# Ammonia Excretion by the Freshwater Prawn *Macrobrachium lar* In Relation to Diet

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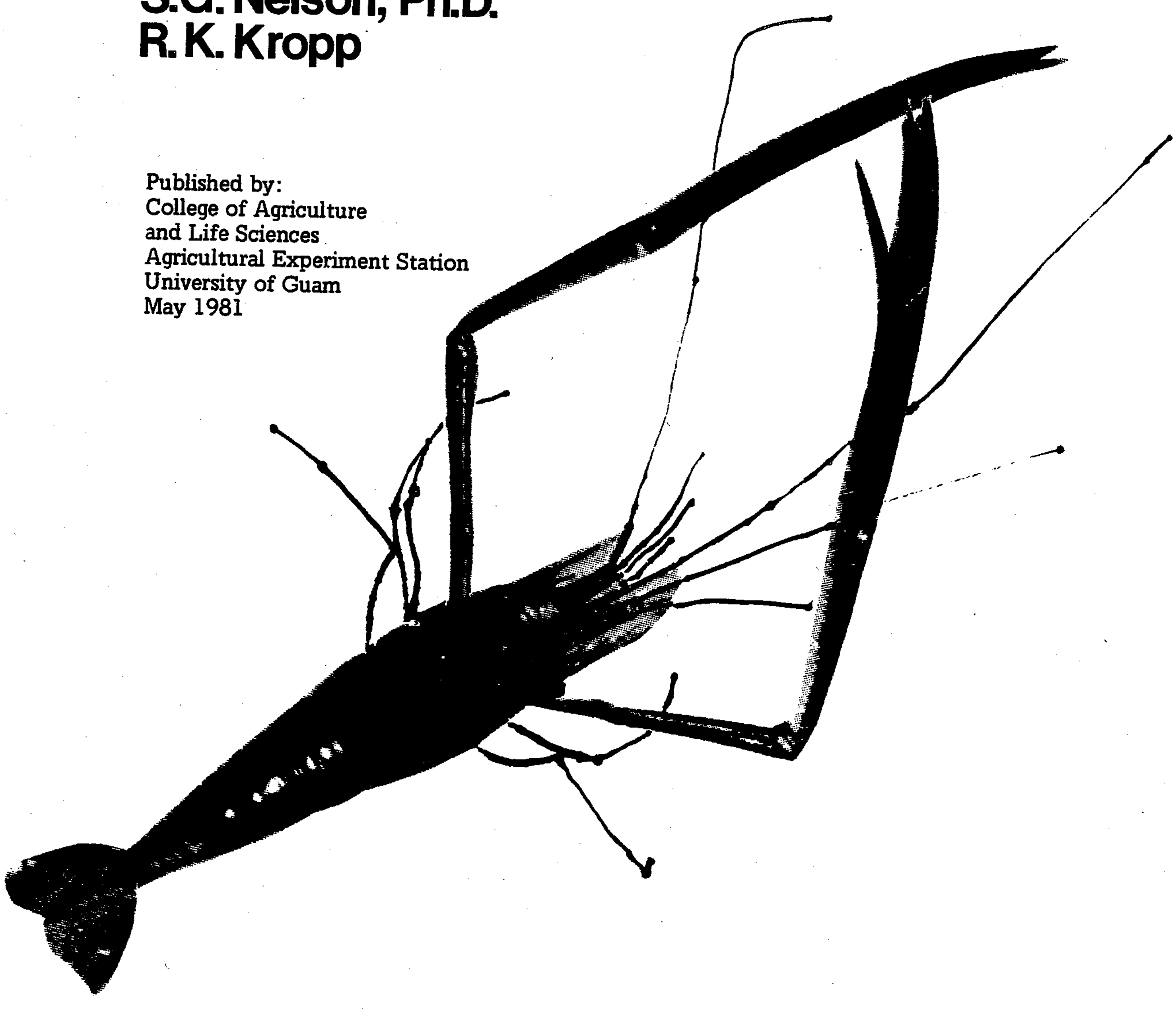

# ABSTRACT

The rates of ammonia excretion by each of 183 freshwater prawns *Macrobrachium lar* were determined. Individual prawns were either starved or fed one of six experimental diets prior to the determination of the excretion rates. The six food items included animal, plant and commercial diets and ranged from 1.5 to 14.4% nitrogen. The relation between the rate of ammonia excretion and prawn weight was influenced by the diet but this was not significantly correlated with the percent of nitrogen in the diet. For all but one diet, the rate of excretion was not correlated with the amount ingested.

# INTRODUCTION

The primary nitrogenous excretory product of aquatic organisms is ammonia (see Campbell, 1973 for review). Ammonia is important in both natural and managed aquatic ecosystems since it serves both as a nutrient for primary production and is, at high levels, a potent toxin to aquatic organisms. Several investigators have examined the role of aquatic organisms in ammonia regeneration in oceanic (Martin, 1972; Jawed, 1969; 1973; Butler et al., 1970; McCarthy and Whitledge, 1972; Biggs, 1977), estuarine (Welsh, 1975; Hale, 1975) and freshwater (Miura et al., 1978) ecosystems. Other studies have focused on ammonia excretion in analysis of nitrogen utilization by aquatic consumers (Nelson et al., 1977; Clifford and Brick, 1979; Capuzzo and Lancaster, 1979). In aquaculture systems ammonia can build up to toxic levels (see Colt and Armstrong, 1979 for review) and maintenance of these levels below critical points where growth is slowed or where mortalities occur contributes to the cost of operations (Liao, 1974).

The purpose of this study was to examine the effects of diet on the rate of ammonia excretion by the freshwater prawn *Macrobrachium lar* (Fabricus). This species was chosen because it is a prominent organism in freshwater habitats throughout Oceania and it is a species which has been suggested for aquaculture development in this region (Maciolek, 1972; Ling and Costello, 1977).

# METHODS

Experimental animals were collected from various streams on Guam. Prior to their use in the experiments the prawns were held in a cement raceway for a period of at least one week.

All excretion experiments were performed with animals held individually in glass containers filled with from 0.25 to 2.0 *l* of water. Each animal was starved for 24 hours prior to the tests. The containers were kept at 28°C ± 0.5°C in a water bath and each was gently aerated through Tygon and glass tubing by means of a diaphragm air-pump. The top of each beaker was covered with aluminum foil to prevent the prawns from escaping and to prevent contamination from external sources.

Prior to determination of $NH_4^+$ excretion rates, each prawn was either allowed to feed for two hours or to continue unfed for the same period of time. Each prawn which was fed was provided with one of six experimental diets: commercial chicken feed, commercial pig feed, aquatic alga (*Microspora* sp.), chopped flesh of a marine gastropod (*Trochus niloticus*), fish (*Scolopsis cancellatus*), or shrimp (*M. lar.*). The prawns were fed to repletion and after two hours were transferred to the containers used for the excretion experiments. The water from each of the containers used during the feeding period was filtered through a pre-weighed, course filter paper which was then dried overnight at 50°C. The filter papers were re-weighed to obtain the dry weight of the uneaten food of each prawn.

These values were corrected for losses as a result of leaching. Correction factors for leaching were determined by comparing the dry and wet weights of 20 samples per diet after they were soaked. The amount lost to leaching was expressed as percentage of the expected dry weights calculated from the wet weights of the diet samples which were fed to the prawn.

Excretion by each prawn was determined by comparing the initial ammonia concentration in each beaker with the concentration after four hours of incubation. Aliquots of 50 ml were used for each determination. The ammonia concentrations were determined with an Orion model 95-10 gas-sensing ammonia electrode.

The Orion probe measures the concentration of un-ionized ammonia ($NH_3$) in the water. Total ammonia includes both the un-ionized form ($NH_3$) and the ionized form ($NH_4^+$). Prior to ammonia determination, 0.5 ml of 10 M NaOH was added to each aliquot. This shifted the ammonia to the ionized form. Therefore, total disolved ammonia was being monitored. This is in accordance with standard procedure for use of the ammonia probe (Orion Research, Inc., 1978). Ammonia excreted by aquatic organisms is in the ionized form ($NH_4^+$), and therefore, excretion rates were expressed in this form.

The prawns were sacrificed by brief immersion in boiling water, dried overnight at 50°C and weighed on an electronic balance to the nearest 0.01 mg. Ammonia excretion rates were determined for individual prawns and expressed as mg $NH_4^+$-$N \cdot g^{-1} \cdot h^{-1}$.

Nitrogen contents of the diets were determined with a microkjedahl procedure with argininge chloride as a standard.

# RESULTS

Since metabolic rate is proportional to a power of body weight, the logarithm of metabolic rate is a linear function of the logarithm of body weight (see Kleiber, 1975 for discussion). The rate of ammonia excretion is related to weight in accordance with equation 1:

$$M/W = A\ W^{B-1} \qquad (1)$$

where M represents the rate of ammonia excretion ($mg\ NH_4^+ \cdot N \cdot g^{-1} \cdot h^{-1}$), W represents the dry weight of an individual shrimp (g), and both A and B are experimentally derived constants. A log transformation results in equation 2:

$$\log (M/W) = \log A + (B - 1) \log W. \qquad (2)$$

Statistics which describe the regression of the log of the ammonia excretion rate ($mg\ NH_4^+ \cdot N\ gdw^{-1} \cdot h^{-1}$) on log dry weight are shown in Table 1. The slope of each regression line is equivalent to the value of the exponent (B-1) in equation 1, while the intercept is equal to the log of A. While the correlation coefficients were significant ($p < 0.05$) they indicated that there was considerable variation in the rates of ammonia excretion attributable to factors other than the dry weight of the shrimp. The variation in the rate of excretion attributable to the regressions ranged from 16 to 49%. The values for A ranged from 0.03 to 0.14 and those for B ranged from 0.19 to 0.68.

Table 2 show the statistics which describe the regression of dry weight on wet weight for each of the diets. These relations were used to determine the dry weights of the meals which were fed to the shrimp. Table 3 shows the summary of data used to correct the amount ingested for the effect of leaching. The amount of leaching ranged from 0 to 12%. Most leaching occurred with the commercial diets.

The correlation of ammonia excretion rate with the amount ingested was significant ($r = -0.545^*$; $n = 20$) for prawns fed the chopped mollusc diet. However, this correlation was not significant for prawns fed any of the other diets. Thus, with the exception of the mollusc diet, the rate of ammonia excretion is independent of the size of the meal. The shrimp which were fed the mollusc diet showed a negative correlation between the rate of ammonia excretion and meal size. It is suspected that this may be an artifact of an effect of body weight on meal size.

The mean nitrogen content of each experimental diet is displayed in Table 4. The diets ranged from 1.5 to 14.4% nitrogen. This is roughly equivalent to a range of 9 to 90% protein on a dry weight basis.

# DISCUSSION

The results of this study showed that the rates of ammonia excretion by fed shrimp are higher than those of starved shrimp and that the magnitude of the increase depends on the diet and on the weight of the shrimp. The constants A and B from equation 1 were also influenced by diet, but this was not correlated with the percent of nitrogen in the diet. In this study, values for A and B varied from 0.03 to 0.14 and from 0.19 to 0.68, respectively.

The values for B found in this study are similar to values which have been reported for other crustaceans. In the present study the values for (B-1) for fed shrimp were higher than those for the unfed shrimp except in the case of the mollusc diet. This is similar to the study of ammonia excretion by the estuarine shrimp *Crangon franciscorum* (Nelson et al., 1979) in which it was reported that the increase in ammonia excretion following ingestion was pronounced in small shrimp. Clifford and Brick (1979), however, reported that these values for *Macrobrachium rosenbergii* were higher for starved shrimp than for those which were fed.

The data of Cappuzzo and Lancaster (1979) on juvenile lobsters *Homarus americanus* and those Clifford and Brick (1979) on *Macrobrachium rosenbergii* demonstrated that both A and B values were influenced by the diet, but this influence appeared to be independent of the protein or nitrogen content of the diets. In the present study, neither the values for A or B were correlated with the nitrogen content of the diet. The factors, then, which influence A and B must be related to either the assimilability of the nitrogen or to the retention of assimilated nitrogen.

Similar results have been reported for other aquatic consumers. Savitz (1971) and Savitz et al. (1977) showed that in largemouth bass *Micropterus salmoides* nitrogen excretion was correlated both to nitrogen ingested and to nitrogen assimilated. Rychly (1980) demonstrated that for rainbow trout *Salmo gairneri* nitrogen excretion increased with increasing protein and decreasing carbohydrate in the feed.

Enviromental factors may also influence the values of A and B. For example, in a study of freshwater shrimp species of Lake Biwa, Iwasa and Miura (1976) showed that both B and log A were correlated with temperature. This study, which included *Macrobrachium nipponense*, indicated that the rate of nitrogen excretion is virtually independent of temperature in water above 20 C. This, with the results of the present studies, suggests that in warm tropical waters the rates of ammonia regeneration by prawns are primarily determined by the size distributions and recent feeding histories of the prawn populations.

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Table 1. Statistics describing the regression of log of the excretion rate ($mg\ NH_4^+ \text{-} N \cdot gdw^{-1} \cdot h^{-1}$) on log of dry weight (g) for groups of *Macrobrachium lar* according to the equation log $M/W = \log A + (B - 1) \log W$.

| Diet | Correlation Coefficient (r) | Slope (B-1) | Y-intercept (Log A) | N |
|---|---|---|---|---|
| Fish | -0.722 | -0.679 | -1.131 | 26 |
| Shrimp | -0.414 | -0.655 | -0.867 | 30 |
| Algae | -0.625 | -0.692 | -1.329 | 32 |
| Pig Feed | -0.595 | -0.317 | -1.062 | 25 |
| Chicken Feed | -0.524 | -0.688 | -0.985 | 23 |
| Mollusc | -0.652 | -0.812 | -0.974 | 20 |
| Starved | -0.458 | -0.518 | -1.541 | 27 |

Table 2. Statistics describing the regression of dry weight on wet weight for experimental diets where dry weight = a(wet weight) + b, with expressed in grams.

| Diet | Correlation Coefficient (r) | Y-intercept b | Slope a | N |
|---|---|---|---|---|
| Fish | 0.993 | -0.000 | 0.218 | 24 |
| Shrimp | 0.998 | -0.005 | 0.233 | 12 |
| Algae | 0.966 | -0.008 | 0.146 | 13 |
| Pig Feed | 0.999 | -0.001 | 0.935 | 24 |
| Chicken Feed | 0.999 | -0.001 | 0.931 | 24 |
| Mollusc | 0.997 | -0.005 | 0.319 | 24 |

Table 3. Mean and standard deviation of percentage dry weight of each diet lost as a result of leaching during the feeding period. N = 12 in each case.

| Diet | Mean % Lost | Standard Deviation |
|---|---|---|
| Fish | 2.57 | 2.40 |
| Shrimp | 4.95 | 0.85 |
| Algae | -0.84 | 2.30 |
| Pig Feed | 10.47 | 1.81 |
| Chicken Feed | 11.98 | 2.47 |
| Mollusc | 7.11 | 0.92 |

Table 4. Mean nitrogen content of replicate samples of each experimental diet.

| Diet | Mean % N | Standard Deviation | N |
|---|---|---|---|
| Fish | 14.4 | 0.64 | 3 |
| Shrimp | 12.3 | 0.52 | 6 |
| Algae | 1.5 | 0.18 | 6 |
| Pig Feed | 7.5 | 0.00 | 2 |
| Chicken Feed | 3.0 | 0.41 | 3 |
| Mollusc | 14.1 | 0.81 | 3 |