Gross, histological and scanning electron microscopic appearance of dorsal fin rot in farmed Atlantic salmon, *Salmo salar* L., parr

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Abstract

The gross, histological and scanning electron microscopic appearance of dorsal fin rot in farmed Atlantic salmon parr, *Salmo salar* L., is described. The lesions were grouped into seven categories: (1) peripheral erosion and ray splitting; (2) peripheral erosion with some nodularity; (3) severe nodularity with differing degrees of tissue loss; (4) extensive to total loss of the dorsal fin; (5) smooth thickening of the dorsal fin; (6) haemorrhagic dorsal fin lesions; and (7) healed dorsal fin rot lesions. The main sign of injury was clefts extending through the epithelium. These injuries were consistent with bites from other parr. During healing from such wounds, damaged cells sloughed from the surface, and there was swelling and hyperplasia in the remaining cells. The majority of the thickening in the fins was the result of epithelial hyperplasia with a variable cellular inflammatory response. The distal epithelium of fins with severe fin rot (i.e. nodular and eroded) was rough, irregular and swollen with superficial nodular extensions. Wounds in all stages of repair were more numerous in such areas. Fin rays were frequently observed protruding from the abnormal epithelium at the distal edge of the fin. With the exception of the isolation of *Aeromonas salmonicida* from a small number of cases, no significant bacterial involvement was detected. Under scanning electron microscopy, bacteria were only detected on the exposed fin rays and not in association with the abnormal epithelium. In the majority of cases, the dorsal fin was either the only fin damaged or the most severely damaged. It is suggested that the hyperplastic response to numerous bite wounds is responsible for the accumulation of abnormal epithelium typical of dorsal fin rot in farmed Atlantic salmon parr.

Introduction

Dorsal fin rot is a well recognized but ill defined condition. In the Scottish Atlantic salmon farming industry, it is perceived as a grey, thickened, nodular lesion on the distal edge of eroded dorsal fins and is considered to be a specific condition occurring in the absence of damage to the other fins. It principally affects the cosmetic appearance, not only of smolts, but also of market size salmon. It is widely accepted that farmed salmonids suffer from damaged fins, indeed this has been suggested as a method for differentiating between farmed and wild fish (Craik, Harvey, Jakupsstovu & Shearer 1987). Fin rot in general, and dorsal fin rot in particular, is not usually associated with high mortalities (Schneider & Nicholson 1980). However, there has been concern that dorsal fin rot might increase susceptibility to secondary infections, especially furunculosis caused by *Aeromonas salmonicida*. The main historical significance of dorsal fin rot has been an effect on the subsequent survival of fish released for ranching or re-stocking. In such fish, loss of or damage to fins affects their swimming ability (Horak 1969; Maheshkumar 1985), and consequently, their capacity to capture prey and avoid predation (Nicola & Cordone 1973).

There is a considerable amount of information available concerning normal fin structure (Haas 1962; Keenleyside & Yamamoto 1962; Maitland...
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1972; Lagler, Bardach, Miller & May Passino 1977; Wedemeyer, Saunders & Clarke 1980; Becerra, Montes, Bexiga & Junqueira 1983; Geerlink & Videler 1987). In contrast, there are few descriptions of dorsal fin rot in the literature. Most authors refer to fin rot as affecting a number of fins. Existing classification of fin rot lesions is inconsistent. The most complete description was by Maheshkumar (1985), who defined fin rot as a characteristic rough white lesion and the healing process as a hyperplastic regeneration of diseased tissue with re-pigmentation. This apparently led to dark-coloured, smooth, healed fins. No distinction was made between the darkening of fins associated with smolification and healing following fin rot. There was also no mention of fins with eroded edges but without changes in coloration. The majority of other reports describe fin rot as an initially ulcerative necrotic lesion, which may result in substantial loss of tissue leading to exposure or loss of the fin rays. It is suggested that the condition may remain necrotic or progress to centripetal epidermal hyperplasia (Bullock & Conroy 1971; Mahoney, Midlidge & Deuel 1973; Murchelano 1975; Wellings, Alpers, McCain & Miller 1976; Schneider & Nicholson 1980; Khan, Campbell & Lear 1981). The hyperplastic tissue has been described as part of the active pathological process; for example, as a response to the presence of bacteria (Bullock & Conroy 1971). Other authors have described translucent hyperplastic tissue as part of the regenerative process (Frantsi, Ritter & Foda 1972; Schneider & Nicholson 1980). Although the appearance of the healed fin is variably described, it is generally agreed that the process involves dermal fibrosis.

The gross, histological and scanning electron microscopic appearance of dorsal fin rot in Atlantic salmon parr is described here and examples of normal fins are included for comparison. The relationship between damage to the dorsal and other fins was also examined.

Materials and methods

Gross and histological appearance of dorsal fin rot

From December 1987 to December 1989, 114 fish from six farms and three streams throughout Scotland were selected for gross and histological examination. The wild fish from the streams were obtained by electro-fishing. The farmed fish demonstrated many stages of dorsal fin condition from fins with no detectable damage to the total absence of the dorsal fin. The fish were anaesthetized, and all fins were examined with the aid of a dissection microscope and the condition recorded. Whole fins were removed with some of the dorsal musculature (Fig. 1), fixed immediately in 10% neutral buffered formalin and subsequently taken to wax. Prior to embedding in wax blocks, the fins were sectioned as shown in Fig. 1, and the cut ends placed towards the face of the block. The microtome sections were taken from distal to proximal fin. The resulting sections were stained with haematoxylin and eosin (H&E) and mounted with pertex (Histo-lab). In addition to the fish sampled specifically for the current study, material from fish with dorsal fin rot submitted to the diagnostic service of the Institute of Aquaculture and material sampled elsewhere (Turnbull 1992) was examined.

Scanning electron microscopy (SEM)

Fish were sampled from three production tanks. These fish included individuals with undamaged dorsal fins, mild erosion, severe nodularity and some eroded but smooth fins. Appropriate fish were selected, and individually netted out and killed by spinal section, which also served to bleed the fish. They were briefly dipped into the water from which they had been removed to remove any blood. The dorsal fin and surrounding tissue was immediately removed and placed into fixative. The processing protocol was adapted from Glauert (1981). Fins were protected during parts of the processing by attaching them to small sheets of dental wax (Anutex, Associated Dental Products Ltd, Swindon, England) by placing tissue paper over the fin and stapling the paper to the wax, avoiding the fin tissue. The fins were critical point dried after 2.5 h impregnation in a Bio-Rad critical point drier (Watford, UK), mounted with EMScope A860 silver conducting.
paint onto SEM stubs so that the fins formed a 45° angle with the surface of the stub. They were then sputter coated with gold in an Edwards 5150B sputter coater (Crawley, UK) and examined in a Phillips PSEM 500 scanning electron microscope (Eindhoven, The Netherlands).

Dorsal fin rot and damage to other fins
A comparison was made between the gross appearance of all the fins of 101 Atlantic salmon parr obtained on 12 dates from six farms. The farms included five freshwater tank sites and one freshwater cage site.

Results
No evidence of fin damage was detected in the fish from wild populations, which were therefore used as examples of normal undamaged fins. An example of a normal undamaged dorsal fin is shown in Fig. 2. The branching, segmented fin rays or lepidotrichia can be seen supporting the membranous tissue of the fin. The surface of the fins was covered with normal teleost epithelium (reviewed by Bullock & Roberts 1975) (Fig. 3). Mucous cells were more numerous near the base of the fin. Examination of a number of sections suggested that there was a progressive thinning of the epithelium towards the distal fin.

Examination of undamaged fins under SEM revealed a smooth distal edge, whilst the surface of the fin consisted of alternating smooth and corrugated areas of epithelium. The smooth areas were situated over the fin rays. The corrugated areas, between the rays, would be capable of expansion during erection of the fin (Fig. 4). The epithelium over the fin had a distinctive microridge pattern (Fig. 5).
Under the epithelium, there was a basement membrane and a layer of dense connective tissue which was continuous with the stratum compactum on the dorsal surface of the fish body. Melanocytes were situated in the deeper portion of this layer. Below the level of the melanocytes, the connective tissue became progressively less dense, forming a wide band of loose tissue comprising a substantial portion of the thickness of the fin between fin rays, this area being continuous with the hypodermis elsewhere. In the centre of a cross section of the fins, between fin rays, there was a thin band of dense cellular connective tissue representing the connection between adjacent rays. In the proximal fin, the less dense fibrous hypodermal layer intervened between the fin ray and the stratum compactum. Distally, the stratum compactum was intimately associated with the outside of the fin ray (Fig. 3).

The damage to the dorsal fin was differentiated into seven main categories listed below.

Peripheral erosion and ray splitting

This was the least severe form of damage observed and consisted of splitting of the tissue between the fin rays, occasionally with some loss of the distal fin rays (Fig. 6).

It was difficult to obtain undamaged histological sections of the area immediately surrounding the erosion because of the fragile nature of the tissue. In cases where the only gross sign was erosion, no cellular inflammatory response was observed. The most common findings were areas of thin epithelium, and defects or clefts extending through the depth of the epithelium. The clefts were only detected with any regularity in fins that had gross signs of fresh rough erosion.

Under SEM, fins with peripheral erosion had variable numbers of clefts in the surface epithelium often surrounded by raised epithelial cells (Fig. 7). Such injuries were easily distinguished from cracks or artefacts produced during processing. Artefacts usually followed the outline of the superficial cells, and even when damaged, the surrounding cells showed no evidence of hypertrophy or other changes. The clefts in the epithelium invariably showed evidence of changes in the surrounding cells with some swelling and sloughing. Round bodies which may have been lymphocytes were also seen near the wounds. In some cases, the superficial cells at the edge of the cleft appeared to extend into the cleft. Some of the clefts observed had mostly smooth edges with large numbers of rounded cells protruding from the wound in a disorganized manner. In some cases, such lesions were surrounded by areas of roughened epithelium with swollen superficial cells (Fig. 8). There were also healed wounds on many of the fins examined. These appeared as shallow depressions in the epithelium often surrounded by rounded swollen or sloughing cells (Fig. 9).

Peripheral erosion with some nodularity

Peripheral erosion was often associated with differing degrees of nodularity. The degree of erosion was not always proportional to the nodularity, i.e. some fish had quite severe nodularity with relatively minor erosion and vice versa. The gross appearance of the nodularity described under this heading was a limited opaque thickening of the distal fin. In some cases, the thickening extended down the edges of larger splits in the fins. The combination of erosion and thickening frequently had a rough, irregular appearance, but occasionally, the edge of the fin was smooth and irregular.

On the eroded and nodular fins examined under SEM, all types of injury were present from large fresh wounds to slight depression as the result of healing. Some of the fresh wounds consisted of a number of parallel gashes or clefts in the superficial epithelium (Fig. 10). The epithelium on the dorsal edge of severely eroded and nodular fins was roughened and nodular with numerous clefts. On closer examination, only a small proportion of these defects appeared to be artefacts. In many cases, the fin rays were observed protruding from the abnormal epithelium (Fig. 11). Some of the exposed fin rays had material adhering to them (Fig. 11). This material was largely composed of bacteria and a granular material that may have been bacterial glycocalyx or retained mucus (Fig. 12). Bacteria were not consistently detected in significant numbers on any other areas of the fins. During preliminary experiments, some fins had retained substantial quantities of mucus and red blood cells on their surface; even on these specimens the fin rays appeared to be the only site of bacterial colonization.

The main defects detected histologically were again clefts through the epithelium (Fig. 13). The nodularity in these sections was largely caused by epithelial hyperplasia; however, there was evidence of a cellular inflammatory response in many
Figure 6 An example of peripheral erosion and ray splitting in a live Atlantic salmon parr.

Figure 7 The lateral surface of a fin with some erosion. There are a number of clefts in the epithelium (bar = 400 µm).

Figure 8 A cleft on the lateral surface of a fin with some erosion. The edge of the lesion is mostly smooth with some rough cells in one area (A). Within the cleft, there are a large number of rounded cells protruding in a disorganized manner (bar = 200 µm).

Figure 9 An area on the lateral surface of a slightly nodular fin. There is a depression in the epithelium running from bottom left to top right. Some of the surrounding cells are swollen or in the process of sloughing (S) (bar = 50 µm).

Figure 10 The leading edge of an eroded and nodular dorsal fin. The dorsal part of the fin has a rough disorganized appearance with clefts (C) and nodules (N). There are several deep parallel clefts in the otherwise normal lateral surface of the fin (bar = 800 µm).

Figure 11 A broken fin ray extending from the dorsal surface of a severely thickened nodular fin. There is material adhering to the fin ray (A) (bar = 100 µm).