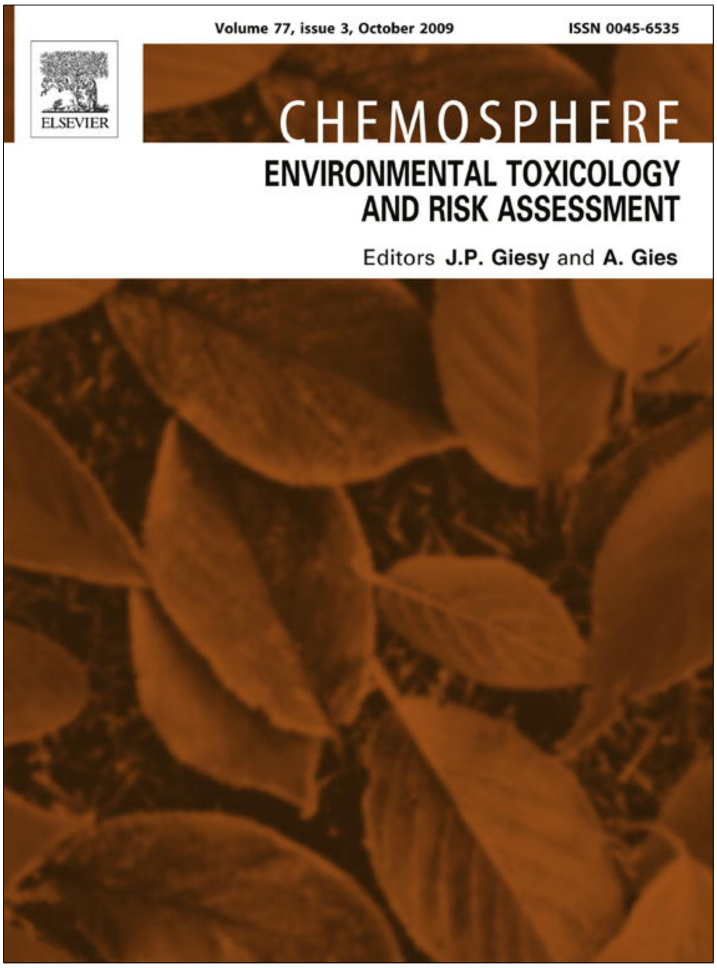
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Identification of some skeletal deformities in freshwater teleosts raised in Egyptian aquaculture

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ABSTRACT

This study examines different forms of skeletal deformities detected in fish species collected from two Egyptian aquaculture facilities during two harvest seasons in 2008. Various patterns of skeletal deformities were observed in 19 of 959 fish collected. Deformities were diagnosed using a number of techniques including clinical, radiographic, sonographic and histopathological evaluations. Observed deformities included: lordosis and kyphosis in African catfish (*Clarius gariepenius*); lateral projection of the mandible, parrot-like head, scoliosis, kyphosis, lordosis and fusion of dorsal with anal fins in Nile tilapia (*Oreochromis niloticus*); and stump body, scoliosis and mandibular joint deformity in common carp (*Cyprinus carpio*). Relative incidences of deformities in fish from a facility located in the Sharkia province were 5.12%, 2.66% and 2.85% among catfish, Nile tilapia and common carp, respectively. At a second fish farm located in the Kafr Elsheikh province, the incidences of deformities were 1.02%, 1.55% and 0% among catfish, Nile tilapia and common carp, respectively. Some of the deformities were confirmed using both sonographic and histopathological evaluations. The reasons for the observed deformities could not be definitively determined, but possible aetiologies are discussed.

1. Introduction

Egypt is geographically bounded by a number of large natural water bodies, including the Mediterranean Sea to the north, Red Sea to the east, Lake Nasser to the south and the Nile River running through the middle of the country. Fish harvests from these sources are insufficient to satisfy Egyptian needs. Thus, aquaculture has been adopted as a logical solution. The aquaculture industry in Egypt is growing rapidly and is currently a top 10 worldwide producer (FAO, 2005). Freshwater fish such as Nile tilapia (*Oreochromis niloticus*), common carp (*Cyprinus carpio*) and African catfish (*Clarius gariepenius*) are the most widely farmed fishes in Egypt.

There are several limiting factors affecting the pisciculture industry in Egypt, including infectious diseases, nutritional disorders, environmental pollution and some managerial factors. Fish deformities are the end product of some or all these factors and as such might be indirectly considered as limiting factors for the industry. Deformities can be the direct or indirect cause of death and low body weight gain in young fish. Because malformed fish are usually rejected by consumers, they are often culled prior to reaching the market (Boglione et al., 2001; Issa, 2008).

Skeletal deformities in fish may be caused by pollutants, nutritional deficiencies and genetics. Moreover, some environmental factors such as thermal shock and overcrowding might play a role in the generation of deformities in certain types of fish during early growth stages (Milton, 1971; Brown and Nuñez, 1998; Vogel, 2000). Deformity mechanisms are not yet well understood (Bengtsson, 1979; Al-Harbi, 2001) but in most cases appear to be linked to the disruption of early developmental processes (Longwell et al., 1992).

Although it is difficult to confirm the aetiology of deformities, it has been suggested that skeletal deformities in fish are good bioindicators of pollution (Bengtsson, 1979; Lien, 1997; Klumpp et al., 2002). The prototypes of such malformations can predict whether long term exposures or acute contact are the underlying mechanism. Long term exposure can be predicted when the incidence of malformation increases with fish age (Slooff, 1982). Conversely, acute exposures can be predicted if anomalies are found in early developmental stages, including when bioaccumulated pollutants are maternally passed to developing eggs (Lien, 1997).

Although numerous publications have discussed the possible causes of skeletal deformities, none have definitively documented

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Table 1
Prevalence of spinal deformities from the investigated fish farms during two harvest seasons of 2008.

Type of fishes	Total number of examined fish		Total number of fish with spinal deformities		Percentage	
	Sharkia	Kafr Elsheikh	Sharkia	Kafr Elsheikh	Sharkia (%)	Kafr Elsheikh (%)
African catfish	39	98	2	1	5.12	1.02
Nile tilapia	300	405	8	7	2.66	1.55
Common carp	70	47	1	0	2.85	0.00
Total	409	550	11	8	2.68	1.45
Cumulative	959		19			
					1.98	

a specific cause. Most of the reported deformities have been attributed to the following causal factors: vitamin C deficiency (Dabrowski et al., 1988); presence of heavy metals (arsenic, cadmium, copper, lead, mercury, zinc) (Bengtsson, 1975, 1979; National Research Council, 1977); high levels of vitamin A (Lim and Lovell, 1978); organophosphate (Mount and Stephen, 1967) and organochlorine chemicals (Couch et al., 1977); genetics (Tave et al., 1983; Mair, 1992); traumatic injury (Breeder, 1953; Gunter and Ward, 1961); strong water currents in very early developmental stages (Backiel et al., 1984); the histozoic parasite, *Myxobolus cerebralis* (Treasurer, 1992; MacConnell and Vincent, 2002); *Icthyophonus hoferi* infection (Bailey, 2004); and bacterial infections (Madsen et al., 2001; Decostere et al., 2004; Pasnik et al., 2007).

Various types of deformities have been reported in different fish species (Easa et al., 1989); these include scoliosis, lordosis, spondylolisthesis, mandibular deformities, semi-opened operculum, stump body, pug-head, double fins, fin fusion and cross bits (Bruno, 1990; Al-Harbi, 2001; Cunningham et al., 2005; Issa, 2008).

In the past, most diagnoses of fish diseases were made through euthanasia and necropsy because clinical or physical evaluations of fish and available diagnostic tests were limited (Love and Lewbart, 1997). Today, diagnostic imaging is used as a complementary technique to further evaluate specimens. Radiography is mainly used for detecting skeletal and swim bladder disorders. Coelomic details are poor in fish, and therefore evaluation of coelomic soft tissues has been limited (Love and Lewbart, 1997). The primary purpose of sonography in fish is to determine the sex, maturational status and general health of commercially important fish (Sandee and Popee, 1995; Martin-Robichaud et al., 2000).

This study reports different patterns of skeletal deformities in important freshwater fishes cultured in Egypt. We predict possible aetiologies of deformities, and describe efficient methods to confirm diagnosis using radiographic and sonographic techniques.

2. Materials and methods

2.1. Fish sampling

A total of 959 market size fish were collected during two harvest seasons from two polyculture, semi-intensive fish farms located in Sharkia and Kafr Elsheikh, Egypt. A total of 409 fish (39 catfish, 300 tilapia and 70 common carp) were collected from the Sharkia fish farm during April 2008. A total of 550 fish (98 catfish, 405 tilapia and 47 common carp) were collected from Kafr Elsheikh fish farm during November 2008.

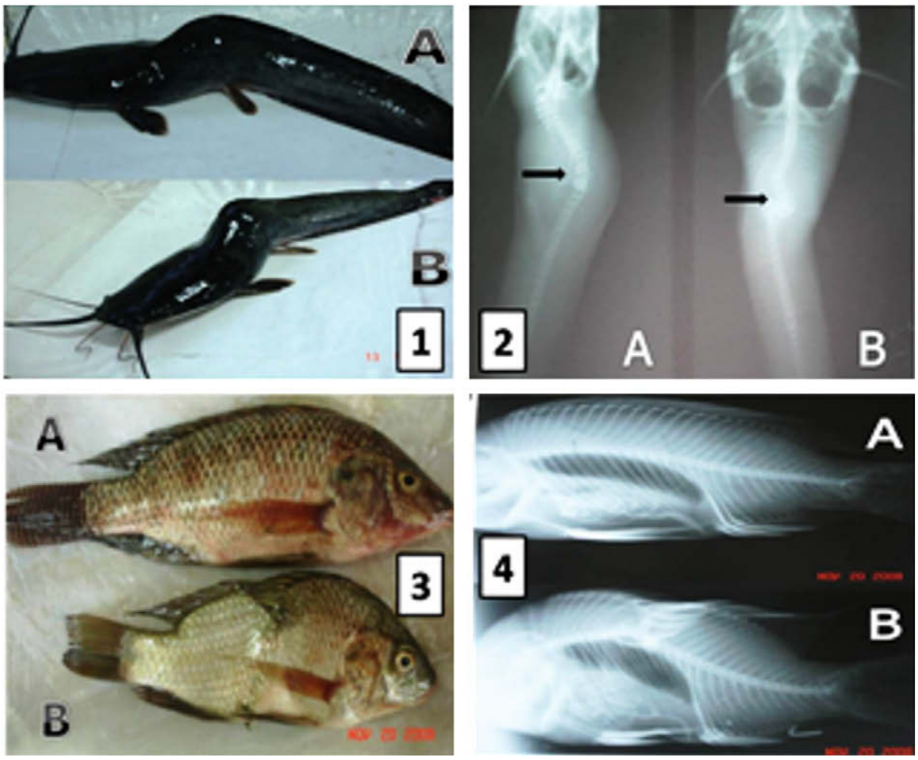

Fig. 1. (1) Lateral (A) and dorsal (B) views of African catfish with hump-like thickening at the antero-dorsal region. (2) X-radiograph of lateral (A) and dorsal–ventral view (B) of African catfish showing S-shaped dorsal spinal curvature (lordosis) with marked separation of one or more vertebrae at the site of S-shaped curvature. (3) Normal (A) and deformed (B) Nile tilapia with body stricture due to marked fusion of dorsal with anal fins. (4) X-radiograph showing normal (A) and deformed (B) Nile tilapia with marked fusion of dorsal with anal fins.

2.2. Clinical examination

Clinical examinations of fishes were performed at the fish farm harvesting areas by gross examination with the unaided eye. The three major anatomical regions of the fish body (i.e., head, trunk and tail) were thoroughly inspected for the presence of any deformities. Deformed and comparably normal fishes were kept on crushed ice and transferred to the Fish Diseases and Management Laboratory (FDML), Cairo University (Cairo, Egypt) for further evaluation.

2.3. Radiographic examination

A radiograph (Toshiba-Varian, TF-6TL6, Utah, USA) with a technique chart utilising 40–50 kV and 10–20 mA was used. For radiographic imaging, fishes were placed onto film cassettes in lateral and/or dorso-ventral positions.

2.4. Sonographic examination

Two-dimensional, real-time sonography was performed using an 8-MHz convex transducer (Toshiba, Just Vision 200 SSA-320 A, Tokyo, Japan). To obtain sonographic images, fish were held in lateral and dorso-ventral positions.

2.5. Histopathology

Tissues samples were fixed in 10% neutral buffered formalin solution, and were then processed and embedded in paraffin. Five-micron sections of tissue were stained with hematoxylin and eosin (H & E) using methods described by Bancroft et al. (1996).

3. Results

During the harvest season, fish were subjected to thorough clinical examination for the detection of any abnormalities. Visual examination of fish from the farms studied revealed the presence of different prototypes of skeletal deformities. In particular, skeletal deformities were detected in 19 of 959 fish sampled in various species. The incidences of skeletal deformities in fish sampled from the Sharkia facility were 5.12%, 2.66% and 2.85% among catfish, Nile tilapia and common carp, respectively. The incidences of deformities in fish from the Kafr Elsheikh facility were 1.02%, 1.55% and 0% among catfish, Nile tilapia and common carp, respectively. The incidences of deformities were significantly lower at the Kafr Elsheikh facility than at the Sharkia farm (Table 1).

For a complete assessment of deformities in African catfish, clinical, radiographic, sonographic and histopathological techniques were used to identify deformity types. Clinical evaluations of catfish indicated a large, tumour-like growth or hump-like thickening at the antero-dorsal region (Fig. 1(1)). Radiographic examination revealed the appearance of S-shaped dorsal spinal curvature (lordosis) concomitant with the separation of one or more vertebrae at the site of the S-shaped curvature (Fig. 1(2)). Sonographic examination of the vertebral column showed a well marked hyperechoic deformity in the form of an S-shaped curve on the back (Fig. 2). Histopathological examination of tissue samples revealed marked infiltration of epidermal inflammatory cells, dermal edema and proliferation of melanophores (Fig. 4(1)), as well as muscular edema and congestion of blood capillaries (Fig. 4(3)).

Clinical examination of harvested Nile tilapia showed variations in the patterns of skeletal abnormalities that were not seen in catfish. However, patterns of deformity in Sharkia tilapia were much more substantial than in tilapia sampled from Kafr Elsheikh. We observed five types of skeletal deformities among Sharkia tilapia, while only two deformity types were clinically determined in the Kafr Elsheikh tilapia (Table 2). Examined fish from both locations presented with lateral projections of the mandible, fusion of the dorsal and anal fins (Fig. 1(3)), scoliosis, kyphosis, lordosis (Fig. 3(1)) and parrot-like heads (Fig. 3(3)). Radiographic examination of the clinically deformed fish confirmed the details of each type of deformity (Figs. 1(4) and 3(2) and (4)).

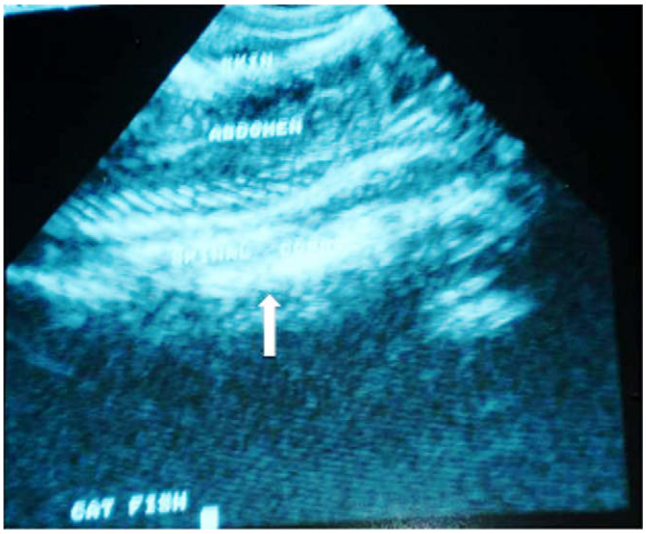

Fig. 2. Sonograph of a deformed African catfish showing S-shaped dorsal spinal curvature (Lordosis) together with separation of spinal vertebrae (arrow).

For common carp, deformities were only found among fish harvested from the Sharkia facility. No skeletal deformities were detected in Kafr Elsheikh fishes (Table 2). Specifically, scoliosis, mandibular joint deformities and stump body (dwarfism) were recorded among the examined Sharkia carp. Histopathology revealed a marked case of spondylolisthesis and bone necrosis (osteosis) (Fig. 4(2)) in which one or more vertebrae were observed to be slipped out of their normal axis. This pathology was concomitant with the proliferation of newly formed intervertebral osteogenous tissue incorporated with mononuclear cells (Fig. 5). Higher magnification (400× power) indicated the occurrence of osteophagia, where osteoclast cells were encircling and phagocytosing the necrotic bony tissue (Fig. 4(4)).

Table 2
The reported types of spinal deformities from the investigated fish farms during two harvest seasons of 2008.

Type of fishes	Total number of examined fish	
	Sharkia	Kafr Elsheikh
African catfish	1. Lordosis 2. Kyphosis	1. Lordosis and kyphosis
Nile tilapia	1. Lateral projections of the mandible	1. Scoliosis
	2. Parrot like head	2. Fusion of dorsal and anal fins
	3. Scoliosis 4. Kyphosis, lordosis 5. Fusion of dorsal and anal fins	
Common carp	1. Stump body 2. Scoliosis 3. Mandibular joint deformity	No deformities were reported

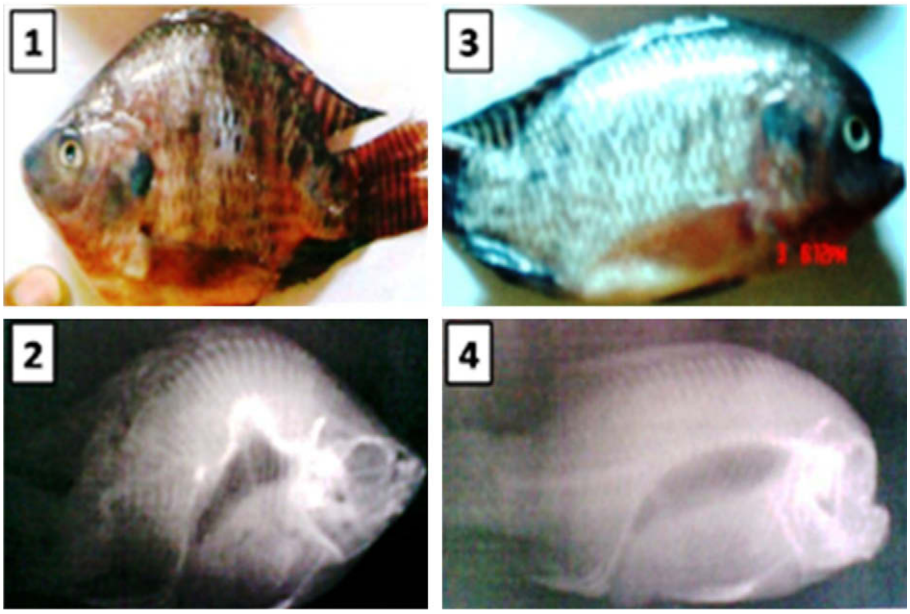

Fig. 3. (1) Nile tilapia with marked lordosis (dorsal spinal curvature) and kyphosis (ventral spinal curvature). (2) X-radiograph showing Nile tilapia with marked lordosis (dorsal spinal curvature) and kyphosis (ventral spinal curvature). (3) Deformed Nile tilapia with marked parrot-like head (anterior mandibulo–maxillary projection). (4) X-radiograph showing deformed Nile tilapia with marked parrot-like head.

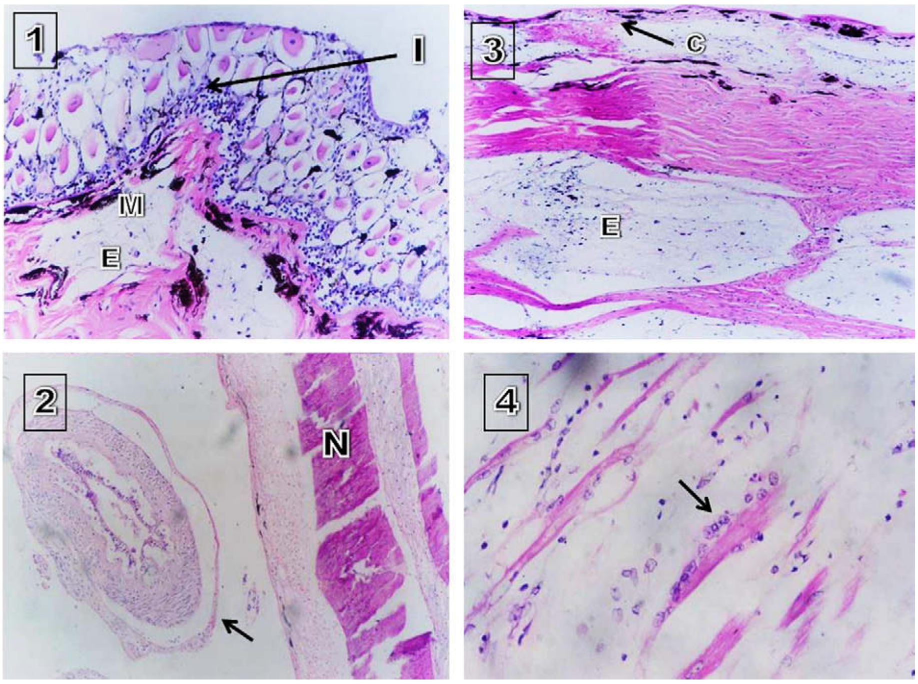

Fig. 4. (1) Photomicrograph of catfish skin showing infiltrations of epidermal inflammatory cells (I), dermal edema (E) and proliferation of melanophores (M) (H & E, 200×). (2) Photomicrograph of common carp showing osteosis (bone necrosis)(n) and severely congested blood vessels (arrow) (H & E, 100×). (3) Photomicrograph of catfish showing congestion of the cutaneous blood capillaries (C) and marked muscular edema (E) (H & E, 100×). (4) Photomicrograph of common carp showing osteophagia (osteoclast cells are encircling and phagocytosing the necrotic bony tissue) (arrows) (H & E, 400×).

4. Discussion

Skeletal deformities in fish farmed by the Egyptian aquaculture industry constitute an economic problem that decreases the market value of fish. Such deformities are rarely observed in natural teleost populations but are quite common in farm-raised fish held in captivity (Sennar, 1980). Several studies have focused on deformities of the skeletal system. Moreover, a wide spectrum of aetiologies have been suggested, including genetics, inbreeding depression, temperature fluctuation during early life stages, low pH, parasitism, nutritional deficiencies and environmental pollution (Orska, 1962; Koumoundouros et al., 1997; Eissa and Moustafa, 2008). A number of diagnostic techniques have been widely employed to detect fish skeletal deformities; these include radiog-

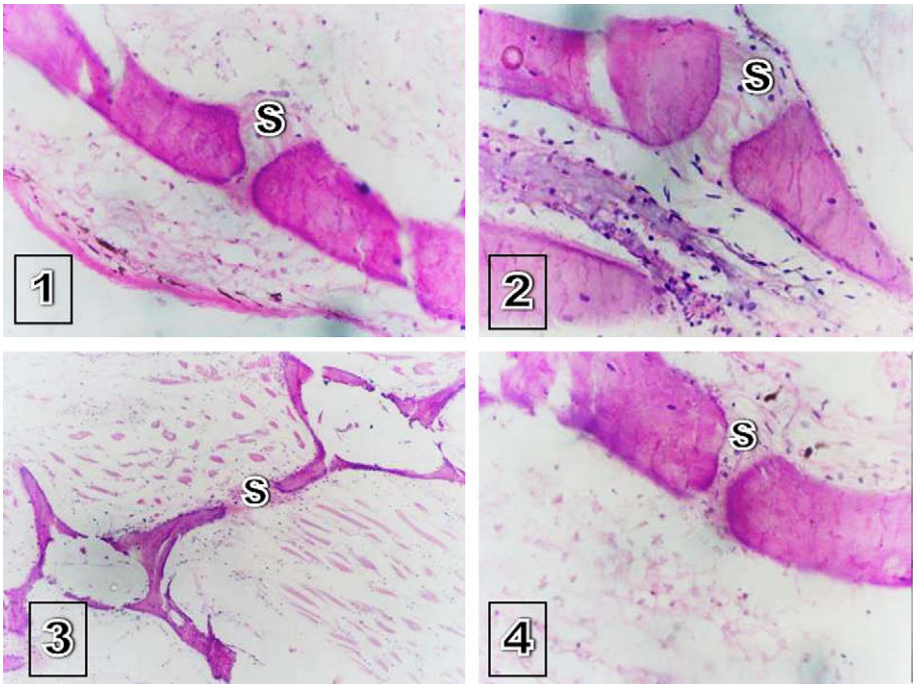

Fig. 5. Photomicrographs of common carp showing spondylolisthesis (slipping out of one or more vertebrae from its normal axis) with the proliferation of newly formed intervertebral osteogenous tissue (S) incorporated with mononuclear cells (arrows) (H & E, 200×).

raphy, sonography and histopathology. Such diagnostic techniques were efficiently used in our study to reveal the type, location and magnitude of deformities in a number of cultured fish (Figs. 1–5).

Skeletal deformities in fish harvested from the two subject facilities were characterised by variations in the incidence and degree of deformities. Also, the identification of deformities among cultured fish coincided with studies reporting that deformities in teleosts are rarely found in natural populations but are common in farmed fishes (Sennar, 1980; Al-Harbi, 2001).

Incidences of skeletal deformities among fishes harvested from Sharkia were higher than those at Kafr Elsheikh (Tables 1 and 2). In accordance with Egyptian agricultural standard regulations, both facilities usually use freshwater from streams that is mixed with agricultural drainage water. However, the Sharkia province is surrounded by a large number of industries including weavers, ceramic manufacturers, oil refineries, charcoal producers and metallurgical industries. These industrial facilities emit significant amounts of pollution into the atmosphere that can accumulate in upper atmospheric layers by condensation, thereby forming acid clouds. This condition produces significant acid rain events across vast areas of the Sharkia province. Fish exposed to acid rain may be susceptible to numerous adverse effects including reproductive failure, reduced growth, poor respiration and notable skeletal deformities (Sarkar and Kapoor, 1956; Andrades et al., 1996). Skeletal deformities in fish exposed to acid rain may be associated with a response to reduced blood pH caused by elevated carbon dioxide. Homeostatic mechanisms will respond to maintain normal serum osmosis and return the blood to a normal pH. This may lead to bone decalcification due to the presence of excessive carbonic acid generated as blood pH normalises (Sarkar and Kapoor, 1956; Andrades et al., 1996). These findings might explain the relatively high incidence of deformities in Sharkia fish as compared to those from Kafr Elsheikh.

It is notable that Kafr Elsheikh province receives more rainfall than Sharkia. This might lead to the hypothesis that skeletal deformities should be more prevalent in Kafr Elsheikh if the atmospheric deposition of pollutants is an important causative factor. However, this conclusion is not supported because there are very few industrial plants near the Kafr Elsheikh aquaculture facility.

Temperature fluctuations during early fish development are another detrimental factor that may induce different prototypes of deformities including skeletal deformities (Ørnsrud et al., 2004). The examined hatcheries were of primitive construction and remotely located. These out-of-date hatcheries could increase embryonated egg exposure to sharp fluctuations in temperature and inappropriate hatching enclosure hydrodynamics, particularly excessive water currents (Zambonino et al., 2005). Such adverse climatic changes might disrupt vital developmental processes during early morphogenesis and might give rise to different deformities (Al-Harbi, 2001; Ørnsrud et al., 2004).

Intensive inbreeding might also lead to a high incidence of spontaneous skeletal deformities such as scoliosis, lordosis, curved neural spines, fused vertebrae and compressed vertebrae (McKay and Gjerde, 1986; Ponyton, 1987). These deformities appear to reflect a genetically defective "lethal gene" rather than an environmental stimulus. This observation is well-supported among land-locked fishes such as *Tilapia zilli*. Natural inbreeding in this species has been observed to cause pyramidal development of genetic defects in subsequent generations (Jawad, 2002). The Nile tilapia, common carp and African catfish examined for this study are also land-locked species. In the current study, male and female broodstocks used in natural (Nile tilapia and African catfish) or artificial (common carp) spawning are the offspring of inbred fish who were land-locked for several generations. This inbreeding pattern could lead to increased development of spontaneous skeletal deformities in Nile tilapia, African catfish and common carp.

Traumatic injury could be another possible cause of skeletal deformities in fish. The muscles of fish are assorted in bands called somites. These somites may be damaged or die if subjected to strong water currents or a bird attack, which can lead to spinal curvature. In our study, dorsal fins were dislocated to fuse with the anal fin. In the most severe cases, the entire fish body had a stricture in the area of the dorsal fin leading to fusion of the dorsal and anal fin (Fig. 1(3)). This fusion could be seen from both sides, suggesting that running hatchery water in the early larval stages might be an important contributor to this deformity. This effect has also been previously reported in Atlantic salmon exposed to similar conditions (Bruno, 1990).

Nutritional deficiencies could play an important role in the development of skeletal deformities. Vitamin C deficiencies might lead to spinal malformations including kyphosis, scoliosis and lordosis (Preziosi et al., 2006). Several individual cases of spinal deformities in catfish were attributed to vitamin C deficiencies (Lim and Lovell, 1978). This deficiency could be due to either an inability to synthesise vitamin C or to vitamin C production that is insufficient for normal cartilage, bone and connective tissue formation (Lim and Lovell, 1978; Preziosi et al., 2006). Moreover, fish exposed to agricultural drainage water containing pesticides might by susceptible to decreases in the physiological functioning of vitamin C. Consequently, these chemical exposures might affect skeletal collagen, resulting in spinal deformations (Preziosi et al., 2006). In our study, heat treatment during pellet production, faulty storage of fish rationing ingredients and direct exposure to agricultural drainage water might lead to reduced availability and efficiency of vitamin C. Thus, vitamin C deficiency could be considered an initial cause of the skeletal deformities observed in African catfish.

Egyptian agriculture drainage water often contains organochlorine and organophosphate pesticides. Such chemicals can induce skeletal deformities in exposed fish by altering biological processes necessary for maintaining the biochemical integrity of bone or neuromuscular tissue. This may lead to deformities without chemical changes in vertebral composition (Chatain, 1994). In Egyptian aquaculture, organophosphate pesticides are regularly used to treat external parasites of fish such as crustaceans (e.g., Argulus and Lernea), leeches and monogeneans. These chemicals must be considered a possible cause of skeletal deformities in fish.

A large number of industrial facilities including textile, ceramic, glass, electrical cable, battery and metallurgical factories surround the Sharkia fish farm. These facilities constitute potential sources of heavy metals, which might reach fish either through direct drainage or atmospheric deposition. For example, cadmium is thought to cause spinal deformities by inducing osteomalacia and neuromuscular damage (Muramoto, 1981; Benaduce et al., 2007). Lead is another heavy metal suspected of causing spinal deformities in a wide spectrum of aquatic animals, including fish (Bengtsson and Larsson, 1986). Both cadmium and lead may be present in Sharkia industrial effluents.

Mycotoxins are another group of chemicals that may cause skeletal deformities (Halver et al., 1969; Couch et al., 1977; Easa et al., 1989; Eissa and Moustafa, 2008). Mycotoxin metabolites such as isocoumarin and related compounds may impair calcium, vitamin C and protein metabolism, all of which are required for healthy connective tissue, cartilage and bone matrices (Halver et al., 1969; Easa et al., 1989). Of particular relevance to our study, faulty storage of fish feed, especially storage under conditions of high humidity and temperatures, may lead to fungal growth and the production of mycotoxins.

Streptococcal infection was implicated in the formation of skeletal deformities observed in Nile tilapia (Pasnik et al., 2007). Mycobacteriosis is another infectious disease that can cause deformities in fish (Decostere et al., 2004). Multiple granulomas might be formed if Mycobacteria spread to skeletal tissue, and this might progress to the formation of spinal deformities such as scoliosis, lordosis and kyphosis. Streptococcus and Mycobacterium species are common inhabitants of sewage effluent, and are associated with human and animal waste contamination. Effluent waters containing these bacteria might reach the water supplies of fish farms.

Minor vertebral changes in fish could be detected radiographically and sonographically, when no observable effects on external morphology were noted. This demonstrates the importance of diagnostic imaging in detecting skeletal deformities in fish. Detected vertebral changes ranged from demineralisation, increased density and slight loss of structure to collapse, fusion and change in intervertebral spacing for multiple vertebrae. This impairment could give rise to lordosis, kyphosis or abnormal vertebrae at several locations.

Radiography and sonography are the two most rapid techniques available for detecting skeletal deformities. Other less rapid diagnostic techniques include histopathology and immunohistochemistry. Histopathology must be considered complementary to clinical examination. Histological evaluations can reveal greater tissue and cellular details than radiography and sonography because of enhanced tissue resolution achieved by staining and microscopic examination.

In catfish, epidermal and muscular edema as well as inflammatory cell aggregation could be caused by chronic irritation linked to erratic movements of deformed vertebrae at sites of lordosis or kyphosis. Such erratic movements from the contraction of muscles during swimming might result in muscular and skin blood vessel injuries. These injuries may in turn lead to the release of blood elements, including plasma and mononuclear cells that result in muscular and epidermal edema as well as the aggregation of mononuclear cells (Fig. 4(1–3)).

In common carp, there were marked cases of spondylolisthesis and bone necrosis (osteosis) (Fig. 4(2)). This injury was diagnosed by observing one or more vertebrae slipping from their normal axis, with resulting chronic injury to surrounding tissues including periosteal and muscular tissues. Chronic inflammatory injury might be the initial event that leads to the proliferation of newly formed intervertebral osteogenous tissue and mononuclear inflammatory cells, as well as osteoclast cells. This tissue and cell proliferation is normally associated with a healing response to repair slipped and necrotised bony tissue of the vertebrae (Fig. 4(4)).

5. Conclusion

Skeletal deformities can impact the normal physiological functioning of fish by disrupting buoyancy, which may ultimately hinder the ability of fish to eat, reproduce and avoid predators. Increased incidences of skeletal deformities among commercial fish species are suggestive of an environmental deterioration that signals the urgent need for timely corrective action.

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