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# Oyster adenylate energy charge: response to levels of food

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**Abstract**

Adenylate Energy Charge (AEC) values of *Crassostrea gigas* (9 and 18 months old) cultured in ponds in Marennes-Oléron Bay (Atlantic coast, France) are reported. Two ponds were loaded with two different initial oyster biomasses in order to obtain different food conditions. The growth of oysters was different in the two ponds which corroborated the effectiveness of attaining different food conditions. AEC level was different depending on the pond and was therefore related to food availability per unit oyster biomass. Well-fed oysters exhibited greater AEC values than poorly-fed oysters, throughout the study. Information on nutritional situations for wild or cultured populations can therefore be obtained by AEC. In each pond, the seasonal AEC variations were similar for both age-groups and were characterized by a minimum value in summer and a maximum value in winter. The influence of reproduction on these variations cannot be ruled out as for all the batches maturation processes were evidenced. Research will be done on a temperature effect to try to distinguish these seasonal variations. A strong correlation between adenylate energy charge (AEC) and guanylate energy charge (GEC) was demonstrated; it was similar for both ponds. The levels of nucleotide concentration were relatively stable during the survey except in September and December for oysters in the low density pond. Among nucleotides, adenylate pool represented the major part (80%). A decrease in the total concentration of adenylates was observed in August. The best growth correlated with the lowest adenylate percentage and a better GTP/ATP ratio, suggesting that this ratio could be a useful index for growth.

**Keywords :** Adenylate energy charge, nucleotides, food, *Crassostrea gigas.*

*Charge énergétique adénylique chez les huîtres : réponse à différents niveaux de nourriture.*

**Résumé**

La charge énergétique adénylique (CEA) des huîtres *Crassostrea gigas* (9 et 18 mois) cultivées à densités différentes dans deux claires, pour obtenir deux conditions nutritives, a été suivie pendant 6 mois (Marennes-Oléron, France). Les croissances ainsi obtenues traduisent bien l'efficacité du protocole expérimental. Les niveaux de la CEA sont différents dans les deux claires et dépendent donc du niveau d'alimentation des huîtres. Les valeurs de la CEA sont toujours supérieures pour les huîtres dans la claire chargée à faible densité. La CEA se révèle donc un indicateur de la situation trophique d'un bassin d'élevage. Les variations saisonnières de la CEA sont semblables pour les deux classes d'âge, dans une même claire : un minimum estival et un maximum hivernal. L'influence de la reproduction sur ces variations ne peut être éliminée puisque tous les lots ont maturé. L'influence de la température sur l'évolution des valeurs de la CEA sera recherchée pour essayer de distinguer l'effet saisonnier. Il existe une forte corrélation entre la charge énergétique adénylique (CEA) et la charge énergétique guanylique (CEG) : elle est similaire dans les deux claires. Les concentrations de nucléotides sont semblables et stables dans les deux claires sauf en septembre et décembre où les valeurs augmentent pour les huîtres qui poussent le mieux. Les adénylates sont les représentants majeurs des nucléotides (80%), cependant leur pourcentage diminue en août au détriment des guanylates. D'un point de vue

général le pourcentage des adénylates est plus faible chez les huîtres qui ont la meilleure croissance et donc le rapport GTP/ATP est plus élevé. Ce rapport pourrait être un indice intéressant pour décrire la croissance.

**Mots-clés :** Charge énergétique adénylique, nucléotides, nutrition, *Crassostrea gigas*.

## INTRODUCTION

The adenylate energy charge defined by Atkinson (1968) as (ATP+0.5 ADP)/(ATP+ADP+AMP) reflects the cell energy equilibrium and has been proposed to control the regulation of metabolic pathways. Theoretically, the AEC values can vary from 0 to 1, but the normal AEC for viable organisms is between 0.8 and 0.9 (Ivanovici, 1980). Any deviation from this normal value can be related to non-optimal equilibrium or to factors which upset homeostasis.

The AEC has been shown to vary seasonally with the general metabolism, in temperate organisms, Numerous species have been studied: oysters *C. gigas* (Moal *et al.*, 1987), mussels *Mytilus edulis*, clams (Giesy and Dickson, 1981; Skjoldal and Barkati, 1982; Sylvestre, 1988; Picado *et al.*, 1988), crayfish (Dickson and Giesy, 1982), copepods (Skjoldal and Bamstedt, 1976). These seasonal variations have been associated with the reproductive cycle for molluscs and marine crustaceans (Bamsted and Skjoldal, 1976), to increases in activity during the breeding period for muscle in crayfish (Dickson and Giesy, 1982). Changes in AEC have also been described in response to salinity and temperature (Sylvestre, 1987). These parameters may contribute to the annual AEC cycle in estuarine areas where oysters are cultured. Few studies have dealt with the effects of food levels on adenylate energy charge, although phytoplankton biomass, which is the main food for filter-feeding organisms, shows a strong seasonal variation in the field.

We report here the influence of food supply on seasonal AEC values for oysters *C. gigas* as they can experience, depending on the area or the bathymetric zone in a given area, highly variable food levels or feeding times. Indeed, in France, *C. gigas* is largely cultured on tidal areas in large enclosed bays with either patchy distributions of phytoplankton or particulate gradients due to currents.

## MATERIAL AND METHODS

Two possibilities were available to obtain different status of *in situ* nutrition for oysters (seasonal variations): either modification of the feeding time (emersion time), or changes in food concentrations in a similar ecologic situation (temperature, salinity, light). The first possibility was not utilized since it also included a secondary effect of anoxia, and anoxia has been demonstrated to influence AEC in bivalves (Sylvestre, 1987; Moal *et al.*, 1989 *b*).

Two food conditions were established in a similar ecologic situation by using two adjacent ponds with the same food input but two different oyster densities. This solution also allowed the rearing of a sufficient oyster population for a seasonal survey.

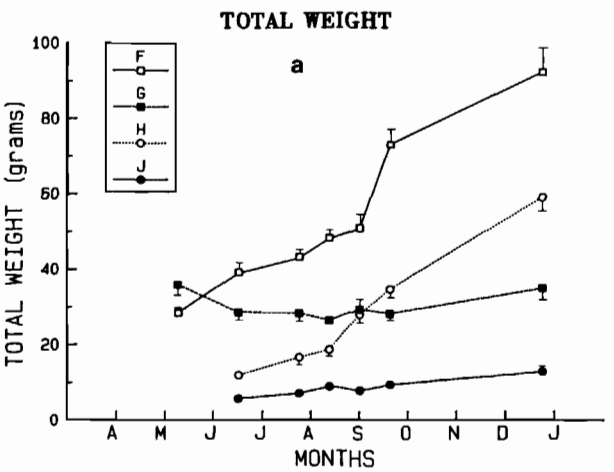
The spat of Japanese oysters, *C. gigas*, from a cultured stock were collected on artificial collectors in the Bay of Marennes-Oléron (Atlantic coast). After periods of either nine or eighteen months, the seeds were scraped off the collectors. The two age groups were then transferred to natural ponds. The ponds were filled with the sea water from the mouth of Seudre river (La Tremblade) at each high tide. These ponds were newly rebuilt. Two adjacent ponds (on the same water channel) were distributed with two oyster densities. Each pond received oysters of both age groups (9 and 18 months). The initial respective density was 1 kg/m$^2$ for the greater density pond and 100 g/m$^2$ for the lesser density pond. The experiment started in spring (April) and samples were taken bimonthly until September. The experiment was ended in December. Oysters were placed in plastic trays on the sea floor and were continuously immersed.

At each sampling date, twelve individual oysters from each set were analysed for growth parameters and nucleotide content. Oysters were removed from the pond, quickly weighed and opened, the flesh plunged into liquid nitrogen (−196°C) and held there until analysis. The frozen flesh weight and shell weight were also recorded. For nucleotide analysis the samples were then extracted as already described (Moal *et al.*, 1989 *a*). The nucleotides were quantified by high performance liquid chromatography (HPLC) on a reverse phase column with a counter-ion. The method allowed separation of the major nucleotides (ATP, ADP, AMP, GTP, GDP, GMP, UTP, UMP). The proteins were measured by the method of Lowry *et al.* (1951). The nucleotides were expressed as nmol/mg protein. Adenylate and guanylate energy charge was calculated as: (NTP+0.5 NDP)/(NTP+NDP+NMP) with N representing adenine or guanine base. Each value presented represents the mean of twelve values.

## RESULTS

Growth of oysters in the two ponds was significantly different (for each age-group Student $t$ test, $p > 95\%$). Growth as determined by flesh or shell weight was good in the pond with the lower stocking density. No growth was observed in the high density pond (*fig.* 1). Low food limited the growth of 18-month-old oysters (G) as well as for the juveniles (J). However, maturation processes took place in both ponds and for both age-groups.

AEC seasonal variations were observed for both ponds (ANOVA; F = 36, F = 19 for low and high density pond respectively: $p > 99\%$). The AEC values were quite similar regardless of age group within a pond. The adenylate energy charge was significantly different (Student $t$ test $p > 99\%$) for each age group in the ponds with the two stocking densities (*fig.* 2).



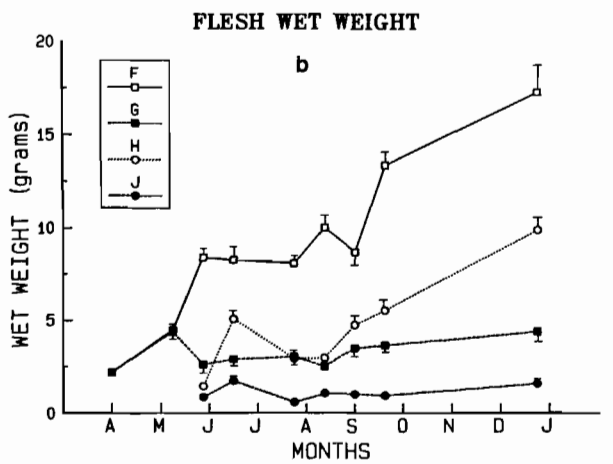




**Figure 1.** – (*a*) Total weight (shell + flesh) of oysters *C. gigas* cultured from April to December in ponds in Marennes-Oléron Bay.

(*b*) Oyster frozen flesh weight. Flesh was separated from the shell and frozen in liquid nitrogen. The flesh was weighed in frozen state.

Square: 18-month-old oysters. Circle: 9-month-old oysters. Empty symbol: Low-density pond. Full symbol: high-density pond. Vertical bar: standard deviation.

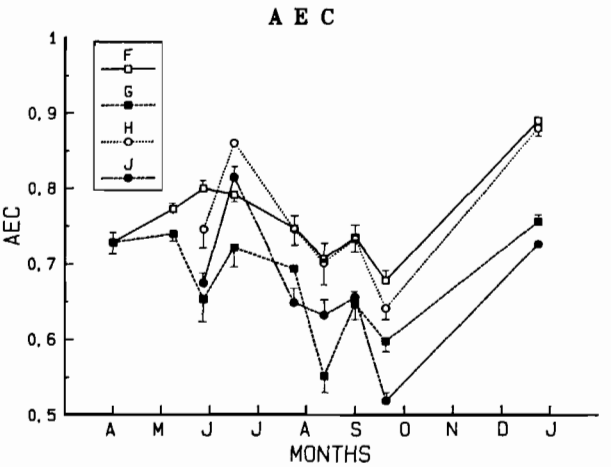



**Figure 2.** – Adenylate energy charge (AEC) of oysters *C. gigas* cultured from April to December at two densities in ponds in Marennes-Oléron Bay.
AEC = ATP + 0.5 ADP/ATP + ADP + AMP (symbols as in *fig.* 1).

The total concentrations of adenylate nucleotides per unit protein exhibited no differences between the ponds except in September and December (*fig.* 3). At these times, values were greater in the low density pond for oysters of both age groups. The percentage of adenylic nucleotides (ATP + ADP + AMP) related to total analysed nucleotides (ATP + ADP

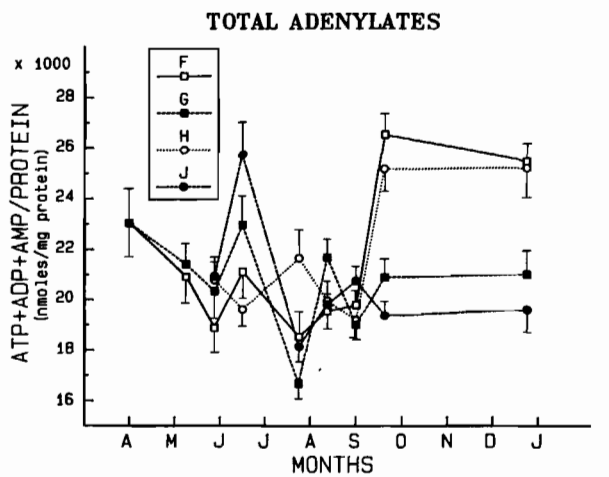



**Figure 3.** – Concentrations of adenylate nucleotides in oysters *C. gigas* cultured at two densities in ponds in Marennes-Oléron Bay. The sum of adenylate nucleotides are expressed relative to proteins (nmol/mg protein).
Adenylate nucleotides = ATP + ADP + AMP (symbols as in *fig.* 1).

+AMP+GTP+GDP+GMP+UTP+UMP) is quite stable (80%) but for all the groups a decrease occurred in mid July (*fig.* 4). This decrease was compensated for by an increase in guanylic nucleotides

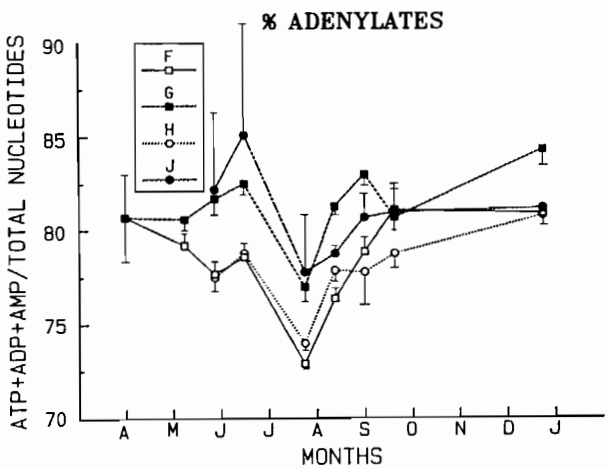



**Figure 4.** – Fraction of the adenylate nucleotides in total analysed nucleotides. The ratio varies between 0 and 1 (symbols as in *fig.* 1).

from 9 to 14% of the total (*fig.* 5). This percentage was also dependent on the pond and was less for the oysters cultured in the pond stocked at the lower density. The oysters which grew more had a greater AEC value but a lesser adenylic nucleotide percentage.

A significative correlation was found between AEC (adenylate energy charge) and GEC (guanylate energy charge) with a correlation coefficient $r=0.906$ ($n=362$).

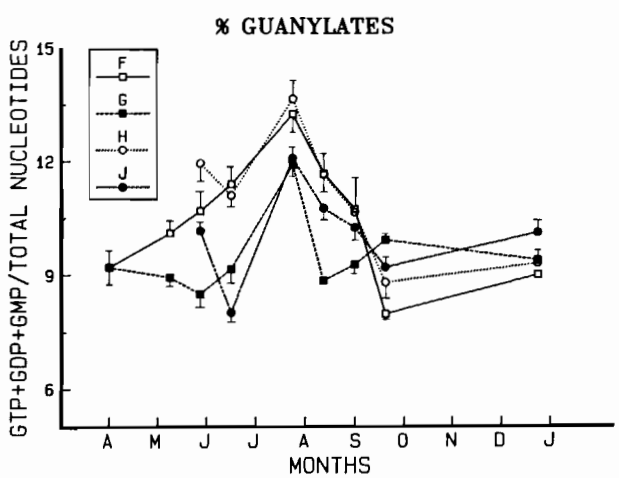



**Figure 5.** – Fraction of the guanylate nucleotides in total analysed nucleotides. The ratio varies between 0 and 1 (symbols as in *fig.* 1).

## DISCUSSION

The results demonstrate the effect of stocking density on the growth of oysters. Both experimental ponds had similar physical and chemical characteristics since they were filled with the same sea water, with the same filling frequency (natural tides). In particular the absolute quantity and quality of the food (mainly phytoplankton) were the same. However, the food availability per oyster was different between the two ponds. So the differences in rate of growth of oysters observed between the two ponds can be related to food levels. We presumed that the two different stocking densities of oysters modified differently the two pond water masses via the oysters' physiology (respiration and, mainly, excretion), leading undoubtedly to different dissolved concentrations of organic matter and thus water quality in the ponds. It is known that bivalves can directly absorb dissolved organic matter but its significance in relation the total energy requirements of the bivalves is not great, given the amounts of dissolved organic material in the sea (Jorgensen, 1966). Thus, this aspect was considered of minor importance, all the more because so it is also related to food conditions.

All the parameters describing the growth (total weight, flesh wet weight) showed no growth in the high density pond (batches G-J) and a spring and autumnal growth in the low density pond (F-H). The shell growth was better for the young oysters (group 0) as they almost reached the same final size as group 1 at the end of the experiment. Considering the flesh, the growth pattern was similar for both age-groups. This is the normal growth pattern in this locality for *C. gigas* cultured in the bay as well as for those in the ponds: shell growth is favoured relatively to flesh growth for the young oysters (Maurer and Borel, 1986; Berthomé *et al.*, 1986). The lack of growth observed in sets G and J was an extreme situation and reflects a continuous limiting trophic situation. It was not a starvation situation since the animals survived without weight losses; no mortality occurred and normal maturation was observed during the experiment. Numerous experimental studies have alread shown the influence of food concentration on the growth of bivalves (Winter and Langton, 1976; Cahalan *et al.*, 1989). *In situ* observations also demonstrated that seasonal growth was related to seasonal variations of food (Valh, 1980; Deslous-Paoli, 1982). At La Tremblade, a North-South growth gradient observed in the bay has heen related to a North-South food gradient consecutive to a continuous impoverishment by the oyster stock of the sea water masses that circulate North to South (Bacher, 1989).

The spawning period (July) for group 1 oysters resulted in a stabilization of wet weight. Weight losses

of genital products are not seen as the water percentage of the gonad also increased at this period (Deslous-Paoli and Héral, 1988). For group 0, the apparent decrease in wet weight in July may be related to a different sample processing in June. Whereas in the normal processing, only flesh was frozen, in June the whole animals were plunged in liquid nitrogen. So the June weight value corresponded to frozen flesh plus intervalve water. Maturation processes were visible for the four oyster batches. Maturation processes and spawning have already been evidenced for young oysters (1 year) in Arcachon Bay (Maurer and Borel, 1986). It seems that most of the energy is channelled to maturation, even in limiting food conditions, which is detrimental to the growth.

The seasonal variations in AEC were very similar for the four sets of data and agreed with values which have been previously reported (Moal *et al.*, 1987) and exhibit a minimum in summer and a maximum in winter. This scheme was valid irrespective of the age group and the pond. In a previous work (Moal *et al.*, 1987) we attributed the low summer values possibly to the maturation processes, which was the reason we chose to work on two age groups in this experiment, hoping that group 0 would not mature. Unfortunately, maturation proceeded in all groups and we cannot corroborate this hypothesis.

However, depending on the pond, two AEC levels can be identified. The AEC values were significantly greater for oysters in the low-density ponds (F-H). As for growth, we attribute the difference in AEC values to different availability of food per individual oyster. Well-fed oysters exhibited greater AEC values than poorly-fed oysters. Differences in AEC quickly appeared and persisted throughout the survey. Few studies are concerned with this relation. Giesy and Dickson (1981) have reported an AEC increase when clams were transferred from the field to the laboratory and attributed this increase to changes both in food and temperature. Wisjman (1976) did not observe differences in AEC values between starved and fed mussels. Several authors working on crustacea demonstrated no clear relation between starvation and AEC but their data concerned short-term experiments (Schirf *et al.*, 1987; Gies *et al.*, 1988; Harms *et al.*, 1990).

Guanylate energy charge (GEC) calculated as (GTP+0.5 GDP/GTP+GDP+GMP) was strongly correlated to adenylate energy charge ($r=0.906$; $n=362$) as already noticed (Wisjman, 1976; Moal *et al.*, 1987). When the ponds were analysed separately the correlation was identical. Derr and Zieve (1972) showed parallel variations of AEC and GEC in fasting rats. AEC and GEC regulations seem strictly related as we have found the same correlation on *C. gigas* (2 surveys) and on *Ostrea edulis*.

Depending on the pond, differences in concentrations of adenylate nucleotides were also observed (*fig.* 3) and consequently related to the food. While the concentrations of nucleotides showed no trend in the high density pond, a sharp increase was observed in autumn in the low density pond (F-H). In contrast Schirf *et al.* (1987) described an increase of nucleotide level with starvation for crustacea. Also changes in the relative concentrations of adenylate nucleotides are given (*fig.* 4). Adenylates represented the major nucleotides but their percentage decreased very sharply in mid July. This decrease in adenylate concentration had been already noticed in August in a previous study at La Tremblade (Moal *et al.*, 1987). It is not clear if this trend was due to spawning. Eggs very rich in adenylate nucleotides, especially in ATP, to satisfy the first energetic needs of the larvae would explain the relative decrease of adenylate. On the other hand, gonadal tissue of *Pecten maximus* is richer in guanylic nucleotides than other organs (data not published).

The percentage of adenylate nucleotides was also different between ponds. Oysters (G-J) that did not exhibit any growth had the greater percentage of adenylate and consequently the smaller percentage of guanylate. GTP/ATP ratio has been related to growth in bacterial community (Karl, 1978). As quoted by Derr and Zieve (1972) GTP is involved in the first step of gluconeogenesis and in protein synthesis and is required for growth but probably not for survival. Also GTP is very important in the adenylate cyclase reaction that controls mitotic activity of the cell.

In this study, oysters were continuously submerged unlike in previous studies where oysters were cultured on a tidal area, but the observed variations in AEC were similar. Although tidal emersion induces a decrease in AEC depending on season (Moal *et al.*, 1989; Sylvestre, 1988), oysters cultured in a tidal area then compensated at the following high tide (also data not published) confirming the high adaptability of oyster to anoxia. However, depending on the culturing mode (tidal, immersed), differences are shown in nucleotide concentration evolution. While in tidal regime oysters exhibited higher nucleotide concentration in summer than in winter, an inverse result is obtained here for the well fed oysters (F-H), the poorly fed oysters showing no changes during the seasonal cycle.

## CONCLUSION

From an ecological point of view, AEC is then an index that responds to trophic variations and might allow information to be obtained about the nutritional situation for wild or cultured oyster populations. However, a previous knowledge of normal seasonal variations is required, as even in good food conditions AEC can decrease momentarily, especially in summer which seems a critical period for oysters. We are studying the role of temperature on adenylate metabolism of the oyster to determine its influence on the seasonal AEC variations.

Adenylate energy charge (AEC) values of Japanese oysters are different depending on the level of food. For management this index would be a useful parameter to obtain information about the trophic capacity of intensive production areas.

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