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Rate of Excretion of Ammonia by the Hard Clam *Mercenaria mercenaria* and the American Oyster *Crassostrea virginica**

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Abstract

Two species of bivalve molluscs, *Mercenaria mercenaria* Linné and *Crassostrea virginica* Gmelin, were maintained in an identical laboratory environment and fed the same diet for 1 month prior to measuring the quantity of ammonia which they excreted per gram dry weight. Data derived from juveniles and adults of these species were fitted to a log-log equation. *M. mercenaria* excreted more ammonia per gram body weight than *C. virginica,* and considerably more scatter is evident in the fit of the data from *M. mercenaria* to the log-log relationship. Experiments showed that neither temperature fluctuation during the experiments nor decomposition of organic nitrogen in the test water account for the scatter of points. Behavioral differences between the two species of organisms may explain these differences.

Introduction

The hard clam (*Mercenaria mercenaria* Linné) and the American oyster (*Crassostrea virginica* Gmelin) are important organisms from an ecological and commercial standpoint. Extremely high densities of these bivalve molluscs can dominate the fauna of local estuarine environments (Newell, 1964), so production of metabolites by these dense populations may represent a significant method of recycling nutrients in intertidal embayments. Since these animals are also important commercial seafoods, research has been underway to develop a prototype of a commercial process for the cultivation of these bivalves in a recirculating seawater environment (Epifanio *et al.*, 1974). An important component of this research is the identification of the type and quantity of nitrogenous wastes produced by these molluscs.

The comparative aspects of nitrogen excretion have been reviewed by Nicol (1960), Potts (1967) and Campbell (1973). Potts (1967) noted the general lack of reliable data on molluscan excretion.

Mercenaria mercenaria and *Crassostrea virginica* are classified as ammonotelic organisms. Ammonia excretion, however, represents only one pathway in which nitrogenous compounds can be lost to the environment by these bivalves. Other terminal products of nitrogen metabolism are often detected in quantities less than 50%.

Bivalves contain large amounts of free amino acids in their extracellular fluids (Allen, 1961). The composition of this free amino acid pool is variable and appears to be regulated by the enzyme glutamate dehydrogenase (Emerson, 1969). These amino acids can either be taken from the environment or lost to it in response to environmental conditions. Free amino acids within these organisms play an important part in the osmoregulation and ionoregulation of euryhaline invertebrates (Schoffeniels and Gilles, 1972). For example, Allen and Awapara (1960) have implicated taurine as a component of the body fluids which assists in osmoregulation in *Mytilus edulis* and *Rangia cuneata*. Jeffries (1972) in studies of *Mercenaria mercenaria* inferred that the composition of the free amino acid pool can be used as a measure of stress induced by oil pollution or laboratory conditions. Hammen (1968) showed that amino acids leak from *M. mercenaria* and *Crasso-*

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strea virginica at a rate of about 0.2 µ M/g tissue/day.

Amino acids can also be directly absorbed by molluscs. The clam *Spisula solidissima* readily removes glycine, glutamic acid, tyrosine, methionine, phenylalanine and arginine from seawater (Stephens and Schinske, 1961).

Hammen *et al.* (1966) reported analysis of nitrogenous metabolites in *Crassostrea virginica* based on excretion by 12 oysters, each weighing approximately 100 g total weight. Hammen (1968) reported further measurements of the quantity of 4 nitrogenous compounds (ammonia, urea, amino acids and uric acid) excreted by adult bivalve molluscs including *C. virginica* and *Mercenaria mercenaria*. Evidence was also presented that the proportions of each nitrogen metabolite excreted may have varied considerably even while an experiment was taking place. Ammonia excreted by *Tagelis plebius* varied from 50 to 30% of the total measured nitrogen excretion products during a 24-h test period. The specific questions of how organism size, and experimental and behavioral factors affect the quantity of individual compounds excreted within the framework of the total nitrogen budget have not been investigated.

This work reports the rate of excretion of ammonia by individual juvenile-through-adult *Mercenaria mercenaria* and *Crassostrea virginica*. These molluscs were maintained in the laboratory under identical conditions of diet, temperature, oxygen, pH and salinity. Experiments were also performed to assess factors which might affect the measurement of ammonia excretion such as temperature changes, the time at which observations were made, and the effect of an individual not pumping during portions of an experiment.

Materials and Methods

Bivalve Molluscs

Clams (*Mercenaria mercenaria*) were obtained from Rehoboth Bay, Delaware (USA), and oysters (*Crassostrea virginica*) came from the Silver Bed in upper reaches of the Delaware Bay on April 11, 1974. Single specimens were graded according to size and held for 1 month at a temperature of 20°C in a large recirculating seawater tank (Epifanio *et al.*, 1974). The clams and oysters were fed an algal diet of equal parts by number of *Phaeodactylum tricornutum, Carteria* sp., *Isochrysis galbana* and *Chryptomonas* sp. on a daily schedule and in sufficient quantities to promote growth (Epifanio and Mootz, in press). Prior to an experiment, molluscs were numbered, weighed and measured. After the experiments they were opened and contents of their shells were placed on pieces of tared aluminum foil. Oyster and clam meats were dried at a temperature of 65°C for 3 days and then weighed.

Chemical Analysis

Ammonia. The Orion 95-10 electrode was used for determination of the concentration of ammonia. We were able to achieve a precision and accuracy of 3% with this technique (Srna *et al.*, 1973).

pH. pH was measured using a Fisher Accumet 520 meter and Fisher probes (Fisher Scientific Corp., King of Prussia, Penna.)

Nitrite and Nitrate Analysis. These analyses were performed according to methods described by Strickland and Parsons (1968).

Salinity. Salinity was measured using a refractometer (American Optical Corp., Keene, New Hampshire, Cat. 10419) which was accurate to ± 1‰ Salinity.

Preliminary Experiments

Preliminary experiments were performed which aided in the design of the final experiments to determine ammonia excretion as a function of dry weight over a 24-h period.

One such preliminary experiment consisted of placing 10 adult oysters (∿ 1.2 g dry weight) in 15 l of artificial seawater and monitoring the ammonia concentrations on an hourly basis. The data showed that the ammonia concentration in the water increased nearly linearly for about 4.0 h, after which time the ammonia concentration in the water began to decline. When this occurred, analysis of the water for nitrite and nitrate ions showed a substantial concentration increase. This suggested that nitrification was occurring in the containers used in the experiment. Since ammonia production data would be more precise if a single analytical method were used to measure ammonia excretion rather than three separate measurements, we attempted to exclude nitrifying bacteria from experiments. The artificial seawater was sterilized in an autoclave prior to using it in an experiment. Each mollusc shell was coated with a thin layer of silicone stopcock grease. Under these conditions the rate of nitrification in the water decreased, but still

proceeded at a measurable rate. Therefore it was necessary in all subsequent experiments to measure nitrite and nitrate concentration in addition to ammonia in the seawater. In a control experiment, ammonium chloride at a concentration of 3.0×10^{-5} M/l gave no evidence of ammonia loss over a 24-h period. Typically, 50% of the nitrogen species measured at the end of a 24-h experiment was nitrate ion. However, none of these nitrogen species ever approached a concentration in the water which was toxic to the molluscs (Epifanio and Srna, 1975).

Procedures

Ammonia Excretion as a Function of Dry Weight. The bivalves were carefully cleaned with a soft bristle brush, rinsed with fresh water, and blotted dry. They were then weighed, measured, and placed in a battery jar containing artificial seawater (Instant Ocean). This water was prepared so that it was the same temperature (20°C ± 1C°) and salinity (30 ± 2‰) as the water in which they had been held. The molluscs were kept in this tank for 16 h without food so that feces or pseudofeces would not be present in the experimental water. After this period, individuals were put in aerated culture dishes containing seawater at the same temperature and salinity as the water to which they had been acclimated. They remained in these dishes for 24 h. Oxygen was kept at saturation by aeration. Temperature was measured in a control dish during the experiment. After 24 h, the molluscs were removed from the dishes and the water was analyzed for ammonia, pH, nitrite and nitrate; analysis of the water was completed within 3 h after removal of the molluscs from the dishes. The dry weight of the molluscs was determined. The amount of ammonia produced by each individual was calculated by adding the ammonia, nitrite and nitrate concentration of the water in the experimental dishes and subtracting from this value the ammonia, nitrite and nitrate concentration of the water in the control dish.

Quantity of Ammonia Excreted by Individual Mercenaria mercenaria at Different Times. Ten adult (∿ 2 g dry weight) and 10 juvenile (∿ 1 g dry weight) clams were placed in individual dishes containing 1.0 or 0.5 l of artificial seawater and the ammonia produced was determined after a 24-h period using the procedure outlined in the previous section.

After each determination, however, the clams were returned to the holding tank until 1 week later when the procedure was repeated. Three determinations, one each week, were made using these individuals.

Effect of Rapid Temperature Change on Ammonia Excretion. Preliminary treatment of the bivalves used was identical to that in experiments described previously. However, temperature control was ± 0.1C°. Groups of 15 clams or oysters which had been maintained at 20°C were placed in 8 l of water of 10°, 15°, 20°, 25°, or 30°C for 24 h. The water was analyzed before and after the 24-h experiments.

Effect of Decomposition of Organic Metabolites and Being Out of Water on Measured Ammonia Excretion. Groups of 15 adult clams and oysters were kept out of water for 0, 8, and 24 h and then placed in 8 l of seawater for 24, 16, and 3 h, respectively. After the bivalves had been removed, samples of this water were analyzed for pH, ammonia, nitrite and nitrate. The water was not discarded, but aerated at room temperature (20°C) for 4 additional days. Analysis of this water for its inorganic nitrogen content and pH were carried out on a daily basis.

Results

Ammonia Excretion as a Function of Dry Weight. Figs. 1 and 2 plot the ammonia excreted during a 24-h period by *Mercenaria mercenaria* and *Crassostrea virginica*, respectively at 20°C, as a function of the dry weight of each mollusc. The data may also be fitted to an equation of the form: $\log Y = k \log x + b$, where Y is moles ($\times 10^{-6}$) of ammonia excreted/day, k is the slope of the line, x is the dry weight in grams, and b is a constant. A linear regression of the data for the clams yields a value for k of 0.94, and for b of 1.33, with a correlation coefficient of 0.67. Similarly, the analysis of data for oysters yields a k value equal to 1.22, with a correlation coefficient equal to 0.84. A Student's t test indicated the slopes of the two regressions were different at the 95% confidence level. The pH of the test water declined during the 24-h experiments but was always within the range 8.2 to 7.7.

Quantity of Ammonia Excreted by Mercenaria mercenaria at Different Times. Table 1 lists the results of determinations of ammonia

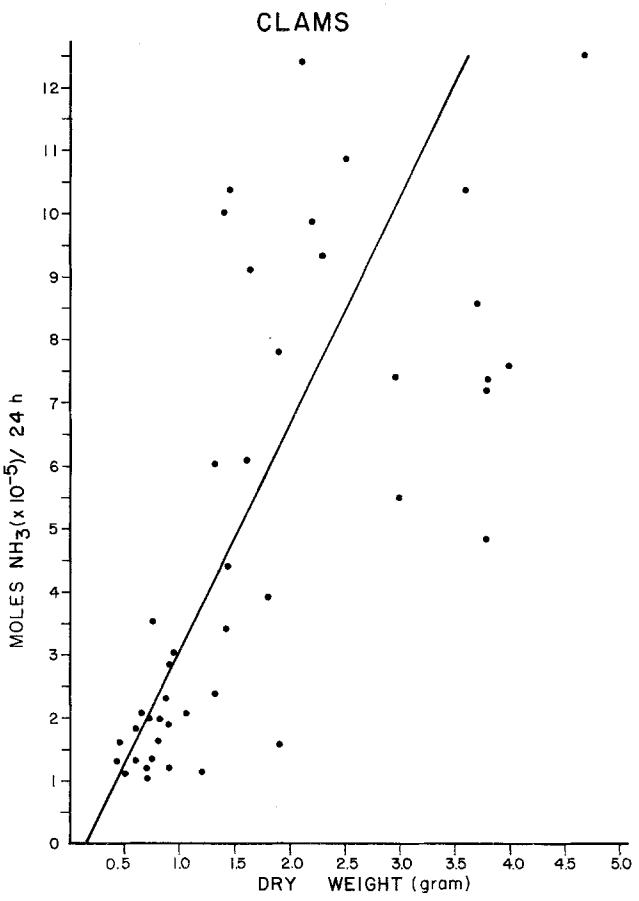

Fig. 1. *Mercenaria mercenaria*. Ammonia excretion (moles/day)

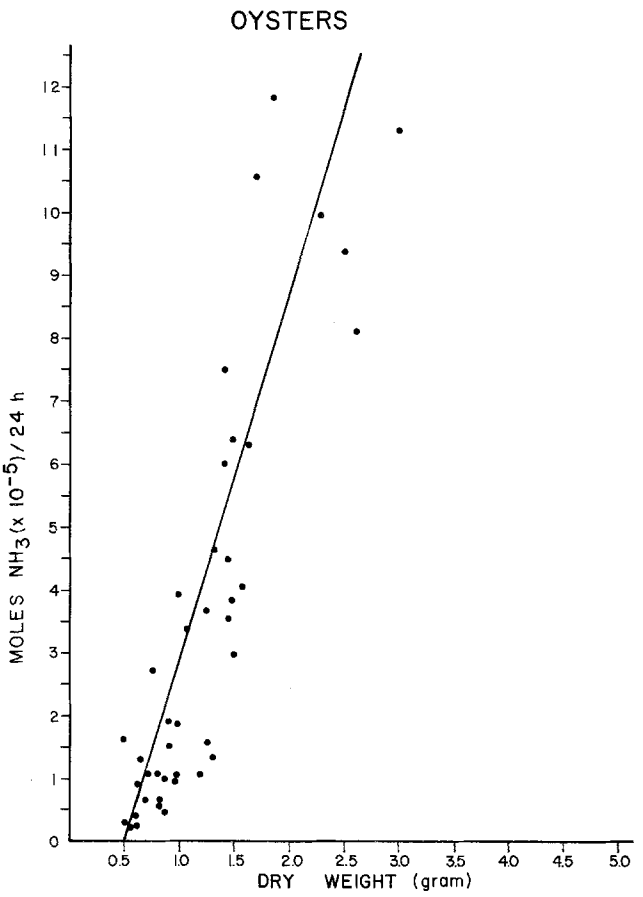

Fig. 2. *Crassostrea virginica*. Ammonia excretion (moles/day)

excreted by identifiable individuals on three separate occasions. An adult and a juvenile size-class of *M. mercenaria* were studied. The range of individual measurements was as high as ± 30% of the average of the three determinations. An *F* test showed the variance of the values of total excretion by the groups on different occasions to be significantly different at the 95% confidence level.

Effect of Rapid Temperature Change on Ammonia Excretion. The average weight of the 15 oysters used in the experiment was 0.5 ± 0.1 g and the average weight of the clams was 0.8 ± 0.2 g. Except for the lowest temperature (10°C), clams and oysters responded to a temperature perturbation by increasing the quantity of ammonia excreted. The group of clams excreted 6 times more ammonia when the temperature was rapidly increased by 10C°. Oysters whose temperature was reduced by 5C° to 15°C excreted nearly 4 times more ammonia than at 20°C. When subjected to a temperature of 10°C both clams and oysters excreted less ammonia than at 20°C. The bivalves at this temperature appeared not to open as frequently as during tests at other temperatures.

Effect of Decomposition of Organic Metabolites and Being Held out of Water on Measured Ammonia Excretion. Since bivalves excrete organic nitrogen containing compounds in addition to ammonia it is possible that decomposition of these organic metabolites will lead to erroneous ammonia excretion measurements. The results of experiments designed to assess this potential error are shown in Table 2.

The data indicate that decomposing organic metabolites caused only small increases in the ammonia concentration if the molluscs were freely pumping and analyses were completed as soon as the molluscs were removed from the water.

Discussion

Florkin and Bricteux-Gregoire (1972) pointed out the importance of examining all nitrogen products excreted by ex-

Table 1. *Mercenaria mercenaria*. Ammonia excretion by individual clams on different dates

Size-class	Dry weight of individuals studied (g)	Ammonia excreted over 24-h period (x 10^{-6} M)		
		Week 1	Week 2	Week 3
Large individuals				
1	3.04	57.2	45.3	49.7
2	2.94	61.5	66.9	59.5
3	3.63	84.2	52.7	96.6
4	3.74	34.7	45.6	85.2
5	4.58	40.6	56.0	120.5
6	3.83	41.4	31.3	42.0
7	4.60	48.4	61.0	40.9
8	3.71	50.4	43.7	67.2
9	2.54	55.6	65.6	102.1
10	2.32	38.2	66.0	87.3
Average	3.5±.8	56±14	53±12	75±27
Small individuals				
1	0.73	9.0	17.1	7.9
2	0.91	15.9	11.1	11.0
3	0.83	13.3	8.6	9.2
4	0.74	19.3	3.7	15.4
5	0.83	17.6	22.8	16.6
6	0.75	10.4	20.7	30.5
7	0.74	17.0	9.1	12.5
8	0.87	19.8	20.7	12.4
9	0.75	32.2	15.1	24.0
10	0.66	18.2	5.9	34.4
Average	0.78±.07	17±6	13±7	17±9

Table 2. *Mercenaria mercenaria* and *Crassostrea virginica*. Effect on ammonia analyses of holding clams and oysters out of water during test period

Hours held out of water	Ammonia excreted during 24-h test period (x 10^6 M/g dry weight)	Ammonia found in water after molluscs were removed (x 10^6 M/g dry weight)		
		Day 2	Day 3	Day 4
Clams				
0	22.2	24.1	25.0	30.7
8	35.3	39.5	55.4	58.9
Oysters				
0	12.2	14.5	13.2	15.5
8	22.3	23.4	21.1	33.0
24	12.5	28.6	45.5	40.0

perimental animals, since organisms have available more than one pathway by which nitrogen can be lost to the environment. The data on nitrogen excretion in *Crassostrea virginica* are illustrative. Hammen *et al.* (1966) have shown that 65% of the nitrogen found in their experimental determinations using this species was ammonia, 13% urea and 5% amino acids; 17% remained unidentified. It is clear, however, that the size (Johannes, 1964), physiological state (Widdows and Bayne, 1971) or environment of the organism measured (Feng *et al.*, 1970) can affect nitrogen excretion. Therefore, we think it important to determine not only the end products of nitrogen metabolism but also the variability in the data for animals of different size when determinations of excretion rates are made under well defined experimental conditions.

Since *Mercenaria mercenaria* and *Crassostrea virginica* occupy similar environmental niches, and can be successfully reared on the same diet, excretion measurements on the two species were made concurrently after these molluscs had been maintained under identical environmental conditions.

The bivalves received a 1-month acclimation period to their laboratory environment so that they could adjust from the environmental conditions experienced in the field to those to be experienced during experiments. This is longer than the 14-day acclimation period used by Bayne (1973) for his work with *Mytilus edulis*. Hammen (1968) did not specify the time elapsed between collection of bivalves and actual experiments, in his study using *Mercenaria mercenaria* and *Crassostrea virginica*.

During this 1-month acclimation period, the molluscs received a four-part diet, which was an equal mixture (by cell numbers) of *Phaeodactylum tricornutum*, *Carteria* sp., *Isochrysis galbana* and *Cryptomonas* sp. in quantities which have been demonstrated to be sufficient for growth from spat to maturity (Epifanio and Mootz, in press). Thus, effects of starvation or inadequate diet did not affect the metabolism of these bivalves. Since decomposing feces can contaminate the water in which the quantity of liquid metabolites is being determined (Duerr, 1968; Florkin and Bricteux-Gregoire, 1972); it was necessary to terminate the feeding schedule 16 h prior to beginning the experiment. During this time the molluscs purged their guts. They were not fed during the experiments due to the problems which result from uptake of metabolites by algae.

Artificial seawater was used in the experiments so that the water would be

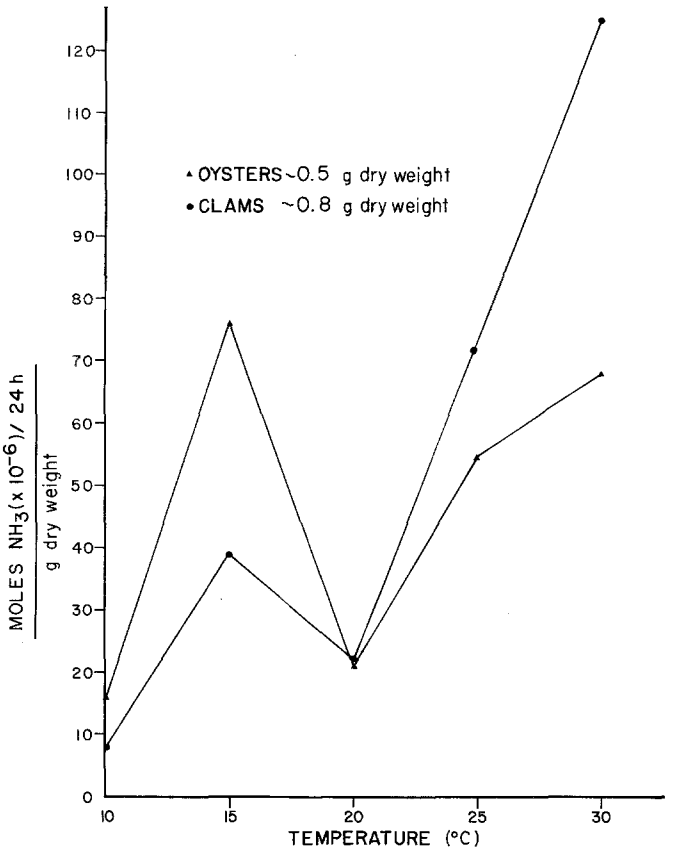

Fig. 3. *Mercenaria mercenaria* and *Crassostrea virginica*. Effect of rapid temperature change on ammonia excretion by bivalve molluscs acclimated to 20°C

free of undesirable compounds which might contaminate coastal waters. The quantity of water used in the experimental dishes was chosen so that the pH did not change beyond a normal seawater pH range of 7.7 to 8.1 during determinations. This volume was also sufficient to prevent accumulation of metabolites to a detrimental level (Epifanio and Srna, 1975). The salinity of the artificial seawater was adjusted (using distilled water) to within 1‰ of the 30‰ S to which the bivalves were acclimated. No data is available which indicates the effect of rapid temperature changes (which might occur during an experiment) on the excretion of ammonia. In order to gauge this effect, the experiments whose results are shown in Fig. 3 were performed. Individuals experiencing even a 5C° temperature perturbation from the 20°C control excreted more ammonia than individuals held under constant temperature conditions. For this reason, the temperature was controlled (± 1C°) during experiments.

The ammonia excretion rates determined for *Mercenaria mercenaria* and *Crassostrea virginica* are shown in Figs. 1 and 2, respectively. Ammonia excretion in moles/day is plotted against the dry weight of the experimental individuals. In order to compare these results with those obtained by Hammen (1968), it is necessary to convert the wet weight of these species to their dry weight. The wet and dry weight data for the bivalves used in the excretion experiments was regressed to linear equations of the form $Y=mx+b$, where Y is the wet weight and m is the slope, and x is the dry weight and b is a constant. The values for m and b were 42.79 and -5.87 for the clams and 84.29 and -45.10 for the oysters. The correlation coefficients of the data to these equations are 0.93 and 0.92, respectively. Using these equations and those which relate ammonia excretion to dry body weight, ammonia excretion rates of 0.55 µ M/g tissue per day are obtained for 51-g wet weight clams and 0.28 µ M/g tissue per day for 101-g oysters. This value for clam excretion is higher than the 0.463 µ M/g tissue per day reported by Hammen (1968). The values for the oyster excretion rates reported by Hammen (1968) ranged from 0.298 to 0.978 µ M/g tissue per day and are higher than the value computed from our data. Since the data points for the clams on Fig. 1 indicate considerable scatter around the regressed line, experiments were performed to determine the effect of time on excretion from 10 clams, measured at weekly intervals (Table 1). Large-size individuals excreted significantly more ammonia (at the 95% confidence level) in Week 3 than in Weeks 1 or 2. The smaller specimens excreted significantly less ammonia (at the 95% confidence level) in Week 2 than in Weeks 1 or 3. The results do not suggest any particular trend in the variation of excretion rates by these samples over the test period. Individual variability measured on the three occasions was also great, the range being frequently as high as ± 30% of the average of the three measurements. It is clear that some parameter in these experiments was not adequately controlled.

One possible source of variation in the measured quantities of ammonia excreted would be the utilization of alternative nitrogen excretion pathways during the experiments; for example, the organisms could have remained closed for a considerable time during the 24-h determination and have utilized anaerobic pathways which yield excretion product mixtures of considerably different composition. No specific method is avail-

able which enables careful control of the behavior of the test organisms. However, ammonia was found in all dishes, indicating that the bivalves opened at least once. Five or six random observations of the molluscs in the dishes confirmed that they opened soon (30 min) after the start of the experiment and were open during most of the experiment. Since most of the bivalves were open at all times, this indicates that there was no specific external stimuli which dictated that an individual remain closed. Also, the bivalves had not been fed for 16 h, and hunger would be expected to cause them to actively pump water.

Another possible source of error in ammonia excretion-rate determinations is decomposition of organically bound nitrogen by bacteria to yield ammonia. This factor would be expected to yield high ammonia readings. Individuals utilizing anaerobic metabolism for portions of the 24-h period might be expected to produce excretion products containing a different percentage of organic nitrogen than specimens freely pumping aerated seawater. For these reasons, bivalves were held out of water for varying periods of time before measuring excretion rates and the ammonia content of the water was monitored over a period of several days so that the affect of bacterial decomposition on measured ammonia could be estimated. Two conclusions can be drawn from the results shown in Table 2: (1) bacterial decomposition of metabolites did not contribute substantially to the ammonia measurements; (2) individuals held out of water produced considerable quantities of substances which decomposed to ammonia, specimens which were permitted to pump freely did not. These data also support the observation that under the conditions of these experiments the bivalves were open and pumping water most of the time. Clams and oysters of the same body weight which were in identical environments showed significant differences in the amount of ammonia excreted. The considerable scatter which is evident in the measured excretion by *Mercenaria mercenaria* appears to be characteristic of this species when the data is compared to the data obtained from *Crassostrea virginica* in this study. The variability in the quantity of ammonia excreted by *M. mercenaria* may be a result of strenuous activity such as occurs when the clam attempts to orient itself with its foot while in an experimental dish. *C. virginica* does not possess the ability to change its orientation.

A more general implication from this work is that when metabolic functions are being studied it is necessary to carefully define conditions prior to and during the experiments. Perturbations from the standard state can then be investigated in a systematic manner so that extrapolations to the natural state of the organisms can be made more meaningful. Subtle differences between species can be recognized and the effect of environmental stresses on their energy budgets can be tested more definitively.

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