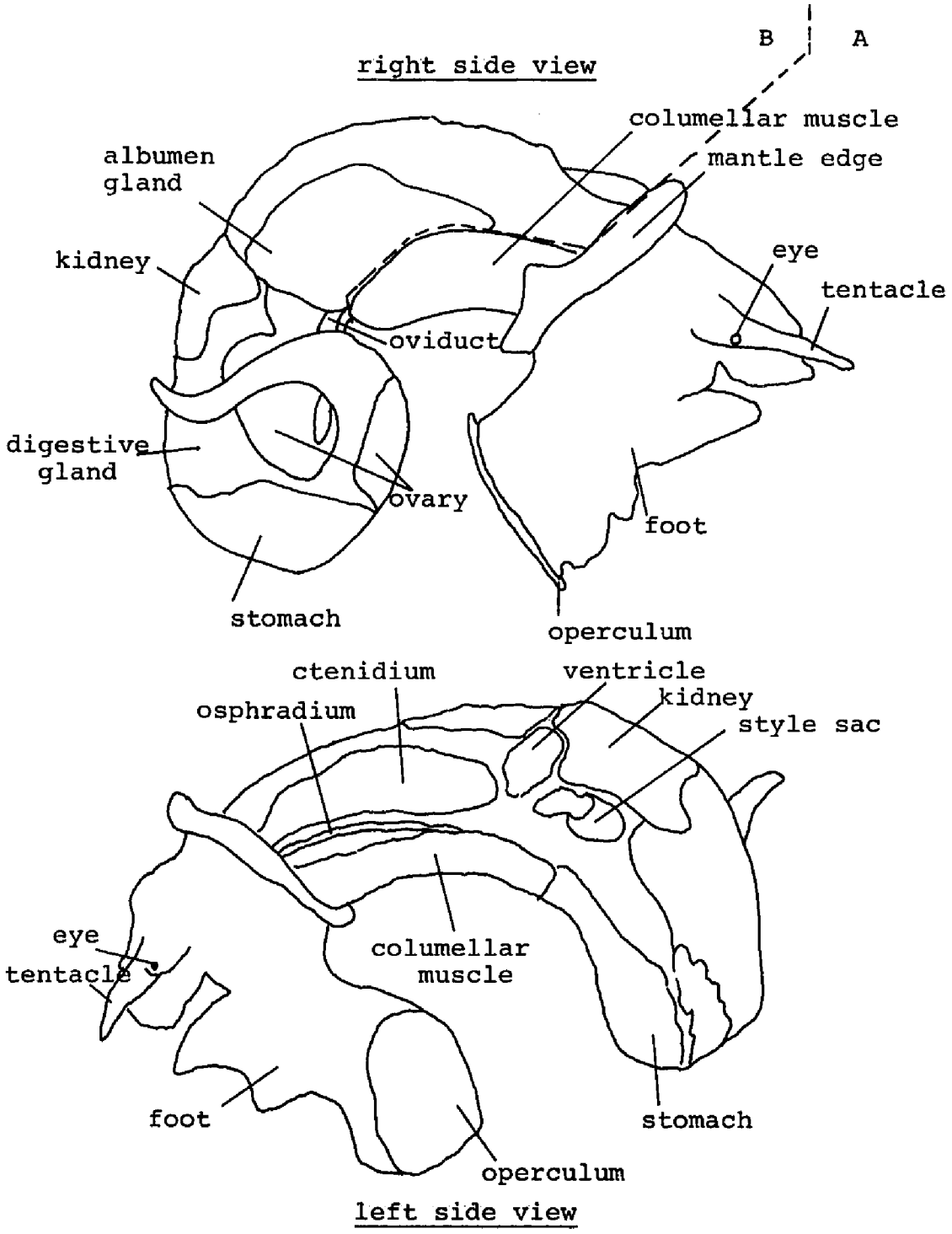
laboratory to sublethal concentrations of benzene and DMN for three weeks. I hypothesized that the AEC would decrease and that there would be reductions in the stored nutrient pool, especially the lipids and carbohydrates.

Biology of Littorina littorea

The body plan of Littorina littorea is presented to illustrate the general organization of this prosobranch (Fig. 1). The gill of L. littorea is located on the left side of the mantle cavity and is composed of ciliated lamellae. Water enters on the left side, passes through the lamellae, and exits on the right side of the mantle cavity. Alongside the gill is the osphradium, which is thought to be a receptor for chemical stimuli or particulate matter in the water. Other sense organs include the tentacles (tactile and olfactory), the eyes (at the base of the tentacles), tactile receptors in the skin, and statocyst (influenced by gravity). The tentacles and eyes are located on the head, which is situated at the anterior of the mantle cavity. Located below the head is the foot and operculum. At the anterior end of the head, situated within the buccal cavity, is the radula, a feeding organ which employs a conveyor belt like action to carry food into the gut. On the right side of the mantle cavity are located the rectum and terminal portion of the reproductive duct.

Littorina littorea is an herbivore capable of grazing Aufwuchs, filamentous algae, and foliose algae (Hunter and

Figure 1. Littorina littorea female removed from the shell. After Fretter and Graham 1962.



Russell-Hunter 1983; Steneck and Watling 1982). Littorina littorea also grazes the shoots and rhizomes of Spartina alterniflora; this grazing activity may effectively reduce the expansion of S. alterniflora (Bertness 1984). Ulva and Enteromorpha appear to be the preferred food of L. littorea (Lubchenco 1978; Watson and Norton 1985).

The estimated onset of sexual maturity for Littorina littorea occurs at a shell height of 10-14 mm (Hughes and Roberts 1980; Fish 1972). Following copulation, the eggs are enclosed in a capsule (1-5 eggs/capsule) and released. Female L. littorea can produce more than 200 egg capsules 2 to 12 hours after copulation. The estimated total number of egg capsules per female per season is approximately 5000 (Thorson 1946). The reproductive apparatus becomes reduced during non-reproductive periods and enlarges with the approach of the next reproductive period.

In Europe, the spawning period of Littorina littorea is variable according to location but has been recorded as being as early as December and as late as September (Alifierakis and Berry 1980; Fish 1972; Tattersall 1920). Fish (1972) compared the spawning activity of an estuarine and open coast population (Wales) of L. littorea and found that the estuarine population matured earlier with maximum spawning activity in January; for the open coast population, maximum spawning was in March. The difference in maximum spawning activity was attributed to the greater concentration of nutrients associated with the estuary.

Using data from plankton hauls, Fish (1979) determined that a semi-lunar periodicity existed in L. littorea reproduction, and that oviposition occurred during full and new moon spring tides. In laboratory studies, Alifierakis and Berry (1980) found females released the largest number of eggs when exposed to a tidal regime and during periods of new and full moons (see also Grahame 1975).

Grahame (1973) investigated the breeding energetics of Littorina littorea and attributed a decline in calories during the late winter and early spring to loss of energy in the form of gametes. He also noted that the cost of sperm production was 60%-80% as great as that of egg production. He speculated that the volume of sperm needed for successful fertilization may be so high that over-production of sperm is a necessity. Williams (1970) has shown that the percentage of lipid and carbohydrate in Littorina littorea varies seasonally. Lipid and carbohydrate reserves increase during the summer feeding-growth phase and are used during the following nonfeeding winter period when gonad maturation occurs. Williams also indicated that the seasonal turnover in total protein was similar to the lipid and carbohydrate levels, and that the pattern was the same in both sexes.

Newell et al. (1971) studied the feeding rate of Littorina littorea under a variety of conditions. They showed that radular activity rates varied with temperature and with the period the snail was exposed to the atmosphere.

Feeding rates of upper shore animals after immersion were faster than the rates of lower shore animals. They measured oxygen consumption in conjunction with radular activity at different temperatures and determined that thermal acclimation was achieved by maintaining a uniform energy expenditure, rather than by modification of the rate of radular activity. Newell and Pye (1970, 1971) determined that the standard rate of respiration in L. littorea was nearly independent of temperature and that active rate was temperature dependent. They also showed that the rate of oxygen consumption of cell-free homogenate was independent of temperature over a portion of the normal environmental temperature range of this snail. L. littorea has also been shown to be capable of respiratory regulation between 20 and 30°C and to remain active when exposed to air (McMahon and Russell-Hunter 1977).

Adenylate energy charge

A relatively new means of evaluating the physiological condition of an organism and estimating whether the animal is under stress or in optimal condition is determination of the adenylate energy charge (AEC). The AEC is the metabolic energy potentially available to an organism and is defined as (ATP + ½ADP)/(ATP + ADP + AMP) (Atkinson and Walton 1967; Atkinson 1968). Adenylate energy charge values of 0.8 - 0.9 represent organisms in optimal physiological condition, i.e., growing and reproducing (Ball and

Atkinson 1975); lower values indicate organisms in sub-optimal condition (Ivanovici 1980a). The dynamics of the adenylate pool have been summarized by Lehninger (1977). The concentrations of ATP, ADP, and AMP over short periods are relatively constant when cells are in steady state. Under normal conditions, ATP concentrations exceed ADP and AMP concentrations, indicating the adenylate system is nearly full of phosphate groups. When a sudden work load is placed on a cell, ATP utilization increases, causing a reduction in ATP concentration and a rise in ADP concentration. This causes the acceleration of the ATP-generating reactions of glycolysis and respiration. When the work load is removed, the reverse occurs.

The AEC has been determined for a number of aquatic invertebrates (Cantelmo-Cristini et al. 1985; Dickson and Giesy 1981; Giesy et al. 1981; Skjoldal 1981; Skjoldal and Bakke 1978) , including freshwater and marine molluscs, subjected to a variety of conditions; Littorina littorea has not yet been studied. Giesy and Dickson (1981) investigated the effects of season on AEC of two freshwater bivalve species. Their findings indicated a seasonal variation in the concentration of the individual adenylates, total adenylate concentration, and AEC, all of which were related to reproductive periods in both species. The effect of reduced salinity on the AEC of three estuarine molluscs was monitored by Rainer et al. (1979). Following immersion into the lower salinity, the AEC of the three

species decreased significantly, although the absolute AEC levels and the size of AEC reduction differed between species. Using the estuarine gastropod Pyrazus ebeninus, Ivanovici (1980b) showed that AEC responded within 24 hours to reductions in salinity. Ivanovici indicated that the measurement of AEC may be useful in monitoring the response of P. ebeninus to environmental perturbation. Skjoldal and Barkati (1982) studied ATP content and AEC of Mytilus edulis. They concluded that seasonal variation of both ATP concentration and AEC ratio were a result of changing weight proportions of the different organs.

Some of the features of AEC have been demonstrated by field studies conducted by Ivanovici (1980a). These include:

1. ease of sample collection in the field
2. precision of AEC under field conditions is comparable with that in the laboratory
3. minimal seasonal and spatial variability
4. reduced AECs correlate with organisms in perturbed environments and at a presumed reproductive disadvantage.

Ivanovici (1980a) indicated that more studies are needed to elucidate the relationship of AEC with growth, reproductive potential, and viability of offspring. Likewise, Skjoldal and Barkati (1982) acknowledged the need for more information concerning the correlation between ATP level, AEC, and environmental and physiological conditions. The Orchard Beach and Breezy Point Littorina littorea populations, because of their differences in frequency of the reproductive cycle, population size, and size of animals,

appear to provide an excellent opportunity to explore and clarify some of these topics.

Hydrocarbon perturbation

In its intertidal habitat, Littorina littorea can be exposed to hydrocarbons adhering to the substrate or mixed in the water column. A potential source of these hydrocarbons is from passing boats or nearby marinas. A perturbed environment can be simulated in the laboratory by exposing L. littorea to sublethal levels of hydrocarbons. The effects of such a stress on L. littorea can be examined by comparing the gross biochemical composition, adenylate concentrations, and AEC of the experimental animals with the control animals. The results of such an experiment will help evaluate the ability of L. littorea to cope with additional environmental stress and the degree to which such a stress is reflected by the AEC.

The toxic effect of hydrocarbons on marine organisms is well documented. Aromatic hydrocarbons have been detected in seawater, polluted and apparently unpolluted (Gordon et al. 1974), and in marine sediments (Farrington and Quinn 1973; Youngblood and Blumer 1975). Benzene and dimethylnaphthalene (DMN) are two aromatic hydrocarbons which have entered the marine environment and have been shown to affect negatively the physiology of a variety of organisms (Anderson et al. 1974; Cantelmo et al. 1982; Whipple et al. 1981). These by-products of petroleum

refinery have many industrial applications and are components of various petroleum products including No. 2 fuel oil (DMN) and pesticides (benzene). Benzene is one of the most toxic components of the water soluble fraction of crude oil (Struhsaker et al. 1974). The effects of petroleum pollutants on marine molluscs and some of the inherent problems of such research was surveyed by Bayne et al. (1982). Hydrocarbon pollution may detrimentally affect the reproductive cycle of molluscs (Bayne et al. 1982; Stekoll et al. 1980) but few studies have yet been reported. Stekoll et al. (1980) studied the sublethal effects of Prudhoe Bay crude oil on Macoma balthica. Growth was inhibited and gametes reabsorbed when these intertidal clams were subjected to the lowest concentration of oil in seawater analyzed (0.03 mg/ml). At higher concentrations, gonads were abnormal when compared to control animals after six months of exposure. They suggested that chronic exposure at the lowest concentration would eventually result in population reductions. These adverse effects of hydrocarbon perturbation should translate into reduced AEC values in Littorina littorea.

Materials and Methods

Sites and collection

Adult Littorina littorea (16-19 mm shell height) were collected from Breezy Point (BP) on Rockaway Inlet (Lat. 40°33'54"; Long. 73°54'10") (November 1983-July 1984) and Orchard Beach (OB) (Lat. 40°52'10"; Long. 73°47'4") on the western end of Long Island Sound (November 1983-June 1985) (Fig. 2) during low tide. Average salinities at the two locations during 1983 and 1984 were 29.2 and 26.1 °/oo respectively (NYC DEP). The section of Orchard Beach where the animals were collected is a rocky intertidal site with scattered patches of Ulva, Enteromorpha, Fucus, and red algal species in residence during the spring and summer months. Snails were collected from rocks either lacking or with minimal macro algal cover. The Breezy Point site is a litter-strewn beach where L. littorea are found attached to old tires and other discarded debris. A portion of the area is covered with Spartina; L. littorea are found there attached to the root systems. Ulva and Enteromorpha are also found in scattered patches at this location during the spring and summer. At this site animals were collected from Spartina stands and a variety of hard substrata.

Average monthly air and water temperatures (November 1983-June 1985) from Willets Point (Willets Pt. is 5.12 miles south of Orchard Beach) are presented in Figure 3 (National Ocean Service, NOAA). According to the NY Harbor

Figure 2. Map of NY Harbor and vicinity indicating the locations of Breezy Point and Orchard Beach.

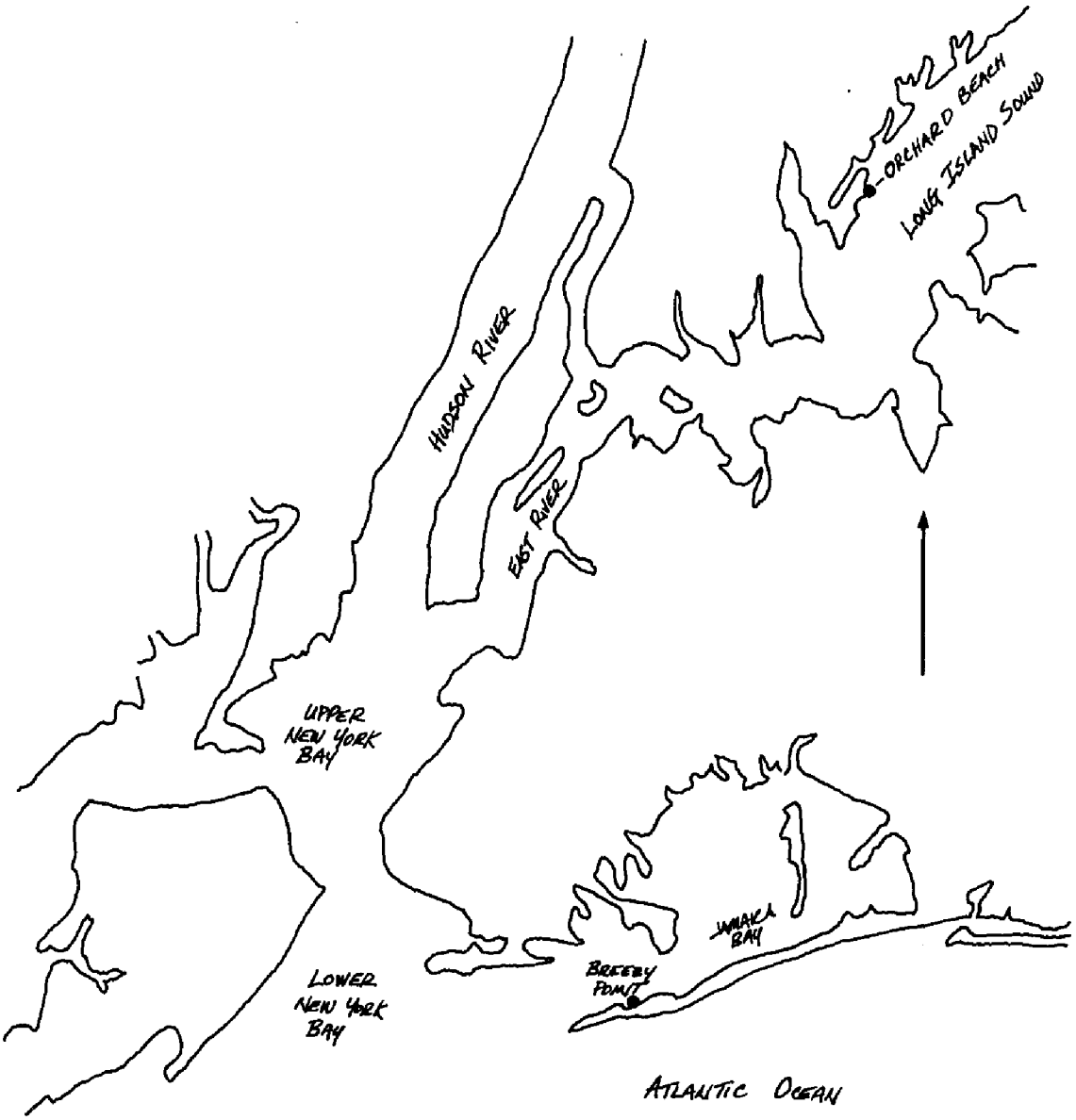
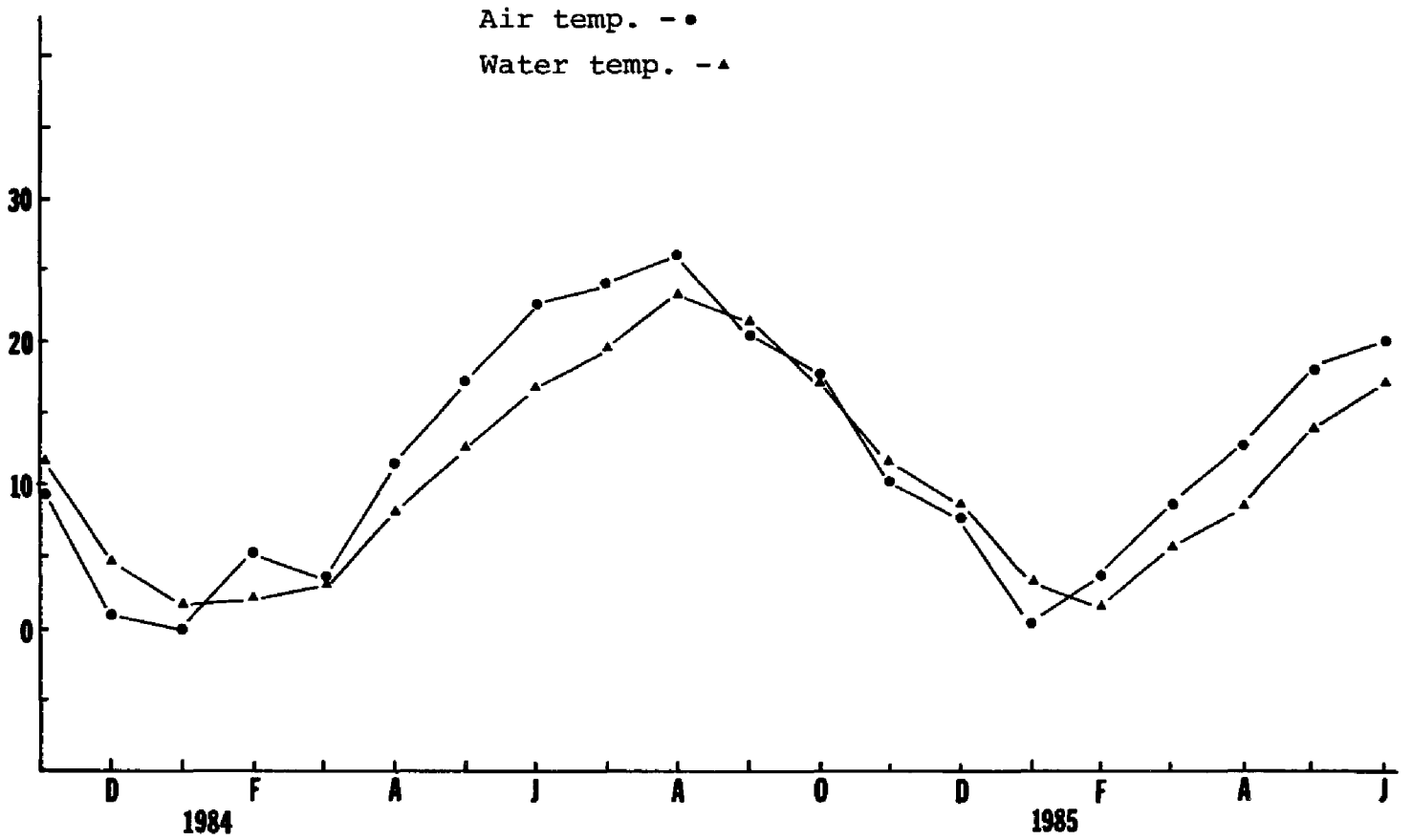


Figure 3. Average monthly air and water temperatures (November 1983-June 1985) from Willets Point based on daily temperatures recorded by the National Ocean Service, NOAA. Willets Pt. is 5.12 miles south of Orchard Beach.



Water Quality Survey (NYC DEP 1983, 1984), average water temperatures at Rockaway Inlet (Breezy Point) do not differ by more that 1° or 2°C from those in the vicinity of Willets Point.

Maintenance of animals

Animals not immediately sacrificed for assay of adenylates or gross biochemicals (total lipid, carbohydrate, protein) were maintained in the laboratory at 18°C with a 10/14 hr light-dark schedule. These snails were used to monitor reproductive condition and for benzene exposure experiments (Orchard Beach animals only). Reproductive condition was determined by observations of egg release and examination of female gonads at time of sacrifice. Laboratory animals were maintained in artificial sea water (28-29°/oo) changed on alternate days and provided with frozen Ulva or Enteromorpha which they also ate. Littorina littorea used in dosing experiments were held in enclosed aerated vessels in which a daily dosing-water change regime was followed (see Cantelmo et al. 1982). Animals were allowed a one week period of acclimation prior to any dosing.

Bioassays

Benzene and dimethylnaphthalene (DMN) were used in preliminary bioassays to examine the effects of various concentrations of these hydrocarbons on Littorina littorea. Bioassays were conducted employing benzene concentrations

of 5,3,2,1 ppm and DMN concentrations of 5,2,1,.02,.01 ppm. Concentrations greater than 1 ppm benzene and 0.01 ppm DMN resulted in mass mortality or total inactivity. At the lowest concentration, animals survived for 21 days or more, thus I chose these levels as a chronic sublethal dose. L. littorea exposed to these latter concentrations (1 ppm benzene, 0.01 ppm DMN) were assayed for adenylates and gross biochemical composition and compared. These preliminary experiments revealed that the toxic effect of benzene was not significantly different than DMN. I chose to use 1 ppm benzene for the remainder of the experiments.

Analyses

Eight field animals from each collection were analyzed in duplicate for adenylates. Three to six animals have been shown to be adequate to detect statistically significant differences (Ivanovici 1980b; Rainer et al. 1979). Twenty animals were pooled in four groups of five per collection and analyzed for biochemical composition starting in April 1984. Prior to that time, only six animals had been used. Samples were run in triplicate for carbohydrates and protein analysis; lipids were duplicated. Analyses were performed on the combined head, foot, and columellar muscle (A), and on the remainder of the animal, called the viscera, including digestive and reproductive organs (B). The snail was bisected in this manner (see Fig. 1) to examine seasonal differences in biochemical

composition of component A (primarily muscle) and the combined visceral tissue of component B. Lipids, carbohydrates, and proteins are presented as percent dry weight.

Dosing experiments of three weeks duration were conducted with animals collected in March, April, and May 1985. A six week dosing experiment was conducted with snails collected in April. At the end of the dosing period, animals were prepared for adenylate and gross biochemical analysis. Six dosed animals and six control animals were used for adenylate analysis. Ten animals in each category were used to determine biochemical composition. The reduced sample size was used to allow the simultaneous assay of controls and experimentals. During all experiments, eggs were collected and examined under a microscope for signs of cleavage. Eggs were considered viable when cleavage was detected.

The AEC analysis, which includes ATP, ADP, and AMP assays, follow the method of Ivanovici (1981), with modifications by Hospod and Cristini (personal communication). Animals were bisected (components A and B) and immediately frozen in liquid nitrogen. Following extraction of nucleotides with perchloric acid, the supernatant was assayed in two stages, first for ATP and then ADP-AMP. The complete procedure is given in the appendix to this paper.

The assay is enzymatic, in which the ATP in the sample phosphorylates glucose to glucose-6-phosphate in the presence of hexokinase. The glucose-6-phosphate dehydrogenase-

mediated reaction of glucose-6-phosphate and NADP+ produces NADPH and an increase in absorbance at 340 nm (monitored in the UV band of a spectrophotometer). Each mole of ATP in the sample produces one mole of NADPH.

The phosphorylation of ADP by phosphoenolpyruvate is catalyzed by pyruvate kinase and produces ATP and pyruvate. The lactate dehydrogenase-mediated conversion of pyruvate to lactate generates one mole of NAD+ from one mole of ADP. The resulting decrease in absorbency at 340 nm is caused by the formation of NAD+ from NADH. This is proportional to the amount of ADP in the sample. Myokinase is then used to catalyze the formation of 2 moles of ADP from one mole of AMP (in the sample) and ATP (in the buffer). Two moles of NAD+ are the result.

The methods for biochemical analyses represent modified versions of the procedures employed by Lambert and Dehnel (1974) and Emerson and Duerr (1967). Total lipids were first extracted from the dried tissue using a chloroform-methanol extraction. Following lipid extraction, hexoses (considered as total carbohydrates) were extracted via the anthrone method (Keleti and Lederer 1974). This method is based on the condensation of anthronol which reacts with the sulfuric acid created furfural derivative of the sugars present. Proteins were determined by the Lowry method (Lowry et al. 1951). The detailed procedures for all of these analyses can be found in the appendix.

Statistical Methods

Data collected were analyzed statistically between months and populations (OB vs BP), and experimentals (benzene dosed) and controls using one-way ANOVA. Percentage and AEC data were arcsine transformed to meet the criteria of ANOVA. Correlation coefficients were used to determine the degree of association between biochemical components and adenylates. A value of $p < .05$ was taken to indicate significant differences.

Results

In addition to the physical differences between the Orchard Beach and Breezy Point sites, differences existed in makeup of the populations of Littorina littorea. At Orchard Beach, L. littorea were always abundant, in contrast to the small population inhabiting Breezy Point. Each population was assessed for size frequency distribution several times during 1984 using ¼ m^2 plots (Table 1). The majority of L. littorea at Breezy Point were in the 16-24 mm shell height range. Orchard Beach snails were of greatest abundance in the 10-19 mm shell height range.

Egg release by Breezy Point animals was monitored in the laboratory from 1982 to July 1984. Until 1984, snails collected from this population either failed to release or released a very small number of eggs in the laboratory. Eggs were released by animals collected between April and July 1984, after which time the number of egg carrying females decreased.

The release of eggs (in the laboratory) by Littorina littorea from Orchard Beach occurred yearly from March through July for the past three years (1983,1984,1985). Eggs have been observed in the reproductive tract and collected from the animals as early as February. In 1983, animals collected in January began releasing eggs in February; in 1984, eggs were not collected until April. During 1985, eggs were first observed in the reproductive

Table 1. Size frequency distributions of Breezy Point and Orchard Beach populations of Littorina littorea, totals of random ¼ m^2 sampling.

			-----Shell height (mm)----------					
Date	site	¼m^2 n	5	6-9	10-15	16-19	20-24	25
5/84	BP	5	0	12	7	29	34	3
	OB	5	11	31	88	100	1	0
6/84	BP	3	0	0	6	6	16	0
	OB	4	1	36	45	100	0	0
7/84	BP	4	0	0	4	7	16	0
	OB	4	0	50	77	50	0	0

tracts of females collected 11 February but actual egg release was first recorded for animals from the March collection. During the typical reproductive period of L. littorea from Orchard Beach, average water temperatures rose from 4°C in March to 20°C by July (Fig. 2). Average air temperatures varied between 4°C and 24°C over this five month period (Fig. 2). Algae such as Enteromorpha sp. and Ulva sp. begin to appear by April and were observed in the intertidal region through October. During periods of extreme cold, L. littorea were found at the lowest intertidal rather than spread throughout the low and mid intertidal regions. Since collections took place during mean low water, animals used for all assays were exposed to ambient air temperatures and other local weather conditions.

Adenylates, AEC, and biochemical composition:

Orchard Beach and Breezy Point

The first eleven months of OB data were analyzed to determine differences between males and females in terms of lipid, carbohydrate, and protein composition, as well as the AEC. There were no significant differences ($p > .05$) between the sexes; therefore, data from males and females were pooled in all analyses (Table 2). The OB data were examined again after 19 months for differences between the sexes (Table 3). Component B (viscera) was compared for differences in lipid, carbohydrate, protein, and AEC.

Table 2. One-way analysis of variance. Comparison of male and female Littorina littorea from Orchard Beach: lipid, carbohydrate, protein and AEC; April-Sept. 1984 (gross biochemical composition), Nov. 1983-Sept. 1984 (AEC).
A - head, foot, columellar muscle; B - viscera
AEC n=4 biochemicals n=10

		F	df	p
lipid	A	.094	1,10	>.05
	B	.054	1,10	>.05
carbohydrate	A	.355	1,10	>.05
	B	.262	1,10	>.05
protein	A	.137	1,10	>.05
	B	.026	1,10	>.05
AEC	A	.447	1,20	>.05
	B	.021	1,20	>.05

Table 3. One-way analysis of variance. Comparison of male and female L. littorea from Orchard Beach. Nov. 1983-June 1985.
B - viscera
AEC n=4 biochemical n=10

		F	df	p
lipid	B	.702	1,38	>.05
carbohydrate	B	.263	1,38	>.05
protein	B	1.419	1,38	>.05
AEC	B	.154	1,44	>.05

Again there were no significant differences ($p > .05$) between male and female Littorina littorea.

Animals from both populations (OB and BP) were assayed for adenylates, lipids, carbohydrates, and proteins over a nine month period (Nov. 1983-July 1984). The Figures (4-11) indicate the general similarities and minor differences between both populations. The overall monthly patterns of AEC and percent protein and lipid are similar for OB and BP animals during the November 1983 to July 1984 period (Figs. 4,6,7,8,10,11). The February 1984 percent lipid (A) of BP Littorina littorea is much greater than the OB value but returns to similar levels by March 1984 (Figs. 2,8). The percent carbohydrate patterns are most different between the two populations (Figs. 5,9). The general trend for the OB population is the reduction in percent carbohydrate over the period. In contrast, the carbohydrate values of the BP animals increase and decrease several times over the same reproductive period. One-way analysis of variance was used to compare both populations over this nine month period (Nov. 1983-July 1984; Table 4). This analysis indicated that there was no significant difference ($p > .05$) between the two populations. At this point, I discontinued collecting and assaying animals from Breezy Point since the two populations were judged to be similar.

Correlation coefficients (Table 5) were calculated for both populations to determine the amount of association between any two of the components assayed. In only the BP

Figure 4. Orchard Beach: Percent lipid (mean) of Littorina littorea, vertical bars indicate ± standard error.

n=6 Nov. 1983-March 1984; n=20 April-July 1984.
▲A - head, foot, columellar muscle
●B - viscera

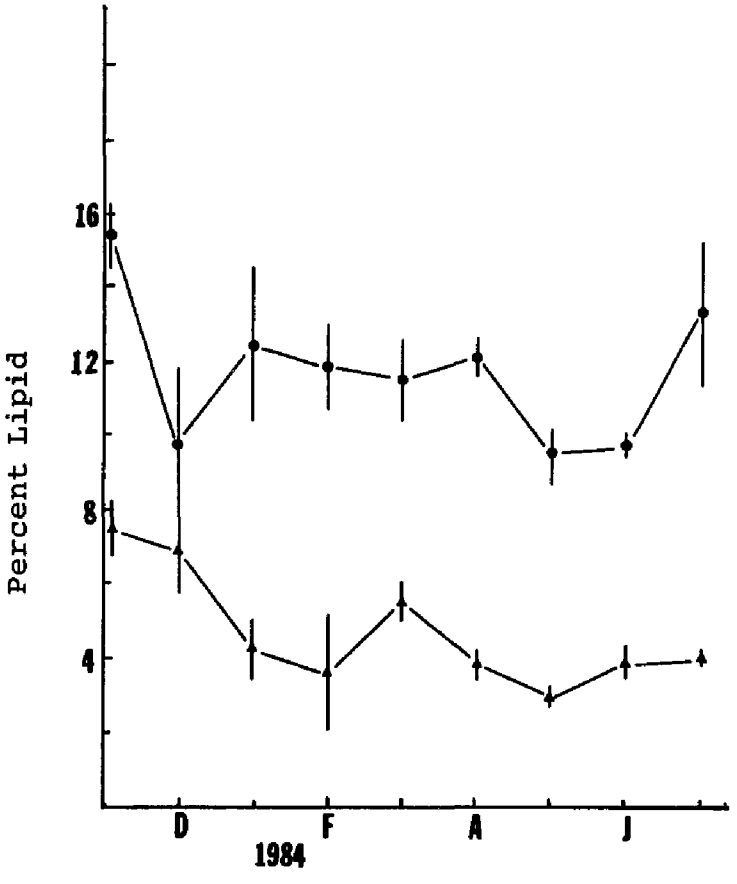


Figure 5. Orchard Beach: Percent carbohydrate (mean) of Littorina littorea, vertical bars indicate ± standard error.

n=6 Nov. 1983-March 1984; n=20 April-July 1984.
▲A - head, foot, columellar muscle
●B - viscera

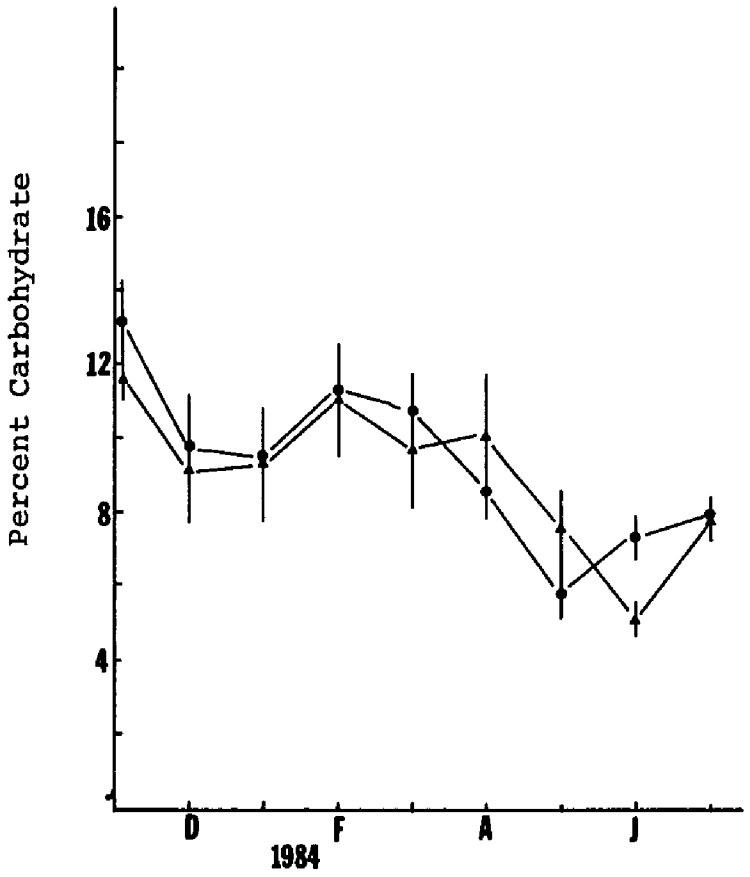


Figure 6. Orchard Beach: Percent protein (mean) of Littorina littorea, vertical bars indicate ± standard error.

n=6 Nov. 1983-March 1984; n=20 April-July 1984.
▲A - head, foot, columellar muscle
●B - viscera

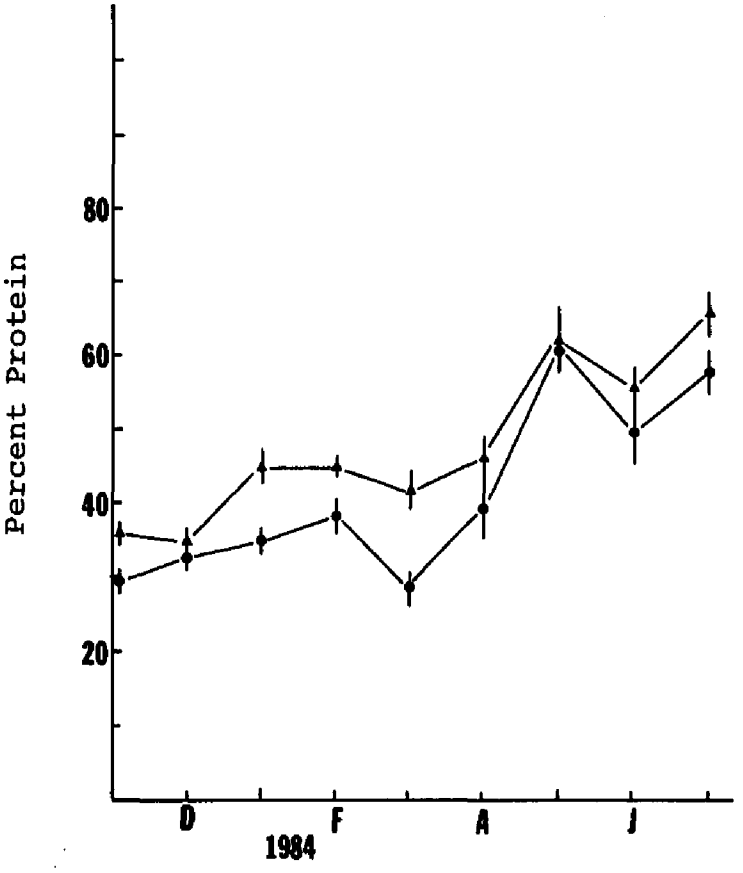


Figure 7. Orchard Beach: Adenylate energy charge (AEC) (mean) of Littorina littorea, vertical bars indicate ± standard error.

n=8
▲A - head, foot, columellar muscle
●B - viscera

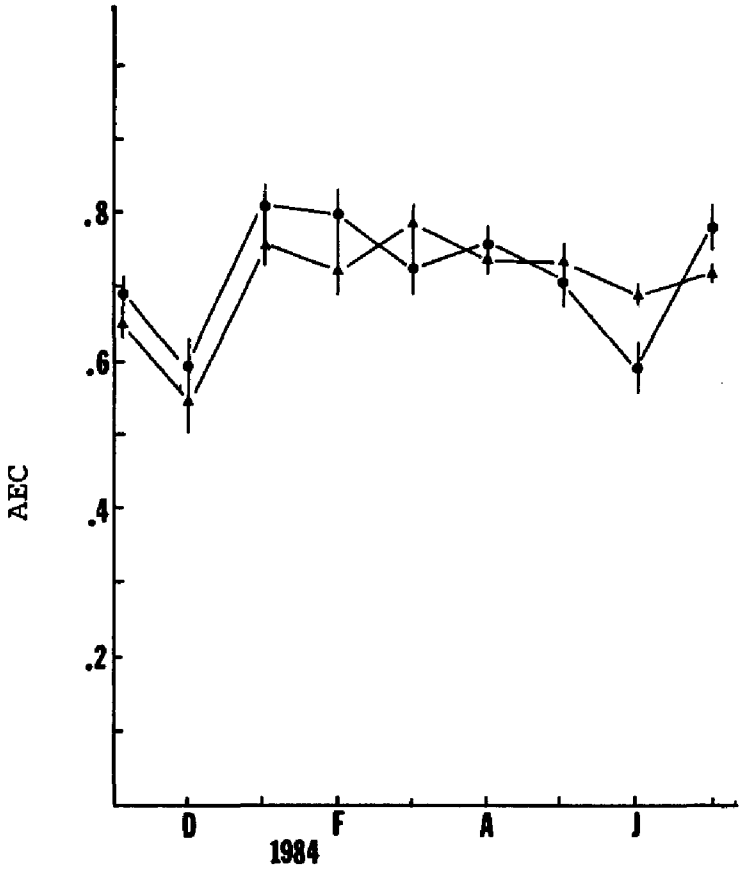


Figure 8. Breezy Point: Percent lipid (mean) of Littorina littorea, vertical bars indicate ± standard error.

n=6 Nov. 1983-March 1984; n=20 April-July 1984.
▲A - head, foot, columellar muscle
●B - viscera

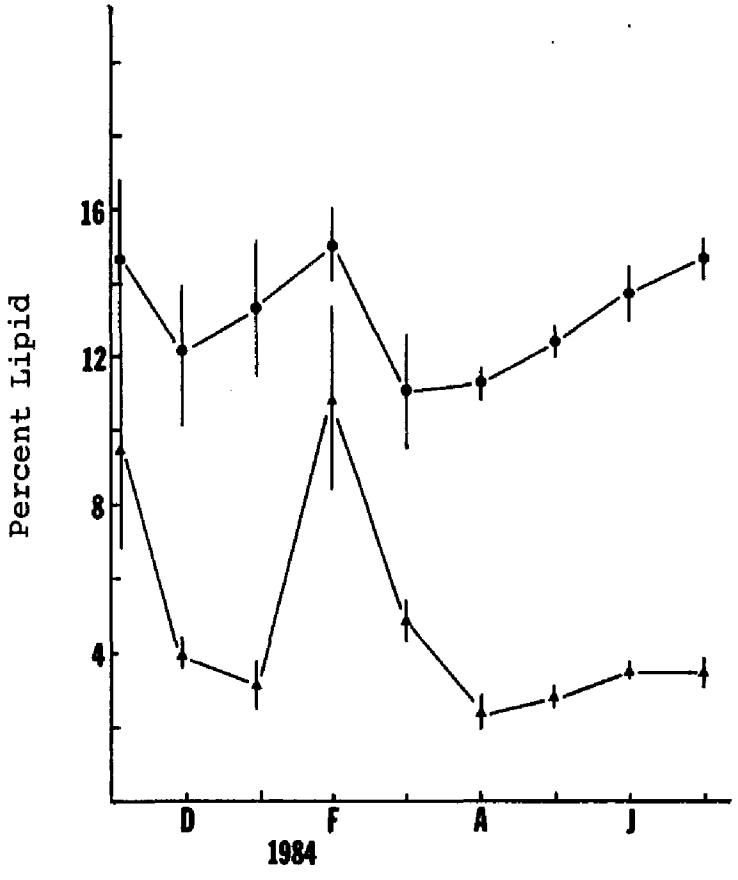


Figure 9. Breezy Point: Percent carbohydrate (mean) of Littorina littorea, vertical bars indicate ± standard error.

n=6 Nov. 1983-March 1984; n=20 April-July 1984.
▲A - head, foot, columellar muscle
●B - viscera

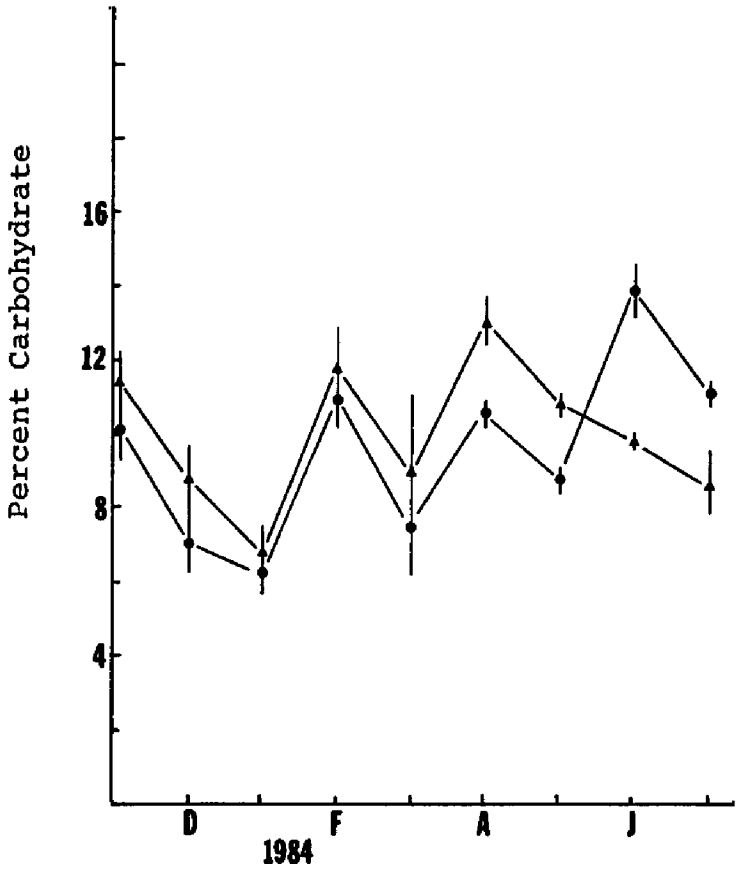


Figure 10. Breezy Point: Percent protein (mean) of Littorina littorea, vertical bars indicate ± standard error.

n=6 Nov. 1983-March 1984; n=20 April-July 1984.
▲A - head, foot, columellar muscle
●B - viscera

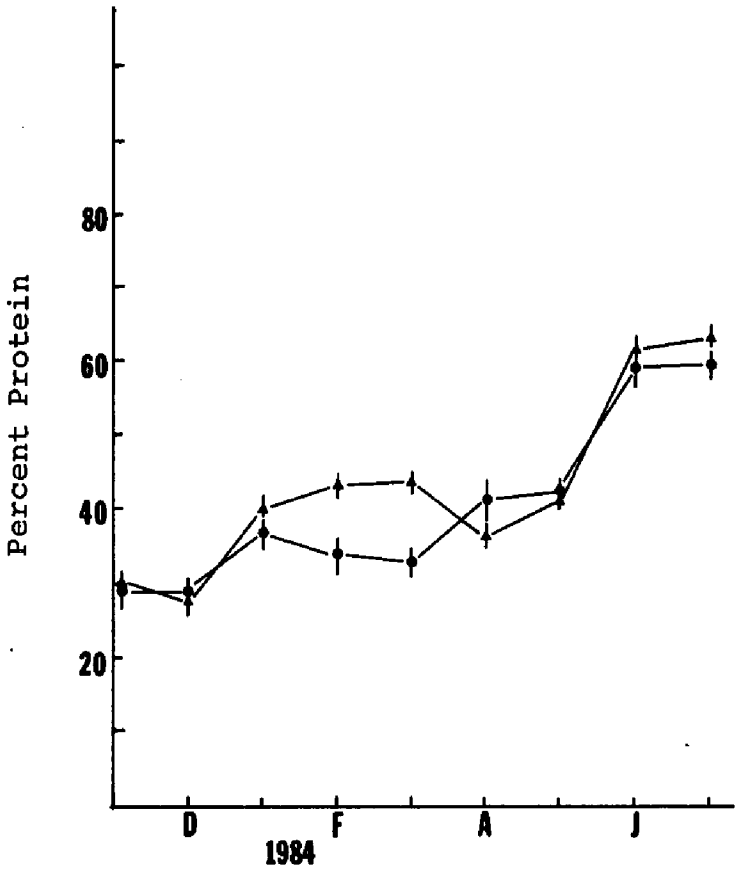


Figure 11. Breezy Point: Adenylate energy charge (AEC) (mean) of Littorina littorea, vertical bars indicate ± standard error.

n=8
▲A - head, foot, columellar muscle
●B - viscera

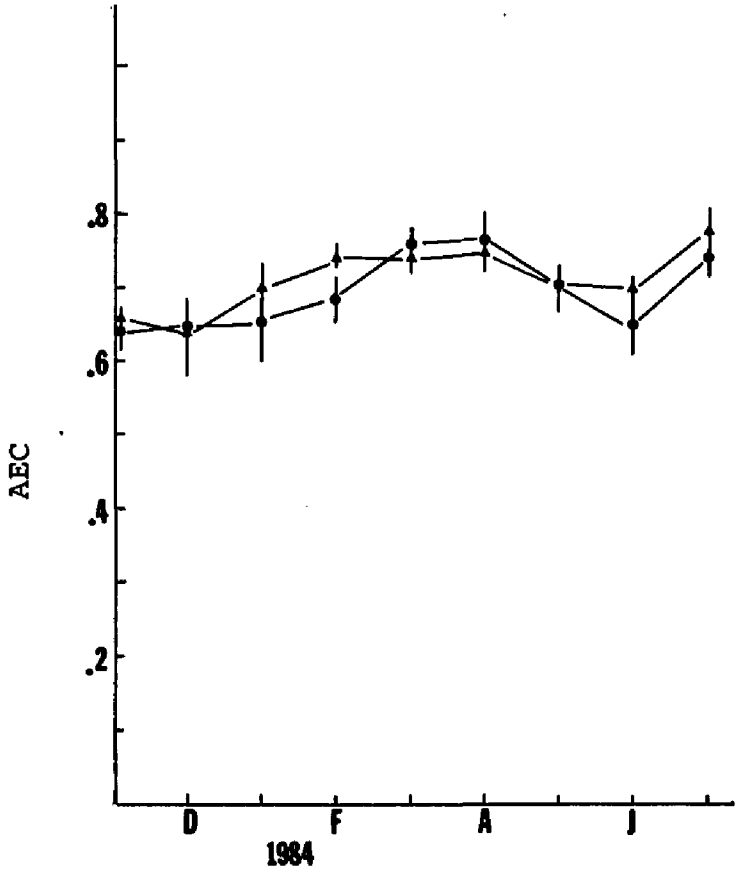


Table 4. One-way analysis of variance: comparison of proteins, lipids, carbohydrates, and AEC between Orchard Beach and Breezy Point *Littorina littorea* (Nov. 1983-July 1984).
AEC n=8 biochemicals n=6 (Nov.-March)
n=20 (April-July)
A - head, foot, columellar muscle
B - viscera

	F	df	p
protein A	.698	1,16	>.05
B	.009	1,16	>.05
lipid A	.003	1,16	>.05
B	1.498	1,16	>.05
carbohydrate A	1.008	1,16	>.05
B	.039	1,16	>.05
AEC A	.014	1,16	>.05
B	.690	1,16	>.05

Table 5. Correlation coefficients between adenylate concentrations, AEC, and gross biochemical compositions of Littorina littorea based on monthly samples during 11 months (OB) and 9 months (BP). Underlined values represent significant ($p < .05$) correlations.
A - head, foot, columellar muscle B - viscera

Orchard Beach

		ATP	ADP	AMP	Lipid	Carb.	Prot.
AEC	A	.279	-.538	-.639	-.478	-.029	.409
	B	-.299	-.539	-.299	.501	.442	-.006
ATP	A		-.131	.155	-.090	.300	.191
	B		-.157	.307	.540	.424	-.171
ADP	A			.360	.601	.025	-.240
	B			.296	-.021	.002	-.236
AMP	A				.418	.399	-.377
	B				.156	.536	-.519
Lip.	A						-.640
	B						-.381
Car.	A						-.506
	B						-.606

Breezy Point

		ATP	ADP	AMP	Lipid	Carb.	Prot.
AEC	A	.607	.271	-.885	.210	.115	.663
	B	.492	.438	-.585	-.478	.025	.202
ATP	A		.757	-.306	-.066	-.096	.581
	B		.690	.280	-.116	-.017	-.159
ADP	A			-.033	-.180	.089	.182
	B			-.099	-.214	.179	.303
AMP	A				.341	-.065	-.504
	B				.399	.138	-.413
Lip.	A					.354	-.238
	B					.466	.232
Car.	A						-.223
	B						.673

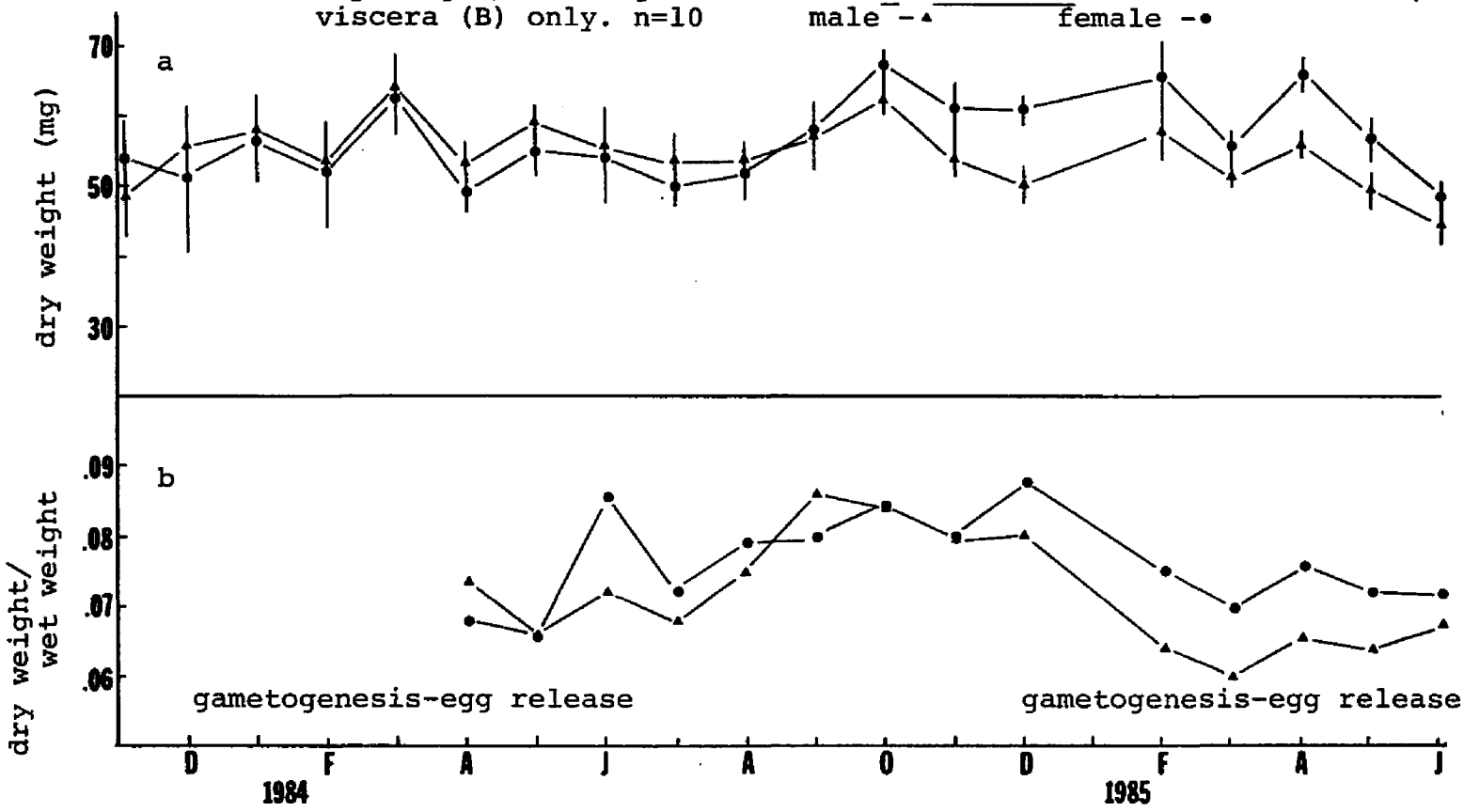
population were there any significant positive correlations; e.g. carbohydrate-protein (B) and ATP-ADP (A,B). Significant negative correlations included OB: AEC-AMP (A), protein-lipid (A), protein-carbohydrate (B), AEC-AMP (A); BP: AEC-AMP (A). The AEC-AMP (A) correlation shared between the two populations indicates that seasonal variation in AEC values may be reflected by the AMP variations. A similar finding was reported by Skjoldal and Barkati (1982), but this was not related clearly to the overall annual reproductive cycle.

Orchard Beach: November 1983-June 1985

Dry weights for the head, foot, columellar muscle (component A) and the viscera (component B) are presented in Figure 12a. Mean dry weights range between 45 and 68 mg for 16 to 19 mm snails collected over the 19 month period. These values do not exhibit any definite seasonal trends, although there is a slight reduction in dry weight that coincides with the period of reproduction. The patterns exhibited by components A and B reflect the almost parallel changes in the dry weights of the viscera and head-foot-columellar muscle. From December 1983 through August 1984, component B is less than A. Starting in September 1984, the average dry weights of B exceed A. The mean dry weight/wet weight ratios of male and female Littorina littorea (component B, viscera) are presented in Figure 12b (April 1984-June 1985). These ratios indicate a reduction

Figure 12a. Dry weights (mean) of Littorina littorea from Orchard Beach, vertical bars indicate ± standard error. n=6 11/83-2/84, n=20 4/84-6/85
▲A - head, foot, columellar muscle •B - viscera

12b. Dry weight/wet weight ratios of L. littorea from Orchard Beach, viscera (B) only. n=10 male -▲ female -•



of dry weight occurring during the reproductive period and an increase following the reproductive phase.

The gross biochemical components exhibit significant differences among months ($p < .05$; Table 6, Figs. 13,14,15). These components display varying patterns attributable to season, with changes in protein composition the least explainable in terms of the release of gametes. The protein content of component A is slightly higher than the other (B) but both display similar patterns over the 19 months (Fig. 13). The greatest percent protein values occurred during March through July 1984 and June 1985. Proteins increased, with the exception of March 1984, during the reproductive period in 1984 (April through July) and in 1985, increased in March and again showed an increase from April through June. Protein composition increases during the period of reproduction followed by a reduction.

The similar patterns of carbohydrate composition in components A and B indicate a reduction through the winter and summer months (Fig. 14). The highest values occurred during September, October, and November 1984 in component B with a slightly reduced concentration in component A. The curious peak in carbohydrate A that occurred in April 1985 is the one point where the patterns are most different. Figure 14 clearly displays the increase in carbohydrates that occurs between August and November 1984 and the subsequent reduction in carbohydrate composition. The reduction

Table 6. One-way ANOVA: comparison of carbohydrates, proteins, and lipids among months of Littorina littorea from Orchard Beach.
n=6 Nov. 1983-March 1984
n=20 April 1984-June 1985
A - head, foot, columellar muscle B - viscera

		F	df	p
carbohydrates	A	5.21	18,67	<.05
	B	11.951	18,67	<.05
protein	A	14.82	18,67	<.05
	B	17.55	18,67	<.05
lipid	A	6.63	18,67	<.05
	B	3.23	18,67	<.05

Figure 13. Orchard Beach: Percent protein (mean) of Littorina littorea, vertical bars indicate ± standard error. n=6 Nov. 1983-March 1984
n=20 April 1984-June 1985
▲A - head, foot, columellar muscle
•B - viscera

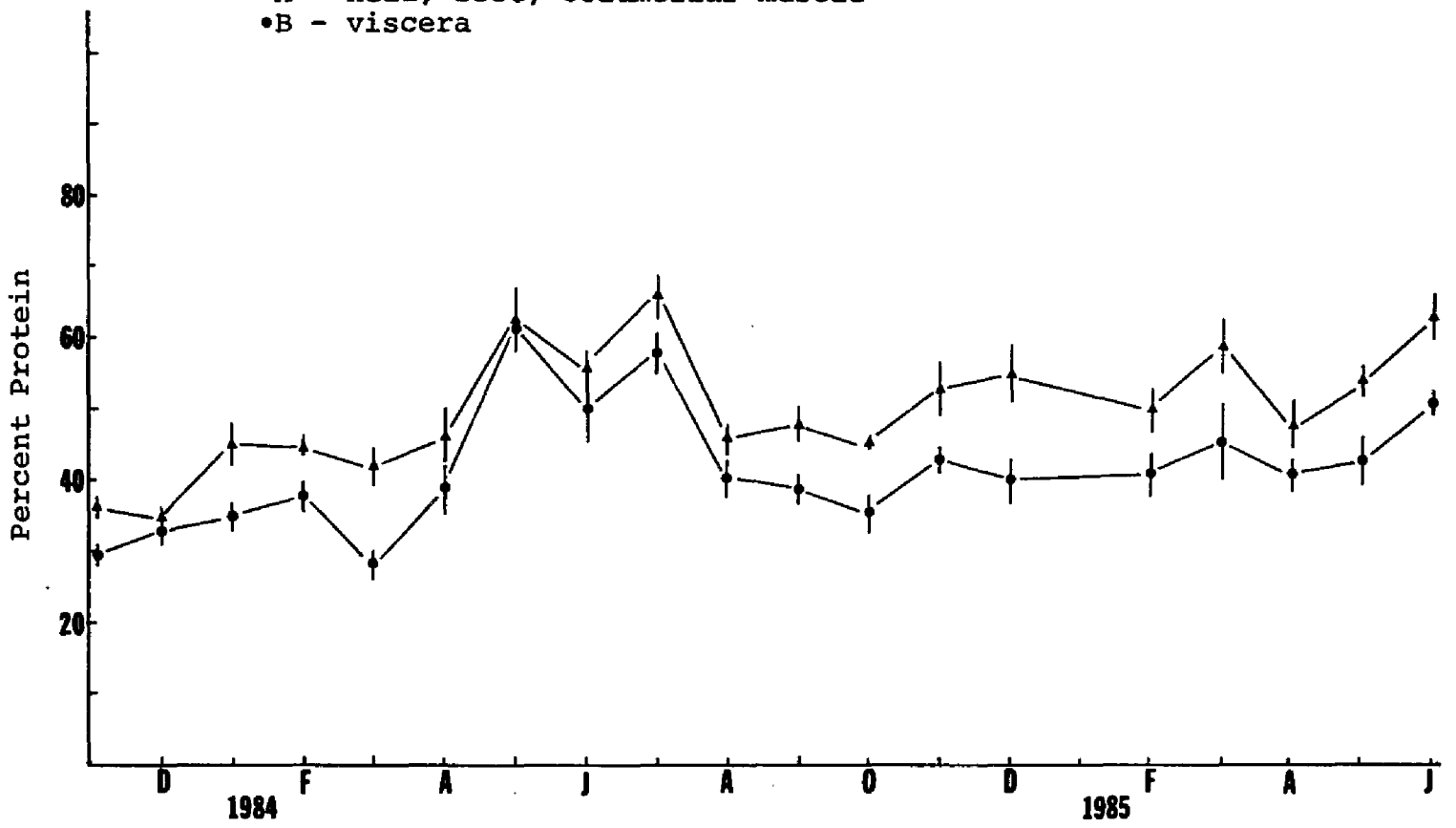


Figure 14. Orchard Beach: Percent carbohydrate (mean) of Littorina littorea, vertical bars indicate ± standard error. n=6 Nov. 1983-March 1984
n=20 April 1984-June 1985

▲A - head, foot, columellar muscle
●B - viscera

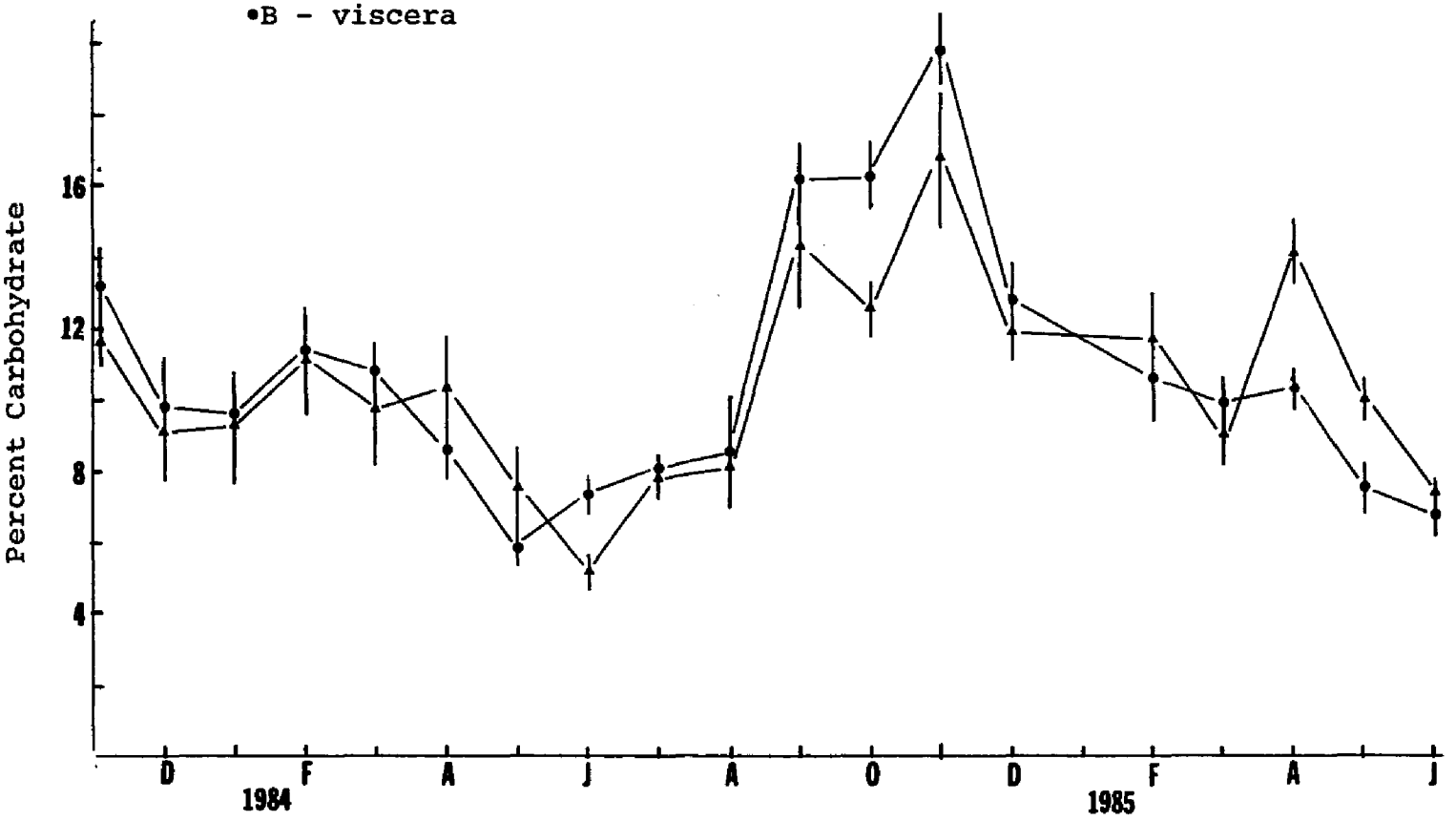
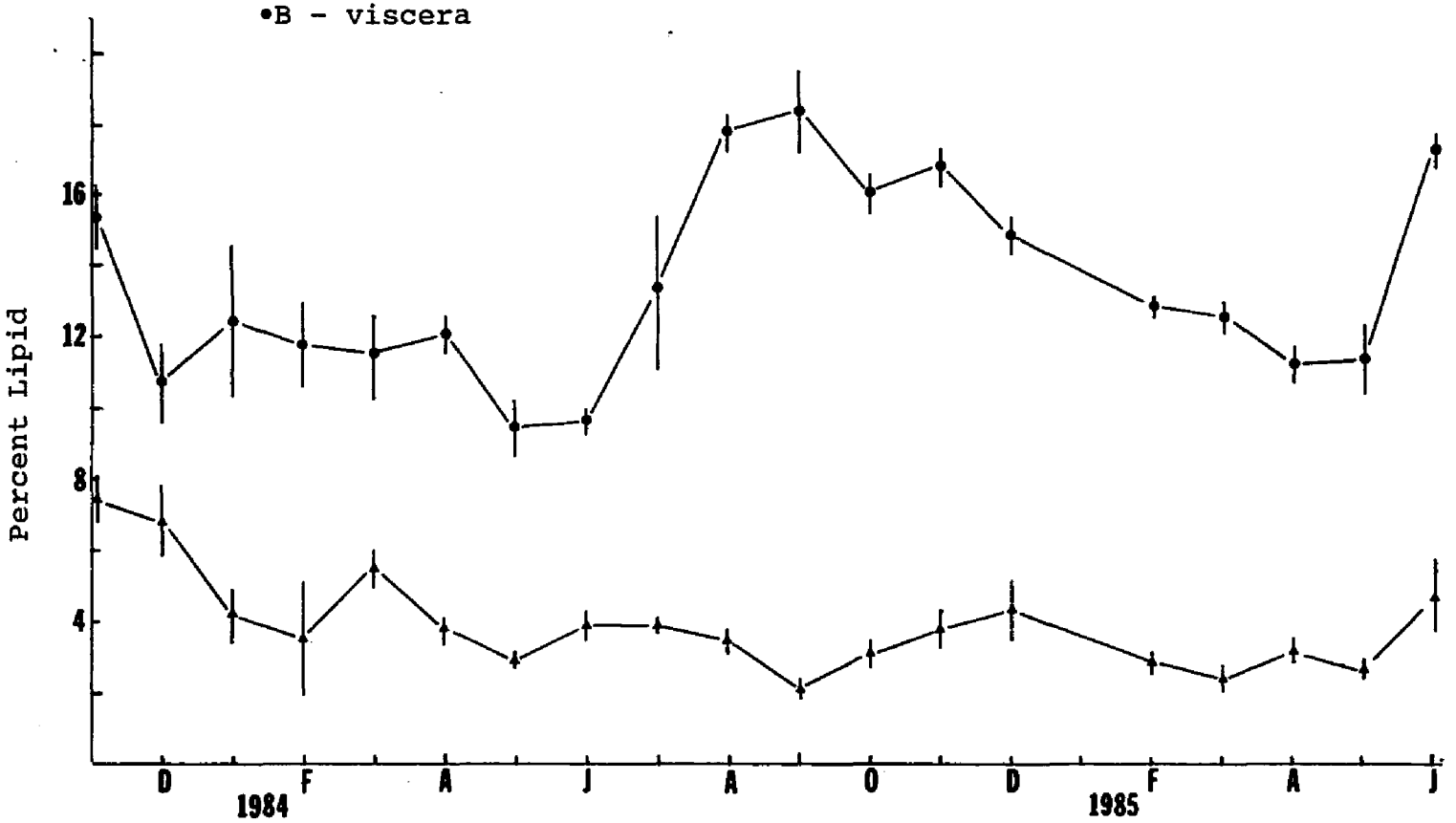


Figure 15. Orchard Beach: Percent lipid (mean) of Littorina littorea, vertical bars indicate ± standard error. n=6 Nov. 1983-March 1984
n=20 April 1984-June 1985
▲A - head, foot, columellar muscle
•B - viscera



in carbohydrates is also apparent after November 1983. The increases and decreases in carbohydrates clearly coincide with gametogenesis, fertilization, and storage of nutrients (see discussion).

The percent lipid values of component B are greater than those in A throughout the 19 months period (Fig. 15). During the period of January through May 1984 and November 1984 through April 1985, a decrease occurs in B, which coincides with gametogenesis and the release of gametes. Similar but less extensive changes occur in component A during 1984 and from December through March in 1985. The highest lipid content in A occurred during November-December 1983 and March 1984. The lowest lipid in B (Sept. 1984) occurred during the same period as the highest lipid in A. It appears that lipid A is not as closely coordinated to the reproductive cycle as B, unlike the situation with the carbohydrates.

The carbohydrate to protein ratio is employed as an indicator of carbohydrate storage and use and has been utilized most recently by Hawkins et al. (1985). The ratios presented for OB (Fig. 16) were calculated from mean monthly percent compositions of carbohydrate and protein. Components A and B follow similar patterns with B exhibiting the most dramatic changes. Figure 16 illustrates the reduction of carbohydrates (relative to protein) during the period of reproduction and the subsequent increase. The lipid/protein ratios (Fig. 17) were also

Figure 16. Orchard Beach: Carbohydrate/protein ratio of Littorina littorea.
▲A - head, foot, columellar muscle
●B - viscera

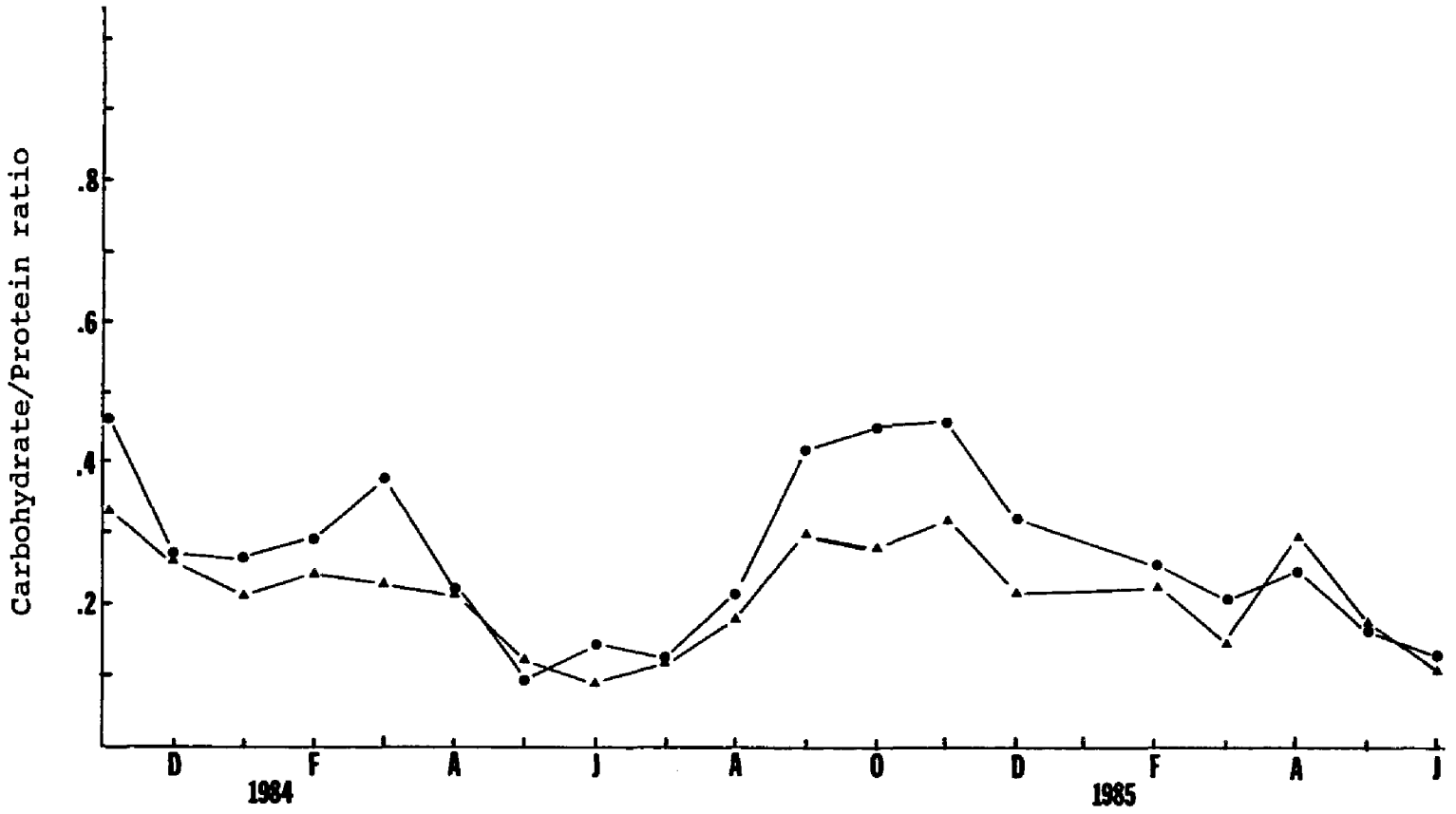
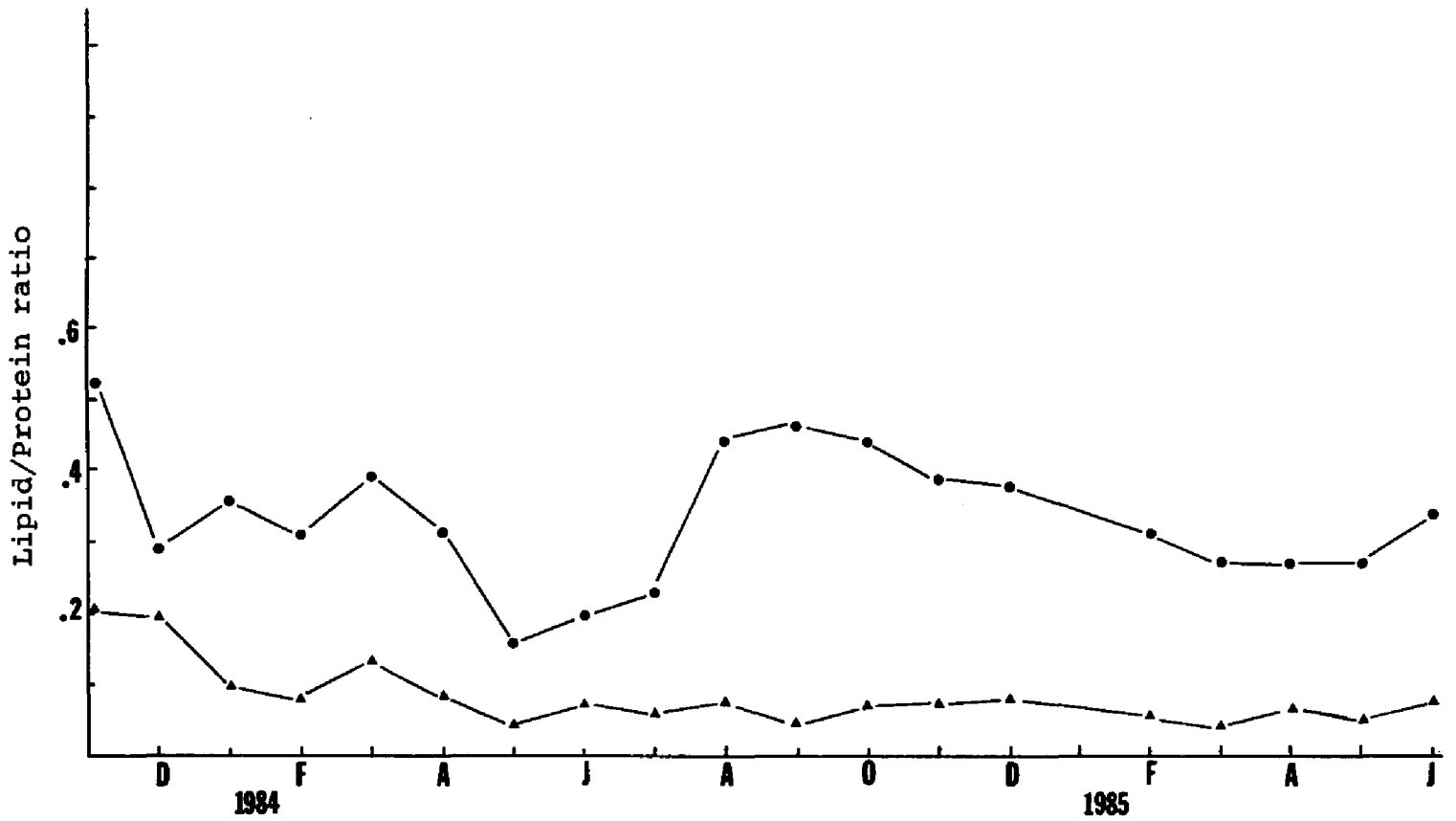


Figure 17. Orchard Beach: Lipid/protein ratio of Littorina littorea.

▲A - head, foot, columellar muscle
●B - viscera



calculated and graphed assuming lipid storage and use is implied in the same manner as the carbohydrate/protein ratio. The monthly lipid/protein ratios of B resemble the pattern of carbohydrate ratios; this pattern does not occur in the lipid ratios of component A (head, foot, columellar muscle).

Adenylates and AEC

Individual adenylates and the adenylate energy charge (AEC) compared over the 19 months displayed statistically significant among-month differences, as did the gross biochemical components (Tables 6,7). Seventy-four percent of the 19 monthly mean AEC values were between 0.7 and 0.85 (Fig. 18). Only November and December 1983, June 1984, February 1985, and April 1985 fell below 0.7. The AEC of components A and B were similar over the 19 months. No seasonal pattern related to the reproductive cycle is evident in the AEC of Littorina littorea. June 1984 and April 1985 were the only reproductive months that fell below 0.7 AEC. During December 1983 and February 1985, the AEC reached or fell below 0.55.

The ATP values for component A were similar to those in component B, with B generally exhibiting the lower concentration (Fig. 19). The recorded ATP values loosely followed the seasonal reproductive pattern during brief periods in 1984 (May, June) and 1985 (Feb., March, April), where ATP concentration decreased from the previous months.

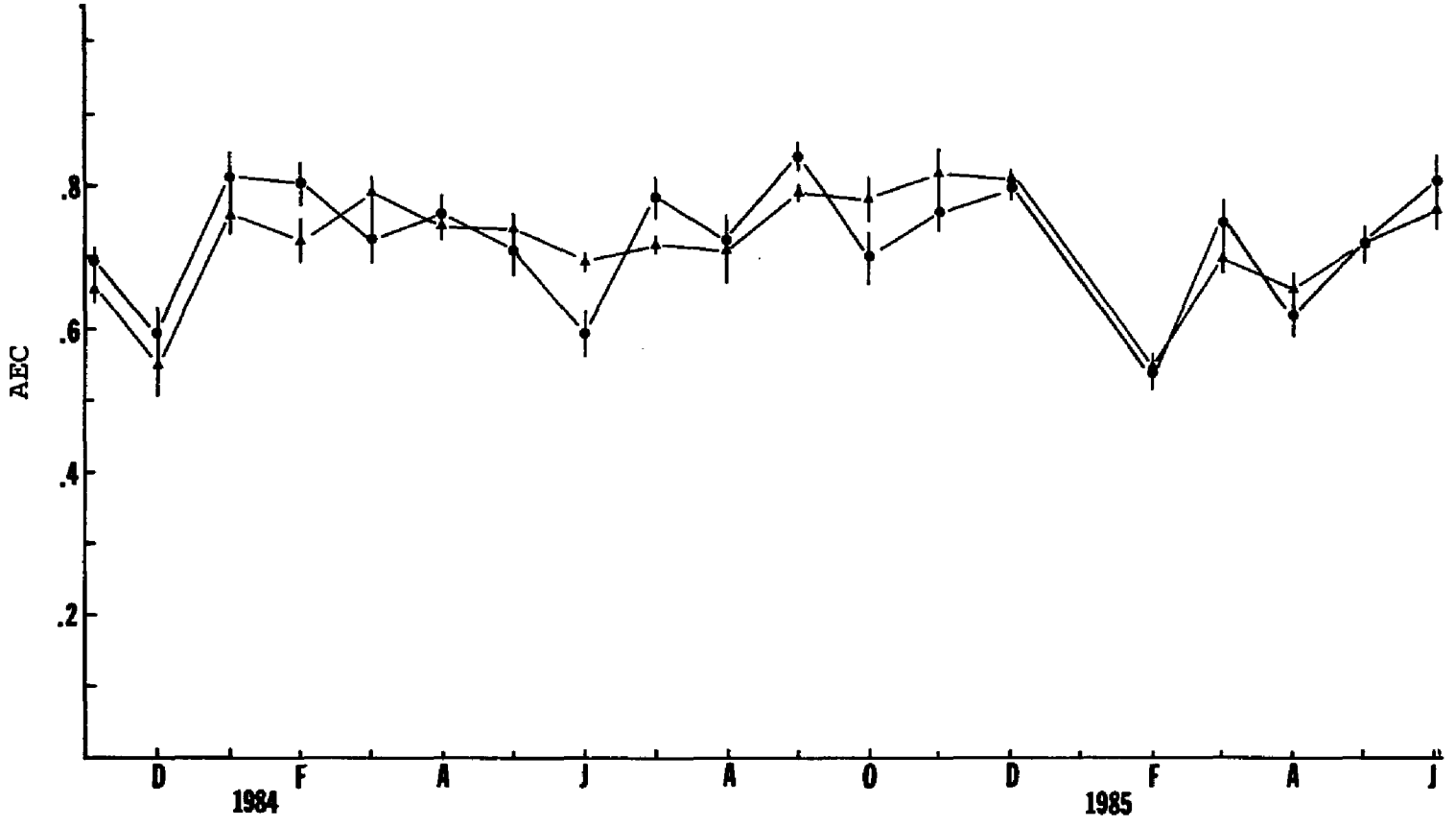
Table 7. One-way ANOVA: comparison of AEC and adenylates among months of Littorina littorea from Orchard Beach.

A - head, foot, columellar muscle
B - viscera

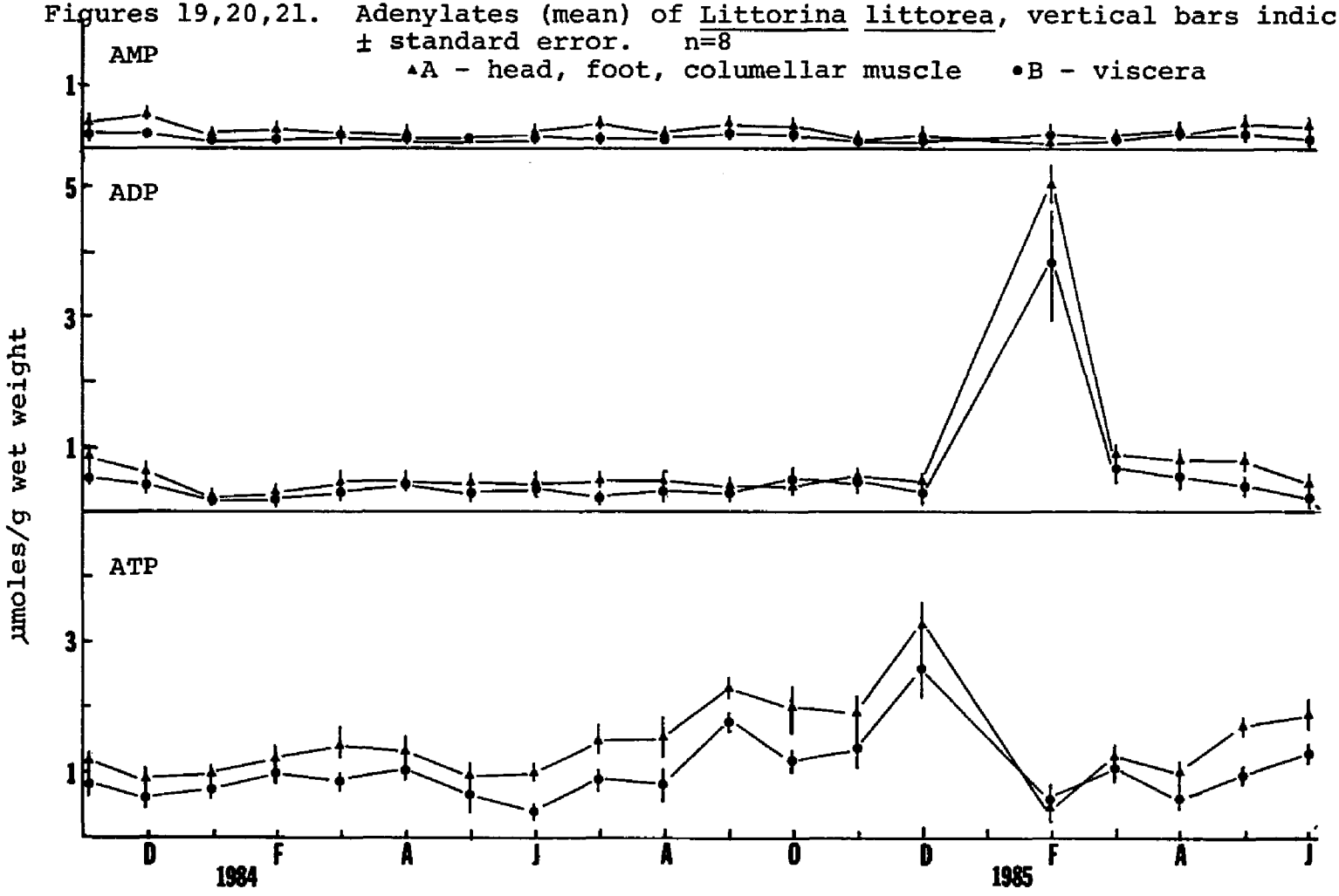
		F	df	p
AEC	A	4.33	18,132	<.05
	B	6.39	18,132	<.05
ATP	A	9.82	18,132	<.05
	B	6.28	18,132	<.05
ADP	A	142.37	18,132	<.05
	B	20.40	18,132	<.05
AMP	A	10.46	18,132	<.05
	B	2.15	18,132	<.05

Figure 18. Orchard Beach: AEC (mean) of Littorina littorea, vertical bars indicate ± standard error. n=8 Nov. 1983-June 1985.

▲A - head, foot, columellar muscle
●B - viscera



Figures 19,20,21. Adenylates (mean) of Littorina littorea, vertical bars indicate ± standard error. n=8
▲A - head, foot, columellar muscle ●B - viscera



December 1983 represents another period of low ATP concentration, which sharply contrasts with December 1984, the highest recorded mean ATP concentration.

Like ATP, the ADP concentrations of component A resembled component B (Fig. 20). The extremely high value recorded during February 1985 is very uncharacteristic of the other 18 months. The exact cause of the high February ADP value is unknown. In general, the ADP level remained relatively constant throughout the study period. Of the adenylates, the monthly AMP concentrations (Fig. 21) remained most consistent over the period. No seasonal trends occurred in the ADP and AMP concentrations of Littorina littorea.

Correlation coefficients (Table 8) were determined for all possible combinations of AEC, adenylates, and gross biochemical components. Significant correlations ($p < .05$) existed between AEC and ATP (A,B), ADP (A,B), and lipid (B). ATP-lipid B and ATP-carbohydrate B were also significantly correlated ($p < .05$).

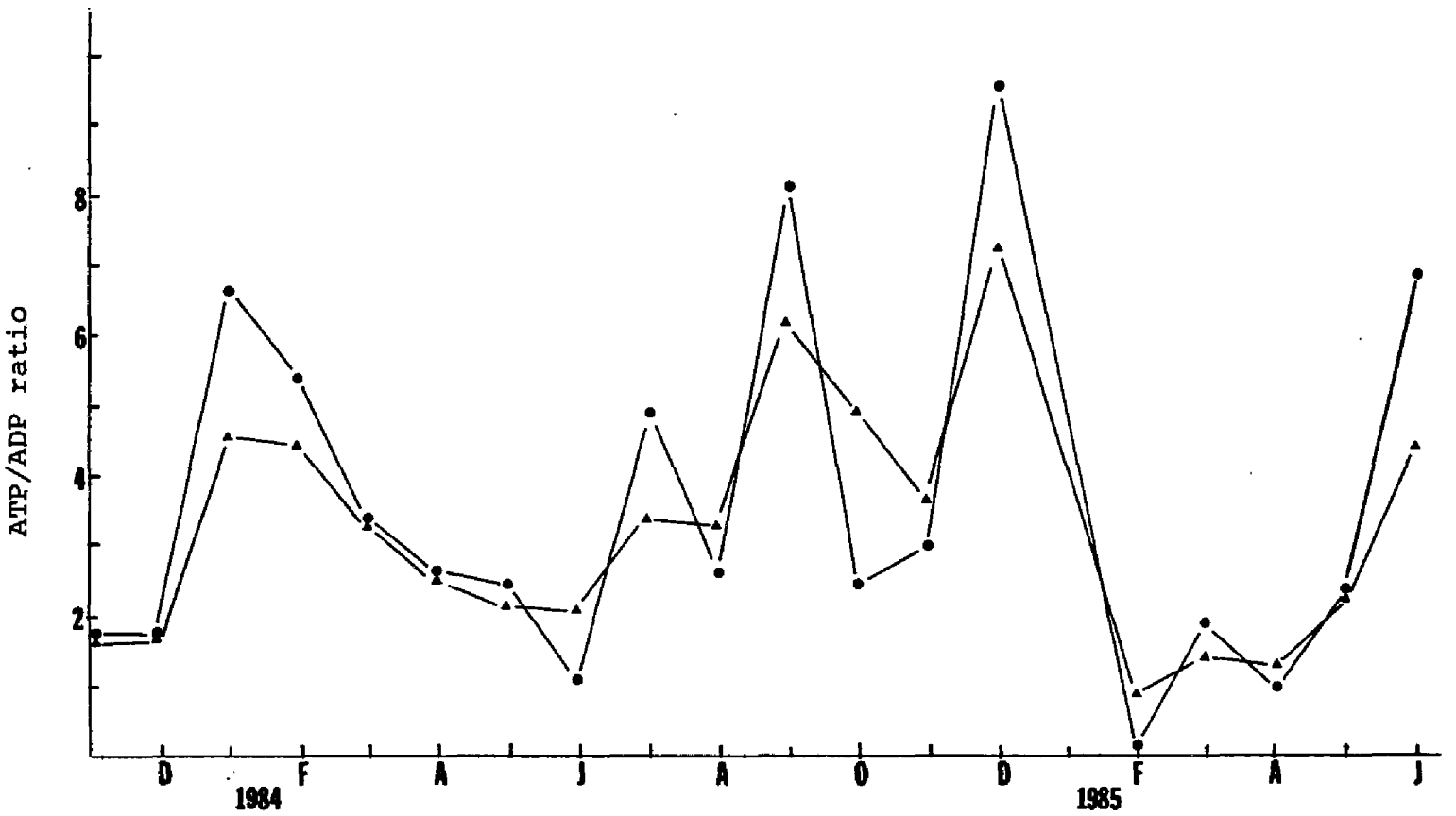
ATP/ADP ratios were determined from the monthly OB data (Fig. 22). This ratio is considered to reflect the metabolic status of a cell (Beis and Newsholme 1975; see also Ivanovici 1980b and Zaroogian et al. 1982). Important components of this pattern exhibited in Figure 22 are the depressed values recorded during the period of gametogenesis and gamete release. During 1984, low values occurred from March through June; for 1985, low values were recorded

Table 8. Correlation coefficients between adenylate concentrations, AEC, and gross biochemical composition of Littorina littorea based on monthly samples (OB, Nov.1983-June 1985). Underlined values represent significant ($p < .05$) correlations.

A - head, foot, columellar muscle B - viscera

		ATP	ADP	AMP	Lipid	Carb.	Prot.
AEC	A	.728	.532	-.093	-.210	.205	.304
	B	.696	.552	-.504	.500	.186	.093
ATP	A		-.384	.144	-.131	.340	.209
	B		-.192	-.267	.546	.499	-.066
ADP	A			.151	-.209	.140	-.001
	B			.420	-.060	.061	-.037
AMP	A				.268	-.020	-.252
	B				.110	.315	-.365

Figure 22. ATP/ADP ratio of Littorina littorea from Orchard Beach. n=8
▲A - head, foot, columellar muscle ●B - viscera



from February through May. During 1984, eggs were not collected until April, which is approximately one month later than in 1985. Low values were also recorded during other brief periods, but it appears that the extended low levels of ATP relative to ADP are consistent with gamete release. The depressed ATP, lipid, and carbohydrate concentrations overlap during the reproductive period (Figs. 16,17,22), which is further corroborated by the significant correlations recorded between ATP-lipid B and ATP-carbohydrate B. Figure 23 is presented for comparison of the ATP/ADP and the carbohydrate/protein ratios. Depressed ATP/ADP and carbohydrate/protein ratios occur from March 1984 through June 1984 and February 1985 through May 1985.

Effects of sublethal doses of hydrocarbon

Animals collected during March, April, and May 1985 were dosed for a period of three weeks or six weeks following one week of acclimation to the laboratory. Mean AEC values of dosed and control animals often exceeded the AEC values of field animals from which the laboratory animals were originally sampled (Fig. 24). The preliminary February dosing experiment (results) is included in Figure 24 to illustrate the increase in AEC that generally occurred after the animals were maintained in the laboratory for four weeks. The 1 ppm concentration of benzene used did not affect the behavior of the animals nor their ability to release eggs in the laboratory. Dosed animals

Figure 23. Comparison of seasonal changes in viscera of Littorina littorea from Orchard Beach: ATP/ADP vs carbohydrate/protein.

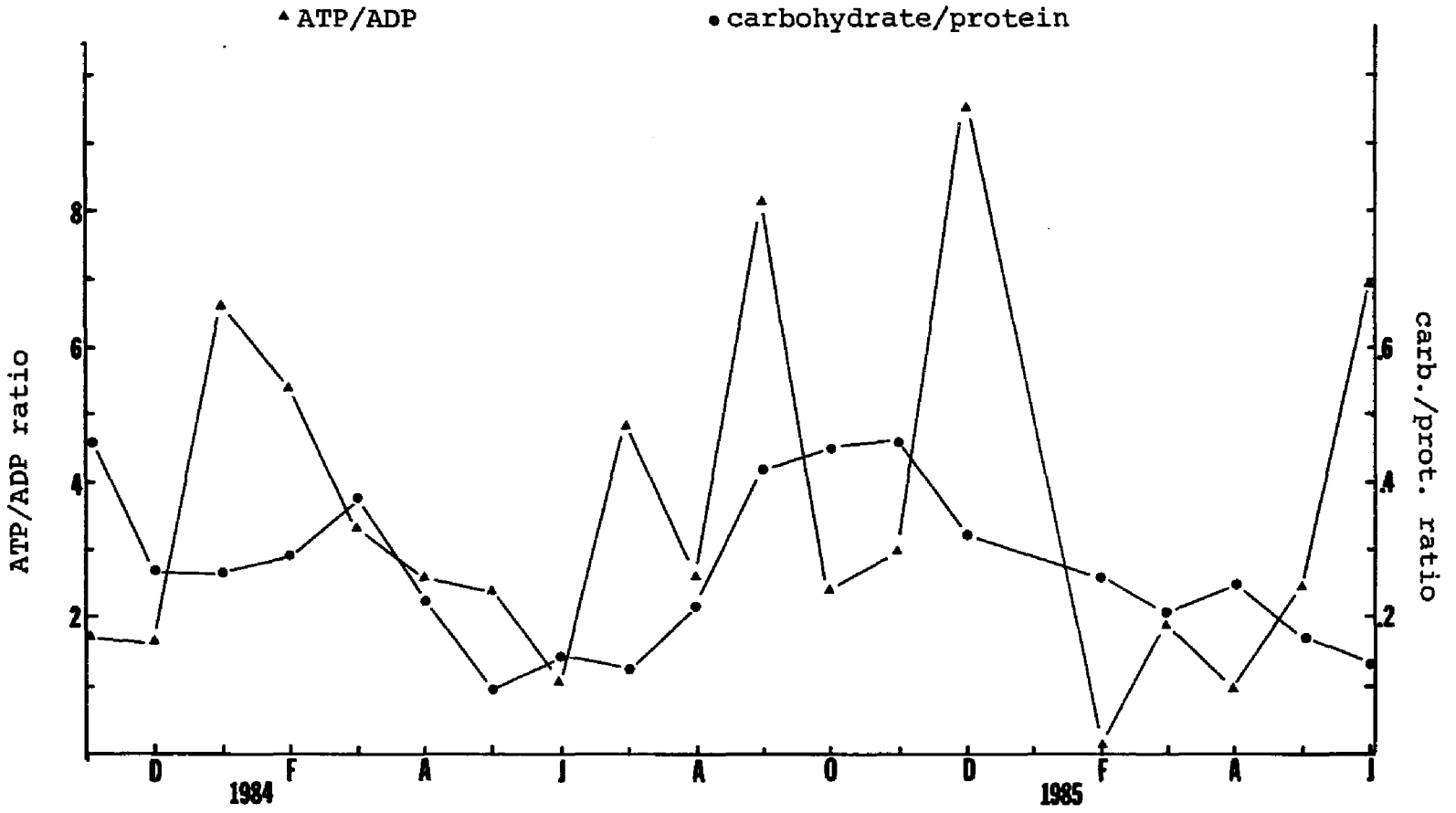
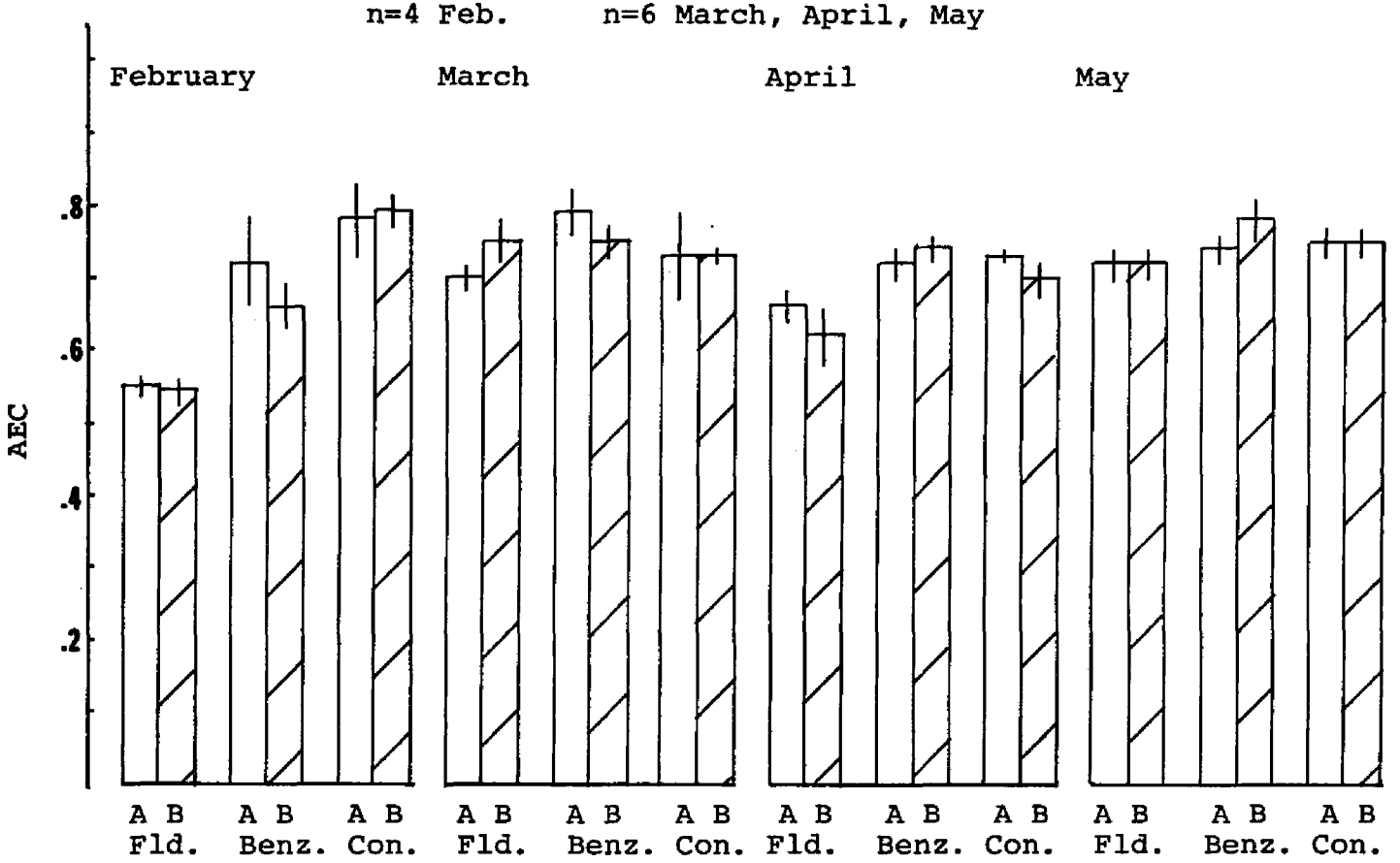


Figure 24. Adenylate energy charge (AEC) of field, benzene dosed, and control Littorina littorea from Orchard Beach collected in February, March, April, and May 1985, vertical bars indicate ± standard error.

A - head, foot, columellar muscle B - viscera
n=4 Feb. n=6 March, April, May



did not appear to act any differently than control animals. The dosages used in the experiments were an order of magnitude greater than the benzene concentrations occurring in the natural environment of Littorina littorea from Orchard Beach (based on NYC DEP sampling sites within 3.4 miles of OB, waters and sediments). Eggs released by experimental animals showed signs of cleavage, as did eggs collected from control animals.

The data collected for the 3 months were pooled after determining between month differences were not significant ($p > .05$; appendix). Lipid and protein content of dosed animals (A,B) were not significantly different from control ($p > .05$; Table 9). Although not significantly different (except carbohydrate A), mean lipid and carbohydrate experimental values exceeded control values. Protein values of control animals were higher than experimental animals.

Pooled AEC values did not exhibit a significant difference between experimental and controls ($p > .05$; Table 10). In all cases, the mean adenylates of dosed animals (Table 11) exceeded the control values, with ATP concentrations of component B experimental snails significantly greater ($p < .05$; Table 10) than controls. The AEC values (component A) of the control group exceeded the experimental group by a very slight margin (Table 11). Pooled total adenylates (March, April, May 1985, Table 11) of dosed animals exceeded controls by a slight margin (2.6 vs 2.34 (A) and 1.64 vs 1.45 (B)).

Table 9. One-way ANOVA: comparison of gross biochemical components of benzene dosed and control Littorina littorea from Orchard Beach. March, April, May 1985 pooled, n=30

A - head, foot, columellar muscle B - viscera
D - dosed con - control

		F	df	p
lipid	A D vs con	.326	1,10	>.05
	B D vs con	.847	1,10	>.05
protein	A D vs con	3.033	1,10	>.05
	B D vs con	2.049	1,10	>.05
carbohydrate				
	A D vs con	17.378	1,10	<.05
	B D vs con	3.682	1,10	>.05

Table 10. One-way ANOVA: comparison of AEC and adenylates of benzene dosed and control L. littorea from Orchard Beach. March, April, May 1985 pooled, n=18.

A - head, foot, columellar muscle B - viscera
D - dosed con - control

		F	df	p
AEC	A D vs con	.100	1,34	>.05
	B D vs con	2.779	1,34	>.05
ATP	A D vs con	2.714	1,34	>.05
	B D vs con	5.415	1,34	<.05
ADP	A D vs con	.224	1,34	>.05
	B D vs con	.048	1,34	>.05
AMP	A D vs con	1.504	1,34	>.05
	B D vs con	.947	1,34	>.05

Table 11. AEC and adenylate concentrations of benzene dosed and control Littorina littorea from Orchard Beach: pooled 3 weeks experiments and 6 weeks experiment.

adenylates - µmol/g wet weight

A - head, foot, columellar muscle B - viscera

	A		B	
	Dosed	Control	Dosed	Control
Pooled expts.				
ATP	1.58	1.41	.97	.82
ADP	.71	.67	.49	.47
AMP	.31	.26	.18	.16
Total	2.6	2.34	1.64	1.45
ATP/ADP	2.23	2.1	1.99	1.72
AEC	.75	.76	.74	.73
6 wks. expts.				
ATP	1.45	1.99		
ADP	.53	.6		
AMP	.28	.23		
Total	2.26	2.82		
AEC	.76	.81		

A six week experiment was conducted with animals collected in April 1985. Only adenylates were determined (n=4) for component A because problems with the constant temperature chamber increased the mortality rate leaving only enough animals to assay a reduced number. The AEC for experimental snails was 0.76 compared to 0.81 for control snails (Table 11). Total adenylates of animals dosed for 6 weeks was less than controls, 2.26 vs 2.82 respectively.

The experimental Littorina littorea were not significantly different from the control snails in all categories measured with the exception of the carbohydrate composition of component A and the ATP concentration of component B. In most instances the measured values of dosed animals exceeded the same values of the control animals. This trend was reversed in the longer 6 weeks experiments in that the adenylates of control snails exceeded the experimental snails (with the exception of AMP concentration of control animals).

Discussion

The seasonal patterns of reproduction and nutrient storage-utilization displayed by Littorina littorea from Orchard Beach during this study are similar to those reported for L. littorea from a variety of locations in Great Britain (Fish 1972; Grahame 1973; Williams 1964, 1970). Typically, molluscs accumulate proteins, lipids, and carbohydrates during periods of abundant food supply, ultimately using these stored nutrients for gametogenesis (Gabbott 1983). The molluscan digestive gland is considered to be a primary site of digestion and storage of ingested food (Lambert and Dehnel 1974; Barber and Blake 1981; Sastry and Blake 1971; Vassallo 1973), but its role as a site of storage has not been proven to be the case in all species (Webber 1970). Glycogen cells located in the mantle edge are another area where glycogen (carbohydrate) is stored (Joosse and Geraerts 1983). The actual cues responsible for the apparently prescheduled events of reproduction and nutrient storage-utilization are not completely known but probably include a complex combination of environmental and physiological factors (Barber and Blake 1981; Gabbott 1983; Newell et al. 1982; Webber 1977).

Starvation studies commonly have been used to determine which nutrient pools are drawn from during periods of stress. Emerson and Duerr (1967) reported that in Littorina planaxis, lipids were the main source of stored

energy. They also reported a reduction in all free amino acids except taurine. Results of a starvation study using Thais lamellosa during the fattening stage of the reproductive cycle indicated that this snail employs a lipid-protein oriented metabolism (Stickle and Duerr 1970). Starvation of T. lamellosa immediately after spawning indicated that protein and carbohydrate were used as energy sources (Stickle 1971). Stickle pointed out the importance of knowing the reproductive conditions when such physiological studies are conducted. Glycogen is probably the major carbohydrate reserve in marine gastropods (Blackmore 1969). A 70% reduction in glycogen and a 44% reduction in neutral lipids was reported for Littorina littorea after 14 weeks of starvation (Holland et al. 1975). Although there was no indication in which season this L. littorea starvation experiment was conducted, it confirms the importance of both carbohydrates and neutral lipids as energy reserves.

Orchard Beach and Breezy Point: Nov. 1983-July 1984

The two Littorina littorea populations were not significantly different in biochemical composition, although observations indicated differences existed in population size, size frequencies, and reproductive patterns. The overall carbohydrate pattern of the BP population increased rather than decreased as occurred in the OB population indicating that the carbohydrate reserves of the BP snails were not being fully used for reproduction. A disrupted

reproductive cycle, as occurred at BP during the previous two years (1982,1983), would result in the redirection of energy production from reproduction to maintenance. This would allow the population to maintain adenylates and biochemicals at a level similar to or greater than a reproducing population. Although the exact cause of the disrupted reproductive cycle remains unknown, it had no apparent effect on the ability of the BP snails to release eggs in 1984. The possibility that the disrupted reproductive cycle of the BP population was a result of some form of contamination is quite possible since the BP site is more polluted than OB in terms of total coliforms, fecal coliforms, volatile organic compounds in the sediments (dichloromethane, bnezene, toluene) and in the water column (chloroform, trichloroethylene, carbon tetrachloride) (based on NYC DEP sampling sites, 1983 and 1984, within 3 miles of the BP and OB sites).

The similarities in AEC between the BP and OB populations are unexpected since the BP site is more polluted and would be expected to elicit a significantly reduced AEC in Littorina littorea. Since L. littorea survives in this environment, it suggests that this snail is resistant to pollution stress. Similar findings have been reported for other marine organisms; for instance, no significant differences in AEC were determined for Mytilus edulis collected from a polluted site impacted by sewage treatment effluent and a control site in Narragansett Bay in May and

August 1979 (Zaroogian et al. 1982). The polychaete Nereis diversicolor was collected from a heavily polluted (organic and industrial waste) estuary along the Belgium - Holland border and from a control site in Belgium and assayed for adenylates and AEC (Verschraegen et al. 1985). No significant differences existed between polluted sites or between the pooled polluted sites and the control site. AEC values from the polluted sites ranged from 0.77 to 0.89; the value for the control site was 0.85. Verschraegen et al. (1985) suggested that at polluted sites, only pollutant resistant species will be found and these can maintain a relatively elevated AEC.

It is interesting to note that the gastropod Crepidula convexa collected at a nearby BP site during the same period as my studies proved to be fecund in the laboratory (Ander, personal communication). Unlike Littorina littorea, Crepidula convexa is a filter feeding, protandrous hermaphrodite, which broods from May-September (Matusiak and Fell 1982). The fact that C. convexa collected from Breezy Pt. were able to reproduce suggests either that the two species are differentially sensitive to pollution and react accordingly or that the level of pollution was not an important factor in disruption of the reproductive cycle of Littorina littorea.

If levels of pollution at the different sites had no significant effect on the physiology of the animals, differences in reproductive success may have been the

result of quality or quantity of food. Other studies have shown this to be an important factor. For example, physiological differences in conspecific populations of Mytilus edulis and Scrobicularia plana were attributed to food quality and quantity (Newell et al. 1982; Worrall et al. 1983). Two Long Island populations of M. edulis examined by Newell et al. (1982) displayed a three month difference in the onset of gametogenesis. This disparity was attributed to the temporal and quantitative differences in energy content of the food supply available to the two populations. Fish (1972) investigated the breeding cycles of an estuarine and open coast population of Littorina littorea in Wales. The estuarine population attained maximum spawning activity in January compared to March on the open coast. He indicated that sea and air temperatures were not directly related to maturation and spawning in L. littorea and attributed the differences to the greater nutrient concentration of the estuary.

Littorina littorea from Orchard Beach

The overall similarities of the monthly changes in carbohydrate composition in the muscular component (A) and viscera (B) of Littorina littorea from Orchard Beach indicate that both components function as storage depots. The reduction in carbohydrates at the time of egg release and the subsequent increase during the summer-fall exhibit the classic pattern of seasonal changes in nutrient composi-

tion as it is related to reproduction. Although the overall patterns are similar for 1984 and 1985, between year differences in amplitude and duration do exist. Carbohydrates stored in the viscera are probably used to provide energy for gametogenesis. The mobilization of carbohydrate reserves in component A is more varied and may be associated with maintenance and other non-reproductive activities such as relative amounts or periodicity of locomotion. Williams (1970) reported low carbohydrate values for a British population of L. littorea from February to June. Following this period, the carbohydrate levels rose to a maximum in September and decreased through April; gonad maturation occurred from December to April (Williams 1964, 1970). In the abalone Haliotis cracheroidii, the foot is apparently an important site of glycogen storage (Webber 1970), while the digestive gland does not appear to act as a site of nutrient storage. The foot of the carnivorous gastropod, Thais lamellosa, is probably not an important site of nutrient storage (Lambert and Dehnel 1974; Stickle 1975). In the marine bivalve Argopecten irradians concentricus, the adductor muscle was shown to be an important energy storage site for glycogen and protein and the digestive gland acted as a short term lipid depot (Barber and Blake 1981). Radiotracer studies of Barber and Blake (1985) using ^{14}C suggested the occurrence of active oogenesis by the loss of lipids, carbohydrates, and protein from storage sites and the accumulation of these labeled compo-

nents in the ovaries of A. irradians concentricus.

A seasonal pattern in lipid composition similar to that of the carbohydrates described above is exhibited by the viscera of Littorina littorea from OB but does not occur in component A, the head, foot, and columellar muscle. The molluscan foot is not generally considered a site of lipid storage (Giese 1969 cited in Stickle 1975) so seasonal changes in lipids would not be expected in the foot of L. littorea.

The protein composition of Littorina littorea from OB, unlike that of the carbohydrates and lipids, reached maximum values toward the end of the reproductive period. Since values are given in terms of percent dry weight, actual protein composition may not really vary as greatly as shown. The increase reflects to a certain degree the decrease in dry weight which coincides with reductions in lipid and carbohydrate. The mean dry weights are low during this period of reproduction. Likewise, the decrease in protein composition after July 1984 is due partly to the increase in dry weight associated with deposition of carbohydrate and especially lipid. For these reasons, the protein composition as depicted in the figures during this period are somewhat exaggerated. Although the percent protein appears to contradict the seasonal reproductive pattern, the carbohydrate/protein ratio coincides with the seasonal reproductive changes.

The increase in protein concentration coinciding with

the period of reproduction may simply reveal the accumulation of protein reserves associated with increased availability of food. The decline in protein is partially due to the loss of gametes. Williams (1970) proposed a seasonal turnover in protein correlated with growth and reproduction for Littorina littorea, as he had shown for lipids and carbohydrates. This is not the case in the sexually mature L. littorea from OB examined in this study. The importance of protein as an energy reserve in L. littorea may be minimal, since 14 weeks of starvation produced little change in protein nitrogen or total nitrogen (Holland et al. 1975).

The figures for dry weight, protein, lipid, and carbohydrate content of Littorina littorea from OB for 1984-1985 were higher immediately prior to and during the period of gametogenesis than during 1983-1984. Eggs were collected one month earlier in 1985 than 1984, which suggests that there was a greater supply of stored nutrient. This increase in storage may have caused the earlier gamete maturation and subsequent release of eggs. Other studies of mollusc populations at different sites have indicated the importance of nutrient status to gametogenesis (Fish 1972; Newell et al. 1982). Major periods of gametogenesis correlated with increases in weight of the digestive gland and other tissues of Mytilus edulis suggested to Hawkins et al. (1985) the important relationship between food availability and gamete maturation.

The carbohydrate/protein ratio (c/p) infers the

relative levels of stored carbohydrate available for use as respiratory substrates (Hawkins et al. 1985). When the ratio is low, the carbohydrate reserves are depleted; when the ratio is high, carbohydrate is in abundance. It was assumed that the lipid/protein ratio (l/p) would convey similar implications. Each of these ratios emphasize the seasonal changes in lipids and carbohydrates. The pattern of c/p for components A and B are again similar, indicating parallel patterns of mobilization and storage. The l/p ratio of A is different from that of B, in that A does not show pronounced seasonal changes. This implies that lipid storage and lipid utilization in muscle is not as important a function as in the viscera. These figures provide added evidence for the seasonal patterns and the strong relationship of Littorina littorea's carbohydrate-lipid oriented metabolism to the reproductive cycle since these reduced ratios coincide with the months of reproduction.

Grahame (1973) examined the breeding energetics of a British population of Littorina littorea by determining caloric content of the entire snail. He reported that the decline in calories during the late winter and spring represented the loss of gametes. The caloric loss was always greater in females. For example, in 17 mm animals, caloric loss for females was estimated to be 23% between the January maximum and spring minimum calorific figures; 19% for males. He calculated that approximately 1/3 of the turnover in animal biomass was attributable to gametes.

Results from the population of L. littorea from OB support the seasonal caloric storage-utilization patterns reported by Grahame, in that the reduction in lipids and carbohydrates of the OB snails coincide with the decline in calories of Grahame's L. littorea.

Adenylates and AEC: Littorina littorea from Orchard Beach

Although significant among-month differences existed in AEC and adenylate concentrations, there were no obvious seasonal patterns such as those displayed by the gross biochemical components. Mean AEC values less than 0.7 occurred during the spring, summer, and winter; thus they were apparently not associated with any particular seasonal event such as reproduction. Littorina littorea is apparently capable of adjusting its adenylate pool to maintain the AEC above 0.7 throughout most of the year.

Examination of the relationship between reproduction and AEC in a variety of aquatic invertebrates has produced conflicting reports. Ivanovici (1980a) suggested that AEC appeared to be independent of season for the gastropod Pyrazus ebeninus, based on data collected in July and October. In a ten week study of Mytilus edulis, Zaroogian et al. (1982) determined that reproductive condition had no significant effect on the adenylates, nor did it significantly alter the response of AEC to stress. However, in the same paper, they reported a low AEC value (0.42, control) for Crassostrea virginica, which they feel may

have been the result of stress from spawning. In two species of freshwater clams, Corbicula fluminea and Anodonta imbecillis, individual adenylates, total adenylate concentration, and AEC varied seasonally and were closely related to the reproductive periods (Giesy and Dickson 1981). Minimum adenylate concentrations and total adenylates corresponded to the expected period of maximum reproduction in C. fluminea. In A. imbecillis, the AEC increased prior to the period of maximum egg development and decreased during reproductive activity.

In a study of the reproductive cycle of Mytilus edulis, Skjoldal and Barkati (1982) reported significant negative correlations between AEC-ADP and AEC-AMP and a positive significant correlation between ATP-carbohydrate and ATP-caloric content; AEC was not correlated to ATP. Giesy and Dickson (1981) suggested that a lack of correlation between AEC and ATP may be more mathematical than biochemical, i.e., ATP appears in the numerator and denominator of the AEC equation. In agreement with the work of Zaroogian et al. (1982), Skjoldal and Barkati (1982) reported that no clear cut relation between AEC and the annual reproductive cycle existed. The highest AEC value occurred after spawning in July, while the lowest values were recorded in September, October, and April. These depressed values were the result of increased levels of AMP. Significant seasonal changes in ATP concentration were related to the overall annual nutritional and reproductive cycle.

For Littorina littorea from Orchard Beach, significant positive correlations existed between AEC-ATP (A,B), AEC-ADP (A,B), AEC-lipid (B), ATP-lipid (B), and ATP-carbohydrate (B). AEC-AMP (B) was significantly negatively correlated. The only correlations common to Mytilus edulis (Skjoldal and Barkati 1982) and L. littorea (OB) are the positive correlation which existed between ATP-carbohydrate and the negative AEC-AMP correlation. This simply indicates the respective association of ATP-carbohydrate and AEC-AMP, i.e., both followed similar qualitative patterns. When ATP levels are high, AEC approaches one; when AMP dominates the adenylate pool AEC approaches 0 (see Rainer et al. 1979). The positive correlation between AEC-ATP and the negative correlation between AEC-AMP agree with this. The positive ATP-carbohydrate and ATP-caloric content correlations for M. edulis (Skjoldal and Barkati 1982) and the positive ATP-lipid (B) and ATP-carbohydrate (B) of L. littorea demonstrate the positive association of ATP with the viscerally stored nutrients and the reproductive cycle.

Animals assayed during February 1985 possessed extremely high ADP concentrations and very low AEC. A possible explanation may be that the extremely cold air temperatures prior to the day of collection (National Weather Service and NOAA) may have essentially shut down the animals' ability to synthesize ATP, resulting in an ADP surplus. Only on two occasions did the daily mean tempera-

ture rise above freezing during the week prior to collection (National Weather Service). The standard deviation of ADP for this month was much higher compared to other months, indicating not all animals were similarly affected.

The two prolonged periods (3-6/84 and 2-5/85) during which the ATP/ADP ratios were relatively depressed coincided with the reproductive period. This suggests that the ATP demand exceeded supply throughout this period. This pattern is very similar to that displayed by the lipids (B) and carbohydrates. During reproduction it is presumed that the decrease in lipids and carbohydrates not only reflects production and release of gametes but the mobilization of these reserves for the eventual conversion to ATP. The ATP is then utilized for the myriad of physiological events associated with this period of the animal's life cycle.

Benzene effects: Littorina littorea from Orchard Beach

The unexpected results of the laboratory dosing experiments, i.e., 1 ppm benzene over a three week period had no significant effect on the AEC of L. littorea, may be due to several factors. After the Littorina littorea were placed in the dosed media, more often than not the snails crawled up the sides of the experimental vessel to a location above the water line, thus escaping the hydrocarbons. This behavior was also common to the control L. littorea maintained in the laboratory. In effect, the majority of experimental animals were subjected to dosing for several

hours rather than the entire day. Possibly a longer period of dosing, as displayed in the six weeks experiment, would be necessary to elicit an effect. Stickle et al. (1984) found the molluscs Thais lima and Mytilus edulis to be very tolerant to short-term oil exposure. A decline in tolerance was associated with the duration of exposure, which suggested to those authors that short term bioassays were not an accurate indication of an invertebrate's sensitivity to chronic oil exposure. Alternatively, the time of year (season) the animals are collected, i.e., animals dosed during gametogenesis may elicit a different response from those collected and dosed before or after. Obviously L. littorea is a very hardy species and is able to cope with low levels of perturbation, which is expressed by a slight elevation in the level of adenylates. This seems to be a logical possibility because of the harsh environment in which this species lives. L. littorea has been shown to be well adapted to the intertidal environment specifically in terms of its ability to regulate its metabolism under a fairly wide range of environmental conditions (McMahon and Russell-Hunter 1977; Newell and Pye 1970).

Lipid and carbohydrate composition of experimental Littorina littorea (OB) exceeded controls for both components although carbohydrate (A) was the only component exhibiting statistical significance. Protein composition of controls (A,B) exceeded the experimental values although not by a significant margin. The possible decreased storage

of protein by the experimental snails may result from an enhanced utilization of protein as a substrate for energy production, as suggested by Bayne et al. (1982). These differences in gross biochemical components may simply reflect the natural variation between animals, but it appears that a pattern does exist. The experimental adenylates exceeded controls, indicating the increased activity of the adenylate pool and probably an increased rate of oxygen uptake, as reported for other molluscan species. The higher lipid and carbohydrate concentrations may indicate a reduction or minimization in use of these nutrient stores by the typical energy extracting pathway in favor of an alternate pathway (see Livingstone 1983).

The adenylate energy charge has successfully reflected the stress of reduced salinities, increased temperatures, and pollutants on a variety of aquatic invertebrates including Corbicula fluminea, Procambarus pubescens, Pyrazus ebeninus, and Saccostrea commercialis (Cantelmo-Cristini et al. 1985; Giesy et al. 1981; Ivanovici 1980b; Rainer et al. 1979). The energy charge of other aquatic invertebrates, for example Procambarus acutus acutus, Mytilus edulis and Nereis diversicolor, for whatever reason, does not appear to be sensitive to environmental perturbation (Dickson et al. 1982; Phelps et al. 1981; Verschraegen et al. 1985; Zaroogian et al. 1982; see also Haya and Waiwood 1983). Littorina littorea can be added to the latter category, at least as it was dosed and assayed

in the present study. The most obvious reason is that L. littorea is very well adapted to its environment and is capable of maintaining metabolic equilibrium even though mildly perturbed.

Summary

1. No significant differences existed between populations of Littorina littorea from Breezy Point and Orchard Beach in terms of gross biochemical composition and AEC during the 1984 reproductive cycle. Snails from both populations released eggs in the laboratory from April to July. The overall patterns of carbohydrate composition differed between the two populations: the OB animals displayed a reduction in carbohydrates from November 1983 through July 1984 while carbohydrate composition of the BP population was much more variable and increased over the same period. The disrupted reproductive cycle of the BP population during the previous two years had no apparent effect on any of the measured biochemical components or the ability of the BP animals to release eggs during 1984. Analysis of the OB population continued from July 1984 through June 1985.

2. Reductions in carbohydrate composition of the head, foot, columellar muscle (A) and the viscera (B) are related to the reproductive cycle in L. littorea from OB. This pattern also occurs in visceral lipid composition. The reproductive cycle begins with gametogenesis in November/December and continues with fertilized egg release from March/April through July. It is during this period that the carbohydrate and lipid reserves are used.

3. Percent protein composition increased during the reproductive cycle. This is related to the reduction in dry weight due to the loss of carbohydrates and lipids. Protein reserves are not used during the reproductive cycle.

4. AEC is independent of season and reproduction. The period of reproduction in L. littorea cannot be considered stressful in terms of AEC. Concentrations of ATP appear to be more closely related to the reproductive cycle than ADP and AMP concentrations.

5. The ATP/ADP ratio is depressed during the reproductive phase, indicating a high demand for ATP during this period.

6. Significant positive correlations exist between: ATP-lipid (B) and ATP-carbohydrate (B).

7. The AEC of L. littorea exposed to 1 ppm benzene for 3 weeks was not significantly different from controls. Adenylate concentrations, lipid and carbohydrate compositions were slightly greater in experimental animals.

7. Protein composition was greater in control L. littorea. Experimental animals released eggs which showed signs of cleavage. It appears that L. littorea is a very resistant organism and that 3 weeks is not a sufficient period of stress.

8. This study of Littorina littorea from Orchard Beach and Breezy Point is the only known analysis of AEC, adenylates, and gross biochemical composition of L. littorea from the northwestern Atlantic.

Appendix

Adenylate analysis: after Ivanovici (1981) with modifications by Hospod and Cristini (personal communication).

Preparation of samples:

1. Sampling and sample storage
   a. snails are returned to the lab and quickly removed from the shell; opercula are removed at this time
   b. the head, foot, and columellar muscle are removed as one piece and compressed between aluminum blocks stored in liquid nitrogen
   c. the remainder of the animal is treated similarly and placed into a Dewar flask filled with liquid nitrogen

2. Extraction and adenylate analysis
   a. tissue is weighed to 0.1 mg
   b. tissue is ground to a coarse powder in liquid nitrogen
   c. 6% perchloric acid (10 µl/mg wet tissue weight) is added and tissue is ground to a fine powder and allowed to defrost on ice
   d. tissue extract is centrifuged (45,000 x g) for 10 min. at 0°C
   e. ATP assay: ATP in the sample phosphorylates glucose to glucose-6-phosphate (G6P) with hexokinase (HK). G6P dehydrogenase (G6P-DH) acts on G6P reducing NADP+ to NADPH causing an increase in absorbance at 340nm. 1 mole of ATP generates 1 mole of NADPH
      1. 50 mM tris-Cl buffer is pipetted into quartz cuvettes and the sample or 0.5 mM ATP standard is added; the blank consists of the buffer (without NADP+)
      2. G6P-DH is added to each cuvette and mixed
      3. absorbance (A_1) is read against the blank after 5 min. on a spectrophotometer
      4. 0.4 M D-glucose and HK are then added and mixed
      5. the absorbance (A_2) is then read after 15-20 min.
      6. the change in NADPH due to ATP in the sample is reflected by $\Delta A_{ATP} = A_2 - A_1$
   f. ADP - AMP assay: employs pyruvate kinase (PK) to catalyze the phosphorylation of ADP by phosphoenolpyruvate (PEP), producing pyruvate and ATP. Lactate dehydrogenase (LDH) then catalyzes the conversion of pyruvate to lactate, oxidizing NADH to NAD+. One mole of ADP in the sample generates one mole of NAD+ resulting in a decrease in absorbance at 340 nm. Myokinase (MK) catalyzes the conversion of AMP (sample) and ATP (buffer) to ADP generating NAD+ by the preceeding reactions. One mole of AMP in the sample produces 2 moles of ADP and 2 moles of NAD+
      1. 50 mM tris-SO_4 buffer is added to each cuvette

2. 0.5 mM ADP + 0.5 mM AMP standard and samples are added separately; the blank consists of the buffer (without NADH) and snail extract
3. LDH is added to each cuvette and mixed
4. after 10 min. absorbance (A_1) is read against the blank
5. PK is added and mixed, A_2 is read after 10 min.
6. MK is added and mixed, A_3 is read after 30 min.
7. $\Delta A_{ADP} = A_1 - A_2$; $\Delta A_{AMP} = A_2 - A_3$

Biochemical analyses: methods represent modified versions of the procedures employed by Lambert and Dehnel (1974) and Emerson and Duerr (1967)

1. snail is removed from the shell; operculum is removed
2. head, foot, and columellar muscle are removed in one piece; this tissue and the remainder of the animal are weighed separately and placed into a vacuum desiccator for a minimum of 3 days
3. tissue is reweighed

Lipids (total):
1. the dried tissue is homogenized in 2 ml of chloroform-methanol (2:1) with a mortar and pestle
2. the homogenized tissue is transferred to a centrifuge tube and centrifuged for approximately 10 min.
3. the pellet is retained for carbohydrate and protein analysis
4. the lipid layer is transferred to a test tube with 1.6% $CaCl_2$ (solution) and separated by shaking
5. after centrifugation, the layer above the lipid is discarded. a wash solution of chloroform methanol (2:1) and 2% $CaCl_2$ (4:1) is carefully layered on the lipid surface. following centrifugation the second wash solution is pipetted off
6. the remaining lipid is poured into a preweighed aluminum pan. 2 ml of diethyl ether is used to rinse out the test tube and added to the aluminum pan
7. the contents of the pan are allowed to evaporate to dryness. the lipid level is expressed as percent lipid/ unit dry weight

Carbohydrates (total hexoses):
1. the pellet (see 3 above) is heated with 4 ml of 80% ETOH
2. this solution is centrifuged for approximately 5 min. and the supernatant is discarded
3. the pellet is heated at 100°C in TCA (10%) for 15 min. to extract the carbohydrate
4. the supernatant resulting from three extractions is placed into a test tube
5. an aliquot is diluted with distilled water and analyzed by the anthrone method

6. anthrone method, modified after Keleti and Lederer (1974)
   a. β-D(+) glucose (100 mg/ml) is used to produce a standard curve
   b. 200 mg anthrone is dissolved in 100 ml conc. H_2SO_4; prepared fresh daily 4 hours prior to use
   c. the aliquot is placed in an ice bath for 45 min.
   d. the anthrone reagent (2 x aliquot) is added dropwise to the test tube while shaking in the ice bath
   e. stoppered tubes are placed into a 92°C water bath for 8 min.
   f. tubes are reimmersed in an ice bath to stop the reaction
   g. solutions are read in a spectrophotometer at 620 mμ

Protein:
1. protein level is determined by the Lowry method (Lowry et al. 1951); bovine albumin provides a standard
2. the tissue is dissolved in 2 ml NaOH (5N) for several hours and diluted with a volume of distilled water
3. a 0.6 ml aliquot is used for protein determination

Equations for calculation of adenylate concentrations and AEC. adenylates - μmol/g wet weight tissue

$$ATP = \Delta A_{ATP} \times AV \times EV / 6.22 \times SV \times TW \times CPL$$

$$ADP = \Delta A_{ADP} \times AV \times EV / 6.22 \times SV \times TW \times CPL$$

$$AMP = \Delta A_{AMP} \times AV \times EV / 6.22 \times SV \times TW \times 2 \times CPL$$

AV = assay volume in cuvette
EV = extract volume
SV = sample volume
TW = tissue weight
CPL = cell path length = 1 cm
6.22 = extraction coefficient
absorbancy at 340 nm and pH 7.6 of a solution of NADPH (or NADH) containing 1 μmol/l

$$AEC = (ATP + \tfrac{1}{2}ADP) / (ATP + ADP + AMP)$$

Orchard Beach: mean gross biochemical composition (± SE) of Littorina littorea.
A - head, foot, columellar muscle B - viscera
Nov. 1983 - March 1984 n=6 April 1984 - June 1985 n=20

month	Protein				Lipid				Carbohydrate			
	A	±SE	B	±SE	A	±SE	B	±SE	A	±SE	B	±SE
N	36	.94	29	1	7.5	.68	15.4	.93	11.7	.58	13.2	.95
D	35	1.06	33	.9	6.9	1	9.7	2.1	9.2	1.38	9	1.43
1984												
J	45	2.6	35	1.7	4.3	.64	12.5	2.3	9.3	1.19	9.5	1.1
F	45	1.5	38	1.5	3.6	1.6	11.9	1.2	11.1	1.42	11.3	.63
M	42	2.3	29	1.4	5.6	.46	11.5	1.2	9.8	1.55	10.9	.8
A	46	2.8	39	3.2	3.9	.15	12.2	.42	10.3	1.4	8.7	.49
M	63	3.7	61	2.5	3	.15	9.5	.65	7.5	1.1	5.9	.28
J	56	2.2	50	3.8	4	.38	9.8	.14	5.2	.4	7.4	.5
J	66	2.6	58	2.5	4	.13	13.3	1.8	7.8	.38	8	.5
A	46	1.5	40	1.8	3.5	.13	17.7	.37	8.2	1	8.6	1.5
S	48	1.9	39	2	2.2	.17	18.1	1.3	14.4	1.5	16.2	1
O	45	.7	36	1.7	3.2	.28	16	.53	12.6	.7	16.3	.7
N	53	3.5	43	1.5	3.9	.49	16.8	.56	16.8	1.9	19.8	1.1
D	55	3.9	40	2.5	4.4	.8	14.9	.5	11.9	.73	12.8	.98
1985												
J												
F	50	2.9	41	2.7	2.9	.2	12.9	.29	11.7	1.25	10.6	1.15
M	59	3.5	46	4.5	2.5	.28	12.6	.36	9	.75	9.5	.6
A	48	3.2	41	.8	3.3	.18	11.3	.43	14.1	.75	10.3	.39
M	54	1.8	43	3	2.8	.15	11.5	.75	10	.53	7.5	.54
J	63	2.7	51	1	4.8	1.15	17.3	.44	7.4	.44	6.8	.47

Breezy Point: mean gross biochemical composition (± SE) of Littorina littorea
A - head, foot, columellar muscle B - viscera
Nov. 1983 - March 1984 n=6 April 1984 - June 1985 n=20

month	Protein				Lipid				Carbohydrate			
	A	±SE	B	±SE	A	±SE	B	±SE	A	±SE	B	±SE
N	31	.92	29	1.3	9.6	2.9	14.7	2.2	11.4	.77	10.1	.77
D	28	1	29	1.9	4.1	.23	12.1	1.8	8.8	1.02	7	.61
1984												
J	41	.77	37	1.2	3.2	.59	13.3	1.6	6.8	.69	6.2	.35
F	44	1.02	34	2.2	10.2	2.5	15.1	.8	11.8	.98	10.9	.49
M	44	1.34	33	1.1	4.9	.37	11.1	1.3	9.1	1.7	7.4	.98
A	37	.91	42	1.8	2.5	.17	11.4	.27	13.1	.54	10.6	.23
M	42	1.1	43	.8	2.9	.06	12.5	.16	10.8	.26	8.8	.11
J	62	2.1	60	1.9	3.6	.04	13.7	.53	9.8	.12	13.9	.63
J	64	1.3	60	1.7	3.6	.07	14.7	.42	8.7	.82	11.1	.09

Orchard Beach and Breezy Point: AEC, ATP, ADP, AMP values (reported as mean ± standard error); n=8, adenylates - µmoles/g wet weight.

Orchard Beach

month		AEC	SE	ATP	SE	ADP	SE	AMP	SE	Total
Nov.	A	.65	.01	1.13	.066	.70	.068	.44	.020	2.27
1983	B	.69	.025	.74	.079	.42	.050	.20	.003	1.36
Dec.	A	.55	.046	.82	.180	.51	.070	.59	.026	1.92
	B	.59	.036	.52	.080	.31	.029	.29	.025	1.12
Jan.	A	.76	.029	.99	.100	.22	.054	.23	.032	1.44
1984	B	.81	.029	.80	.130	.12	.040	.14	.029	1.06
Feb.	A	.72	.029	1.20	.160	.27	.007	.35	.050	1.82
	B	.80	.030	.97	.086	.18	.043	.18	.001	1.33
Mar.	A	.79	.020	1.30	.130	.34	.050	.21	.020	1.85
	B	.72	.029	.73	.080	.22	.030	.20	.029	1.15
Apr.	A	.74	.020	1.26	.070	.49	.044	.27	.039	2.02
	B	.76	.020	1.03	.082	.40	.046	.18	.002	1.61
May	A	.74	.020	.93	.070	.44	.050	.19	.025	1.56
	B	.71	.036	.60	.140	.25	.032	.14	.020	.99
Jun.	A	.69	.013	.94	.068	.43	.046	.31	.036	1.68
	B	.59	.032	.34	.050	.31	.050	.18	.020	.83
Jul.	A	.72	.014	1.47	.140	.43	.063	.40	.029	2.30
	B	.78	.029	.82	.090	.17	.030	.16	.013	1.15
Aug.	A	.71	.046	1.50	.200	.45	.030	.25	.030	2.20
	B	.72	.043	.74	.140	.28	.047	.17	.037	1.19
Sep.	A	.79	.11	2.30	.100	.37	.096	.48	.049	3.10
	B	.84	.022	1.77	.100	.22	.070	.26	.019	2.24
Oct.	A	.78	.032	1.96	.300	.39	.040	.42	.055	2.77
	B	.70	.043	1.14	.160	.36	.130	.29	.057	1.79
Nov.	A	.82	.025	1.87	.200	.51	.107	.20	.036	2.58
	B	.76	.025	1.38	.317	.47	.079	.17	.050	2.02
Dec.	A	.81	.036	2.58	.300	.44	.055	.39	.068	3.40
	B	.80	.063	1.76	.340	.20	.039	.21	.060	2.17
Feb.	A	.55	.010	.47	.062	5	.257	.05	.020	5.50
1985	B	.54	.020	.58	.135	3.9	.775	.29	.132	4.77

month		AEC	SE	ATP	SE	ADP	SE	AMP	SE	total
Mar.	A	.70	.016	1.20	.149	.82	.115	.28	.046	2.29
	B	.75	.030	1.05	.167	.55	.079	.12	.032	1.72
Apr.	A	.66	.019	1.03	.057	.74	.185	.35	.042	2.11
	B	.62	.034	.52	.082	.18	.050	.24	.046	.95
May	A	.72	.020	1.70	.110	.75	.082	.44	.085	2.89
	B	.72	.030	.90	.089	.37	.042	.11	.025	1.38
Jun.	A	.77	.030	1.84	.170	.42	.053	.40	.064	2.66
	B	.81	.030	1.22	.079	.17	.060	.20	.043	1.59
Breezy Point										
Nov.	A	.66	.013	1.4	.14	.63	.08	.58	.053	
	B	.64	.027	1	.096	.4	.07	.45	.061	
Dec.	A	.63	.05	1.03	.14	.32	.053	.5	.032	
	B	.64	.036	.68	.08	.19	.039	.332	.032	
Jan.	A	.7	.029	1.2	.17	.37	.06	.39	.043	
1984	B	.65	.053	.7	.16	.25	.06	.28	.05	
Feb.	A	.74	.018	1.15	.06	.223	.03	.32	.053	
	B	.68	.032	.67	.054	.103	.029	.31	.053	
Mar.	A	.74	.019	1.26	.057	.5	.05	.3	.043	
	B	.76	.02	.98	.096	.35	.032	.18	.018	
Apr.	A	.74	.018	1.33	.07	.53	.05	.29	.034	
	B	.76	.036	.98	.054	.39	.068	.3	.064	
May	A	.7	.029	1.08	.17	.43	.049	.315	.053	
	B	.7	.029	.55	.08	.299	.032	.15	.025	
Jun.	A	.7	.011	1.25	.082	.398	.05	.43	.032	
	B	.65	.032	.62	.05	.3	.06	.28	.05	
Jul.	A	.78	.025	1.97	.12	.65	.14	.257	.05	
	B	.74	.02	.92	.04	.38	.053	.215	.032	

One-way ANOVA: comparison of AEC, ATP, lipids, carbohydrates, and protein of benzene dosed Littorina littorea (OB) collected in March, April, and May (3,4,5) 1985.
n = 10 (lip.,carb.,prot.) n = 6 AEC, ATP
A - columellar muscle, foot, head
B - viscera
D - dosed

		F	df	p
AEC	A 3D vs 4D vs 5D	1.721	2,15	>.05
	B 3D vs 4D vs 5D	.921	2,15	>.05
ATP	A 3D vs 4D vs 5D	1.034	2,15	>.05
	B 3D vs 4D vs 5D	.742	2,15	>.05
lipid	B 3D vs 4D vs 5D	1.662	2,3	>.05
protein	A 3D vs 4D vs 5D	.533	2,3	>.05
carbohydrate				
	A 3D vs 4D vs 5D	.616	2,3	>.05
	B 3D vs 4D vs 5D	4.49	2,3	>.05

Benzene dosed Littorina littorea from Orchard Beach, adenylate concentrations and AEC for animals collected in March, April, May 1985. adenylates - µmol/g wet weight
A - head, foot, columellar muscle B - viscera

Three weeks experiments. n=6

		Dosed				Control			
		A	±SE	B	±SE	A	±SE	B	±SE
March	AEC	.78	.032	.75	.019	.73	.065	.73	.009
	ATP	1.73	.147	.99	.050	1.39	.106	.87	.085
	ADP	.77	.134	.58	.095	.47	.047	.53	.113
	AMP	.22	.093	.20	.046	.19	.027	.16	.014
April	AEC	.72	.019	.74	.014	.73	.006	.70	.023
	ATP	1.45	.134	.9	.072	1.35	.049	.63	.05
	ADP	.8	.067	.6	.057	.86	.089	.44	.065
	AMP	.32	.049	.12	.02	.25	.020	.14	.02
May	AEC	.74	.02	.78	.029	.75	.02	.75	.019
	ATP	1.54	.138	1.02	.065	1.49	.162	.96	.096
	ADP	.55	.04	.38	.053	.69	.053	.46	.055
	AMP	.36	.03	.22	.02	.32	.039	.17	.032

Six weeks experiment. n=4

AEC	.76	.019	.81	.007
ATP	1.45	.24	1.99	.13
ADP	.53	.075	.6	.04
AMP	.28	.042	.23	.019
tot.adenyl.	2.26		2.82	

References

Alifierakis, N.S. and A.J. Berry. 1980. Rhythmic egg-release in Littorina littorea (Mollusca: Gastropoda). J.Zool., Lond. 190:297-307.

Anderson, J.W., J.M. Neff, B.A. Cox, H.E. Tatem and G.M. Hightower. 1974. Characteristics of dispersions and water-soluble extracts of crude and refined oils and their toxicity to estuarine crustaceans and fish. Mar.Biol. 27:75-88.

Atkinson, D.E. 1968. The energy charge of the adenylate pool as a regulatory parameter: interaction with feed-back modifiers. Biochem. 7:4030-4034.

Atkinson, D.E. and G.M. Walton. 1967. Adenosine triphos-phate conservation in metabolic regulation: rat liver citrate cleavage enzyme. J.Biol.Chem. 242:3239-3241.

Ball, W.J. and D.E. Atkinson. 1975. Adenylate energy charge in Saccharomyces cerevisiae during starvation. J.Bacteriol. 121:975-982.

Barber, B.J. and N.J. Blake. 1981. Energy storage and utilization in relation to gametogenesis in Argopecten irradians concentricus (Say). J.Exp.Mar.Biol.Ecol. 52: 121-134.

_____. 1985. Intra-organ biochemical transformations asso-ciated with oogenesis in the bay scallop, Argopecten irradians concentricus (Say), as indicated by ^{14}C incorporation. Biol.Bull. 168:39-49.

Bayne, B.L., J. Widdows, M.N. Moore, P. Salkeld, C.M. Worrall and P. Donkin. 1982. Some ecological consequences of the physiological and biochemical effects of petroleum compounds on marine molluscs. Phil.Trans.R.Soc.Lond.B. 297:219-239.

Beis, I. and E.A. Newsholme. 1975. The contents of adenine nucleotides, phosphagens and some glycolytic intermedi-ates in resting muscles from vertebrates and inverte-brates. Biochem.J. 152:23-32.

Bertness, M.D. 1984. Habitat and community modification by an introduced herbivorous snail. Ecology 65:370-381.

Blackmore, D.T. 1969. Studies of Patella vulgata L. II. Seasonal variation in biochemical composition. J.Exp.Mar.Biol.Ecol. 3:231-245.

Brenchley, G.A. and J.T. Carlton. 1983. Competitive displacement of native mud snails by introduced periwinkles in the New England intertidal zone. Biol.Bull. 165:543-558.

Cantelmo, A., L. Mantel, R. Lazell, F. Hospod, E. Flynn, S. Goldberg and M. Katz. 1982. The effects of benzene and dimethylnaphthalene on physiological processes in juveniles of the blue crab, Callinectes sapidus. In Physiological mechanisms of marine pollutant toxicity (eds. W.B. Vernberg, A. Calabrese, F.P. Thurberg, F.J. Vernberg), pp. 349-389. Academic Press, N.Y.

Cantelmo-Cristini, A., F.E. Hospod and R.J. Lazell. 1985. An in situ study on the Adenylate Energy Charge of Corbicula fluminea in a freshwater system. In Marine Pollution and Physiology: recent advances (eds. F.J. Vernberg, F.P. Thurberg, A. Calabrese, W.B. Vernberg), pp. 45-62. Univ. of South Carolina Press, Columbia, SC.

Dickson, G.W. and J.P. Giesy. 1981. The effect of season and location on phosphoadenylate concentrations and adenylate energy charge in two species of freshwater clams. Oecologia 49:1-7.

Dickson, G.W., J.P. Giesy and L.A. Briese. 1982. The effect of chronic cadmium exposure on phosphoadenylate concentrations and adenylate energy charge of gills and dorsal muscle tissue of crayfish. Environ.Toxic.Chem. 1:147-156.

Emerson, D.N. and F.G. Duerr. 1967. Some physiological effects of starvation in the intertidal prosobranch Littorina planaxis (Phillippi, 1847). Comp.Biochem.Physiol. 20:45-53.

Farrington, J.W. and J.G. Quinn. 1973. Petroleum hydrocarbons in Narragansett Bay. I. Survey of hydrocarbons in sediments and clams (Mercenaria mercenaria). Estuar.Cstl.Mar.Sci. 1:71-79.

Fish, J.D. 1972. The breeding cycle and growth of open coast and estuarine populations of Littorina littorea. J.Mar.Biol.Assoc.U.K. 52:1011-1019.

_____. 1979. The rhythmic spawning behaviour of Littorina littorea (L.). J.Moll.Stud. 45:172-177.

Fretter, V. and A. Graham. 1962. British prosobranch molluscs: their functional anatomy and ecology. 755pp. Ray Society, London.

Gabbott, P.A. 1983. Developmental and seasonal metabolic activities in marine molluscs. In The Mollusca: environmental biochemistry and physiology (eds. K.M. Wilbur and P.W. Hochachka), vol. 2, pp. 165-217. Academic Press, N.Y.

Giese, A.C. 1969. A new approach to the biochemical composition of the mollusc body. Oceanogr.Mar.Biol.Annu.Rev. 7:175-299.

Giesy, J.P. and G.W. Dickson. 1981. The effect of season and location on phosphoadenylate concentrations and adenylate energy charge in two species of freshwater clams. Oecologia 49:1-7.

Giesy, J.P., S.R. Denzer, C.S. Duke and G.W. Dickson. 1981. Phosphoadenylate concentrations and energy charge in two freshwater crustaceans: responses to physical and chemical stressors. Verh.Internat.Verein.Limnol. 21: 205-220.

Gordon, D.C., P.D. Keizer and J. Dale. 1974. Estimates using fluorescence spectroscopy of the present state of petroleum hydrocarbon contamination in the water column of the Northwest Atlantic Ocean. Mar.Chem. 2:251-261.

Grahame, J. 1973. Breeding energetics of Littorina littorea (L.)(Gastropoda: Prosobranchiata). J.Anim.Ecol. 42:391-403.

_____. 1975. Spawning in Littorina littorea (L.)(Gastropoda: Prosobranchiata). J.Exp.Mar.Biol.Ecol. 18:185-196.

Hawkins, A.J.S., P.N. Salkeld, B.L. Bayne, E. Gnaiger and D.M. Lowe. 1985. Feeding and resource allocation in the mussel Mytilus edulis: evidence for time averaged optimization. Mar.Ecol.Prog.Ser. 20:273-287.

Haya, K. and B.A. Waiwood. 1983. Adenylate energy charge and ATPase activity: potential biochemical indicators of sublethal effects caused by pollutants in aquatic animals. In Aquatic toxicology (ed. J.O. Nriagu), pp. 307-334. Wiley, N.Y.

Hayes, F.R. 1927. The effect of environmental factors on the development and growth of Littorina littorea. Trans. Nova Scotian Inst.Sci. 17:6-13.

Holland, D.L., R. Tantanasiriwong and P.J. Hannant. 1975. Biochemical composition and energy reserves in the larvae and adults of the four British periwinkles Littorina littorea, L. littoralis, L. saxatilis and L. neritoides. Mar.Biol. 33:235-239.

Hughes, R.N. and D.J. Roberts. 1980. Reproductive effort of winkles (Littorina spp.) with contrasted methods of reproduction. Oecologia 47:130-136.

Hunter, R.D. and W.D. Russell-Hunter. 1983. Bioenergetic and community changes in intertidal Aufwuchs grazed by Littorina littorea. Ecology 64:761-769.

Ivanovici, A.M. 1980a. Application of adenylate energy charge to problems of environmental impact assessment in aquatic organisms. Helg.Meeres. 33:556-565.

_____. 1980b. The adenylate energy charge in the estuarine mollusc, Pyrazus ebeninus. Laboratory studies of responses to salinity and temperature. Comp.Biochem.Physiol. 66A:43-55.

_____1981. A method for extraction and analysis of adenine nucleotides for determination of adenylate energy charge in molluscan tissue. CSIRO, Div.Fish.Oceanog., Report 118, 27pp. Cronulla, Australia.

Joosse,J. and W.P.M. Geraerts. 1983. Endocrinology. In The Mollusca: physiology I (eds. K.M. Wilbur and A.S.M. Saleuddin), vol. 4, pp. 317-406. Academic Press, N.Y.

Keleti, G. and W.H. Lederer. 1974. Handbook of micro-methods for the biological sciences. 166pp. Van Nostrand Reinhold Co., N.Y.

Lambert, P. and P.A. Dehnel. 1974. Seasonal variations in biochemical composition during the reproductive cycle of the intertidal gastropod Thais lamellosa Gmelin (Gastropoda, Prosobranchia). Canadian J.Zool. 52:305-318.

Lebour, M.V. 1937. The eggs and larvae of the British prosobranchs with special reference to those living in the plankton. J.Mar.Biol.Assoc.U.K. 37:229-239.

Lehninger, A.L. 1977. Biochemistry: the molecular basis of cell structure and function. 1104pp. Worth Publishers, Inc., N.Y.

Livingstone, D.R. 1983. Invertebrate and vertebrate pathways of anaerobic metabolism: evolutionary considerations. J.Geol.Soc.London 140:27-37.

Lowry, O.H., M.J. Rosebrough, A.L. Farr and R.J. Randall. 1951. Protein measurement with the folin phenol reagent. J.Biol.Chem. 193:265-275.

Lubchenco, J. 1978. Plant species diversity in a marine intertidal community: importance of herbivore food preference and algal competitive abilities. Am.Nat. 112:23-39.

_____. 1982. Effects of grazers and algal competitors on fucoid colonization in tide pools. J.Phycol. 18:544-550.

_____. 1983. Littorina and Fucus: effects of herbivores, substratum heterogeneity, and plant escapes during succession. Ecology 64:1116-1123.

Lubchenco, J. and B.A. Menge. 1978. Community development in a low rocky intertidal zone. Ecol.Monogr. 48:67-94.

Matusiak, J.P. and P.E. Fell. 1982. Reproductive cycle of Crepidula convexa (Say) within a New England eelgrass community. Internat.J.Invert.Repro. 5:253-260.

McMahon, R.F. and W.D. Russell-Hunter. 1977. Temperature relations of aerial and aquatic respiration in six littoral snails in relation to their vertical zonation. Biol.Bull. 152:182-198.

Newell, G.E. 1958. The behaviour of Littorina littorea (L.) under natural conditions and its relation to position on the shore. J.Mar.Biol.Assoc.U.K. 37:229-239.

Newell, R.C. and V.I. Pye. 1970. Seasonal changes in the effect of temperature on the oxygen consumption of the winkle Littorina littorea (L.) and the mussel Mytilus edulis L. Comp.Biochem.Physiol. 34:367-383.

_____. 1971. Quantitative aspects of the relationship between metabolism and temperature in the winkle Littorina littorea (L.). Comp.Biochem.Physiol. 38B: 635-650.

Newell, R.C., V.I. Pye and M. Ahsanullah. 1971. Factors affecting the feeding rate of the winkle Littorina littorea. Mar.Biol. 9:138-144.

Newell, R.I.E., T.J. Hilbish, R.K. Koehn and C.J. Newell. 1982. Temporal variation in the reproductive cycle of Mytilus edulis L. (Bivalvia, Mytilidae) from localities on the east coast of the United States. Biol.Bull. 162:299-310.

New York City Dept. Environ. Protection. 1983, 1984. New York Harbor Water Quality Survey. Bureau of water pollution control, Wards Island, N.Y.

Petraitis, P.S. 1982. Occurrence of random and directional movements in the periwinkle, Littorina littorea (L.). J.Exp.Mar.Biol.Ecol. 59:207-217.

_____.1983. Grazing patterns of the periwinkle and their effect on sessile intertidal organisms. Ecology 64: 555-523.

Phelps, D.K., W. Galloway, F.P. Thurberg, E. Gould and M.A. Dawson. 1981. Comparison of several physiological monitoring techniques as applied to blue mussel, Mytilus edulis, along a gradient of pollutant stress in Narragansett Bay, Rhode Island. In Biological monitoring of marine pollutants (eds. F.J. Vernberg, A. Calabrese, F.P. Thurberg and W.B. Vernberg), pp. 335-355. Academic Press, N.Y.

Rainer, S.F., A.M. Ivanovici and V.A. Wadley. 1979. Effect of reduced salinity on adenylate energy charge in three estuarine molluscs. Mar.Biol. 54:91-99.

Sastry, A.N. and N.J. Blake. 1971. Regulation of gonad development in the bay scallop, Aequipecten irradians Lamarck. Biol.Bull. 140:273-283.

Skjoldal, H.R. 1981. ATP concentration and adenylate energy charge of tropical zooplankton from waters inside the Great Barrier Reef. Mar.Biol. 62:119-123.

Skjoldal, H.R. and T. Bakke. 1978. Relationship between ATP and energy charge during lethal metabolic stress of the marine isopod Cirolana borealis. J.Biol.Chem. 253:3355-3356.

Skjoldal, H.R. and T. Barkati. 1982. ATP content and adenylate energy charge of the mussel Mytilus edulis during the annual reproductive cycle in Lindaspollene, Western Norway. Mar.Biol. 70:1-6.

Stekoll, M.S., L.E. Clement and D.G. Shaw. 1980. Sublethal effects of chronic oil exposure on the intertidal clam Macoma balthica. Mar.Biol. 57:51-60.

Steneck, R.S. and L. Watling. 1982. Feeding capabilities and limitation of herbivorous molluscs: a functional group approach. Mar.Biol. 68:299-319.

Stickle, W.B. 1971. The metabolic effects of starving Thais lamellosa immediately after spawning. Comp.Biochem. Physiol. 40A:627-634.

_____. 1975. The reproductive physiology of the intertidal prosobranch Thais lamellosa (Gmelin). II. seasonal

changes in biochemical composition. Biol.Bull. 148: 448-460.

Stickle, W.B. and F.G. Duerr. 1970. The effects of starvation on the respiration and major nutrient stores of Thais lamellosa. Comp.Biochem.Physiol. 33:689-695.

Stickle, W.B., S.D. Rice and A. Moles. 1984. Bioenergetics and survival of the marine snail Thais lima during long-term oil exposure. Mar.Biol. 80:281-289.

Struhsaker, J.W., M.B. Eldridge and T. Echeverria. 1974. Effects of benzene (a water-soluble component of crude oil) on eggs and larvae of pacific herring and northern anchovy. In Pollution and physiology of marine organisms (eds. F.J. Vernberg and W.B. Vernberg), pp. 253-284. Academic Press, N.Y.

Tattersall, W.M. 1920. Notes on the breeding habits and life history of the periwinkle. Sci.Invest.Fish.Br. Ireland 1:1-11.

Thorson, G. 1946. Reproduction and larval development of Danish marine bottom invertebrates with special reference to the planktonic larvae of the Sound (Oresund). Meddr.Kommn.Danm.Fisk-og Havunders, (plankton) 4:1-523.

Vassallo, M.T. 1973. Lipid storage and transfer in the scallop Chlamys hericia Gould. Comp.Biochem.Physiol. 44A:1169-1175.

Verschraegen, K., P.M.J. Herman, D. Van Gansbeke and A. Braeckman. 1985. Measurement of the adenylate energy charge in Nereis diversicolor and Nephtys sp. (Polychaeta: Annelida): evaluation of the usefulness of AEC in pollution monitoring. Mar.Biol. 86:233-240.

Watson, D.C. and T.A. Norton. 1985. Dietary preferences of the common periwinkle, Littorina littorea (L.). J.Exp.Mar.Biol.Ecol. 88:193-211.

Webber, H.H. 1970. Changes in the metabolite composition during the reproductive cycle of the abalone Haliotis cracheroidii (Gastropoda: Prosobranchia). Physiol.Zool. 43:213-231.

_____. 1977. Gastropoda: Prosobranchia. In Reproduction of marine invertebrates, Molluscs: gastropods and cephalopods (eds. A.C. Giese and J.S. Pearse), vol. 4, pp. 1-97. Academic Press, N.Y.

Whipple, J.A., M.B. Eldridge and P. Benville. 1981. An ecological perspective of the effects of monocyclic aromatic hydrocarbons on fishes. In Biological monitoring of marine pollutants (eds. F.J. Vernberg, A. Calabrese, F. Thurberg and W.B. Vernberg), pp. 483-551. Academic Press, N.Y.

Williams, E.E. 1964. The growth and distribution of Littorina littorea (L.) on a rocky shore in Wales. J.Anim.Ecol. 33:413-432.

_____. 1970. Seasonal variations in the biochemical composition of the edible winkle Littorina littorea (L.). Comp.Biochem.Physiol. 33:655-661.

Worrall, C.M., J. Widdows and D.M. Lowe. 1983. Physiological ecology of three populations of the bivalve Scrobicularia plana. Mar.Ecol.Prog.Ser. 12:267-279.

Youngblood, W.W. and M. Blumer. 1975. Polycyclic aromatic hydrocarbons in the environment: homologous series in soils and recent marine sediments. Geochim.cosmochim.acta. 39:1303-1314.

Zaroogian, G.E., J.H. Gentile, J.F. Heltshe, M. Johnson and A.M. Ivanovici. 1982. Application of adenine nucleotide measurements for the evaluation of stress in Mytilus edulis and Crassostrea virginica. Comp.Biochem.Physiol. 71B:643-649.