A preliminary histological study of normal and pathological tissues in cultivated turbot (*Scophthalmus maximus* L.)

D. Bucke

Ministry of Agriculture, Fisheries and Food, Directorate of Fisheries Research, Fish Diseases Laboratory, Weymouth, Dorset DT4 8UB, England

A histological study has been made of tissues and organs from cultivated turbot *Scophthalmus maximus* L. originating from four sites in the United Kingdom. Necrosis and septicaemia associated with bacterial invasion were commonly identified: other observed abnormalities included glomerulonephritis, nephrocalcinosis, a granulomatous disease and a possible herpesvirus infection. Many of these conditions were found in otherwise apparently 'normal' turbot and it is believed that the clinical disease state often arises after fish are moved or handled.

Introduction

There is a paucity of information on diseases of turbot, particularly histopathological descriptions of abnormalities induced under farming conditions. Significant contributions have been made by Anderson et al. (1976) with their description of the hepato-renal syndrome in cultured turbot and by Horne et al. (1977) on peracute vibrosis in juvenile turbot introduced into a culture system. Also of importance is a series of papers describing a herpesvirus infection in cultured turbot (Richards and Buchanan, 1978; Buchanan et al., 1978; Buchanan and Madeley, 1978).

This presentation describes a histological study of tissues from cultivated turbot which were apparently normal or which showed clinical evidence of disease. The objective was to determine a baseline of normal histology for reference to facilitate the description and diagnosis of pathological changes and the aetiologies of diseases.

Materials and methods

Turbot samples were obtained from four marine sites in the UK. Ages ranged from 5 days post-feeding larvae through newly metamorphosed fry to broodstock.

After a macroscopical examination, samples were taken for bacteriological, virological, and histological examination, using methods in routine use at the MAFF, Fish Diseases Laboratory.

Case histories and results

Case 1. Samples of early feeding fry were reported to be dying in a commercial hatchery. The fry, which were hatched on site from eggs from wild fish, had been kept in a continuous flow system at 18°C. No details of diet were given. The fry became lethargic, subsequently dying in 'large' numbers after 5 to 10 days post feeding. No macroscopic lesions were observed. Bacteriological and virological tests were not made. Histological changes revealed intestinal mucosal cells dilated with amorphous acidophilic material and renal tubules unusually dilated. No diagnosis was made.

Case 2. Ten to twelve days post-feeding fry on an experimental fish farm were reported to be dying in 'large' numbers. These fish had been held at 18°C and were hatched from ova derived from wild fish. The intestines appeared blanched, otherwise no other abnormalities were observed. Details of diet were not given. Bacteriological tests revealed Gram-positive rods on direct plating only. Virological tests were negative. Histological examination showed intestinal lumens to be packed with masses of Gram-negative bacteria, and in some sections these were seen to infiltrate the mucosal epithelium (Fig. 44 A). Occasionally, necrotic foci were evident in the hepatopancreas. The condition was presumptively diagnosed as a bacterial infection of an unknown type.

Case 3. Mortalities in 5 days post-feeding fry on an experimental fish farm eventually exceeded 80 %. The ova had been hatched from brood fish, originally wild,
held on another site. Larvae from the same ova stock, reared on a second site, did not show any unusual abnormalities. The affected fry developed distended abdomens, failed to feed and eventually died. The abdomens contained a small amount of water fluid. Histological examination failed to reveal any major pathological changes. Fry were obtained from the second site and examined histologically. It was possible by examining samples at 5, 10, 15, 22, and 40 days post feeding to follow the sequential development of the visceral organs (Fig. 44 B). Diagnosis was 'water-belly' disease, cause unknown.

Case 4. Juvenile turbot on an experimental site was reported to be dying at a rate of 20 to 50 fish daily. A dietary-related problem was suspected by the owners. The fish were in two-sized groups of 5 and 10 cm, and both were equally affected. The fish had previously been treated with antibiotics, both in the food and as a bath, but mortalities had continued. Material was not presented for microbiological examination, but samples from 32 fish were received fixed for histology. This examination showed enormous variation in the size of vacuoles in the hepatocytes, and minimal numbers of focal areas of degeneration. The vacuoles contained lipid. All fish showed evidence of degrees of mineralization in the renal tubules. Diagnosis was inconclusive. Nephrocalcinosis and varying amounts of lipid were present, but neither of these changes were thought to be sufficient to account for the mortalities.

Case 5. Juvenile turbot on a commercial fish farm held in heated effluent water (18°–22°C) were reported to be dying at a low rate. The fish were fed on a commercially prepared diet. In the 20 fish examined, the main external sign was a pale dorsal surface. Additionally, a few fish had swollen abdomens and the occasional fish exhibited exophthalmia. Internal examination revealed enlarged pulpy kidneys and an oedematous area in the periorbital cavity. Bacteriological tests from individual fish revealed mixed cultures of *Vibrio* and *Aeromonas* spp. *Vibrio anguillarum* was isolated from two fish. Virology tests were negative. Histological
examination showed bacterial colonies present in visceral organs from most fish samples. These colonies were associated with a septicaemic condition of cellular infiltration, hyperaemia, and necrosis (Fig. 44 C). Minimal degrees of glomerulonephritis and nephrocalcinosis were present in all samples. A presumptive diagnosis of vibriosis was made.

Case 6. Brood fish originating from wild stock held on an experimental site were reported to be in a moribund condition. The fish were held in a flow of filtered sea water and fed on sprat. As a bacterial infection was suspected, and these were valuable brood fish, a course of I/M antibiotic (100 mg/kg tetracycline) had been prescribed. However, after 10 days treatment 3 fish died and 5 fish weighing between 1800 and 3200 g were presented for examination. Of these, 2 were suitable for bacteriological tests and 3 for virological tests. Tissues were taken from all the fish for histology. Each fish was covered in haemorrhagic petechiae. Haemorrhages were also present on the surface of the intestine and gonads. Liver colours varied from cream to bright red, and in two fish approximately 35 ml of pale red fluid was present in the abdominal cavity. The bacteriological tests revealed a mixture of Vibrio species; virological tests were negative. The results of histological examination were:

(a) epidermal lesions varied between minimal proliferation of the epithelia and dilation of the underlying blood vessels to marked infiltrating lesions involving a loss of epidermis and sub-epidermal connective tissue layers. These gross lesions revealed an extensive oedematous and infiltrating area affecting the skeletal muscle bundles. In these instances, muscle bundles showed fibromyitis and Zenker's necrosis. Gram-negative bacteria were present in these lesions;
(b) marked degrees of glomerulonephritis, proliferation of the haematopoietic cells with areas of necrosis (Fig. 44 D). Dilated renal collecting ducts filled with calcified material (Fig. 45 A);
(c) splenic changes involved enlarged ellipsoids and cellular infiltration with areas of necrosis where Gram-negative bacteria were prominent;
(d) necrotic lesions were present throughout the hepatic architecture;
(e) multiple granulomatous lesions occurred in the myocardium. These granulomas consisted of a mineralized centre and necrotic cellular material and were surrounded by fibrous connective tissue. The cellular material was negative for acid-fast organisms but Schiff positive. Myxidium spp. and Rhabdospora thelohani were observed in the visceral organs. Trichodina spp. and Costia spp. were present attached to the gill epithelium, but there was no tissue reaction.

Diagnosis was that the primary cause was unknown; but a secondary bacterial infection was the likely cause of death.

Case 7. A population of 100 × 1+ turbot had been reared for 6 months in tanks containing sea water at ambient temperature, pumped in from a disused dock. Food was a commercially prepared diet. A continuous low mortality rate had occurred for two months and 6 fish were submitted fixed in 10 % buffered formalin for
histological examination only. External signs were 3 to 10 mm raised nodules on both surfaces and, internally, multiple granulomatous lesions were present on the visceral organs. Histological examination showed the external lesions to consist of multiple whorls of reticuloendothelial cells surrounded by fibrous connective tissue. These lesions were infiltrated with fungal hyphae (Fig. 45 B). Lesions in the visceral organs showed some variation; some were similarly arranged in cell whorls with necrotic cellular centres, but no fungus was obser-

Figure 45 B. A whorl of fibrous tissue infiltrated by fungal hyphae (arrowed) (H & E $\times$ 800).

Figure 45 C. Kidney section showing fibro-reticulate material replacing haematopoietic cells (H & E $\times$ 800).

Figure 45 D. 'Giant' cells in gill filaments (H & E $\times$ 800).

ved. Other lesions, mainly in the kidney, consisted of a fibro-plastic condition in which areas of fibro-reticulate tissue had replaced the haematopoietic tissue (Fig. 45 C).

Diagnosis was unknown primary cause, but presumptively a secondary fungal infection. No further investigations as the site was closed down. However, it was learned that there had been algal blooms and salinity problems.

Case 8. A batch of 5 to 10 cm turbot was obtained from a commercial farm site for laboratory investigation. The fish had been hatchery reared and were apparently healthy. A histological examination was instigated before investigations were to begin. This revealed grossly enlarged cells, often as much as 50 to 70 $\mu$m diameter, present in the epidermal epithelium and the epithelium of the gill filaments. These 'giant' cells contained a single nucleus and a granular acidophilic cytoplasm. No tissue reaction was evident (Fig. 45 D). Ultrastructural examination has not to date revealed any evidence of viral particles.

Diagnosis was cause uncertain, but case histologically similar to a described herpesvirus infection.

General observations in healthy turbot obtained from the four marine sites showed that *Myxidium* spp., *Rhabdospora thelohani*, *Trichodina* spp., and *Euboethrium* spp. were fairly common. Minimal degrees of nephrocalcinosis was also seen in most fish examined.

Discussion

It was not necessarily the purpose of these studies to reach a diagnosis of the cause of the described diseases
in cultivated turbot, but to record some of the abnormal conditions encountered. Since Koch's postulates could not be fulfilled, it was not possible to make more than two presumptive diagnoses of microbial infections. In the experience of the author, fry and juvenile turbot are difficult to handle without allowing access for waterborne micro-organisms. Austin (personal communication) has identified several species of marine bacteria other than *Vibrio* spp. which are capable of inducing pathological changes in turbot or other marine fish species. Work on the control of such organisms is under current investigation in our laboratory.

Apart from opportunist bacteria, there are many other organisms such as ciliates, internal protozoa, and cestodes, all of which are considered to be ubiquitous, but may be secondary or tertiary agents of disease if suitable conditions occur (Purdom and Howard, 1971; Pearse, 1972). It is most likely that the fungus noted in Case 7 occurred because the fish were kept in poor conditions.

The difficulties in making a diagnosis have been highlighted by the fact that there is little published information on 'normal' histology. Cowey et al. (1976) described a hepato-renal condition similar to that described here, and observed that it was present in both wild and cultivated stocks, with biochemical parameters also similar in the two groups. Anderson et al. (1976) considered that the hepato-renal syndrome was associated with infiltrations of *Myxidium incurvatum* and *Rhabdospora thelohani*, although they did not consider them specifically to be the causative agents. In these investigations, and others in different species, the organisms were present even in 'normal' fish and were not thought to be the cause of disease. Richards and Buchanan (1978) reported that 'giant' cells in the skin and gills of turbot were induced by a herpesvirus infection which was the cause of mortalities, yet in the author's investigations similar 'giant' cells did not even evoke a tissue response and there was no evidence of a disease or of losses.

Because commercial turbot farming has shown modest development in the UK and France, it is necessary to have knowledge of any pathological conditions which may lead to losses in this species. Experience in pathology of other cultivated species (mainly salmonids) has shown that it is essential to establish the 'normal' or accepted range of conditions in tissues, and to know which organisms are capable of being pathogens. This must mean experimental work, comparing the effect of conditions, diets and, most of all, fulfilling Koch's postulates before making a diagnosis.

References