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The Effects of Eyestalk Ablation on Oxygen Consumption and Ammonia-N Excretion of Juvenile Shrimp *Penaeus monodon*

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Fan-Hua Nan, Shyn-Shin Sheen, You-Tsu Cheng and Shiu-Nan Chen (1995) The effects of eyestalk ablation on oxygen consumption and ammonia-N excretion of juvenile shrimp *Penaeus monodon*. *Zoological Studies* **34**(4): 265-269. The effects of eyestalk ablation on oxygen consumption and ammonia-N excretion of *Penaeus monodon* were investigated for 24-hr period. The oxygen consumption and ammonia-N excretion were high in shrimp treated bilaterally, intermediate in shrimp treated unilaterally and low in intact shrimp. Eyestalk ablation resulted in an increase of oxygen consumption and ammonia-N excretion, but a decrease in the O:N ratio (by atoms), indicating a shift from lipid-dominated to protein-dominated metabolism. Lethal dissolved oxygen was low in shrimp treated unilaterally, intermediate in intact shrimp and high in shrimp treated bilaterally. Lethal time was longest in intact shrimp, intermediate in shrimp treated unilaterally and shortest in shrimp treated bilaterally.

Key words: Penaeids, Respiration, Nitrogen excretion, Metabolism.

Oxygen consumption and ammonia-N excretion are good indicators of the general physiological state and energetic needs of crustacea. These indicators are affected by various abiotic (temperature, salinity, and pollutants) and biotic (body size, life cycle, feeding and activity) factors (Lei et al. 1989). Although the effects of abiotic and biotic factors on oxygen consumption and ammonia-N excretion of crustaceans have been investigated extensively (Florkin 1960, Vernberg 1983, Chen and Nan 1992a), information on the effects of eyestalk ablation on oxygen consumption and ammonia-N excretion of *Penaeus monodon* is limited. The relationship of a molt-inhibiting hormone to elevation of oxygen consumption following eyestalk ablation was reviewed by Charniaux-Cotton and Kleinholz (1964). However, no subsequent investigations of oxygen consumption and ammonia-N excretion after eyestalk ablation have been offered.

The relationship between oxygen consumption and ammonia-N excretion is usually expressed as the O:N ratio (atoms of oxygen consumed per atom of nitrogen secreted) (Harris 1959, Snow and Williams 1971). This is considered a good parameter of the state of general metabolism and it reflects the protein metabolism of the animal (Mayzaud 1973). The O:N ratio indicates what biochemical fraction of the animal is being used as an energy source, and the main metabolic pathways involved. This ratio has proved useful in assessing the physiological response of bivalves and shrimp to various stresses (Ansell and Sivadas 1973, Bayne 1975, Lei et al. 1989).

Penaeus monodon is a tropical penaeid shrimp and it is the most intensively cultivated shrimp in Taiwan (Liao and Chao 1983). Although technology of eyestalk ablation is a useful method to induce shrimp ovarian maturation, little information has been available regarding the neuroendocrine regulation of oxygen consumption and ammonia-N excretion of unilaterally and bilaterally ablated *P. monodon*. The present study therefore attempted to examine the effects of eyestalk ablation on the oxygen consumption and ammonia-N excretion of juvenile *P. monodon*.

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MATERIALS AND METHODS

Penaeus monodon juveniles obtained from a private nursery farm were shipped to the laboratory and allowed to acclimate for 1 week before use. Seawater was filtered through a gravel and sand bed by air-lifting. Salinity was adjusted to 30 ppt with municipal fresh water dechlorinated with sodium thiosulphate and aerated for 3 days prior to use. Salinity was determined with an AO refractometer. The average wet weight of shrimp was 0.373 ± 0.032 g.

This experiment was conducted by placing animals in 308 ml BOD bottles kept in a water bath (25 ± 1°C). At the initiation of the experiment, juveniles were randomly selected from the holding tanks. Each shrimp was placed in an individual BOD bottle. There were three forms of treatment (control, unilateral and bilateral eyestalk ablation) and four sampling times (6, 12, 18, and 24 hr). For each treatment 5 individuals was tested. The experiment lasted 24 hr with renewal of the test solutions every 6 hr. Dissolved oxygen in the 6 hours water samples was measured with a DO meter and electrode probe (Model 58, YSI Incorporated, Yellow Springs, Ohio, U.S.A.) attached to a battery powered stirrer. The DO meter was air calibrated and salinity compensation was used (Weiss 1970). Ammonia-N was determined by the phenolhypochlorite method (Solorzano 1969) applied to water samples taken when the test solution was renewed. Water temperature was maintained at 25 ± 0.5°C, and pH was kept at 8.23 ± 0.12. Lethal DO was measured with the DO meter at the time when the shrimp had died; lethal time was taken as the time from start of the test until the shrimp died (Chen et al. 1991, Chen and Nan 1992). To observe the physiological effects of eyestalk ablation, experimental shrimp were ablated by using iridectomy scissors under seawater. Intact shrimp were used as a control group.

All data were subjected to a one-way analysis of variance (Steel and Torrie 1980). If the significant differences were indicated at the 0.05 level, then Duncan's multiple range test was used to identify significant differences among treatments (Duncan 1955).

RESULTS

Comparisons of oxygen consumption between the experimental and intact shrimp are shown in Table 1. The results reveal that bilateral-eyestalk-ablated shrimp had a high oxygen consumption, an intermediate oxygen consumption in shrimp treated unilaterally and a low oxygen consumption in intact shrimp during 24 hr period. The effect of eyestalk ablation on ammonia-N excretion of *Penaeus monodon* is shown in Table 2. Eyestalk ablation significantly ($p < 0.05$) increased ammonia-N excretion of the shrimp and ammonia-N excretion was highest in shrimp treated bilaterally, intermediate in shrimp treated unilaterally and lowest in intact shrimp. The O:N ratio (atoms of oxygen consumption relative to atoms of N excreted) of *P. monodon* was high in intact shrimp, inter-

Table 1. Mean oxygen consumption of eyestalk ablated (unilateral and bilateral) and eyestalk-intact *Penaeus monodon* shrimp at various periods

Treatment	Oxygen consumption (mg/(g × shrimp)) Time elapsed (hr)			
	6	12	18	24
Control	0.58[a] (0.09)	0.49[a] (0.05)	0.55[a] (0.08)	0.46[a] (0.05)
UEA	0.80[b] (0.05)	0.68[b] (0.08)	0.80[b] (0.04)	0.66[b] (0.08)
BEA	0.87[c] (0.01)	0.83[c] (0.03)	0.89[c] (0.02)	0.82[c] (0.03)

Data in parentheses are standard error of mean.
UEA: unilateral eyestalk ablation.
BEA: bilateral eyestalk ablation.
[a,b,c]Within each column, mean oxygen consumption with different letters are significantly different ($p < 0.05$).

Table 2. Mean ammonia-N excretion of eyestalk-ablated (unilateral and bilateral) and eyestalk-intact *Penaeus monodon* shrimp at various periods

Treatment	Ammonia-N excretion (ug/(g × hr)) Time elapsed (hr)			
	6	12	18	24
Control	18.35[a] (4.15)	20.35[a] (2.75)	18.44[a] (4.34)	24.04[a] (5.51)
UEA	25.99[b] (2.08)	30.31[b] (2.16)	32.01[b] (4.13)	35.24[b] (3.33)
BEA	45.53[c] (3.54)	45.35[c] (5.74)	42.73[c] (3.15)	45.14[c] (5.74)

Data in parentheses are standard error of mean.
UEA: unilateral eyestalk ablation.
BEA: bilateral eyestalk ablation.
[a,b,c]Within each column, mean ammonia-N excretion with different letters are significantly different ($p < 0.05$).

mediate in shrimp treated unilaterally and lowest in shrimp treated bilaterally (Fig. 1). The mean O:N ratio of bilaterally ablated, unilaterally ablated and intact shrimp during the 24 hr period were 16.94, 21.35, and 23.63, respectively. Bilateral-eyestalk-ablated shrimp had significantly lower O:N ratios ($p < 0.05$) than intact or unilateral-eyestalk-ablated shrimp.

Lethal DO concentrations and lethal times were significantly different ($p < 0.05$) among treatment groups. The bilaterally ablated shrimp had the highest lethal DO level, while the unilaterally ablated shrimp had the lowest (Fig. 2). The intact shrimp had the highest lethal time, while the bilaterally ablated shrimp had the lowest (Fig. 3).

DISCUSSION

Rosas et al. (1991) indicated that eyestalk ablation caused an increase in the oxygen consumption of the crab *Callinectes similis*. The present study also has demonstrated that shrimp without eyestalks had a higher oxygen consumption than intact shrimp. Respiratory-depressing hormones or other factors were found to exist in the eyestalks of the fiddler crab *Uca pugilator* (Silverthorn 1975). Therefore, intact shrimp may secrete unknown substances to regulate respiratory rates. It has been reported that eliminating eyestalks of shrimp by ablation allows a respiratory-stimulating hormone to increase respiratory rates, which changes the general metabolism and causes an increase in the oxygen consumption (Kleinholz 1976).

Unilateral-eyestalk-ablated shrimp showed a higher Na^+,K^+-ATPase activity in the gills than that of intact shrimp (Nan et al. 1993). Chen and Nan (1992b) also demonstrated that an increase of Na^+,K^+-ATPase activity in the gills of *Penaeus chinensis* may increase ammonia-N excretion. The present study has demonstrated that bilateral and unilateral eyestalk ablation accelerate ammonia-N excretion in *P. monodon*. Therefore, it is suggested that intact shrimp may secrete unknown

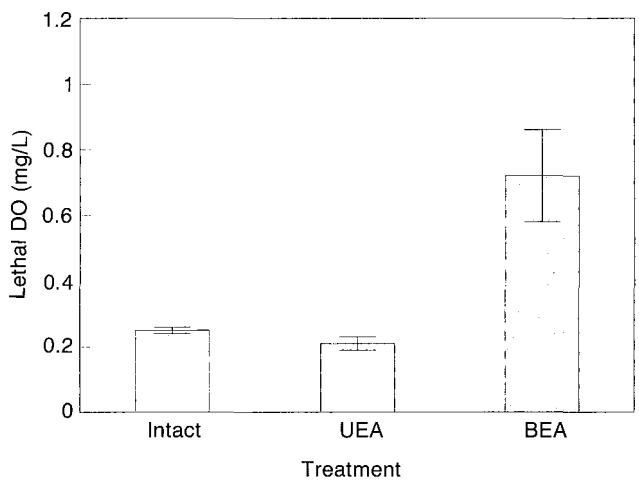
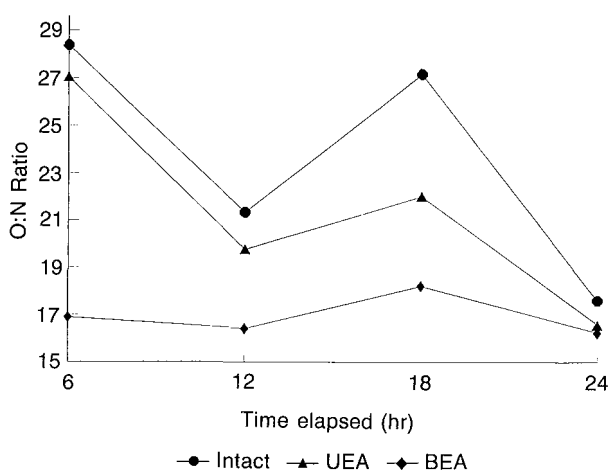


Fig. 2. Lethal DO of intact, unilateral-(UEA) and bilateral-(BEA) eyestalk-ablated *Penaeus monodon* during the experimental period.



Figs. 1. The O:N ratio of intact (●), unilateral-(UEA) (▲), and bilateral-(BEA) (◆) eyestalk-ablated *Penaeus monodon* during the experiment period.

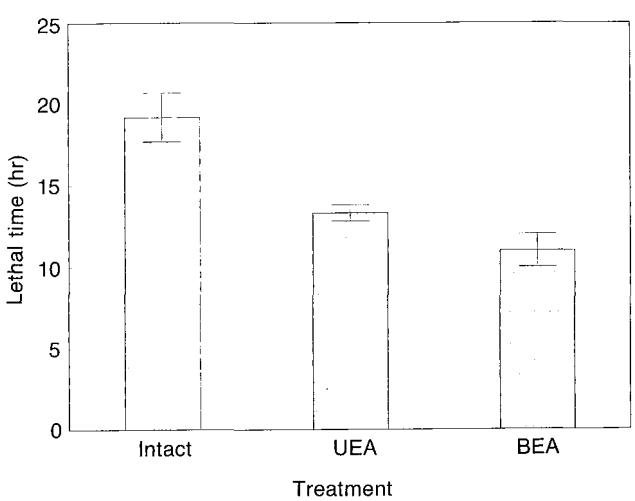


Fig. 3. Lethal time of intact, unilateral-(UEA) and bilateral-(BEA) eyestalk-ablated *Penaeus monodon* during the experimental period.

factors to control ATPase activity of gills which, in turn regulates ammonia-N excretion. The physiological effects of neuroendocrine factors in the eyestalks of shrimp need to be further investigated.

Corner and Cowey (1968) indicated that the O:N ratio is useful to evaluate the characteristics of nutrients utilization by animals. A value of the O:N ratio lower than 24 implies that protein is being used as the main energy source, and an O:N ratio above 24 indicates the breakdown of fat or carbohydrates for this purpose (Taniguchi 1975). In the present study, bilateral- and unilateral-eyestalk-ablated shrimp had lower O:N ratios than intact shrimp. Koshio et al. (1992) indicated that the protein requirements of American lobster *Homarus americanus* without eyestalks were higher than those of intact lobster. Therefore, it is suggested that shrimp without eyestalks need higher dietary protein levels than intact shrimp.

Carter (1962) first used the lethal dissolved oxygen method to detect environmental toxins. Ballard and Oliff (1969) also indicated that lethal dissolved oxygen levels could be used as an indicator to reflect the effects of intrinsic and extrinsic stresses on physiological regulation mechanisms of animals. The present study showed that bilateral-eyestalk-ablated shrimp had a higher lethal DO level than both the intact and unilateral-eyestalk-ablated shrimp. Furthermore, bilateral-eyestalk-ablated shrimp had a shorter lethal time than both the intact and unilaterally ablated shrimp. The high lethal DO level and shorter lethal time of shrimp without eyestalks indicate that the basal metabolism of these shrimp should be higher than that of intact shrimp and high water quality should be required for shrimp without eyestalks.

Rosas et al. (1991) indicated that ablation of eyestalks caused an increase in respiratory rates in the crab *Callinectes similis*. Ablation of eyestalks of the lobster *Panulirus argus* altered its physiological state, such as inducing hyperglycemia, a decrease of glycogen and an increase of lipids in the hepatopancreas (Diaz-Iglesia et al. 1987). Sochasky et al. (1973) and Castell et al. (1976) demonstrated that the nutritional level of the diet plays an important role in the growth of the lobster *Homarus americanus* after its eyestalks have been ablated. The present study clearly indicates that nutrient utilization of eyestalk-ablated shrimp shifted from lipid dominated to protein dominated. The shrimp without eyestalks may accumulate lipids in the hepatopancreas and efficiently use protein as an energy source. It is therefore suggested that sufficient protein is essential for normal growth of shrimp *Penaeus monodon* without eyestalks. However, changes of enzyme activity in the digestive glands and the optimum feed formula for eyestalk-ablated shrimp need further investigation.

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眼柄剪除後對草蝦耗氧和排氨之影響

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將草蝦眼柄單眼柄和雙眼柄剪除後，於鹽度 30 ppt，水溫25°C下，研究草蝦幼蝦24小時的耗氧和排氨。在耗氧量方面，以雙眼柄剪除的草蝦最高，其次是剪單眼柄草蝦，最低的是對照組草蝦，顯示眼柄剪除會增加草蝦的耗氧量。而經剪眼柄處理後，會促進草蝦的氨－氮排泄，其中以剪雙眼柄草蝦最高，其次是剪單眼柄草蝦，最低的爲對照組草蝦。經剪眼柄處理後，草蝦的氧：氮原子比值顯著降低，由此可知，剪眼柄草蝦的能量來源由脂質依賴型轉變爲蛋白質依賴型。在致死溶氧的實驗中，以剪單眼柄草蝦最低，其次是對照組，最高的是剪雙眼柄草蝦；而致死時間以對照組草蝦最高，其次爲剪單眼柄草蝦，最低的爲剪雙眼柄草蝦。

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