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Effects of Ammonia, Salinity and Oxygen on the Growth and Energetics of the Grass Shrimp, *Palaemonetes pugio*

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EFFECTS OF AMMONIA, SALINITY AND OXYGEN ON THE GROWTH AND ENERGETICS OF THE GRASS SHRIMP, PALAEMONETES PUGIO

by
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DEDICATION

This work is dedicated to my mother. She wanted to see this work finished, but the wish of The Almighty, Allah occurred faster.

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I express my sincere thanks and appreciation for the financial support of my study and research by the Arabian Gulf University, Bahrain. I value this help and hope that I will be given the opportunity to serve the University with what I have learned.

Warmest gratitude and appreciation is expressed to my family for their love and support. Special and warm thank belong to my wife for the emotional sustenance and prayer she provided. Thanks to my father, sisters, and my children for their patience and sacrifices.

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INTRODUCTION

Population growth near major estuaries, and the use of these systems as receiving waters for sewage and industrial discharges may endanger estuarine flora and fauna (Odum 1970; Wastler and Wastler 1972). While estuaries are characterized by wide and rapid fluctuation in concentrations of ammonia and other nitrogenous wastes, dissolved oxygen (D.O.) and salinities, man's activities near these systems may intensify these changes. Ammonia may cause serious ecological problems to aquatic animals at high concentrations (Appendix A). Changes in D.O. levels and salinities may further complicate the effects of ammonia on natural systems.

In evaluating the potential ecological effects of a toxicant, the types of information which can be drawn from short-term single-factor toxicity tests, although useful, are insufficient to establish "reliably" the levels that produce little or no chronic effects on processes such as growth (Mount 1968; Sprague 1971 and 1972). The development of chronic toxicity tests designed to study the long-term effects of sublethal levels of multiple stresses on productivity may remedy the shortcomings of acute tests.

Appropriate decisions regarding release of pollutants into estuarine systems should be based upon a series of

relatively long-term tests. In order to be of value, a measurement must have some relevance to ecological fitness. In addition, these tests should have a predictive weight which would permit estimation of the degree of ecological change which may take place. These tests should, also, acknowledge the effects of combinations of stresses which may considerably exceed the effect of one agent.

One assessment technique which focuses on the estimation of the chronic effects of stress is the "scope for growth" (SFG) (Warren and Davis 1967; Bayne 1975; Nelson et al. 1985). The SFG technique employs short-term (usually 96 hr or less) measurements of a few physiological processes (such as respiration and food consumption) to estimate the energy potentially available in a stressed population for growth and reproduction. To calculate the energy budget, the values of many physiological processes are estimated based upon average values reported in the literature. Therefore, for the purpose of the present study, this type of measurement of productivity potential will be referred to as the "estimated growth".

A more powerful assessment technique which examines the chronic effects of stress involves the determination of the biological energy balance. Reasonable correlation between changes in energy balance and changes in population fitness have been reported (Edwards 1978; Capuzzo and Lancaster 1981; Reitsema 1981; Johns and Miller 1982; Llobrera 1983). This technique directly measures most, if not all, of the

major physiological processes of a population to predict the energy available for maintenance, growth, and reproduction. In the present study, this type of measurement of the productivity potential of a stressed population will be referred to as the "predicted growth" of a population.

The most comprehensive type of assessment techniqu› follows a population through a sufficiently long period of time to measure the production rate directly. In this study, this type of direct measurement of the productivity potential will be referred to as the "actual growth" of a population.

Most of the population assessment studies that have focused on productivity potential, whether "estimated", "predicted" or "actual", have been conducted under controlled laboratory conditions to evaluate the effects of a single stress. Clearly, a more realistic experimental design would evaluate the effects of a toxicant under a combination of conditions similar to those found in estuarine systems.

The present study was designed to examine the combined effects of ammonia, salinity and dissolved oxygen conditions on the energy budget of a population of juvenile grass shrimp, Palaemonetes pugio, a vital component of many estuarine food webs (Appendix A). The study also allowed comparisons of the "estimated" and "predicted" measurements to the "actual" determination of growth rates of the grass shrimp population exposed to various conditions of ammonia,

salinity and dissolved oxygen. The results of the present study should provide insight into the usefulness of the three techniques in determining the impact of combinations of natural and man-made stresses on the secondary productivity of estuarine ecosystems.

METHODS AND MATERIALS

To assist in analyzing the bioenergetics of an animal, various models have been used to represent the processes involved. The following equation has been employed to describe the energy budget of aquatic animals (Warren and Davis 1967; Brett and Groves 1979; Brafield and Llewellyn 1982):

$$G = C - (F + R + U + M) \quad \ldots (1)$$

Where,

G = energy gain as growth;

C = energy input through food consumption;

F = energy lost through feces;

R = energy lost through respiration;

U = energy lost through excretion;

M = energy lost through molting.

The experimental regime of the study examined all component of the energy budget described in equation 1. This approach allowed "actual" population growth values (G or secondary production) to be balanced against the "predicted" growth values from the right side of the equation. The actual growth values could also be compared to "estimated"

values calculated with assumptions used in traditional SFG studies.

The "actual growth" was calculated by direct determination of the energy gain during the experimental period. This energy gain was a result of the increase in dry weight of the shrimp.

The "predicted growth" was calculated by equation 1 (i.e, by subtracting the total energy expenditures for various physiological processes from the energy consumed through food). The various values of the equation were directly determined.

The "estimated growth" was calculated by the SFG equation presented by Nelson et al. (1985):

$$SFG = (C \times A) - (R + E) \quad .... \quad (2)$$

The absorption efficiency (A) value required to calculate the SFG also was calculated by the equation of Nelson et al (1985): $A = C - F/ (1-F) \times C$. The SFG equation requires information about the food consumption, fecal production, excretion rates and respiration rates. Nelson et al measured the food consumption and respiration directly. They used conversions factors to get the energy lost through respiration, fecal material and excretion. The food consumption and respiration rates were measured in the present study and utilized in the calculation. The values for the remaining processes were estimates from the

literature.

Caloric conversion factors were required to estimate the energy in each of these processes. These factors were estimated as a percentage of the ingested energy. Estimates of 10 and 6% of the ingested energy were lost through fecal production and excretion, respectively (Winberg 1971; Welsh 1975; Llobrera 1983).

In the present study, the energetic processes were examined for a full factorial combination of ammonia, salinity and D.O. conditions.

A flow-through system was designed to be used in this study. The system was capable of delivering up to 16 combinations of ammonia and salinity. A series of trial runs were made to test the performance of the system. The results of these runs indicated close agreement with the nominal concentrations, with variability that never exceeded five percent. This level of accuracy was viewed as acceptable, considering the many levels of mixing of the system.

The study was conducted in two phases. The first phase was a 21-day experiment to determine the effects of ammonia, salinity and D.O. on the growth of juvenile shrimp. During this phase, the effect of ammonia on molting frequency was also investigated. The second phase was also a growth experiment to determine the effect of ammonia toxicity on the energetics of the grass shrimp. Specific processes examined were food consumption, oxygen consumption, feces production, and excretion.

Juvenile grass shrimp, P. pugio were utilized in this study. Shrimp of a known age (between four and seven weeks old) were utilized. Shrimp were cultured in the laboratory or were obtained from a commercial source. The shrimp were acclimated to laboratory conditions for four to ten days prior to the experiments. During the acclimation and experimental periods, juvenile shrimp were fed pre-weighed rations of dry fish flakes (Tetra Min, Staple Food, West Germany) on a daily basis. The nutritional values of the flakes were: 45% protein, 5% fat, 7% fiber, and 3% sodium chloride.

Prior to phase one of the experiments, pilot studies were conducted to estimate the growth rate of the grass shrimp under control conditions. These conditions were: a salinity of 25 ppt, high dissolved oxygen (near saturation level), a temperature of 20 $\pm$ 1 ^{o}C, and the flow-through system set to a flow rate of 2.0 $\pm$ 0.3 l/hr. The primary objectives of these studies were to estimate the growth rates and to determine the minimum period of time required for a measurable amount of growth to occur. Four experiments were performed. In each experiment, there were four replicates. Each replicate consisted of three jars, each containing six shrimp. Estimation of growth rates (increases in wet weight over time) were made every five days by sacrificing the contents of one jar. The experiment was terminated when the increase in wet weight approached about 25% of the initial wet weight.

Experiments were performed at four treatment levels of ammonia: 12, 6, 3, and 0 (control) mg/l. Two levels of D.O. and four salinity levels were evaluated. The two D.O. levels were high and low. High D.O. (> 5 mg/l) was maintained as close as possible to the saturation level by continuous aeration. Low D.O. (1.7 $\pm$ 0.5 mg/l) conditions were achieved by gently pumping nitrogen gas into the solution and were maintained by a continuous flow of nitrogen just over the surface of the solution. The salinities were 20, 25, 30, and 35 ppt. There were four replicates of each treatment.

Juvenile shrimp were selected and randomly divided into 64 groups of 18 shrimp each. Each group was weighed and the wet weight recorded to the nearest 0.1 mg. Each group was randomly assigned to growth chambers receiving the specific conditions of ammonia-D.O.-salinity. Six shrimp from each group were randomly selected to determine the initial wet and dry weights. Wet weights of shrimp were determined to the nearest 0.1 mg. Water adhering to the shrimp was blotted with absorbent paper before weighing. To determine the dry weights, shrimp were sacrificed by brief immersion in boiling water. Dead shrimp were placed in aluminum pans, dried for 24 hrs at 60°C, and weighed to the nearest 0.1 mg. The ratios between dry and wet weights were determined. The initial weights of experimental populations were estimated from wet weights multiplied by these ratios.

Each growth chamber contained approximately 950 ml of

the solution. Water was delivered to the growth chambers continuously at 2.0 $\pm$ 0.3 l/hr. This flow rate corresponded to approximately 95 percent replacement of water in each growth chamber every hour. To allow sufficient time for acclimation, no ammonia was added to the system for the first 24 hr.

After 10 days of exposure to the various treatment levels, all shrimp were reweighed. Shrimp from two of the replicates were sacrificed for dry weight determinations. The other replicates were returned to their respective chambers for an additional 10 day growth period.

The specific growth rate was determined by difference between actual dry weights at the end of the experiment and estimated initial dry weights. Specific growth rates (G) as percent weight gained per day were calculated for each shrimp population using the formula (Brett 1979; Ricker 1979):

$$G = [\ln DW_2 - \ln DW_1 / t_2 - t_1] \quad \ldots (3)$$

Where,

$\ln DW_2$ = natural logarithm of final dry weight;

$\ln DW_1$ = natural logarithm of initial dry weight;

t_2 = termination time in days, and

t_1 = starting time in days.

During the course of the phase one growth experiment, molting casts were collected from each growth chamber. Collected casts were rinsed with deionized water, dried for

24 hrs at 60 °C, and stored in a desiccator for subsequent determination of dry weight, and energy content.

Flow-through respirometers were utilized to determine the rate of oxygen uptake for each experimental condition. Wide-mouth 0.5 l glass jars were used as respiration chambers. A water flow rate of 2.0 ± 0.3 l/hr was maintained by peristaltic pumps. The D.O. concentrations were measured by a Beckman Model 0260 oxygen analyzer. The oxygen analyzer was standardized according to the manufacturer's instructions at the beginning of each set of readings. Close attention was paid to the stability of the meter. In the event of unstable readings, the meter was either replaced with a stand-by meter of the same type, or the probe was changed and re-standardized. Six juvenile shrimp were utilized for each measurement. The shrimp were transported from the specific solutions to each respiration jar 30 min before the oxygen measurements were started. The wet weight of each group was determined immediately after the oxygen readings and just before transporting them back to the growth chambers.

Triplicate readings were made for each chamber and a total of three replicate chambers were estimated for each treatment. During the oxygen consumption experiments, the shrimp were fed their pre-weighed daily rations immediately after the oxygen readings were taken.

Upon termination of the oxygen consumption experiment, the shrimp were utilized to estimate food consumption and

feces production rates. Wide-mouth one-liter glass jars were utilized as feeding and living chambers. Six juvenile grass shrimp were utilized for each feeding experiment. The shrimp were starved for 48 hr prior to the experiment. The wet weight of each group of shrimp was determined immediately before the beginning of the experiment. The shrimp were placed in clean feeding chambers in the flow-through system and allowed a two hour acclimation period. Flow of water to each feeding chamber was reduced to 1.0 $\pm$ 0.20 l/hr. At the initiation of the experiment, each group of shrimp was provided with pre-weighed dry food. Shrimp were permitted to feed for two hours. Thereafter, shrimp were transferred into clean living chambers for feces collection.

Water from each feeding chamber was filtered through oven-dried and pre-weighed paper filters. Filters containing uneaten food were dried for 24 hr. at 60 ^{o}C, weighed, and the amount of uneaten food determined. Loss of food from chambers without shrimp never exceeded two percent of the initial food supplied. Food consumption rates were calculated as the weight of dry food offered minus dry weight of uneaten food.

Shrimp in the living chambers were utilized for the estimation of feces production. Flow of water to each living chamber was maintained at 1.0 $\pm$ 0.2 ml/hr to minimize loss of fecal matter in the flow-through system. Shrimp were permitted a period of 24 hr to completely evacuate their guts. Water flowing from each living chamber was

filtered during these 24 hr. The filters were dried for 24 hr at 60°C. The weight of dried filters with fecal material minus the initial weight of these filters represent the amount of fecal material that was produced over 24 hr.

This procedure of alternative feeding and fecal collection was repeated for a period of seven days to determine the rates of food consumption and feces production on the same populations of shrimp.

In order to calculate excretion rates, shrimp of known wet weight were selected and confined in 200 ml of the specified ammonia /salinity solutions. The test was carried out for all salinities of the study. The shrimp were confined for a period of four hr. Thereafter, they were sacrificed for dry weight determination. The solutions were preserved for analysis by autoanalyzer. Ammonia was analyzed colorimetrically by the automated Phenate Method (OSIC 1981). The method detection limit is 0.01 mg/l.

Caloric content of food, molt casts, and whole animals were directly evaluated using a Philipson Oxygen Microbomb Calorimeter. Three to four samples of each material were pelletized, dried, weighed to 0.1 mg, and ignited in the bomb calorimeter. Corrections were applied for wire combustion.

The frequency of molting and the average caloric content of the molts, were used to calculate the portion of the energy budget associated with the molting process.

Calories lost due to respiration were estimated from

oxygen consumption measurements utilizing a conversion oxycalorific factor of 4 kcal/g. O_2 (Welsh 1975). The caloric conversion factor for excretion was calculated from the measured ammonia production, an estimate of the amount of ammonia produced per unit of protein metabolized (Welsh 1975), and the caloric equivalents of protein metabolism (Winberg 1971).

Caloric content of feces was determined by cutting a one centimeter circle from the filter paper used to filter the water from living chambers. These pieces were weighed, pelletized, and ignited. Similar pieces from blank filter were treated in the same way to determine the caloric content of the filter paper. The caloric content of feces were determined by subtracting the caloric content of blank filters from the caloric content of the filters with fecal material.

Statistical Methods

A stepwise regression analysis (SAS 1985a) was used to evaluate the significance of different treatments and interactions on the components of the energy budget of the juvenile grass shrimp. Three dimensional plots of the regression models for growth, oxygen consumption, food consumption and feces production were produced by the SAS-Graph computer package (SAS 1985b). The regression models selected were those which represented a major portion of the

"explained" variance, but contained only highly significant ($p<0.01$) variables. If necessary, logarithmic transformations of the independent variables were employed to improve the R-square values of regression models.

RESULTS

No mortalities were observed among shrimp in the pilot studies. The means of the absolute increase of dry weight and growth rates of the shrimp populations over the ten-day periods are shown in Appendix B.

The oxygen consumption rates and the result of the regression analysis are presented in Table 1 and Figure 1. High ammonia, salinity and D.O. conditions significantly increased the oxygen consumption rates. Low D.O. condition suppressed the oxygen consumption rates, regardless of other conditions. In most cases, the oxygen consumption rates of the shrimp held in low D.O. conditions were 40% less than rates of shrimp in high D.O. conditions (Table 1). The most important interaction for oxygen consumption rates was ammonia-salinity-D.O. This interaction alone accounted for about 73% of the total variance of the data.

Molting frequencies of the grass shrimp are presented in Figure 2. Molting frequencies were mainly affected by the ammonia treatment and that salinity did not greatly affect molting frequencies. Molting appeared to be completely inhibited by ammonia concentrations of 12 mg/l, regardless of other conditions. Molting was not observed in 6 mg/l ammonia under low D.O. condition.

Table 1. Mean oxygen consumption rates (mg O_2/g dry wt./hr) and the regression analysis results of the grass shrimp, Palaemonetes pugio, exposed to different treatments. Numbers in parenthesis represent the standard errors. (n=4).

Salinity ppt	Ammonia mg/l	Oxygen consumption High D.O.	Low D.O.
20	0	2.37 (0.24)	1.61 (0.18)
	3	3.01 (0.15)	1.64 (0.20)
	6	4.07 (0.28)	1.93 (0.21)
	12	5.02 (0.27)	2.01 (0.18)
25	0	2.84 (0.31)	1.83 (0.27)
	3	2.74 (0.20)	1.94 (0.17)
	6	4.49 (0.20)	2.02 (0.15)
	12	4.91 (0.27)	1.82 (0.20)
30	0	2.11 (0.16)	2.00 (0.21)
	3	2.59 (0.22)	1.74 (0.30)
	6	3.28 (0.31)	1.82 (0.19)
	12	4.24 (0.40)	1.68 (0.19)
35	0	2.46 (0.23)	2.00 (0.17)
	3	2.29 (0.22)	1.93 (0.20)
	6	3.90 (0.37)	1.93 (0.20)
	12	4.86 (0.40)	1.93 (0.29)

Variable	B Value	Partial R-square	P>F
Intercept	1.86575000		
NH_3-salinity-D.O.	0.00718528	0.7355	0.0001
Salinity-D.O.	-0.06577937	0.0516	0.0001
D.O.	2.35501667	0.0506	0.0001
Model R-square		0.8378	

Figure 1. Regression model predictions of oxygen consumption rates (mg O_2/mg dry wt day/hr) of the grass shrimp, Palaemonetes pugio, exposed to different ammonia concentrations (mg/l) and salinities levels (ppt) under high dissolved oxygen conditions.

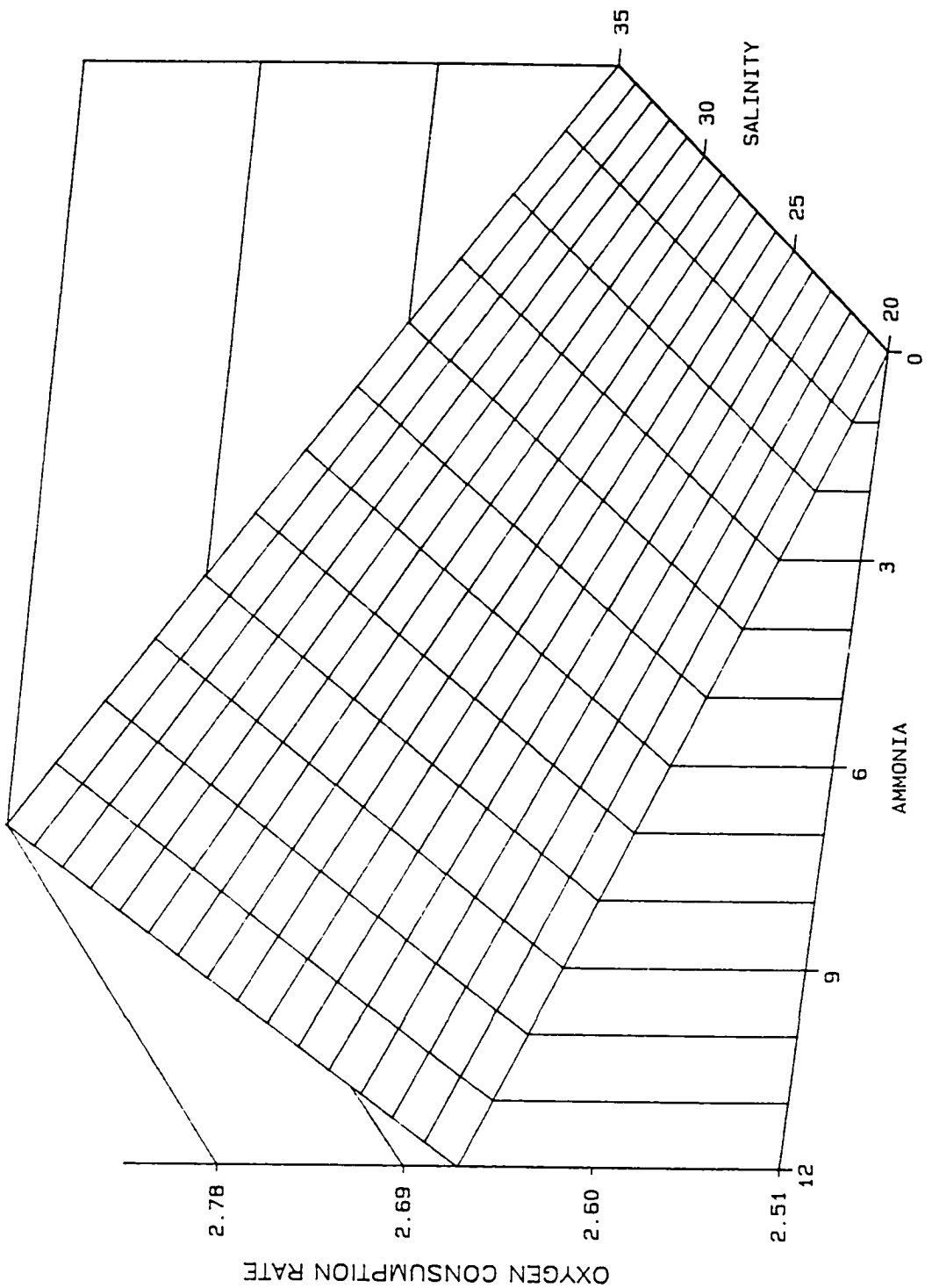
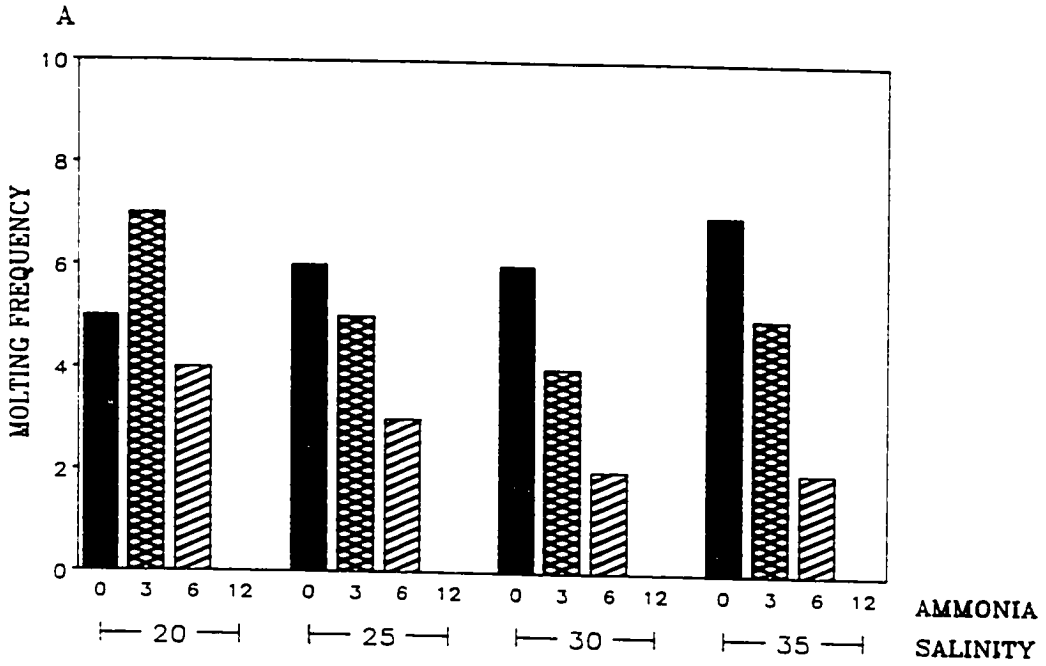
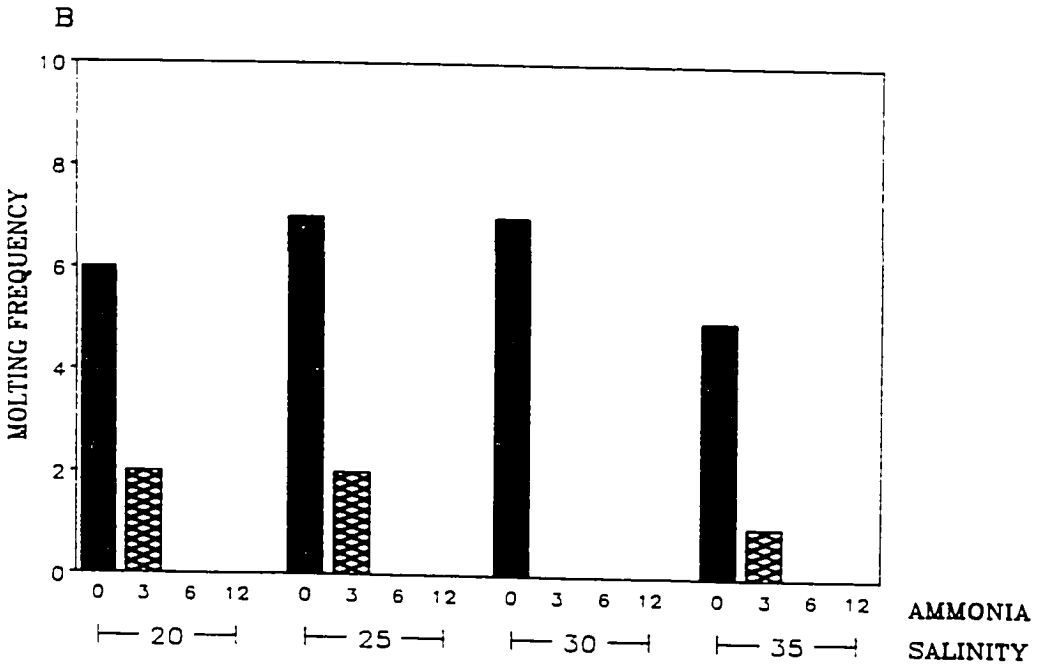


Figure 2. Molting frequencies of the grass shrimp, Palaemonetes pugio, exposed to different combinations of ammonia concentrations (mg/l), and salinity (ppt) and a high dissolved oxygen (mg/l) conditions for a period of 21 days. The values are for populations under: A) high dissolved oxygen and B) low dissolved oxygen.





The molting rates, in most cases, were highest in treatments of no ammonia.

It was impossible to observe individual shrimp in each treatment for molting events. The animals probably feed upon their own molt casts and, thus, some of the molts may not have been detected. However, this sort of error probably was randomly distributed across all treatments.

The results of the feeding experiment are reported in Table 2 and Figure 3. Food consumption rates were significantly higher in high salinities. About 67% of the variance of the food consumption data was explained by change in salinity. The ammonia-salinity interaction was also significantly and directly related to higher food consumption rates.

The results of the fecal production experiments are shown in Table 3 and Figure 4. The amount of fecal material produced by juvenile grass shrimp was significantly higher in high salinities. The interactive effects of ammonia-salinity and salinity-D.O. were also directly related to higher fecal production. The first interaction alone accounted for about 33% of the variance of the feces production data.

While food consumption and fecal production were both elevated for high ammonia and high salinity conditions, the

Table 2. Mean food consumption rates (mg/mg dry wt./day) and the regression analysis results of the grass shrimp, Palaemonetes pugio, exposed to different treatments. Numbers in parenthesis represent standard errors. (n=4).

Salinity ppt	Ammonia mg/l	food consumption High D.O.	Low D.O.
20	0	1.19 (0.012)	1.19 (0.006)
	3	1.21 (0.008)	1.22 (0.010)
	6	1.19 (0.010)	1.22 (0.009)
	12	1.22 (0.009)	1.25 (0.008)
25	0	1.21 (0.006)	1.25 (0.014)
	3	1.20 (0.020)	1.23 (0.010)
	6	1.22 (0.022)	1.26 (0.016)
	12	1.23 (0.008)	1.27 (0.019)
30	0	1.32 (0.010)	1.33 (0.014)
	3	1.36 (0.012)	1.32 (0.016)
	6	1.33 (0.012)	1.36 (0.015)
	12	1.33 (0.012)	1.36 (0.017)
35	0	1.39 (0.008)	1.28 (0.009)
	3	1.41 (0.015)	1.34 (0.010)
	6	1.42 (0.009)	1.37 (0.020)
	12	1.44 (0.009)	1.36 (0.017)

Variable	B Value	Partial R-square	P>F
Intercept	0.96437500		
Salinity	0.01133747	0.6670	0.0001
NH_3-salinity	0.00009558	0.0216	0.0001
Model R-square		0.6885	

Figure 3. Regression model predictions of food consumption rates (mg/mg dry wt/day) of the grass shrimp, Palaemonetes pugio, exposed to different ammonia concentrations (mg/l) and salinities (ppt) under high dissolved oxygen for a period of seven days.

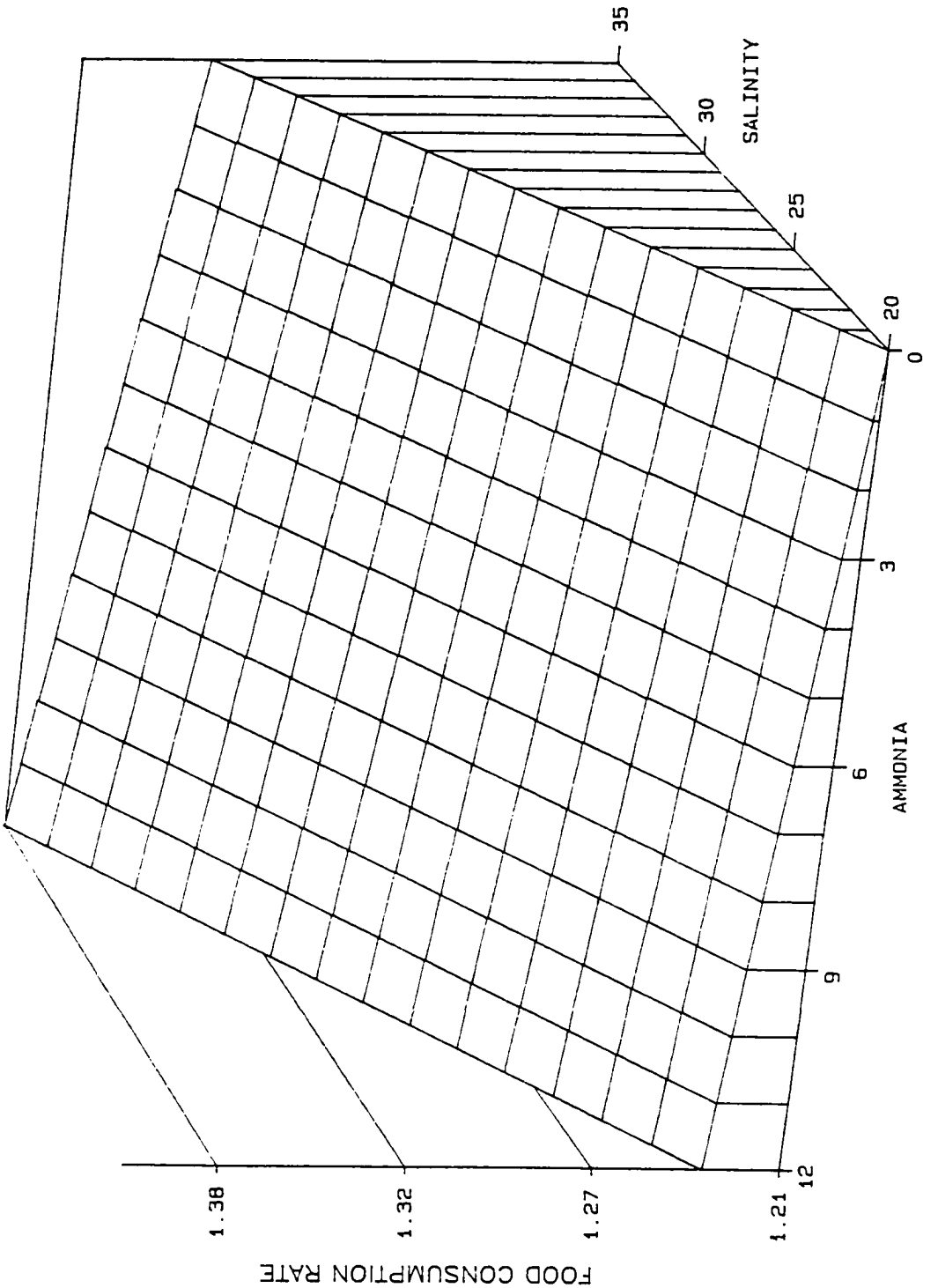
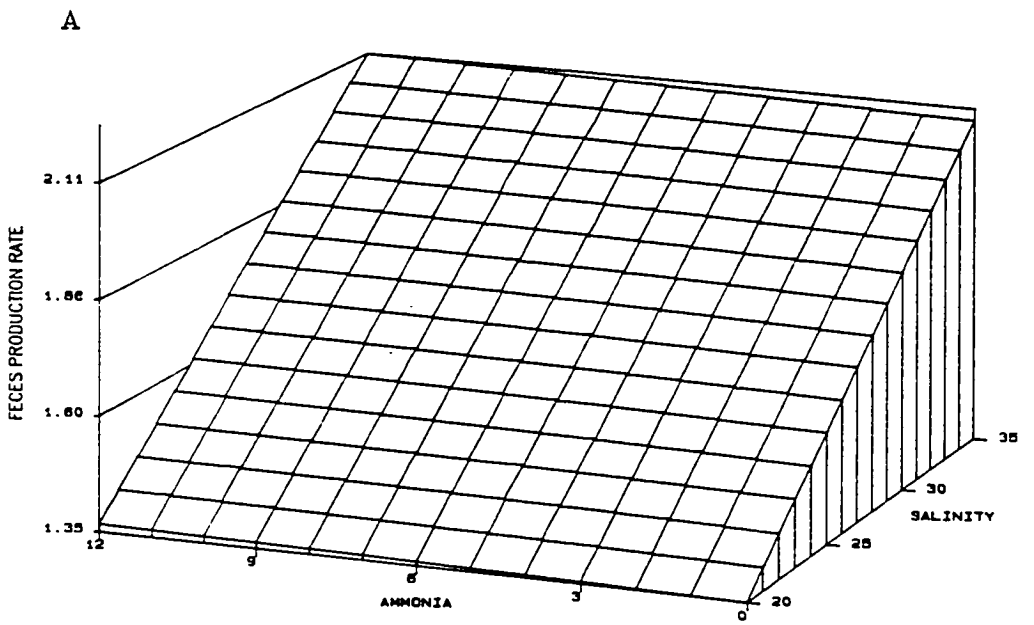


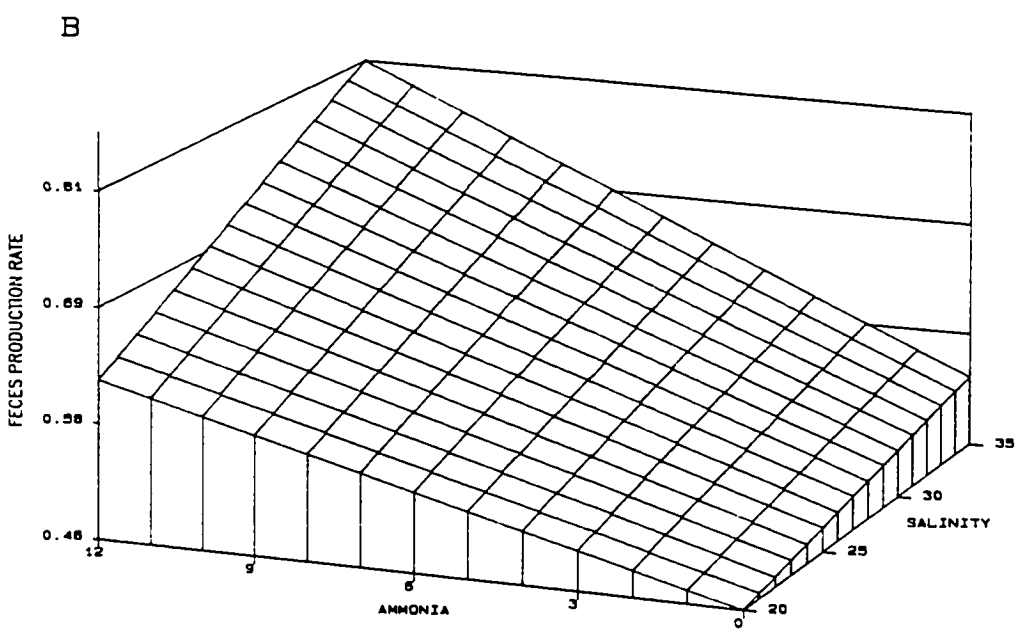
Table 3. Mean fecal production rates (mg/mg dry wt/day) and the regression analysis results of the grass shrimp, Palaemonetes pugio, exposed to different treatments. Numbers in parenthesis represent standard errors. (n=4).

Salinity ppt	Ammonia mg/l	feces production High D.O.	feces production Low D.O.
20	0	0.59 (0.010)	0.63 (0.006)
	3	0.62 (0.011)	0.63 (0.009)
	6	0.52 (0.011)	0.73 (0.010)
	12	0.54 (0.009)	0.77 0.008)
25	0	0.57 (0.009)	0.60 (0.007)
	3	0.60 (0.013)	0.60 (0.009)
	6	0.50 (0.010)	0.70 (0.010)
	12	0.56 (0.008)	0.78 (0.010)
30	0	0.57 (0.012)	0.59 (0.012)
	3	0.58 (0.010)	0.55 (0.010)
	6	0.63 (0.007)	0.67 (0.009)
	12	0.59 (0.009)	0.67 (0.010)
35	0	0.79 (0.008)	0.63 (0.012)
	3	0.72 (0.009)	0.69 (0.012)
	6	0.67 (0.010)	0.75 (0.010)
	12	0.89 (0.010)	0.75 (0.009)

Variable	B Value	Partial R-square	P>F
Intercept	0.37083929		
Salinity	0.00465686	0.1508	0.0001
Salinity-D.O.	0.00429116	0.0327	0.0001
NH_3-salinity	0.00065313	0.3326	0.0001
NH_3-salinity-D.O.	-0.00058337	0.1452	0.0001
Model R-square		0.6613	

Figure 4. Regression model predictions of fecal production rates (mg/mg dry wt./day) of the grass shrimp, Palaemonetes pugio, exposed to different ammonia concentration (mg/l) and salinity (ppt) for a period of seven days. The values are for populations under A) high dissolved oxygen and B) low dissolved oxygen.





former effect could not account for the magnitude of the latter. In another words, the shrimp did not appear to be utilizing food efficiently under high ammonia and high salinity conditions.

The excretion rates of the shrimp were significantly higher in low ammonia concentrations (Table 4 and Figure 5). There was a marked reduction of the excretion rates in the 12 mg/l ammonia treatments across all salinities. It appears that the interaction of ammonia and salinity significantly affected the excretion rates. Lower excretion rates were observed for higher ammonia and high salinity conditions.

The increase in dry weight and the survival rate of the 21-day growth experiment are given in Appendix B. Calculated growth rates and the results of the regression analysis are shown in Table 5 and Figure 6. An examination of the R-square values indicates that the effects of ammonia accounted for about 17% of the total variance in the growth rate data. High ammonia and salinity conditions significantly reduced the growth rates of the shrimp. No shrimp were observed to survive the entire experimental period at 12 mg/l ammonia and 35 ppt salinity under either high or low D.O. conditions.

Table 4. Mean excretion rates (mg NH_3/mg dry wt/day) and the regression analysis results of the grass shrimp, Palaemonetes pugio, exposed to different ammonia concentrations (mg/l) and salinities (ppt) under high dissolved oxygen conditions.

Salinity	NH_3	Excretion rate
20	3	0.17 (0.010)
	6	0.14 (0.020)
	12	0.09 (0.005)
25	0	0.16 (0.005)
	3	0.13 (0.008)
	6	0.13 (0.009)
	12	0.10 (0.005)
30	3	0.08 (0.010)
	6	0.08 (0.010)
	12	0.05 (0.005)
35	3	0.16 (0.020)
	6	0.06 (0.004)
	12	0.05 (0.020)

Variable	B Value	Partial R-square	P>F
Intercept	0.15726005		
Salinity-NH_3	-0.00027764	0.53	0.0001
Model		0.53	

Figure 5. Regression model predictions of excretions rates (mg NH_3/mg dry wt/day) of the grass shrimp, Palaemonetes pugio, exposed to different ammonia concentrations (mg/l) and salinities (ppt) under high dissolved oxygen.

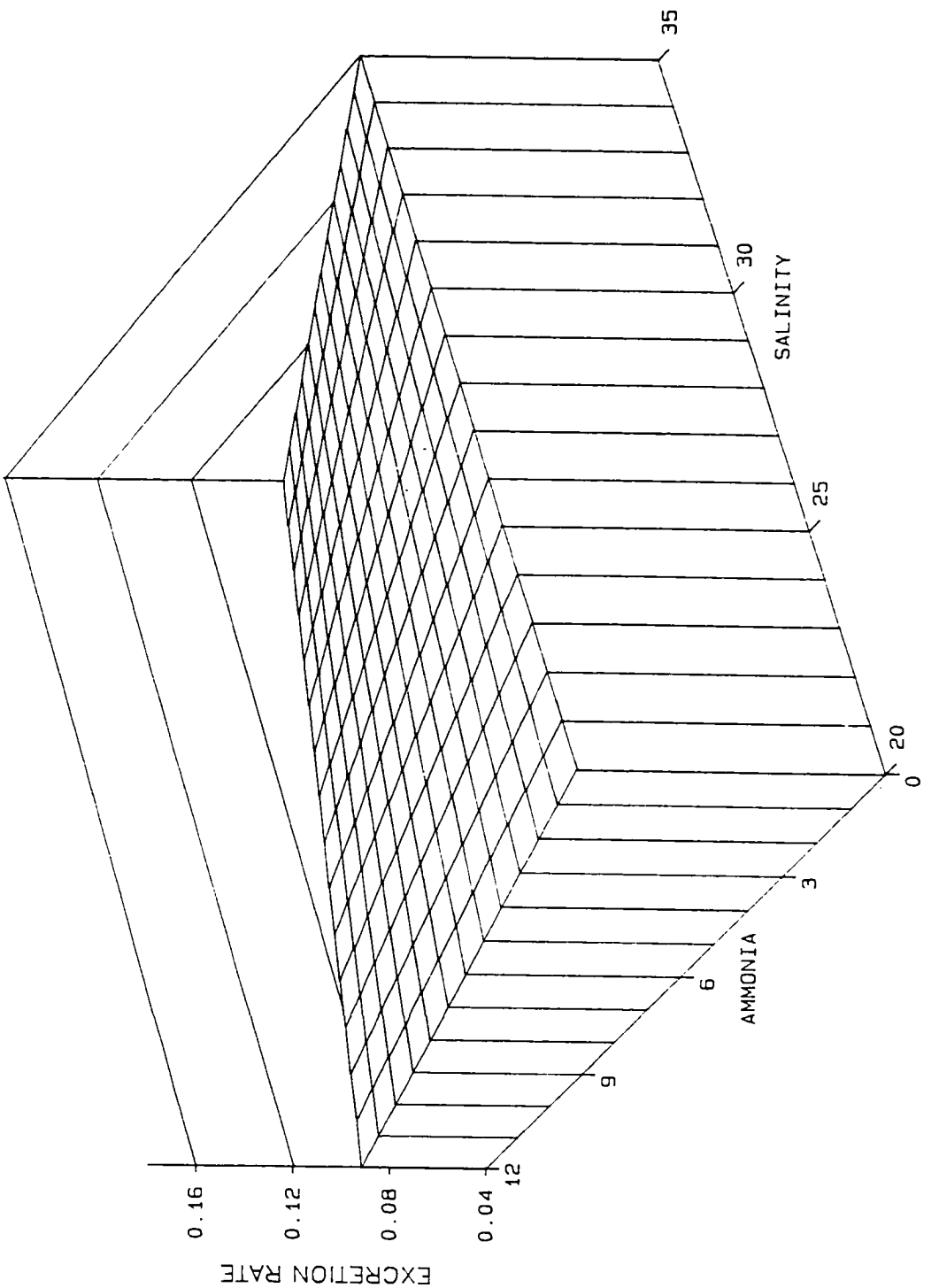


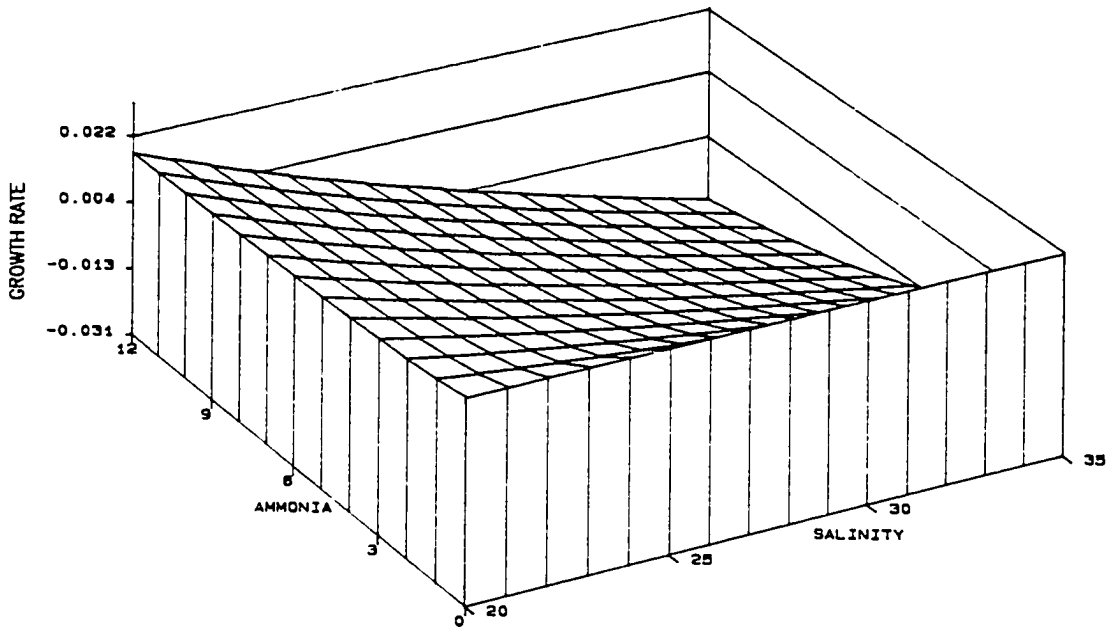
Table 5. Mean growth rates (mg d wt/day) and the results of the regression analysis of the grass shrimp, Palaemonetes pugio, exposed to different treatment conditions. Numbers with minus signs indicate negative growth. T.M. refers to total mortality where growth rate could not be calculated. Logarithmic value of (ammonia+1) and (salinity) were used in the models. (n=4).

Salinity	Ammonia	Specific Growth Rate			
ppt	mg/l	11 Days		21 Days	
		High D.O.	Low D.O.	High D.O	Low D.O
20	0	0.0133	0.0144	0.0134	0.0116
	3	0.0141	0.0062	0.0120	0.0043
	6	0.0098	0.0105	0.0169	0.0097
	12	0.0055	0.0066	0.0070	0.0041
25	0	0.0138	0.0217	0.0129	0.0140
	3	0.0202	0.0067	0.0115	0.0095
	6	0.0105	0.0144	0.0112	0.0116
	12	0.0070	-0.0040	0.0060	-0.0047
30	0	0.0092	0.0105	0.0117	0.0075
	3	0.0187	0.0075	0.0132	0.0067
	6	0.0082	-0.0035	-0.0008	0.0065
	12	0.0008	-0.0035	-0.0006	-0.0079
35	0	0.0119	0.0101	0.0155	0.0105
	3	0.0171	0.0072	0.0138	0.0073
	6	-0.0104	-0.1427	-0.0211	T.M.
	12	-0.6642	T.M.	T.M.	T.M.

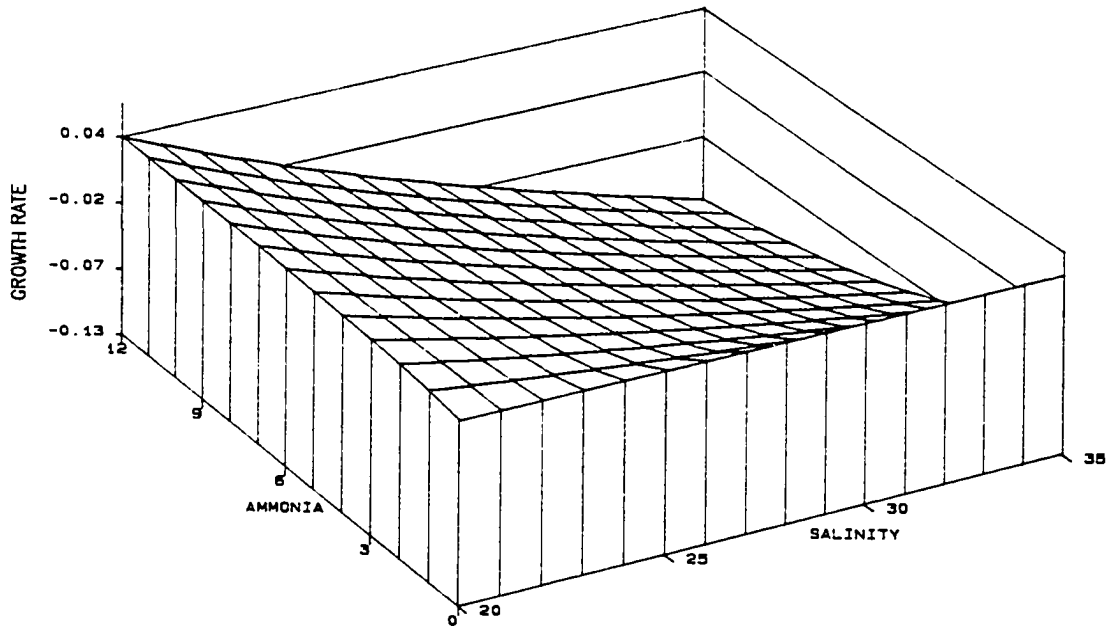
Variable	B Value	Partial R-square	P>F
Intercept	0.02200818		
NH_3	0.83803374	0.1786	0.0001
NH_3-D.O.	-0.60830288	0.0585	0.0001
NH_3-salinity	-0.63083778	0.1312	0.0001
NH_3-salinity-D.O.	0.45111604	0.0611	0.0001
Model R-square		0.4294	

Figure 6. Regression model predictions of growth rates (mg dry wt/day) of the grass shrimp, Palaemonetes pugio, exposed to different ammonia concentrations (mg/l), salinity levels (ppt) and dissolved oxygen (mg/l) conditions for a period of 21 days. The values are for populations under A) high dissolved oxygen and B) low dissolved oxygen conditions.

A



B



The energy budget calculations are presented in Tables 6 and 7. The energy lost through respiration ranged between 38 and 60% of the ingested energy. The controls lost about 39% of the ingested calories through respiration. The shrimp exposed to 12 mg/l ammonia, 35 ppt salinity and high D.O. lost about 60% of the total ingested calories through respiration. The energy lost through respiration by the shrimp exposed to low D.O. conditions was about 25% of the ingested calories, regardless of other treatments.

The energy lost through molting was calculated to reflect the frequency of the molting. The amount of calories lost through molting ranged between 8 and 20% of the total ingested energy.

The energy lost through fecal material was between 10 and 15% of the ingested energy. These percentages were similar for shrimp exposed to high and low D.O. conditions.

The amount of calories lost through ammonia excretion ranged between 3 and 5% of the ingested energy. Shrimp exposed to high ammonia excreted less ammonia and, therefore, lost less energy through excretion. Shrimp exposed to treatments with no ammonia lost 5% of their ingested energy through excretion.

The results of the three techniques for measuring the productivity potential of the shrimp are presented in Tables 6 and 7 and Figures 7 to 9. "Estimated growth" was always higher than both the "predicted" and "actual" growth, regardless of the test of conditions.

Table 6. The energy budget of populations of the grass shrimp, Palaemonetes pugio, exposed to different ammonia concentrations (mg/l), salinities (ppt) and high dissolved oxygen conditions. Values are in calories/mg dry wt./day. Output = (R+M+F+E+MR). The Balance = (C/Output)x100.

SALINITY	NH_3	C	Energy Expenditures					ACTUAL	PREDICTD	OUTPUT	%BALANCE
			R	M	F	E	MR				
20	0	153	57	10	18	8	0	46	61	139	90
	3	156	72	13	19	8	0	43	44	156	99
	6	169	98	8	20	6	0	55	38	187	110
	12	172	120	—	24	5	0	22	23	171	99
25	0	173	68	13	21	9	0	45	63	156	90
	3	160	66	11	19	8	0	53	56	157	98
	6	183	108	7	22	6	0	38	41	180	98
	12	185	118	—	26	6	0	19	36	169	91
30	0	207	51	15	25	10	0	44	106	145	70
	3	214	62	10	26	11	0	48	106	157	73
	6	200	79	5	28	6	0	21	82	139	70
	12	200	102	—	29	6	25	-2	39	160	80
35	0	213	59	15	26	11	0	54	103	165	77
	3	231	55	11	32	12	0	46	122	155	67
	6	218	94	4	30	7	46	-2	38	179	82
	12	195	117	—	29	6	142	—	—	—	—

C = Ingested energy
R = Energy lost through respiration
M = Energy lost through molting
F = Energy lost through fecal material
E = Energy lost through excretion
MR = Mortality

Table 7. The energy budget of populations of the grass shrimp, Palaemonetes pugio, exposed to different ammonia concentrations (mg/l), salinities (ppt) and low dissolved oxygen conditions. Values are in calories/mg dry wt./day. Output = (R+M+F+E+MR). The Balance = (C/Output)x100.

SALINITY	NH_3	C	Energy Expenditures					ACTUAL	PREDICTD	OUTPUT	%BALANCE
			R	M	F	E	MR				
20	0	155	39	15	19	8	0	38	75	119	76
	3	151	39	5	18	8	0	15	80	86	57
	6	161	46	—	22	5	0	29	88	102	63
	12	163	48	—	23	5	0	12	87	88	54
25	0	158	44	16	19	8	0	47	72	133	84
	3	160	47	5	19	8	0	32	81	110	69
	6	166	49	—	21	5	0	38	92	112	67
	12	169	44	—	24	5	28	20	68	121	71
30	0	155	48	10	19	8	0	22	70	107	69
	3	162	42	—	19	8	0	24	92	93	57
	6	171	44	—	22	5	0	5	100	76	45
	12	179	40	—	25	5	21	20	87	112	62
35	0	161	48	17	19	8	0	37	68	129	80
	3	169	46	3	20	9	0	25	90	103	61
	6	180	46	—	25	5	182	—	—	259	—
	12	183	46	—	26	6	170	—	—	—	—

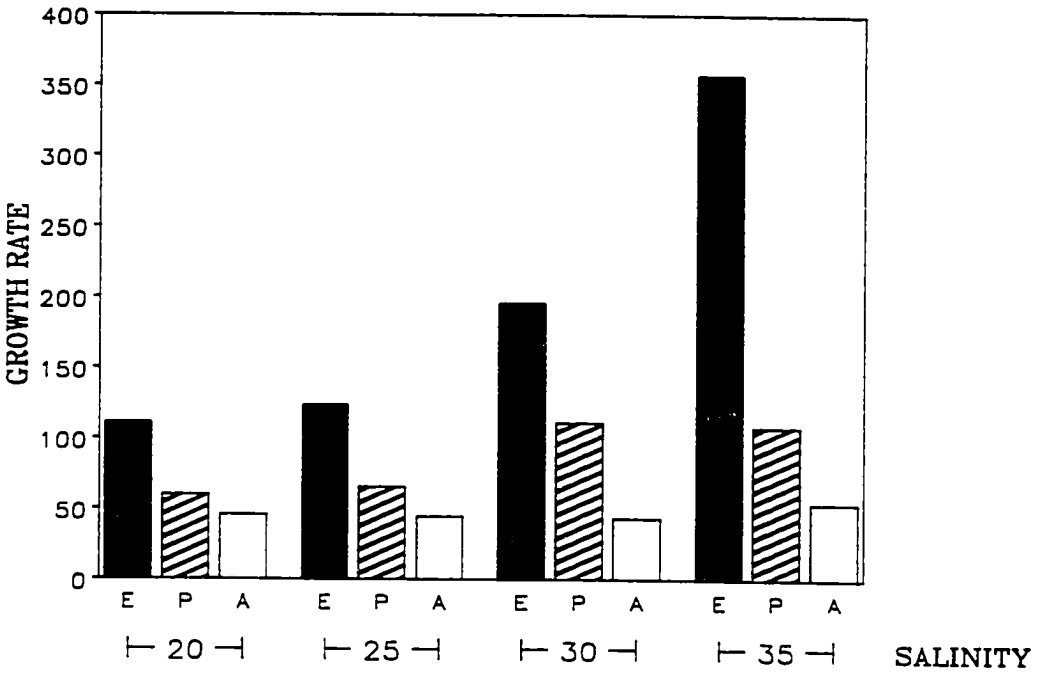
C = Ingested energy
R = Energy lost through respiration
M = Energy lost through molting
F = Energy lost through fecal material
E = Energy lost through excretion
MR = Mortality

In the absence of ammonia, the "estimated growth" values were about 80% higher than the "predicted growth" values across all salinities and D.O. conditions (Figure 7). The differences varied with ammonia, salinity and D.O. treatments but "predicted growth" was always at least 80% greater than the "actual growth" (Figures 8 and 9).

The "predicted growth" of the shrimp was also higher than the "actual growth" across all conditions. In the absence of ammonia, the discrepancies between "predicted growth" and "actual growth" values were highly affected by D.O. conditions (Figure 7). The differences between the "predicted" and "actual" growth were about 30% in 20 ppt salinity and high D.O. conditions. The discrepancies increased with increases in ammonia concentrations and salinities, reaching a maximum for high ammonia and high salinity treatments (Figures 8 and 9).

Figure 7. Productivity potential of the grass shrimp, Palaemonetes pugio, under zero ammonia concentration, and different salinities (ppt). The data were calculated for: A) high dissolved oxygen conditions and B) low dissolved oxygen. Three productivity values were calculated: E for "estimated growth", P for "predicted growth"; and A for "actual growth". (See text for description of calculations).

A



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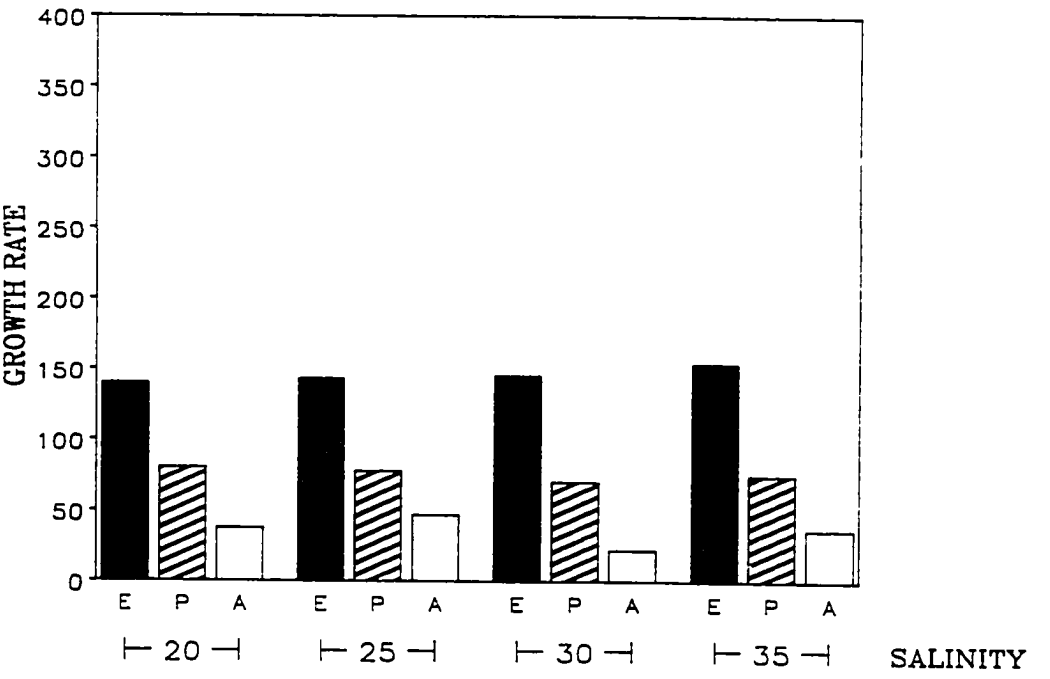


Figure 8. Productivity potential of the grass shrimp, Palaemonetes pugio, exposed to different ammonia concentrations (mg/l), and salinities (ppt) under high dissolved oxygen conditions. The data were calculated for salinities A) 20, B) 25, C) 30 and D) 35). Three productivity values were calculated: E for "estimated growth", P for "predicted growth"; and A for "actual growth". (See text for description of the calculations).

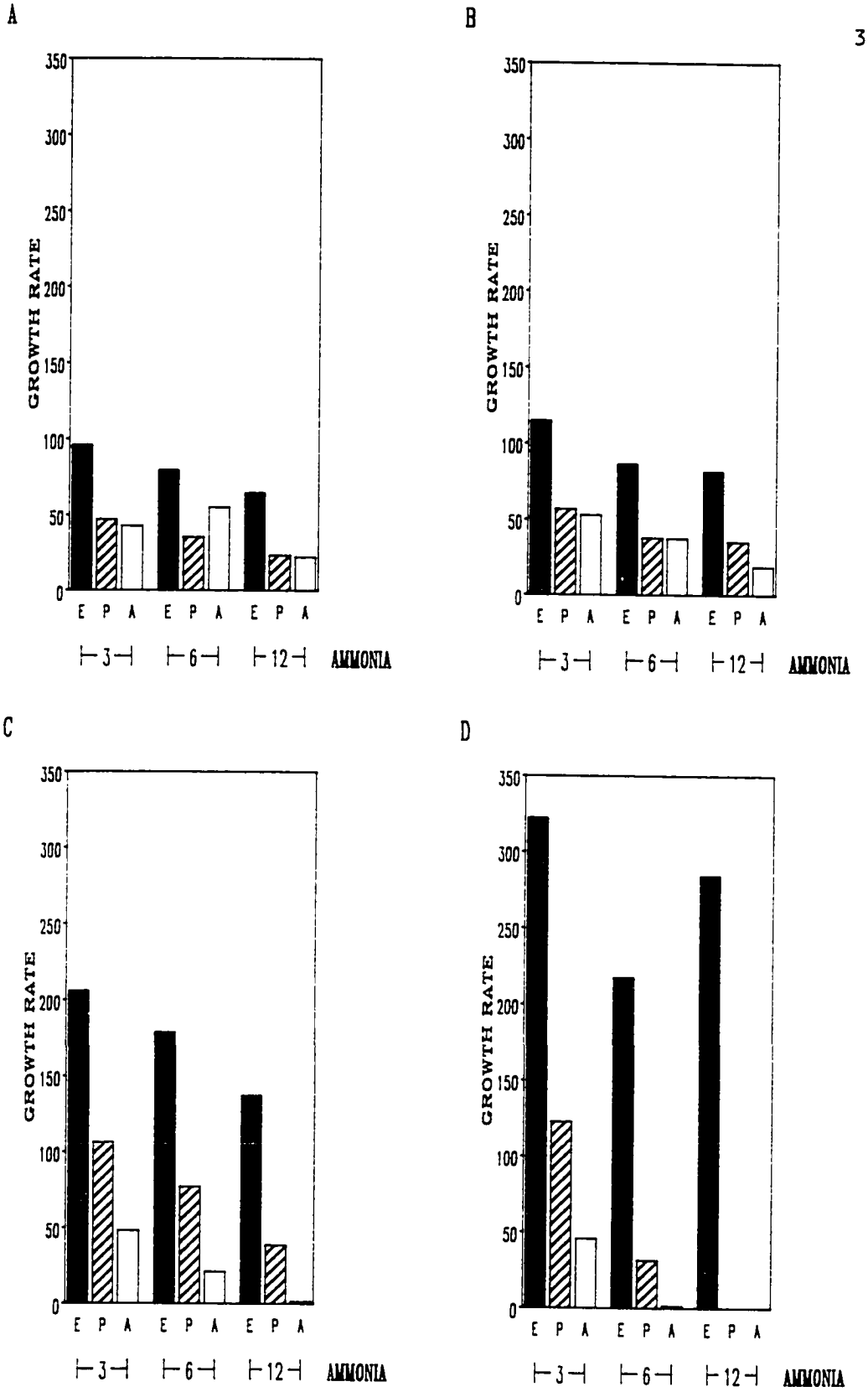
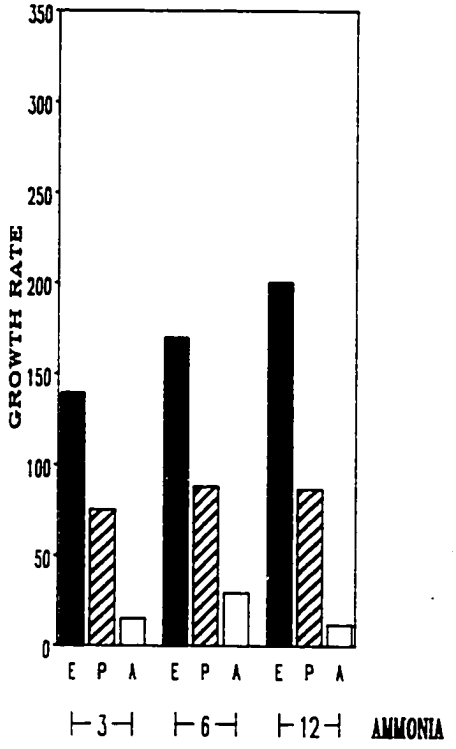


Figure 9. Productivity potential of the grass shrimp, Palaemonetes pugio, exposed different ammonia concentrations (mg/l), and salinities (ppt) under low dissolved oxygen conditions. The data were calculated for salinities of A) 20, B) 25, C) 30 and D) 35. Three productivity values were calculated: E for "estimated growth"; P for "predicted growth"; and A for "actual growth". (See text for description of the calculations).

A



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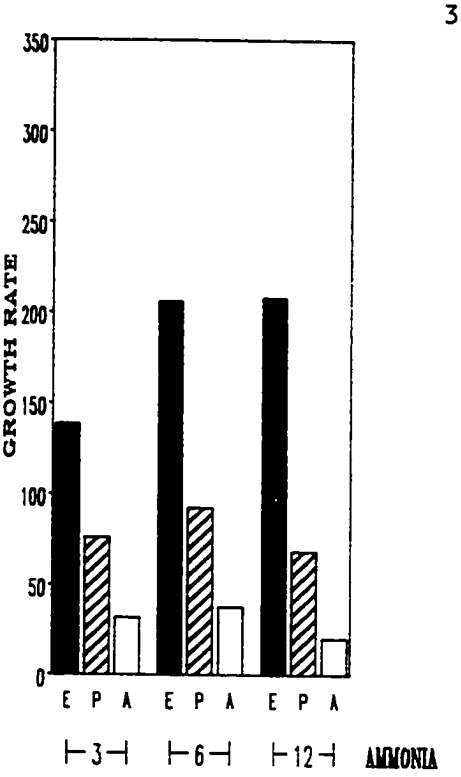
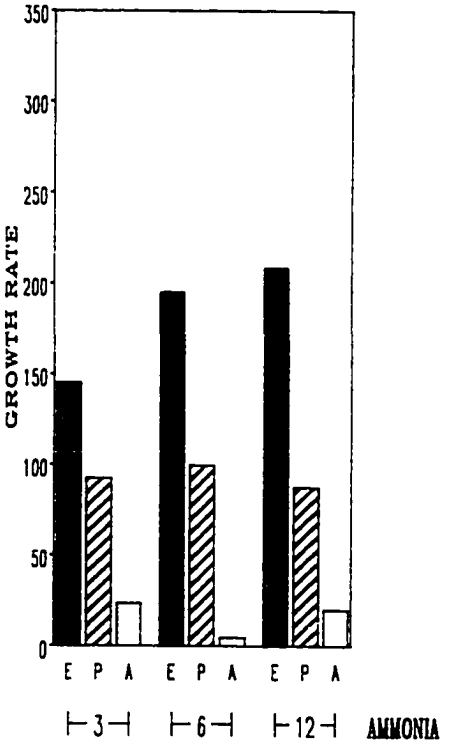


C



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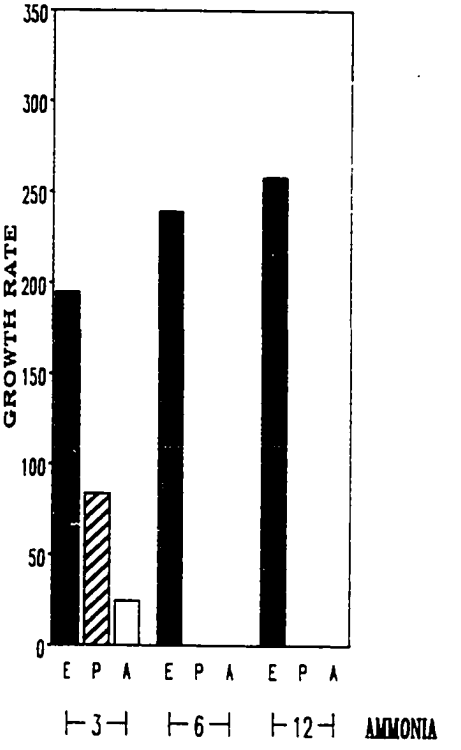

DISCUSSION

Effects of Multiple Stresses on Physiological Processes

Ammonia concentrations, salinities and D.O. conditions are important factors which affect the physiology of the grass shrimp. The interaction of these environmental factors were also observed to influence physiological processes of the shrimp. Combinations of these environmental factors significantly affected respiration, molting, feeding, fecal production and excretion of the shrimp.

In crustaceans, the initial response to low D.O. conditions includes rapidly increased ventilation (hyperventilation). Hyperventilation enhances the oxygen supply to the body (McMahon 1988), and is usually associated with increased pH of the internal body fluids. In natural, unpolluted environments this increase in the pH will lead to an increase in the oxygen affinity of the blood. However, in an environment with high ammonia concentrations, a small increase in the pH of the body fluids could cause harmful effects to the animal. The high pH levels in the body fluids would shift the equilibrium from the ionic form of ammonia (NH_4^+) to the more toxic unionized form of ammonia (NH_3). In addition, the high environmental concentrations of ammonia would slow or reverse the diffusion process, which

is typically considered to be the primary excretion mode for most aquatic animals. The ion exchange processes by which NH_4^+ ions are excreted would tend to be inhibited due to the pH shift in the equilibrium. Therefore, an increase in pH would not only increase the toxicity of the ammonia in the animal's body fluids, but it would also interfere with the excretion processes, leading to ammonia accumulation in the body.

As an adaptation to avoid this problem, the animal may shut down or substantially reduce ventilation or oxygen consumption. This, of course, means that the animal must find an alternate means of satisfying metabolic needs. One solution may be a conversion to anaerobic respiration. Indeed, this may be the case for many aquatic animals under stress conditions (Burke 1979; Herreid 1980; deZwann 1983; Ellington 1983; Field 1983; Livingstone and deZwann 1983; Booth et al. 1984; McMahon 1988). Anaerobic metabolism would satisfy the critical energy requirements needed to cope with the stress, while reducing the oxygen demand, without increasing the pH of the blood.

There were indications that the grass shrimp in this study may have utilized anaerobic metabolism under certain conditions. These shrimp were able to survive long periods of time in low D.O. conditions, even with moderately high ammonia concentrations. Even under the most stressful conditions of high ammonia concentrations, high salinities and low D.O. conditions, the juvenile shrimp were able to

survive for up to ten days. The grass shrimp were apparently able to shut down, or at least reduce aerobic respiration to a minimum. The conditions which lead to this kind of response are not unusual in the natural environment of estuarine shrimp. Palaemonetes pugio inhabits organically rich estuarine environments which periodically experience low D.O. (Moore 1974; Welsh 1975) and high ammonia concentrations (Haines 1979). Therefore, the grass shrimp might be expected to have developed the ability to switch to anaerobic metabolism.

The lack of balance in the energy budgets of the shrimp particularly, under low D.O. conditions may be attributed to anaerobic metabolism. Shrimp under high and low D.O. conditions exhibited an average energy balance of 85 and 65% respectively. Since various components of the energy budget were directly measured, the lack of balance under low D.O. conditions may attributed to errors in the measurements or to an unmeasured energy expenditures. The first probability was unlikely with the direct measurement technique. Since the values measured for the various components of energy expenditure were similar to those reported in the literature, it is unlikely that analytical error contributed significantly to the lack of balance. Furthermore, the magnitude of the energy expenditure, other than respiration, are much smaller (i.e. 6-10% of input) than would be necessary to explain the lack of balance.

The second possibility is more likely to explain the

large discrepancies in the energy balances. Respiration is a major part of the energy budget. It is not unusual for an aquatic animal to lose up to 60% of ingested energy in respiration. Therefor, the respiration process is as a more likely candidate to be the unmeasured energy expenditure that resulted in the lack of energy balance for shrimp exposed to low D.O. conditions.

Molting is an extremely important phenomenon associated with the growth of crustaceans. Many external and internal factors are involved in the initiation of molting. Light and temperature are among the most important external factors. The internal factors consist of the organic and inorganic reserves of the animal. The organic and inorganic reserves are primarily stored in the hepatopancreas. Molting may be prolonged or prevented when these reserves are depleted or altered by stress (Passano 1960).

The organic reserves are used to meet the extra energy demands for material and energy during molting. Under normal conditions, they are usually not utilized for general tissue growth. Lipids are the major component of the organic reserves.

The inorganic reserves of the hepatopancreas are essential in the molting process. These inorganic reserves consists of Ca, Mg, P, Cu, Na, K and some other minor ions. These reserves must be at their optimum levels and any alteration in these reserves may alter the molting cycle of the animal (Passano 1960).

There is an inverse relationship between the balance of Na^+ to K^+ and ammonia concentrations of the blood. An increase of the Na^+ and a decrease of the K^+ concentrations in the tissues are among the effects of high ammonia concentrations in the blood. These effects have been attributed to the competition between ammonia and K^+ receptor sites of the Na-K ATPase (Post and Jolly 1957).

Under natural conditions, the ability of the Na-K ATPase to remove Na^+ from the tissue exceeds the ability of low ammonia concentrations of the environment to enhance Na^+ influx. However, when the NH_4^+ concentrations of the environment are equal or greater than the external K^+ concentrations, the NH_4^+ successfully competes with K^+. At high levels of environmental ammonia, K^+ does not enter the tissue, but Na^+ entry increases and its removal from the tissue is very much reduced, resulting in low K^+ and high Na^+ concentrations. This also results in a change in the balance of ions of the inorganic reserves.

Absence of molting may be attributed to one or more of the following possibilities. The energy required to cope with the most stressful conditions may have depleted the organic reserves to the point that molting could not take place. The loss of weight (negative growth rates) of shrimp in these stressful conditions tend to confirm this possibility. The disruption in the balance of the inorganic ion reserves associated with the high ammonia concentrations and the active ion exchange excretion processes could also

inhibit molting.

In addition, several physiological changes have been noticed in premolt crustaceans which may be of significance to the observations concerning the suppression of molting by stress. A number of investigators have observed that mechanisms of gas exchange and transport are disrupted and the concentrations of hemocyanin in the blood of crustaceans decreased during the premolt period (Truchot 1980; Mangum et al. 1985). Other investigators have reported an increase in oxygen uptake during this period (Edwards 1950; Bliss 1953). The stresses of the experimental conditions (e.g., low D.O.) and the presumed switch to anaerobic respiration may disrupt or complicate these premolt changes.

The influence of ammonia, salinity and D.O. conditions on food consumption was examined. Food consumption rates of the shrimp significantly increased with increasing environmental salinity. Effects of salinity alone accounted for about 66% of the total variance of the data. The effects of ammonia alone on the food consumption were overshadowed by the strong effects of salinity. However, the interaction of salinity and ammonia was significantly associated with increased food consumption. The increased food consumption under high salinity and high ammonia conditions may represent an adaptive response to meet the increased energy demands of stress. However, weight loss followed by massive mortalities among shrimp populations exposed to high salinity and high ammonia conditions suggests that the

elevated food intake was either not sufficient to meet the high energy demands or that the consumed food was passing through the shrimp's gut without being efficiently utilized.

The latter suggestion was supported by the pattern of fecal production of shrimp held in high salinity and high ammonia conditions. Fecal production was also directly related to salinities. However, fecal production in shrimp populations exposed to combinations of high salinity and high ammonia concentrations was greatly enhanced (Figure 6). Thus, high food intake was accompanied by even greater fecal production rates for shrimp exposed to the most stressful conditions. This phenomenon can only be explained by inadequate food assimilation. While never reported before in shrimp, higher animals appear to display low food assimilation efficiencies under stress or disease (Hainsworth 1981). Grass shrimp populations could lose a sizable portion of their energy through unassimilated food. This sort of loss may have contributed to the weight loss and massive delayed mortalities in populations in high salinity and high ammonia treatments.

There are two proposed theories of the methods of ammonia excretion by aquatic animals. The first theory is the ammonia diffusion theory. According to this theory, ammonia is primarily excreted by passive diffusion. The majority of ammonia excreted by this method is in the anionic NH_3 form and Na^+ uptake is presumably coupled to H^+ loss to maintain electrostatic stability.

The second theory deals with active exchange of NH^+_4 with Na^+. According to this theory, the internal ionized ammonia is exchanged with external Na^+. Active transport of NH_4^+ may start when diffusion processes are interrupted or no longer possible (Prosser 1973). Active transport requires energy, therefore the presence of the active transport can be detected by measuring the energy balance and the process can be blocked by specific inhibitors.

The results of this study indicated that ammonia excretion was sharply reduced under high environmental ammonia (12 mg/l) conditions. This reduction may be a result of an ammonia influx into the body fluids. However, no decreases in the ammonia concentrations of water in the small test chambers were observed. Instead, slight increases in the ammonia concentrations of the test media were noticed in the highest ammonia conditions. This sort of increase indicates that the shrimp were still actively excreting ammonia (in NH_4^+ form) against the diffusion gradient.

Active ammonia excretion has been reported in aquatic animals when diffusion is no longer possible. Maetz (1973), for example, noticed active ammonia transport in goldfish exposed to environmental ammonia concentrations exceeding those of the blood.

Comparison of Productivity Potential Measurement Techniques

Traditionally, the productivity potential of a stressed population has been estimated utilizing experiments in which the exposure period is short (often 96 hr or less). The SFG is an example of a technique that employs this kind of approach (Nelson et al. 1985). The SFG technique, although useful for rapidly providing a rough approximation of the chronic effects of a toxicant, may not always produce reliable results. Scope for growth studies usually employ direct measurements of a few physiological processes, with estimated values being used to calculate the effects of other processes. Estimated values are collected from literature to provide energy conversion factors for key physiological processes. Since these factors are used as constants in SFG calculations, the effects of variations in environmental conditions on these physiological processes may greatly influence the results. Shifts in oxygen and salinity conditions that are well within the range of natural values may influence physiological processes to such a degree that the SFG values become invalid.

Many of these weaknesses of the SFG approach were obvious in the present study. The "actual growth" rates were direct measurements of the productivity of the juvenile grass shrimp populations. Thus, these measurements represent reference values against which the accuracy of the other techniques may be assessed. It is clear that the "estimated

growth" values calculated from the SFG equations were much greater than the "actual growth" rates.

Even with no ammonia treatments, the "estimated growth" values were at least double that of the "actual growth" rates. While the "actual growth" rates declined slightly with increased salinities, the "estimated growth" rates steadily rose, primarily due to the influence of elevated food consumption rates on the calculations. Likewise, low D.O. conditions tended to depress "actual growth" rates, but the "estimated growth" rates remained high due to decreased oxygen consumption. Thus, the discrepancies between SFG and "true" productivity values appear to be due to the fact that the overall effects of natural stresses on all energy budget processes are not considered with the SFG approach.

The "predicted growth" rates were based upon a comprehensive energy budget model employing direct measurements of most physiological processes. These values were closer to the "actual growth" rates than were those values produced by the SFG approach. The effect of natural stress on the physiological processes were generally taken into account. However, the "actual growth" and the "predicted growth" values did not balance as well under low D.O. conditions. It is speculated that an unmeasured output process such as anaerobic respiration may be responsible for the overestimated productivity predictions under these conditions.

When the effects of ammonia toxicity were added to the

comparison of the three techniques, the divergences become greater. Under the most stressful combinations of ammonia, salinity and D.O. conditions, the "estimated growth" values were up to an order of magnitude higher than "actual growth" rates which were either greatly reduced, negative, or not calculable due to high mortalities. Clearly, the SFG technique greatly overestimated the productivity potential of the grass shrimp populations that have been strongly stressed.

While the "predicted growth" rates appear to overestimate the true productivity potential of the shrimp populations under stress, they clearly produced a more accurate picture than the "estimated growth" rates. Under the most stressful combinations of ammonia, salinity and D.O. conditions, the average increase of the "predicted growth" rates were, on the average, only 20% higher than "actual growth" rates.

Future studies designed to produce rough approximations of the productivity potentials of populations should include direct measurements of as many key physiological processes as possible. Ideally, the effects of natural stresses on these processes should be directly measured, or at least estimated through regression models such as those produced by the present study. In addition, the potential for aquatic populations to respond to stresses such as low D.O. by switching to anaerobic respiration should be explored further. If confirmed as a common response, this process

should be considered as a component of future energy budget models.

An important outcome of the energy budget evaluation involves the implications to pollution studies designed to determine the effects of stress on the productivity of aquatic populations. An energy budget is a sensitive measurement of an animal's fitness only when adequate physiological information is available. The present study indicated that the SFG approach is not always as accurate as was previously believed. This type of indirect approach may overestimate the true productivity potential of a stressed population and underestimate the potential impacts of the stress. Therefore, stressful conditions capable of significantly depleting an animal's energy reserves may go undetected. This possibility would be especially true if the animal is capable of utilizing anaerobic metabolism, which represents an unmeasured drain on its productivity potential. These limitations of the SFG technique should be explored for other aquatic populations and other stresses before SFG is accepted as a standard"condition index" in environmental studies.

CONCLUSIONS

The results of this study provided insight into the tolerance of juvenile grass shrimp to ammonia, salinity and D.O. conditions. The growth rates of the shrimp were determined for each combination of conditions. THe growth rates were higher in shrimp populations exposed to low ammonia, low salinity and high D.O. conditions. High ammonia concentrations suppressed the growth of the shrimp, particularly in combination with high salinities and low D.O. conditions. No shrimp were able to survive the full experimental period when exposed to 12 mg/l ammonia and 35 ppt salinity.

Physiological processes were also greatly affected by different combinations of ammonia, salinity and D.O. conditions. The respiration rates of the shrimp exposed to low D.O. conditions were significantly lower than the rates of shrimp exposed to high D.O. conditions. The interactions of ammonia, salinity and D.O. significantly reduced the respiration rates of the shrimp.

The molting of the grass shrimp was inhibited by high ammonia and high salinity treatments. Molting was more affected by these treatments under low D.O. conditions than under high D.O. conditions.

The food consumption rates of the grass shrimp were

highly affected by salinity. High salinity levels caused the shrimp to consume more food. The interaction between high salinities and high ammonia also significantly increased the food consumption rates. Food consumption rates were highest for treatments of high ammonia concentrations, high salinities and high D.O. conditions.

The fecal production rates of the grass shrimp were highest in high salinity and high ammonia conditions. The interaction of D.O. with salinity and ammonia was also highly significant in increasing the fecal production of the shrimp.

The excretion rates of the grass shrimp were sharply reduced by high ammonia concentrations. Excretion rates were also significantly reduced by highest salinities and the ammonia-salinity interaction.

The effects of the interaction of multiple stresses due to high ammonia, high salinities and low D.O. conditions significantly affected the physiological processes used to determined the energy budget and the secondary production of the shrimp. The importance of interactions of natural and man-made stresses demonstrate the limitation of single factor toxicity tests.

Energy budgets were calculated for the shrimp population exposed to each set of test conditions. The ingested energy balanced the energy expenditures of shrimp exposed to low ammonia and salinity under high D.O. conditions. High ammonia and high salinity conditions produced unbalanced

energy budgets.

A general lack of balance of the energy budgets of the shrimp exposed to low D.O. conditions was obvious across all ammonia and salinity conditions. The lack of balance may be attributed to anaerobic respiration. This type of response by the shrimp may be considered an adaptation of shrimp populations that are routinely exposed to low D.O. conditions in natural ecosystems.

The SFG approach produces high "estimated growth" rates across all test conditions. The "predicted growth" rates were closer to the "actual growth" rates. However, the "predicted growth" rates and the "actual growth" rates diverged for populations exposed to low D.O. conditions, probably due to an unmeasured energy expenditure such as anaerobic respiration.

The present study indicated that the SFG approach is not always as accurate as was previously believed. Limitation in the SFG approach may mean that stressful conditions capable of significantly depleting an animal's energy reserves may go undetected.

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APPENDICES

Appendix A

LITERATURE REVIEW

A review of the available information on ammonia toxicity to marine animals indicates that most studies have been conducted on fishes. Details concerning the toxicity of ammonia to estuarine crustaceans and to shrimp in particular, are very limited (Shaw 1960; Mangum et al. 1976; Wickins 1976; Delistraty et al. 1977; Armstrong et al. 1978; Fava et al. 1985). In the case of grass shrimp Palaemonetes pugio, only limited data could be found concerning ammonia toxicity (Fava et al, 1985). The present study examines the effects of ammonia on the growth rates and general energy budget of juvenile grass shrimp, for a range of oxygen and salinity conditions which may be expected in an estuarine ecosystem.

Ammonia is normally found in most natural waters as a product of microbial-decomposition, as well as the primary excretory product of many aquatic animals. Because of the continuous conversion to nitrite and nitrate, ammonia is usually found in low concentrations in most natural waters. Ammonia also enters natural waters as a result of human activities. The main anthropogenic sources of ammonia include, but are not restricted to, industrial wastes,

municipal sewage effluent, and agricultural runoff. Ammonia is also a major problem in intensive aquaculture systems, often representing a limiting factor in the productivity of these systems.

Ammonia, under standard conditions, is a colorless gas with three hydrogen atoms bound to nitrogen (NH_3). The molecular weight of NH_3 is 17.03. Ammonia has special chemical properties in natural water. The chemistry of ammonia in solution has been discussed by Whitfield (1974) and Colt and Tchobanoglous (1976). Ammonia exists in solution as the NH_4^+ ion and unionized NH_3 molecule. The proportion of each of these is highly influenced by pH and, to a lesser extent dependent on temperature and salinity (Belding 1927; Grindley 1946; Whitfield 1974; Emerson et al 1975; Armstrong et al 1978). An increase of one pH unit raises the NH_3 concentration about tenfold. The NH_3 form is a weak base which can take up H^+ to form NH^{4+} (Goldstein and Forster 1970):

$$NH^3 + H+ <=====> NH_4^+$$

The term ammonia in this study refers to the total of both the ionized and unionized ammonia form.

Downing and Merkens (1955) and Tomasso et al. (1980) have reported a clear correlation between environmental pH and ammonia toxicity, primarily due to the greater concentration of the more toxic unionized form at higher pH.

Lloyd (1961) and Smart (1978) reported a negative correlation in fish between D.O. concentration and ammonia toxicity.

Bicarbonate alkalinity affects ammonia toxicity only to the extent that it affects the pH of the water and free carbon dioxide (CO_2) present in the water (Lloyd 1961). Ammonia toxicity has been reported to be influenced by CO_2 and D.O. concentrations. Increasing CO_2 concentration of the water may decrease the ammonia toxicity (Alabaster and Herbert 1954). The main effect was due to free CO_2 lowering the pH of the water and therefore reducing the unionized ammonia.

The effect of temperature on the equilibrium is not as important. A ten degree decrease is required before the proportion of the total ammonia in solution in the form of NH_3 is reduced by approximately one-half. Whereas increases of both pH and temperature favor the formation of NH_3 at the expense of NH_4^+, an increase in the ionic strength of the medium reduces the proportion of total ammonia in the form of NH_3.

While environmental ammonia can be biologically detoxified through nitrification, the by-products of this process can have negative impacts on water quality. In the first of the two steps of the nitrification process, the oxidation of ammonia to nitrite takes place according to the following equation (Mead 1974):

$$2NH_4^+ + 3O_2 \leftrightarrows 2NO_2^= + 2H_2O + 4H^+$$

Two hydrogen ions are produced, and four oxygen atoms are consumed for every ammonia molecule completely oxidized. According to Meade (1974), about 4.56 kg of oxygen are required to meet the demand for oxidation of one kilogram of ammonia-nitrogen. Hence, waters where nitrification rates are high tend to become acidic and low in D.O. High concentrations of ammonia may remain in natural systems for a relatively long time due to the slow growth rate of the nitrifying bacteria and the extended process of nitrification (Colt and Tchobanoglous 1976).

Ammonia can be highly toxic to aquatic life at comparatively low concentrations and may reach a high level in polluted waters. Low concentrations of ammonia may produce direct effects on the growth and histopathological changes in aquatic animals (Smart 1976; Colt and Tchobanoglous 1978; Alderson 1979). Elevated ammonia concentrations can be lethal to aquatic animals (Burrows 1964; Smart 1976 and 1978). High Environmental ammonia concentrations have been shown to limit the excretion of internally produced ammonia in fishes (Larmoyeux and Poper 1973). The relationship between salinity and ammonia toxicity of fish were reported by Herbert and Shurben (1965).

Among the signs observed in ammonia toxicity were the effects on the nervous system. However, there is disagreement among authors about the cause of death in ammonia in-

toxication. Wilson et al. (1968) suggested that sheep and dogs injected with ammonia died as a result of heart failure. There are increasing indications that the CNS is the more likely site of ammonia effects. Disagreement also exists about the concentrations of ammonia in the blood necessary to produce the effects on the CNS. The natural ammonia levels in the blood of vertebrates are low. These levels range from less than 0.34 mg/l in mammals to 25.5 mg/l in fishes. The natural levels of ammonia in the blood of the invertebrata Decapoda ranges from 2 to 25 mg/l.

Ammonia is toxic and, therefore, must be removed from the body or converted into a less toxic form (Campbell 1973). Excess amounts of amino acids usually result from the consumption of protein by aquatic animals. These amino acids are utilized as energy sources by these aquatic animals (Campbell 1973). Before the carbon skeletons of amino acids can be oxidized, the amino groups must be removed. The removal of these groups results in the formation of ammonia (Goldstein 1972). The principal nitrogenous product of protein and nucleic acid catabolism is ammonia. The nitrogenous waste product excreted by an organism is related to its need for water. These needs may change during the different development stages of the organism. The common nitrogenous excretory products of animals, in order of toxicity, are ammonia, urea and uric acid (Goldstein and Forster 1970).

Teleost fishes and aquatic crustaceans, which have a

large amount of water available to them for dilution, primarily excrete ammonia, the most highly toxic nitrogenous waste product. These aquatic organisms depend on: (1) rapid diffusion of ammonia, mainly in its unionized form (Goldstein 1972), through gill membranes (Fromm and Gillette 1968) or (2) on the active exchange of ammonium ions (NH_4^+) with sodium ions (Na^+) (Shaw 1960; Maetz and Garcia-Romeu 1964; Campbell 1973; Mangum et al. 1976; Mangum and Towle 1977). The former process (diffusion) is believed to dominate over the latter, because of the greater lipid-solubility of NH_3 and the known ease by which lipid soluble substances pass through membranes (Goldstein and Forster 1970).

The mechanism of ion exchange in aquatic animals has been recognized for a long time. Krogh (1939) demonstrated that the gill membranes in freshwater teleosts are the site of independent active uptake of Na^+ and Cl^-. Twenty five years later, Maetz and Garcia-Romeu (1964) confirmed this independent uptake, when he observed that the rate of Na^+ uptake often differed from that of Cl^- in goldfish, Carassius auratus, and that the injection of these fish with ammonia salts increased Na^+ uptake. The phenomena of increased Na^+ uptake with the increased of internal ammonia concentrations was again confirmed with the eel, Angilla anguilla, (Garcia-Romeu and Motais 1966), and with rainbow trout (Kerstetter et al. 1970). Increases in ambient ammonia concentrations were also found to inhibit Na^+ uptake

(Maetz 1972). Maetz (1973) calculated that ammonia to sodium ratios exceeding 5.6:1 inhibited sodium uptake in goldfish.

The formation and excretion of ammonia for elimination of nitrogenous wastes may have several biological advantages. The free base, NH_3, could transported easily across body surfaces by diffusion into the aqueous medium, facilitating removal from the animal. Although it is estimated that only one percent of the total ammonia is the free base form at the normal pH of the animal, the conversion of NH_4^+ to NH_3 is thought to occur readily and therefore not to be rate-limiting (Goldstein 1972). Many of the chemical reactions leading to the formation of ammonia ultimately lead to the production and capture of free energy. The conversion of ammonia to less toxic forms, such as urea, requires a metabolic expenditure (Campbell 1973). In addition, ionized ammonia may be incorporated into physiological processes that play a role in excretion, osmoregulation, and maintenance. This theory has not received wide acceptance as yet, but a growing array of indirect evidence may support it (Payan 1978).

Responses to stress vary widely between animals. For example, mammals response to stress in a fairly consistent manner (Selye 1955). There are three phases of response in mammals consisting of an alarm reaction, a resistance stage and adaption stage. The purpose of these stages is to maintain a steady-state, or homeostasis. The responses of

aquatic invertebrates to stress, on the other hand, cannot be described as consistent, although they may respond in a triphasic fashion like mammals (Kinne 1976). Invertebrates generally have a different strategy to cope with environmental changes. Rather than maintaining a constant internal steady-state, they adjust one or more of the internal parameters, resulting in physiological stability but not necessarily steady-state (Mangum and Towle 1977). Therefore, deviations from steady-state in invertebrates cannot be used as a stress indication. This sort of response makes it difficult to define stress in these organisms. Bayne (1975) attempted to solve this dilemma by defining stress in aquatic invertebrates to be:

"a measurable alternation of a physiological (or behavioral, or biochemical, or cytological) steady- state which is induced by an environmental change, and which renders the individual (or the population, or the community) more vulnerable to further environmental change."

The grass shrimp, Palaemonetes pugio was utilized in current study for several reasons. It is an ample and cosmopolitan member of the estuarine community (Welsh 1975; Wood 1967). The grass shrimp are very abundant in the summer months. Reproductive activity begins about April and young shrimp mature in a few weeks (Welsh 1975; Wood 1967).

This shrimp serves a vital role in the flow of energy by ingesting meiofaunal organisms (Bell and Coull 1978; Nelson 1979; Morgan 1980) and particulate organic matter, including

fecal pellets (Johannes and Satomi 1966; Adams and Angelovic 1970; Nixon and Oviatt 1973; Welsh 1975). Palaemonetes pugio, in turn, serves as food to consumers at various trophic levels (Darnell 1958; Wood 1967; Nixon and Oviatt 1973; Stickney et al.,1975). The shrimp are easy to collect and maintain in the laboratory for physiological experiments. Palaemonetes pugio is notably recommended as a standard test species for pollution studies by many agencies (e.g., APHA 1975; ASTM 1980). Palaemonetes pugio is a eurytolerant organism which exhibits a high degree of regulation of both consumption rates and osmotic pressure of its body fluids (McFarland and Pickens 1965; Thorp and Hoss 1975; Roesijadi et al. 1976).

Appendix B

Table B-1. The mean dry weights (mg) and growth rates (Gr) rates (mg d wt/day) of populations of the grass shrimp, Palaemonetes pugio, for 4 runs of the pilot studies conducted in 25 ppt salinity and 20 oC. Numbers between parenthesis are S.E. There was no significant difference between the runs. (n=4)

Run No	Dry Wt (mg) Initial	Day 5	Day 10	Gr
1	39.50 (1.73)	43.00 (1.95)	45.00 (1.75)	0.013
2	36.25 (1.33)	38.70 (0.95)	40.50 (1.50)	0.011
3	38.25 (2.00)	41.50 (1.30)	43.90 (1.20)	0.014
4	32.50 (1.09)	36.00 (1.32)	37.50 (0.77)	0.011
Mean	36.88 (1.54)	39.80 (1.38)	41.73 (1.31)	0.012

Table B-2 The mean dry weights (mg) and growth rates (Gr) rates (mg d wt/day) of populations of the grass shrimp, Palaemonetes pugio, exposed to different combinations of ammonia and salinity under high D.O. conditions. Numbers between parenthesis are S.E. (n=4)

		Initial	Day 11		Day 21	
Sal	HN_3	Dry wt.	Dry wt.	% Sur	Dry wt.	% Sur
20	0	31.75 (3.01)	36.25 (1.03)	100	41.50 (2.60)	100
	3	32.99 (0.71)	38.00 (0.82)	100	41.00 (1.08)	100
	6	29.25 (1.55)	32.25 (1.79)	100	39.00 (2.40)	100
	12	31.75 (0.85)	33.00 (0.58)	100	36.50 (1.33)	100
25	0	32.00 (1.22)	36.75 (1.43)	100	41.50 (0.65)	100
	3	29.00 (1.82)	35.50 (1.19)	100	40.25 (0.48)	100
	6	31.75 (0.75)	35.25 (1.11)	100	39.75 (0.75)	100
	12	31.25 (1.11)	33.50 (0.50)	100	35.25 (1.18)	100
30	0	35.25 (2.50)	38.65 (2.29)	100	44.50 (1.94)	100
	3	34.00 (1.73)	41.00 (2.34)	100	44.25 (3.35)	100
	6	32.25 (2.53)	35.00 (3.13)	100	36.75 (3.04)	100
	12	31.25 (2.02)	31.50 (1.32)	100	30.91 (1.32)	96
35	0	31.75 (2.32)	35.75 (2.29)	100	43.25 (2.93)	100
	3	30.75 (1.93)	36.50 (1.71)	100	40.50 (0.86)	100
	6	29.00 (2.65)	26.16 (1.14)	67	29.00 (1.28)	67
	12	30.25 (3.59)	10.00 (1.14)	37	- -	0

Table B-3. The mean dry weights (mg) and growth rates (Gr) rates (mg d wt/day) of populations of the grass shrimp, Palaemonetes pugio, exposed to different combinations of ammonia and salinity under low D.O. conditions. Numbers between parenthesis are S.E. (n=4)

		Initial	Day 11		Day 21	
Sal	HN_3	Dry wt.	Dry wt.	% Sur	Dry wt.	% Sur
20	0	30.50 (1.04)	35.25 (1.75)	100	38.50 (1.55)	100
	3	35.50 (2.60)	37.75 (1.65)	100	38.70 (1.63)	100
	6	29.25 (2.21)	32.50 (1.55)	100	35.50 (2.25)	100
	12	29.50 (0.86)	31.50 (0.65)	100	32.00 (0.71)	100
25	0	31.00 (1.87)	38.50 (2.66)	100	41.00 (3.13)	100
	3	32.25 (1.10)	34.50 (1.71)	100	39.00 (0.41)	100
	6	30.75 (0.75)	35.50 (1.04)	100	38.75 (1.97)	100
	12	36.00 (1.47)	34.00 (1.55)	100	34.25 (1.03)	96
30	0	29.25 (2.17)	32.50 (2.40)	100	34.00 (2.31)	100
	3	34.75 (2.06)	37.50 (1.85)	100	39.75 (1.89)	100
	6	32.75 (1.31)	32.50 (0.86)	100	33.75 (1.97)	100
	12	36.50 (1.55)	35.25 (1.88)	100	34.25 (2.72)	96
35	0	33.44 (1.84)	37.00 (1.87)	100	41.25 (1.49)	100
	3	33.50 (2.99)	36.00 (3.88)	100	38.75 (3.73)	100
	6	38.75 (1.75)	8.79 (0.74)	37	- -	0
	12	36.25 (1.45)	- -	0	- -	0

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ABSTRACT

EFFECTS OF AMMONIA, SALINITY AND OXYGEN ON THE GROWTH AND ENERGETICS OF JUVENILE GRASS SHRIMP, *Palaemonetes pugio*

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Old Dominion University, 1988
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The growth and energy budget of juvenile grass shrimp, *Palaemonetes pugio*, were examined under various combinations of ammonia, salinity and dissolved oxygen. Experiments were performed to determine the effects of multiple stresses on the growth and physiological processes of the shrimp. The experimental design also allowed an evaluation of the effectiveness of several techniques for determining the productivity potential of aquatic animals exposed to stress.

A flow-through system was used to produce combinations of ammonia concentrations (0, 3, 6 and 12 mg/l) and salinities (20, 25, 30 and 35 ppt) for 21-day tests. The tests were conducted at high (> 5 mg/l) and low (1.7 mg/l) dissolved oxygen (D.O.) conditions.

The oxygen consumption rates were elevated for shrimp exposed to high ammonia and high salinity combinations under high D.O. conditions. However, under low D.O. conditions the oxygen consumption was depressed for all treatments involving low D.O. Molting was prevented by high ammonia concentrations, but did not appear to be affected by salinity. Feeding rates were directly affected by salinity

and, to a lesser degree, by ammonia. Feces production rates were directly affected by ammonia concentrations and, to a lesser degree, by salinity. Excretion rates were lower under high ammonia and high salinity conditions.

The growth rates of the shrimp were significantly reduced in treatments with high ammonia and high salinity combinations. The adverse effects of the ammonia-salinity interaction were particularly evident under low dissolved oxygen conditions.

The findings also indicated that commonly used "scope for growth" estimates may significantly overestimate the productivity potential of stressed populations, particularly when natural stresses are not taken into account. Even more comprehensive energy budget models may underestimate actual growth rates under stressful conditions, particularly those involving low D.O. Processes such as anaerobic respiration, which are not addressed in these models are probably responsible for the lack of balance in the energy budgets of stressed populations.