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X-cell pseudotumors in a hardhead catfish *Arius felis* (Ariidae) from Lake Pontchartrain, Louisiana, USA

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ABSTRACT: X-cell epidermal lesions are described from a single specimen of the hardhead catfish *Arius felis* (Ariidae). The lesions exhibited an unusual growth pattern but did not involve any visceral organs. Histologically, the lesions resembled those previously described for coldwater fishes. This is the first report of X-cell lesions from Lake Pontchartrain, Louisiana, USA, and the hardhead catfish represents the first warmwater species affected with the disease.

KEY WORDS: X-cell · *Arius felis* · Hardhead catfish

INTRODUCTION

The nature and origin of X-cells are unknown. These ovoid, pale-staining cells with a large, round nucleus and prominent nucleolus are known to occur only in certain lesions, termed X-cell pseudotumors, reported only from marine teleosts. X-cells may produce large aggregations that superficially resemble true neoplasms (Brooks et al. 1969, Harshbarger 1984), or less commonly, they may also occur in small, isolated clusters of individual cells (Diamant & McVicar 1987). They usually occur in such organs as skin, pseudobranch, and gills (e.g. Peters et al. 1978, Morrison et al. 1982, Diamant & McVicar 1990), but have also been observed in renal, splenic and gonadal tissue (Diamant & McVicar 1987), and from an internal pseudotumor (Kent et al. 1988). X-cells have been studied with conventional histology, electron microscopy, microfluorometric DNA analysis, varous DNA-binding dyes and isozyme analyses.

It has been suggested that there is a correlation between X-cell incidence and environmental pollution (Stich et al. 1976) or that X-cells are virally transformed fish cells (Peters et al. 1978, 1981). Alternatively, X-cells may be invasive unicellular parasites embedded in the fish host's tissue (Alpers et al. 1977, Dawe 1981). Although an increasing body of evidence supports the latter hypothesis, conclusive proof of the parasitic origin of X-cells has yet to be provided.

In this paper, we report on the occurrence of an extensive epidermal X-cell lesion in hardhead catfish *Arius felis* from Lake Pontchartrain, Louisiana, USA. This record extends the condition to include a new teleost taxon (Order Siluriformes, Family Ariidae) as well as a new zoogeographic region.

MATERIALS AND METHODS

Fish were collected in eastern Lake Pontchartrain, using a custom-designed high rise 16 ft (ca 4.9 m) otter trawl with a chain sweep. Two trawls were made with tow durations of 10 min and a towing speed of 2 to 3 knots against the prevailing current. Following capture, fish were counted and examined for evidence of gross pathological abnormalities. A total of 21 hardhead catfish were fixed in Dietrich's solution for histo-

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pathological examination. Water quality data were collected at the site and sediment samples were taken for subsequent toxicity tests and contaminant analyses. Tissue samples from 10 additional catfish were taken for chemical analysis.

Representative samples of gill, spleen, liver, kidney, skin, fin and operculum were processed for routine paraffin histology. Sections were cut at 6 µm and stained with Harris' hematoxylin and eosin. For electron microscopical observation, Dietrich's solution-fixed tissue samples of skin and operculum were rehydrated to water from 70 % ethanol and then transferred to 0.1 M Millonig's phosphate buffer. These tissues were minced into 0.5 to 1 mm^3 pieces, postfixed in 1 % osmium tetroxide in phosphate buffer at 4 °C for 4 h, dehydrated in a graded acetone series, and embedded in Spurr low viscosity resin. Semi-thin sections (1 µm) were cut using a Reichert Ultracut® microtome[1] and stained with toluidine blue. Ultrathin sections (50 to 60 nm) were stained with uranyl acetate and lead citrate (Hayat 1981) and examined using a Zeiss EM902 transmission electron microscope.

RESULTS

The affected fish had a total length of 177 mm and preserved weight of 64.6 g. Grossly, the fish exhibited diffuse, yellowish, slightly elevated epidermal growths on the ventrolateral body surface extending to and involving the pelvic fins and perianal region (Fig. 1). The lesions also involved the inner surfaces of the opercula (Fig. 2) and the pectoral fin bases.

Histologically, both skin and opercular lesions appeared as numerous membrane-enveloped epidermal nodules delimited by a thin external layer of epithelial cells. Nodules were made up of rounded or oval-shaped X-cells that measured up to 35 µm in greatest dimension and exhibited pale cytoplasm and vesicular nuclei. The eosinophilic nuclei contained a single, prominent nucleolus but lacked heterochromatin. Ventral skin lesions were 30 to 40 X-cells in thickness and were restricted to the epidermis. They were covered by a thin layer of epithelium, and occasionally alarm substance cells were situated around or between X-cell nodules (Fig. 3). A mild inflammatory infiltrate was present at the base of some nodules. Opercular lesions were considerably bulkier and formed distinct rugae (Fig. 2). These folds consisted of about 100 X-cells in width and measured approximately 1.55 mm in thickness. The inflammatory response was more extensive in the opercular lesions. Numerous neutrophils and lymphocytes infiltrated connective tissue near the nodule and extended up to the folds of the lesion. In both the skin and opercular lesions, the size of the X-cells tended to decrease from the center of each nodule, with the smallest occurring at the periphery. Additionally, numerous irregularly shaped, basophilic nuclei were dispersed among the X-cells within the nodules (Fig. 4). No mitotic figures or any other evidence of cell division was seen. Gill, liver, spleen and kidney appeared unaffected.

Ultrastructurally, nuclei dispersed among the X-cells were identified as those of envelope cells which are typically associated with X-cell lesions. Slender cytoplasmic extensions of these cells that separated neighboring X-cells were visible in 1 µm plastic sections (Fig. 4). Apart from confirming the identity of envelope cells, ultrastructural observations did not reveal any additional features. The quality of the reprocessed tissue samples was not adequate for observation of any sub-cellular structures so no details of the cytoplasmic structures could be discerned.

Water quality data taken at the time of specimen collection included a temperature of 29 °C, salinity of 4.3 ppt, pH of 6.9, and dissolved oxygen of 6.8 ppm. The depth was 2.0 m. None of the levels of chlorinated pesticides, polychlorinated biphenyls, polycyclic aromatic hydrocarbons or trace metals detected in the sediment exceeded low level concentration limits (Long & Morgan 1990). Sediment toxicity tests were also negative.

DISCUSSION

X-cells have been reported from a wide range of marine teleost species, most of them in the families Pleuronectidae and Gadidae and mainly from cold water environments. Reports have also included members of the Gobiidae, Zoarcidae, Sciaenidae and Nototheniidae (Ito et al. 1976, Desser & Khan 1982, Franklin & Davison 1988, Kent et al. 1988, Bucke & Everson 1992). This, however, is the first report of X-cell disease in a hardhead catfish *Arius felis* (Order Siluriformes, Family Ariidae). The Ariidae include mainly marine catfish that inhabit tropical or subtropical regions. The only other warmwater species known to be affected with X-cell disease was an aquarium specimen of *Cheilotrema saturnum* (Family Sciaenidae) from the southern California (USA) coast which developed an internal X-cell lesion (Kent et al. 1988). The present case is the first where X-cell lesions have been found in a wild fish at 29 °C, by far the highest temperature for this disease.

Although the X-cell lesion in the hardhead catfish was extensive, it was diffuse and did not form prominent verrucal pseudotumors. This growth pattern was

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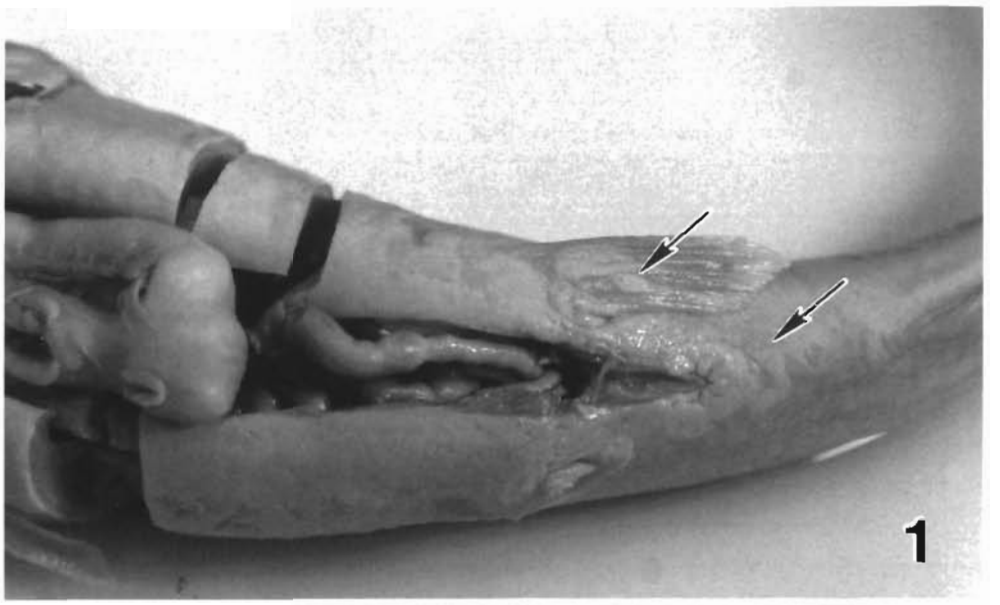
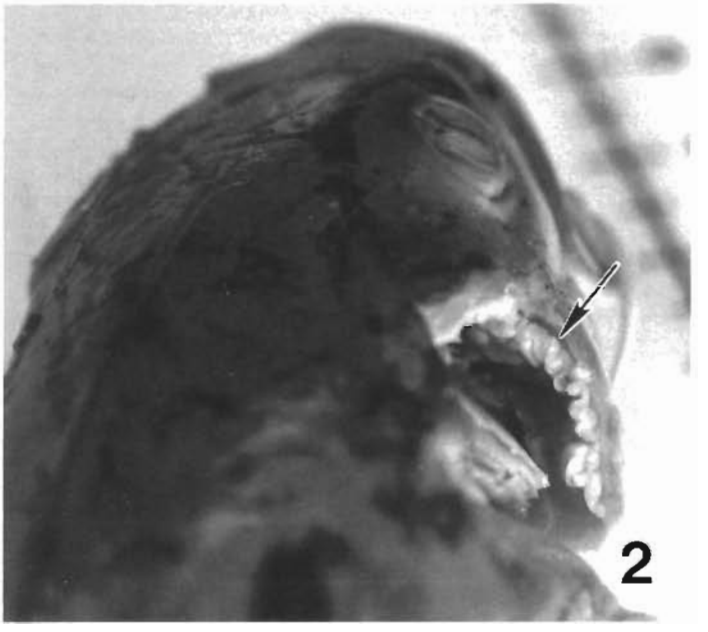
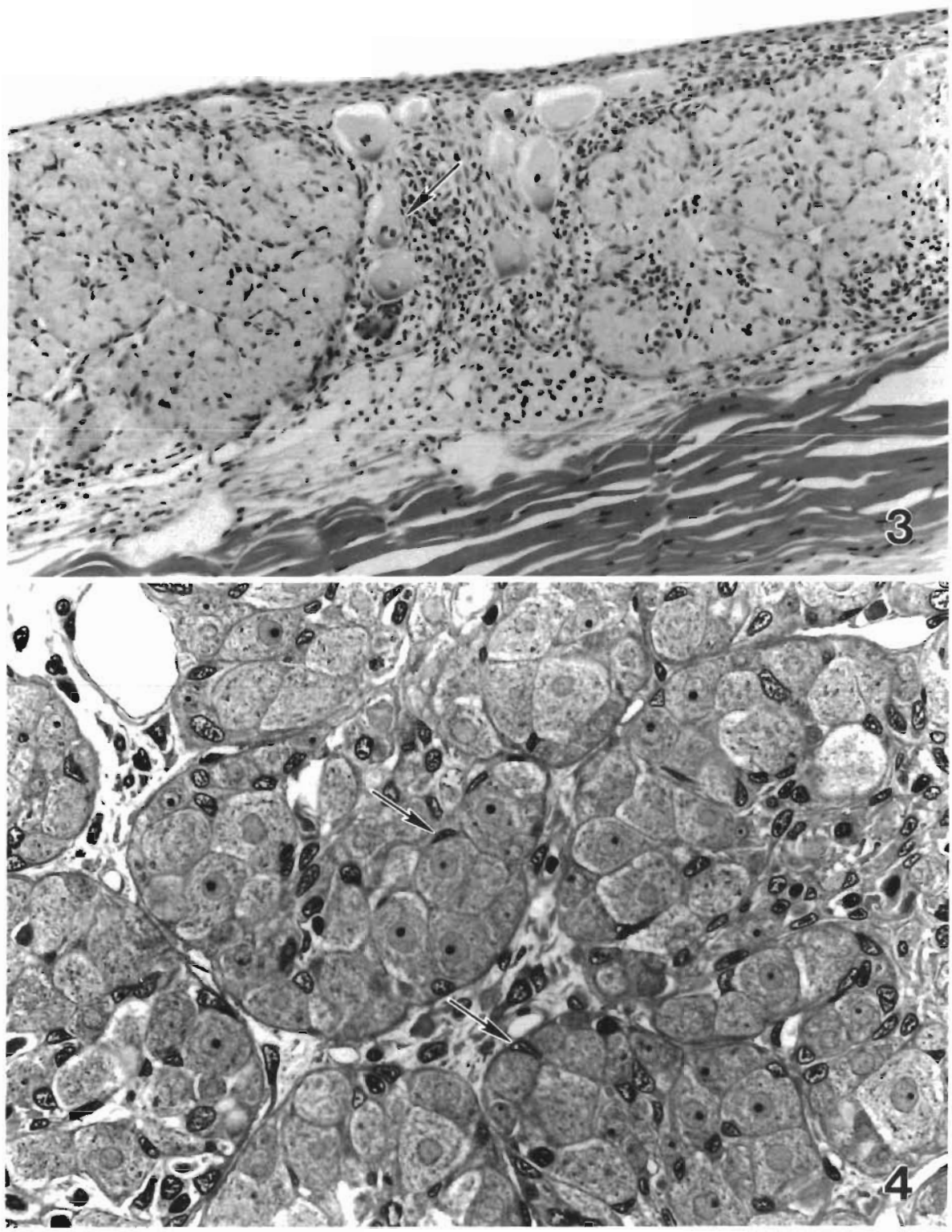




Figs. 1 & 2. *Arius felis*. X-cell lesions in the hardhead catfish. Fig. 1. Ventral view showing diffuse growth pattern. Note elevated epidermal growth on pelvic fin and perianal region (arrows); ×1.7. Fig. 2. View of branchial chamber showing rugose appearance of opercular lesion (arrow); ×2.2

similar to that reported for Pacific Ocean perch *Sebastes alutus* in which lesions appeared as multiple raised and/or sheet-like masses (Myers 1981, in press). These lesions occurred on some of the same locations, including the underside of the operculum, on the fins, and on lateral skin surfaces. In both species, the lesions were composed of multiple, discrete, ovoid nests of X-cells surrounded by connective tissue. Additionally, the hardhead catfish lesion is apparently not related to environmental pollution, based on the low levels of contaminants identified at this site and the negative sediment toxicity tests.

Lesions in the hardhead catfish differ somewhat from those reported in Pacific and Atlantic flatfish (Peters et al. 1978, Watermann 1982). In dab *Limanda limanda* from the North Sea, X-cell lesions were eroded, swollen, contained dilated, congested blood vessels and extended into the underlying stratum compactum and muscle tissue (Watermann 1982). Involvement of gills and internal organs with X-cells is also known in dab (Diamant & McVicar 1987). In Pacific flatfish with dermal X-cell lesions, 'ovoid cells' (X-cells) have been found in the dermal connective tissue underlying the basal lamina (Peters et al. 1978). Even though we also observed a heavy leukocytic inflammatory response, no invasion of X-cells could be found beneath the basal membrane in the hardhead catfish lesions.



Figs. 3 & 4. *Arius felis*. Histologic sections of X-cell lesions. Fig. 3. Skin lesion showing nodular arrangement of X-cells, thin epidermal covering, and alarm substance cells between nodules (arrow); H&E, ×240. Fig. 4. Plastic section showing details of X-cells and nuclei of envelope cells (arrows); Toluidine blue, ×715

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