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Responses and adaptations of adenylate energy charge and digestive enzyme activities to tidal emersion of *Crassostrea gigas* population in Marennes-Oleron Bay

J. MOAL, J. F. SAMAIN, J. R. LE COZ & J. Y. DANIEL

IFREMER. B. P. 70. 29263 Plouzané. France

SUMMARY: *Crassostrea gigas* oysters cultivated in Marennes-Oleron Bay (France), face seasonal variations in the environment: salinity, turbidity, phytoplanktonic production, and the tidal rhythm of emersion. The oysters are maintained at different depths to reduce competition for space (e.g. by settlement of mussel spat). The physiological responses of two groups of oysters to different periods of emersion were studied using a seasonal sampling strategy: one group was on a flat part of the area, and the effect of emersion was studied in a short time survey (less than three hours emersion at low tide); the other was located in a very narrow place with a steep slope. This group was subdivided into three, according to bathymetric position, to study the long term effect of different periods of emersion. Adenylate energy charge (AEC), total nucleotide concentrations and digestive enzyme activities were recorded in each experiment. The effect of short term emersion on energy charge was dependent on season: energy charge stayed high and stable for three hours after emersion in winter (January), but decreased in May and July. AEC did not differ in the long term among groups subjected to daily emersion at different tidal heights, but growth rates of these groups were different. The decrease of AEC after a short emersion would be an indication of the rate of ATP utilization, and is thought to be related to seasonal differences in the metabolism of oysters. This decrease is compensated at each tide, as in a long term survey no differences were observable. So animals from the same location subjected to different amounts of emersion adapt to maintain their energy charge. The effects of emersion and feeding on growth and AEC are discussed with reference to the activities of digestive enzymes in oysters.

Key words: Crassostrea, physiology, emersion, AEC, digestive enzimes.

INTRODUCTION

Crassostrea gigas oysters cultivated in Marennes-Oleron Bay (France), face the seasonal variations in salinity (input of fresh waters from river), turbidity, phytoplanktonic production and the tidal cycle. The oysters are also distributed at different tidal levels on the shore according to the breeding strategy or space availability. They thus experience pronounced variations in oxygen supply during a tidal cycle.

During exposure to air, the bivalves exhibit anaerobic metabolism (AHMAD & CHAPLIN, 1977). In energetic terms, this metabolism is less efficient (7 times) than aerobic metabolism even if bivalves have developed particular compensatory mechanisms, e.g. a dramatic drop in energy demand during anaerobiosis (DE ZWANN, 1977). So the study of the short and long term effect of emersion on energy metabolism may provide insights into how oysters adapt their energy metabolism during such periods, and whether cultivated populations can maintain a satisfactory energy balance in such a variable environment. The physiological response to aerial exposure was studied using the adenylate energy charge (AEC, ATKINSON, 1968), which indicates the energy status of the organisms.

The effect of aerial exposure was assessed on two sets of animals according to a seasonal sampling strategy: one was on a flat part of the shore and the effect of emersion was studied in a short-term survey; the other was located in a very narrow place,with a steep slope and subdivided into three, according to bathymetric position, to study the long-term effect of different periods of emersion.

MATERIAL AND METHODS

The oysters *Crassostrea gigas* originated from Marennes-Oleron where the spat was abundant and easily collected. The oysters were bred in plastic boxes (0,5 m^2, above the bottom in two areas in Marennes-Oleron Bay (Fig. 1). The oysters cultivated at Dagnas were at a tidal coefficient of 75 and were derived from spat collected in 1983, separated from the collector 18 months later and placed in boxes in May 1985. The short-term effect of emersion on energy charge value was analysed on these oysters in January and May 1986.

At the mouth of the Seudre, oysters were distributed at 3 different tidal coefficients of 60, 75, 90, respectively named C, D, E. These oysters, from spat collected in 1984, were placed in boxes in May 1986 and used to study the short and long term effects of emersion in July 1986.

Oysters were sampled when they had just emerged at low tide (zero time) and after one, two and three hours of emersion. Twelve oysters were collected at zero time and analysed individually. For the other sampling times, five oysters were pooled for the nucleotide and enzyme analysis.

Immediately after collection, the soft body was separated from the shell and the whole flesh plunged in liquid nitrogen (—196 °C) as quickly as possible. The samples were kept frozen in liquid nitrogen until analysis. They were then crushed (30 sec) to a fine powder by a Dangoumau ball-mill whose metal recipients were previously cooled in liquid nitrogen to avoid the thawing of samples (final temperature of powder —100 °C). An aliquot of this frozen powder (200 mg) was mixed with 2 ml cooled TCA solution (0,5 M) in a Potter homogenizer, allowed to stand 15 min and centrifuged 10 min at 4500 rpm.

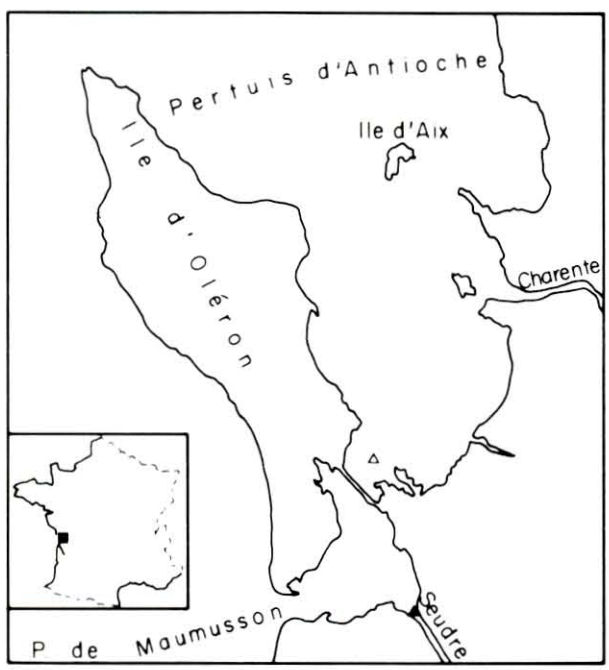

FIG. 1. — Chart of Marennes-Oleron area showing Dagnas (empty triangle) and Seudre (full triangle) experimental sites.

The TCA precipitate was dissolved in 2 ml N NaOH for the protein analysis by the LOWRY *et al.* (1951) method. The supernatant was neutralized (V/V) with an amine freon solution (KHYM, 1975). The neutralized extracts were analysed by a high performance liquid chromatography (HPLC) method for the separation and detection of the nucleotides. The method was adapted from HOFFMAN & LIAO (1977) with a C18 column, eluted by a NaH_2PO_4 (0.15 M), ammonium tetrabutyl (4.2 mM), methanol 5.4 % (V/V) buffer at a 1 ml/min flow rate. The detection was at 254 nm.

The adenylic nucleotides, GTP, GDP, GMP, UTP, UMP and CTP were separated in less than 25 minutes. The nucleotide concentrations were measured as the pic surfaces on a SHIMADZU integrator. The results were expressed in µmole/g protein.

The adenylate energy charge (AEC) (ATKINSON, 1968) was then calculated as the ratio (ATP + 1/2 ADP) / (ATP + ADP + AMP). The ratio value varies between 0 and 1 and represents the energy status of the organisms. In healthy animals, AEC varies between 0.8-0.9; in partial stress the value drops to 0.5-0.75 (IVANOVICI, 1980).

Enzyme analyses were performed on the lyophylised powder. An aliquot (50 mg) of the lyophylised powder was crushed in polytron with 2 ml distilled water. Amylase activity was estimated on supernatant (SAMAIN *et al.*, 1977) and expressed as international unit per mg protein. Proteins were measured as total proteins after NaOH extraction (LOWRY *et al.*, 1951).

RESULTS

The short term emersion effect (tidal cycle) was assessed during three different months (January, May and July 1986). The AEC results are shown on figure 2. Two kinds of response are observed. The adenylate energy charge was not modified by exposure to air in the winter month (January). The initial value 0.77 ± 0.06 ($P < 1$ %) was maintained after two hours emersion. On the contrary, the AEC ratio dropped quickly in May and July when animals stayed out of the water.

These modifications of AEC occurred at a constant total adenylic nucleotide (ATP + ADP + + AMP) level (Fig. 3). So interconversions occurred in the adenylic nucleotide pool. The AEC drop in May was explained by the ATP conversion into ADP (13 %) and AMP (6.5 %), but in July all ATP was

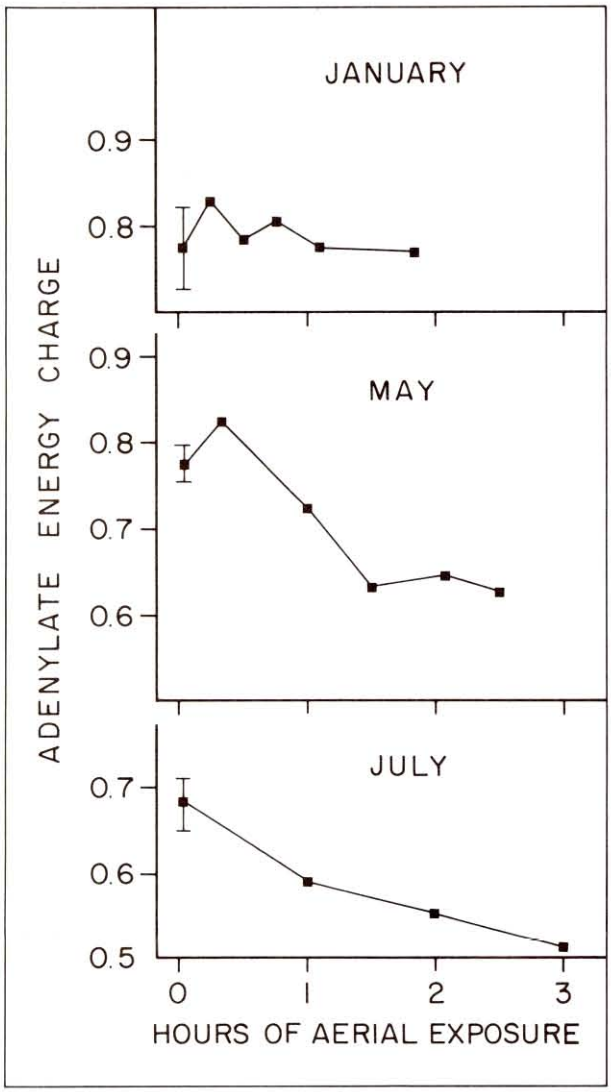

FIG. 2. — Adenylate energy charge during emersion in January, May and July 1986. The zero value is the mean of twelve individual measures and the bar represents the standard error at 95 % probability level. The other points are the value of five pooled oysters.

transformed into AMP; ADP levels stayed constant (Fig. 4).

May and July responses also differed when considering their evolution in time. In May after a sharp 18 % initial decrease in 1 1/2 hour, the AEC stabilized around 0.64. In July, a linear decrease was observed and AEC was 25 % less after 3 hours (AEC = 0.51).

The long term effect of emersion was studied on the same oyster population bred at three different bathymetric levels, so that the cumulative time of emersion was different for the three sets. After one month (July) cultivation at these three tide levels, no distinction (t-test, $P < 95$ %) can be made on the AEC index (Fig. 5). The initial value of AEC was significantly lower in July (0.68 ± 0.04) than in January (0.77 ± 0.06) and May (0.77 ± 0.03).

After twelve months spent at the three different depths, large differences were observed in growth (Table I). Animals fed for a longer time (E) were significantly bigger. The digestive enzyme amylase activity measured in July, when the AEC was the most sensitive to emersion time, was significantly higher ($P < 95$ %) for oysters that had experienced longer aerial exposure (Fig. 5).

DISCUSSION

Our results show that AEC response to aerial exposure is different according to the period of the year (Fig. 2) probably because of seasonal variation in temperature. BARTHEL (1984) showed that the ini-

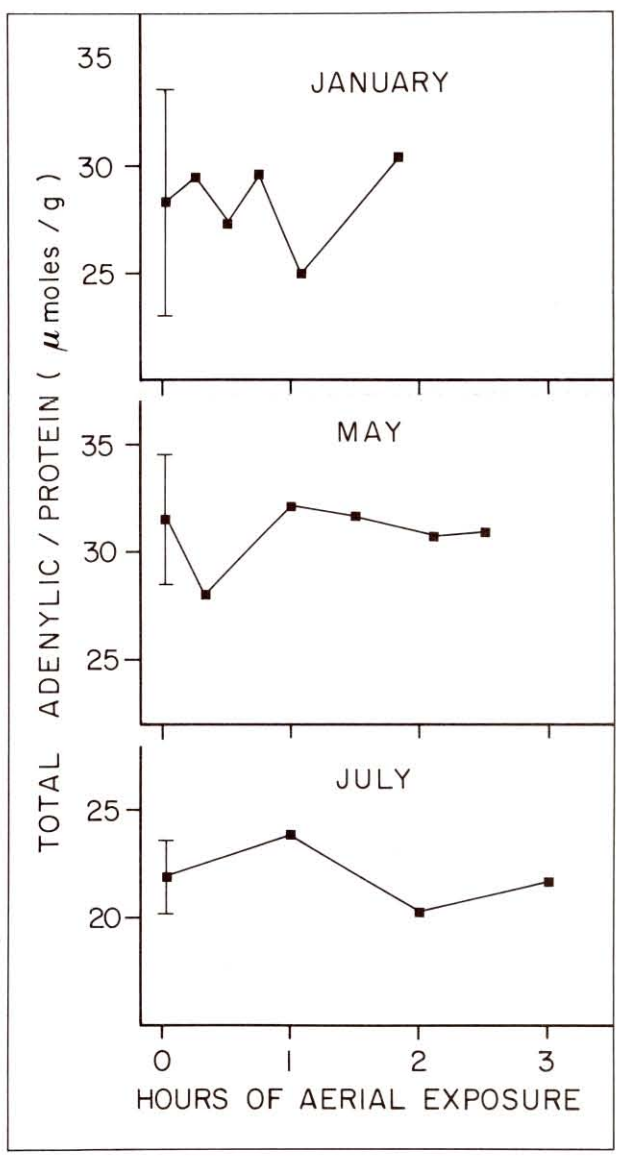

FIG. 3. — Total adenylic nucleotides (ATP + ADP + AMP) expressed as µmoles nucleotides/g protein during emersion in January, May, July 1986. Symbols as in figure 2.

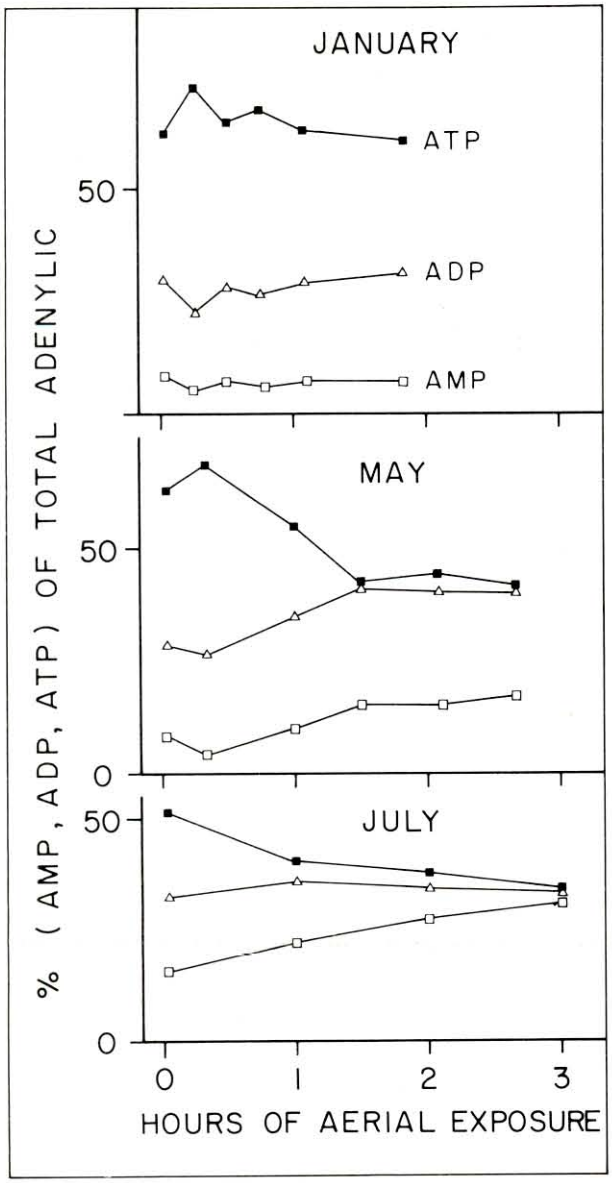

FIG. 4. — Relative variations of ATP, ADP, AMP expressed as percentages of the total adenylic nucleotides during emersion in January, May and July 1986.

tial anoxia induced AEC decrease was more pronounced at higher temperatures for *Macoma calcarea*. The absence of an AEC response to air exposure in January is new as an AEC decline for the molluscs during anoxia and aerial exposure in experimental conditions has been always noticed (WISJMAN, 1976; IVANOVICI, 1977, BARTHEL, 1984; SYLVESTRE, 1987). The AEC value is the result of an equilibrium between ATP inflow and outflow since animals do not store ATP. So the equilibrium is maintained in winter, whether the oysters were exposed or submerged. This is not true in May and July when AEC decreased during exposure to air, which clearly indicates and energy outflow (ATP utilization) superior to the energy inflow (ATP production). We assume then that two different metabolic levels characterized the oysters: an active one in summer which is also the reproductive period and a resting one in winter.

From an energetic point of view, the tidal cycle would affect two important physiological pathways that are directly linked to the AEC regulation because they modify the ATP inflow: the periodic interruption of the feeding processes and the absence or reduction of the oxidative phosphorylation during emersion time. Elsewhere, ATP outflow is highly dependent on the metabolic activity.

Although several bivalve species are able to respire aerobically in air (BAYNE *et al.*, 1976; DE ZWAAN, 1977; WIDDOWS *et al.*, 1979), we do not know to what extent an aerobic pathway can operate on exposure to air for the species *C. gigas*. These results indicate that the anaerobic pathway is utilized during exposure to air, leading to a reduction in ATP production by a factor of approximately 7.

In winter the anaerobic ATP production would compensate for the low energy demand. On the contrary, in summer the decrease in AEC during emersion would be related to higher metabolic needs (ATP utilization) unbalanced by anaerobic ATP production.

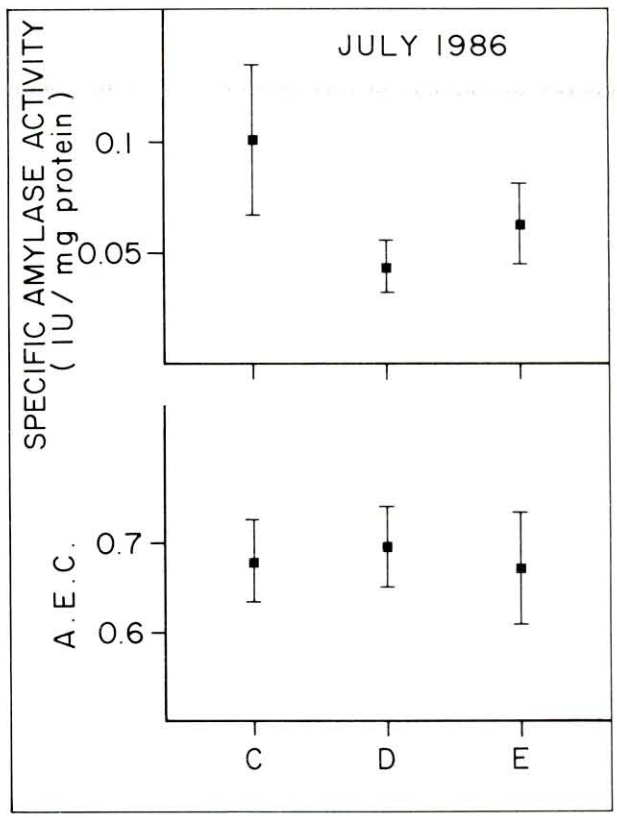

FIG. 5. — Adenylate energy charge and specific amylase activity for *C. gigas* after one month at three depths C, D, E, are in descending order of duration of aerial exposure. Mean and standard error at a probability level of 95 %; n = 12.

TABLE I. — AEC and flesh wet weight values of *C. gigas* oysters bred during one year at three bathymetric levels in mouth of R. Seudre. C, D and E are respectively the 60, 75 and 90 tide levels.

		C	D	E
AEC	m̄	0.87	0.88	0.84*
	s	0.02	0.02	0.04
Flesh wet weight		3.15*	5.16	6.78
		0.59	1.45	2.56

m̄ = mean; s = standard deviation; n = 12.

The adenylate energy charge modifications take place at a constant adenylic nucleotide level (Fig. 3) (SYLVESTRE, 1987; WIJSMAN, 1976). Interconversions between adenylic nucleotides can operate by the mean of adenylate kinase enzymes. In May and July the changes in AEC levels are not explained by the same transformations. In May ATP is mostly transformed in to ADP, whereas in July ATP is essentially recovered as AMP. The more advanced degradation of ATP (to AMP) in July can be related to the greater "oxygen debt" observed by WIDDOWS (1979) in *Mytilus edulis* during emersion when temperature increases. The summer months also differ with regard to the kinetics. The continuous decrease in AEC in July compared with the sharp but short decrease in May would demonstrate that the highest energy demand is in this period. July is the main spawning period in this area and corresponds also to the highest temperatures in the field. From figure 2, it is evident that aerial exposure influences the AEC, and one can speculate about the long term effects of aerial exposure on the ability of oysters to overcome the energy disequilibrium induced by the tidal cycle in summer. The cumulative exposure experienced had no effect on the AEC value. From figure 5, it can be seen that oysters from three different depths exhibited the same AEC value one month after being laid. It means that the more or less important AEC decrease induced by different periods of exposure to air is compensated by the following immersion period. WIDDOWS (1979) showed that "oxygen debt" is repayed immediatly after immersion, and GADE & MEINARDUS (1981) and PORTNER *et al.* (1986) that anaerobic end products are reused after reimmersion.

However, the differences in shell growth shown after one month in such conditions (BODOY, personal communication), and the significant differences in flesh weight observed after one year (Table I), demonstrate that emersion had little effect on AEC, but that growth rates differed according to depth.

The growth rate is also related to food conditions. The oysters from the upper level have less time for feeding but exhibit a higher amylase activity (Fig. 5). Such an increase in digestive enzyme has been observed for copepods or *Artemia* when food is limiting (SAMAIN, 1985; SAMAIN *et al.*, 1985; HARRIS *et al.*, 1986). As a result, a better assimilation of the ingested food compensates the lower food availability. This enzyme compensatory mechanism could be effective for molluscs and could contribute to the AEC recovery during immersion. The food availability is too low at this period however, related to growth needs, and the digestive capacities cannot compensate for the differences in feeding duration among the three sets of experimental oysters.

In a previous paper (MOAL *et al.*, 1987), we reported that in summer AEC level is lower than in winter. Such results are confirmed here (values at time zero). This indicates that the trophic environment and/or physiological cycles (maturation) can affect the AEC value. However, as AEC was compensated for oysters at the three tide levels, differences in the feeding status related to the aerial exposure are probably less than the seasonal variations of the trophic environment. Referring to IVANOVICI's (1980) table (Fig. 6), it can be emphasized that our results show no direct relation between the growth capacity and a defined AEC level for the oysters *C. gigas* at Marennes-Oleron. Other experiments are designed to evaluate effects of the seasonal and reproductive cycles on AEC and digestive enzymes in *C. gigas*.

A.E.C.	ENVIRONMENTAL CONDITION	ORGANISMS CHARACTERIZED BY
0.8 - 0.9	NON - LIMITING (NO - STRESS)	HIGH GROWTH RATE REPRODUCTION VIABILITY
0.5 - 0.75	LIMITING (PARTIAL STRESS)	SLOW OR ZERO GROWTH RATE NO REPRODUCTION VIABILITY MAINTAINED
≃0.5	SEVERELY LIMITING (SEVERE STRESS)	NO GROWTH NO REPRODUCTION VIABILITY LOST EVEN AFTER TRANSFERT TO NON-STRESS CONDITIONS

FIG. 6. — Relation between environmental condition, organismic features and AEC (from IVANOVICI, 1980).

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