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EXCRETION D'AMMONIAQUE : INDICE DE L'UTILISATION DE L'AZOTE PURIQUE CHEZ ARTEMIA

par

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R E S U M E

— La quantité d'ammoniaque excrétée par Artemia élevé dans des conditions axéniques, dépend de la nature qualitative et quantitative des nucléotides puriques utilisés pour satisfaire le besoin essentiel de ce crustacé en dérivés puriques. Les résultats obtenus dans les conditions expérimentales rigoureuses de culture sur milieu synthétique permettent de poser le problème de la signification métabolique de l'activité de l'adénylate désaminase. —

A B S T R A C T

— The quantity of ammonia excreted by Artemia reared under axenic conditions depends on the quality and quantity of purine nucleotides used to satisfy the essential purine requirement of this crustacea. Results obtained under the rigorous conditions of culture in synthetic media, rise the problem of the metabolic significance of the adenylate deaminase activity. —

M O T S - C L E S : Artemia, élevage axénique, excrétion, ammoniaque, adénylate désaminase.

K E Y W O R D S : Artemia, axenic culture, excretion, ammonia, adenylate deaminase.

Dérivés pyrimidiques	Nucléotides puriques mg %								
	AMP			GMP			IMP		
CMP = 20 mg % thymidine = 5 mg %	60	100	140	60	100	140	60	100	140
Indice de croissance	11.5	12.2	12.8	10.8	11.4	12.1	11.1	12.3	12.8
µg NH_4 - N excrété	13.62 ± 0.52	17.99 ± 0.65	22.10 ± 2.34	4.34 ± 0.4	5.92 ± 0.26	7.27 ± 0.26	5.44 ± 0.24	5.94 ± 0.55	7.58 ± 0.58

Tableau I. Quantités d'ammoniaque excrétées par Artemia en fonction de la qualité et de la quantité des nucléotides puriques alimentaires (données pour le 14ème jour)

Nucléotides puriques Calbiochem A grade	AMP acide libre mono-hydraté			GMP disodique 1,5 hydraté			IMP acide libre anhydre		
Poids moléculaire	365.24			434.2			348.2		
Pourcentage d'azote	19.21			15.88			16.08		
Concentration mg/100 ml milieu nutritif	60	100	140	60	100	140	60	100	140
N purique mg/100 ml milieu nutritif	11.5	19.2	26.9	9.5	15.9	22.2	9.6	16.1	22.5

Tableau II. Quantités d'azote purique correspondant aux concentrations des nucléotides puriques alimentaires

INTRODUCTION

Une composante essentielle de l'étude de la productivité primaire serait l'estimation de l'excrétion d'ammoniaque par le zooplancton puisque l'ammoniaque constitue une source importante d'azote pour le phytoplancton (JAWED, 1973). Etant donné la nature complexe du phénomène de l'excrétion d'ammoniaque par le zooplancton, ce paramètre est difficile à estimer dans les conditions du laboratoire, de plus les résultats difficiles à interpréter et à extrapoler aux conditions du milieu naturel (IKEDA, 1977). En accord avec PROVASOLI (1977) nous pensons que des informations importantes du point de vue écologique peuvent être acquises paradoxalement grâce à l'utilisation de conditions totalement artificielles.

La participation du métabolisme purique à la production d'ammoniaque chez les Crustacés a été considérée comme impossible à estimer (SCHOFFENIELS et GILLES, 1970). Compte tenu de l'incapacité d'Artemia à synthétiser le noyau purique (CLEGG et Coll., 1967; WARNER et McCLEAN, 1968), notre méthode d'élevage sur milieu synthétique nous a permis d'entreprendre une étude sur les effets de la qualité et de la quantité des nucléotides puriques alimentaires sur l'excrétion d'ammoniaque.

MATERIEL ET METHODES

La méthode mise au point par PROVASOLI et d'AGOSTINO (1969) pour l'élevage axénique de la race Utah est utilisée (salinité 24 ‰ ; température 25° ± 0.5° ; 5 animaux/10 ml de milieu nutritif ; 50 larves pour chacune des conditions expérimentales).

Le besoin d'Artemia en dérivés pyrimidiques est couvert par l'acide cytidylique (CMP : 20 mg %) et par la thymidine (5 mg %). Le besoin en dérivés puriques est couvert soit par l'acide adénylique (AMP), soit par l'acide guanylique (GMP), soit par l'acide inosinique (IMP) à 3 concentrations différentes (60, 100 et 140 mg %). L'index de croissance des larves est établi selon PROVASOLI et d'AGOSTINO (1969) pour le 14ème jour de développement. Le même jour, les mesures d'excrétion d'ammoniaque par Artemia ont été effectuées selon la méthode du dosage colorimétrique à l'indophénol adaptée à l'analyse automatique décrite antérieurement (HERNANDORENA et KAUSHIK, 1981).

RESULTATS ET DISCUSSION

Les résultats de cette expérience sont reportés dans le tableau I. Nous savions (HERNANDORENA, 1972) que l'indice de croissance d'Artemia augmente avec la concentration du milieu en nucléotides puriques quel que soit le mode de couverture de ce besoin par l'AMP, le GMP ou l'IMP. Il en est de même de la quantité d'ammoniaque excrétée qui augmente avec la concentration pour chacun des nucléotides. Lorsque les besoins sont couverts par l'AMP, l'excrétion ammoniacale sur 14 jours est de 13,6, 18,0 et de 22,1 µg N/Artemia, pour des concentrations respectivement de 60, 100 et 140 mg% dans le milieu nutritif. Par contre,

il ressort de ces résultats que l'excrétion d'ammoniaque pour des taux de croissance analogues, est très supérieure lorsque le besoin en nucléotides est couvert par l'AMP. Les différences observées ne peuvent pas s'expliquer par les pourcentages d'azote contenus dans chacun des nucléotides, pourcentages reportés dans le tableau II.

Le fait que le besoin alimentaire d'Artemia en nucléotide purique puisse être satisfait par l'un des trois nucléotides, suppose qu'Artemia dispose des activités enzymatiques nécessaires à l'interconversion des nucléotides puriques. L'interconversion AMP ↔ GMP selon le schéma classique met en oeuvre des réactions de désamination (AMP désaminase et GMP réductase) alors que la production d'AMP et de GMP à partir de l'IMP n'implique aucune réaction de désamination. Il apparaît effectivement que le taux d'excrétion d'ammoniaque est le plus faible quand le besoin en nucléotide est satisfait par l'IMP ; mais les différences très significatives observées entre les taux d'excrétion chez les animaux dont les besoins sont couverts par l'AMP ou le GMP posent le problème de l'existence d'un cycle purique (BISHOP et BARNES, 1971 ; GIBBS et BISHOP, 1977). Compte tenu des taux de croissance analogues obtenus chez les animaux élevés en présence des différents nucléotides, le fonctionnement du cycle purique ne serait pas lié au taux métabolique.

Le catabolisme des acides aminés contribue également à la production d'ammoniaque. L'estimation de cette part due au catabolisme des acides aminés sera le préalable à la vérification de la signification métabolique de l'activité de l'AMP désaminase

1. au niveau de la régulation de la charge énergétique (CHAPMAN et ATKINSON, 1973)

2. au niveau du fontionnement du cycle purique (BISHOP et BARNES, 1971)

3. au niveau du catabolisme purique.

Nous savons que la nature quantitative du besoin alimentaire d'Artemia en AMP varie en fonction de la concentration du milieu nutritif en albumine, en fonction de la salinité (HERNANDORENA, 1974) et en fonction de la température (HERNANDORENA, 1976). Nos conditions expérimentales vont nous permettre de sérier les facteurs susceptibles de varier dans les conditions naturelles et susceptibles de modifier le métabolisme azoté.

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THE COMPLICATED PHYSIOLOGY OF CRABS AND ITS RELATIONSHIP TO ECOLOGY AND MORPHOLOGY

by

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R E S U M E

— Mes travaux sur les crabes permettent d'explorer les problèmes liés aux études "in situ".

Les facteurs importants sont : la consommation d'oxygène, les rythmes, le comportement, les races locales, les différences liées au sexe, la taille et la variabilité individuelle.

Le caractère singulier de toute expérience isolée est souligné, mais le besoin de réaliser des observations à la fois sur l'individu et sur des moyennes établies à grande échelle est rappelé. —

A B S T R A C T

— My work on crabs is explained as a basis for exploring the problems encountered during in situ studies.

Oxygen consumption, rhythms, behaviour, local races, sex differences, size and individual variability are presented as factors of importance.

The local nature of any single experiment is emphasized, and the need for both individual studies, and averages on a broad basis, is stressed. —

M O T S - C L E S : In situ, individualité, excitibilité, formes locales, crabes.

K E Y W O R D S : In situ, individuality, excitability, local races, crabs.

INTRODUCTION

Studies of animals *in situ* are liable to many difficulties. Special equipment must often be prepared, transportation and accommodation must be organized, and many days of labour must be performed under difficult conditions. These problems can be overcome with persistence and inspiration.

Another class of problems is more difficult to surmount; the organisms and their environment are variable and, except by selection, beyond the control of the experimenter. I have studied a number of aspects of physiology and morphology in crabs and this paper is a summary of some of the complications and uncertainties that I have found in this work. I look at the problem of *in situ* studies from the consideration of animals that span an immense range of size in their growth, are long-lived, and can be studied from physiological, morphological and behavioural viewpoints, including individuality.

MATERIALS AND METHODS

I review a variety of work here, both my own and that of others. Most of this work has not been done *in situ*, but approximates this condition by using animals that were freshly collected and measured before any acclimation could take place. My work with *Libinia emarginata* (Leach), the american spider crab, was done in Woods Hole, U.S.A., in 1971-1972. These crabs were examined and dissected immediately upon collection. *Cancer pagurus* (L.) were maintained in the laboratory in London for up to two months, in 1973. *Carcinus maenas* (L.) were used for all of my recent experiments and have been freshly collected and measured immediately at their ambient temperature. *C. maenas* were measured in Roscoff, France, in 1979; in Wicklow, Ireland, in 1979-1980; and in Portaferry, N. Ireland, in 1981. Some *in situ* recordings of oxygen consumption rhythms in *C. maenas* were made in 1978 in Wicklow. The oxygen consumption rates shown in Figures 1 and 3 are excited rates produced by handling the crabs. Excitement caused by feeding is also shown in Figure 1.

RESULTS AND DISCUSSION

1. EXCITED RATES, RHYTHMS, AND DIFFERENCES RELATED TO NUTRITION

1.1. Overall responses of individual crabs

One of the first complications that I encountered in crabs was their marked behavioural control of oxygen consumption (Figure 1). The results here are for *Cancer pagurus* kept in the laboratory, but similar responses are found in freshly collected *Carcinus maenas*. Figure 1 is a facsimile of an individual recording, showing the general shape of the cycle (Aldrich, 1975A; Aldrich and McMullan, 1979). Due to individual asynchronies the average of many such cycles approaches the form of a sinusoidal curve. There was an overall semi-lunar rhythm for both starved and fed crabs, and this persisted for nearly two months in the laboratory. Such rhythms can invalidate experiments that do not account for them. Over several weeks the difference in nutritive levels caused the rates of starved and fed crabs to diverge, but the rhythmicity remained the same.

1.2. Excited and quiescent rates of oxygen consumption

Within the semi-lunar rhythm there was a marked difference between the rates of oxygen consumption of crabs that were undisturbed (quiescent) and those that were disturbed or excited by feeding (F) or handling (H). The excited rate lasted longer when it was caused by feeding (Aldrich, 1975B). Both starved and fed crabs doubled their rates of oxygen consumption when excited, possibly by switching from the use of one scaphognathite and set of gills, to the use of both sets (Cumberlidge and Uglow, 1977). This doubling appears to be a function of the quiescent rate because higher quiescent rates towards the peak of the semi-lunar rhythm are correlated with higher excited rates. Some freshly collected Carcinus maenas have shown very low rates of oxygen consumption and no rhythms nor any excited rates. I presume that these animals were very starved under their natural conditions (Aldrich, 1977). This possibility is represented by the lowest line in Figure 1.

1.3. Differences between laboratory and in situ experiments

The general level of oxygen consumption rates found in Carcinus maenas measured over 24 hours was twice as high in the laboratory as in situ (Aldrich, 1979). I have assumed that this was due to excitement caused by the change in surroundings, but in this case there must be at least two levels to the excited rate found in the laboratory. Freshly collected crabs exhibit the excited rate when handled, but this has a short duration (Figure 1). If the rate then falls to a lower excited rate compared with crabs in situ, then all laboratory work may be based on unnaturally high rates. The recordings of rates in situ did not reveal any overt rhythms, except for a suspected correlation between the sudden change in temperature with the incoming tide (Aldrich, 1979). Depledge (1978) also did not find any rhythms in Carcinus maenas recorded in situ. Since I plan to make more recordings of in situ rhythms in this crab using better respirometers I will not speculate further until then.

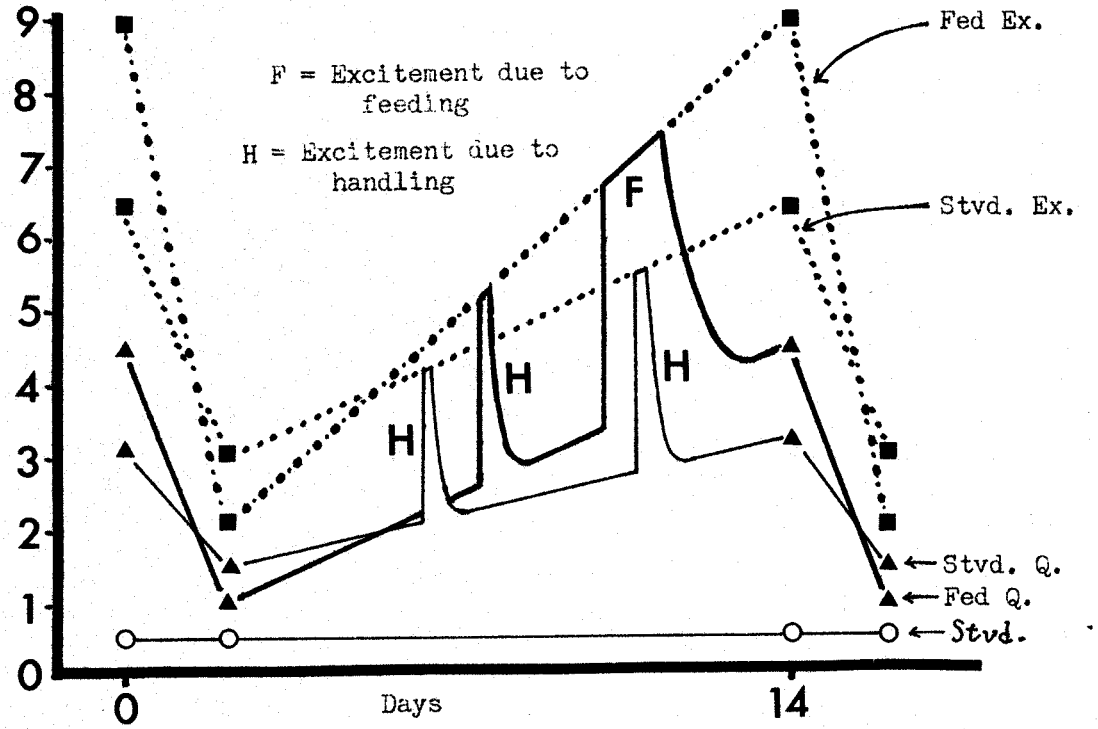

FIGURE 1. Excited (Ex.) and quiescent (Q.) rates of oxygen consumption in fed or starved Cancer pagurus. (See 1.1. - 1.3.)

1.4. The effects of animal behaviour upon experiments

Animals can see, hear, smell, or various combinations of these. For this reason investigations that aim to discover the normal state of an animal must be performed without disturbing it. It is not easy to make such measurements. Oxygen consumption is usually recorded with the animal in a closed container which restricts normal motion. The container can be put in the normal environment, but the animal remains caged. Heart beat can be remotely recorded, but biochemical measurements on living animals require some form of non-destructive sampling, and the materials will probably have to be processed in a laboratory. How to do these things without restricting or disturbing an animal is an open question.

2. THE INFLUENCE OF LOCAL RACES AND SIZE DIFFERENCES ON PHYSIOLOGY

2.1. Local races

The existance of morphological differences in local races has been well documented by Teissier (1936). My own recent work has been devoted to the physiological differences in local races of Carcinus maenas (Aldrich, in press; also work in preparation). Looking first at the relative weights of organs, crabs from Roscoff had larger digestive glands and larger gills than in crabs from Wicklow, when both populations were measured in 1979 (Figure 2). Carcinus subsequently measured in Portaferry had the largest gills of all. The average oxygen consumption of the Roscoff crabs was greater than in the Wicklow crabs, but consumption was highest in Portaferry. Thus the size of the gills was ranked with the rate of oxygen consumption. This correlation could be expected. Gray (1957) found a correlation between the gill area and the relative metabolic rate of several american crabs.

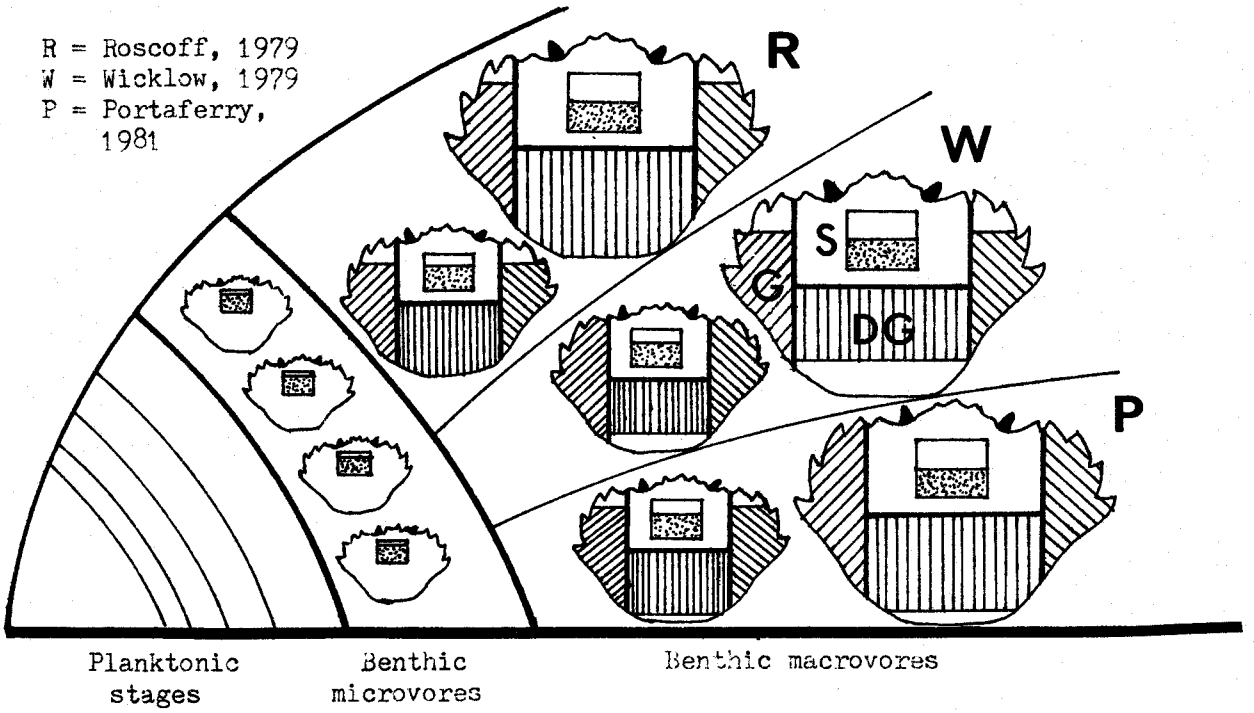

FIGURE 2. Local differences in the proportional sizes of organs in Carcinus maenas. Gills (G), digestive gland (DG), and stomach (S) are shown. Differences in the relative weights of gills and digestive gland were measured in the three populations of Carcinus maenas, (See 2.1.- 2.3.)

2.2. Size differences

I have drawn Figure 2 to emphasize the different ecological niches of the same species as it passes through the immense range of size common to many marine animals. These different niches are a consequence both of the increase in size and morphological changes, and must lead to changes in diet and general energetics (Aldrich, 1972). I found that larger specimens of the american spider crab, Libinia emarginata, were using less of their stomach capacity than were the smaller rapidly growing sizes (Aldrich, 1974). I have not looked for this in Carcinus but show it in the figure as a suggestion for further studies. In the same paper on Libinia I showed the similar slopes for the size regression of oxygen consumption, and for the weight of the digestive gland. I am now working on this relationship in Carcinus. In situ studies can be used to discover the extremes, both high and low, to which physiological functions are used. They can also discover whether the organisms are limited at any stage in their growth by their design. These studies can lead to estimations of the suitability of an organism for its niche, and whether behavioural adaptations are more important than morphological ones.

2.3. Experiments based upon one population

Because of local races and local peculiarities in ecological factors, each population studied will differ from all other populations in subtle ways. It may not be possible to generalize as broadly as one wishes from in situ work because of this limitation. It is even more likely that many laboratory studies have been unintentionally biassed by the assumption that all populations of the same species are equivalent. There is no need to despair over this, many local differences must be slight, but experiments should take these variations into account.

3. INDIVIDUAL VARIABILITY

3.1 Homogeneity of the excited rate

Despite using what should be a well defined upper or excited rate of oxygen consumption to minimise variability, there is still a great range of individual variation in rates (Figure 3). This variation is not the difference between the excited and quiescent rates. The excited rates were plotted on probability paper (Cassie, 1950, 1954) and formed a single normal distribution (Aldrich, 1975C, also in press). Thus for a given experiment the rate was homogeneous. A mixture of both the quiescent and excited rates gives a marked bi-modal plot (Aldrich, 1975C).

3.2. Scope for individual variation

Because of the above analysis, the two to three-fold range of values for the excited rate represents the scope for individual variation within this upper rate. This variation could comprise asynchronies in rhythms, differences between crabs that were well or poorly fed in situ, parasitism, and individual differences in behaviour. In short, this part of the variation is unexplained.

3.3. Sexual differences

A sexual difference is apparent in Figure 3, only males weigh more than 60g and the average respiratory rate of this group is below that expected by extrapolating from smaller crabs. I have found this decrease in male functions in Libinia emarginata as well. Here smaller digestive glands were

found in large males than extrapolations from smaller sizes would predict (Aldrich, 1974). It is therefore important to know something about size-related changes in physiological functions before predictions from studies on a restricted size range are made.

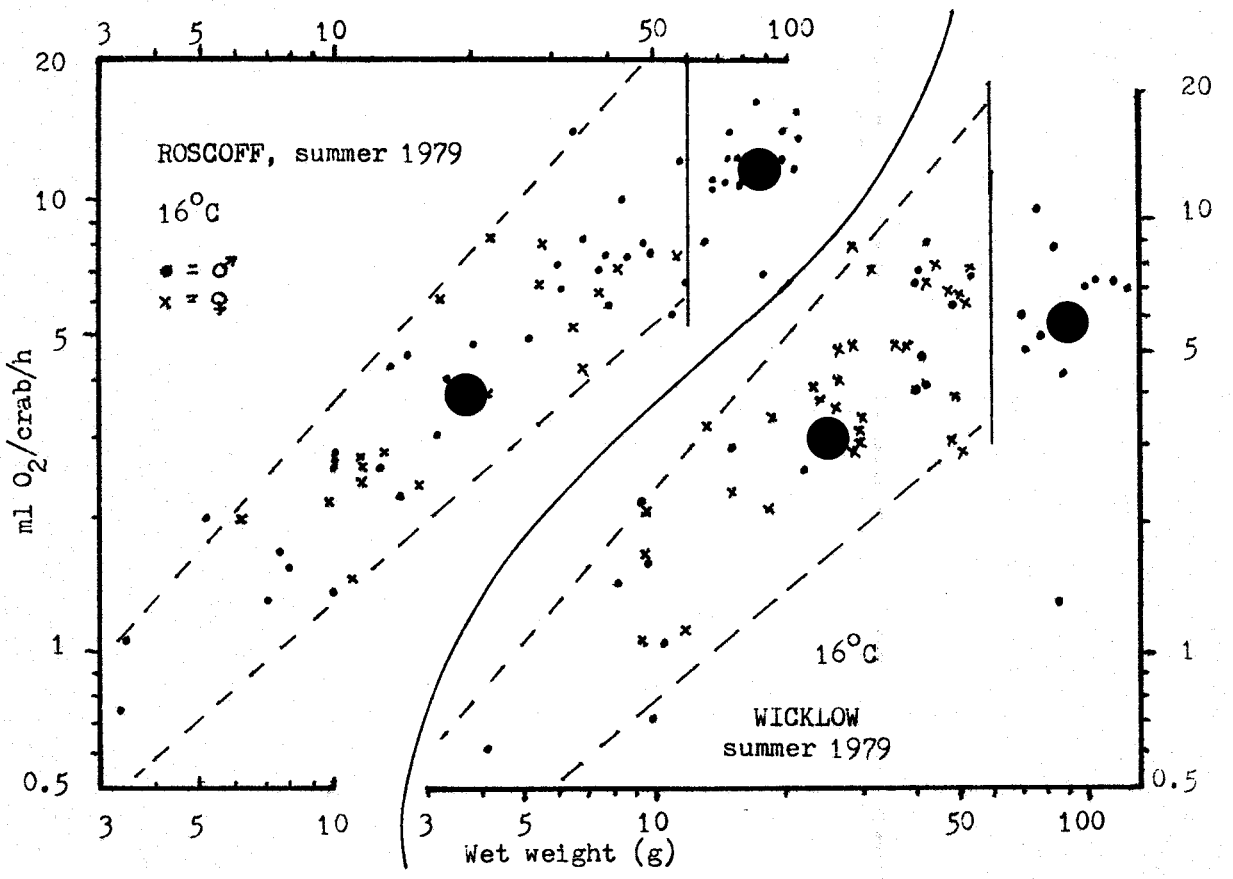

FIGURE 3. Local races, individual variability, and size effects in the oxygen consumption of Carcinus maenas.

(See 3.1.-3.3.)

3.4. The importance of analizing the individual

Because long recordings of individual animals may have to form the basis of some in situ work (E.g., Aldrich, 1979), much must be done with a small amount of data. Thus every experiment may be a collection of individual data, the story of a day and an animal repeated several times. Each day and each story must be treasured for what it is, the best that chance allowed. Extracting one analysis from these stories is a great art, perhaps equivalent in some respects to the convoluted arguments of taxonomy. A crude analysis would be to compare the ecological background with body dimensions, as I have done. A much more defined analysis would be to compare hormonal and enzymatic changes which must reflect the instantaneous state of the individual animals.

The range of variables encountered, their unique combinations, and the maximum and minimum rates exhibited are not given by averages, nor can they be derived from them (Sollberger, 1965). Remember that most invertebrates are eaten by other creatures, that survival to reproductive age is unusual from a statistical standpoint, that local populations of small animals may never develop enough to breed, and that the old successful breeder may contribute

disproportionately to the total stock. Further, there are good and bad years for all such stocks. Individual success is thus a phenomenon.

4. TEMPERATURE AND BIOCHEMISTRY

4.1. Temperature

Newell (1979) discusses his own and other work to the effect that in the natural environment, many species compensate for the variation in temperature with only slightly altered rates of oxygen consumption. I can amplify this conclusion from my work with Carcinus maenas. The response to temperature can be masked by local differences, so that crabs measured at lower in situ temperatures can have higher metabolic rates than crabs measured at higher in situ temperatures.(Aldrich, in press). Note that in Figure 3 crabs measured at the same in situ temperature (16°C) had different average rates of oxygen consumption. The question of local compensations for temperature, or just great local differences in metabolic rates requires much further work in situ.

As a further complication, I suspect that the maximum capacity for growth may rarely be realized in temperate climates. Cold temperatures in winter interrupt growth that would continue in warmer climates. The rates that we measure are therefore those that do exist, not those that can exist. Perhaps it is best to assume as a starting point, that all conditions are unsuitable for maximum rates, and that all animals have been forced into sub-optimal environments by predation and competition.

4.2. Biochemistry

There has been much recent laboratory work on biochemical mechanisms (for review see Newell, 1979) and in situ work is increasing. Boucher et al., (1975) measured the natural production of digestive enzymes in zooplankton. Langton (1977) has investigated the natural digestive rhythms in Mytilus edulis. I have already alluded to the problems in sampling from an excitable animal (1.4) but I hope that studies of motile and large species will become more common in the future. The relative role played by biochemical and behavioural control has not been quantified.

5. SEASON

5.1. Seasonal changes in size-related differences

There appear to be seasonal differences in the size-related slopes of oxygen consumption in Carcinus maenas, although these differences are often slight (Aldrich, in preparation). Klein Breteler (1975) found seasonal differences in these slopes for small Carcinus but these differences were not statistically significant, although the levels were. I am not convinced that the lack of statistical proof of these differences in slope should be accepted without further study. It seems surprising that a great range of sizes of the same species should be equally successful or not, as the seasons change. Such size ranges may span more than one feeding niche. Seasonal differences in slope have been found in the Iceland scallop, Chlamys islandica (Vahl, 1978).

5.2. Seasonal changes in the ecological background

Variables in the field will be far more numerous than in the laboratory, and there is no choice in this. As an example consider the changes in tides and weather in the intertidal zone. Noon or midnight, and the highest tides correspond twice each month so one might expect great activity in nocturnal species that also have a tidal rhythm. But tides are also controlled by wind, and temperatures can vary considerably between bright and cloudy days. The chances of having the best conditions for activity at the same time, or even the same day in each month, are remote. Months pass quickly and the ecological background of in situ experiments changes accordingly. I think it is no exaggeration to say that in the North Atlantic environment there will be no two days alike in the entire year, when we consider the totality of background factors.

Invertebrates grow quickly, and with this growth their relative metabolic rates and their requirements change. They pass through successive niches according to their size and abilities. An energetic planktonic larva quickly becomes a slower benthic juvenile and finally an adult that may feed rarely. Thus the environment and the animals change. We must learn to see the world as our subjects do.

CONCLUSIONS

The major problem with in situ studies is in my opinion, that very few of the natural variations in the environment can be selected at will. The organisms found in situ can be manipulated in various ways but in the end result their natural behaviour and physiological experience may override all other factors. I can imagine in situ studies taking two directions. One direction will use many animals in each experiment, disregarding their individuality entirely, and concentrating on average results. These large experiments can be repeated in many environments and seasons. Their analysis will require some multivariate technique. The other direction will lead to very defined analyses of individual behaviour in a small number of specimens, and in a neccessarily restricted range of natural conditions. These two approaches will then be combined on a statistical basis to predict the individual mechanisms by which a population survives. This will not reveal the entire story, but the limitations of time, money and natural complexity must be acknowledged.

"What is the first business of the philosopher? To cast away conceit: for it is impossible for a man to begin learning what he thinks he knows."

Epictetus

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