

Dietary Copper Requirement of *Penaeus vannamei*

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The dietary copper requirement of *Penaeus vannamei* juveniles was investigated using casein/gelatin based semi-purified diets under controlled laboratory conditions. Twenty-day-old *P. vannamei* postlarvae were fed the basal diet lacking copper supplementation but containing 2.0 mg Cu/kg for a 10-day period to reduce tissue stores of copper. After conditioning, six replicate groups of juvenile shrimp (mean weight 0.057 g) were fed each diet containing graded levels of supplemental copper (0, 4, 8, 16, 32, 64, or 128 mg Cu/kg diet) for the duration of a 42-day feeding trial. Weight gain (percent weight gain) ranged from 2.88 g (5,064%) for shrimp fed the basal diet without supplemental copper (2 mg total Cu/kg diet) to 3.5 g (6,177%) for shrimp fed the basal diet supplemented with 64 mg Cu/kg. Weight gain of shrimp increased in response to copper supplementation up to 32 mg Cu/kg diet, indicating a dietary copper requirement of no more than 34 mg total Cu/kg diet. In addition to depressed growth, shrimp fed copper-deficient diets had enlarged hearts and depressed copper levels in the hemolymph, carapace, and hepatopancreas. There was no clear indication that supplementation of up to 128 mg Cu/kg diet adversely affected shrimp growth or survival.

Although substantial research has been conducted on the toxicity of dissolved copper to marine species,^{1,2} the dietary essentiality of this nutrient in marine species has received little attention. Copper functions in hematopoiesis and in numerous copper-dependent enzymes including cytochrome *c* oxidase (EC 1.9.3.1; CCO) and superoxide dismutase (EC 1.15.1.1; SOD).^{3,4} Some marine invertebrates such as crustaceans utilize the copper-containing pigment hemocyanin as the oxygen-carrying pigment of their blood.^{5,6} Depledge^{4,7} estimated that, on a fresh-weight basis, 40% of the whole-body-copper load of shrimp is found in hemocyanin, implying a considerable increase in physiological demand above that required by vertebrates.

Although dietary deficiencies of copper have been documented in freshwater fish,⁸⁻¹¹ the dietary essentiality of copper has not been evaluated for marine fish, and only one study has been conducted with shrimp. Kanazawa *et al.*¹² found that dual deletion of iron and copper did not significantly affect growth and survival of *Penaeus japonicus*. However, in that series of experiments, the percent weight gain was very low

(40%) and survival was very poor (57%); hence, the nutritional stress and/or quality of the diet may not have been adequate to discern a dietary copper deficiency for that species. The present study was designed to evaluate the dietary requirement for copper and to characterize copper deficiency signs in *P. vannamei*.

Materials and Methods

Feeding Trial

The basal diet (Table 1) was formulated to contain 40% crude protein, a gross energy of 3.5 kcal/g, and contained 2.0 mg Cu/kg. This diet was supplemented with seven levels of copper (0, 4, 8, 16, 32, 64, or 128 mg Cu/kg), replacing cellulose with cupric sulfate heptahydrate. Diets were prepared by mixing the dry ingredients in a V-mixer. Menhaden fish oil and an appropriate amount of hot deionized water, required for pelleting, were then added to the dry ingredients and homogenized. Each diet was then pelleted using a meat grinder and a 3 mm die. After pelleting, the diets were dried by forced air at 60°C for 3 h and forced ambient air for 12 h to

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Table 1. Composition of the basal diet*¹
(% Dry weight)

Ingredient	(% Dry weight)
Casein* ²	35.0
Gelatin* ²	8.0
Wheat starch* ²	20.2
Menhaden fish oil* ³	7.0
Lecithin (purified)* ²	1.0
Cholesterol* ²	0.5
Mineral mixture* ⁴	15.0
Vitamin mixture* ⁵	8.0
Stay-C* ⁶ (3000 mg active vitamin C/kg)	3.3
Alpha-cellulose* ⁷	2.0

*¹ Basal diet contained 2 mg Cu/kg diet as determined by atomic absorption spectrophotometry.

*² I.C.N. Nutritional Biochemicals Inc., Cleveland, Ohio, USA.

*³ Zapata Haynie Corp., Reedville, Virginia, USA. Supplemented with 125 ppm santonin.

*⁴ Contains (as g/kg): CaHPO₄, 500.0; NaCl, 74.0; K₂C₂H₅O₇·H₂O, 220.0; K₂SO₄, 52.0; MgO, 24.0; MnSO₄·H₂O, 5.16; FeSO₄·7H₂O, 4.95; ZnSO₄·7H₂O, 3.67; KIO₃, 0.01; Na₂SeO₄, 0.0072; KCr(SO₄)₂·12H₂O, 0.55; cellulose, 115.65.

*⁵ Contains (as g/kg): vitamin A palmitate, 1.8; vitamin E (250 u/g), 22.0; inositol, 180; choline chloride, 75; menadione, 2.3; *p*-aminobenzoic acid, 30; niacin, 26; riboflavin, 8; pyridoxine HCl, 3; thiamine mononitrate, 5; D-calcium pantothenate, 15; vitamin D₃ (40,000 u/g), 1; biotin, 1; folic acid, 5; vitamin B₁₂ crystalline, 1; sucrose, 623.9.

*⁶ Vitamin Technologies International, Buhl, Idaho, USA.

*⁷ Sigma Chemical Company, Cleveland, Ohio, USA.

achieve a moisture content of 8 to 10%. Feeds were mechanically crumbled and sieved to the desired size, then frozen at -10°C until use.

The feeding trial was conducted in 136-*l* rectangular tanks (bottom surface area 0.34 m²), each receiving 4 *l*/min of pre-filtered (rapid-rate sand filter, 45–55 mm silica sand) water. These tanks were part of a 50-m³ recirculating system receiving a 10% daily water exchange and was designed to maintain a constant environmental temperature (28±1°C) and salinity (30±1 ppt). The photoperiod was set for a 12:12 h light:day cycle. To ensure that adequate water quality parameters were maintained, the system's temperature, dissolved oxygen, and salinity were measured daily. Ammonia nitrogen and nitrite were measured weekly utilizing spectrophotometric methods.¹³⁾

Prior to initiation of the feeding trial, 20-day-old postlarval *P. vannamei* were fed the basal diet lacking supplemental copper for a 10-day conditioning period. At the start of the growth trial, conditioned juvenile shrimp were hand-graded to a uniform size (0.057±0.019 g) and stocked at a density of 30 shrimp per tank. To

estimate the initial weight, 30 shrimp were randomly selected during stocking, set aside, towel-dried and weighed individually to determine the mean initial wet weight and standard deviation.

Dietary treatments were each fed to six replicate groups of shrimp for a 42-day period. Shrimp were fed 12 times a day in excess of satiation for the duration of the experiment. At the conclusion of the growth trial, the shrimp were enumerated and an average wet weight per tank determined. After weighing, seven shrimp per tank were held for subsequent enzyme analyses and the determination of heart and hepatopancreas indices. In addition, hemolymph samples from the remaining live shrimp were taken by heart puncture technique utilizing 15 μ l capillary tubes. After bleeding, the shrimp and hemolymph samples were immediately frozen for subsequent mineral analyses.

Tissue Analyses

Forty-eight hours after termination of the feeding trial, individual shrimp were weighed and then the heart and hepatopancreas removed and weighed. The heart and hepatopancreatic indices [wet organ weight (mg)/wet shrimp weight (g)] were determined for 30 shrimp per treatment. For enzymatic analyses, the heart and hepatopancreatic tissues from six shrimp were pooled into five composite samples per dietary treatment.

Heart and hepatopancreas samples were homogenized in 3 ml of cold Triton X-100 (0.2%). Heart CCO activity was determined in homogenates by measuring the oxidation of ferrocytochrome *c* at 550 nm in 10 mM potassium phosphate buffer, pH 7.0.¹⁴⁾ The initial concentration of ferrocytochrome *c* in the reaction mixture was 40 μ M. One unit of activity was defined as the oxidation of 1 μ M of ferrocytochrome *c*/min at 25°C.

Hepatopancreas homogenates were centrifuged at 10,000×*g* for 30 min. The resulting supernatant was assayed for SOD activity by its ability to inhibit the reduction of ferricytochrome *c* by the xanthine oxidase reaction.¹⁵⁾

Frozen shrimp were rinsed with deionized water, then the carapace and hepatopancreas were dissected from 10 shrimp per tank (60 shrimp per treatment) and oven-dried at 90°C to a constant weight. Hepatopancreas, carapace, and hemolymph samples were wet-washed and copper was analyzed by atomic absorption spectrophoto-

metry according to procedures described by the Association of Official Analytical Chemists.¹⁶⁾ Data were analyzed using a one-way analysis of variance to determine significant ($P < 0.05$) differences among treatment means. Data for which there were significant differences were analyzed by regression to determine the effects of dietary treatments on the dependent variables. Student-Newman-Keuls' multiple range test¹⁷⁾ was used to evaluate significant differences among treatment means. All statistical analyses were conducted using the Statistical Analysis System.¹⁸⁾

Results

Mean \pm standard deviation total ammonia nitrogen, nitrite nitrogen, and dissolved oxygen were 0.07 ± 0.05 mg N/l, 0.05 ± 0.04 mg N/l, and 6.4 ± 0.28 mg O₂/l, respectively. These values are consistent with those considered adequate for the normal growth and survival of shrimp.¹⁰⁻²¹⁾ Based on atomic absorption spectrophotometric analysis of a full strength sea water sample, the copper content of the system's water was below 0.2 mg Cu/l.

Wet weight gain (percent weight gain) ranged from 2.88 g (5,064%) for shrimp fed the basal diet without supplemental copper (2 mg total Cu/kg) to 3.50 g (6,177%) for shrimp fed the diet containing 66 mg total Cu/kg. Weight gain of shrimp increased in response to supplemental copper up to 34 mg Cu/kg diet after which there was no significant difference in wet weight gain (Fig. 1). Survival of shrimp maintained on the treatment diets ranged from 92.2% for shrimp fed the basal diet to 86.1% for shrimp fed the diet containing 130 mg Cu/kg. Although not significant, there was a general linear decrease in survival associated with increasing dietary supplementation of copper ($P = 0.1017$). Estimated feed conversion (wet weight gain/dry weight feed offered) ranged from 2.0 for shrimp fed diets without copper supplements to 1.6 for shrimp fed diets containing 66 mg Cu/kg, and inversely corresponded to the growth data.

Heart CCO activity ranged from 5.99 units/g wet tissue for shrimp fed diets containing 6 mg Cu/kg to 8.01 units/g wet tissue for shrimp fed diets containing 66 mg Cu/kg. Hepatopancreas index ranged from 32.4 to 41.7 for shrimp fed diets containing 130 and 34 mg Cu/kg, respectively. There were no significant differences or general trends observed in the

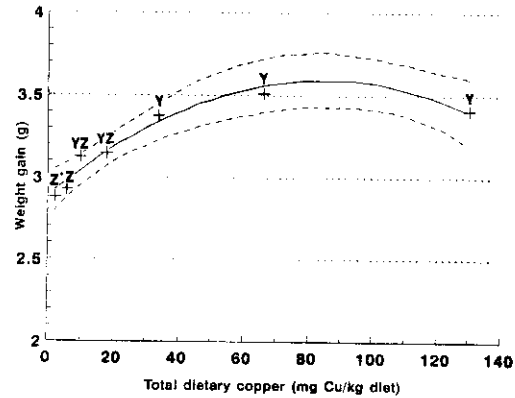


Fig. 1. Relationship [predicted value \pm 95% confidence interval for the expected value (mean) of the dependent variable] between dietary copper and the final weight gain of *P. vannamei*. The regression line is described by $y = 0.0167x - 0.000098x^2 + 2.89$ ($n = 42$; Adj. $R^2 = 0.4316$).

* Based on SNK test, treatment means (+) with the same letter are not significantly different.

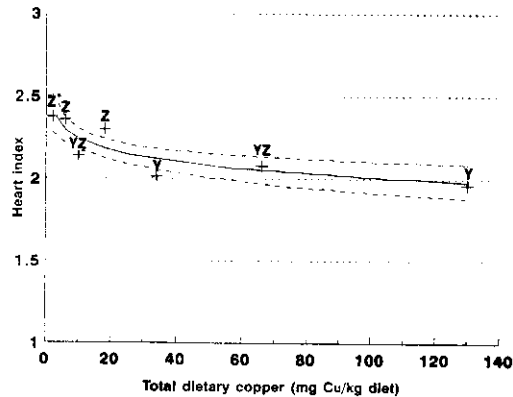


Fig. 2. Relationship [predicted value \pm 95% confidence interval for the expected value (mean) of the dependent variable] of the heart index [wet weight heart (mg)/wet weight shrimp (g)] to dietary copper.

The regression line is described by $y = -0.099(\log x) + 2.466$ ($n = 208$; Adj. $R^2 = 0.0831$).

* Based on SNK analyses treatment means (+) with the same letters are not significantly different.

heart CCO activity (ANOVA $P > F = 0.9976$) or hepatopancreas index (ANOVA $P > F = 0.3800$). Heart index ranged from 2.38 for shrimp fed the basal diet to 1.96 for shrimp fed the diet containing 130 mg Cu/kg. The heart index decreased logarithmically with dietary copper supplementation (Fig. 2).

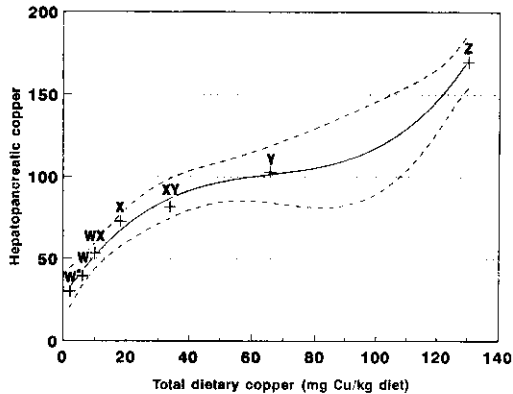


Fig. 3. Relationship [predicted value \pm 95% confidence interval for the expected value (mean) of the dependent variable] between dietary copper supplementation and hepatopancreatic copper ($\mu\text{g Cu/g}$).

The regression line is described by $y = 2.929x - 0.0406x^2 + 0.000205x^3 + 26.32$ ($n=42$; Adj. $R^2 = 0.8360$).

* Based on SNK test, treatment means (+) with the same letter are not significantly different.

Total SOD activity was measured in the presence of 0.01 mM KCN which was used to inhibit cytochrome oxidase.²²⁾ This method has been successfully employed with fish liver.¹⁰⁾ However, due to an interfering substance or substances (e.g. proteolytic enzymes), the baseline was not stable and hence the SOD levels could not be determined in shrimp hepatopancreas.

The copper content of the hepatopancreas ranged from 30.1 $\mu\text{g Cu/g}$ dry weight for shrimp fed the basal diet to 170.0 $\mu\text{g Cu/g}$ dry weight for shrimp fed the diet supplemented with 130 mg Cu/kg. The copper content of the hepatopancreas increased sigmoidally in relation to increasing dietary copper (Fig. 3).

Copper levels of the hemolymph ranged from 6.49 g Cu/dl for shrimp fed the basal diet to 14.38 g Cu/dl for shrimp fed the diet containing 130 mg Cu/kg. Copper content of the hemolymph increased with dietary copper content up to 34 mg Cu/kg and then levelled out (Fig. 4). Based on the spline model presented in Fig. 4, the supplementation of diets with more than 34 mg Cu/kg did not result in a significant increase in copper content of the hemolymph.

The copper content of the carapace from shrimp fed diets containing 2, 6, 10, and 18 mg Cu/kg were 13.64, 14.46, 14.44, and 15.79 $\mu\text{g Cu/g}$ dry weight, respectively. These values were sig-

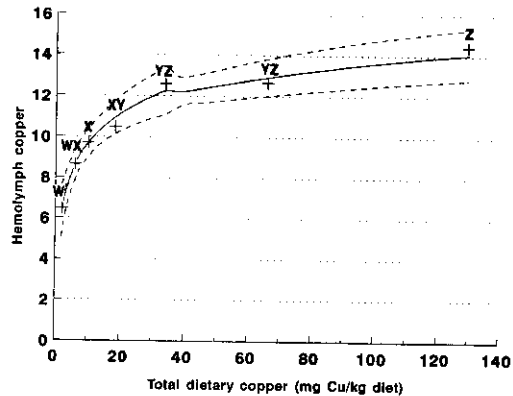


Fig. 4. Relationship [predicted value \pm 95% confidence interval for the expected value (mean) of the dependent variable] between dietary copper supplementation and hemolymph copper content (g Cu/dl).

The regression line is described by $y = 2.087(\log z) - 0.249(\log z) + 5.057$; if $x < 34$ then $z = 2$; if $x \geq 34$ then $z = x - 32$ ($n=42$; Adj. $R^2 = 0.6447$).

* Based on SNK test, treatment means (+) with the same letters are not significantly different.

nificantly lower than those of shrimp fed diets containing 34, 66, and 130 mg/kg, which were 22.94, 22.79, and 26.30 $\mu\text{g Cu/g}$ dry weight, respectively.

Discussion

A dietary essentiality of copper has been reported for several species of freshwater fish⁵⁻¹¹⁾ but has not been identified in marine fish or shrimp. It is clear that many marine organisms are obligate accumulators of certain heavy metals and that exposure to high environmental levels results in elevated body stores.^{1,4)} The essential nature of metals such as copper is acknowledged in some environmental monitoring studies.^{23,24)} However, it is often implicitly assumed that the total amount required for metabolic processes is negligible compared with total measured body concentrations.²⁵⁾

Djangmah and Grove²⁰⁾ demonstrated that the shrimp *Crangon vulgaris* can absorb copper from saltwater. Kanazawa *et al.*¹²⁾ was unable to demonstrate a dietary essentiality of copper for the shrimp *P. japonicus*, which had a final weight gain of 40%. They concluded that the shrimp may have been able to satisfy their physiological need through absorption from the water. Based on these observations it has generally been con-

cluded that shrimp may obtain adequate copper from seawater.

Under the reported experimental conditions, the lack of supplemental dietary copper resulted in a significant depression in growth of *P. vannamei*. Weight gain increased with dietary supplementation up to 32 mg Cu/kg diet (34 mg total Cu/kg), indicating that the basal diet was not adequate to meet the physiological copper requirement of the shrimp.

In the current experiment, the survival of the shrimp was slightly depressed at supplemental levels of 64 and 128 mg Cu/kg diet; however, there were no clear indications that dietary supplementation of up to 128 mg Cu/kg diet (130 mg total Cu/kg) adversely affected the growth or survival of the shrimp. Previous research with other crustaceans has shown that that excessive amount of dissolved copper may be lethal;²⁷⁾ however, the effects of excessive dietary supplementation had not been investigated with shrimp.

The hepatopancreas, carapace, and hemolymph of crustacea have been identified as containing a significant portion of the body burden of copper.^{1,4,25,28-30)} The hepatopancreas functions in the absorption, storage, metabolism, and detoxification of minerals;³¹⁾ thus, it should be a good indicator of the nutritional status of shrimp. In the current experiment, the copper content of the hepatopancreas increased with dietary copper up to 34 mg Cu/kg and then plateaued in shrimp fed diets containing 34 and 66 mg Cu/kg. This corresponded to the plateauing of weight gain (Fig. 1) and copper contents of the hemolymph (Fig. 4) and carapace. Plateauing of the copper content of these tissues would indicate that tissue levels were saturated once 32 mg supplemental Cu/kg diet (34 mg total Cu/kg) was fed, and that the copper content of these tissues appeared to be regulated. The additional increase in the copper content of the hepatopancreas in shrimp fed diets containing 130 mg Cu/kg may indicate that the hepatopancreas was acting as a site for copper storage and/or excretion.

In addition to depressed tissue mineralization, enlargement of the heart has been identified in several vertebrates as an indicator of copper deficiency.²⁾ In the current experiment shrimp fed diets containing low levels of copper had larger hearts (Fig. 2). This morphological alteration appeared to be alleviated through adequate copper supplementation.

Under the reported conditions, a dietary deficiency of copper was observed for *P. vannamei* fed diets supplemented with less than 32 mg Cu/kg diet (34 mg total Cu/kg diet). The dietary copper deficiency in *P. vannamei* was characterized by producing enlargement of the heart, lower growth, and depressed copper contents of the carapace, hepatopancreas, and hemolymph. Based on the observed results, nominal values for the hepatopancreas, carapace, and hemolymph of the shrimp maintained under the reported conditions were 75–118 μg Cu/g, 17–27 μg Cu/g, and 11–15 g Cu/dl. These results indicate that juvenile *P. vannamei* cannot meet their physiological needs for copper from seawater and that a dietary source is required for maximum growth and tissue mineralization.

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References

- 1) G. W. Bryan: Heavy metal contamination in the sea, in "Marine Pollution" (ed. by R. Johnston), Academic Press, New York, New York, USA, 1976, pp. 185–302.
- 2) B. L. O'Dell: Biochemistry of copper. Symposium on trace elements. *Med. Clin. North Am.*, 60, 697–703 (1976).
- 3) C. P. Mangum: Oxygen transport in the blood, in "The Biology of Crustacea" (ed. by D. E. Bliss), Vol. 5, Internal Anatomy and Physiological Regulation, Academic Press, New York, New York, USA, 1983, pp. 373–429.
- 4) M. H. Depledge: Re-evaluation of metabolic requirements for copper and zinc in decapod crustaceans. *Mar. Environ. Res.*, 27, 115–126 (1989).
- 5) C. Ogino and G.-Y. Yang: Requirement of carp and rainbow trout for dietary manganese and copper. *Nippon Suisan Gakkaishi*, 46, 455–458 (1980).
- 6) T. Murai, J. W. Andrews, and R. G. Smith, Jr.: Effects of dietary copper on channel catfish. *Aquaculture*, 22, 353–357 (1981).
- 7) S. Satoh, H. Yamamoto, T. Takeuchi, and T. Watanabe: Effects on growth and mineral composition of rainbow trout of deletion of trace elements or magnesium from fish meal diet. *Nippon Suisan Gakkaishi*, 49, 425–429 (1983).
- 8) S. Satoh, H. Yamamoto, T. Takeuchi, and T. Watanabe: Effects on growth and mineral composition of carp of deletion of trace elements or magnesium from fish meal diet. *Nippon Suisan Gakkaishi*, 49, 431–435 (1983).
- 9) S. Satoh, H. Yamamoto, T. Takeuchi, and T. Watanabe: Effects of deletion of several trace elements from fish meal diets on growth and mineral composition of rainbow trout fingerlings. *Nippon Suisan Gakkaishi*, 49, 1909–1916 (1983).
- 10) D. M. Gatlin III and R. P. Wilson: Dietary copper requirement of fingerling channel catfish. *Aquaculture*, 54, 277–

- 285 (1986).
- 11) K. Julshamn, K. O-J, Andersen, O. Ringdal, and J. Brenna: Effect of dietary copper on the hepatic concentration and subcellular distribution of copper and zinc in the rainbow trout (*Salmo gairdneri*). *Aquaculture*, **73**, 143-155 (1988).
 - 12) A. Kanazawa, S. Teshima, and M. Sasaki: Requirements of the juvenile prawn for calcium, phosphorus, magnesium, potassium, copper, manganese, and iron. *Mem. Fac. Fish., Kagoshima Univ.*, **33**, 63-71 (1984).
 - 13) S. Spotte: Fish and Invertebrate Culture, in "Water Management in Closed Systems", 2nd ed., Wiley, New York, New York, USA, 1979.
 - 14) D. C. Warton and A. Tzagoloff: Cytochrome oxidase from beef heart mitochondria, in "Methods in Enzymology" (ed. by R. W. Estabrook and M. E. Pullman), Vol. 10, Academic Press, New York, New York, USA, 1967, pp. 245-250.
 - 15) J. M. McCord and I. Fridovich: Superoxide dismutase. An enzymatic function for erythrocuprein (hemocuprein). *J. Biol. Chem.*, **244**, 6049-6055 (1969).
 - 16) Association of Official Analytical Chemists: Official Methods of Analysis. Association of Official Analytical Chemists, Arlington, Virginia, USA, 1984.
 - 17) R. G. D. Steel and J. H. Torrie: Principles and Procedures of Statistics: A Biometrics Approach. McGraw-Hill, New York, New York, USA, 1980.
 - 18) SAS Institute Inc., SAS/STAT User's Guide, Release 6.03 Edition, Cary, North Carolina, USA, 1988.
 - 19) J. F. Wickins: The tolerance of warm-water prawns to recirculated water. *Aquaculture*, **9**, 19-37 (1976).
 - 20) T.-S. Chin and J.-C. Chen: Acute toxicity of ammonia to larvae of the tiger prawn, *Penaeus monodon*. *Aquaculture*, **66**, 247-253 (1987).
 - 21) J.-C. Chen and T.-S. Chin: Acute toxicity of nitrite to tiger prawn, *Penaeus monodon*, larvae. *Aquaculture*, **69**, 253-262 (1988).
 - 22) M. L. Salin and S. M. Bridges: Absence of the iron-containing superoxide dismutase in mitochondria from mustard (*Brassica campestris*). *J. Biochem.*, **195**, 229-233 (1981).
 - 23) G. Walker, P. S. Rainbow, P. Foster, and D. J. Crisp: Barnacles: possible indicators of zinc pollution? *Mar. Biol.*, **30**, 57-65 (1975).
 - 24) D. J. H. Phillips: The use of biological indicator organisms to monitor trace metal pollution in marine and estuarine environments-a review. *Envir. Pollut.*, **13**, 281-317 (1977).
 - 25) S. L. White and P. S. Rainbow: On the metabolic requirements for copper and zinc in molluscs and crustaceans. *Mar. Environ. Res.*, **16**, 215-229 (1985).
 - 26) J. S. Djangmah and D. J. Grove: Blood and hepatopancreas copper in *Crangon vulgaris* (Fabricius). *Comp. Biochem. Physiol.*, **32**, 733-745 (1970).
 - 27) G. W. Bryan: Concentration of zinc and copper in the tissues of Decapod crustaceans. *J. Mar. Biol. Ass. U. K.*, **48**, 303-321 (1968).
 - 28) W. Johnston and A. A. Barber: Reconstitution of functional hemocyanin from apohemocyanin: the hepatopancreas as copper donor. *Comp. Biochem. Physiol.*, **28**, 1259-1273 (1969).
 - 29) K. L. Shewbart, W. L. Mier, and P. D. Ludwig: Nutritional requirements of brown shrimp, *Penaeus aztecus*. Department of Marine Resources Information, Center for Marine Resources, Texas A & M Univ., College St., Texas, USA, 1973.
 - 30) J. D. Icely and J. A. Nott: Accumulation of copper within the "hepatopancreatic," caeca of *Corophium volutator* (Crustacea: Amphipoda). *Mar. Biol.*, **57**, 193-199 (1980).
 - 31) W. Dall and D. J. W. Moriarty: Functional aspects of nutrition and digestion, in "The Biology of Crustacea" (ed. by D. E. Bliss), Vol. 5, Academic Press, New York, New York, USA, 1983, pp. 215-261.