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Histopathology of red-sore disease (*Aeromonas hydrophila*) in naturally and experimentally infected largemouth bass *Micropterus salmoides* (Lacépède)

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Abstract. The histopathology of red-sore disease, caused by the gram-negative bacterium, *Aeromonas hydrophila*, is described for largemouth bass, *Micropterus salmoides*. Externally, lesions range from those affecting a few scales (pin-point), to those associated with extensive chronic ulcerations; there is focal haemorrhage, oedema and dermal necrosis which exposes underlying muscles producing infiltration of mononuclear and granulocytic inflammatory cells. Internally, the liver and kidneys are foci for toxic products produced by *A. hydrophila* with, in the most severe cases, complete destruction of the structural integrity of both organs. Pathological changes were not serious in either the spleen or heart, even in cases with massive damage in the liver and kidney. Internal and external lesions were similar in both natural and experimentally induced infections. The pathobiology of red-sore disease in bass is postulated to be linked to elevated water temperature stimulating increased metabolism, decreased body condition and stress, leading to the increased production of corticosteroids and the concomitant rise in susceptibility to infection.

Introduction

Red-sore disease is a common infection of warm-water fish, caused by the gram-negative bacterium *Aeromonas hydrophila*. In recent years, several massive epizootics have caused considerable mortality among a variety of fish species in reservoirs throughout the south-eastern United States (Shotts, Gaines, Martin & Prestwood 1972; Miller & Chapman 1976; Esch & Hazen 1978).

Esch, Hazen, Dimock & Gibbons (1976) and Esch & Hazen (1978) studied the epizootiology of red-sore disease among largemouth bass, *Micropterus salmoides* (Lacépède), in Par Pond, a 1012 ha cooling reservoir located on the Savannah River Power Plant, Aiken, South Carolina. They reported higher infection percentages among bass taken from thermally altered sites within the reservoir and that bass with lower body conditions (<2·0) were more likely to have the disease than bass in good condition (2·0–3·0). Throughout the study, representative tissues and organs from infected and non-infected bass, as well as from those in which the disease was induced

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experimentally in the laboratory, were fixed for subsequent microscopic examination.

The main purpose of this paper is to describe and compare the histopathology of naturally acquired, and experimentally induced, red-sore disease in largemouth bass. In addition, we present a hypothetical scheme to explain the interactions of thermally induced stress, the development of lesions and the epizootiology of red-sore disease in largemouth bass from Par Pond.

Materials and methods

Par Pond is a 1012 ha cooling reservoir located on the Savannah River Power Plant, Aiken, South Carolina. Its biotic and abiotic characteristics have been extensively described by Lewis (1974), Parker, Hirshfield & Gibbons (1973) and Hazen (1978).

Largemouth bass used in this study were collected by a combination of electrofishing and angling in thermally altered and ambient temperature areas of Par Pond, as part of a larger study of the epizootiology of red-sore disease (Esch *et al.* 1976; Esch & Hazen 1978). Representative tissues were excised from approximately 100 bass, some of which exhibited characteristic external lesions and from others which did not appear to be diseased. Six disease-free bass were held in laboratory aquaria for 30 days and then exposed to *Aeromonas hydrophila* added in the water; the final concentration of *A. hydrophila* was 10^6 cells/ml. Water temperature in the aquaria was 20°C. The presence and abundance of *A. hydrophila* were determined by the bacteriological culture procedures described by Shotts & Bullock (1975).

All tissues taken for study were rapidly fixed in 10% buffered formalin, or Bouin's fixative, and processed by routine methods (paraffin sectioning, haematoxylin and eosin staining). Selected tissues were stained with Giemsa–Jenner, Masson's trichrome, Ziehl–Nielson and periodic acid Schiff. In the field investigation, tissues were examined (by HWH) without knowledge of the collecting site and attempts were then made to correlate the degree of pathology with the location from which the bass were collected, i.e. thermally altered versus ambient temperature areas. In the experimental laboratory infections, selected red-sore lesions possessing the attached ciliate, *Epistylis* sp., were fixed in glutaraldehyde, post-fixed in osmium tetroxide and processed by standard methods for examination with a scanning electron microscope.

Results

(1) *Bacteriology*

Using the diagnostic scheme of Shotts & Bullock (1975) *A. hydrophila* were isolated in large numbers from the liver, gill, skin, kidneys, heart and intestinal tract of all experimentally infected fish. Naturally acquired infections produced positive *A. hydrophila* cultures from surface lesions, kidneys and intestinal tracts. Non-infected fish produced positive cultures only from the intestine. *A. hydrophila* was confirmed

to be dense in surface lesions by staining impression slides of lesions directly with anti-*A. hydrophila* fluorescent antibody (see Hazen, Raker, Esch & Fliermans 1978 for details of protocol).

(2) *Histopathology*

(a) *Par Pond bass*

Macroscopic lesions. Red-sore lesions were observed on all external locations,

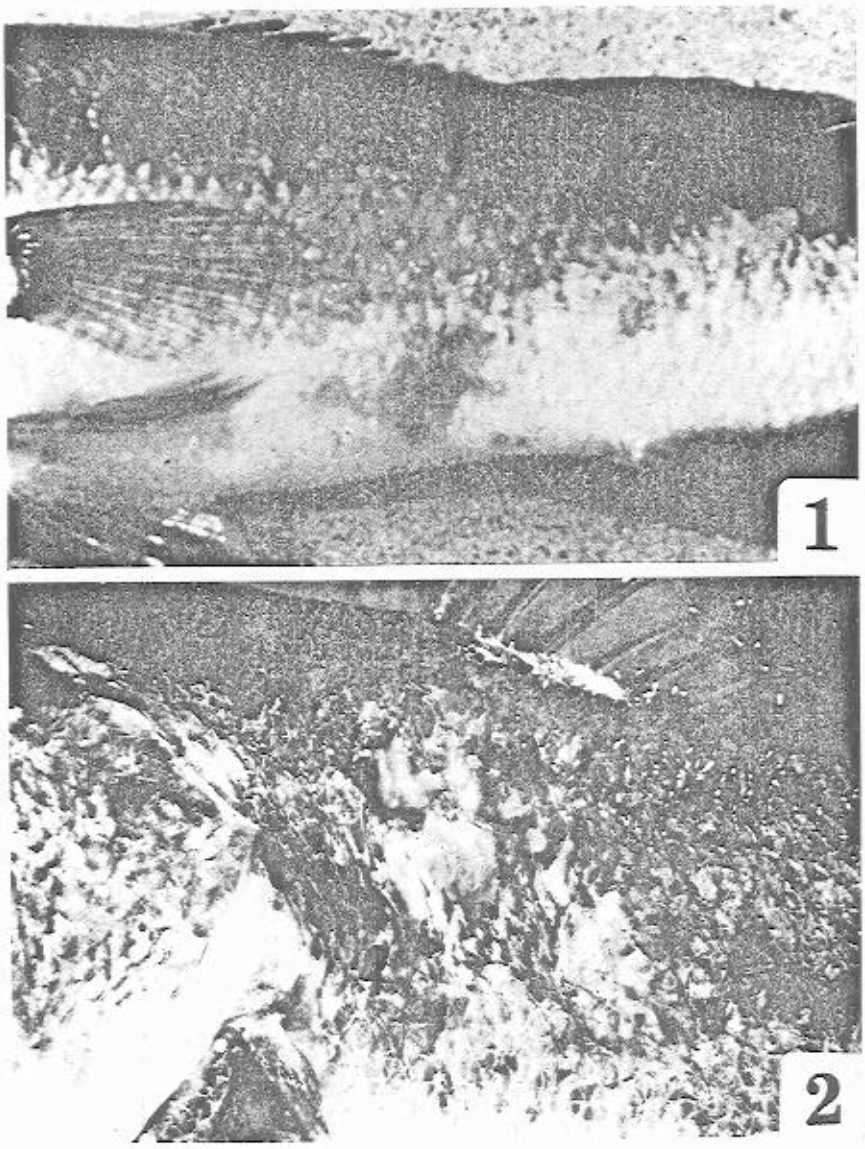


Figure 1. Acute red-sore lesions on skin of largemouth bass.
Figure 2. Chronic red-sore lesions on skin of largemouth bass.

including fins and gills, but the majority were found in a zone along the body surface from the anterior tip of the mandible, paralleling the lateral line and continuing to the end of the tail (Esch *et al.* 1976). Skin lesions were variable in size and progressive through three stages (Figs 1 & 2): (1) *early pinpoint lesion* – confined to one or two scales; haemorrhagic, oedematous and with peripheral capillary congestion: (2) *acute lesion* – white to yellow in colour, with raised, oedematous areas diffusely spread over a few to 30 scales; early epidermal and dermal necrosis in centre of lesion with haemorrhage, capillary congestion and central necrotic exudate; some lesions with secondary masses of ectocommensal ciliates: (3) *chronic ulcerative lesion* – crater shaped and 10–30 (or more) scales in area; characterized by loss of epidermis, dermal scales and dermis in centre of lesion, with resulting exposure of necrotic, underlying muscles; epidermis thickened around margins of ulcer; serofibrinous exudate mixed with secondary agents (fungi, stalked ciliates). Individual fish possessed one to several skin lesions progressing through stages 1–3.

Microscopic lesions (1) *Skin*. The early lesion (subacute) is characterized by epidermal necrosis and oedema (Fig. 3). After the necrotic epidermis is lost, haemorrhage, inflammation and oedema are observed (Fig. 4). The dermis undergoes extensive hyperplasia; proliferations of fibrocytes and infiltrating inflammatory cells extend upward from the dermis (Fig. 5). The dermis and underlying muscles show early signs of necrosis and oedema. Infiltrations of inflammatory cells (mononuclear cells and granulocytes) are observed throughout the lesion (Fig. 6).

If the infection progresses to the chronic stage, the epidermis and dermis are lost, exposing underlying muscles which become severely necrotic (Fig. 7). Inflammatory cells are not present in the exposed muscle. The adjacent epidermis undergoes extensive hyperplasia to form the raised margin of the crater-shaped ulceration. At this phase, the infection becomes systemic.

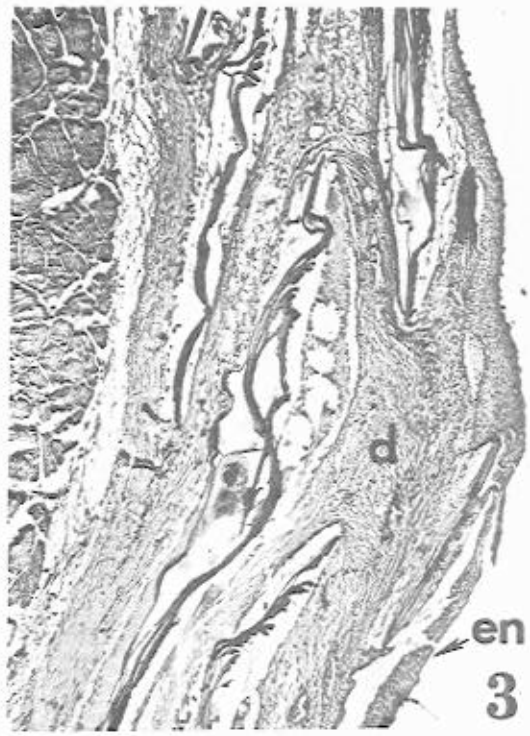
(2) *Liver*. An acute, diffuse hepatocytic necrosis was caused, presumably by the toxic products known to be released by *A. hydrophila*. Hepatocytic necrosis was variable and apparently associated with the degree of skin infection. The most severe liver damage was observed in fish with chronic skin lesions. A mild stage of necrosis was characterized by swollen cell membranes, hyperaemia and cell enlargement. In the intermediate stage of necrosis, the cell membranes were damaged; cells were enlarged and undergoing fatty changes (Figs 8 & 9). The severe stage of necrosis resulted in complete destruction of structural integrity of hepatocytes (Fig. 10). Inflammatory cells were absent, except in livers also infected with encysted trematode metacercariae.

Figure 3. Acute lesion showing early epidermal necrosis (en); oedema and hyperplasia of dermis (d) (×35).

Figure 4. Acute skin lesion showing epidermal necrosis; inflammation, oedema and hyperplasia of dermis; oedema and early necrosis of subdermal muscles (sm) (×63).

Figure 5. Acute skin lesion showing epidermal necrosis; dermal inflammation, haemorrhage and hyperplasia (dh) (×35).

Figure 6. Higher magnification of right margin of Figure 5 showing loss of epidermis; dermal scale (ds) surrounded by inflammation, haemorrhage and dermal hyperplasia (×160). Figs 3–17 and 19–26. Sections stained with H + E.



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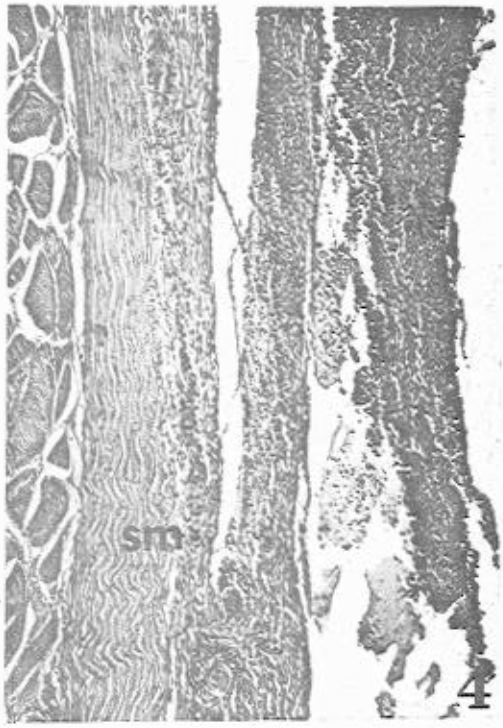

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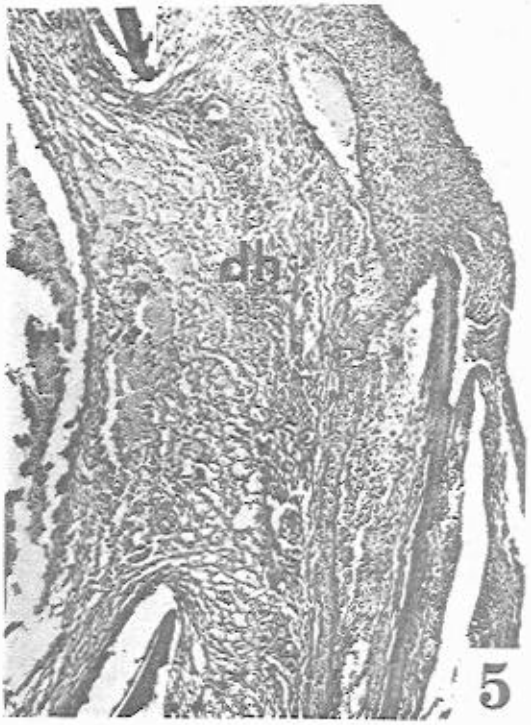

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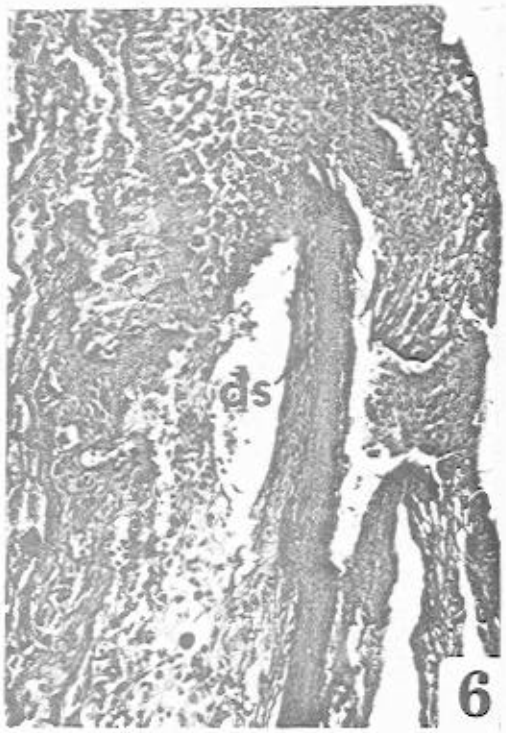

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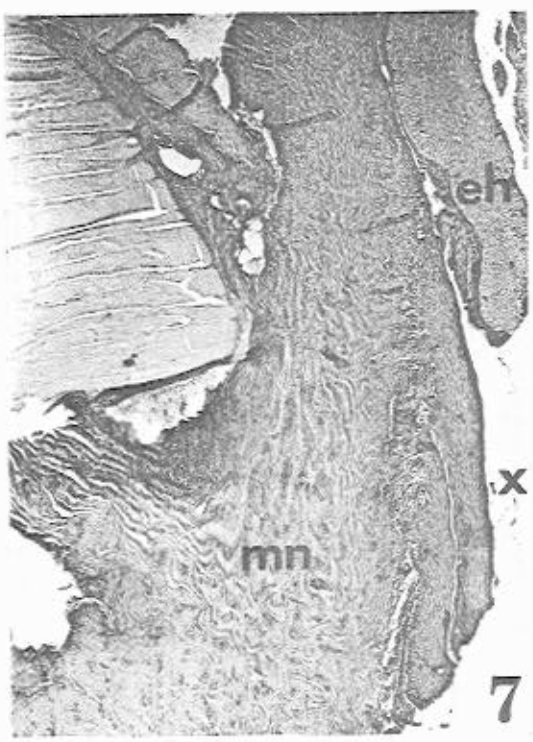
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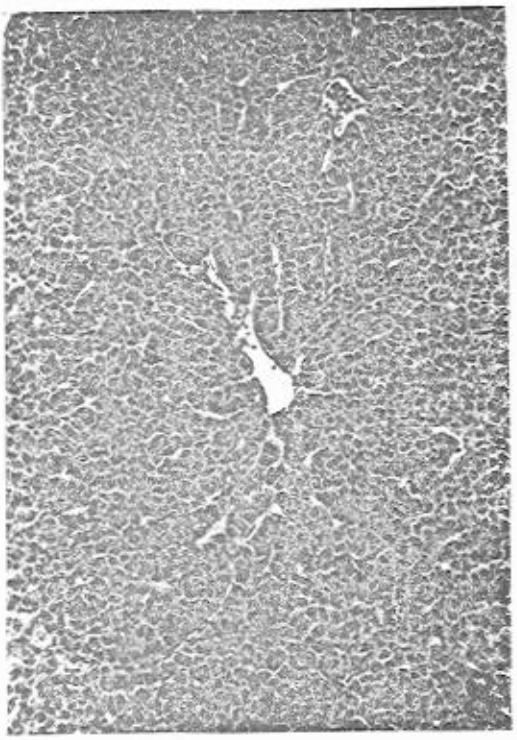

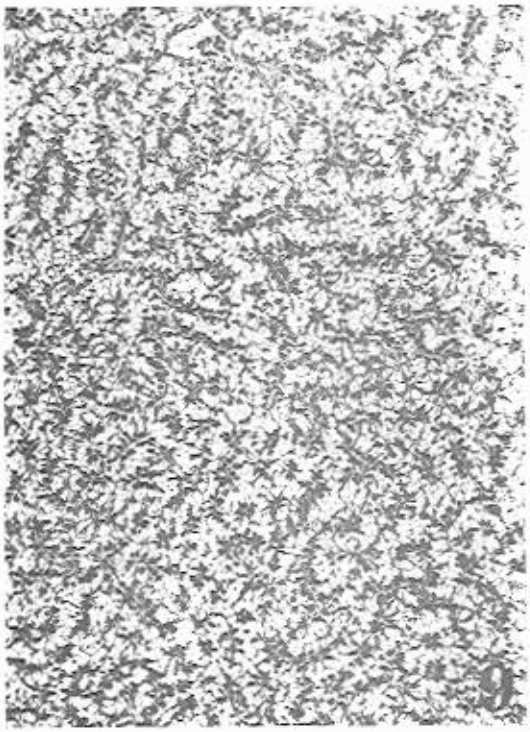
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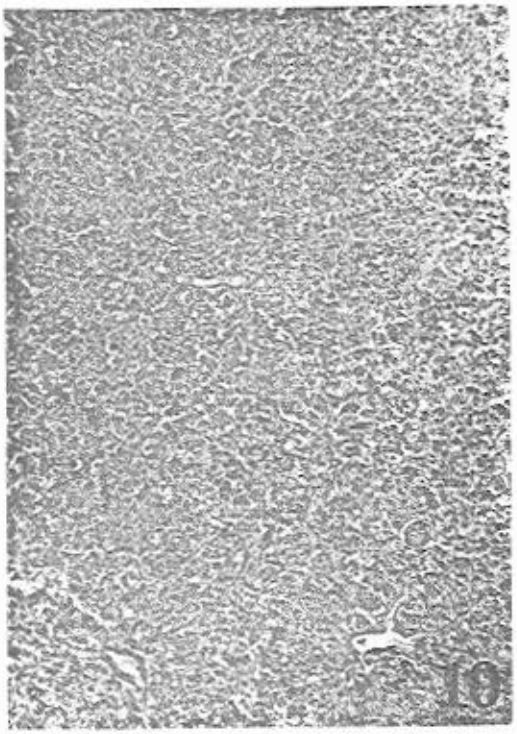
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(3) *Mid-kidney*. An acute, diffuse, tubular and glomerular necrosis of variable severity was observed in kidneys of most infected fish (Figs 11, 12 & 13). Haemorrhages were apparent in some kidneys. The necrosis was of rapid onset and, in some fish, a form of coagulation necrosis was present (Fig. 14). Inflammatory cells were absent, except in kidneys also infected with encysted trematode metacercariae.

(4) *Spleen*. A series of spleens from infected bass were examined, but there was no clear pattern of pathogenesis. In most infected bass having liver and kidney lesions, the spleen appeared normal. However, in a few bass, a diffuse necrosis of the red pulp and structural trabeculae was observed.

(5) *Heart*. There was a conspicuous absence of heart muscle necrosis in fish that exhibited severe skin, liver, or kidney lesions. Only a few isolated necrotic foci were observed in a single fish.

(b) *Experimental bass*

Macroscopic lesions. Lesions produced by *A. hydrophila* in laboratory infections were similar to those observed among naturally-infected largemouth bass in Par Pond.

Microscopic lesions. (1) *Skin*. The early, pin-point lesion is characterized by hyperaemia, epidermal necrosis and hyperplasia. The underlying dermis and outer muscle layer is inflamed and slightly oedematous (Fig. 15). The acute lesion shows reduced thickness of the epidermis and epidermal necrosis in the centre of the lesion, exposing dermal scales. Oedema, haemorrhage, inflammation and diffuse necrosis are present in the dermis and subdermal muscles (Fig. 16). In some experimental fish, proliferations of the stalked ciliate, *Epistylis* sp. were attached to the surfaces of exposed scales (Figs 16, 17 & 18). The attachment organelles of *Epistylis* sp. did not extend below the scales (Fig. 18). The ciliates were designated as facultative ectocommensals (Hazen *et al.* 1978), apparently feeding upon bacteria associated with skin lesions.

The chronic lesion is characterized by loss of scales, inflammation, haemorrhage and diffuse necrosis of dermis and underlying muscles. Hyperplastic epidermis forms the margin of the crater-like lesion (Fig. 19). The late chronic lesion shows complete loss of epidermis and dermis in the centre of the lesion, with severe necrosis of exposed muscles (Fig. 20).

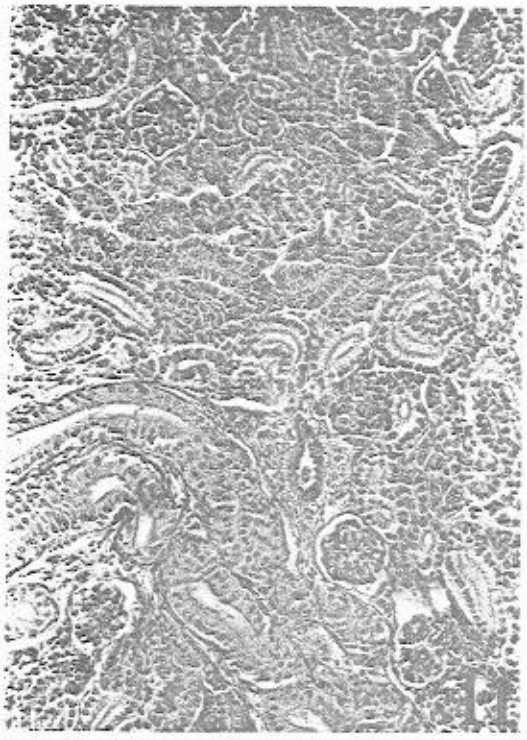
(2) *Gills*. There was severe haemorrhagic necrosis of gill epithelium, with destruction of gill lamellae and filaments, exposure of supporting cartilage, and loss of functional integrity of gill tissue. Diffuse oedema, profuse haemorrhage, mononuclear infiltration and extensive necrosis were observed in the underlying tissues (Fig. 21).

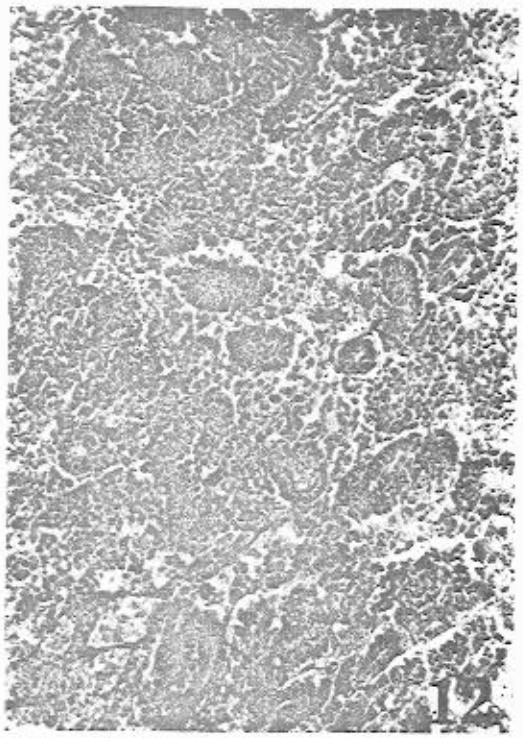
Figure 7. Chronic skin lesion showing muscle necrosis (mn) below missing epidermis and dermis (x). Note lateral epidermal hyperplasia (eh) (×35).

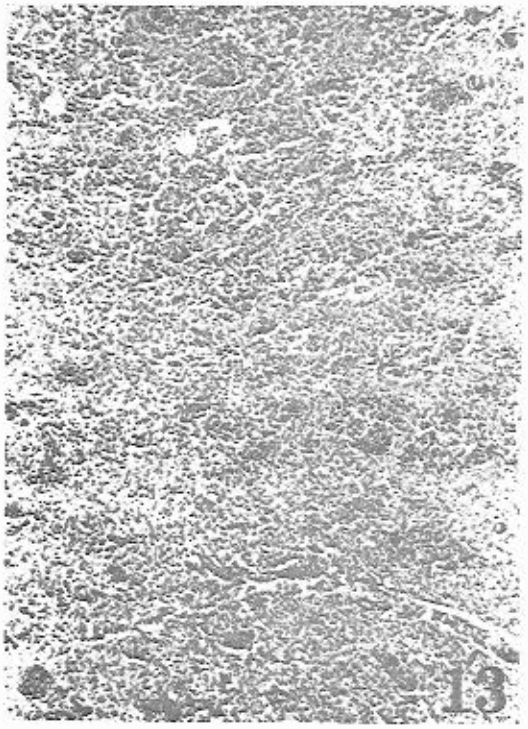
Figure 8. Liver section from non-infected largemouth bass (×160).

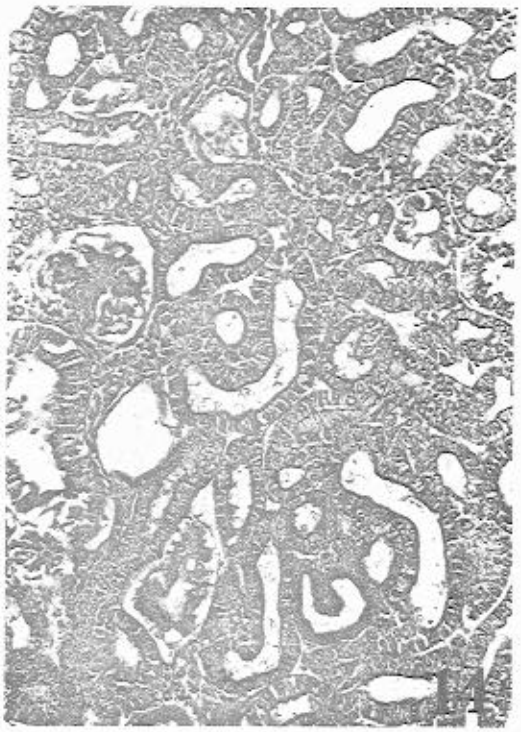
Figure 9. Early, diffuse hepatocytic necrosis with damage to cell membranes, swollen cytoplasm and fatty degeneration (×160).

Figure 10. Severe hepatocytic necrosis with destruction of cell structure (×160).









(3) *Fins*. In some fish, there was severe haemorrhagic necrosis and destruction of epidermis and connective tissues, exposing the bone supports (Fig. 22).

(4) *Liver*. The degree of hepatocytic necrosis observed between fish was variable. The early, acute form of necrosis was characterized by diffuse damage of cell membranes and swollen cytoplasm, with conspicuous lysis of hepatocytes adjacent to liver capillaries (Fig. 23). The severe form of necrosis was characterized by loss of structural integrity of tissue (Fig. 24). Melanin-macrophage centres were abundant (Fig. 23). There was an absence of inflammation, except surrounding encapsulated trematode metacercariae.

(5) *Mid-kidney*. An acute, diffuse tubular and glomerular necrosis caused extensive tissue damage and loss of structural integrity (Fig. 25). There was an absence of inflammation, except surrounding encapsulated trematode metacercariae.

(6) *Spleen*. Pathogenesis varied from mild to severe necrosis of red pulp, white pulp and structural trabeculae (Fig. 26).

(7) *Heart*. There was slight inflammation, oedema and minor focal necrosis in a few bass, but most were normal even though some individuals had severe liver and kidney necrosis.

Melanin-macrophage centres were abundant in the liver, kidney and spleen, but not in skin, of red-sore diseased largemouth bass. These centres stained positively with PAS and Ziehl–Nielson stains. Melanin-macrophage centres were also observed in lesser numbers in the liver, kidney and spleen of non-diseased bass, and they were particularly abundant immediately surrounding encapsulated trematode metacercariae.

Discussion

As one of the most important diseases of freshwater fishes, there is an extensive literature on the pathogenesis of red-sore disease (Amlacher 1970; Gaines 1972; Mawdesley-Thomas 1969; Schäperclaus 1965; Wolke 1975). However, the histopathology of red-sore disease has been most completely studied in carp *Cyprinus carpio* L. (Amlacher 1970) and channel catfish *Ictalurus punctatus* (Rafinesque) (Gaines 1972). The pathogenesis of furunculosis, a closely related disease caused by *Aeromonas salmonicida*, has been described in brown trout *Salmo trutta* L. (Ferguson & McCarthy 1978) and goldfish *Carassius auratus* (L.) (Mawdesley-Thomas 1969).

The histopathology of red-sore disease in largemouth bass conforms closely to descriptions in other fish species. In largemouth bass, red-sore is an acute disease, characterized by haemorrhagic necrosis and ulceration of the surface and, to a lesser extent, the gills. It is further marked by septicaemia and severe haemorrhagic necrosis

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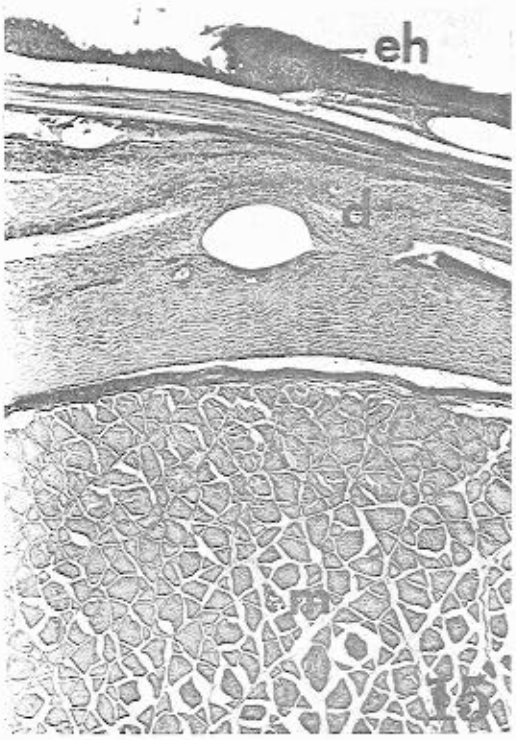
Figure 11. Section of mid-kidney from non-infected largemouth bass (× 160).
Figure 12. Diffuse necrosis of glomeruli and tubules of mid-kidney (× 160).
Figure 13. Severe necrosis of mid-kidney with loss of structural integrity (× 160).
Figure 14. Coagulation necrosis of mid-kidney (× 160).



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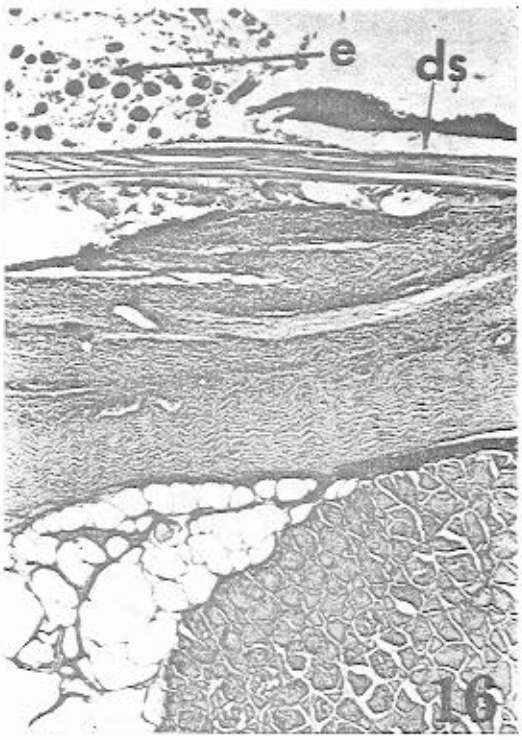

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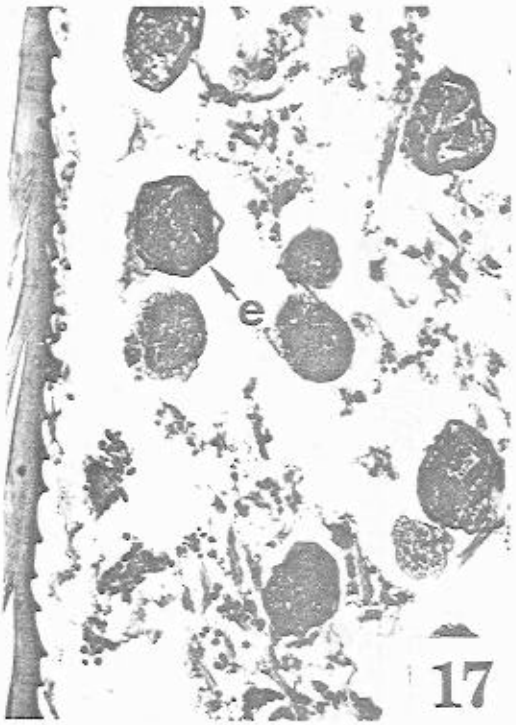

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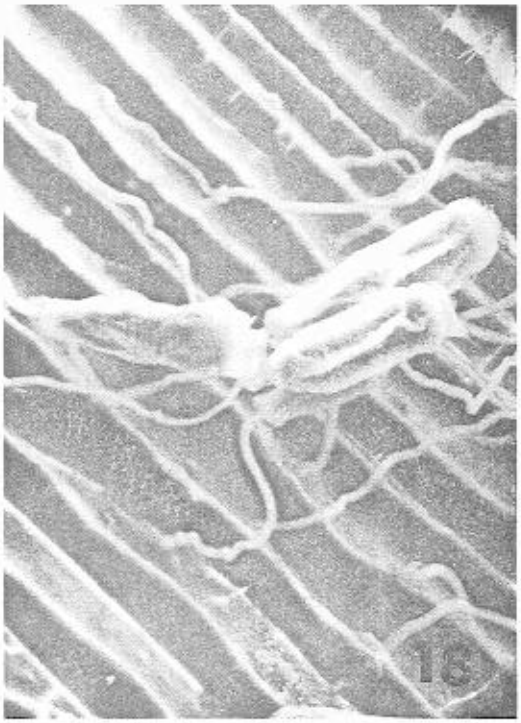

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of major internal organs, most importantly the liver and kidney. Heart muscle is only mildly affected, even in fish showing severe haemorrhagic necrosis of liver and kidney. This contrasts with furunculosis in brown trout (Ferguson & McCarthy 1978).

The rapid onset and severe pathogenesis of *A. hydrophila* infection in the largemouth bass are apparently caused by toxic bacterial products which have been partially characterized by Liu (1961) and Wretlind, Mollby & Wadstrom (1971). Recently, Donta & Haddow (1978) have demonstrated that *A. hydrophila* toxins are cytotoxic. The increased numbers of active melanin-macrophage centres observed in red-sore diseased largemouth bass may play a role in the phagocytosis of haemoglobin breakdown products released following haemorrhagic necrosis caused by *A. hydrophila* toxins, as suggested by Roberts (1975). Death of an infected largemouth bass would be expected to follow haemorrhagic necrosis and loss of structural and functional integrity of major organs, primarily the liver and kidneys.

Close agreement was observed between the pathogenesis in naturally and experimentally infected bass. The only exception was the presence of lesions on gills of experimental bass exposed to massive doses of *A. hydrophila* grown in culture. The course of infection was rapid in experimental bass, with surface lesions observed 48 h post-exposure. It was anticipated that bass collected in thermally altered areas of Par Pond might have more severe lesions than bass collected in ambient temperature locations. While sample sizes were small and histopathological comparisons somewhat subjective, it is our opinion that the pathogenesis of red-sore disease in bass from the two areas was not substantially different. However, it should be emphasized that significantly more bass are infected in thermally elevated areas (Esch *et al.* 1976; Esch & Hazen 1978) and that body conditions of bass in these areas are significantly lower (Gibbons, Bennett, Esch & Hazen 1978; Esch & Hazen 1978).

Rogers (1971) implicated *Epistylis* sp. as the causative agent for red-sore disease. He proposed that a motile telotroch settles on the surface of a fish and induces local scale erosion with the subsequent creation of pit-like lesions and local haemorrhage. *A. hydrophila* was then said to enter the tissues, producing haemorrhagic septicaemia and death of the host. Based on recent studies by Hazen *et al.* (1978), and confirmed through examination of surface lesions by light microscopy in the present investigation, *Epistylis* sp. can be designated as nothing more than an ectocommensal which attaches to the surface of fish and feeds opportunistically on bacteria. One possible role for the colonial ciliate in the disease process is that it provides a matrix for attachment of bacteria and may exacerbate the lesion-producing capabilities of *A. hydrophila*.

Figure 15. Early pin-point lesion with epidermal hyperplasia (eh) and necrosis; inflamed dermis (d) and oedematous underlying muscle (m) (×35).

Figure 16. Acute lesion with epidermal necrosis and exposed dermal scales; inflammation and diffuse necrosis of dermis and subdermal muscles. *Epistylis* (e) attached to exposed dermal scales (ds) (×35).

Figure 17. Higher magnification of *Epistylis* (e) seen in Figure 16 (×400).

Figure 18. SEM photomicrograph of *Epistylis* attached by stalks to denuded dermal scales (×500).

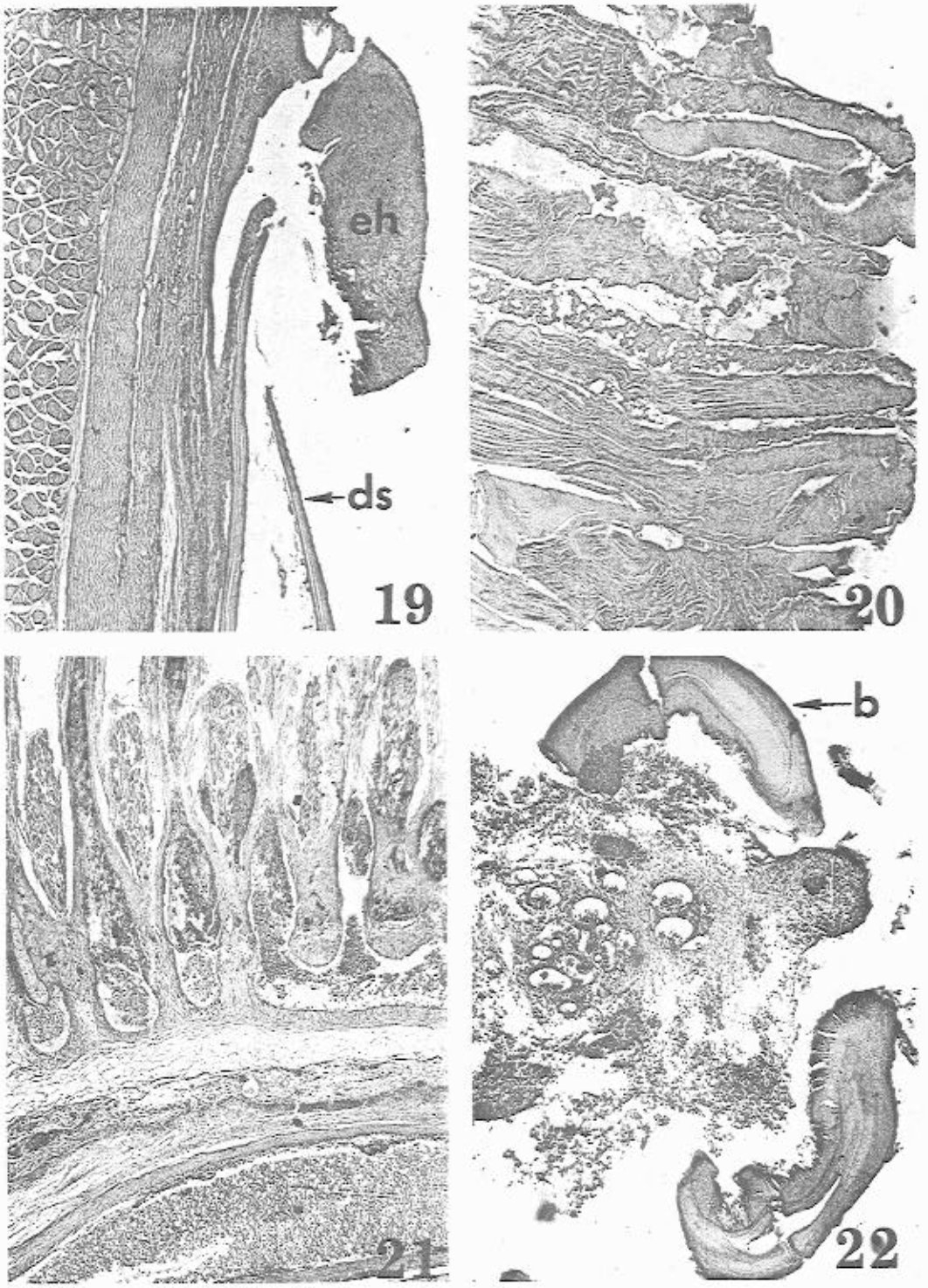

Figure 19. Chronic skin lesion with exposed dermal scales (ds) and epidermal hyperplasia (eh) extending from margin of crater-shaped lesion (×35).

Figure 20. Late chronic lesion with exposed necrotic muscle (×63).

Figure 21. Severe haemorrhagic necrosis of gill lamellae and filaments; haemorrhagic necrosis and inflammation of underlying supporting tissues (×35).

Figure 22. Severe haemorrhagic necrosis of epidermis and connective tissues of fin, exposing bony fin rays (b) (×35).

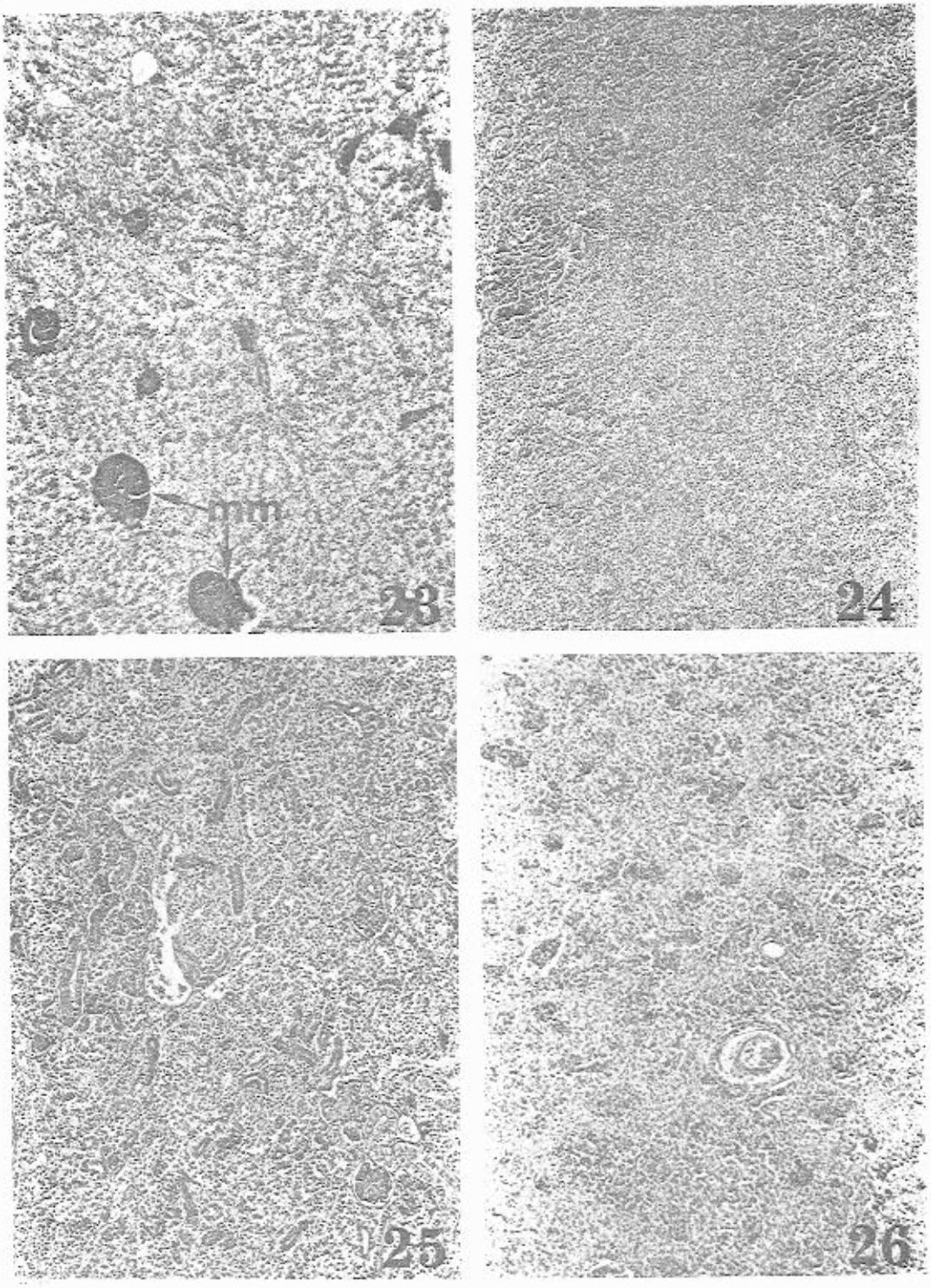

Figure 23. Diffuse hepatocytic necrosis with damaged hepatocytes paralleling liver capillaries. Note numerous melanin-macrophage centres (mm) (×63).
Figure 24. Severe hepatocytic necrosis with loss of structural integrity (×63).
Figure 25. Diffuse haemorrhagic necrosis of kidney tubules (×63).
Figure 26. Diffuse haemorrhagic necrosis of red and white pulp of spleen (×63).

Based on the infection scheme proposed by Esch & Hazen (1978), and on observations generated in the present study, we believe that the pathobiology of red-sore disease among largemouth bass in Par Pond follows a clear pattern: namely, elevated water temperature stimulates increased metabolism of bass, resulting in increased catabolism of stored fat and protein; body conditions of these bass then decline according to the duration and magnitude of exposure to elevated temperature (Gibbons *et al.* 1978; Hazen, Esch, Glassman & Gibbons, 1978). Increased temperatures either directly, or indirectly for bass with lowered body conditions, cause increased production and release of ACTH which, in turn, stimulates production and release of corticosteroids, primarily cortisol. An extensive literature (see views by Wedemeyer 1970; Esch, Gibbons & Bourque 1974) indicates that corticosteroids have a suppressive effect on cellular and humoral immune responses of fish. In Par Pond, the result would be that largemouth bass stressed by elevated temperature become more susceptible to the ubiquitous, facultative bacterium, *Aeromonas hydrophila*. Recent evidence (Esch & Hazen, in preparation) indicates that there is a significant correlation between levels of circulating cortisol and body condition of largemouth bass. After initial penetration by *A. hydrophila*, the red-sore lesion rapidly develops through acute and chronic phases, with the resultant exposure of subdermal muscles. At this point, *A. hydrophila* enters the blood stream and multiplies to produce systemic bacteraemia in the immunologically suppressed bass. Release of bacterial toxins causes acute haemorrhagic necrosis of vital organs (primarily liver and kidneys), leading to rapid death of some fish due to organ failure. Chronic infections, lasting several weeks to months, are also observed. In some cases, naturally infected bass are known to recover from red-sore disease; studies now in progress may yield information regarding the nature of immunity to red-sore disease.

Acknowledgments

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