

## **The Pathology of Cadmium and Nickel Toxicity in the Banana Shrimp (*Penaeus merguensis* de Man)**

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### **Abstract**

The pathology of the banana shrimp (*Penaeus merguensis* de Man) exposed to 0.0, 0.1, 0.3 and 0.5 mg/l cadmium and 0.0, 0.4, 1.2 and 2.0 mg/l nickel for thirty days is described. Cellular hyperplasia followed by degeneration and necrosis was evident in the gills of shrimp exposed to each metal, although such changes were more pronounced with cadmium. Degeneration, necrosis and nuclear inclusion bodies possibly of viral origin were also present but only in the hepatopancreas of shrimp exposed to cadmium. Erosion and necrosis with inflammation were found in appendages of shrimp exposed to a cadmium concentration of 0.5 mg/l. Each metal caused similar signs though gill damage was more apparent in the cadmium group.

### **Introduction**

Above certain concentrations, all heavy metals adversely affect aquatic organisms. Their effect varies between species, depending upon the permeability of the organisms and their detoxification mechanisms. The pathological changes caused by cadmium toxicity in

some species of shrimp have been reported mainly in the gills (Nimmo et al. 1977; Couch 1977; Papathanassiou 1983; Papathanassiou and King 1983).

The investigations described herein examined the pathological changes occurring in banana shrimp after exposure to subacute concentrations of cadmium and nickel. These metals were chosen for study because they exhibit different toxicity patterns and because of their potential industrial significance as pollutants in the related area as well as in other parts of the world. The pattern of metal uptake will be described separately.

### Materials and Methods

Juvenile banana shrimp were captured at low tide from Three Mile Creek and the Bohle River estuary near Townsville (19° 13'S; 146° 30'E). Those selected for testing ranged in body length (post-orbital margin to telson tip) from 45 to 70 mm and weighed 1.0-2.5 g.

All tests were conducted in high density polyethylene tanks containing 50 l of seawater. The tanks were artificially aerated to maintain oxygen levels above 90% saturation at all times. The temperature and salinity adopted for testing were  $25 \pm 1^\circ\text{C}$  and  $36 \pm 1$  ppt, respectively. A similar experiment was carried out to monitor uptake of the metals.

Stock solutions (100 g/l) of cadmium and nickel were prepared by dissolving the analytical grade salts of either cadmium chloride ( $\text{CdCl}_2 \cdot 2\text{H}_2\text{O}$ ) or nickel chloride ( $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ ) in glass-distilled water. These were further diluted with distilled water as required and checked against standards using atomic absorption spectroscopy. The test concentrations were 0.1, 0.3 and 0.5 mg Cd/l and 0.4, 1.2 and 2.0 mg Ni/l and were selected on the basis of acute toxicity data previously reported (Denton and Burdon-Jones 1982).

Forty shrimp were placed at random in each of the test tanks and were allowed to acclimate to the experimental conditions for five days prior to treatment. The required toxicant concentrations were pipetted into each tank and were rapidly dispersed by water currents generated by the aerators. The tests were continued for a period of 30 days with 10 prawns being removed from each tank for analysis after 0, 5, 15 and 30 days exposure.

The shrimp were fed throughout the investigation with finely chopped, blanched squid. The test water was changed every three

days by slowly siphoning the water from each tank simultaneously to within 2-3 cm of the bottom and refilling with saline containing the same dose of metal. Water was collected from each tank for metal analysis immediately prior to changing, and was consistently found to be within 95% of the required concentrations for each metal.

Seven out of ten shrimp per group were selected at intervals and immediately fixed in 10% buffered formalin. Each shrimp was partially cut transversely into three parts to allow penetration of fixative and kept at 4-5°C for about three days. Paraffin blocks of tissue were sectioned at 7  $\mu$ m thickness and stained mainly with hematoxylin and eosin.

## Results

After five days exposure to all cadmium levels the shrimp appeared to be in normal condition, but thereafter those in medium and high concentration tanks (0.3 and 0.5 mg/l Cd) lost their appetite, and much food was still uneaten one to two hours after feeding. After six days at high concentration, six prawns (24%) died, and after day ten some surviving animals showed abnormal swimming movements in a vertical position followed by weakness. They later lay at the bottom of the tanks with hyperactive movements of the extremities. More than 50% of the animals showed erosion and necrosis of the telson, rostrum and dorsal cuticle on day 15 and about 60% of surviving animals showed blackening of their gills. The cumulative survival rate of animals during this experiment up to day 30 can be seen in Fig. 1A. All surviving animals exposed to the medium and high concentration were killed for histological examination before day 30.

As in the cadmium toxicity studies, after five days exposure to nickel the shrimp appeared normal, but animals in medium and high concentration tanks (1.2 and 2.0 mg/l Ni) later lost their appetite and some died between day 5-15. Before death the shrimp showed weakness, poor swimming ability and they lay at the bottom of the tank with hyperactive movement of the extremities. After day 15, however, only two shrimp were found to have blackened gills.

The cumulative survival rate of shrimp during this treatment up to day 30 can be seen in Fig. 1B. As in the cadmium experiment all surviving animals at concentrations of 1.2 and 2.0 mg/l Ni were killed for histological examination before day 30.

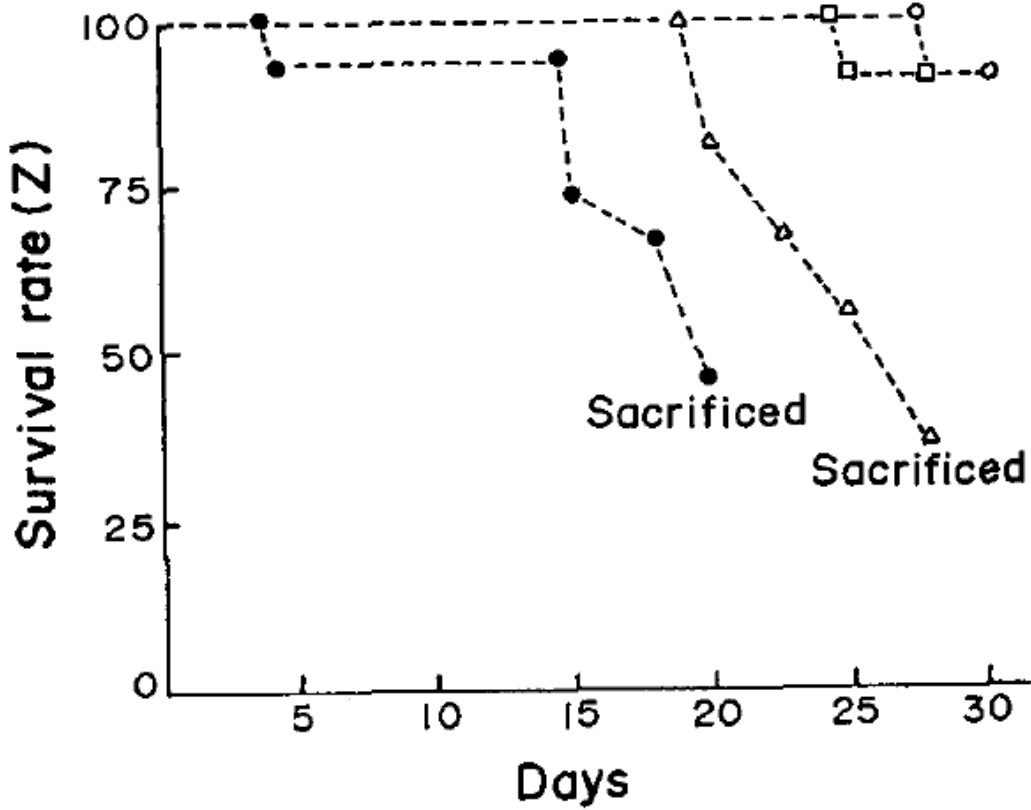


Fig. 1A. Cumulative survival rate of *Penaeus merguensis* in cadmium. (o = Control, □ = 0.1 mg/l, Δ = 0.3 mg/l, • = 0.5 mg/l).

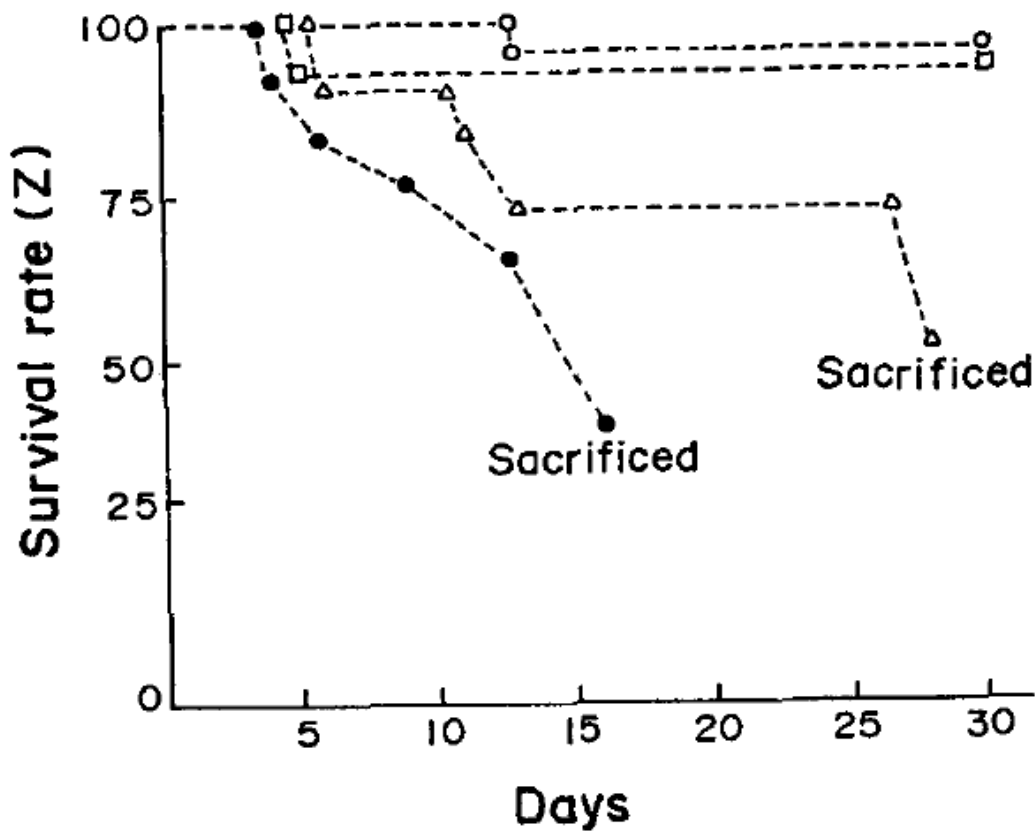


Fig. 1B. Cumulative survival rate of *Penaeus merguensis* in nickel. (o = Control, □ = 0.4 mg/l, Δ = 1.2 mg/l, • = 2.0 mg/l).

### ***Histopathological Changes***

Histological changes occurred mainly from days 15 to 30 and at the higher concentrations (Table 1). Lesions were most severe in the gills, hepatopancreas and gut, but only mild changes were found in the cuticle.

The severe black-pigmented lesions which occurred in the gills of shrimp after 15 days exposure to the highest cadmium concentration consisted of lamellar hemocyte aggregation and melanization of branchial lamellae, accompanied by necrosis, especially in the distal gill filament (Fig. 2). In some shrimp almost all gill processes were affected, while in others changes mainly occurred unilaterally. Large numbers of hemocytes accumulated in some lamellae, causing blockage of hemolymph channels. In some gills, the lamellae were hyperplastic.

The hepatopancreas, where most heavy metals accumulate, also showed changes in tubular epithelium and intertubular tissues as irregular degeneration and necrosis. Cell debris and eosinophilic material occurred inside some tubules which were surrounded by fibrous tissue. After 15 days exposure to 0.5 mg/l cadmium, hepatopancreatocytes of some shrimp were hypertrophic and found to contain eosinophilic and some basophilic nuclear inclusion bodies (Fig. 3). The size of the eosinophilic inclusion bodies was variable, both smaller or larger than the hepatopancreatocytic nucleus. One cell often contained more than one inclusion and sometimes a large inclusion was surrounded by basophilic cytoplasm which was difficult to differentiate from the nucleus. Basophilic intranuclear inclusions were sometimes found concomitantly with chromatic margination, especially in the medullary area.

Changes in the gut were hemocyte accumulation (hemorrhage) in the lumen (Fig. 4) and into the muscularis mucosa. The epithelium of the intestine was hypertrophic and irregular.

Clinically, more than 50% of animals at high concentration exposure showed localized erosion and necrosis in the appendages, and hemocyte accumulation and melanization in these areas.

Few histopathological changes were caused by nickel toxicity (Table 2). All animals at the high concentration were killed by day 15 as only 10 animals survived at that time. The most severe changes observed on day 30 were in the hepatopancreas of the medium concentration group.

In the gills, lesions were similar to those in cadmium toxicity, with accumulation of hemocytes, pigmented gill filaments and

Table 1. Number of shrimp (*Penaeus merguensis*) with histopathological changes after exposure to cadmium (C = 0 mg/l; L = 0.1 mg/l; M = 0.3 mg/l; H = 0.5 mg/l).

Organs	Changes	Day 5			Day 15			Day 30			H**
		C	L	M	C	L	M	C	L	M*	
Gills	1 Epithelial hyperplasia	1	2	1	1	1	3	0	0	1	5
	2 Congestion	0	0	0	0	0	4	0	0	1	3
	3 Necrosis	0	0	0	0	0	4	0	0	1	3
Hepatopancreas	1 Vacuolation	1	1	1	2	0	0	0	0	1	1
	2 Congestion	0	0	0	0	0	0	0	0	1	1
	3 Necrosis	0	0	0	0	0	0	0	0	2	3
	4 Inclusion bodies	0	0	0	0	0	0	0	0	2	3
	5 Helminths	1	2	1	1	3	3	1	1	2	2
Gut	1 Congestion	0	0	0	0	0	0	0	1	1	2
	2 Epithelial hypertrophy	0	0	0	0	0	0	0	1	1	2
Appendages	1 Erosion and necrosis	0	0	0	0	0	0	0	0	1	2
Number of shrimp		7	7	7	7	7	7	7	7	7	5

\* = killed at day 28

\*\* = killed at day 24

Table 2. Number of shrimp (*Penaeus merguensis*) with histopathological changes after exposure to nickel (C = 0 mg/l; L = 0.4 mg/l; M = 1.2 mg/l; H = 0.5 mg/l).

Organs	Changes	Day 5			Day 15			Day 30			H**
		C	L	M	C	L	M	C	L	M*	
Gills	1 Epithelial hyperplasia	0	0	1	1	1	1	1	2	1	n
	2 Congestion	0	0	1	0	0	1	0	1	0	n
	3 Necrosis	0	0	0	0	0	0	0	0	0	n
Hepatopancreas	1 Vacuolation	0	0	0	0	0	0	0	0	0	n
	2 Congestion	0	0	0	0	0	0	0	0	2	n
	3 Necrosis	0	0	0	0	0	0	0	0	1	n
	4 Inclusion bodies	0	0	0	0	0	0	0	0	0	n
	5 Helminths	1	0	1	2	0	0	0	0	0	n
Gut	1 Congestion	0	0	0	0	0	1	1	0	1	n
	2 Epithelial hypertrophy	0	0	0	0	0	0	0	0	1	n
Appendages	1 Necrosis	0	0	0	0	0	0	0	0	0	n
Number of shrimp		7	7	7	7	7	7	7	7	7	0

\* = killed at day 25

\*\* = killed at day 15

n = not observed

necrosis, but their incidence was much lower, being found in only two animals on day 15 at the high concentration.

In the hepatopancreas, pathological changes occurred in only two animals as focal hematocyte aggregation, degeneration and necrosis of the tubules. Cell debris in some tubules was surrounded by a slight increase of fibrous tissue. In the gut, moderate epithelial hypertrophy and hemorrhage in the lumen were also found.

### Discussion

Similar clinical signs were seen in shrimp due to the effect of each metal except that in nickel toxicity, erosion of the appendages did not occur up to 30 days. The clinical toxic effects of both metals appeared five days after treatment in animals given mid and higher concentrations. The effects then intensified as shown by the numbers of deaths in shrimp given a high concentration of these metals.

#### *Cadmium*

The toxic pathology of cadmium in the gills of *P. merguensis* in this study showed initial hypertrophy of epithelial cells, hemocyte aggregation and necrosis occurring at a concentration of 0.5 mg/l Cd on day 15. In *P. duorarum* Burkenroad these changes occurred also on day 15 at a concentration of 0.7 mg/l Cd (Couch 1977). In *P. duorarum* and *Palaemonetes vulgaris* at a concentration of 1 mg/l Cd changes occurred on day 21 and 25 (Nimmo et al. 1977). Lesions due to cadmium toxicity in the gills of shrimp may vary according to time, concentration of exposure, temperature, salinity and species.

Few authors have described the toxic effect of cadmium on the hepatopancreas of shrimp but in the pumpkin seed, *Lepomis gibbosus* (L.), it inhibited deposition of vitamin B<sub>12</sub> in the liver (Merlini 1978), and in the mummichog, *Fundulus heteroclitus* (L.), this metal inhibited some liver enzymes (Jackim et al. 1970). In the present study changes due to cadmium toxicity in the hepatopancreas varied with dose and time of exposure, but were more prevalent at the higher concentration. Congestion, hemorrhage and fibrosis in the interstitium of the hepatopancreas indicated other effects and necrotic tubules with cell debris inside their lumen had a similar toxic origin. The changes were similar to those in *Penaeus stylirostris* Stimpson and *P. vannamei* Boone suffering from aflatoxicosis (see

Lightner et al. 1982), where a marked intertubular hemocyte infiltration and inflammation also occurred, followed by fibrous encapsulation of affected tubules, indicative of subacute damage.

Eosinophilic and basophilic nuclear inclusion bodies were found in the hepatopancreas of some shrimp, usually in the medulla. The size of the eosinophilic inclusions was variable. These changes were similar to those described in *P. merguensis*, *P. semisulcatus* De Haan, *P. orientalis* Bate and *P. monodon* Fabricius in some Asian countries suffering from parvovirus-like disease (Lightner and Redman 1985), although inclusion bodies in this study commonly occurred in medullary rather than cortical tubules. Damage to cells caused by cadmium toxicity or other factors, for example stress conditions, may increase sensitivity to viral or bacterial infection (Snieszko 1974). In the present study inclusions only occurred in a proportion of the cadmium-treated animals and isolation of concurrent viral infection was not attempted. Their absence from the nickel group may indicate a specific predisposing effect of cadmium.

Histological changes in the gut were not marked except for hemocyte accumulation in the lumen of the intestine; this was similar to that observed in *Penaeus duorarum* and *Palaemonetes vulgaris* exposed to subacute toxicity by cadmium (Nimmo et al. 1977). Hypertrophy of the epithelial cells and basal membrane of the gut in *P. merguensis* in this study resembles that in kuruma shrimp (*P. japonicus* Bate) suffering from an idiopathic proliferative disease syndrome (Lightner et al. 1984), but in that condition the basal membrane of the intestinal epithelium was thicker than in cadmium toxicity.

Erosion and necrosis in the cuticle, rostrum and antennula (appendages) were similar to those in *P. japonicus* placed in moderate and high density tanks in which an idiopathic proliferative syndrome occurred (Lightner et al. 1984). In the present experiment this lesion occurred only at high concentrations from day 15. Cadmium may have acted as a corrosive element to the chitin in the animals, as lesions included hemocyte accumulation and necrosis associated with cellular inflammation.

### **Nickel**

Although the mortality rate from the highest nickel concentration was somewhat faster than in the comparable cadmium



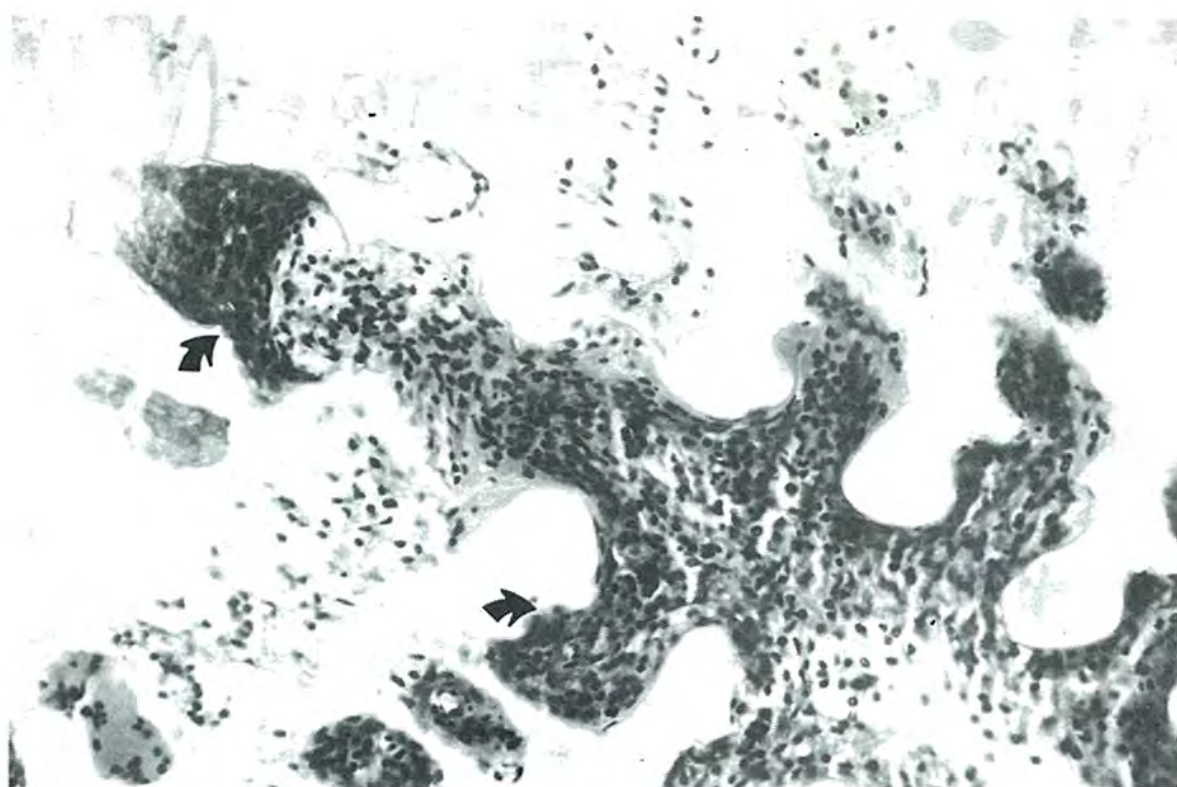


Fig. 2. Hemocyte infiltration, degeneration and melanization of the gill lamellae, accompanied by necrosis (0.5 mg/l Cd at day 15). 135X magnification; hematoxylin and eosin.

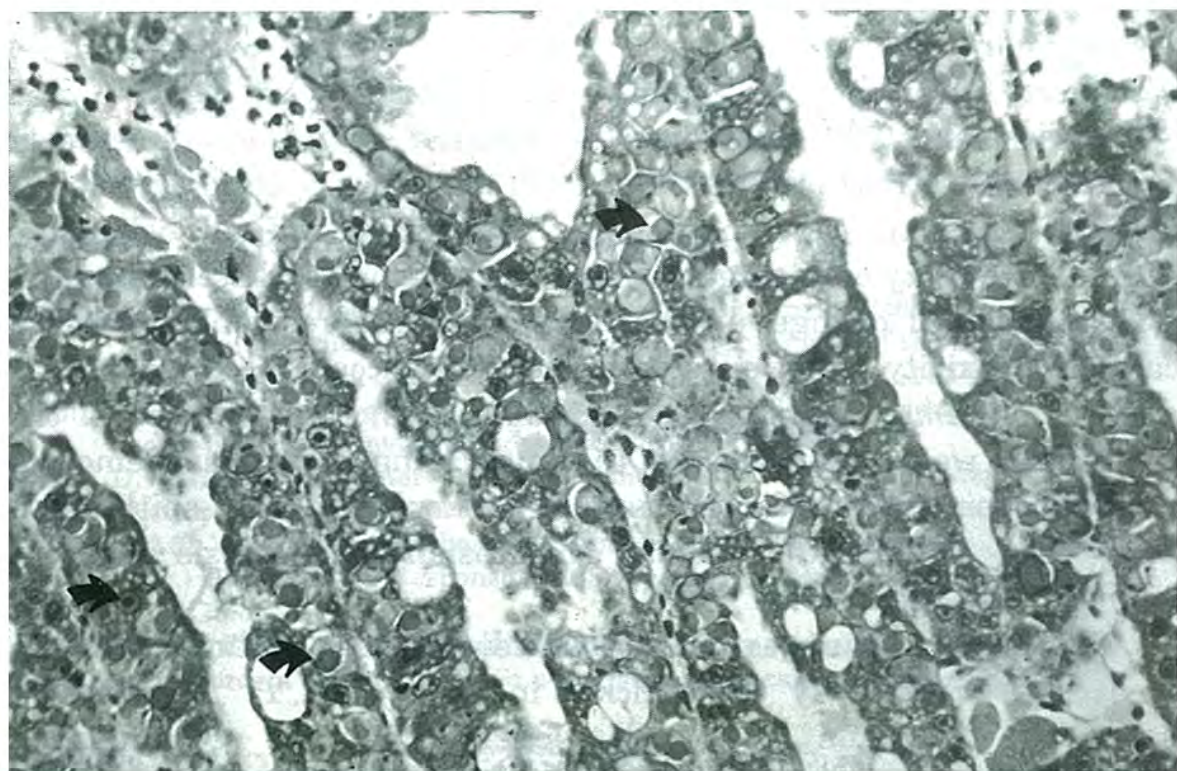


Fig. 3. Eosinophilic and basophilic intranuclear inclusion bodies and hypertrophy of epithelial cell tubules of the hepatopancreas (0.5 mg/l Cd). 340X magnification; hematoxylin and eosin.

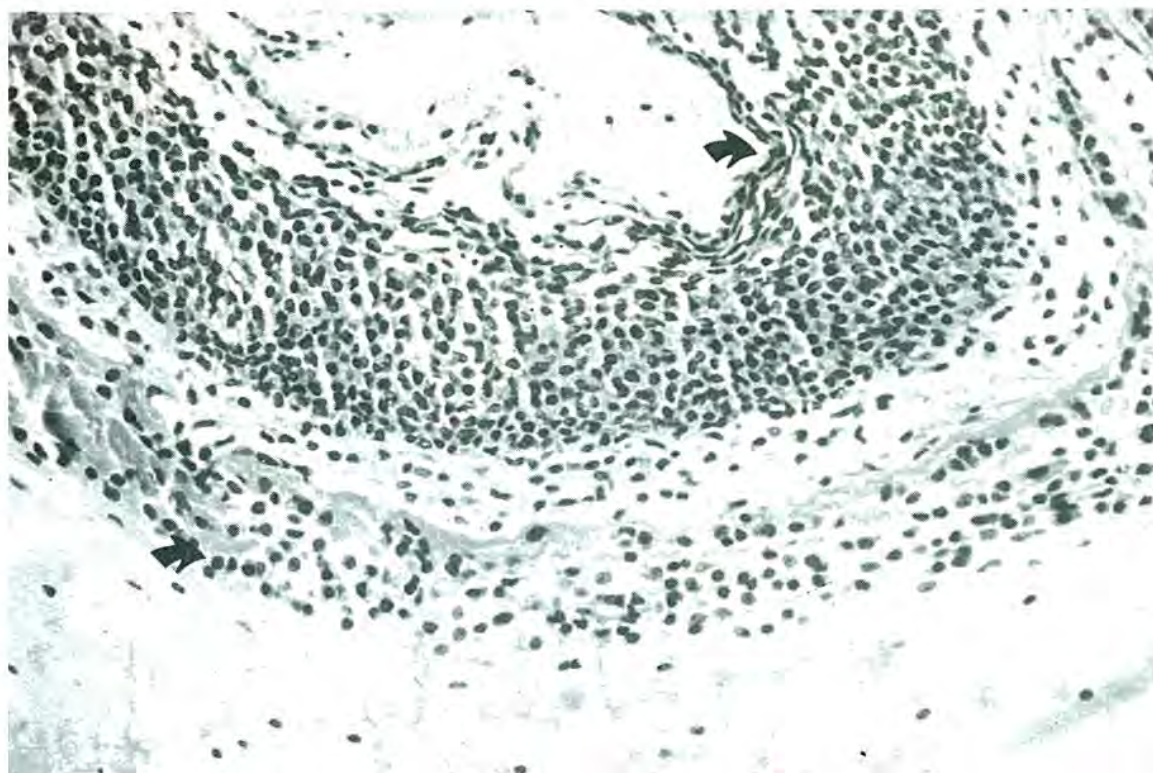


Fig. 4. Accumulation of hemocytes in the lumen of intestine and hemocyte leakage in the tunica propria (0.5 mg/l at day 15) 135X magnification; hematoxylin and eosin.

group, the pathological effect of nickel on the gills was less severe. Hypertrophy of the gill filament was similar in rainbow trout (*Salmo gairdneri* Richardson) exposed to nickel at a dose of 0.32 mg/l (Hughes et al. 1979), and this also occurred when animals were in polluted water (Hughes and Perry 1979). Seemingly all heavy metals dissolved in water cause pathological changes in the gills when the degree of severity of damage is related to the level of dissolved metal. The histological changes in the hepatopancreas resembled those of cadmium toxicity and were similar to aflatoxicosis in *P. stylirostris* and *P. vannamei* (Lightner et al. 1982) but inclusion bodies in the epithelium were not found. In the gut, changes such as hemocyte accumulation in the lumen and hypertrophy of the epithelium, occurred as in cadmium toxicity, but erosion and necrosis were not found in the nickel group.

Histological changes therefore indicate that cadmium had a greater tissue toxicity than nickel in the prawn *P. merguensis*. According to Denton and Burdon-Jones (1982), the recommended safety levels of Cd and Ni for *P. merguensis* are 0.1 mg/l and 0.4 mg/l, respectively. These were similar to the results of our study, where in the lowest level experiments, pathological changes did not

occur. That estimate can be used for comparison when monitoring prawns for heavy metal pollution in marine waters or under conditions of aquaculture.

### Acknowledgements

The authors would like to thank staff at Tropical Veterinary Science and Marine Biology, James Cook University, Townsville, Australia, for their help. The work was supported by the James Cook University-Balivet Collaborative Project sponsored by The Australian International Development Assistance Bureau.

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