THE EFFECT OF EYESTALK ABLATION ON GROWTH, HAEMOLYMPH COMPOSITION AND GILL Na^+ , K^+ -ATPase ACTIVITY OF *PENAEUS MONODON* JUVENILES

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Abstract—1. Eyestalk unablated and unilaterally ablated *Penaeus monodon* juveniles had survival rates after 5 months of 75–72.5 and 67.5–60%, respectively.

2. Unilaterally ablated shrimps had significantly higher ($P < 0.05$) growth rate than unablated shrimps.

3. Eyestalk-ablation resulted in a decrease in the haemolymph sodium concentration and an increase in the potassium and calcium concentration of shrimps.

4. The osmolality of haemolymph and total protein concentration of unablated shrimps were demonstrated to be higher than those of unilaterally ablated shrimps.

5. The eyestalk-ablated shrimps possess higher total ATPase and Na^+ , K^+ -ATPase activities in the gill than those of unablated shrimps.

INTRODUCTION

During the growing period, exuviation of the old exoskeleton followed by replacement with a new one is a common metabolic procedure for crustaceans. The periodic shedding of the old exoskeleton is accomplished by molting, the procedures of which consist of numerous biochemical, physiological and morphological changes that temporally occupy much of the preceding molt cycle. Experiments performed on crab showed that crustacean have linear growth in a discontinuous step-wise manner (Freeman *et al.*, 1983). However, tissue increment reveals a continuous process that occurs throughout the molt cycle (Anger *et al.*, 1989).

It is known that the x-organ plays a major role in the inhibition of the molting process for decapod crustaceans (Chang and O'Connor, 1987). Ablation of the eyestalk may remove or destroy the function of x-organ sinus gland complex and may accelerate molt or growth of the crustacean. However, studies with American lobster (*Homarus americanus*) and western Atlantic spiny lobster (*Panulirus argus*) eyestalk ablation did not result in an acceleration of their molting cycle (Travis, 1952; Sochasky *et al.*, 1973). In contrast, Castell *et al.* (1976) reported that eyestalk ablation may enhance the growth rate of *H. americanus*. Koshio *et al.* (1992) also demonstrated that growth of the freshwater prawn, *Macrobrachium rosenbergii* may be accelerated by unilateral eyestalk ablation. The molting cycle of spiny lobster (*Panulirus argus*) and red king crab (*Paralithodes*

camtschatica) may also be significantly improved by ablation of eyestalk (Quackenbush and Herrnkind, 1981; Molyneaux and Shirley, 1988).

In addition to the above described metabolic processes, the operation of eyestalk ablation may also result in a decrease of blood glucose (Keller *et al.*, 1985), promotion of vitellogenesis (Adiyodi, 1985), accumulation of water (Jackson *et al.*, 1987), increase in respiratory rate (Rosas *et al.*, 1991) and lowering of sodium concentration in haemolymph (McNamara *et al.*, 1990). The mechanisms of osmotic and ionic regulation have been demonstrated to be under neuroendocrine control (Kamemoto and Tullis, 1972; Kleinholtz, 1976; Mantel and Farmer, 1983). Kamemoto and Tullis (1972) injected a cerebral ganglion extract derived from *M. rosenbergii* which resulted in an increase of haemolymph chloride concentration and sodium influx in the freshwater crayfish, *Procambarus clarkii*. The importance of the eyestalk ganglionic system in the regulation of water and ion movement has also been demonstrated in a variety of crustaceans (Heit and Fingerma, 1975; Davis and Hagadorn, 1982). Moreover, Charmanier *et al.* (1984) reported that eyestalk neurohormonal factor may regulate haemolymph osmosis, sodium and chloride concentration in *H. americanus*. It was also concluded that eyestalk removal may initiate a lowering of haemolymph sodium (Heit and Fingerma, 1975) due to increased sodium efflux, and result in an increase of sodium permeability in crustaceans (Davis and Hagadorn, 1982).

The active transport of Na^+/K^+ requires a specific carrier enzyme, Na^+ , K^+ -ATPase (Towle *et al.*, 1976). Na^+ , K^+ -ATPase, a ubiquitous component

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from cell membrane of animals (Towle, 1981), is a prime mediator of ion transport across cellular membranes (Neufeld *et al.*, 1980) which may regulate ion balance at the organ or cell level for marine animals (Towle, 1981). ATP reservoirs are reduced by increasing energy demand in response to physiologically stressful conditions (Chen and Nan, 1992). Therefore, examination of ATPase activity could be an indicator for the physiological well-being of an animal.

Little information is available regarding the neuro-endocrine mediation of growth and haemolymph composition of ablated *P. monodon*. The present study, therefore, attempts to examine the effects of unilateral eyestalk ablation on the process of growth and haemolymph composition of juvenile *Penaeus monodon*.

MATERIALS AND METHODS

Penaeus monodon juveniles obtained from a private nursery located in southern Taiwan, Kaohsiung were shipped to the laboratory and acclimated for 1 week before use. Sea-water pumped from the Keelung coast was filtered through gravel and sand beds by air-lifting. Salinity was adjusted to 30.5–31.5 ppt with municipal water dechlorinated with sodium thio-sulphate and aerated for 3 days prior to use.

Duplicate experiments were conducted outdoors with 1500 l FRP tanks (200 × 120 × 75 cm). At the initiation of each experiment, a number of randomly selected juveniles were obtained from the holding tanks. Water was lightly blotted from the animals before they were individually weighed. The mean wet weight of *P. monodon* juveniles for the first and second experiment was 0.59 g. Each tank was stocked with 40 shrimps and aerated with an air stone connected to a blower.

Salinity was determined daily with an AO refractometer. Dissolved oxygen was measured with a YSI model 58 DO meter (YSI Inc., Yellow Spring, Ohio). During the course of the experiment, water temperature fluctuated from 25 to 30°C and salinity was kept at 33 ± 1 ppt. Dissolved oxygen ranged from 6 to 6.6 mg/l.

To observe the physiological effect of eyestalk ablation, the eyestalks of experimental shrimps were ablated using iridectomy scissors under sea-water. For comparison, intact shrimps were used as control groups. The shrimps were fed commercial shrimp ration (Tairoun products Co. Ltd, Taipei) twice daily at 9:00 am and 17:00 pm and at 8% of body weight per day level. Wet weights of live individuals in each experimental group were measured and recorded at 30 day periods. The duration of the experiments was 5 months.

At the end of the experiments, the haemolymph were sampled and centrifuged at 15,000 rpm for 5 min and collected samples were then used for osmotic, ionic and total protein analysis. Osmotic concentration was determined by the freezing point

depression method as described by Salomao (1980) with a Model 2430 Automatic Osmometer (Precision System, U.S.A.). The ionic concentrations were determined by flame spectrophotometry, and the total protein was measured by the Bradford method (Bradford, 1976).

The gills were excised at the end of the experiment, transferred to an ice bath and homogenized. Total ATPase and Na⁺, K⁺-ATPase activities were determined following the method described by Tucker (1979). The amount of inorganic phosphate (Pi) liberated in the reaction of ATP → ADP + Pi was expressed as μmol Pi mg⁻¹ protein hr⁻¹ (Bartlett, 1958). The protein content of the homogenate was determined (Lowry *et al.*, 1951). The detection limit for the total ATPase and Na⁺, K⁺-ATPase was 0.02.

All data were subjected to 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 difference between two treatments (Duncan, 1955).

RESULTS

To investigate the growth rate of experimental shrimps after eyestalk ablation, a comparison between experiment and control shrimps was made (Fig. 1). The results showed that the eyestalk ablated shrimps had higher mean wet body weight than the intact shrimps throughout the experimental period. The mean wet weight of initial and final weight gain, total weight and survival of shrimps are shown in Table 1. The final wet weight, weight gain and total weight of shrimp in experiment I were less than those of shrimps in experiment II. Weight gain and total weight of intact shrimps were significantly lower than those of eyestalk ablated shrimp. However, the survival of intact shrimps was higher than that of eyestalk ablated shrimps.

Fig. 1. Growth (mean wet weight) of intact and eyestalk ablated juvenile *Penaeus monodon* during the experiment period.

Table 1. Mean (standard deviation) weight (g), total weight and survival of eyestalk ablated and eyestalk intact *Penaeus monodon* reared for 5 months

Treatment	Body weight		Weight gain	Total weight (g)	Survival (%)
	Initial	Final			
Experiment I					
Intact	0.59 ^a (0.16)	8.79 ^a (1.38)	8.2 ^a	307.8	75
EA	0.60 ^a (0.14)	18.76 ^b (3.71)	18.2 ^b	506.5	68
Experiment II					
Intact	0.59 ^a (0.11)	13.70 ^a (3.03)	13.1 ^a	424.6	73
EA	0.58 ^a (0.22)	23.94 ^b (5.08)	23.4 ^b	574.5	60

EA: Eyestalk ablation.

Within each experiment, means with different letters are significantly different ($P < 0.05$).

A significant correlation was found between wet weight and time. The regression equations in experiment I were $W = 0.7295 e^{0.5247 T}$ ($r = 0.98$), for intact shrimp, $W = 0.9447 e^{0.6576 T}$ ($r = 0.94$) for eyestalk ablated shrimp and in experiment II were $W = 0.7675 e^{0.6478 T}$ ($r = 0.95$) for intact shrimp, $W = 0.939 e^{0.7329 T}$ ($r = 0.93$) for eyestalk ablated shrimp, where W = wet weight (mg), and T = experimental period (days).

The results after 5 months of eyestalk ablation on osmolality, ion concentrations and total protein in the haemolymph of *P. monodon* are shown in Table 2. The haemolymph osmolality, potassium, calcium and total protein concentrations obtained from the intact shrimps were lower than those from the eyestalk ablated shrimps. In comparison, haemolymph sodium concentrations in the intact shrimps were higher than those of the eyestalk ablated shrimps. The analysis of variance suggested that eyestalk ablation significantly ($P < 0.05$) affected the haemolymph composition in the experimental shrimps.

Total ATPase activity and Na^+ , K^+ -ATPase activity in the gills of *P. monodon* after each treatment are given in Figs 2 and 3, respectively. The eyestalk-

ablated shrimps showed significantly higher ($P < 0.05$) total ATPase activity and Na^+ , K^+ -ATPase activity in the gills than those obtained from the intact shrimps.

DISCUSSION

Growth of *M. rosenbergii* and *M. lancesteri* was also enhanced by eyestalk ablation (Ponnuchamy *et al.*, 1981; Koshio *et al.*, 1992), but the growth of the red king crab (*P. camtschatica*) and the mud crab (*Rhithropanopeus harrisi*) was not enhanced by unilateral eyestalk ablation (Costlow, 1966; Molyneux and Shirley, 1988). Although, studies on an inconsistent effect of unilateral eyestalk ablation on growth of crustacean have been reported, the present study demonstrated that unilateral eyestalk ablation may accelerate the growth of *P. monodon*. Since most previous studies showed that a reduction of the intermolt period and growth of tissue depends on nutritional condition (Koshio *et al.*, 1989, 1990). It is, therefore, suggested that except for physiological triggers, nutrition is also a key factor for the promotion of growth of the shrimp.

Table 2. Mean (standard deviation) haemolymph contents of eyestalk ablation and eyestalk intact *Penaeus monodon* reared for 5 months

Treatment	Hemolymph contents				
	Osmolality (mOsm/kg)	Na^+ (mEq/l)	K^+ (mEq/l)	Ca^{2+} (mEq/l)	Protein (μg)
Experiment I					
Intact	666.86 ^a (8.24)	374.05 ^a (6.05)	7.76 ^a (0.07)	40.79 ^a (0.27)	181.12 ^a (6.13)
EA	757.23 ^b (8.99)	339.51 ^b (1.90)	9.07 ^b (0.15)	52.50 ^b (3.29)	228.44 ^b (7.25)
Experiment II					
Intact	717.74 ^a (10.36)	413.25 ^a (3.49)	8.14 ^a (0.05)	43.11 ^a (0.47)	213.49 ^a (8.68)
EA	786.13 ^b (5.45)	353.86 ^b (3.66)	9.95 ^b (0.27)	66.26 ^b (1.33)	254.46 ^b (6.95)

EA: Eyestalk ablation.

Within each experiment, means with different letters are significantly different ($P < 0.05$).

Fig. 2. Total ATPase activity in the gill of intact and eyestalk ablated juvenile *Penaeus monodon* after 5 months.

It was also reported that high mortality of crustacean may be caused by eyestalk ablation (Mauviot and Castell, 1976; Nakatani and Otsu, 1979, 1981; Trider *et al.*, 1979; Fairfull, 1980; Freeman *et al.*, 1983). Similarly, the present study further confirmed that eyestalk ablated shrimps revealed higher mortality than the intact ones. The high mortality of eyestalk ablated shrimps may be caused by cannibalism of animals that are confined in a limited environment with unsuitable stocking density or an incorrect rearing method and inferior food quality.

Physiologically, the present study also showed that *P. monodon* treated by eyestalk ablation had a significantly lower ($P < 0.05$) sodium concentration than that of the intact ones. This result is similar to those obtained by McNamara *et al.* (1990) in a study with the freshwater prawn, *M. olfersii*. Ehrenfeld and Isaia (1974) showed that the freshwater crayfish, *Astacus leptodactylus*, diminished sodium influxes by 30% in eyestalk-ablated animals while effluxes remained unchanged. These results may suggest that reduction of sodium concentration in the haemolymph of shrimps by eyestalk ablation could be initiated by an increase of sodium efflux. Furthermore, Heit and Fingerman (1975) demonstrated that a neurofactor existing in the eyestalk of fiddler crabs (*Uca pugilator*), may play

a major role in the prevention of sodium loss in hyposmotic media by promoting active transport of sodium. The results obtained from the present study incorporated with the previous studies, therefore, suggest that eyestalk may play a major role in either regulating sodium uptake mechanism or increasing sodium permeability.

It was reported that the chloride cells of teleost gill contain a large amount of Na^+, K^+ -ATPase (Sargent *et al.*, 1975). The efflux of sodium ion out of the chloride cell requires energy which could be indirectly supplied by Na^+, K^+ -ATPase (Sargent *et al.*, 1980). The increase in activity of gill Na^+, K^+ -ATPase is, therefore, always accompanied by an efflux of Na^+ . Heit and Fingerman (1975) indicated that a hormone present in eyestalks may inhibit the loss of haemolymph Na^+ in hyposmotic sea-water. The results obtained in the present study demonstrated that following eyestalk ablation the experimental shrimps have a higher ATPase activity in the gill and lower haemolymph sodium ion than those of the intact ones. It is reasonable to assume that the intact shrimp may secrete unknown substances to inhibit ATPase activity and prevent Na^+ loss from haemolymph. On the other hand, ATPase activity in the eyestalkless shrimp may initiate a removal of Na^+ from the haemolymph. The physiological effects of neuroendocrine factor in the eyestalk of the shrimps needs to be further investigated.

The ablation of *P. argus* lobster (Diaz-Iglesia *et al.*, 1987), *Varuna litterata* crab (Madhyastha and Rangneker, 1976) and *H. americanus* lobster (Mauviot and Castell, 1976) eyestalks caused a decrease of glycogen and an increase of lipids in the hepatopancreas, muscle glycogen, oxygen consumption and growth of the animals. Sochasky *et al.* (1973) and Castell *et al.* (1976) also demonstrated that the nutritional status of the diet may play an important role in growth and survival of the ablated lobsters. The results obtained from the present study clearly indicate that eyestalk-ablated shrimp show a better growth than the intact ones. It is, therefore, suggested that eyestalk ablation may eliminate the molt inhibiting hormone and consequently accelerate the metabolism of crustacean growth. To maintain a normal growth of eyestalkless *P. monodon* or broodstock, sufficient nutritional supply is equally important. However, the standardization of the feed for eyestalk ablated shrimps needs further investigation.

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REFERENCES

- Adiyodi R. G. (1985) Reproduction and its control. In *The Biology of Crustacea* (Edited by Bliss D. E.), Vol. 9, pp. 147–215. Academic Press, New York.

Fig. 3. Na^+, K^+ -ATPase activity in the gill of intact and eyestalk ablated juvenile *Penaeus monodon* after 5 months.

- Anger K., Harms J., Puschel C. and Seeger B. (1989) Physiological and biochemical changes during the larval development of a brachyuran crab reared under constant conditions in the laboratory. *Helgolander Meeresunters* **43**, 225–244.
- Bartlett G. R. (1958) Phosphorus assay in column chromatograph. *J. biol. Chem.* **234**, 466–468.
- Bradford M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein using the principle of protein-dye binding. *Analyt. Biochem.* **72**, 248–254.
- Castell J. D., Mauviot J. C. and Covey J. F. (1976) The use of eyestalk ablation in nutrition studies with American lobster (*Homarus americanus*). Proceedings of the 7th Annual Meeting, World Mariculture Society, San Diego, California. 12–15 January, pp. 431–441.
- Chang E. S. and O'Connor J. D. (1987) Crustacea: molting. In *Invertebrate Endocrinology* Vol. 2 (Edited by Laufer H. and Downer R.), pp. 181–200. Alan R. Liss, New York.
- Charmantier G., Chermantier-Daures M. and Aiken D. E. (1984) Neuroendocrine control of hydromineral regulation in the American lobster (*Homarus americanus*). 1. Juveniles. *Gen. comp. Endocrinol.* **54**, 8–19.
- Chen J. C. and Nan F. H. (1992) Effect of ambient ammonia on ammonia-N excretion and ATPase activity of *Penaeus chinensis*. *Aquat. Toxicol.* **23**, 1–10.
- Costlow J. D. (1966) The effect of eyestalk extirpation on larval development of the mud crab, *Rhithropanopeus harrisi* (Gould). *Gen. comp. Endocrinol.* **7**, 255–274.
- Davis C. W. and Hagadorn I. R. (1982) Neuroendocrine control of sodium in the fiddler crab (*Uca pugilator*). *Am. J. Physiol.* **242**, 505–513.
- Diaz-Iglesia, E., Brito R. and Hernandez I. (1987) Efecto de la ablacion del complejo neurosecretor peduncular en juveniles de langosta *Panulirus argus* II. Algunos aspectos metabolicos. *Revista de Investigaciones Marinas* **3**, 81–93.
- Duncan D. B. (1955) Multiple-range and multiple F test. *Biometrics* **11**, 1–24.
- Ehrenfeld J. and Isaia J. (1974) The effect of ligaturing the eyestalks on the water and ion permeabilities of *Astacus leptodactylus*. *J. comp. Physiol.* **93**, 105–115.
- Fairfull R. W. (1980) The genetics and early growth of lobster (*Homarus americanus*) as related to rearing under artificial conditions. Ph.D. Thesis, Dalhousie University, Halifax, N.S., Canada.
- Freeman J. A., West T. L. and Costlow J. D. (1983) Post-larval growth in juvenile *Rhithropanopeus harrisi*. *Biol. Bull.* **165**, 409–415.
- Heit M. and Fingerhman M. (1975) The role of an eyestalk hormone in the regulation of the sodium concentration of the blood of the fiddler crab (*Uca pugilator*). *Comp. Biochem. Physiol.* **50A**, 277–280.
- Jackson S. A., Bruce M. J., Chang E. S. and Clegg J. S. (1987) Effects of eyestalk ablation upon water relations in the American lobster, *Homarus americanus*. *J. exp. Zool.* **244**, 389–393.
- Kamemoto F. I. and Tullis R. E. (1972) Hydromineral regulation in decapod crustacea. *Gen. comp. Endocrinol. Suppl.* **3**, 299–307.
- Keller R., Jaros P. P. and Kegel G. (1985) Crustacean hyperglycemic neuropeptides. *Am. Zool.* **25**, 207–221.
- Kleinholz L. H. (1976) Crustacean neurosecretory hormones and physiological specificity. *Am. Zool.* **16**, 151–166.
- Koshio S., Haley L. E. and Castell J. D. (1989) The effect of two temperatures and salinities on growth and survival of bilateral eyestalk ablated and intact juvenile American lobsters, *Homarus americanus*. *Aquaculture* **76**, 373–382.
- Koshio S., O'Dor R. K. and Castell J. D. (1990) The effect of varying dietary energy levels on growth and survival of eyestalk ablated and intact juvenile lobsters, *Homarus americanus*. *J. World Aquacult. Soc.* **21**, 160–169.
- Koshio S., Teshima S. I. and Kanazawa A. (1992) Effects of unilateral eyestalk ablation and feeding frequencies on growth, survival and body compositions of juvenile freshwater prawn *Macrobrachium rosenbergii*. *Nippon Suisan Gakkaishi* **58**, 1419–1425.
- Lowry O. H., Rosebrough N. J., Farr A. L. and Randall R. J. (1951) Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193**, 265–275.
- Madhyastha M. M. and Rangneker P. V. (1976) Metabolic effects of eyestalk removal in the crab *Varuna litterata* (Fabricius). *Hydrobiology* **48**, 25–31.
- Mantel L. H. and Farmer L. (1983) Osmotic and ionic regulation. In *The Biology of Crustacea* Vol. 5 (Edited by Bliss D. E.), pp. 54–161. Academic Press, New York.
- Mauviot J. C. and Castell J. D. (1976) Molt and growth enhance effects of bilateral eyestalk ablation on juvenile and adult American lobster (*Homarus americanus*). *J. fish. Res. Bd Can.* **33**, 1922–1929.
- McNamara J. C., Salomao L. C. and Ribeiro E. A. (1990) The effect of eyestalk ablation on haemolymph osmotic and ionic concentrations during acute salinity exposure in the freshwater shrimp *Macrobrachium olfersii* (Wiegmann). *Hydrobiologia* **199**, 193–199.
- Molyneux D. B. and Shirley T. C. (1988) Molting and growth of eyestalk-ablated juvenile red king crabs, *Paralithodes camtschatica* (Crustacea: Lithodidae). *Comp. Biochem. Physiol.* **91A**, 245–251.
- Nakatani I. and Otsu T. (1979) The effects of eyestalk and uropod removal on the molting and growth of young crayfish, *Procambarus clarkii*. *Biol. Bull.* **157**, 182–188.
- Nakatani I. and Otsu T. (1981) Relations between the growth and the molt interval in the eyestalkless crayfish, *Procambarus clarkii*. *Comp. Biochem. Physiol.* **68A**, 549–553.
- Neufeld G. J., Holliday C. W. and Pritchard J. B. (1980) Salinity adaptation of gill Na^+/K^+ -ATPase in the rock crab, *Cancer irroratus*: response to DDT. *Comp. Biochem. Physiol.* **62C**, 165–172.
- Ponnuchamy R., Reddy S. R. and Katre S. (1981) Effects of eyestalk ablation on growth and food conversion efficiency of the freshwater prawn *Macrobrachium lanchesteri* (De Man). *Hydrobiologia* **77**, 77–80.
- Quackenbush L. S. and Herrnkind W. F. (1981) Regulation of molt and gonadal development in the spiny lobster *Panulirus argus* (Crustacea: Palinuridea): effect of eye-stalk ablation. *Comp. Biochem. Physiol.* **69A**, 523–527.
- Rosas C., Vanegas C., Alcaraz G. and Diaz F. (1991) Effect of eyestalk ablation on oxygen consumption of *Callinectes similis* exposed to salinity changes. *Comp. Biochem. Physiol.* **100A**, 75–80.
- Salomao L. C. (1980) Determinacao da concentracao osmotica de micro-amostras de fluidos atraves do pont de congelamento. *Bolm Fisiol. Animal USP* **4**, 133–141.
- Sargent J. R., Bell M. V. and Kelly K. F. (1980) The nature and properties of sodium ion plus potassium ion-activated adenosine triphosphatase and its role in marine salt secreting epithelia. In *Epithelial Transport in the Lower Vertebrates* (Edited by Lahlou B.), pp. 251–267. Cambridge University Press, London.
- Sargent J. R., Thomson A. J. and Bonancin M. (1975) Activities and localization of succinic dehydrogenase and Na^+/K^+ -activated adenosine triphosphatase in the gills of fresh water and sea water eels (*Anguilla anguilla*). *Comp. Biochem. Physiol.* **51B**, 75–79.
- Sochasky J. B., Aiken D. E. and Mcleese D. W. (1973) Does eyestalk ablation accelerate molting in the lobster *Homarus americanus*? *J. fish. Res. Bd Can.* **30**, 1600–1603.

- Steel R. G. D. and Torrie J. H. (1980) *Principles and Procedures of Statistics*. McGraw-Hill, New York.
- Towle D. W. (1981) Role of Na^+ , K^+ -ATPase in ionic regulation by marine and estuarine animals. *Mar. Biol. Lett.* **2**, 107–122.
- Towle D. W., Palmer G. E. and Harris J. L. (1976) Role of gill Na^+ , K^+ -dependent ATPase in acclimation of blue crab, *Callinectes sapidus*, to low salinity. *J. exp. Zool.* **196**, 315–322.
- Travis D. F. (1952) The control of the sinus gland over certain aspects of calcium metabolism in *Panulirus argus*. *Anat. Rec.* **11**, 503 (abstr).
- Trider D. J., Mason E. G. and Castell J. D. (1979) Survival and growth of American lobster (*Homarus americanus*) juvenile after eyestalk ablation. *J. fish. Res. Bd Can.* **36**, 93–97.
- Tucker R. K. (1979) Effects of *in vivo* cadmium exposure on ATPase in gill of the lobster (*Homarus americanus*). *Bull. envir. contam. Toxicol.* **23**, 33–35.