

Evaluation of the Dietary Iron Requirement of *Penaeus vannamei*

D. ALLEN DAVIS¹ AND ADDISON L. LAWRENCE

Texas Agricultural Experiment Station, Texas A&M University System, P.O. Drawer Q,
Port Aransas, Texas 78373 USA

DELBERT M. GATLIN III

Department of Wildlife and Fisheries Sciences, Texas A&M University System,
College Station, Texas 77843 USA

Abstract

Two experiments were conducted to evaluate the dietary iron requirement of juvenile *Penaeus vannamei*. Prior to initiation of 28 day (Experiment I) and 35 day (Experiment II) feeding trials, 17-20-day-old postlarvae were fed the basal diet lacking iron supplementation but containing 12 mg Fe/kg for seven days. After conditioning, juvenile shrimp (mean weight: 0.038 g Experiment I, 0.047 g Experiment II) were fed one of four diets supplemented with 0, 20, 40 or 80 mg Fe/kg from ferrous sulfate heptahydrate. Final weight gain of shrimp ranged from 1.03 to 1.28 g in Experiment I and 1.62 to 1.94 g in Experiment II. Although percent weight gain was in excess of 2700% in Experiment I and 3400% in Experiment II, there were no significant differences in growth or survival of shrimp fed the different levels of iron. Iron levels of the hepatopancreas and carapace increased linearly with dietary iron; however, there were no significant differences in the iron content of muscle tissue. Based on these results, the iron content of the basal diet appeared to satisfy physiological needs of the shrimp. Therefore, practical diets containing protein sources of animal origin which are rich in iron should not require iron supplementation.

Iron is a trace element that is a component of cytochromes, catalases and peroxidases of all aerobic cells, and is essential for the production and normal functioning of hemoglobin and myoglobin (Prosser 1973). Iron deficiencies have been documented for several species of marine and freshwater fish (National Research Council 1983). Hypochromic microcytic anemia without any influence on growth has been reported for brook trout *Salvelinus fontinalis* (Kawatsu 1972), yellowtail *Seriola quinqueradiata* (Ikeda et al. 1973), red sea bream *Chrysophrys major* (Sakamoto and Yone 1976, 1978a), and carp *Cyprinus carpio* (Sakamoto and Yone 1978b) fed diets without iron supplementation. Gatlin and Wilson (1986) found that a minimum dietary supplement of 20 mg Fe/kg diet (30 mg total

Fe/kg) was required by channel catfish *Ictalurus punctatus* for best growth and hematological values.

Decapod crustaceans possess the copper-based respiratory pigment, hemocyanin, in their hemolymph. The essential role played by copper in the metabolism of decapods may explain the apparent lack of interest in studying the essentiality of other metallic constituents (Martin 1973). Although dietary essentiality of iron has not been reported for shrimp, supplementation of over 70 mg Fe/kg had an adverse effect on the growth of juvenile *Penaeus japonicus* (Deshimaru and Yone 1978; Kanazawa et al. 1984).

In addition to iron's potential toxicity, it is one of the major destabilizing agents in feeds. For example, due to the prooxidant nature of iron, it is one of the primary metals involved in lipid oxidation in feeds and feedstuffs with ferrous iron acting as a more potent catalyst of lipid peroxidation than ferric iron (Chvapil et al. 1974; Lee et al.

¹ Corresponding author's present address: The University of Texas at Austin, Marine Science Institute, P.O. Box 1267, Port Aransas, TX 78373 USA.

1981; Desjardins et al. 1987). Iron has also been found to decrease the stability of ascorbic acid (Hilton 1989). To meet the physiological needs of shrimp, but to avoid the adverse effects of oversupplementation, this study was designed to evaluate the dietary requirement for iron by *Penaeus vannamei*.

Methods

Two experiments were conducted to evaluate the dietary requirement of iron by *P. vannamei*. The basal diet (Table 1) was formulated to contain 40% protein and gross energy of 3.5 kcal/g. The basal diet had an intrinsic iron level of 12 mg Fe/kg and was supplemented with four levels of iron (0, 20, 40 and 80 mg Fe/kg) by replacing cellulose with ferrous sulfate heptahydrate. Diets were prepared by mixing dry ingredients in a V-mixer. Menhaden oil and an appropriate amount of deionized water (required for pelleting) were added to the dry ingredients and homogenized. Each diet was then pressure pelleted using a meat grinder with a 3 mm die. After pelleting, diets were dried by forced air at 60 C for 3 h followed by forced ambient air for 12 h to a moisture content of 8 to 10%. Portions, as needed for feeding, were mechanically crumbled and sieved to the desired size and frozen at -10 C until fed.

The feeding trial was conducted in 19 L culture chambers (bottom surface area of 0.06 m²) which are part of a 50 metric ton recirculating system which received a 5% water exchange daily and was designed to maintain a constant environmental temperature (28 ± 1 C) and salinity (30 ± 1 ppt). Iron levels of the seawater at the termination of Experiment II were below readable levels (<1 ppm) for a seawater sample being read on the atomic absorption spectrophotometer. Due to the large volume of water in the system (50 mt) and low biomass (i.e., low feed input), minerals leaching from feeds into the water would have a negligible effect on the iron content of the water; additionally, any effect would have been equal

TABLE 1. Composition of basal diet.^a

Ingredient	% Dry weight
Casein ^b	35.0
Gelatin ^b	8.0
Wheat starch ^b	20.6
Menhaden fish oil ^c	7.0
Lecithin (purified) ^b	1.0
Cholesterol ^b	0.5
Mineral mixture ^d	15.0
Vitamin mixture ^e	8.0
Stay-C ^f (3,000 mg active vitamin C/kg)	2.9
Alpha-cellulose ^g	2.0

^a Basal diet contained 12 mg Fe/kg diet as determined by atomic absorption spectrophotometry.

^b I.C.N. Nutritional Biochemicals Inc., Cleveland, Ohio, USA.

^c Zapata Haynie Corp., Reedville, Virginia, USA. Supplemented with 125 ppm Santoquin.

^d Contains (as g/kg): CaHPO₄, 500.0; NaCl, 74.0; K₃C₆H₅O₇·H₂O, 220.0; K₂SO₄, 52.0; MgO, 24.0; MnSO₄·H₂O, 5.16; CuSO₄·5H₂O, 0.68; ZnSO₄·7H₂O, 3.67; KIO₃, 0.01; Na₂SeO₄, 0.0072; KCr(SO₄)₂·12H₂O, 0.55; cellulose, 100.47.

^e Contains (as g/kg): Vitamin A palmitate, 1.8; Vitamin E (250 μg), 22.0; Inositol, 180; Choline chloride, 75; Menadione, 2.3; PABA, 30; Niacin, 26; Riboflavin, 8; Pyridoxine HCl, 3; Thiamine mononitrate, 5; D calcium pantothenate, 15; Vitamin D₃ (40,000 μg), 1; Biotin, 1; Folic acid, 5; Vitamin B₁₂ crystalline, 1; sucrose, 623.9.

^f Vitamin Technologies International, Buhl, Idaho, USA.

^g Sigma Chemical Company, Cleveland, Ohio, USA, replaced with ferrous sulfate heptahydrate to provide 0, 20, 40 and 80 mg Fe/kg diet.

for all treatments. The photoperiod was set for a 12:12 h light:dark 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 (Spotte 1979).

One week prior to initiation of the experiments, 17-20-day-old postlarval *P. vannamei* having a mean weight of 0.0026 g (Experiment I) and 0.0032 g (Experiment II) were fed the basal diet lacking iron supplementation. At the start of the growth trial, conditioned juvenile shrimp were hand

graded to a uniform size and stocked at a density of 10 shrimp/chamber. To estimate the initial weight of the shrimp, four groups of shrimp (10 shrimp/group) were randomly selected during stocking and set aside. These shrimp were then towel-dried and weighed individually to determine the mean initial weight \pm standard deviation (Experiment I, 0.038 ± 0.018 g; Experiment II, 0.047 ± 0.016 g).

Each dietary treatment was fed to eight replicate groups of shrimp in 28 day (Experiment I) and 35 day (Experiment II) feeding trials. Shrimp were fed in excess twelve times a day for the duration of the experiments. At the midpoint of each experimental period, the density of shrimp was reduced to six shrimp per chamber. At the conclusion of the experimental period, all shrimp were weighed and immediately frozen for subsequent mineral analyses.

Frozen shrimp were rinsed with deionized water and then the carapace and hepatopancreas dissected. In Experiment II, muscle tissue from the first segment of the tail was also dissected. Tissue samples from shrimp in each experiment were pooled into four composite samples per dietary treatment, oven-dried, and wet-ashed according to procedures described by the Association of Official Analytical Chemists (1984). Iron levels were determined by atomic absorption spectrophotometry (Association of Official Analytical Chemists 1984). Data were analyzed using one-way analysis of variance to determine significant ($P < 0.05$) differences among treatment means. Data for which there were significant differences among treatment means were subjected to regression analysis to determine effects of the dietary treatments on the dependent variables. Statistical analyses were conducted using the Statistical Analysis System (SAS Institute, Inc. 1988).

Results

Weekly ammonia, nitrite and dissolved oxygen levels (mean \pm standard deviation) were 0.13 ± 0.06 mg N/L, 0.11 ± 0.12 mg

N/L and 6.43 ± 0.24 mg O₂/L, respectively, in Experiment I and 0.08 ± 0.03 mg N/L, 0.13 ± 0.01 mg N/L, and 6.40 ± 0.14 mg O₂/L, respectively, in Experiment II. Based on established values for Penaeid shrimp (Wickins 1976; Chin and Chen 1987; Chen and Chin 1988), the water quality observed in these experiments should not have adversely affected growth and survival of the shrimp.

Iron supplementation of the diet did not have a significant effect on weight gain or survival of the shrimp (Table 2). In both experiments, survival during the first half of the experiment (65–87%) was considerably lower than that observed during the second half of the experiment (90–100%). This poor initial survival may have been due to handling stress during stocking, or due to stress associated with the molting frequency of the smaller shrimp. The overall survival observed during these experiments were within the normal range seen within this laboratory for shrimp maintained on a casein/gelatin-based semi-purified diet.

The relationship of dietary iron supplementation to the iron content of hepatopancreas from shrimp is represented in Figs. 1 (Experiment I) and 2 (Experiment II). In both experiments, the iron levels of the hepatopancreas responded linearly with dietary iron supplementation.

In Experiment I, iron level of the carapace was positively correlated with iron supplementation (Fig. 3). In Experiment II, however, iron levels of the carapace ranged from 29.4 to 36.6 μ g Fe/g dry tissue for shrimp fed diets supplemented with 0 and 20 mg Fe/kg, respectively, and there was no significant correlation among dietary iron supplementation and iron levels of the carapace.

Iron levels of muscle tissue from shrimp fed diets containing various levels of iron ranged from 12.1 μ g Fe/g dry tissue for shrimp fed diets supplemented with 20 mg Fe/kg to 17.9 μ g Fe/g for shrimp fed diets supplemented with 40 mg Fe/kg, with no

TABLE 2. Percent survival and weight gain of juvenile *P. vannamei* fed diets containing various levels of iron.^a

Supplemental Iron (mg/kg)	Experiment I			Experiment II		
	Percent Survival		Weight Gain (g) (Percentage)	Percent Survival		Weight Gain (g) (Percentage)
	Day 0-15	Day 16-28		Day 0-16	Day 17-35	
0	71.3	95.0	1.08 (2,864.9)	85.0	100.0	1.71 (3,634.0)
20	65.0	100.0	1.28 (3,409.2)	87.5	95.8	1.80 (3,820.2)
40	66.3	95.0	1.03 (2,776.8)	73.8	95.8	1.62 (3,439.9)
80	72.5	90.0	1.08 (2,877.9)	78.8	93.7	1.94 (4,134.0)
ANOVA ($P > F$)	0.6439	0.2120	0.5084	0.1135	0.1026	0.2476
Pooled S.E.	4.52	3.31	0.080	3.89	2.40	0.126

^a Means of eight replicates.

significant differences observed among the treatment means.

Discussion

Nutritional deficiencies due to inadequate dietary iron have not been reported in Penaeid shrimp; however, Kanazawa et

al. (1984) found that diets supplemented with 0 and 70 mg Fe/kg produced better growth of *P. japonicus* than diets supplemented with 140 or 270 mg Fe/kg, suggesting an iron toxicity. There was no indication that iron supplementation of the basal diet at levels as high as 80 mg Fe/kg

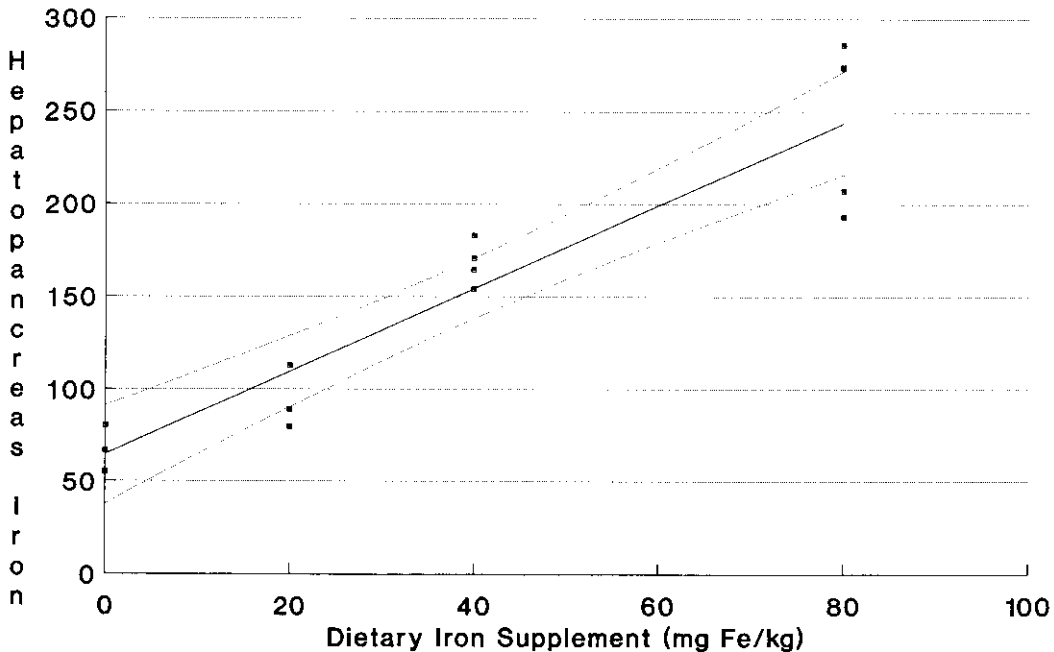


FIGURE 1. Relationship [predicted value \pm 95% confidence interval for the expected value (mean) of the dependent variable] between dietary iron supplementation and hepatopancreas iron ($\mu\text{g Fe/g dry weight}$) in Experiment I. The regression line is described by $Y = 2.24X + 64.5$ ($N = 14$; Adjusted $R^2 = 0.8577$).

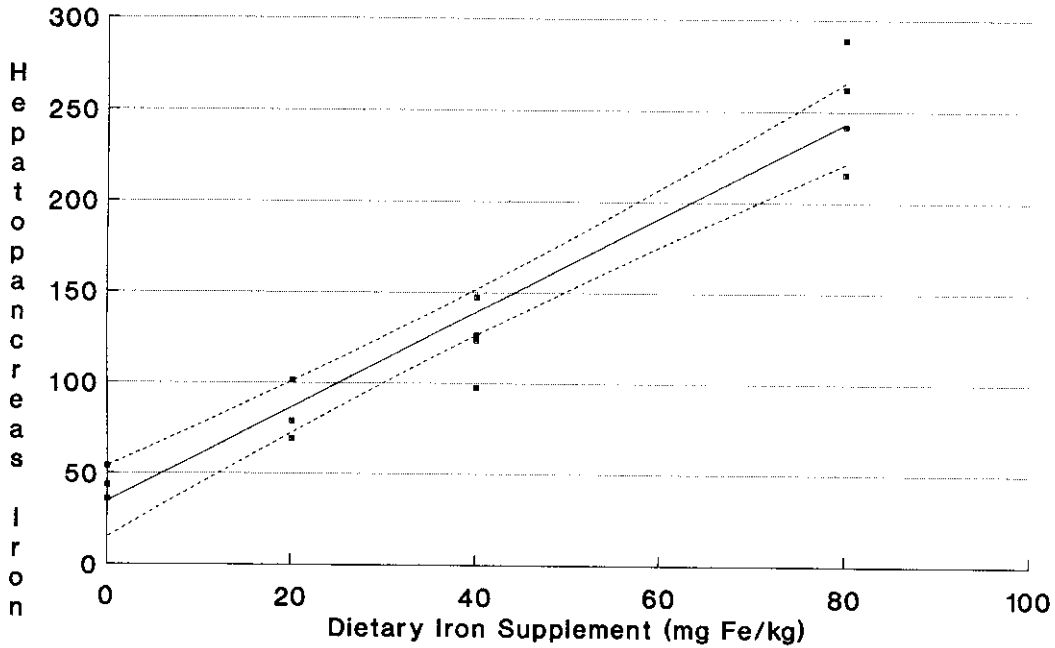


FIGURE 2. Relationship [predicted value \pm 95% confidence interval for the expected value (mean) of the dependent variable] between dietary iron supplementation and hepatopancreas iron ($\mu\text{g Fe/g dry weight}$) in Experiment II. The regression line is described by $Y = 2.61X + 34.6$ ($N = 15$; Adjusted $R^2 = 0.9298$).

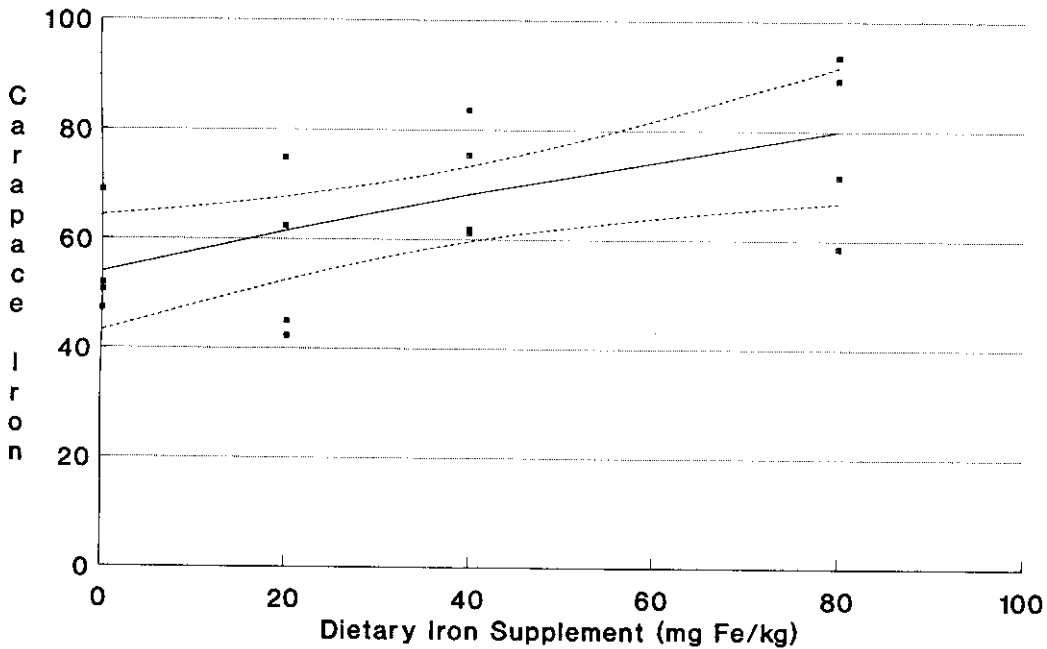


FIGURE 3. Relationship [predicted value \pm 95% confidence interval for the expected value (mean) of the dependent variable] between dietary iron supplementation and carapace iron ($\mu\text{g Fe/g dry weight}$) in Experiment I. The regression line is described by $Y = 0.32X + 53.75$ ($N = 16$; Adjusted $R^2 = 0.3417$).

had adverse effects on growth or survival of the shrimp in the present study.

Vertebrates minimize toxic effects of free iron ions by regulating transport via transferrin. Regulation of iron absorption is controlled by intestinal ferritin stores which are reduced during periods of low iron intake and increased during periods of high iron intake (Ganong 1985). Iron transport proteins with properties similar to transferrin have been identified in the hemolymph of the crabs *Macropipus puber* (Linne) (Ghidalia et al. 1972), *Cancer magister* (Huebers et al. 1982), and *Scylla serrata* (Depledge et al. 1986). In addition to functioning as a site for the excretion of digestive enzymes, absorption of nutrients and storage of metabolic reserves, the hepatopancreas functions in the absorption, detoxification and excretion of minerals (Dall and Moriarty 1983) and has been found to be the organ richest in iron stores (Martin 1973; Depledge et al. 1986). Iron is also found in the exoskeleton, hemolymph, gills and muscle tissues (Ghidalia et al. 1972; Martin 1973; Icely and Nott 1980; Brown 1982; Depledge et al. 1986).

In the present experiments, iron levels of the hepatopancreas increased linearly with dietary supplementation. If tissue stores were not satisfied, a disproportionate increase (i.e., sigmoidal, asymptotic or broken-line response) would be expected. Since the response was linear it may be concluded that under the described conditions, physiological iron needs were satisfied by the basal diet water and the hepatopancreas was serving as an excretory/storage organ. This conclusion is further supported by the minimal or lack of iron deposition in the carapace and muscle tissue in response to iron supplementation.

In the crab *Scylla serrata* the shell has been found to contain 50% of the body iron (Depledge et al. 1986). Injection of radioactive iron into hemolymph of the crab *Cancer magister* demonstrated that highest rates of uptake occurred in the hepatopancreas and carapace lining (Huebers et al.

1982), confirming that these tissues are active in iron metabolism. The minimal increase in carapace iron deposition in response to increased dietary iron supplementation in the present study would indicate that the hepatopancreas was functioning to regulate the transfer of dietary iron into the circulatory system.

Iron uptake and utilization in species that have copper-based hemocyanin as a respiratory pigment are influenced principally by environmental availability and general cellular demands rather than specific requirements for hemoglobin synthesis (Depledge et al. 1986). Since hemoglobin constitutes the bulk of body iron and because of the very high requirements of red cell forming tissues, vertebrates would be expected to have a higher iron requirement than invertebrates utilizing a copper-based respiratory pigment (Huebers et al. 1982). In addition to a potentially lower iron requirement, iron may be absorbed from the water. Although seawater typically contains a low concentration of iron, Martin (1973) found the ratio of iron concentration in the gills to that of seawater (0.018 mg/L) indicated a strong absorptive ability of this organ. Consequently, the physiological demand for iron may be met from the water. Thus, the lack of evidence for dietary essentiality of iron is not surprising.

Conclusion

Under the experimental conditions, there were no clear deficiency signs observed in *P. vannamei* fed a basal diet containing 12 mg Fe/kg diet and no apparent toxicity for iron supplementation of up to 80 mg/kg diet. Hepatopancreas iron levels increased linearly with dietary iron supplementation, while carapace levels responded minimally to dietary iron supplementation. Based on the results of the current study, it appears that the dietary iron requirement of *P. vannamei* is lower than that of fish.

Although the availability of iron from various feed sources has not been determined for shrimp, current formulations

containing animal protein sources such as fish meal should supply enough iron to meet the dietary requirement of shrimp. Since previous research has demonstrated that excess supplementation of iron may inhibit the growth of shrimp and could adversely affect feed stability through iron-catalyzed oxidation, iron supplementation of practical diets does not appear warranted.

Acknowledgments

The authors would like to express their thanks to F. Castille, R. Sis and W. Neill for their critical reviews of the manuscript. We would also like to thank K. Hall for her technical assistance. This research was supported by the Texas Agricultural Experiment Station under project H-6325.

Literature Cited

- Association of Official Analytical Chemists.** 1984. Official methods of analysis. Association of Official Analytical Chemists, Arlington, Virginia, USA.
- Brown, B. E.** 1982. The formation and function of metal-containing 'granules' in invertebrate tissues. *Biological Review* 57:621-667.
- Chen, J.-C. and T.-S. Chin.** 1988. Acute toxicity of nitrite to tiger prawn *Penaeus monodon* larvae. *Aquaculture* 69:253-262.
- Chin, T.-S. and J.-C. Chen.** 1987. Acute toxicity of ammonia to larvae of the tiger prawn *Penaeus monodon*. *Aquaculture* 66:247-253.
- Chvapil, M., A. L. Aronson and Y. M. Peng.** 1974. Relation between zinc and iron peroxidation of lipids in liver homogenate in Cu EDTA-treated rats. *Experimental Molecular Pathology* 20:216-227.
- Dall, W. and D. J. W. Moriarty.** 1983. Functional aspects of nutrition and digestion. Pages 215-261 in D. E. Bliss, editor-in-chief. *The biology of crustacea*, volume 5. Internal anatomy and physiological regulation. Academic Press, New York, New York, USA.
- Depledge, M. H., R. Chan and T. T. Loh.** 1986. Iron distribution and transport in *Scylla serrata* (Forsk.). *Asian Marine Biology* 3:101-110.
- Deshimaru, O. and Y. Yone.** 1978. Requirement of prawn for dietary minerals. *Bulletin of the Japanese Society of Scientific Fisheries* 44:907-910.
- Desjardins, L. M., B. D. Hicks and J. W. Hilton.** 1987. Iron catalyzed oxidation of trout diets and its effect on the growth and physiological response of rainbow trout. *Fish Physiology and Biochemistry* 3:173-182.
- Ganong, W. F.** 1985. Review of medical physiology, 12th edition. Lange Medical Publications, Los Altos, California, USA.
- Gatlin, D. M., III and R. P. Wilson.** 1986. Characterization of iron deficiency and the dietary iron requirement of fingerling channel catfish. *Aquaculture* 52:191-198.
- Ghidalia, W., J. M. Fine and M. Marneux.** 1972. On the presence of an iron-binding protein in the serum of a decapod crustacean [*Macropipus puber* (Linne)]. *Comparative Biochemistry and Physiology* 41B:349-354.
- Hilton, J. W.** 1989. The interaction of vitamins, minerals and diet composition in the diet of fish. *Aquaculture* 79:223-244.
- Huebers, H. A., E. Huebers, C. A. Finch and A. W. Martin.** 1982. Characterization of an invertebrate transferrin from the crab *Cancer magister* (Arthropoda). *Journal of Comparative Physiology* 148B:101-109.
- Icely, J. D. and J. A. Nott.** 1980. Accumulation of copper within the "hepatopancreatic" caeca of *Corophium volutator* (Crustacea: Amphipoda). *Marine Biology* 57:193-199.
- Ikeda, Y., H. Ozaki and K. Uematasu.** 1973. Effect of enriched diet with iron in culture of yellowtail. *Journal of Tokyo University of Fisheries* 59:91-99.
- Kanazawa, A., S. Teshima and M. Sasaki.** 1984. Requirements of the juvenile prawn for calcium, phosphorus, magnesium, potassium, copper, manganese and iron. *Memoirs of the Faculty of Fisheries, Kagoshima University* 33:63-71.
- Kawatsu, H.** 1972. Studies on the anemia of fish. V. Dietary iron deficient anemia in brook trout, *Salvelinus fontinalis*. *Bulletin of Freshwater Fisheries Research Laboratory* 25:59-67.
- Lee, Y. H., D. K. Laymann, R. R. Bell and H. W. Norton.** 1981. Responses of glutathione peroxidase and catalase to excess iron in rats. *Journal of Nutrition* 111:2195-2202.
- Martin, J.-L. M.** 1973. Iron metabolism in *Cancer irroratus* (crustacea decapoda) during the intermoult cycle, with special reference to iron in the gills. *Comparative Biochemistry and Physiology* 46A:123-129.
- National Research Council.** 1983. Nutrient requirements of warmwater fishes and shellfishes. National Academy Press, Washington, D.C., USA.
- Prosser, C. L.** 1973. *Comparative animal physiology*, 3rd edition. W. B. Saunders Co., Philadelphia, Pennsylvania, USA.
- Sakamoto, S. and Y. Yone.** 1976. Requirement of red sea bream for dietary Fe-I. Report of Fishery Research Laboratory, Kyushu University 3:53-58.
- Sakamoto, S. and Y. Yone.** 1978a. Requirement of red sea bream for dietary iron—II. *Bulletin of the Japanese Society of Scientific Fisheries* 44:223-225.

- Sakamoto, S. and Y. Yone.** 1978b. Iron deficiency symptoms of carp. *Bulletin of the Japanese Society of Scientific Fisheries* 44:1157-1160.
- SAS Institute, Inc.** 1988. SAS/STAT user's guide, 6.03 edition. SAS Institute, Inc., Cary, North Carolina, USA.
- Spotte, S.** 1979. *Fish and invertebrate culture: water management in closed systems*, 2nd edition. Wiley, New York, New York, USA.
- Wickins, J. F.** 1976. The tolerance of warm-water prawns to recirculated water. *Aquaculture* 9:19-37.