Asian Fisheries Science **13**(2000): 161-168
Asian Fisheries Society, Manila, Philippines

https://doi.org/10.33997/j.afs.2000.13.2.007

Changes in the Epidermal Ultrastructure of Atlantic Herring (*Clupea harengus*) Yolk-sac Larvae Exposed to Copper

A.R. ABBASI[1], S.E. SHACKLEY and P.E. KING

[1]*Department of Fresh Water Biology & Fisheries*
University of Sindh
Jamshoro, Sindh
Pakistan

University College
Swansea, South Wales
U.K. SA2 8PP

Abstract

The effects of copper on the yolk-sac larvae of *Clupea harengus* (Clupeidae) hatched from eggs previously exposed to copper were examined in terms of epidermal ultrastructure. The dermal cells of the larvae hatched in 0.03 or 0.05 mgl^{-1} copper showed the following changes; extensive vacuolation, degeneration of mitochondria and golgi complexes, dilation of endoplasmic reticulum and perinuclear spaces, disruption of the nuclear membrane, disappearance of felt-like fibrous plate and increase in the size and number of microvilli. The chloride cells in copper-exposed larvae had fewer and smaller mitochondria. These ultrastructural changes probably affect respiration and osmoregulation, thus the survival of herring larvae exposed to copper.

Introduction

The fish epidermis consists of epithelial cells, mucus cells and neuromasts (Jones et al. 1966; Somasundaram 1985; Mohammad Nagib 1987). The function of the epidermal layer, especially of the mucus cells, is to produce mucin, a glycoprotein substance with which water forms mucus. The mucus facilitates swimming and protects fish from parasites, bacteria and other small organisms (Lagler et al. 1977). The chloride cells function in ion secretion and absorption (Lubin et al. 1989).

The skin also function in respiration, osmoregulation, and absorption during the early larval stages when gills are not functional (Holliday 1964;

Blaxter 1969; Ferreira et al. 1984). Disruption of the epidermal layer may affect osmoregulation and respiration or increase the susceptibility to microorganisms.The skin of fish larvae is highly susceptible to heavy metal toxicity (Oronsaye and Brafield 1984; Somasundaram 1985).

Somasundaram et al. (a, b 1984) reported cellular disruption and deformities in the larvae of *C. harengus* exposed to zinc. Gardner and La Roche (1973) also found cellular damage in Fundulus heteroclitus exposed to copper solutions.

Ultrastructural and pathological changes in the gills, liver, kidney, lateral line and olfactory organs of fish due to copper have been reported (Baker 1969; Segner 1987; Beckman and Zaugg 1988). The cytological changes in muscle cells, brain cells and deformities in vertebrae and jaws in the larvae of herring, *C. harengus* exposed to copper have been previously reported (Abbasi et al. 1995; Abbasi and Shackley 1995). This paper describes the effects of copper on the ultrastructure of the epidermal layer of herring larvae, *C. harengus* L.

Materials and Methods

Adult *C. harengus* that were about to spawn were caught by gillnet from Castle Beach, Milford Haven, South Wales in March 1989. Eggs were stripped from these fish and artificially fertilized.

Samples of about equal numbers of fertilized eggs were placed in 2 l glass jars containing clean artificial sea water (Tropical Marine Salts) diluted to 20% (ambient salinity) with or without copper. Test solutions contained either 0.01, 0.03, 0.05 mgl^{-1} copper prepared from a stock solution of $CuS_O4 .5H_2O$. The jars were continuously aerated at 8ºC ambient temperature. Control and test solutions were renewed every two days to counter possible adsorption of copper by glassware and uptake by the eggs.

Upon hatching 15d after fertilization, the yolk-sac larvae were fixed for electron microscopy. Samples of ten larvae were removed from both control and test solutions and fixed for 2 h at 4ºC in 5% cacodylate-buffered glutaraldehyde with sucrose added. These were then washed in several changes of the buffer solution at 4ºC for 24 h, post-fixed in chilled osmium tetroxide for 90min, washed 1h further in buffer solution, then dehydrated in a graded series of cold acetone and finally embedded in TAAB embedding resin.

Gold/silver interference color sections were obtained using knives on a Cambridge Huxley Mark I ultramicrotome. Sections were mounted on copper grids and double-stained in 80% uranyle acetone in methanol (20 min) and lead citrate (10 min) and viewed in a Joel 1200 transmission electron microscope. A morphometric analysis of the electron micrographs was carried out according to Weible et al. (1966). Electron micrographs were enlarged to a final magnification of 39 x 10 and analysed using a multipurpose test system consisting of 100 points enclosing 50 short test lines in order to determine the relative volume of the mitochondria and the surface-to-volume ratio of the mitochondrial cristae in chloride cells.

Results

The epidermis of the larvae of *C. harengus* consists of the outer epidermal cells, the inner epidermal cells and the chloride cells. Each outer epidermal cell contains an elongated nucleus, a few mitochondria, golgi complexes, and vesicles (Fig. 1). The distal surface of these outer cells are extended into microvilli or microridges (Figs. 1 and 2). Under these cells, there is a felt-like mass of fibers forming a plate. The inner epidermal cells have extensive rough endoplasmic reticulum (Fig. 2) but the other organelles are similar to those in the outer cells. The innercells are connected to each other by desmosomes. The chloride cells contain a large number of mitochondria with an electron-dense matrix and numerous cristae (Fig. 3), ribosomes, osmiophilic vesicles, tubules, extensive smooth and rough endoplasmic reticulum (SER and RER) that ramify throughout the cell except in the narrow apical cytoplasm. The nucleus, spherical or ovoid in shape, lies in the periphery of the cell. Part of the cell (about 6.2 μm) lies exposed in a depression on the skin surface, flanked by epidermal cells.

Significant morphological changes occurred in both epidermal and chloride cells of larvae hatched from eggs incubated in 0.03 or 0.05 mgl^{-1} copper. Larvae in 0.03 or 0.05 mgl^{-1} copper developed vacuoles among the mitochondria while the felt-like fibrous plate in the outer epidermal cells disappeared (Fig. 4). In the inner cells, the RER and perinuclear spaces were dilated (Fig. 5). In the larvae in 0.05 mgl^{-1} copper, the cytoplasm of the outer epidermal cells was vacuolated (Fig. 6), the organelles disappeared except some of the remnants of mitochondria and golgi complexes; the nuclear envelope was disrupted (Fig. 7) and the microvilli became more numerous (Fig. 8). The nuclei of the inner epidermal cells had low electron density, and the mitochondrial cristae were fragmented or missing. The chloride cells of the larvae in 0.03 mgl^{-1} copper had swollen SER and mitochondria. The chloride cells from larvae incubated in 0.05 mgl^{-1} copper were smaller than those in control, contained fewer mitochondria, formed nodular masses and contained granular and osmiophilic inclusions (Fig. 9). The mitochondrial cristae degenerated (Fig. 10) leaving a granular mass in the matrix.

The relative volumes of the mitochondria and the surface-to-volume ratios of their cristae in the chloride cells are shown in Table 1. Both measures significantly decreased in larvae hatched from eggs incubated in 0.03 and 0.05 mgl^{-1} copper.

Discussion

In the present study necrosis and sloughing off of the epidermal cells were observed in the herring larvae hatched from eggs incubated in high concentrations of copper. The epidermal cells became vacuolated and were finally sloughed off. Somasundaram (1985) also observed the vacuoles, the swelling of mitochondria and the increased intracellular space in the epithelial cells in *C. harengus* larvae exposed to zinc. Eisler and Gardner (1973)

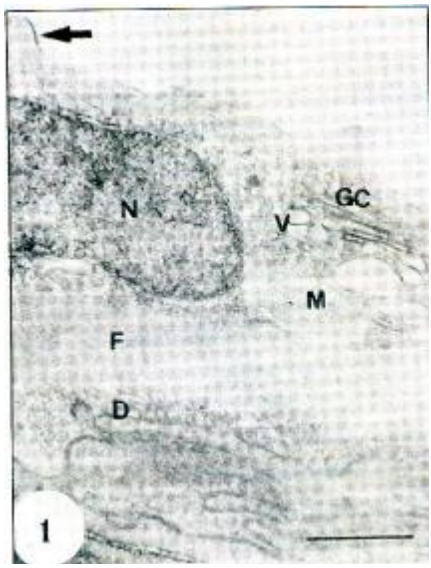

Fig. 1. The epidermal cell of *C. harengus* larvae at hatching, showing nucleus (N), mitochondria (M), golgi complexes (GC), vesicles (V), felt-like mass of fibers (F) forming a plate and desmosomes. Arrow head shows microvilli. Scale bar 1 m.

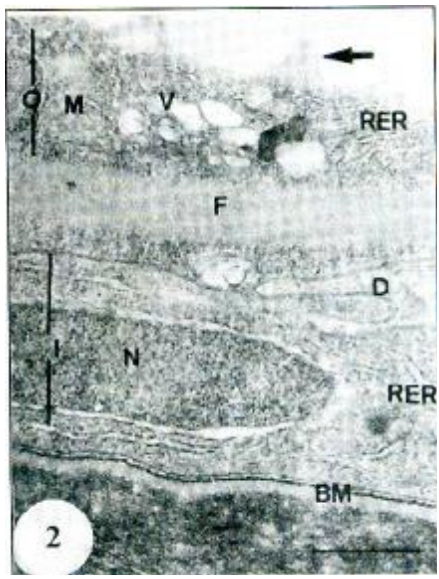

Fig. 2. Epidermal cells of *C. harengus* larvae showing the outer (O) and inner (I) epidermal cells, rough endoplasmic reticulum (RER), and nucleus (N). The epidermal cells are connected together by desmosomes (D). BM = basement membrane. Scale bar 1 m.

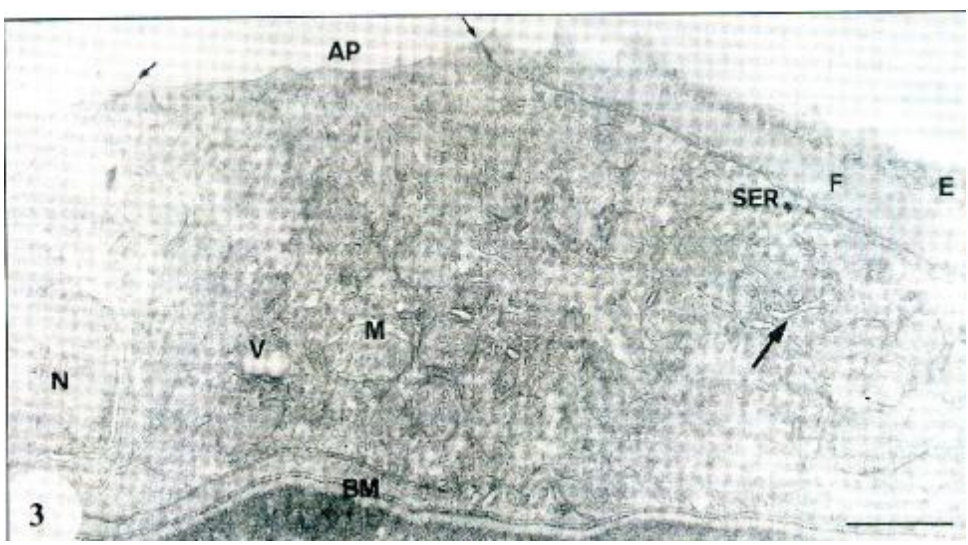

Fig. 3. The chloride cell in the epidermis of *C. harengus* larvae at hatching. The epidermis contains branches of smooth endoplasmic reticulum (SER), rough endoplasmic reticulum (big arrow), and numerous mitochondria (M). Note the chloride cells extending beneath the epidermal cell (E) and connected to the surface (small arrow) via the apical pit (AP). V = Vesicles; BM = Basement Membrane. Scale bar 1 m.

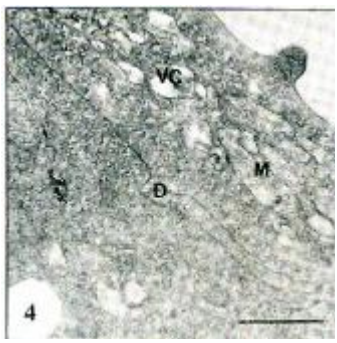

Fig. 4. The outer epidermal cells of *C. harengus* larvae hatched from eggs previously exposed to 0.03 mgl^{-1} copper,showing the vacuoles (VC),degenerationof mitochondria (M) and disappearance of felt-like fibrous mass. D = desmosomes. Scale bar 0.5 m.

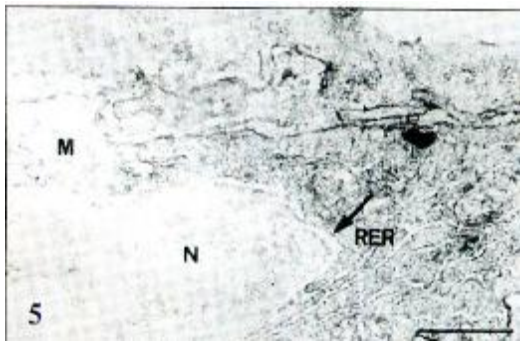

Fig. 5. The inner epidermal cells of *C. harengus* larvae hatched from eggs previously exposed to 0.03 mgl^{-1} copper, showing fragmentation of rough endoplasmic reticulum (RER), mitochondria (M), and the perinuclear space (arrow). Scale bar 1 m.

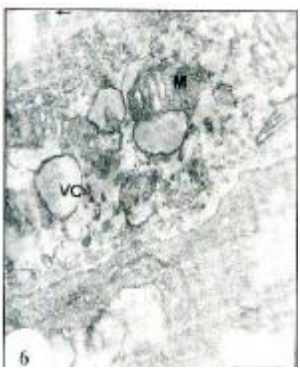

Fig. 6. The outer epidermal cells of *C. harengus* larvae hatched from eggs previously exposed to 0.05 mgl^{-1} copper, showing many vacuoles (VC), distorted mitochondria (M), numerous microvilli (arrow), and no felt-like fibrous mass. Scale bar 2 m.

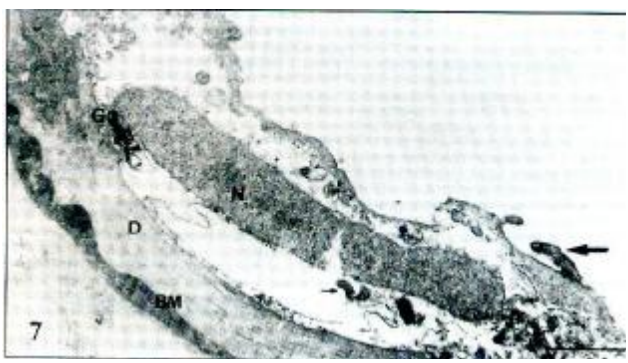

Fig. 7. The outer epidermal cells of *C. harengus* larvae hatched from eggs previously exposed to 0.05 mgl^{-1} copper, showing degeneration of outer epidermal cells, remnants of mitochondria (small arrow) and golgi complexes (GC) disrupted nuclear membrane (N), and more microvilli (large arrow). Scale bar 2 m.

Fig. 8. The outer epidermal cell of *C. harengus* larvae from eggs previously exposed to 0.05 mgl^{-1} copper, showing increased abundance and size of microvilli (MV). Scale bar 0.5 m.

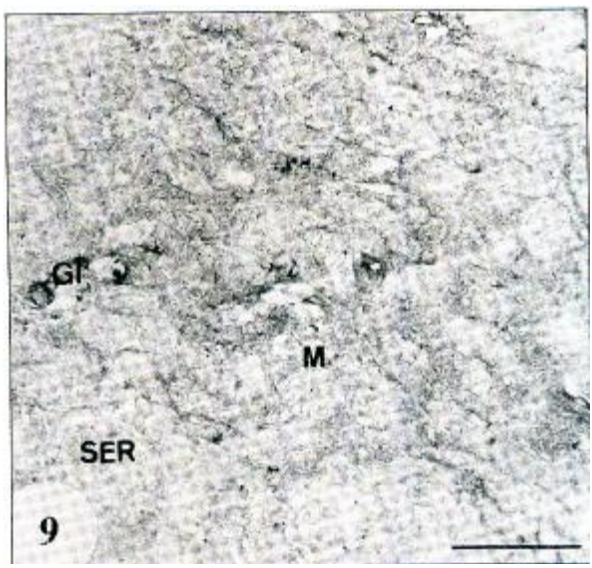

Fig. 9. The chloride cell of *C. harengus* larvae hatched from eggs previously exposed to 0.05 mgl^{-1} copper, showing the reduced size and abundance of mitochondria (M) and granular inclusions (GI) in the cytoplasm.SER(smooth endoplasmic retculum). Scale bar 1 m.

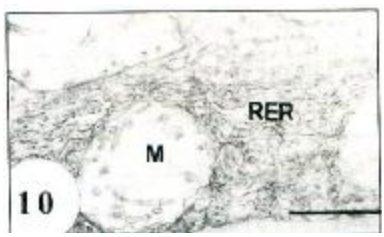

Fig. 10. The Chloride cell of *C. harengus* larvae hatched from eggs previously exposed to 0.05 mgl^{-1} copper showing the degeneration of mitochondria cristae (M), RER = rough endoplasmic reticulum. Scale bar 1 m.

Table 1. Morphometric analysis of the chloride cells of *C. harengus* L. yolk-sac larvae hatched from eggs previously exposed to copper for 15 days. For each group 25 larvae were examined.

Copper concentration	Mean Relative Volume of mitochondria	Mean surface–to–volume ratio of cristae
0 (Control)	0.60	0.42
0.01	0.61	0.44
0.03	0.53	0.34
0.05	0.46	0.20

observed the necrosis of the epithelial lining in the oral cavity of Fundulus heteroclitus exposed to zinc. Nuclear pyenosis, necrosis and sloughing off of the epidermal cells were also reported in *C. harengus* larvae reared in acidic and aluminum solutions (Mohammad Nagib 1987). In Winter flounder exposed to copper, a cytological breakdown of the gill epithelium occurs and chloride cells expand into bubbles (Baker 1969). Rainbow trout exposed to mercury showed severe epithelial necrosis as well (Wobeser 1975). Benedetti et al. (1989) reported that due to copper poisoning the epidermis of brown bullhead, Ictalurus nebulosus appeared thinner in patches which showed shrunk cytoplasm, dissolution of the nuclear envelope and dispersed chromatin structure. Similar results have also been observed in the present study. Baker (1969) suggested that copper may be absorbed by the gill epithelium

and act primarily on the cell enzymes resulting in the formation of lysosomes, vacuoles and vesicles. In the present study, nodular masses or granular inclusions on the epithelial cell surface may be due to the copper acting as a coagulant on the mucus secreted by the cells within the epithelial layer. The present study suggests that the granular matter may effect oxygen/carbon dioxide exchange through the epithelial cells.

The presence of microridges or microvilli on the skin have been reported in many teleost larvae including *C. harengus* (Jones et al. 1966; Mathy et al 1980; Lubin et al 1989; Foscarini 1989). Microridges which are respiratory and excretory in function, are also sensitive to stimuli from the surrounding environment (Baker 1969; Foscarini 1989). In the present study, the copper treated larvae had numerous microvilli that were longer than those in the control larvae, but the microvilli disappeared when the outer epidermal cells degenerated. High microridges were also observed in the larvae of *C. harengus* exposed to zinc (Somasundaram 1985) and mercury (Jastania 1989). Mercuric chloride or methyl mercuric chloride damaged the microvilli of gills before damaging the epithelia (Olson et al 1973). In the present study, copper caused cellular changes in the chloride cells of *C. harengus*. Similar degeneration of chloride cells was also observed in the larvae of *C. harengus* in acidic solutions (Mohammad Nagib 1987). The appearance of chloride cells in the epidermis of *C. harengus* may be an adaptation to environmental changes and their disruption may be due to changes in the metabolic activity (Somasundaram 1985).

In Winter flounder exposed to copper, the mucus cells replaced by chloride cells functioned in adaptation to and excretion of copper (Baker 1969). The present study is in agreement with Somasundaram (1985) and Baker (1969) that in the chloride cells, the surface to volume ratio of the mitochondrial cristae increase in 0.01 mgl^{-1} copper suggests an increase in metabolism, hence the cells may be involved in the excretion of copper, but in 0.05 mgl^{-1} copper reduced mitochondria and the cristae indicates low metabolism due to cellular metabolic disruption. In Stickleback exposed to cadmium, the chloride cells increased in number as ionic regulation increased at the gills, but later declined in number as they were poisoned and their effectiveness in removing cadmium was reduced (Oransaye and Brafield 1984). Disruption of the ultrastructure and the sloughing off of the epidermal cells of *C. harengus* in copper solutions may be expected to adversely affect respiration, osmoregulation and the survival of larvae and would increase the chances of fungal and bacterial infections.

References

Abbasi, A.R. and S.E. Shackley 1995. Effects of copper on the Newly hatched larvae of herring, Clupea harengus L. Pakistan Journal of Zoology 27:285-290.

Abbasi, A.R., S.E. Shackley and P.E. King 1995. Effects of copper on the ultrastructure of brain cells of Atlantic herring, *Clupea harengus* L. Pakistan Journal of Zoology 27:203-206.

Abbasi, A.R., S.E. Shackley and P.E. King 1995. Effects of copper on the ultrastructure of muscle cells of herring, *Clupea harengus* L. Pakistan Journal of Zoology 27:83-87.

Baker, J.T.P. 1969. Histological and electron microscopical observations on copper poisoning in the winter flounder, *Pseudopleuronectes americanus*. Journal Fish Research Board Canada, 26: 2785-2793.

Beckman, B.R. and W.S. Zaugg. 1988. Copper intoxication in chinook salmon, *Oncorhynchus tshawytshe* induced by natural spring water. Effects on gill Na+ ,K+ ATPase, hematocrit and plasma glucose. Canadian Journal of aquatic science, 45:1430-1435.

Benedetti, I., A.G. Albano and L. Mola 1989. Histomorphological changes in some organs of the brown bullhead, *Ictalurus nebulosus Le Sueur*, following short and long-term exposure to copper. Journal of Fish Biology 34:273-280.

Blaxter, J.H.S. 1969. Development: eggs and larvae. In: Fish Physiology vol.3. (eds. W.S. Hoar and D.J. Randall) pp.177-252. Academic Press: New York.

Eisler, P.E. and G.R. Gardner 1973. Acute toxicology to an estuarine teleost of mixtures of cd, cu and zn salts. Journal of Fish Biology 5:131-142.

Ferreira, J.T., H.J. Schoonbee and G.L. Smith. 1984. The up take of the anaesthetic benzocaine hydrochloride by the gills and the skin of three freshwater fish species. Journal Fish Biology. 25:35-42.

Foscarini, R. 1989. A comparative study of the skin and gill structure in oviparous and viviparous freshwater fish larvae. Journal Fish Biology. 34:31-40.

Gardner, G.R. and G. LaRoche. 1973. Copper induced lesions in estuarine teleosts. Canadian Fish Research Board Journal. 30:363-368.

Jastania, H.A. 1989. Studies on South Wales fish stocks and the possible effects of heavy metals. Ph.D Thesis, University of Wales, U.K. pp.450.

Jones, M.P., F.G.T. Holliday and A.E.G. Dunn. 1966. The ultrastructure of the epidermis of larvae of the herring, *Clupea harengus* L. Journal Marine Biological Association U.K. 46:234-239.

Lagler, K.F., J.E. Bardach and R.R. Miller. 1977. Ichthyology. John Wily and Sons, Inc., New york, pp 506.

Lubin, R.T., A.W. Rourke and T.M. Bradley. 1989. Ultrastructural altrations in branchial chloride cells of Atlantic salmon, *Salmo salar*, during parrsmolt transformation and early development in sea water. Journal Fish Biology 34:259-272.

Mathy, D.L., M. Morgan and D.E. Wright. 1980. A scanning electron microscope study of the Pseudobranch of two marine teleosts. Journal Fish Biology 18:331-343.

Mohammad Nagib, F.J.B. 1987. Effects of heavy metals on the biological activities of marine fishes, with special reference to their reproduction and development. Ph.D. Thesis, University of Wales PP 330.

Olson, K.R., P.D. Fromm and W.L. Frants. 1973. Ultrastructure changes of rainbow trout gills exposed to methyl mercury or mercuric chloride. Fed. Proc. 32:261-267.

Oronsaye, J.A.D. and A.E. Brafield. 1984. The effect of dissolved cadmium on the chloride cells of the gills of the stickle back, Gasterosteus aculeatus. Journal Fish Biology 25:253-258.

Segner, H. 1987. Response of fed and starved roach, Rutilus rutilus, to sublethal copper concentration. Journal Fish Biology 30:423-437.

Somasundaram, B. 1985. Effects of zinc on epidermal ultrastructure in the larvae of *Clupea harengus*. Marine Biology 85:199-207.

Somasundaram, B., P.E. King and S.E. Shackley. 1984a. The effects of zinc on the ultrastructure of the brain cells of the larvae of *Clupea harengus* L. Aquatic Toxicology 5:323–330.

Somasundaram, B., P.E. King and S.E. Shackley. 1984b. Some morphological effects of zinc upon the yolk sac larvae of *Clupea harengus* L. Journal Fish Biology 25:333-343.

Somasundaram, B., P.E. King and S.E. Shackley. 1985. The effects of zinc on the ultrastructure of the posterior gut and pronephric ducts of the larvae of *Clupea harengus* L. Comparative Biochemical Physiology 81:29-37.

Steel, C.W.1985. Latent behavioural toxicity of copper to sea catfish, Arius felis and sheephead, *Archosargus probatocephalus*.Journal of Fish Biology 7:227-282.

Weibel, E.R., G.S. Kistler and W.F. Scherle. 1966. Practical streological methods for morphometric cytology. Journal Cell Biology 30:23-38.

Wobeser, G. 1975. Acute toxicity of methyl mercury chloride and mercuric chloride for rainbow trout, *Salmo gairdneri*, fry and fingerlings.Journal Fish Research Board Canada 32:2005-2013.

Manuscript recieved 12 May 1995; Accepted 24 March 1998.