# EFFECTS OF SALINITY ON THE OXYGEN CONSUMPTION AND AMMONIA-N EXCRETION OF YOUNG JUVENILES OF THE GRASS SHRIMP, PENAEUS MONODON FABRICIUS<sup>1</sup>

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(Accepted May 16, 1989)

Chi-Hsiang Lei, Li-Yung Hsieh and Chao-Kuan Chen (1989) Effects of salinity on the oxygen consumption and ammonia-N excretion of young juveniles of the grass shrimp, *Penaeus monodon* Fabricius. *Bull. Inst. Zool.*, *Academia Sinica* 28(4): 245-256 Young juveniles (wet body weight, 8.80-264.3 mg) of *P. monodon* acclimated to salinity 29 ppt at 25°C, 600 lux and 16L:8D photoperiod for over 2 weeks were subjected to different salinities (15, 20, 25, 29 and 35 ppt), and their rates of oxygen consumption and ammonia-N excretion were measured simultaneously at 25°C in dark.

Within the range of salinity tested, the rates of oxygen consumption  $(R, \mu g/animal/hr)$  and ammonia-N excretion  $(E, \mu g/animal/hr)$  increased with increasing body sizes. The rates of oxygen consumption and ammonia-N excretion as a function of wet body weight (W) were best expressed as  $\log R = \log a + b \log W$  and  $\log E = \log a + b \log W$ , respectively. There was no significant difference among the b values for both oxygen consumption (F=1.28; df=4.50; p>0.05) and ammonia-N excretion (F=0.91; df=4.50; p>0.05) determined at different salinities, but the position of intercept  $(\log a)$  varied significantly for both oxygen consumption (F=1.28; df=4.50; p<0.001) and ammonia-N excretion (F=27.86; df=4.50; p<0.001).

The mean oxygen consumption rate and mean ammonia-N excretion rate at different salinities were adjusted to a common wet body weight of 76.64 mg, and the difference among the adjusted means were tested by covariance analysis. There was significant difference among adjusted means of the rates of oxygen consumption and ammonia-N excretion determined at different salinities. The oxygen consumption rate increased at salinities deviating from acclimating salinity (29 ppt), and ammonia-N excretion rate increased with decreasing salinity. O:N ratios (by atoms) decreased from 27.7 at 35 ppt to 8.1 at 15 ppt indicating a shift from lipid-dominated to protein-dominated metabolisms when salinity decreased.

**Key words:** Salinity effect, Oxygen consumption, Ammonia-N excretion, Grass shrimp.

Respiratory rates (Lockwood, 1967; Marshall, 1973; Vernberg, 1983; Wolvekamp and Waterman, 1960) and nitrogen excretion rates (Corner and Davies, 1971; Needham, 1957; Parry, 1960) of crustaceans are affected by various intrinsic (e.g. body size, stage of life cycle and

<sup>1.</sup> Paper No. 326 of the Journal Series of the Institute of Zoology, Academia Sinica.

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physiological states) and extrinsic (e.g. temperature, light, oxygen concentration, salinity, pH and pollutants) factors. Although effects of salinity on oxygen consumption (Florkin, 1960; Vernberg, 1983; Wolvekamp and Waterman, 1960) and ammonia excretion (Needham, 1957; Parry, 1960) of aquatic crustaceans have been investigated extensively, comitant recordings of the rates of oxygen consumption and ammonia excretion under various salinities are very scarce.

The relationship between oxygen consumption and ammonia excretion may be expressed as the  $\mu g$ -atom ratio between oxygen consumed and ammonia-N excreted (e.g. Corner, 1972; Harris, 1959; Snow and Williams, 1971). The O:N ratio should be a good indicator of the state of general metabolism, reflecting the level of activity of the oxidative and protein metabolism of the animal studied (Mayzaud, 1973). The value of the atomic 0:Nratio shows what biochemical fraction of the body is used as an energy source, and what main metabolic pathways are involved. This ratio has proved useful in assessing the physiological response of bivalves to various stressful environments (Ansell and Sivadas, 1973; Bayne, 1973, 1975).

Penaeus monodon is a tropical penaeid shrimp of considerable commercial importance, and is the most intensively cultured shrimp in Taiwan (Liao and Chao, 1983). The purpose of this study was to measure the rates of oxygen consumption and ammonia-N excretion of the young juveniles of *P. monodon* under various salinities, and to assess their physiological response to salinity changes.

#### MATERIALS AND METHODS

The young juveniles of *P. monodon* used for the measurement of the rates

of oxygen consumption and ammonia-N excretion were obtained from the private hatchery in Tainan. The shrimps were cultured in 8 liter plexiglass tanks containing 6 liters of filtered (0.45 µm Millipore membrane filter) and aerated (at least to 95 per cent saturation) seawater (hereafter this water will be referred to as filtered seawater) of 29 ppt salinity, and fed frozen chironmid (Chironomus sp.) larvae daily for over two weeks before the experiment. tanks were kept in a growth chamber with the conditions of 25°C, 600 lux and 16L:8D photoperiod. The seawater (29) ppt) in the tanks was aerated lightly with air stone continuously and renewed daily.

Prior to the experiments, shrimps were transferred into other plexiglass tanks containing 6 liters of filtered seawater of 25°C and 29 ppt salinity, and starved for one day to clear their gut of excess food. This was done in order to eliminate the possibility of deriving ammonia and other nitrogenous products undigested food. During process of starvation plexiglass tanks were also kept in the growth chamber. After the starvation one to three shrimps (depending on size) of similar size were first gently picked up with a net from the plexiglass tank, washed several times with fresh filtered seawater, and carefully placed in a 300-ml BOD bottle containing filtered seawater of 25°C and test salinities (15, 20, 25, 29 and 35 ppt). The BOD bottles with shrimps were then set aside without disturbance in the growth chamber to allow shrimps to acclimate to experimental conditions. After one hour of acclimation fresh filtered seawater of 25°C and test salinities was gently siphoned with a rubber tube. reaching down to the bottom of the bottle without touching the shrimps, from a 10 liter plastic jar into the BOD bottle to replace used seawater.

volume of filtered seawater equals to at least one time the volume of BOD bottle was allowed to overflow from the top of the BOD bottle to assure complete replacement of used seawater. The BOD bottle was then carefully stoppered at once to avoid capturing air bubbles. This was done to eliminate or reduce the possible effect of handling stress. Concurrently, several control bottles containing filtered seawater of 25°C and test salinities only were also prepared in the same manner. The experimental and control bottles were then wrapped with aluminum foil, and placed in a growth chamber with temperature maintained at 25°C and light turned off, and incubated for 3 hours. All experiments were carried out at the same period of a day, usually started at 9 to 10 am, to avoid the effect biological rhythm. Experimental below 30 ppt seawater salinity prepared by dilution of full strength seawater with deionized water, and of higher salinities was prepared by adding concentrated seawater obtained by evaporation at 100°C. At the end of incubation the amount of dissolved oxygen in the experimental and control bottles was measured with a polarographic oxygen meter (YSI Model 58). The difference between the oxygen content of each experimental and mean oxygen content of controls of respective salinity was taken as the amount of oxygen consumed by the animals. The amount of oxygen consumed was then converted to a per animal per hour value (µg O2/animal/hr).

After the measurement of dissolved oxygen concentration the content of experimental bottles were strained through a \$00 silk bolting net (mesh size 0.752 mm) to collect animals for the determination of wet body weight. Oxygen consumption (µg O<sub>2</sub>/animal/hr) was then divided by the mean wet body weight, and converted to weight-specific rate of oxygen consumption (µg O<sub>2</sub>/mg

wet weight/hr). The rate of oxygen consumption as a function of body weight was expressed as

log  $R=\log a+b \log W$  (or  $R=aW^b$ ) where R is the rate of oxgen consumption ( $\mu$ g O<sub>2</sub>/animal/hr), W is the wet body weight (mg), b is an exponential constant, and a is a constant of proportionality.

For determination of ammonia excretion rate a 25 ml aliquot was removed from each experimental and control bottles, and assayed for ammonia-N using a modification of the procedure of Solorzano (1969). The difference between the mean ammonia-N content of controls and the ammonia-N content of each experimental bottle was taken as the amount of ammonia-N excreted by the animals. The amount of ammonia-N excreted was then converted to a per animal per hour value (µg N/animal/hr). Ammonia-N excretion (µg N/animal/hr) was then divided by the mean wet body weight and converted to weightspecific rate of ammonia-N excretion ( $\mu$ g N/mg wet wt/hr). The rate of ammonia-N excretion as a function of body weight was expressed as

 $\log E = \log a + b \log W$  (or  $E = aW^b$ ) where E is the rate of ammonia-N excretion ( $\mu$ g N/animal/hr), W is the wet body weight (mg), and b and a are constants.

#### RESULTS

### Rates of oxygen consumption

The rate of oxygen consumption ( $\mu$ g O<sub>2</sub>/animal/hr) of the young juveniles of P. monodon increased with increasing body size under test salinities (Tables 1 and 2). There was no significant difference among the b values of regression equations determined at each salinity (F=1.28; df=4,50; p>0.05); b

Table 1 Oxygen consumption (OC as  $\mu$ g O<sub>2</sub>/animal per hr) of various sized (mean wet weight, MW. in mg) young juveniles of *Penaeus monodon* at 25°C and different salinities

15 ppt		20 ppt		25 ppt		29 ppt		35 ppt	
MW	OC	MW	OC	MW	OC	MW	OC	MW	OC
14.60	14.08	12.10	10.83	19.40	18.87	8.80	7.32	19.43	20.82
17.67	18.42	19.13	13.48	20.83	20.50	8.88	6.82	22.30	21.88
27.50	22.77	30.13	30.38	26.63	24.63	11.20	10.82	24.50	17.83
32.33	27.72	34.00	35.27	37.37	36.87	16.04	10.32	34.67	36.53
43.60	38.94	53.15	49.06	47.95	44.00	16.15	8.82	39.50	35.27
68.55	52.83	59.55	51.73	53.60	51.51	19.16	10.15	48.00	50.79
80.40	72.03	67.90	45.85	56.15	38.37	22.30	11.82	51.65	45.24
88.95	64.26	68.60	46.64	58.00	50.80	27.66	15.82	54.25	50.26
89.80	86.94	154.70	124.21	132.80	135.31	53.37	31.32	73.90	68.98
157.50	155.06	170.90	129.29	160.90	142.30	67.51	36.57	106.70	97.29
206.10	175.50	177.90	106.22	172.80	146.99	174.10	70.65	142.60	74.18
233.20	264.31	250.40	187.50	217.40	196.25	224.82	155.15	168.50	138.29
Means:									
88.35	82.74	91.54	69.20	83.65	75.53	54.17	31.30	65.50	54.78
Adjusted	i								
mean	73.51		57.47		70.01		49.01		63.56

Adjusted mean: mean adjusted for size by covariance analysis to a common size of 76.64 mg wet body weight.

varied from 0.856 to 1.008. The position of intercepts (log a) varied significantly (F=14.36; df=4,50; p<0.001). However, weight-specific rate of oxygen consumption (Fig. 1) was not related to body size (correlation coefficients, r's are not significant, p>0.05).

To test if there was a significant difference in the rates of oxygen consumption among animals studied at different salinities, the mean rate of oxygen consumption was first adjusted by covariance analysis to remove the effect caused by difference in size (Table 1). The variance ratio (F=4.24) was highly significant (p<0.001; df=4.54), implying that the young juveniles of P. monodon had different rates of oxygen consumption under different salinities. For shrimps with wet body weight of

Table 2
Regression equations for the oxygen consumption (R, μg O<sub>2</sub>/animal/hr) as a function of wet body weight (W, mg) of young juveniles of Penaeus monodon under 25°C and different salinities

	Salinity (ppt)	Regression equation	Correlation coefficient (r)	Level of significance for r
	15	$\log R = -0.0583 + 1.0077 \log W$	0.9907	p<0.01
	20	$\log R = 0.0596 + 0.9123 \log W$	0.9852	p < 0.01
٠.	25	$\log R = 0.0089 + 0.9719 \log W$	0.9921	p < 0.01
, .	29	$\log R = -0.0359 + 0.8819 \log W$	0.9795	p < 0.01
	35	$\log R = 0.1931 + 0.8558 \log W$	0.9620	p<0.01

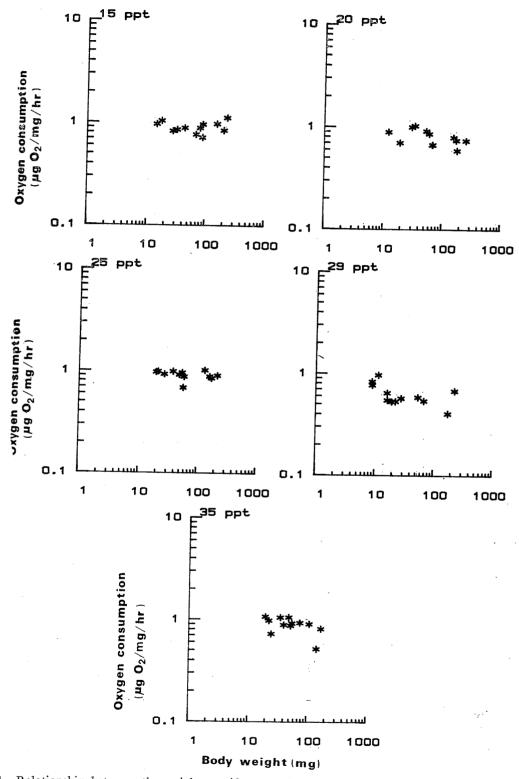


Fig. 1. Relationship between the weight specific rate of oxygen consumption (μg O<sub>2</sub>/mg wet wt/hr) and wet body weight (mg) of young juveniles of *Penaeus monodon* under 25°C and different salinities.

Table 3
Ammonia-N excretion (ANE as µg N/animal/hr) of various sized (mean wet weight, MW. in mg) young juveniles of *Penaeus monodon* at 25°C and different salinities

15 ppt		20 ppt		25 ppt		29 ppt		35 ppt	
MW	ANE	MW	ANE	MW	ANE	MW	ANE	MW	ANE
14.60	1.38	12.10	0.92	19.40	1.26	8.80	0.27	19.43	0.57
17.67	1.81	19.13	1.16	20.83	0.46	8.88	0.24	22.30	0.64
27.50	3.96	30.13	3.25	26.63	1.24	11.20	0.80	24.50	0.56
32.33	2.69	34.00	3.31	37.37	1.67	16.04	0.41	34.67	0.38
43.60	5.02	53.15	2.98	47.95	3.04	16.15	0.48	39.50	0.55
<b>68.</b> 55	4.12	59.55	4.64	53.60	4.18	19.16	0.76	48.00	1.87
80.40	4.61	67.90	5.69	56.15	1.33	22.30	0.52	51.65	0.38
88.95	9.47	68.60	5.75	58.00	3.60	27.66	1.81	54.25	0.95
89.80	6.59	154.70	12.62	132.80	12.80	53.37	1.18	73.90	2.52
157.50	16.90	170.90	13.06	160.90	9.20	67.51	2.16	106.70	3.48
206.10	14.72	177.90	10.18	172.80	6.62	174.10	7.34	142.60	1.21
233.20	33.51	250.40	15.11	217.40	11.46	224.82	13.15	168.50	2.16
Means:									
88.35	8.73	91.54	6.56	83.65	4.74	54.17	2.43	65.50	1.27
Adjusted									
mean	7.96		5.57		4.28		3.91		2.01

Adjusted mean: mean adjusted for size by covariance analysis to a common size of 76.64 mg wet body weight.

76.64 mg, the rate increased at salinities deviating from acclimating salinity (29 ppt; Table 1).

#### Rates of ammonia-N excretion

The rate of ammonia-N excretion ( $\mu$ g N/animal/hr) increased with increasing body size (Tables 3 and 4). There was no significant difference among the b values of regression equations de-

termined at each salinity (F=0.91; df=4,50; p>0.05); b varied from 0.757 to 1.137. The position of intercepts (log a) varied significantly (F=27.86; df=4,50; p<0.001); therefore, salinity probably affected the rate of ammonia-N excretion. The weight-specific rate of ammonia-N excretion (Fig. 2) was not related to body size (correlation coefficients, r's are not significant, p>0.05).

Table 4
Regression equations for the ammonia-N excretion (E, μg N/animal/hr) as a function of wet body weight (W, mg) of young juveniles of Penaeus monodon under 25°C and different salinities

Salinity (ppt)	Regression equation	Correlation coefficient (r)	Level of significance for r	
15	$\log E = -0.9723 + 0.9658 \log W$	0.9456	p<0.01	
20	$\log E = -1.0111 + 0.9370 \log W$	0.9753	p<0.01	
25	$\log E = -1.5464 + 1.1372 \log W$	0.9132	p<0.01	
29	$\log E = -1.5629 + 1.0827 \log W$	0.9494	p < 0.01	
35	$\log E = -1.3118 + 0.7571 \log W$	0.7007	p<0.05	

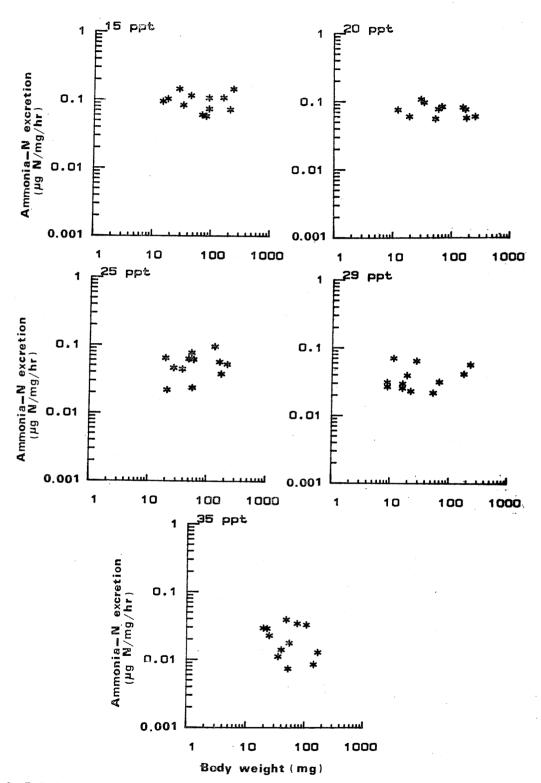


Fig. 2. Relationship between the weight specific rate of ammonia-N excretion (µg N/mg wet wt/hr) and wet body weight (mg) of young juveniles of *Penaeus monodon* under 25°C and different salinities.

Table 5
The rate of oxygen consumption and ammonia-N excretion,
and O:N ratios for young juveniles of Penaeus monodon
under 25°C and different salinities

Salinity (ppt)	Oxygen consumption (µg O <sub>2</sub> /animal/hr)	* Ammonia-N excretion* (µg N/animal/hr)	O:N ratio (by atoms)
 15	73.51	7.96	8.08
20	57.47	5.57	9.03
25	70.01	4.28	14.31
29	49.01	3.91	10.97
 35	63.56	2.01	27.67

<sup>\*</sup> Adjusted mean: mean adjusted for size by covariance analysis to a common size of 76.64 mg wet body weight.

To test further if salinity significantly affected ammonia-N excretion. the mean rate of ammonia-N excretion was adjusted by covariance analysis to remove size-effects (Table variance ratio (F=6.68) was highly significant (p < 0.001, df = 4.54); therefore, salinity significantly affected the rate of ammonia-N excretion of the young juveniles of P. monodon. For shrimps with a common size of 76.64 mg wet body weight, the rate of ammonia-N excretion increased with the decrease of salinity (Table 3).

#### Oxygen and nitrogen relationship

The O:N ratios of the young juveniles of *P. monodon* tended to decrease with the decrease of salinity from 27.67 at 35 ppt to 8.08 at 15 ppt (Table 5).

#### DISCUSSION

The rates of oxygen consumption of the young juveniles of *P. monodon* increased with increasing body size (Table 1), as in other species of Crustacea (for references, see Lockwood, 1967; Wolvekamp and Waterman, 1960). This relationship can be expressed by the equation (Wolvekamp and Waterman, 1960)

 $\log R = \log a + b \log W \text{ (or } R = aW^b)$ where R is oxygen consumption per

animal per unit time, W is body weight, b is the regression coefficient (or slope), and  $\log a$  is the y intercept. The bvalues for Crustacea generally vary between 0.67 and 1.00 (Wolvekamp and Waterman, 1960). For zooplankton, the value of b ranges from 0.62 to 1.14 (Conover. 1968). Liao and Huang (1975) reported a b value of 0.8811 for the young P. monodon with the weight ranging from 0.1 to 1.5 g, and Liao and Murai (1986) reported a mean b value of 0.866 (range: 0.853-0.881) for *P. monodon* weighing 0.4-80 g at 20-30°C. The *b* values (0.856-1.008)obtained for the young juveniles of P. monodon (wet body weight, 0.0088-0.2643 g) in this study were variable and not statistically different. The mean b value 0.926 is slightly larger than those reported above for the same species of shrimp but is in the range reported for zooplankton by Conover (1986). Because there was no significant difference among b values in this study, we cannot demonstrate salinity effects on the relationship of body size and oxygen consumption rate. Although salinity did not significantly affect b, it may have been the most important factor affecting the significant difference among values of the intercept a.

For Crustacea, in general, the smaller individuals within a species, or a small-sized species within a genus, have a

higher metabolic rate per unit weight and time than have larger individuals or species (Lockwood, 1967). Liao and Huang (1975) and Liao and Murai (1986) found an inverse relationship between weight-specific rate of oxygen consumption and body weight for *P. monodon*, but in this study a significant relationship was not found. The reasons for this discrepancy is not known.

Four general types of metabolic response to salinity changes have been described for estuarine animals by Vernberg and Vernberg (1972): (1) The rate of oxygen consumption is not influenced by salinity changes (e.g. the crab Eriocheir sp. and fish Fundulus heteroclitus). (2) The rate of oxygen consumption increases in reduced salinity and decreases in high salinities (e.g. polychaete Nereis diversicolor and the crab Carcinus maenas). (3) Oxygen consumption rate increases in both low and high salinities (e.g. the ghost crab Ocypode quadrata and the shrimp Metapenaeus sp.). (4) Oxygen consumption rate decrease in both low and high salinities. (e.g. the anthozoan Metridium marginatum and the bivalve Mytilus edulis). These categories are based on the new steady metabolic state the organisms reach when exposed to a new salinity (Kinne, 1967). A species initially shows a transitory metabolic response (an over- and under- shoot responses) upon exposure to a new salinity; varying periods of time are required to reach the steady state level. In this study the oxygen consumption rate of the young juveniles of P. monodon increased at salinities deviating from acclimating salinity; this pattern of metabolic response seems to match the third type of metabolic response described above. Ting (1970) also found that the oxygen consumption rates of P. monodon and Metapenaeus monoceros were inversely related to the salinity. These results

seem to be in contradiction to the results reported by Gaudy and Sloane (1981), Liao and Huang (1975), and Liao and Murai (1986) for the same species of shrimp. In their studies, the oxygen consumption rates of P. monodon were stable over the test range of salinities. However, in Ting's (1970) and our studies non-acclimated animals were used for the determination of oxygen consumption rates at different salinities, and the results obtained probably represent acute response to salinity change, whereas in the studies of Gaudy and Sloane (1981). Liao and Huang (1975) and Liao and Murai (1986) acclimated animale (7 day acclimation) were used for the determination, and their results probably represent a chronic response to salinity change.

As reported for other aquatic animals (Bayne and Scullard, 1977; Corner et al. 1965; Kremer, 1977; Needham, 1957; Nelson et al., 1977, 1979) the rate of ammonia-N excretion of the young juveniles of *P. monodon* increased with increasing body size (Table 3). This relationship can be expressed by the equation (Bayne and Scullard, 1977)

 $\log E = \log a + b \log W$  (or  $W = a W^b$ ) where E is ammonia-N excretion per animal per unit time, W is body weight, and a and b are fitted parameters.

Nelson et al. (1977) studied the ammonia excretion of juvenile Macrobrachium rosenbergii fed with different diets, and obtained a mean b value of 0.72379 (range: 0.67899-0.76863). Nelson et al. (1979) reported a mean b value of 0.4879 (range: 0.1315-1.1906) for the benthic estuarine shrimp Crangon franciscorum fed different diets. The b value (0.7571-1.1372) obtained for the young juveniles of P. monodon in this study were variable and not statistically different. The mean b value 0.9760 is higher than those

reported for M. rosenbergii and C. franciscorum.

Weight-specific rates of ammonia-N excretion of *M. rosenbergii* and *C. franciscorum* were significantly and inversely correlated with body weight (Nelson *et al.*, 1977, 1979) but in this study a significant relationship between weight-specific rate of ammonia-N excretion and body weight was not found for the young juveniles of *P. monodon*.

In this study, the rate of ammonia-N excretion of the young juveniles of P. increased with decreasing monodon salinity. Several papers have reported increase rates of ammonia excretion on transfer of several crustacean species to diluted media (Haberfield et al., 1975; Mangum et al., 1976; Regnault, 1984; Spaargaren, 1982; Spaargaren et al., 1982) during periods when tissue FAA (free amino acids) were declining. While part of the increase may be due to ion pump exchange of NH<sub>4</sub> for medium Na<sup>+</sup> (Mangum et al., 1976), it may also reflect an accelerated catabolism of amino acids or other nitrogenous compound (Calybrook, 1983).

A measure of the atomic ratio oxygen consumed: nitrogen excreted provides useful data on the nature of the substrate oxidized by animals, a low value implying that protein is mainly used and a high value characterizing the breakdown of fat or carbohydrate (Corner and Cowey, 1968). When an organism is oxidizing protein exclusively the O:N ratio will be low, less than 7 (Snow and Williams, 1971). The O:N ratio higher than 24 indicates lipid-dominated metabolism, and the O:N ratio lower than 24 indicates proteindominated metabolism (Taniguchi, 1975). In the present study, the O:N ratio (by atoms) decreased from 27.7 at 35 ppt to 8.1 at 15 ppt indicating a shift from lipid-dominated to protein-dominated metabolisms when salinity decreased (Table 5).

Acknowledgements: This work was supported by grant NSC 77-0201-B001-35 from the National Science Council of the Republic of China. The authors wish to thank Mr. Shih-Rong Kuo of Tainan Branch, Taiwan Fishery Research Institute for assistance in obtaining the young juveniles of *P. monodon* for the study.

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# 鹽度對草蝦幼蝦耗氫率及氨態氮排泄率之影響

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本研究利用經於溫度  $25^{\circ}$ C,鹽度 29 ppt 及光週期 16 小時亮、 8 小時暗之狀況馴養兩星期以上之不同大小草蝦( $Penaeus\ monodon\ Fabricius$ )幼蝦,於溫度  $25^{\circ}$ C 及鹽度 15, 20, 25, 29 及 35 ppt 之狀況下分別測定其耗氧率及氨態氮排泄率,以探討個體大小及鹽度對其耗氧率及氨態氮排泄率之影響。在所有測試之鹽度下,耗氧率  $(R; \mu g\ O_2/animal/hr)$  及氨態氮排泄率  $(E; \mu g\ N/animal/hr)$  皆隨個體濕體重 (W) 之增加而增加。 耗氧率與濕體重,以及氨態氮排泄率與濕體重之關係,分別以  $10g\ R=10g\ a+b\ 10g\ W$  及  $10g\ E=10g\ a+b\ 10g\ W$  之廻歸方程式表示者爲最佳。 在不同鹽度下所得到之廻歸係數或斜率  $10g\ E=10g\ a+b\ 10g\ E=10g\ a+b\ 10g\ E=1.28$ , $10g\ E=1.28$   $10g\ E=1.28$  10