GROSS PATHOLOGY
AND
ATAXIA
5. GROSS PATHOLOGY AND ATAXIA

5. 1. INTRODUCTION

Nutrition and pathology are related disciplines which should be considered together. “Every disease has a nutritional aspect. Nutrition thus has relations with all branches of medicine”. The pathology that occurs in fishes incident to oral ingestion of foods and, in some instances, to branchial (gill) absorption may be termed nutritional as opposed to pathology induced by noningested bacteria, viruses, parasites, or other nonnutritional disease entities.

The major beneficiaries of dietary and in particular those nutrient research on dietary vitamin C levels and deficiency syndromes are aquaculturists and feed manufacturers. In fish farming, from the stress induced behavioural abnormalities, if one can diagnose the cause and initiate early remediation, saving the crop would be possible. The external symptoms and behavioural abnormalities are indicative of inner injuries discernible by histopathology. Herein the ataxic symptoms of the scorbutic fish elucidated from studies as the present one and the comparative symptomology discussed in the following pages aid in early diagnosis while monitoring the health of the farmed aquatics, initiation of remediation, saving the stock and consequently prevention from financial loss.

The behavioural abnormalities and gross morphological defects as induced from vertebral, caudal and muscular defects observed in the scorbutic fishes both in the earlier works and in the present study and also those recorded for other toxicities and stress factors are compared and discussed in this section.
5.2. RESULT

5.2.1. GROSS PATHOLOGICAL OBSERVATIONS

The externally detectable gross pathological symptoms of the scorbutic *P. latipinna* were mainly noticed in the fish fed diets 1 and 2. The spinal deformities kyphosis (dorsal spinal curvature) (Fig. 2), kyphoscoliosis (dorsolateral spinal curvature (fig. 2), scoliosis (lateral spinal curvature) (Fig. 2) and lordosis (ventral spinal curvature) (Fig. 3) -- unilateral and bilateral exophthalmia (protruding eyes) (Fig. 4), external haemorrhage usually at the base of the fins (Fig. 5), internal haemorrhage (Fig. 6), petechiae (pin point haemorrhages) throughout the surface of the body, caudal fin erosion (Fig. 7), loss of scales, scale erection (Fig. 8), dermal ulceration (Figs. 9 & 10), paler gills, oedema (Fig. 10), lethargy, anorexia (loss of appetite), feed refusal, emaciation (Fig. 10), and stunted growth were the clinical signs observable during the present study.

A tadpole like pin head fish (Fig. 10) with emaciated trunk, possibly having spent more of its energy in muscular activity especially for swimming and avoidance of feed due to anorexia was observable. In the dorsal view, the trunk showed a very lean appearance and the fish was tadpole like. A part of the belly was depressed slightly and exhibited shrunken appearance.

In a fish, a perforation developed at the lateral side of the belly with haemorrhage (Fig. 11). Then the bleeding stopped but a kind of mucous cord was found extruding from the pore very slowly and continuously. Hence the fish had to drag the mucous cord while swimming. Day by day the body size decreased because of anorexia. A slight external haemorrhage just behind the skull was also observed. The swimming activity was confined to the bottom. It avoided schooling. After a few days it almost stopped swimming and
Fig. 1. Normal clinically healthy *Poecilia latipinna*. Male & Female

Fig. 2. Kyphoscoliosis.

Fig. 3. Lordosis.

Fig. 4. Bilateral exophthalmia.

Fig. 5. External haemorrhage at the base of the pectoral fins.

Fig. 6. Internal haemorrhage in the mandibulo opercular region of head.
GROSS PATHOLOGY (CLINICAL SIGNS)

Fig. 7. Caudal fin erosion.

Fig. 8. Scale erection.

Fig. 9. Dermal ulceration (Lateral view) and oedema in the caudal peduncle with intramuscular haemorrhage and scale loss in that region.
Dermal ulceration (Dorsal view) and oedema in the caudal peduncle with intramuscular haemorrhage.

Fig. 10. A tadpole like pin head fish with emaciated trunk and slightly depressed belly.

Fig. 11. A deficient fish with a perforation (Ulcer) at the lateral side of the belly with internal haemorrhage.

Fig. 12. Aborted sacfries of a scorbutic mother compared with a normal fish (centre).
remained motionless at the bottom almost without beating the pectoral fins. The rate of opercular beat was found to be increased. The fish died having lived after the development of the said symptoms for about 5 days.

A scorbutic mother gave birth to 23 premature abnormal (aborted) baby fish termed as sac fries (Fig. 12). The yolk sac which had given the nourishment for the developing embryo, having not yet been fully utilized, was found still attached to the ventral side of the fry as a balloon. All of them showed exophthalmia like condition, which in fact was due to premature (embryonic) state of the sac fries. All the sac fries died soon after birth. From this, it could be concluded that ascorbic acid was indispensable for the normal embryogeny.

5.2.2. SCALE OBSERVATIONS

5.2.2.1. Normal cycloid scale

Like the majority of teleostean fishes, *P. latipinna* too had cycloid scales which are the characteristic external covering of fishes. The cycloid scales were almost completely dermal and protect the skin. They had the advantage of being imbricate, i.e., overlapping like the tiles on a roof. They were thin, flexible, round and transparent structures (Fig. 13). Since they exhibited characteristic ridges alternating with grooves, they were termed as the bony ridge scales. Normally, the ridges were in the form of concentric rings called the circuli, the growth rings. The central part of the scale was called the focus and developed first.

A scale comprised of two portions, viz., a hard surface layer having organic framework impregnated with salts, mainly of calcium phosphate as hydroxyapatite and calcium carbonate; and a deeper fibrous layer or fibrillar plate, composed largely of
SCALE DAMAGE

Fig. 13. Normal scale of *Platipinna*. Focus (F), Circulus (C), Embedded portion (EP), Exposed portion (EXP), Rows of teeth (RT). X 40.

Fig. 14. An aberrant small scale of a scorbutic fish with poor circuli count and the central portion eroded. X 40.

Fig. 15. An abnormal scale with the loss of scale focus and a few circuli eroded. X 40.

Fig. 16. An abnormal bean shaped very small scale with a deep depression up to the focus, due to resorption. X 40.

Fig. 17. The focal corrosion (FC) was found affecting the circulus. The circuli near the centre were wavy in appearance. The teeth number in each row was very low. X 40.

Fig. 18. Focal corrosion (FC) of circuli. The gap between the circuli was reduced in one side and more in the other side. Bifurcated teeth row (TR). X 40.
collagen. The cycloid scales can be divided into 4 fields: anterior which is smooth and embedded under the preceding scale, horny posterior, dorsal, and ventral.

5.2.2.2. Scale damage due to vitamin C deficiency

The scales of scorbutic fish expressed a number of aberrations with a general reduction in size and much damage in many scales. The scales of varying sizes were noticed. There was a drastic reduction in the circuli count (7-13) compared to the 16 to 20 circuli count in the normal fish. Many scales of abnormal shape occurred in the deficient fish.

The focus region along with the adjacent surrounding circuli was found to have been obliterated (Figs. 14 & 15) and so a very large empty area was identified in the scales. Only the embedded portion of the scale was preserved and the exposed portion with the rows of teeth like structures was totally absent. Erosion of the central portion was noticed in the scales (Fig. 14). The focal corrosion of circuli was observed (Fig. 15). An abnormal shaped scale had much reduced size with a deep depression up to the focus, due to the resorption and it gave a bean shape in appearance (Fig. 16). In one scale, the exposed portion of the scale was found, but it had only 6 rows of teeth, compared to nearly 11 rows of teeth in the normal scale (Fig. 17). The circuli near the centre were wavy in appearance. The number of teeth in each row was very less and the teeth did not have perfect outline. The gap between the circuli was less on one side and more on the other side (Fig. 18) and so it seemed that the central portion was slightly eccentric. The focal erosion of circuli was distinguishable. One row of teeth in the middle had bifurcation.
5. 2. 3. ATAXIA

The fish which were fed 0 mg and 12 mg/kg feed of vitamin C diets displayed defective neuro-sensory symptoms, such as ataxia, hyperirritability, increased handling stress, listlessness, loss of balance and muscular fibrillation, in contrast to those fed 25 mg and 50 mg/kg levels of vitamin C. In fish fed 100 mg, 200 mg and 400 mg/kg level of vitamin C diets, no behavioural abnormalities were visible.

The ataxia of the scorbutic fish was varied and all of them avoided schooling and swam alone without any coordination. A few days before death, they exhibited surface swimming. They had already developed anorexia and so the feed refusal was noticed. The fish were also affected by muscular dystrophy. Those thin and anorexic fish developed tapering shape resulting in pin head conformation. Such fish displayed snake like swimming movement with obvious discomfort, and preferred to swim at the surface. The swimming activity was highly lethargic and the fish were much exhausted. The opercular beat was arhythmic and sometimes the fish also gasped heavily with laborious breathing movements. Almost a day before the death, after settling at the bottom, though the fish showed undulatory movement of the trunk and tail, they failed to move and remained static. Loss of equilibrium was also noticed. The swimming efficiency was probably impaired. The diseased fish showed respiratory distress and could not maintain the horizontal equilibrium.

In the moribund condition, the pectoral fins of the affected fish stopped moving and were kept parallel to the long axis of the body, behind the place of attachment. The fish displayed loss of balance and recovery alternately. The loss of balance progressed slowly from occasional instability to complete inability to maintain the normal position. Now and
then convulsions were also observed in the scorbutic fish. Slowly the fish lost its balance and lay on its side. At this stage, many fish manifested muscular fibrillation. Though the fish tried to swim up in the normal manner, they could not and they remained at the bottom until death. A few fish spent in comatous condition for a few hours just before death.

The bottom scrapping was observable in a few scorbutic fish. Sometimes the fish rolled on their long axis and showed the erratic swimming behaviour. As it remained at the bottom, it squirted abruptly and settled at the bottom. Swirling (spiral) movement was also observed in some sick fish.

The fish with extreme degree of scoliosis, displayed an abnormal swimming behaviour. The tail along with the posterior portion of the trunk was turned to the left side. The fish swam in slanting and inclined position. It took effort to correct its position every now and then during propulsion, but all such attempts were in vain, because the scoliosis had completely altered the general shape. Since the body along with the tail was turned to the left side, the fish also swam turning left. Sometimes it moved like a pestle moved within a mortar. But it still swam actively nibbling the bottom, even when not feeding.

Both kyphosis and scoliosis developed in the vitamin C deficient fish. But the kyphosis was more prominent than the scoliosis. Since these two conditions were found in one and the same fish, it was called kyphoscoliosis (Fig. 2). The long axis of the body was short showing a condition called short body dwarfism.

The fish with kyphoscoliosis (Fig. 2) swam with its head down and tail up position, that is, the long axis of the body was almost perpendicular to the bottom. The fish
showed this postural difference not only during swimming but also during rest. It might be probably due to the loss of stability caused by change in position of the dorsal fin. The fish died after four days, since a slight haemorrhage was noticed at the base of right pectoral fin (Fig. 2).

5. 3. DISCUSSION

The fish fed vitamin C deficient diets displayed the clinical signs, such as kyphoscoliosis (Fig. 2), lordosis (Fig. 3), bilateral exophthalmia (Fig. 4), external haemorrhage (Fig. 5), internal haemorrhage (Figs. 6 & 11), caudal fin erosion (Fig. 7), scale erection (Fig. 8), dermal ulceration and oedema (Fig. 9) and emaciation (Fig. 10). A major fruitful discovery from the present investigation was that the fish reared in vitamin C deficient diets, displayed defective neurosensory symptoms represented by ataxia, hyperirritability, imbalance and muscular fibrillation. In this investigation, P. latipinna nourished sufficiently with vitamin C exhibited normal neurosensory activities such as balanced agile swimming, and active feed intake in contrast to those fed vitamin C deficient diet. Some of the aberrant behaviours of the fish in association with the deficiency of vitamin C in the diet were observed to have solitary swimming, surface swimming, snake like swimming movement, bottom scrapping and lethargic swimming behaviour. The arrhythmic opercular beat and laborious breathing seemed to be an indication of total anaemic condition of the fish.

The behavioural abnormalities as observed in the present study on scorbutic P. latipinna, seemed to be associated with neuromuscular disorder resulting from any one of or in combination of the following causes, viz., cranial injuries, neural lesions, myopathy, myolysis and skeletal deformities due to genetics, nutritional deficiencies particularly minerals, and vitamins, various environmental pollutants, parasitic/pathogenic infections
and also at times due to unknown causes. On these, the detailed bibliography had been compiled by Dawsan (1964 and 1971), Dawson and Heal (1976).

Erratic swimming behaviour known as spinning tilapia and whirling disease had been found due to infection of virus (Bohle virus) (Ariel and Owens, 1997) and myxosporeans of many species particularly Myxobolus cerebralis (Halliday, 1976), M. buri (Egusa, 1985, Sakaguchi et al., 1987) M. sandrae (Jirilom et al., 1991) Triangula percae (Langdon, 1987) and T. nikolskii (Moshu and Molnar, 1997). All these parasites caused cranial lesions, spinal deformities, myolysis, myopathy, asymmetrical change in muscle tone and secondary compression of vertebrae especially in vertebrae 1-6, and abnormal spinal curvature notably scoliosis and lordosis which in turn made the fish to swim abnormally either due to partial damage to the neuromuscular activity or consequent to the unnatural body shape so attained.

The occurrence of external haemorrhage observed at the base of the fins (Fig. 5), the internal haemorrhage (Fig. 6), the pinpoint haemorrhages noticed over the general body surface, the scale loss and the dermal ulcerations (Fig. 9) confirmed that P. latipinna could not tolerate vitamin C deficiency. These clinical signs should not be ignored since many ornamental fish farms culturing P. latipinna experience with similar problems, because of the inadequate addition of vitamin C during the preparation of supplementary diet.

An interesting report of vitamin C deficiency in the study was the occurrence of tadpole like pin head fish in P. latipinna with emaciated trunk (Fig. 10). This has also revealed that P. latipinna slowly developed anorexia that was responsible for a negative response to feed and then to muscle loss. The development of perforation in the abdominal wall (Fig. 11) was found to be mainly due to malsynthesis of collagen, a protein cement essential for the maintenance of tissue integrity. Invariably P. latipinna fed ascorbic acid
deficient diet expressed capillary fragility as indicated by haemorrhage problems. Biochemical reports said that it was mainly due to underhydroxylation of collagen which caused the rupture of blood capillaries in deficient fish.

It could be seen from Fig. 12 that even the developing embryos of *P. latipinna* were not exempted from the influence of scorbutic mother and the report was of a first hand information to the ornamental fish culturist. In fact the deficiency did not permit the sac fries to fully utilize the yolk as nourishment. This was an expression of exophthalmia which could be emphasized as a premature disorderly birth of *P. latipinna* fries. Further the investigation emphasized that the scorbutic mother fish could not give birth to normal young ones unless the diet was incorporated with vitamin C sufficiently.

The toxic effects of insecticides on the metabolism, especially that of neural and muscular too had been reported to cause not only the defective swimming behaviour but also the spinal defects. Murty et al. (1984) had reported erratic movement, swimming head down, rolling, jabbing of snout, and bending backwards due to parathion, which were similar to the observations made during the present study with *P. latipinna*. The insecticides, kepone and malathion and the herbicide, trifluralin were too reported to produce similar abnormalities (Weis and Weis, 1976; Couch et al., 1977; 1979) and the herbicide in addition cause vertebral dysplasia. In general, most of the environmental toxicants at sublethal level had been found to produce vertebral damage (Bengtsson, 1975). Kepone was also found to induce scoliosis in *Cyprinodon variegates* (Weis and Weis, 1976).

The abnormal swimming behaviour in European smelt, *Osmerus operlanes* due to lead and copper was due to the inhibitory effect (Pohl, 1990). The pulp mill effluent had
been chiefly found to cause fin erosion and fin rot (Srivastava, 1984; Bengtsson, 1991; Lindesjoo and Thulin, 1994; Sharples et al., 1994; Sharples and Evans, 1996).

Vitamin deficiency particularly riboflavin, (Amezaga and Knox, 1990) cyanocobalamin, folic acid, (John and Mahajan, 1979) and ascorbate commonly induce lethargy, pin headness, fin erosion and abnormal swimming behaviour. Moreover as discussed below among the vitamins, the ascorbate deficiency had been reported to produce a variety of abnormalities including acute skeletal damage (Ashley et al., 1975; King, 1975; Lovell, 1975). Interestingly Hilger (1992) in Gadus morhua had identified spinal deformities and spinal compression due to unknown cause, which were similar to congenital defect reported by Tave et al. (1982) in tilapias namely stump body syndrome. The symptoms were hemivertebra luxation and spina bifida, kyphoscoliosis, stenosis of body cavity, reduction in the number of vertebrae to 15 from the normal more than 29 due to hypoplasia or aplasia (agenesis) resulting in vertebral bodies with multiple pairs of ribs as opposed to fusion defect affecting multiple endochondral ossification centres.

The gross morphological abnormalities and the swimming behaviour observed in the present study were more in common with those produced by tannery waste in carps (David and Roy 1960) chiefly vibratory gill movements, loss of balance and overturns, alternating loss of equilibrium and recovery, laborious breathing, swimming in slanting manner, quick jumping movements, surfacing and gulping for air.

P. latipinna with the extreme degree of scoliosis expressed lethargic swimming behaviour as noticed by disorderly pushing of the tail along the left side and also by whirling movement of the fish in a circular direction. Interestingly in the present study, kyphosis and kyphoscoliosis were observed in addition.
A dual syndrome of kyphosis and scoliosis as observed in *P. latipinna* in this study was a first hand report of vitamin C deficiency (Fig. 2). The clinical sign as recognized by shortening of the long axis of the body with the loss of stability because of change in the position of the dorsal fin which is the attractive external structure of the molly for hobbyists was due to vitamin C deficiency in this kyphoscoliotic fish. Besides the indication of haemorrhage in the right pectoral fin (Fig. 5) showed brevity of the vitamin C deficiency and the victimization of the scorbutic fish.

Sadler (1990) pointed out that scoliosis at times was initiated during embryonic development. Incomplete dorsal fusion of vertebrae around spinal cord known as spina bifida was another spinal deformity originating during embryogenesis. The causes for spinal deformities had been reported to be the product of genetics (Lodi, 1978; McKay and Gjerde, 1986), defective feed (Zitzow and Millard, 1988), essential aminoacid deficiency especially of tryptophan (Akiyama et al., 1986; Post, 1993b), environmental physical parameters like temperature and light and also salinity (Bolla and Holmefjord, 1988; Langdon, 1988; Polo et al., 1991; Liu et al., 1994), hormonal imbalances (Barbaro et al., 1991; Brown and Nunez, 1994; Brown and Kim, 1995) and pollutants (Thatcher, 1979; Sindermann, 1979; 1988; Hiraoka and Okuda, 1983; Wunder and Schmeller, 1988; Roberts, 1989; Sen et al., 1991). As earlier herein discussed, not only the infectious agents were responsible but also the prophylatic agents and the antibacterial disinfectants were the reasons for the development of spinal deformities (Takashima et al., 1976; Koo and Johnston, 1978; Meyers and Jorgensen, 1983; Shrestha, 1985; John and Kloppman, 1989; Bergh and Jelmert, 1990; Daoulas et al., 1991; Oyen et al., 1991; Tave and Handwerker, 1994). Accidental injury to fishes from man made structures was also another cause (DeVore and Eaton, 1983).
It was evident on observing detectable symptoms of scurvy in *P. latipinna* that the fish fed diet deficient of vitamin C were the actual victims. Syndromes related to spinal curvature (Figs. 2 & 3) were observed to be the confirmation of insufficient content of vitamin C in the test diet and the subsequent clinical sign of the deficiency.

Vitamin C deficiency seemed to be the chief cause when the fishes were fed formulated feed, wherein the reason for deficiency was leaching of water soluble ascorbate from the feeds, on which the reports speak of mainly scoliosis and lordosis (Halver et al., 1969; Lovell, 1973; Lim and Lovell, 1978; Post, 1993b). Ascorbate is essential for spinal development as well as for effective maintenance and repair of spinal tissues. The deficiency causes osteoporosis and consequently leading to brittleness of bony elements notably in the vertebral column and distortion of the supporting cartilage. In acute cases as in the present study along with scoliosis and lordosis, the distress was compounded by kyphosis and kyphoscoliosis which could be considered as a diagnostic symptom pointing to ascorbate deficiency.

The focal scale damage resulting in a hole or intrusion of the osseous scale layer into the underlying fibrillary plate, found among chum salmon (*Oncorhynchus keta*) and the potential use of these traits for stock identification were reported by Bigler (1988). It was described by Crichton (1935) that in Atlantic salmon (*Salmo salar*), the scale resorption commenced, when the fish stopped feeding at the onset of the spawning migration and following the cessation of feeding, cells which had previously functioned to enlarge the scale reversed the function and commenced the removal of the ossified layer. Several authors noted that focal scale damage (resorption) had resulted following experimentally induced periods of starvation in carp (*Cyprinus carpio*) (Ichikawa, 1953) and gold fish (*Carassius auratus*) (Yamada, 1956; Ikeda et al., 1974). These authors concluded that the
resorption of material from the scale focus, as well as from the margin, represented the extreme and occurred under conditions of reduced diet.

A significant reduction in the number of circuli of the scales of scorbutic *P. latipinna* expressed quite a good number of aberrations as shown by a general reduction in size and damage of a considerable number of scales and such factors were clear indication of vitamin C deficiency (Figs. 14-18).

Several previous studies had found that changes in feeding level highly influenced circuli patterns (Grey and Setna, 1931; Major and Craddock, 1962; Bilton and Robins, 1971a; Suzuki and Wada, 1985). Bigler (1988c) reported that focal resorption occurred at a constant rate within a given spawning population and populations in close geographic proximity showed similarities in the frequency of occurrence. The mechanism responsible for focal scale resorption in chum salmon and the distribution of this trait among chum salmon throughout the North Pacific Ocean, the distribution of focal resorption among the scale of an individual fish, the occurrence in other species of salmon and the use of this trait as a population identification character were addressed by Bigler (1989).

While concluding from the above comparative symptomology of external signs, it is amply clear that the behavioural and trait associated symptoms are solely due to the altered physical frame which in turn is reflective of internal neuromuscular and skeletal injuries. The available information and also in conformity with the present study indicates that any one of the toxicants among the diverse spectrum of environmental insults which corrupt external environment and the parasitic (microbial, protozoan and metazoan) infections and the nutritional deficiencies including vitamin C which vitiate the internal organ systems could be the cause for the neuromuscular skeletal injuries. Thus the symptoms do not lead us to any explicit cause and effect inference. The following observed symptoms, viz., external haemorrhage, petechiae, anaemia, perforation in the abdomen and emaciated tadpole like appearance (pin head fish) seem more specifically indicative of non availability of vitamin C. This is useful for aquacultural application.