Studies on dorsal fin rot

in farmed Atlantic salmon (*Salmo salar* L.) parr

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A thesis submitted to the University of Stirling

for the degree of Doctor of Philosophy

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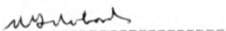
To the memory of my Father

DECLARATION

I hereby declare that this thesis has been composed by myself and is the result of my own investigation. It has neither been accepted nor is being submitted for any other degrees. All the sources of information have been duly acknowledged.

Candidate's signature 

Principle supervisor's signature 

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ABSTRACT

Aspects of dorsal fin rot in farmed Atlantic salmon (*Salmo salar* L.) parr were investigated and the associated pathology described. Substantial evidence was obtained which suggested that the condition was caused and maintained by repeated bites from other parr. The gross, histological and scanning electron microscopic appearance of the lesions were consistent with bite wounds and the typical pathology was reproduced by repeated simulated bites. During behavioural experiments the parr were observed to both bite and damage each others dorsal fins. The typical nodular lesions were more prevalent and took longer to heal at lower temperatures. Dorsal fin rot was found to occur in the absence of damage to the other fins and was more severe in smaller fish. The bacteria associated with the natural lesions and following controlled damage were studied,but not found to play a significant role in the aetiology. They were capable of neither initiating nor maintaining the lesions. The main site of bacterial colonisation appeared to be on exposed fin rays. It was demonstrated that the natural lesions started to resolve as soon as the fish were placed in isolation. A limited study failed to demonstrate a definite relationship between dorsal fin rot and increased susceptibility to *Aeromonas salmonicida* infection. The implications of all these findings for control of the condition are discussed.

INTRODUCTION

INTRODUCTION

The initiative for this study came from the perception of dorsal fin rot as a significant problem in commercial Atlantic salmon (*Salmo salar* L.) smolt production.

Dorsal fin rot is a well recognised 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 an eroded dorsal fin.

Dorsal fin rot was considered to be a specific condition occurring in the absence of damage to the other fins (Robertson,D.A. pers' comm'). One of the main effects of the condition was thought to be on the cosmetic appearance, not only of the smolts, but also the 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 and Shearer, 1987) In addition there was concern that dorsal fin rot might increase susceptibility to secondary infections, especially furunculosis, caused by *Aeromonas salmonicida*. Fin rot in general, and dorsal fin rot in particular, is not usually associated with high mortalities (Schneider and Nicholson, 1980). Its main historical significance has been an effect on the subsequent survival of fish released for ranching or restocking. In such fish, loss of or damage to fins affects their swimming ability (Horak, 1969; Maheshkumura, 1985) and consequently their capacity to avoid predation or capture prey (Nicola and Cordone, 1973).

Scottish salmon farmers consulted during the course of the study were mostly of the opinion that dorsal fin rot was primarily a bacterial infection. Virtually the only method employed to control the condition was bath applications of the surface disinfectant chloramine T, which produces chlorine as the active substance. There was no definite evidence that such treatments were effective.

There are very few references dealing specifically with dorsal fin rot, the majority refer to all types of fin rot as aspects of the same condition. Fin rot will be used here as a term to cover all forms of erosive and hyperplastic fin pathologies.

Classification of fin rot lesions is far from complete, the most comprehensive description by Maheshkumar (1985) followed the procedures of Frantsi, Ritter and Fode (1972). Maheshkumar defined fin rot as a 'characteristic rough white lesion' and the healing process as the hyperplastic regeneration of diseased tissue with re-pigmentation. This apparently led to dark coloured, smooth, healed fins. No distinction was made between the change in colouration associated with smoltification and the healing process. In addition no mention was made of fins with eroded edges but no change in colouration.

The majority of other reports describe fin rot as, initially, an ulcerative, necrotic lesion, which apparently resulted in substantial loss of tissue leading to exposure or loss of the fin rays. The condition either remained necrotic or progressed to centripetal

epidermal hyperplasia (Bullock and Conroy, 1971; Mahoney, Midlige and Deuel, 1973; Murchelano, 1975; Wellings, Alpers, McCain and Miller, 1976; Schneider and Nicholson, 1980; Khan, Campbell and 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 and Conroy, 1971). Other authors have described translucent hyperplastic tissue as part of the regenerative process (Frantsi et al, 1972; Schneider and Nicholson, 1980; Maheshkumar, 1985). Although the appearance of the healed fin is in some doubt, it is generally agreed that the process involves dermal fibrosis. Fin regeneration following controlled damage has been studied in considerable depth. The fins of teleosts heal from severe tissue loss by epimorphosis, the process by which lower vertebrates regenerate limbs. The healing process does not always result in a normal fin (Nabrit, 1929; Lindesjöö and Thulin, 1990) and the fin may fail to regenerate from total amputation (Slater, 1947; Nicola and Cordone, 1973).

There are reports of fin pathologies affecting specific fins other than the dorsal. Peduncle disease affecting the caudal fin is caused by a *Flexibacter psychrophila* infection (chapter III). Other forms of non-specific tail rot have been regularly reported in marine and fresh water fish (Oppenheimer, 1958; Sindermann and Rosenfield, 1958; Conroy, 1964; Bullock, 1968; Raisanen and Behmer, 1982; Maheshkumar, 1985). Bullock and Conroy (1971) also reported a commonly observed erosion or loss of the pectoral fins associated with fish cultured in tanks or raceways with rough surfaces.

In view of the lack of information specifically relating to dorsal fin rot, the proposed aetiologies of all forms of fin pathology were considered.

Many authors mention the presence of bacteria in association with fin pathology. Only two papers propose a primary pathogenic role for these organisms (Oppenheimer, 1956; Bullock and Conroy, 1971). The majority suggest that the bacteria are opportunist or secondary invaders (Bullock, 1966; Schneider and Nicholson, 1980). For example, Mahoney *et al* (1973) reproduced fin rot lesions in marine fish by damaging the fins and then introducing bacteria, however they also implicated pollution in the aetiology. Whether these secondary invaders subsequently have an influence on the pathological process is also debatable. Bullock (1966) suggested they might have a significant effect by the production of proteolytic enzymes. Others have postulated that the bacteria merely reflect the bacterial populations in the host environment (Ghittino, 1972; Horsley, 1973) and do not significantly affect the disease process (Amend, 1970). The bacteria most frequently reported in association with fin pathology in salmonids are the Cytophaga-like bacteria (CLB). Although fungi may invade fin rot lesions they are not thought to be a causal agents (Bullock and Conroy, 1971). Bacterial involvement in fin rot is discussed in greater detail in Chapter III.

Many types of damage including those of physical, toxic and nutritional origin have been implicated as the primary cause of fin rot. Physical damage may result from rough tanks (Bullock and Conroy,

1971), biting from other fish (Abbott and Dill, 1985) or handling (Snieszko, 1958). Overcrowding has been blamed without defining the exact mechanisms (Davis, 1953; Maheshkumar, 1985), however Soderberg and Mead (1987) claimed that overstocking in isolation had no effect on fin rot. Other physical agents which have been implicated include ultra violet radiation from sunlight (Bullock and Roberts, 1981), low temperature (Schneider and Nicholson, 1980; Maheshkumar, 1985) and parasites (Bullock and Conroy, 1971; Pulsford and Matthews, 1984).

Many forms of inappropriate nutrition have been described as causes of fin rot. The nutritional deficiencies have included unspecified forms of malnutrition in salmonids (Schneider and Nicholson, 1980), a diet of zooplankton in lake whitefish (*Coregonus clupeaformis*) (Raisanen and Behmer, 1982). Lack of several essential nutrients have been implicated *e.g.* folic acid (Halver,1954), niacine (Andrews and Murai, 1978), tryptophan (Poston and Rumsey, 1982) and essential amino acids (Ketola, 1983). In one case hypervitaminosis A was suggested as a cause (Poston, 1986).

The final group of aetiological agents are the toxic pollutants. Although these have mostly been discussed in the context of marine fish, there are some fresh water examples. Haneen, Goodman and Wilson, (1977) described fin rot in sheephead minnows (*Cyprinodon variegatus*) exposed to the insecticide Kepone. Reash and Berra (1989) detected an increased incidence of fin erosion in a number of species from a stream polluted with ammonia and heavy metals. Lindesjöö and Thulin (1990) observed fin erosion as the main pathological change in

perch (*Perca fluviatilis*) and ruffe (*Gymnocephalus cernua*) subjected to pulp mill effluent. Many of the reports in marine fish deal with benthic species and a range of industrial pollutants, including crude oil (Haensley Neff, Sharp, Morris, Bedgood and Boem, 1982), polychloro-bi-phenols or PCBs (Wellings et al, 1976), dichloro-diphenyl-trichloroethane or DDT (Mearns and Sherwood, 1974; McDermott, Ehrlich,Sherwood, Heesen, Young and Mearns, 1977) and cadmium (Westerhagen, Dethefsen and Rosenthal, 1980). Still others reported fin rot in association with unspecified combinations of pollutants (Mahoney et al, 1973; Murchelano, 1975; Murchelano and Ziskowski, 1977). As with many studies in the area of aquatic pollution no causal relationships were demonstrated in these papers. It is unlikely that interaction between marine benthic fish and serious industrial pollution has much relevance to dorsal fin rot in farmed fresh water Atlantic salmon parr.

In many of the studies, fin rot was only one of many signs that were observed. It is possible that fin damage was a secondary condition, either indirectly related or totally unrelated to the parameter under study. For example the fin damage observed may have been the result of an indirect effect on the bacterial flora in the system (Roash and Berra, 1989) or changes in the behaviour of the fish. In view of the volume of conflicting evidence many authors have concluded that the aetiology of fin rot is still uncertain (Anderson and Conroy, 1969; Bullock and Conroy, 1971; Murchelano, 1975; Schneider and Nicholson, 1980; Maheshkumar, 1985).

At the outset of this study the intention was to address three main topics :

1. The description of the pathology associated with dorsal fin rot.
2. The development of a method to objectively assess the degree of dorsal fin rot in an individual and the prevalence in a population.
3. The investigation of possible aetiologies, initially concentrating on three main aspects *ie* endogenous factors (*eg* stock origin), environmental conditions and the bacteria associated with the lesions.

Subsequently, three additional areas were also included in the study, *ie* the effects of physical damage on the fin, the healing process and a brief behavioural study.

The work is presented according to subject area and is therefore not necessarily in chronological order.

A variety of statistical methods were employed, unless stated otherwise they were derived from Sokal and Rohlf (1981) and Snedecor and Cochran (1972). The calculations were either performed on a TI - 31 calculator (Texas Instruments) or a Hewlett-Packard mainframe computer using the Minitab statistical package. Some fo the frequently used statistical methods are described in Appendix I.

CHAPTER I

Gross and histological appearance of dorsal fin rot in Atlantic salmon (*Salmo salar* L.) parr.

CHAPTER I

INTRODUCTION

The gross and microscopic appearance of dorsal fin rot in Atlantic salmon up to the stage of smoltification will be described in this chapter. Examples of normal fin structure are also included for comparison.

There is a considerable amount of information available concerning fin structure. The salmonids are malacopterygii or soft rayed fish as apposed to the spiny rayed or acanthopterygii. The membranous tissue of the dorsal fin is supported by 10-12 distally branching cartilaginous rays (Maitland, 1972) or lepidotrichia. These segmented and bifurcated collagenous skeletal units consist of two apposed half rays or hemitrichia, which meet on the mid line. The rounded ends of the hemitrichia segments abut at joints which are supported by collagen fibres running between the segments. The two apposed hemitrichia and adjacent rays are connected by collagenous fibres (Becerra, Montes, Bexiga and Junqueira, 1983). Blood vessel and nerves run through the loose connective tissue between the hemitrichia. The rays branch distally, the branches become thinner and originating between the last few segments of the rays is a double palisade of thin rods or fibrils, the actinotrichia. These stiff actinotrichia are composed of elastoidin (a collagen-like protein) and extend to support the edge of the fin membrane (Haas, 1962).

I
(Introduction)

The membranous tissue is composed of epithelium and varying amounts of dermal and subcutaneous tissue. The thin distal membrane becomes thicker towards the dorsum of the fish. In parr only the thicker tissue near the base of the fin is pigmented. The pigmentation changes during smoltification when an increase in the number of melanocytes causes the distal edge of the fin to appear black. This peripheral melanisation occurs in all the fins during smoltification (Wedemeyer, Saunders and Clarke 1980).

The dorsal fin rays extend ventrally into and are supported by the pteryiophoral muscles which allow a wide range of finely tuned movements. In addition to the ability to erect and collapse the fin the fish can cause a degree of shift between the hemitrichia resulting in a lateral bending of the fin allowing a hydrofoil effect (Geerlink and Videler, 1987). The dorsal fin is used for maintaining lateral stability, manoeuvering and as a brake (Lagler, Bardach, Miller and May Passino. 1977). The dorsal fin is also raised in some forms of aggressive behaviour, for example the 'lateral display' (Keenleyside and Yamamoto, 1962)

There are few, if any, 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 confusing, the most complete description by Maheshkumar (1985) followed the procedures of Frantsi *et al* (1972). Maheshkumar 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 smoltification and healing following fin rot. There was also no mention of fins with eroded edges but without change in colouration.

The majority of other reports describe fin rot as an, initially, ulcerative necrotic lesion which often resulted in substantial loss of tissue leading to exposure or loss of the fin rays. They claimed the condition either remained necrotic or progress to centripetal epidermal hyperplasia (Bullock and Conroy, 1971; Mahoney *et al*, 1973; Murchelano, 1975; Wellings *et al*, 1976; Schneider and Nicholson, 1980; Khan *et al*, 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 and Conroy, 1971) by contrast other authors have described translucent hyperplastic tissue as part of the regenerative process (Frantsi et al, 1972; Schneider and Nicholson, 1980). Although the appearance of the healed fin is variably described, it is generally agreed that the process involves dermal fibrosis. The regeneration of fin tissue is discussed in chapter IV.

The objective of this initial part of the study was to describe the gross and histological appearance of dorsal fin rot. The claim that dorsal fin rot can occur in the absence of damage to other fins was also investigated.

CHAPTER I

MATERIALS AND METHODS

1. GROSS AND HISTOLOGICAL APPEARANCE OF THE UNDAMAGED DORSAL FIN AND DORSAL FIN ROT IN *Salmo salar* L. PARR

1.1. Development of histological processing methods.

A small number of dorsal fins with different stages of fin rot were obtained from farm 1 and processed by a variety of techniques to determine the most effective processing method. The fins were sectioned in vertical, horizontal and a variety of angled planes. The sectioning was conducted before fixation, after fixation and after wax embedding and a variety of orientations to the microtome blade were also examined.

1.2. Gross description of dorsal fin rot.

From December 1987 to December 1989, 114 fish were specifically selected for histological examinationfrom six farms and three burns. The wild fish from the burns were obtained by electro-fishing. The fish represented all the stages of fin rot from normal fins to the total absence of a dorsal fin. All the fins were examined with the aid of a dissection microscope and their condition recorded. Particular attention was paid to the dorsal fins, which were measured with callipers and the details recorded.

In addition to the samples described above, material from fish with dorsal fin rot submitted to the Diagnostic service (Institute of Aquaculture, University of Stirling) was monitored during the remainder of the project. Fish used in other parts of the study (*e.g.* Chapter IV, 4.) were also examined to corroborate the conclusions of this part of the study.

Additional information obtained from the fish used in this chapter is described in chapter II.

1.3. Histological description of dorsal fin rot.

A range of tissues, from these fish, including the internal organs, gills and all the fins were fixed in 10% neutral buffered formalin. A representative selection of the tissues were processed conventionally, stained with haematoxylin and eosin (H & E) and examined under the light microscope.

The dorsal fins were processed by the most informative technique from 1.1. *i.e.* :

A. Whole fins were removed with some of the dorsal musculature (Figure 1.1.) and fixed in 10% neutral buffered formalin and taken to wax.

B. Prior to embedding in the wax blocks, the fins were sectioned as shown in Figure 1.1. (two or three times) and the cut ends placed down towards the cutting face of the block.

C. The cut surfaces were placed so that the distal fin was cut first by the microtome blade.

D. The resulting sections were stained with H & E and mounted with Pertex (Histo-Lab).

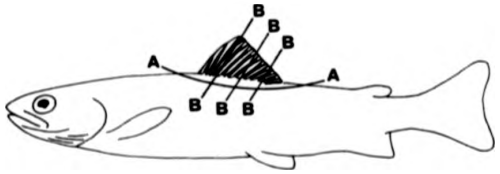


Figure 1.1. Diagrammatic representation of the area sampled (A-A) and the sections examined (B-B) in 1.3.

1.3.1. Description of the histological appearance of the undamaged dorsal fin.

A range of undamaged fins from 1.2. were examined to decribe the normal appearance of the dorsal fin.

1.3.2. Description of the histological appearance of dorsal fin rot.

The material from diseased fins (1.2.) were examined and compared to the recorded gross pathology, to provide a description of the dorsal fin rot lesions.

1.4. Additional stains.

A range of alternative stains were also applied to sections representative of the different stages of dorsal fin rot. These included periodic acid-Schiff (PAS), Gomori's rapid one step trichrome and Gordon and Sweets' reticulin method (Dury and Wallington, 1980).

1.5. The relationship between dorsal fin rot and damage to the other fins.

A gross description of all the fins was recorded for 101 of the fish, to evaluate the relationship between dorsal fin rot and damage to the other fins (Table 1.1).

Table 1.1.
Details of the number and origin of fish sampled specifically for histology.

Site	Date	Population	Number of fish with dorsal fin rot
FARM 1 (tank 5)	18/12/87	1	2
FARM 2	7/1/88	2	4
FARM 1 (tank 5)	11/1/88	3	4
FARM 1 (tank 5)	2/2/88	5	5
FARM 3 (tank 17)	9/2/88	6	2
FARM 3 (tank 17)	10/2/88	7	8
FARM 4 (cage 2)	11/2/88	8	10
FARM 1 (tank 4)	16/2/88	9	4
FARM 1 (tank 12)	16/2/88	10	3
FARM 1 (tank 4)	1/3/88	11	2
FARM 1 (tank 12)	1/3/88	12	3
FARM 5	10/3/88	13	5
FARM 6 (tank A21)	22/3/88	14	6
FARM 6 (tank B8)	22/3/88	15	5
FARM 1 (tank 5)	30/3/88	17	2
			66

With the exception of FARM 4 all the farms were fresh water smolt farms where the fish were reared in glass fibre tanks. FARM 4 was a fresh water cage site.

CHAPTER I

RESULTS

1. GROSS AND HISTOLOGICAL APPEARANCE OF DORSAL FIN ROT IN *Salmo salar* L. PARR

1.1. Development of histological processing methods.

Many of the techniques investigated resulted in artifacts in the epithelium and separation of the deeper tissues. The least processing artifacts were produced by the technique described in Materials and Methods 1.3. This technique also allowed examination of all the main components of the fin including the fin rays the distal edge of the fin. However the oblique section employed occasionally produced longitudinal (Figure 1.16.) or cross sections (Figure 1.13.) of the fin rays due to slight alterations in the position of the fin.

1.2. Gross description of dorsal fin rot.

No evidence of fin damage was detected in the wild or escaped fish, which were therefore used as examples of normal undamaged fins.

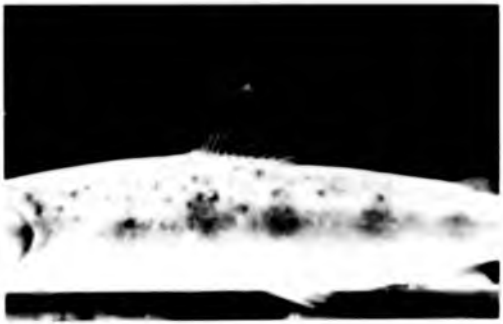


Figure 1.2. Normal undamaged dorsal fin.

The branching, segmented fin rays or lepidotrichia can be seen supporting the membranous tissue of the fin (Figure 1.2.). The shape and the size of the undamaged fins appeared to vary between fish of the same size, this variation was investigated further in chapter II.

It was possible to differentiate damage to the dorsal fin into seven main categories listed below (A-G).

A. Peripheral erosion and ray splitting.

B. Peripheral erosion with some nodularity.

C. Severe nodularity with differing degrees of tissue loss.

D. Extensive to total loss of the dorsal fin.

E. Smooth thickening of the dorsal fin.

F. Healed dorsal fin rot lesions.

G. Haemorrhagic dorsal fin lesions.

Further evidence for the relationship between these forms of damage is discussed in Chapter IV.

A. Peripheral erosion and ray splitting (Figure 1.3.).

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.

Figure 1.3. An example of peripheral erosion and ray splitting in a live Atlantic salmon parr.

B. Peripheral erosion with some nodularity.

Peripheral erosion was often associated with differing degrees of nodularity. The degree of erosion was not proportional to the nodularity in every case, that is, some fish had quite severe nodularity with relatively minor erosion and some had extensive erosion with little nodularity. The nodularity described under this

heading was merely 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. No satisfactory photographs were obtained of this form of fin damage.

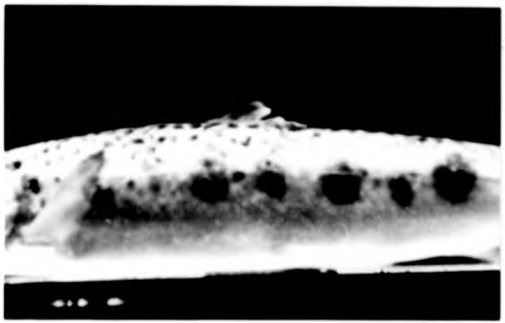


Figure 1.4. An example of severe nodularity with tissue loss in a live Atlantic salmon parr.

Figure 1.5. An example of severe nodularity with tissue loss in a formalin fixed Atlantic salmon parr.

C. Severe nodularity with differing degrees of tissue loss (Figures 1.4. and 1.5.).

Severe nodularity is the lesion usually described by fish farmers as fin rot. This lesion is easily seen when the fish are inspected in the production tanks or cages. Under such circumstances the lesion appears as a grey or white nodular line on the dorsal fin. When examined under a dissection microscope the lesions appeared to be an area of thickened tissue overlaying the remains of the distal fin rays. The distal ends of the damaged and shortened rays were often observed protruding from the nodular tissue. In the majority of cases the nodularity was confined to the distal portion of the remaining fin. Even in severe cases there was usually some normal membranous tissue

remaining in the proximal areas of the fin. The loss of tissue was often more severe towards the posterior edge of the fin.

The nodularity was the result of both the excess tissue and the position of the fin. Slight extension of the fin reduced the nodularity in some fins. However in the most severe cases the large amount of excess tissue reduced the capacity for movement. Attempts to extend such fins with forceps tore the tissue between the fin rays.

Figure 1.6. An example of extensive loss of dorsal fin tissue in a formalin fixed Atlantic salmon parr.

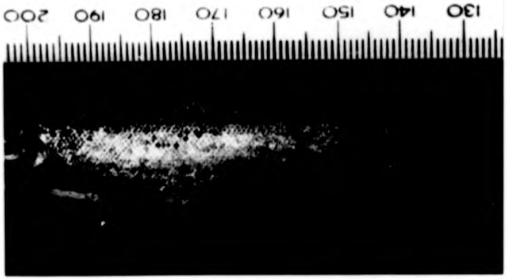


Figure 1.7. An example of extensive loss of dorsal fin tissue in a formalin fixed Atlantic salmon parr.

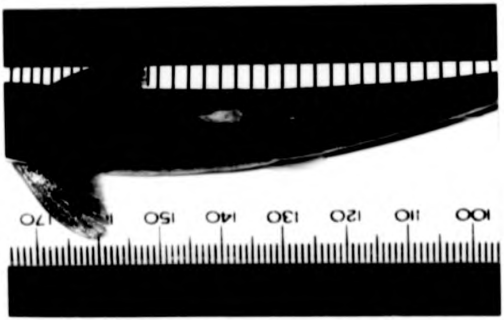


Figure 1.8. An example of extensive loss of dorsal fin tissue and ulceration of the surrounding area, in a formalin fixed Atlantic salmon parr.

D. Extensive to total loss of the dorsal fin (Figures 1.6., 1.7. and 1.8.).

In the most severely affected populations individuals were observed with almost total loss of the dorsal fin. There remained either a very short thick smooth edged fin or a thin nodular 'fin rot' lesion adjacent to the dorsal surface of the fish.

On two of the sites examined a number of fingerlings and parr were observed with an open wound on their back and no remaining external dorsal fin tissue (Figure 1.8.). There is no evidence to confirm or deny that these lesions are part of the disease condition referred to, here, as dorsal fin rot.

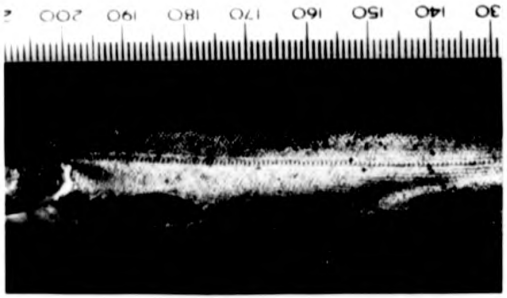


Figure 1.9. An example of smooth thickening of the dorsal fin in a formalin fixed Atlantic salmon parr.

Figure 1.10. An example of smooth thickening of the dorsal fin of a formalin fixed Atlantic salmon parr.

E. Smooth thickening of the dorsal fin (Figures 1.9. and 1.10.). This mildest form of nodularity consisted of a slight, opaque, smooth thickening of the peripheral fin. These smooth lesions did not severely restrict the movement of the fins.

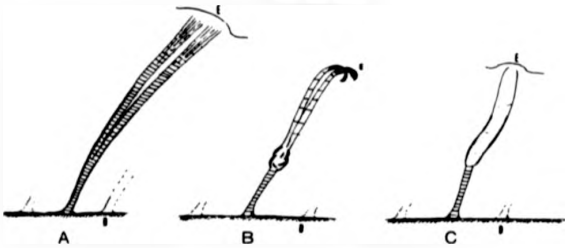


Figure 1.11. A diagrammatic representation of a fin ray from an undamaged fin and a fin thought to have healed from naturally occurring dorsal fin rot. A = Normal fin ray. B = Healed fin ray. C = Healed dorsal fin ray which failed to fork. D = Dorsal surface of the fish. E = Edge of the fin.

Figure 1.12. An example of a healed and regenerated dorsal fin in a live Atlantic salmon parr.

F. Healed dorsal fin rot lesions (Figure 1.11 and 1.12.).

Fins were observed and tentatively classified as healed fins. The proximal portion of such fins was often normal. The distal portion however contained thin or twisted fin rays often with an abnormal branching pattern. These areas of the fin were twisted and incapable of full extension and the areas of normal and abnormal tissue were sharply defined.

G. Haemorrhagic dorsal fin lesions.

On a number of occasions fish from populations suffering concurrently from dorsal fin rot and furunculosis (*A.salmonicida* infection) were examined. Several fish from such populations had haemorrhagic lesions associated with the dorsal fin. A variety of these lesions were observed from diffuse haemorrhages over the fin to focal areas of haemorrhage and swelling at the base of the fin. Bacteriological sampling of these lesions often, but not invariably, recovered large numbers of *A.salmonicida*. No suitable photographs of these lesions were obtained.

1.3. Histological description of dorsal fin rot.

No consistent pathology was detected in any of the gills or internal organs examined.

1.3.1. Description of the histological appearance of the undamaged dorsal fin.

In order to determine the changes occurring in dorsal fin rot it was necessary to describe the normal histology. Sections of normal fin are presented in Figures 1.13. to 1.17. The structure of the normal fin is discussed in the introduction to this chapter.

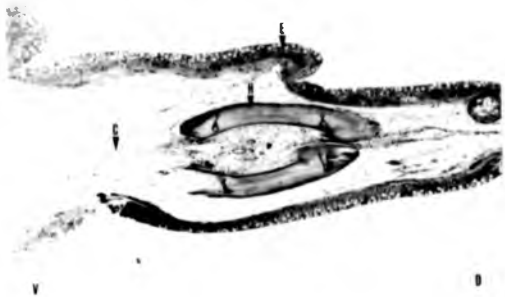


Figure 1.13. A transverse section through a fin ray of an undamaged dorsal fin. D = Dorsal. V = Ventral. E = Epithelium. H = Hemitrichia. C = Central dense connective tissue joining adjacent fin rays. (H & E, x 40).

Figure 1.14. A section through an undamaged dorsal fin. C = Central dense cellular fibrous tissue. H = Hypodermal connective tissue. CM = Connective tissue containing melanocytes. SC = Stratum compactum. B = Basement membrane. E = Epithelium. M = Mucous cell. (H & E, x 100).

The surface of the fin is covered with an epidermis of normal teleost epithelium, the mucous cells being more numerous near the base of the fin and rare in the distal epithelium. It is difficult to determine the exact thickness of the epithelium in cross section, since the angle of the section and any folding in the fin alters the observed depth. However the examination of a number of sections would suggest that there is a progressive thinning of the epithelium towards the distal fin. Despite all attempts to minimise processing artifacts, areas with thin or no epithelium were observed in some of the sections.

Under the epithelium there is a basement membrane and a layer of dense connective tissue continuous with the stratum compactum on the dorsal surface of the fish's body. The melanocytes are situated in the deeper portion of this layer. Below the level of the melanocytes the connective tissue becomes 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 is continuous with the hypodermis elsewhere. In the centre of a cross section of a fin between fin rays there is a thin band of dense cellular connective tissue. This represents the collagenous fibres connecting adjacent rays and is continuous with the dense connective tissue surrounding and separating the two halves of the fin rays (Figures 1.13. and 1.14.). The rays or lepidotrichia, which are dense acellular bundles of collagen fibres are intimately associated with the surrounding connective tissue.

In cross section the fin rays are two closely apposed concave bundles, the central gap formed by these structures containing neural and vascular elements surrounded by cellular connective tissue (Figures 1.13 and 1.15.).

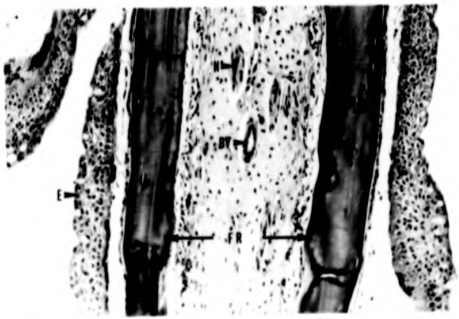


Figure 1.15. A cross section of an undamaged dorsal fin. FR = Fin ray. BV = Blood vessel. N = Nerve. E = Epithelium. (H & E × 100).

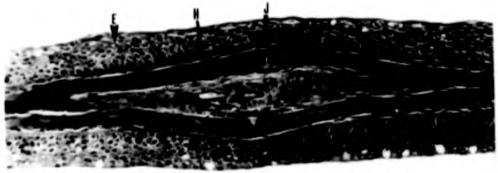


Figure 1.16. A cross section of an undamaged dorsal fin. H = Hemitrichia. J = Joint. E = Epithelium. (H & E × 100).

In longitudinal section the hemitrichia are made up of a number of joined sections (Figure 1.16.). The sections are connected by a cuff of fibrous tissue. In the proximal fin the less dense fibrous hypodermal layer intervenes between the fin ray and stratum compactum. Distally the stratum compactum is intimately associated with the outside of the fin ray.

Figure 1.17 A cross section of the distal edge of an undamaged dorsal fin. (H & E × 200).

The extreme edge of the fin consists of a layer of epithelium (Figure 1.17). This epithelium is supported by the actinotrichia although no suitable photographs were obtained of these slender fibrils.

1.3.2. Description of the histological appearance of dorsal fin rot.

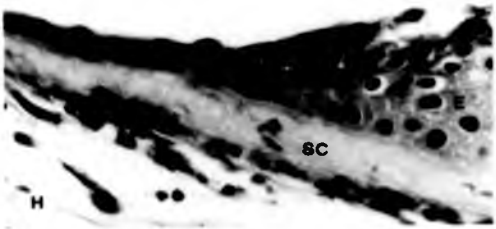


Figure 1.18. A section from an eroded dorsal fin. Part of the epithelium is abnormally thin and there is an adjacent area of necrosis. E = Epithelium. SC = Stratum compactum. H = Hypodermal connective tissue. (H & E = 400).

A. Peripheral erosion and ray splitting (Figures 1.18. and 1.19.).

It was difficult to obtain good sections of the area immediately surrounding the erosion due to the fragile nature of the tissue, however in cases where the only gross sign was erosion, no cellular inflammatory response was observed. The most common findings were areas of thin or attenuated epithelium (Figure 1.18.) and defects or clefts extending through the depth of the epithelium (Figure 1.19.).

It is difficult to differentiate such lesions from artifacts due to processing. However these clefts were only detected with any regularity, in fins that had gross signs of fresh rough erosion.

Figure 1.19. A section from a nodular and eroded dorsal fin. There are clefts in the epithelium and mild nodular hyperplasia E = Epithelium. CL = Cleft. (H & E = 40).

B. Peripheral erosion with some nodularity. (Figures 1.19., 1.20. and 1.21.).

Again the main defects were clefts through the epithelium (Figure 1.19.). The nodularity in these sections was largely due to epithelial hyperplasia (Figure 1.20.), there was however evidence of a cellular inflammatory response in many specimens. In the early stages the cellular response appeared to be associated with the presence of necrotic tissue (Figure 1.21.).

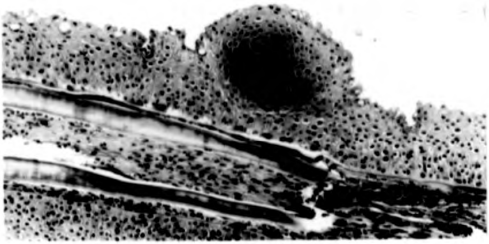


Figure 1.20. A section of a dorsal fin with erosion and mild nodular thickening. A hyperplastic epithelial nodule is obvious in the centre of the photograph. (H & E × 100).

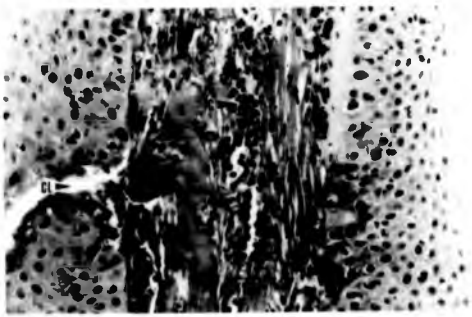


Figure 1.21. A section of an eroded dorsal fin with mild nodular thickening. There is necrotic tissue and a mild cellular inflammatory response. E = epithelium. CL = Cleft. N = Necrotic tissue. IC = Inflammatory cells. (H & E = 200).

C. Severe nodularity with differing degrees of tissue loss (Figures 1.22. to 1.28.).

Changes in the epithelium were common to all the sections of thickened fin examined. The gross nodular appearance was reflected histologically, in milder cases by epithelial nodules (Figure 1.22.) and in more severe cases by dermal extensions surrounded by hyperplastic epithelium (Figure 1.23.). The bulk of thickening in the fin appeared to be the result of a hyperplastic response in the epithelium (Figure 1.23.). The thickened epithelium stained irregularly with severe vacuolation in some areas indicating cytopathology and intercellular

oedema or spongiosis. In the hyperplastic epithelium the presence or absence of mucous cells is similar to the normal fin with fewer in the distal epithelium. There were also necrotic cells and leucocytes throughout the abnormal epithelium (Figures 1.24. and 1.25.). In the areas of greatest hyperplasia the structure of the epithelium was confused, with whorls, areas of cells in different stages of development and dermal extensions into the epithelium (Figure 1.23.). In some areas portions of necrotic fin rays were observed protruding through the epithelium (Figure 1.26.).

Figure 1.22. A section of a dorsal fin with mild nodular thickening, with hyperplastic epithelial nodules and a distinct sub-epidermal cellular inflammatory response. N = Nodule. CI = Cellular inflammatory response. (H & E = 40).

Figure 1.23. A section from a dorsal fin with severe nodular thickening. The fin has irregular dermal projections surrounded by extensive hyperplastic epithelium. The majority of the increase in thickness of the fin is due to epithelial hyperplasia. (H & E × 40).

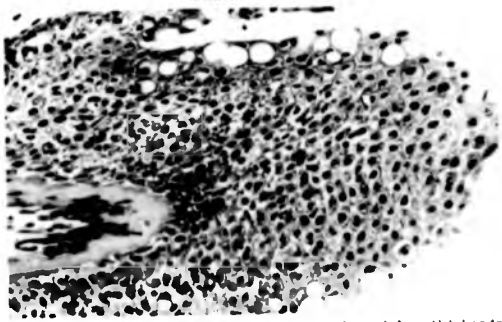


Figure 1.24. A section from a dorsal fin with nodular thickening. The epithelium shows extensive vacuolation and spongiosis. (H & E = 200).

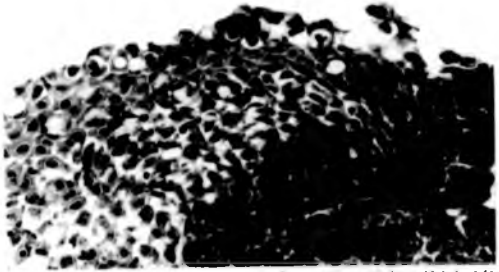


Figure 1.25. A section from a dorsal fin with nodular thickening. The epithelium is necrotic in some areas and has stained very irregularly. Leucocytes are also present within the epithelium. L = Leucocyte. (H & E = 200).

Figure 1.26. A section of a severely eroded and thickened dorsal fin. There is extensive epithelial hyperplasia. The fin rays extend out through the epithelium and are separated by inflammatory tissue displacing the normal vascular and neural elements. FR = Fin ray. (H & E × 20).

The changes in the deeper tissue were far less consistent and varied considerably between individuals with similar degrees of gross change. In all cases there was an increase in cellularity in the deeper connective tissue with some migration of leucocytes through the epithelium (Figure 1.25.). The degree of cellularity varied from mild (Figure 1.23.) to very severe and extensive (Figures 1.27. & 1.28.). In most but not all cases a dramatic increase in cellularity was associated with large accumulations of necrotic tissue. In some cases where there was extensive fibro-cellular reaction and the internal

structure of the fins was severely disrupted with separation of the fin rays and damage to the vascular and neural elements (Figure 1.26.).

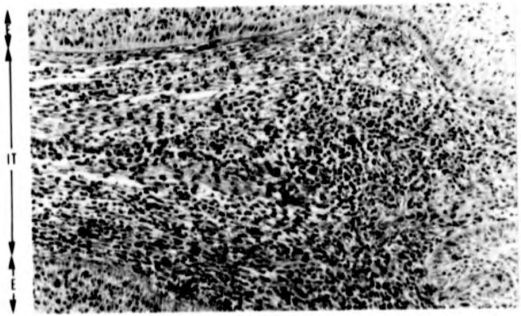


Figure 1.27. A section of a dorsal fin suffering from extensive tissue loss and smooth thickening. The dermal tissues are obscured by a fibro-cellular inflammatory response. E = Epithelium. IT = Inflammatory tissue. (H & E × 100).

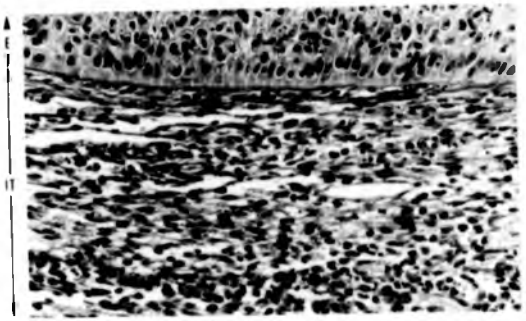


Figure 1.28. A section from a dorsal fin with extensive tissue loss and smooth thickening. The dermal tissues are obscured by a cellular inflammatory response. E = Epithelium. IT = Inflammatory tissue. (H & E × 200).

D. Extensive or total loss of the dorsal fin.

These lesions had the typical appearance of superficial ulcers with various degrees of secondary infections, inflammation and healing.

E. Smooth thickening of the dorsal fin (Figures 1.29. and 1.30.).

Again the gross appearance was reflected histologically, the epithelial hyperplasia presented a smoother external surface (Figure 1.29.). These lesions frequently had extensive dermal inflammatory tissue (Figures 1.27. & 1.28.). Necrosis and sloughing of the superficial epithelium was also a common feature (Figure 1.30.)

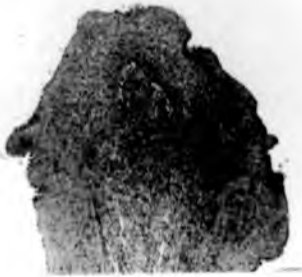


Figure 1.29. A section from a dorsal fin with extensive loss of tissue and smooth thickening. There is substantial epithelial hyperplasia and the dermis is obscured by inflammatory tissue. E = Epithelium. IT = Inflammatory tissue. (H & E = 40).

Figure 1.30. A section from a dorsal fin with limited smooth thickening. The superficial epithelial cells are in the process of sloughing off. (H & E = 100).

F. Healed lesions.

Fins with evidence of healed lesions occasionally had distorted fin rays but histologically were similar to normal fins.

G. Haemorrhagic dorsal fin lesions.

There was no definite pattern to these lesions. Red blood cells were observed free in the tissues in some sections but not all. In the tissue around the base of some fins there was necrotic tissue and bacterial colonies, similar to the appearance of 'furuncles' in *A.salmonicida* infections. Still other sections did not differ in any respect from dorsal fin lesions without haemorrhages.

1.4. Additional stains.

No significant additional information was obtained from the other stains. The PAS emphasised the position of the mucous cells but did not reveal any additional information about their distribution. The Gomori's trichrome emphasised the collagenous elements of the fins which were consistent with published descriptions (Introduction). The reticulin method revealed very little if any of this tissue in the fins.

1.5. Relationship between dorsal fin rot and damage to the other fins.

A comparison was made between the condition of the dorsal fin and the other fins for all twelve farmed populations. With two exceptions all of these populations had a number of fish with dorsal fin rot but no damage to the other fins (Table 1.2.).

Table 1.2.
Proportion of populations with damage to the dorsal and other fins.

Popul^n	Fish with dorsal fin rot only: % dorsal fin remaining 60-90	30-60	10-30	Total	Fish with fin rot on dorsal and other fins
1	-	1	-	1	1
2	3	1	-	4	0
3	2	-	2	4	0
5	1	1	2	4	1
7	-	1	-	1	1
8	-	-	-	0	8
9	1	2	2	5	5
10	-	1	1	2	5
11	1	2	-	3	2
12	1	2	-	3	2
13	3	3	1	7	4
14	-	-	-	0	2
			Total	34 (52.3%)	31 (47.7%)

From Table 1.2. it can be seen that 52.3% of the total number of fish with dorsal fin rot examined did not have any appreciable damage to any of their other fins. All the fish with dorsal fin rot from populations 8 and 14 had significant damage to the caudal fin. In all but four of the fish examined the dorsal fin was the most severely damaged. Three fish each from separate populations, had most damage on their caudal fin and the left pelvic fin of another fish was the most severely damaged.

CHAPTER I

DISCUSSION

The fins were covered by teleost skin of normal histological appearance (Reviewed by Bullock and Roberts, 1975). Fish epidermis differs from higher vertebrates in several respects. The epithelium is covered by a thin mucous layer or cuticle which is virtually impossible to retain during histological processing (Roberts and Bullock, 1976). This layer is produced by a combination of epithelial debris and the products of the mucous or goblet cells, which originate in the middle layers of the epithelium and migrate distally to discharge their mostly glycoprotein secretions. The epithelial cells or malpighian cells are not keratinised and even the most superficial are capable of mitotic division. The relatively thin epithelium is composed principally of similarly shaped cells which only become flattened distally - there is no true stratification. The epithelium contains other cell types including club or alarm cells, granule or eosinophilc cells, leucocytes and occasionally the enigmatic rodlet cells. The epithelium is based on a thin basement membrane separating it from the stratum spongiosum of the dermis which merges with the stratum compactum. The dermis is also the site of the various pigment cells and in some areas of the body, the scales. Underlying and intimately connected with the dermis is the hypodermis, a looser structure containing adipose and vascular elements.

Healing of the teleost epithelium is exceptional in that it occurs largely independently of temperature. The main process following damage to the epithelium is cellular migration, proliferation only playing a secondary role (Bereiter-Hahn,1986). The closure of wounds is also more rapid than in mammals and serves to protect the fish from pathogens and adverse osmotic effects (Anderson and Roberts, 1975).

In the fins examined there were three main types of superficial damage, *ie* areas lacking epithelium, areas of thin or attenuated epithelium and clefts through the epithelium. The areas both lacking and with thin epithelium histologically were detected in fins with all types of gross appearance, from apparently undamaged to the most severely thickened. Although some of these lesions may have represented pathology in the living fish, their distribution would suggest they were mostly artifacts. The areas of thin epithelium were more commonly associated with rough eroded fins. This may imply that the condition of such fins predisposed them to this form of artifact. However, presence of such areas should not be completely discounted since attenuated epithelium has been observed following the epithelial migration associated with superficial wounds (Phromsuthirak, 1977). The most significant evidence of damage observed was the clefts, which were confined to eroded and nodular fins and were not detected in smooth, healed or undamaged fins.

A sequence of events is proposed accepting the limitations imposed by isolated samples.

I
(Discussion)

The peripheral erosion may represent both initial injury, prior to any host response and the latter stages of healing from mild damage. The peripheral erosion with nodularity probably represented a progression from the initial lesion since at this stage some host response was detected *i.e.* epithelial hyperplasia and a limited cellular inflammatory response.

Similar but more extreme host responses were observed in cases of severe nodularity with tissue loss. The tissue loss was presumed to be an indication of the progression of the condition with total absence of the dorsal fin representing the most advanced cases.

Smooth thickening of the dorsal fin was associated with chronic inflammation and a severe fibro-cellular reaction. These were thought to be fins in the early stages of repair. In some of the cases of smooth thickening there was extensive loss of tissue but the fins were invariably capable of a wider range of movement than the roughly thickened fins. There was also evidence of sloughing epithelial cells at the surface of the tissue. This may be similar to the process observed during healing from bacterial gill disease, where repair occurs as the result of necrosis and sloughing of the hyperplastic epithelium (Kudo and Kimura, 1983a and b; Ferguson, Ostland, Byrne and Lumsden, 1991). Bacterial gill disease is discussed in greater detail in Chapter III.

The fins referred to as healed had a distinct area of grossly abnormal tissue. At present there is no other explanation except recovery from

fin rot to account for these changes. Nabrit (1929) reported similar abnormal regeneration of fin rays in the tail of goldfish (*Carassius auratus*) and the killifish (*Fundulus heteroclitus*). Studies into fin amputation, in order to mark fish, suggest that the dorsal fin is unlikely to regenerate following complete removal (Slater, 1947). Therefore the fins described here most likely represented recovery from moderate tissue loss

The haemorrhagic lesions were associated with *A.salmonicida* infections in some cases, otherwise there was no relationship with the type or extent of the fin rot lesions. Schneider and Nicholson (1980) proposed that *A.salmonicida* may be significant in the aetiology of fin rot though this was based on an occasional association rather than the demonstration of a causal relationship.

These observations did not produce any definite evidence for the aetiology of the condition. However the clefts observed in the epithelium may be the result of physical damage which is one of the possible aetiologies. It has been proposed by several authors that fin rot is a response to physical injury including handling (Snieszko, 1958) rough tanks (Bullock and Conroy, 1971) and biting by other fish (Abbott and Dill, 1985).

Despite frequent references to bacteria in association with fin rot, no bacteria were detected histologically. However this finding should be regarded with caution since any superficial bacteria may have been removed during processing.

I

(Discussion)

The major pathological change observed in this study was epithelial hyperplasia. This is a more frequent response in fish than higher vertebrates. It can result from physical, infectious or toxic stimuli and is more common at lower temperatures (Roberts and Bullock, 1976). The precise mechanism by which hyperplasia is induced has not been adequately studied. It may result from the action of bacterial toxins (Kudo and Kimura, 1983c) or as a consequence of cell necrosis (Ostland,Ferguson and Stevenson, 1989).

In some respects the fin rot lesions resembled epidermal papillomas. Both epithelial hyperplasia and cellular inflammation are features of the latter stages of papillomas (Carlisle and Roberts, 1977). However papillomas in salmon tend to be regular lesions, more widely distributed over the surface of the fish and are often most severe in the summer, resolving in the winter.

The leucocytes observed in the epidermis are a normal feature, which increase in numbers under certain circumstances in salmonids *e.g.* sexual maturation (Peleteiro and Richards, 1985) and sloughing of papillomas (Carlisle and Roberts, 1977). In this case the higher numbers of leucocytes observed in some sections probably reflected of the dermal cellular inflammatory response. Although such cells can be involved in local immune responses, their exact role has yet to be defined (Lobb, 1987).

There are obvious discrepancies between the published descriptions of fin rot and the observations made during this study. Maheshkumar

(1985) defined fin rot as the 'characteristic rough white' lesion. This is perhaps equivalent to the cases of severe nodularity observed in this study and the lesions referred to as fin rot by farmers. Maheskumar then described hyperplastic regeneration, resulting in re-pigmentation. The present findings suggested that hyperplasia was part of the active pathology and recovery involved sloughing of that tissue. Pigmentation is confined to the proximal areas of the dorsal fins in parr and distal pigmentation only occurs during smoltification (Wedemeyer, *et al* 1980).

Other authors have described the initial lesion as ulcerative and necrotic (Introduction). In this study there was very little evidence of active ulceration. Necrosis of the tissue was observed irregularly through the specimens with epithelial necrosis being more common in the latter stages. Substantial portions of the dorsal fin were lost in some of the cases of dorsal fin rot examined, however the evidence suggested that the tissue loss was not primarily due to necrosis.

Centripetal epithelial hyperplasia, as reported by other authors, was found consistently, and was the major pathological change in most samples. Frantsi *et al* (1972) and Schneider and Nicholson, (1980) described translucent hyperplastic tissue as part of the healing process and this may be equivalent to the smooth thickening observed in this study. The healing of fins following loss of tissue is discussed in chapter IV.

I
(Discussion)

The stains *i.e.* PAS for mucopolysaccharides, Gomori's trichrome for collagen and Gordon and Sweets' method for reticulin, were employed to emphasise the relevant tissue elements. Although they achieved this objective they did not provide any additional information.

Dorsal fin rot was found in the absence of damage to the other fins in over half of the samples examined and the dorsal fin was the most severely damaged fin in most of the fish. This limited study would suggest that even if dorsal fin rot has the same cause as damage to the other fins it may be the most severely or only fin effected. However the limited number of samples do not necessarily reflect all aspects of fin rot in farmed Atlantic salmon.

The objective of this part of the study was to define and describe the condition. The gross and histological description presented here is a more complete and detailed account of dorsal fin rot than was previously available. Although examination of histological specimens provided a considerable amount of information, an additional technique was required to study the surface of the epithelium in greater detail. Scanning electron microscopy was selected as an appropriate technique and its use is described in chapter V. Other areas that required further investigation included the role of bacteria, including *A.salmonicida*, the response to physical injury and the healing process. These areas were investigated and are reported in subsequent chapters.

CHAPTER II

Methods for assessing the incidence of dorsal fin rot within populations of Atlantic salmon (*Salmo salar* L.) and the relationship of that incidence to other parameters.

CHAPTER II

INTRODUCTION

In order to study any disease it is useful if it is first defined and then a method is developed to determine its incidence. Ideally these aspects should be addressed before further study of the condition. However dorsal fin rot was reported by farm staff, at the sites being investigated, to occur from Autumn until smolt transfer in the following Spring. As the project commenced in January it was considered necessary to obtain as much information as possible over the first four months, since the condition appeared to be seasonal in nature and would not have been available for study until the following October or November. Therefore several aspects of dorsal fin rot were addressed simultaneously ie.

The description and definition (Chapter I).

The development of an objective method for assessing the incidence and severity.

The assessment, by a subjective method, of the incidence and severity within selected populations.

The monitoring of the stocking and environmental parameters affecting those populations.

A study of the bacteria associated with the condition (Chapter III).

II
(Introduction)

The development of an objective technique is described first in this chapter, although it was not the first part of the study to be completed. The development of an objective technique was planned in four main stages. The first was the development of a method for objectively assessing the size and shape of the dorsal fin. The second was the determination of the accuracy and precision of the technique developed. Then an attempt was made to predict the size of the dorsal fin of a fish in order to determine the amount of tissue lost due to fin rot. Finally the objective technique was compared with the subjective visual assessment, used concurrently in other areas of the study.

The development of a technique to determine the loss of tissue due to dorsal fin rot depended on the existence of a consistent relationship between the size of the fish and the size of dorsal fin. It was therefore necessary to determine the relative size of the dorsal fin within and between populations. It has been suggested that the height of the fin grows in proportion with the length of the fish throughout its life and also that this proportion may be similar for all members of a species (Kindachi, 1987). Kindachi did not however publish any experimental corroboration for this theory. Conversely other workers have demonstrated morphometric differences between populations of Atlantic salmon. These differences, including the length and height of paired fins, have been used to differentiate between different stocks. Such variation has genetic and environmental origins (MacCrimmon and Claytor, 1985 and 1986). However published information regarding the variation in size of the dorsal fin is limited.

II
(Introduction)

Concurrent studies monitored the incidence and severity of dorsal fin rot within four tanks on a commercial farm, by a subjective method. It had been reported by farm staff that the incidence of fin rot varied between tanks. The object was to study tanks with different incidences of dorsal fin rot in order to relate that incidence to stock origin, environmental parameters or management practices.

The subjective method was based on visual assessment and a provisional description of the condition. This approach was derived from the method described by Frantsi *et al.* (1972) and subsequently adopted by other workers (Schneider and Nicholson, 1980; Maheshkumar, 1985). These workers classified fin rot as 1°, 2° or 3°. When the distal third of a fin was affected by fin rot it was described as 1°, when the medial third was affected as 2° and 3° when the proximal third was affected. The results from the subjective assessment were subsequently re-assessed in light of improved descriptions and methods of determining the incidence of the varying stages of fin rot.

There were several reasons for examining the relationship between dorsal fin rot and environmental parameters. Previous publications had implicated environmental conditions in the aetiology of dorsal fin rot and in addition many fish farmers maintained that such a link existed. There is also a related condition known as bacterial gill disease which is associated with cytophaga-like bacteria and in many cases initiated by a deterioration in environmental conditions (chapter III).

II
(Introduction)

Published information regarding the role of environmental conditions in the aetiology of dorsal fin rot is far from conclusive. Many of the contradictions have arisen from the interaction between various environmental conditions. Schneider and Nicholson (1980) and Maheshkumar (1985) working on the same hatcheries found both positive and negative correlations between fin rot and temperature. In the same study Maheshkumar (1985) found a higher incidence of fin rot associated with tanks plumbed in series. It was reported that incidence of fin rot increased as the water quality deteriorated.

The effect of stocking density on the incidence of fin rot is also debatable. Westers and Copeland (1973), Schneider and Nicholson (1980) and Maheshkumar (1985) all found a positive correlation between fin rot and population density, however the effect could have been due to concurrent variation in other environmental parameters. Under more controlled conditions, no relationship was found between population density and fin rot in Atlantic salmon (Soderberg and Meade, 1987), in lake trout *Salvelinus namaycush* (Soderberg and Krise, 1987) or in Chinook salmon (*Oncorhynchus tschawytscha*) (Moring, 1982). However the work in Atlantic salmon and lake trout was based on the possibly misleading fin factor proposed by Kindschi (1987) (see discussion).

Since the available information was conflicting and limited, an attempt was made to analyse as wide a range of relevant parameters as possible.

II
(Introduction)

The populations of four tanks were studied, all the tanks having an identical structure and water supply with a similar flow. The four populations received the same type of food as a percentage of body weight, according to commercial charts. The populations under study originated from three strains of broodstock and included three diploid and one all female triploid.

It was accepted that the study of a large number of related factors would not produce any definitive evidence for the aetiology of dorsal fin rot. Definitive evidence would require manipulation of the appropriate parameters under controlled conditions. The aims of this part of the project were to develop an objective method for monitoring the incidence and severity of dorsal fin rot in a population and to identify factors associated with dorsal fin rot worthy of further investigation.

CHAPTER II

MATERIALS AND METHODS

1. METHODS FOR ASSESSING THE PREVALENCE OF DORSAL FIN ROT IN POPULATIONS OF *Salmo salar* L. PARR

This area of the study was carried out in three parts :

1.1. The development of a technique to objectively record the condition of dorsal fins.

1.2. Examination of the relationships between the size of individual fish and the size of their undamaged dorsal fin.

1.3. A comparison between subjective visual assessment and a photographic technique for determining the incidence and severity of dorsal fin rot within a population.

1.1. The development of a technique to record the condition of dorsal fins.

The object was to find a technique that would be suitable for recording the condition of dorsal fins and the length of the fish under field conditions. The following three techniques were assessed :

1.1.1. Measurement with callipers.

1.1.2. Marking of the relevant points onto paper.

1.1.3. Obtaining measurements from photographs of the fish.

With all three techniques the fish were killed by immersion in 2-phenoxyethanol (Sigma chemical Co) prior to recording the details.

The information recorded in each case was (Figure 2.1.) :

FL= Fork length

A = Height of the leading edge of the dorsal fin, taken from the junction of the fin with the dorsal surface of the fish to the highest point of the fin.

B = Height of the trailing edge of the dorsal fin, taken from the junction with the dorsal surface of the fish to as near the beginning of the dorsal edge of the fin as possible.

C = Length of the base of the dorsal fin, taken from the junction of the leading to the junction of the trailing edges with the dorsal surface of the fish.

The fish used were obtained from a commercial farm, and had an average weight of 15g.

1.1.1 Measurement of the fish and fins with callipers (Camlab Ltd). Five fish were used and included one fish with an undamaged dorsal fin, one with severe dorsal fin rot and three with mild fin rot.

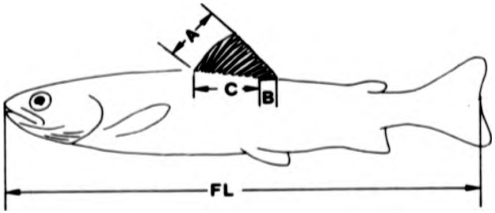


Figure 2.1. Measurements recorded from each fish during the evaluation of objective techniques for determining the extent of damage due to dorsal fin rot.

1.1.2. Marking the relevant points onto paper.

Five fish with similar fin condition to those used in 1.1.1 were selected. The anaesthetised fish were placed onto water-proof paper (Aquascribe, Hawkins and Manwaring). In a similar manner to 1.1.1 a series of ten replicate measurements were taken from each fish.

1.1.3. Measurements from photographs of the fish.

All the photographs were taken with a pentax SLR camera and a 100mm macro lens using Pan-f black and white film (Ilford).

A series of photographs were taken to determine an effective means of recording details of the dorsal fin photographically. A fish with mild dorsal fin rot was selected and thirty six photographs were taken, including combinations of the following parameters :

16 backgrounds, including several colours and textures,

3 shutter speeds,

4 aperture settings and

3 light sources, (camera flash, tungsten spot-lights and colour adjusted bulbs).

A ruler and an identification number were included in each photograph. Both printed photographs and the negatives were examined from this series of 36 photographs, thereafter the details were obtained from the negatives. The film was processed normally and the negatives projected onto a sheet of white paper. The size of the projected image was adjusted to that of the original material with the aid of the photographed ruler. The relevant details were then marked onto the paper with a sharp pencil.

This technique was further refined by constructing a measuring board based on the information obtained from the preliminary photographs (Figures 2.2.). It consisted of a sheet of clear perspex with a perpendicular plate to position the front end of the fish and a 0.5cm scale running at right angles. There was an additional piece of perspex mounted on top of the first, beside this scale. The edge of the second piece was undercut to allow the top edge to be closely applied to the dorsal surface of the fish, this edge was marked with a scale aligned with that on the lower portion. The second piece of perspex was mounted on screws with underlying springs allowing its height from the first plate to be varied. The whole structure was mounted on four rubber feet. In addition to the measuring board a frame was constructed to hold the board, a camera and two tungsten spot lights (Figure 2.3)

This board allowed fish to be placed on their side with the dorsal fin supported by the upper piece of perspex, regardless of the thickness of the fish. When a sheet of white paper was placed under the board it reproduced the most successful of the backgrounds from the preliminary photographs. It was discovered that the photographs of undamaged fins were more easily examined if they were positioned over a piece of masking tape stuck to the board.

A series of five fish with similar fin condition to those used in 1.1.1. were individually identified and photographed repeatedly in random order to produce ten sets of replicate measurements.

Figure 2.2. End view of the measuring board used for photographing the fish. A = Sheet of perspex, B = Perpendicular plate allowing from of the fish to be positioned, C = Undercut additional piece of perspex allowing positioning of the dorsal fin.

Figure 2.3. Stand allowing reproducible positioning of A = Camera, B = Lights, C = Measuring board.

Separate fish had to be used for each technique due to deterioration in the condition of the fins during repeated measuring.

1.2. Comparison of photographic and calliper techniques.

The following experiment was conducted to compare the accuracy and precision of the photograph and calliper technique for recording the condition of dorsal fins.

Twenty-four fish with virtually undamaged fins and twenty one with differing degrees of dorsal fin rot were used. Each fish was killed by immersion in a strong solution of 2-phenoxyethanol. The fish were photographed using the board and frame described in 1.1.3. and then measured with callipers. The photographs all contained an individual identification number allowing the photographic and calliper measurement to be compared for each fish.

The data generated for each parameter (*ie* FL, A B & C) by the two techniques was analysed by a t test for matched pairs since normality is generally assumed for lengths within biological populations.

Since there was a significant difference between the photographic and calliper measurements, the photographic measurement was adjusted by the average difference between the two measurements. The sum of the differences between the photographic and calliper techniques divided by the number of fish was calculated for each parameter, this was added to the individual photographic measurements. The resulting adjusted photographic measurement and the original calliper

measurements were again analysed by a t test for matched pairs *eg* :

For the fork length measurements where $FL(cal)_1$ is the calliper measurement for the first fish and $FL(pho)_1$ is the photographic measurement for the first fish and n = 45.

$$a = \Sigma [(FL(cal)_1 - FL(pho)_1) + (FL(cal)_2 - FL(pho)_2) + \ldots + (FL(cal)_n - FL(pho)_n)] \div n$$

The t test for matched pairs was then performed on the adjusted data.

$FL(cal)_1 : FL(pho)_1 + a$

$FL(cal)_2 : FL(pho)_2 + a$

$FL(cal)_3 : FL(pho)_3 + a$

.....

$FL(cal)_n : FL(pho)_n + a$

This procedure was repeated for all the measured parameters (*ie* FL, A, B and C).

1.3. Correlations between the size of the individual fish and the size of their undamaged dorsal fin.

A total of 135 fish with undamaged dorsal fins were photographed on five occasions over a period of two months. The fish were all taken from one production tank. This population had been derived from the eggs from three hens and the milt from two cocks. The population had been repeatedly graded to select the potential SI's *ie* the largest fish. Therefore the size of the fish was relatively homogeneous.

II

(Materials & Methods)

The fish were anaesthetised with 2-phenoxyethanol prior to being photographed in the manner described in 1.1. The negatives were developed and the same measurements (*ie* FL, A, B & C) taken from the negatives.

The data was grouped into two sets. The first consisted of 54 fish photographed on one occasion and the second included all 135 fish. For each data set, the following regression analysis were performed by the least squares method :

FL on A+B+2

FL on A

FL on B

FL on C

C on A+B+2

C on A

C on B

1.4. Correlation between wild fish and the size of their dorsal fins. In addition to the farm population, fish from four natural burns were also obtained by electro-fishing. The fork length and height of the leading edge of the fin was measured for all the fish. From this data the % fin factor (Kindachi, 1987) was calculated *ie* :

% Fin factor = Height of fin * 100 ÷ fork length of fish

The differences between the populations were analysed by the Mann-Whitney U test.

1.4.1. Twelve parr were obtained from Finchairn and Balliemeanach burns which flow into Loch Awe in Argyll. There were no salmon farms on this water system.

1.4.2. Six parr were obtained from the river Allen, in Bridge of Allen, Stirlingshire. At the time there were no salmon farms on the river system but some farmed fish had been used for re-stocking. There was no evidence to indicate that any of the fish sampled originated from farm stocks.

1.4.3. Six parr were examined from the Howietoun burn, Sauchieburn, Stirlingshire. This burn supplied the water to farm 1. In the area sampled there was no wild run of salmon, therefore all of the fish sampled were farm escapees. There was no evidence of fin damage on any of the fish sampled from this or the other sites.

1.4.4. Six fish with undamaged dorsal fins were also randomly selected from the population studied in 1.4. and 1.5.

1.5. A comparison between subjective visual assessment and a photographic technique for determining the incidence and severity of dorsal fin rot within a population.

A suitable regression from 1.2 (*ie* FL : A+B+2 for the total 135 fish) was used as the basis of a comparison between the photographic technique described above and subjective visual assessment.

II

(Materials & Methods)

The regression line from 1.3. (FL on A+B+2) was taken to represent the 'average undamaged' height of the dorsal fin for a fish of given fork length, within the population under study. The y coordinates were then divided by 90, 60, 30, and 10. The resulting points were plotted with the original regression line (Figure 2.4.). The areas between the resulting lines represented :

a. = 90% remaining to undamaged dorsal fin.

b. = 60% - 90% of dorsal fin remaining.

c. = 30% - 60% of dorsal fin remaining.

d. = 10% - 30% of dorsal fin remaining.

e. = 0% - 10% of dorsal fin remaining.

The areas 'a' to 'e' correspond to the categories used in studies that ran concurrently with this experiment (Chapter II, 2, 3 and 4).

Subjective visual assessment was carried out by examining conscious fish individually. The dorsal fin of each fish was categorised as above (*ie* 90% remaining to undamaged dorsal fin, *etc*).

On each of four occasions approximately fifty fish from the population used in 1.2 were selected for examination by both the subjective and photographic techniques described above.

Measurements were taken from the photographic negatives and the average fin height (A+B+2) was plotted against fork length (FL) on a graph of the 100% - 10% regression lines. The position of the the points on the graph allowed the results from the photographic technique to be converted into the same form as the subjective data

(*ie* number of fish with 90% remaining to undamaged fins *etc*). The resulting data was analysed by a X^2 analysis.

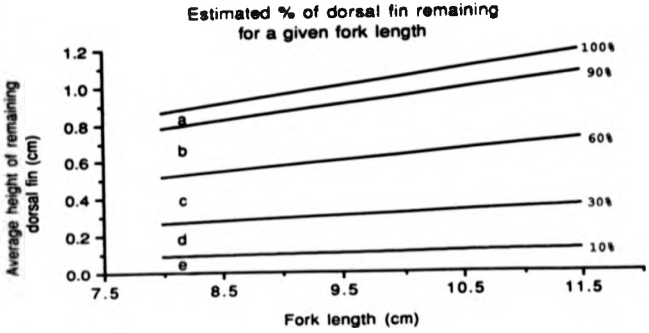


Figure 2.4. Estimate of fin size from fork length. 100% = estimated height of undamaged dorsal fin, 90% = estimated height of fin following 10% loss *etc*.

2. MONITORING DORSAL FIN ROT IN FOUR POPULATIONS OF FARMED *Salmo salar* L. PARR

2.1. Four production tanks on farm 1 were selected for study, these were contained in 1.2 × 3.7m glass fibre tanks, supplied with water

from a loch-fed burn and covered with netting to provide shade and protection from predators. The four tanks consisted of three populations of diploids and one all female triploid, the stock originated from the following farms :

Tank 3, Joseph Johnson and Sons, Triploids.

Tank 4, Seafarm Poly, diploids.

Tank 5, Joseph Johnson and Sons, diploids.

Tank 12, Landcatch, diploids.

The triploids had been induced from an all female stock by heat shock.

Between 43 and 125 fish were examined from each tank on six occasions from January to April 1988. The fish were netted from the tank and as they were individually transferred from one bucket to another, the condition of their dorsal fin was categorised into one of the following five groups.

a. = 90% remaining to undamaged dorsal fin.

b. = 60% - 90% of dorsal fin remaining.

c. = 30% - 60% of dorsal fin remaining.

d. = 10% - 30% of dorsal fin remaining.

e. = 0% - 10% of dorsal fin remaining.

The group of fish were then weighed and returned to the production tanks. In an attempt to improve the precision of the technique all the examinations were conducted by the author and diagrammatic representations of the different categories were referred to before each session. The resulting data was analysed by the Games and Howell method.

2.2. In order to produce a statistic that would represent the degree of dorsal fin damage within a population the mid point value of each 'fin remaining' group was multiplied by the percentage of sample population within that group. The results were divided by 100 to convert the result back to a percentage. The total was the sum of the results of all the groups.

3. WATER QUALITY AND STOCKING PARAMETERS IN THE FOUR POPULATIONS OF FARMED *Salmo salar* L. PARR

From January to April 1988 a range of parameters were measured weekly in the four tanks selected for monitoring dorsal fin rot (*ie* tanks 3, 4, 5 & 12, farm 1). The parameters measured were :

Number of fish

Average weight and total biomass

Dissolved oxygen and % saturation

Water temperature

pH

Alkalinity

Conductivity

Suspended solids

NH_4 and % unionised ammonia

NO_2

Occasionally the sampling day was altered to obtain samples from periods of spate.

II

(Materials & Methods)

3.1. Number of fish.

The initial number of fish in the tank were counted by the farm staff when the tank was stocked and the daily mortalities were recorded.

3.2. Biomass.

The average weights of the four populations were estimated on sixteen occasions over the period of study. Approximately one hundred fish were netted, weighed and counted, producing an average weight for the sample.

3.3. Dissolved oxygen.

The dissolved oxygen was measured at the farm inflow and in the centre of the tank adjacent to the outflow with a Phox 82 TDS O_2 meter (pHOX, Ltd). The % O_2 saturation was calculated with the following formula

$$\% O_2 \text{ Saturation} = (C_1 + C_2) \times 100$$

Where C_1 = The temperature and pressure dependent oxygen solubility and C_2 = The dissolved oxygen concentration of the sample

3.4. Water temperature.

The maximum and minimum daily water temperature was measured and recorded by the farm staff.

For the remaining parameters a litre of water was collected from the centre of each tank near the outflow. The samples were collected in thoroughly cleaned polyethylene bottles which had been rinsed three times with the water to be sampled. Each bottle was identified and

returned to the laboratory for analysis. Most of these parameters were analysed at seventeen regular intervals over the twenty week period.

3.5. pH.

On returning to the laboratory the pH of each sample was measured with a Phillips PW 9409 digital pH meter.

3.6. Alkalinity.

The total alkalinity was measured as described by Stirling (1985).

3.7. Conductivity.

Conductivity was measured with a pHOX 52 conductivity meter (pHOX Ltd).

3.8. Suspended solids.

Suspended solids were measured by the filtration method described by Stirling (1985).

3.9. NH_4 and NO_2.

100 ml the filtrate resulting from the suspended solids measurement was frozen in a cleaned and rinsed polyethylene bottle. These samples were retained until the end of the study period. They were analysed spectrophotometrically using a Technicon II autoanalyser. Ammonia was determined using the phenol-hypochloride, indole blue method (Solorzano, 1969) and nitrite by the sulphanilamide/N-(1-naphthyl)-ethylenediamine dihydrochloride or NNED reaction (Golterman, Clymo and

Ohnstad, 1978). The % unionised ammonia was calculated from the following formula :

% Unionised ammonia = 100 ÷ 1 + antilog (pKa − pH)

Where pKa = Negative logarithm of the temperature dependent ionisation constant

The data for all these criteria was plotted against the degree of fin rot within the relevant population.

4. THE RELATIONSHIP BETWEEN DORSAL FIN ROT AND SIZE IN *Salmo salar* L. PARR

A total of 86 fish from farmed populations were examined in this part of the study. They included 30 fish with virtually undamaged dorsal fins and 56 fish with varying degrees of dorsal fin rot. These fish were sampled on 13 occasions from 6 farms (Table 2.1.).

The information recorded for each fish consisted of fork length, body weight and amount of abdominal fat. This information was compared to the condition of the dorsal fins as calculated in 2.2 (*ie* 90% remaining to undamaged dorsal fin, 60% − 90% of dorsal fin remaining, *etc*).

The coefficient of condition (K factor) was calculated from the weight (W) and fork length (L) (Pickering and Pottenger 1988) :

$$K \text{ factor} = 100\ W \div L^{3}$$

Where the sample sizes were large enough to justify analyses, (*ie*

populations 2, 7, 8, 9, 10, 12 and the total 66 fish) Spearman rank correlation coefficients were calculated for the following parameters :

% of dorsal fin remaining and length

% of dorsal fin remaining and weight

% of dorsal fin remaining and condition factor.

Table 2.1.
Populations and number of fish sampled.

Site	Date	Population	Number of fish with Undamaged dorsal fins	/ Dorsal fin rot
FARM 1 (tank 5)	18/12/87	1	2	2
FARM 2	7/1/88	2	6	4
FARM 1 (tank 5)	11/1/88	3	2	2
FARM 1 (tank 5)	2/2/88	5	3	3
FARM 3 (tank 17)	9/2/88	6	-	2
FARM 3 (tank 17)	10/2/88	7	-	8
FARM 4 (cage 2)	11/2/88	8	-	10
FARM 1 (tank 4)	16/2/88	9	1	4
FARM 1 (tank 12)	16/2/88	10	-	3
FARM 1 (tank 4)	1/3/88	11	2	2
FARM 1 (tank 12)	1/3/88	12	2	2
FARM 5	10/3/88	13	2	4
FARM 6 (tank A21)	22/3/88	14	4	4
FARM 6 (tank 88)	22/3/88	15	4	4
FARM 1 (tank 5)	30/3/88	17	2	2
			30	66

All the farms were fresh water smolt farms where the fish were reared in glass fibre tanks with the exception of FARM 4 which was a fresh water cage site.

CHAPTER II

RESULTS

1. METHODS FOR ASSESSING THE PREVALENCE OF DORSAL FIN ROT IN POPULATIONS OF *Salmo salar* L. PARR

1.1. The development of a technique to record the condition of dorsal fins.

1.1.1. Measurement of the fins with callipers was not absolutely reproducible, due to the flexible nature of the fins. The widest distribution around the mean was seen in the measurements of the trailing edge of the fin and the technique was less accurate for fins with severe fin rot (Table 2.2.)

Table 2.2.

Mean, standard deviation (SD) and the standard deviation as a percentage of the mean (%SD) of the measurements by callipers and photographic techniques.
A = Height of leading edge of fin B = Height of trailing edge of fin

Fish No	Mean (cm)	SD	%SD	Mean (cm)	SD	%SD
			Callipers			
C1	1.402	.0589	4.19%	0.671	.0638	9.42%
C2	1.394	.0433	3.11%	0.659	.0376	6.71%
C3	1.273	.0427	3.36%	0.443	.0333	7.52%
C4	1.433	.0831	5.80%	0.861	.0436	7.77%
C5	0.901	.0713	7.91%	0.143	.0324	22.66%
	Mean %SD = 4.87%				Mean %SD = 10.82%	
			Photographic			
P1	1.388	.0308	2.22%	0.340	0.004	1.18%
P2	1.407	.0320	2.27%	0.455	0.004	0.88%
P3	1.199	.1106	9.22%	0.242	0.043	17.77%
P4	1.053	.1228	11.63%	0.00		
P5	0.871	.0717	8.23%	0.00		
	Mean %SD = 6.71%				Mean %SD = 6.61%	

Different fish were used for the two techniques.

1.1.2. It proved difficult to hold the fish in position whilst marking the relevant points onto paper. The technique resulted in poor reproducibility and was therefore discarded.

1.1.3. Measurements taken from photographs of the fish.

The prints and negatives were examined from the preliminary series of photographs. Of the combinations examined the best photographic representation of the fin was produced when the fish was illuminated by two light sources and photographed from a focal distance of 45cm, with a shutter speed of 0.1S to 0.8s and a f-stop of 4-6. The most suitable background was clear perspex over white paper.

This combination was used with the board and frame described in Material and Methods for all the subsequent photographs of fins in this chapter. However occasionally high ambient light necessitated a reduction in shutter speed and f-stop setting (Figures 2.5. and 2.6.).

The preliminary negatives were examined to determine if the information could be taken directly to avoid printing all the photographs. It was discovered that the details of the fish and dorsal fin were visible on a projected image of the negative. When this image was projected onto a flat piece of paper the appropriate points could be marked with a sharp pencil.

Figure 2.5. Photograph taken on the perspex measuring board, of a fish affected by dorsal fin rot.

Figure 2.6. Photograph taken on the perspex measuring board, of a fish with an undamaged dorsal fin.

II
(Results)

Ten replicate photographs were taken from each of five fish with different degrees of fin damage and measurements taken from the negatives. The distribution of the replicates was similar to that obtained by the use of callipers. The distribution of measurements around the mean was smaller for the trailing edge of the fin but larger for the leading edge (Table 2.2.).

1.2. Comparison of photographic and calliper techniques

The photographic technique had several advantages over the calliper technique in that it was less time consuming under field conditions and produced a permanent record. In order to confirm that it was a sufficiently accurate technique it was necessary to conduct a direct comparison between the calliper and photographic results on the same fish. The mean values of the data produced by the photographic and calliper techniques are recorded in Table 2.3.

Table 2.3.
Mean of n values (X̄), standard deviation (SD) obtained by measuring fish by both the photographic and calliper techniques and the mean difference between the techniques.

	Fin rot n=21		Undamaged n=24		Total n=45		
	Photo'	Callip'	Photo'	Callip'	Photo'	Callip'	R % C-P
FL R	10,72	10,86	11,23	11,33	11,00	11,11	1%
SD	0,36	0,32	0,44	0,47	0,47	0,47	
A R	1,20	1,15	1,84	1,89	1,38	1,39	0,4%
SD	0,13	0,13	0,10	0,09	0,21	0,25	
B R	0,08	0,12	0,62	0,69	0,37	0,42	10%
SD	0,09	0,12	0,13	0,14	0,30	0,31	
C R	1,42	1,32	1,80	1,44	1,46	1,38	6%
SD	0,09	0,08	0,08	0,08	0,10	0,10	

In order to determine accuracy and precision of the photographic technique for measuring the incidence of fin rot within a whole

population the data obtained by the photographic and calliper techniques was analysed by a t test for matched pairs. With n = 45 and 44 degrees of freedom :

FL t = 12.136. p = 0.05 t = 2.0168

A t = 0.477 p = 0.01 t = 2.6952

B t = 5.387

C t = 5.970

Therefore the null hypothesis is rejected for FL, B and C. The conclusion from this is that there is a significant difference between the photographic and calliper techniques for FL, B and C.

The initial results suggested that the photographic technique was not accurate compared with the calliper technique. The mean difference between the two techniques was then added to the photographic results to compare the precision or reproducibility of the photographic and calliper results. A t test for matched pairs then conducted on the adjusted data. In this case t did not exceed 0.00001 for any of the four parameters (FL, A, B or C). Therefore the null hypothesis is accepted for the adjusted data, that is there is no significant difference between the sets of data.

1.3. The correlations between the size of individual fish and the size of their undamaged dorsal fin. The results are recorded in Table 2.4.

Table 2.4.
R^2 values from regressions performed on 54 fish on one occasion and 135 fish over five occasions.

Regression of: -	Single date n=54 R^2	Five dates n=135 R^2
FL on A+B÷2	28,9%	49,2%
FL on A	27,8%	54,5%
FL on B	11,5%	16,6%
FL on C	43,1%	
C on A+B÷2	21,5%	30,4%
C on A	17,6%	44,7%
C on B	9,9%	3,8%

t values $P < 0,001$

1.4. Correlation between the size of wild fish and their dorsal fins. The % fin factors were calculated as described in materials and methods. The results are recorded in Table 2.5. and Figure 2.7.

Table 2.5.
Mean ($\bar{x}$) and standard deviation (SD) of fork length and % fin factor from five populations.

Population	n	Fork length $\bar{x}$	Fork length SD	% Fin factor $\bar{x}$	% Fin factor SD
Finchairn	6	8,06	0,86	19,03	3,95
Ballimeanach	5	10,54	1,16	17,22	2,25
River Allen	6	12,72	0,74	15,52	0,41
Howietoun	6	10,25	1,81	16,15	1,19
Farm 1	6	9,24	0,58	9,16	1,47

The % fin factor of the farmed fish was obviously lower than all the other populations. A Mann-Whitney U test was performed on the data from the wild fish from Finchairn burn and the farm escapees from the Howietoun burn. The test indicated that the median % fin factor of the two populations were significantly different.

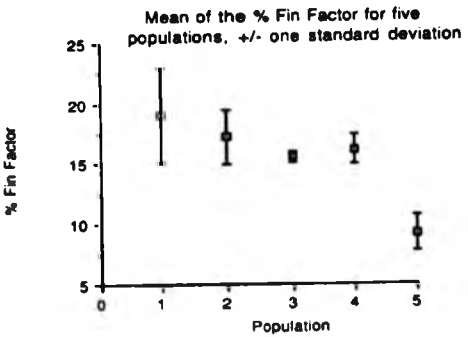


Figure 2.7. Mean ± 1 standard deviation of the % fin factor for five populations. 1 = Finchairn. 2 = Ballimeanach. 3 = River Allen. 4 = Howietoun. 5 = Farm 1.

1.5. A comparison between subjective visual assessment and a photographic technique for determining the incidence of fin rot in within a population.

The regression of FL on A+B+2 was used to estimate the average size of the undamaged dorsal fin of a fish with a known fork length. From this regression line the estimated sizes of fins with 90%, 60%, 30% and 10% of the fin remaining were calculated and plotted (Figure 2.4.). When the measurements from the photographic technique were plotted onto this graph the fish were classified into groups similar to those used for subjective assessment of the amount of fin remaining (Figure 2.8.). This data are recorded with the data from the subjective

assessment in Tables 2.6. and 2.7. The difference in the numbers of fish examined by the two techniques was due to an equipment fault resulting in the loss of some of the photographic negatives.

Table 2.6.
Number of fish categorised into fin remaining groups by the photographic technique (Photo') and subjective visual assessment (Subj').

Sample day	1		2		3		4	
	Subj'	Photo'	Subj'	Photo'	Subj'	Photo'	Subj'	Photo'
90%-100%	9	9	9	4	6	4	3	5
60%-90%	13	25	10	6	5	9	3	4
30%-60%	27	21	35	18	23	34	21	39
10%-30%	7	0	5	0	19	0	24	1
n=	66	55	59	28	53	47	81	49

Table 2.7.
% of fish categorised into each fin remaining group by the photographic technique (Photo') and subjective visual assessment (Subj').

Sample day	1		2		3		4	
	% Subj'	Photo'	% Subj'	Photo'	% Subj'	Photo'	% Subj'	Photo'
90%-100%	16.1	16.4	16.2	14.3	11.3	8.5	6.9	10.2
60%-90%	23.2	45.4	16.9	21.4	9.4	19.1	6.9	8.2
30%-60%	48.2	38.2	59.3	64.3	43.4	72.3	41.2	79.6
10%-30%	12.5	0.0	8.5	0.0	35.8	0.0	47.0	2.0

The data from Table 2.6. were examined by X^2 analyses. These were performed on the total data set and the total excluding the second sample date, since on this occasion a large proportion of the photographic records were lost (Tables 2.6. and 2.9.). The X^2 analyses on the individual days have not been included since they produced more than 20% of the expected counts under five.

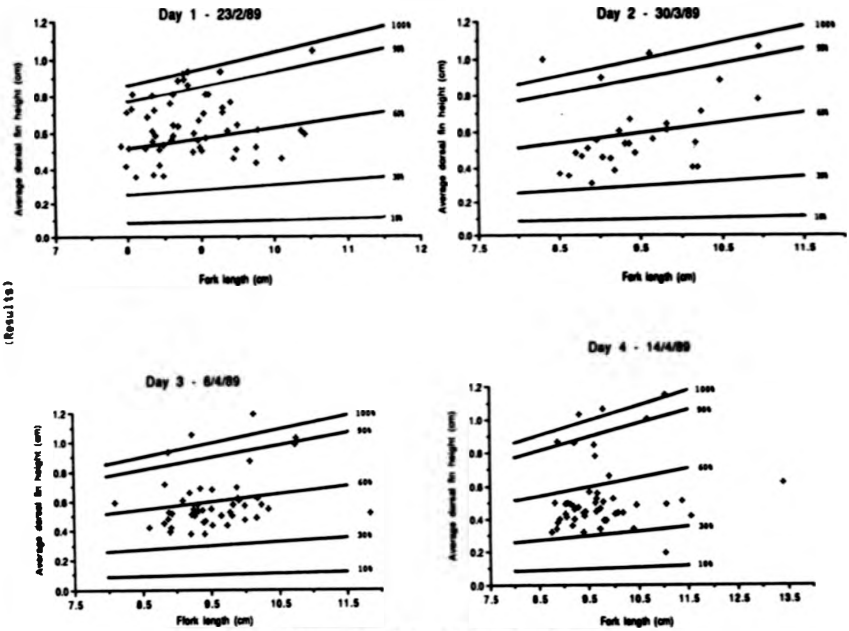


Figure 2.8. Average dorsal fin height and fork length, measured from photographic negatives and plotted against the estimated size of fin for a given fork length.

Table 2.8.

X^2 Analysis on the total subjective and photographic. The expected counts are printed below the observed counts.

	90-100%	60-90%	30-60%	10-30%	Total
Subjective	22	44	112	50	228
	28.6	43.7	126.0	29.7	
Photographic	27	31	104	1	163
	20.4	31.3	90.0	21.3	
Total	49	75	216	51	391

$X^2 = 1.51 + 0.00 + 1.55 + 13.80 +$
$2.11 + 0.00 + 2.16 + 19.31 = 40.45$

Expected counts < 5 = 0
Degrees of freedom = 1
At p = 0.05, $X^2 = 3.84$

Degrees of freedom = 3
At p = 0.05, $X^2 = 7.81$
At p = 0.01, $X^2 = 11.34$

Table 2.9.

X^2 Analysis on the total subjective and photographic excluding the data from the second sample day. The expected counts are printed below the observed counts.

	90-100%	60-90%	30-60%	10-30%	Total
Subjective	18	21	71	50	160
	18.5	30.4	84.9	26.2	
Photographic	18	38	94	1	151
	17.5	28.6	80.1	24.8	
Total	36	59	165	51	311

$X^2 = 0.01 + 2.88 + 2.27 + 21.52 +$
$0.02 + 3.05 + 2.41 + 22.80 = 54.97$

Expected counts < 5 = 0
Degrees of freedom = 1
At p = 0.05, $X^2 = 3.84$

Degrees of freedom = 3
At p = 0.05, $X^2 = 7.81$
At p = 0.01, $X^2 = 11.34$

Both X^2 analyses produced similar results. The subjective visual assessment of the extent of fin loss only differed significantly from the photographic results in its estimation of the number of fish with 10%-30% of their dorsal fin remaining. Significantly more fish were classified in this group by the subjective technique.

2. MONITORING DORSAL FIN ROT IN FOUR POPULATIONS OF FARMED *Salmo salar* L. PARR

2.1. The data obtained from assessing the condition of the dorsal fins of the sampled fish are displayed graphically in Figure 2.9. The number of fish within each 'fin remaining' category is displayed as a proportion of the sample size to facilitate comparison between tanks and over time.

The variance between samples was found to be heterogeneous using the F_{max} test. Therefore the method used was that of Games and Howell for comparisons among pairs of means with heterogeneous variance. The Comparison of the means with the minimum significant differences produced the results tabulated in Tables 2.10. and 2.11.

Table 2.10.
Statistically significant ($p = 0.05$) differences in mean dorsal fin remaining between days in each tank, according to the Games and Howell method.

Tank		1	3	4	5	12
Days	1&2		-	-	+	+
	1&3		+	-	-	-
	1&4		-	-	-	+
	1&5		-	-	+	+
	1&6		/	-	-	-
	2&3		-	-	-	-
	2&4		-	-	-	-
	2&5		+	+	-	-
	2&6		/	+	-	-
	3&4		-	-	-	-
	3&5		+	+	+	-
	3&6		/	+	-	-
	4&5		-	-	-	-
	4&6		/	-	-	-
	5&6		/	-	-	-

- = not significant, + = significant, / = no data.

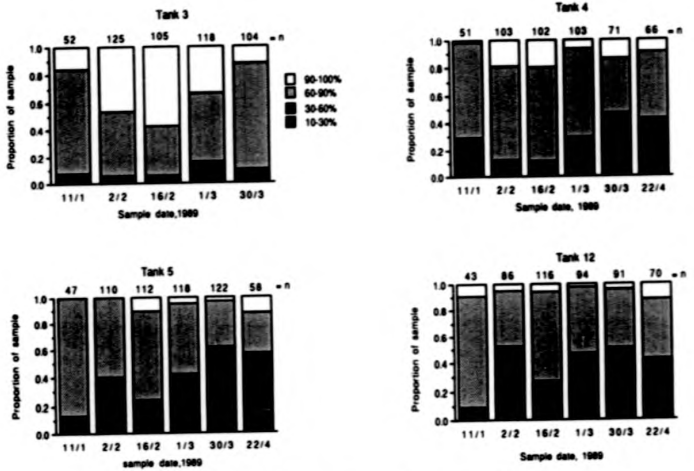


Figure 2.9. Four populations sampled to estimate the degree of dorsal fin rot. The number of fish within each category (*eg* 90%-100%) is expressed as a proportion of the total sample size. n = number of fish in the sample.

Table 2.11.
Statistically significant ($p = 0.05$) differences in mean dorsal fin remaining between tanks on individual days, according to the Games and Howell method.

Day	1	2	3	4	5	6
Tanks 3&4	-	+	+	+	+	/
3&5	-	+	+	+	+	/
3&12	-	+	+	+	+	/
4&5	+	-	-	-	-	-
4&12	+	+	-	-	-	-
5&12	-	-	-	-	-	-

- = not significant, + = significant, / = no data.

2.2. A single statistic was calculated to represent the average degree of fin loss within each population, *ie* for sample 1 tank 3, 15.4% of the population had 90%-100% of the dorsal fin remaining, average 95% remaining

$$\text{and for the remaining groups} \quad 55 \times 15.4 \div 100 = 14.6\%$$

$$75 \times 76.9 \div 100 = 57.7\%$$

$$45 \times 7.7 \div 100 = 3.5\%$$

$$\text{Total} = 75.8\%$$

Therefore the extent of the fin damage in the population could be expressed as - the average fish within the population had 75.8% of its dorsal fin remaining.

This calculation was performed for all the tanks on each sample day. The resulting estimates of average dorsal fin remaining in the four populations on the six sample days is displayed in Figure 2.10. These statistics were used in 3 to compare the water quality parameters with the fin condition in the tanks over time.

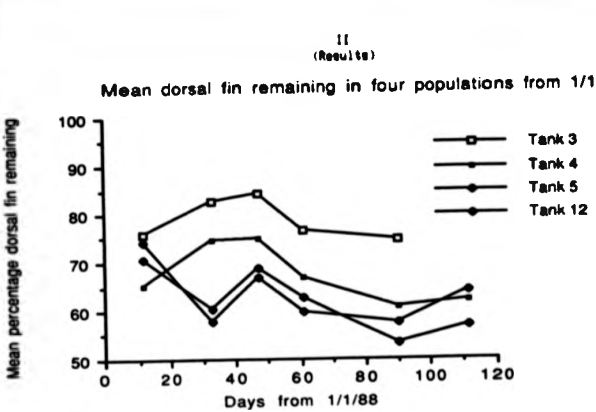


Figure 2.10. Mean percentage dorsal fin remaining within the four populations under study from 1/1/88.

3. WATER QUALITY AND STOCKING PARAMETERS IN FOUR POPULATIONS OF FARMED *SALMO SALAR* L.PARR

The water quality and stocking data is represented graphically in figures 2.11. to 2.22. with the exception of the numbers of fish which are recorded in Table 2.12. The water flow in the burn which supplied the farm varied over the study period but there was only one serious spate on 23/3/88.

Table 2.12.
Number of fish recorded in the tanks over the period of the survey.

Date	tank 3	tank 4	tank 5	tank 12
11/1/88	6345	5994	6014	5990
19/1	"	5990	6013	"
1/2	6344	"	6012	"
9/2	6341	5989	6006	"
16/2	"	5988	"	"
24/2	6340	5983	"	5985
8/3	6339	5978	"	5981
16/3	6338	5977	"	"
23/3	6333	"	"	"
6/4	"	5976	"	"
22/4	6329	"	5981	"

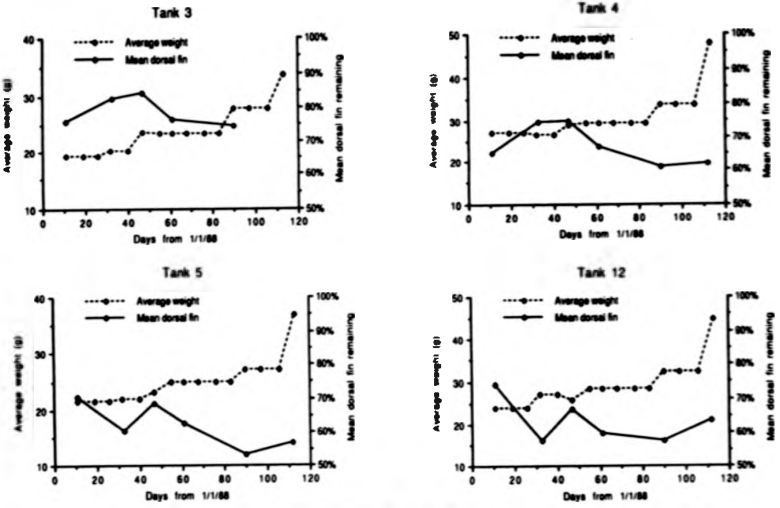


Figure 2.11. Average weight and mean dorsal fin remaining within the four populations under study for the duration of the survey.

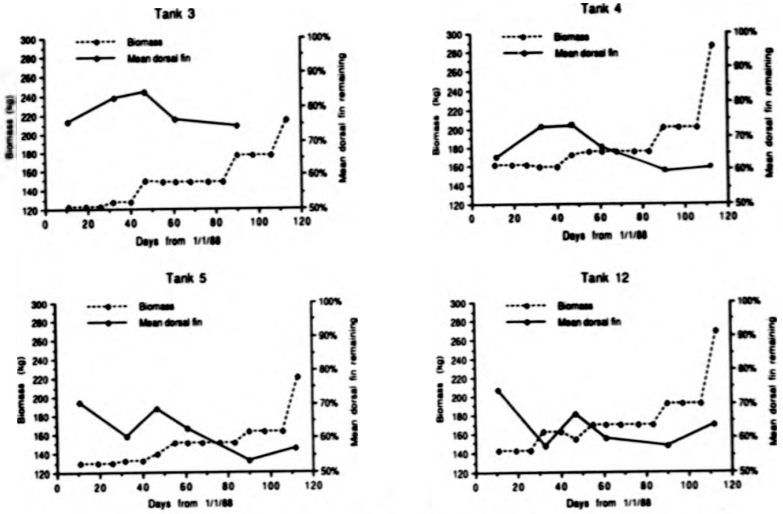


Figure 2.12. Biomass and mean dorsal fin remaining within the four populations under study for the duration of the survey.

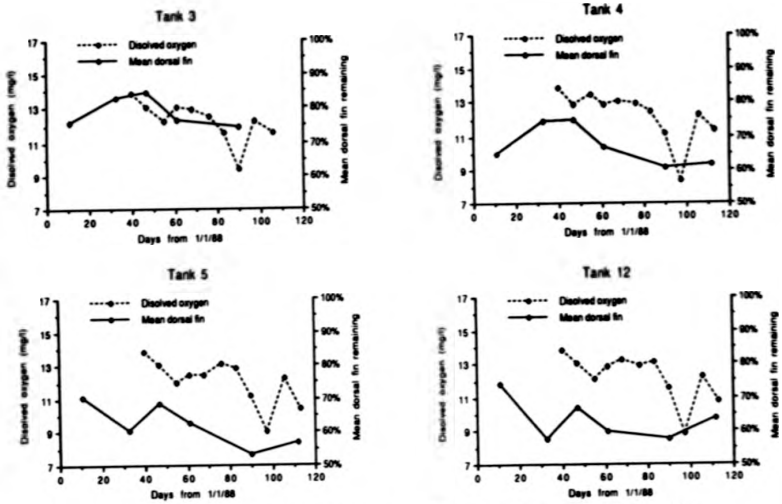


Figure 2.13. Dissolved oxygen and mean dorsal fin remaining within the four populations under study for the duration of the survey.

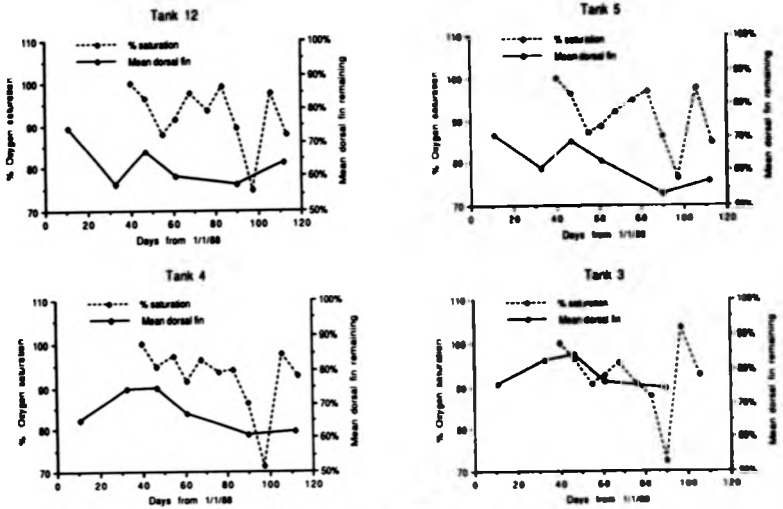


Figure 2.14. % oxygen saturation and mean dorsal fin resaining within the four populations under study for the duration of the survey.

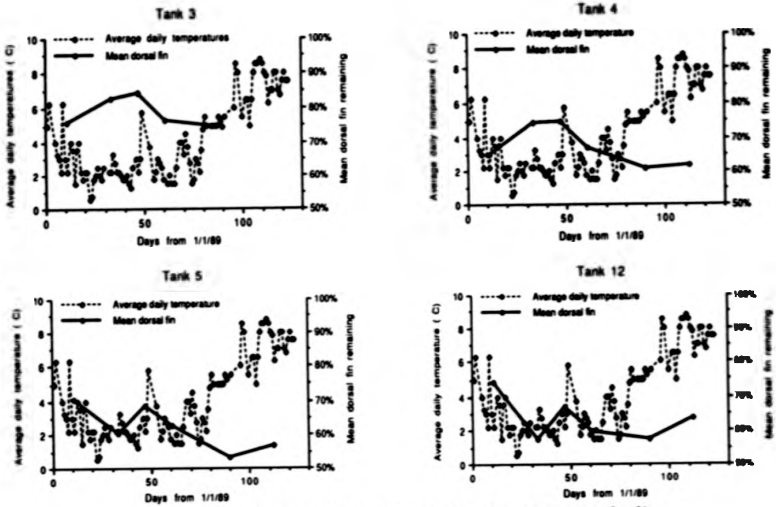


Figure 2.15. Average daily temperatures and mean dorsal fin remaining within the four populations under study for the duration of the survey.

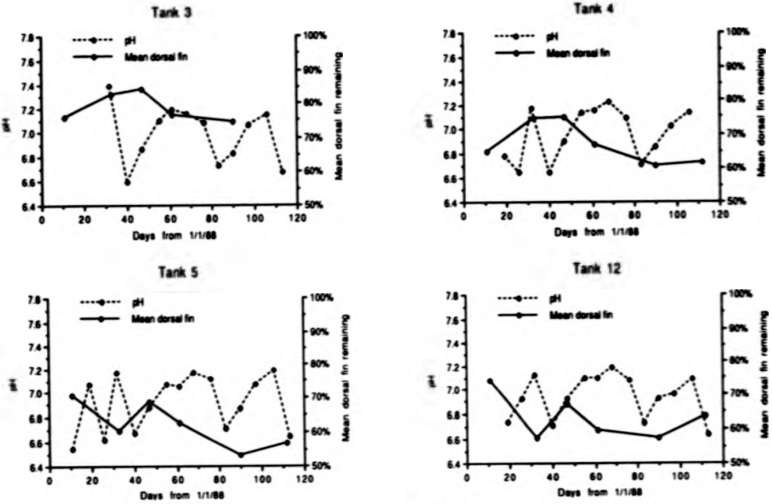


Figure 2.16. pH and mean dorsal fin remaining within the four populations under study for the duration of the survey.

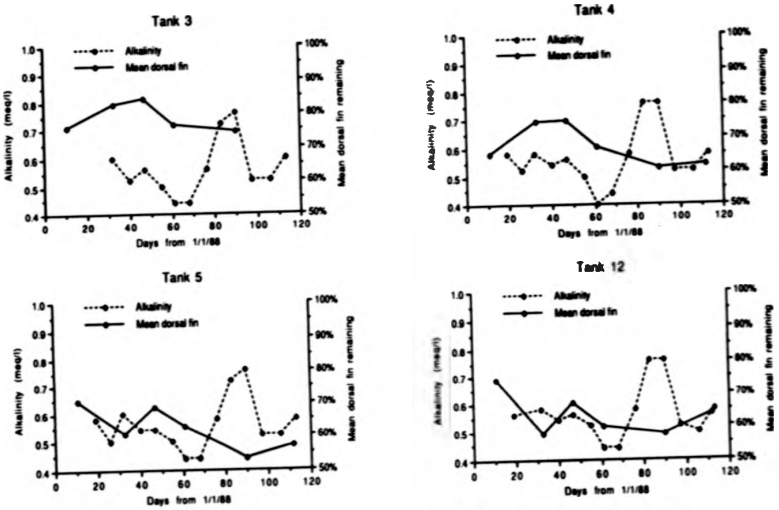


Figure 2.17. Alkalinity and mean dorsal fin remaining within the four populations under study for the duration of the survey.

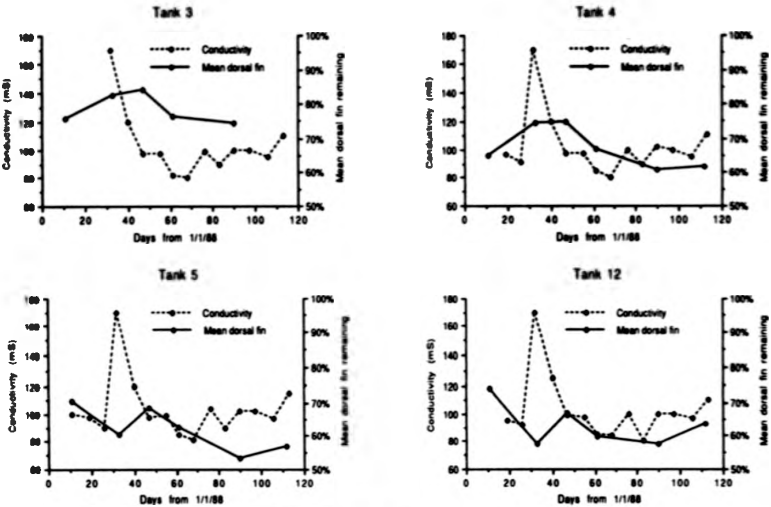


Figure 2.18. Conductivity and mean dorsal fin remaining within the four populations under study for the duration of the survey.

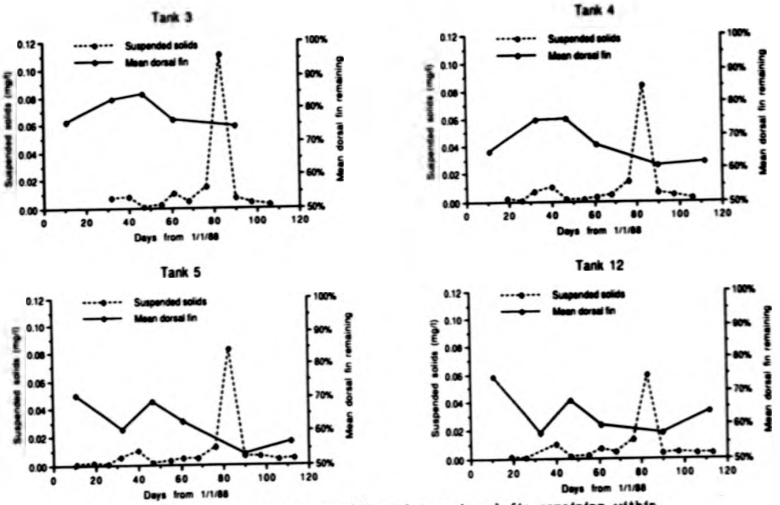


Figure 2.19. Suspended solids and mean dorsal fin remaining within the four populations under study for the duration of the survey.

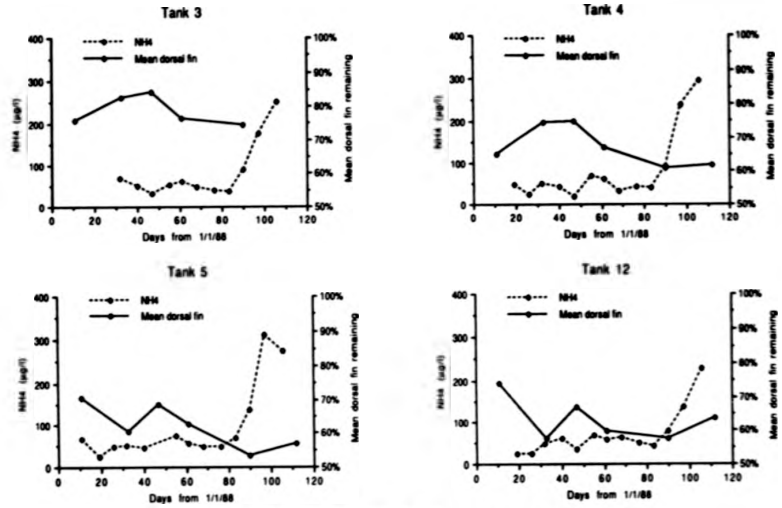


Figure 2.20. Ammonia and mean dorsal fin remaining within the four populations under study for the duration of the survey.

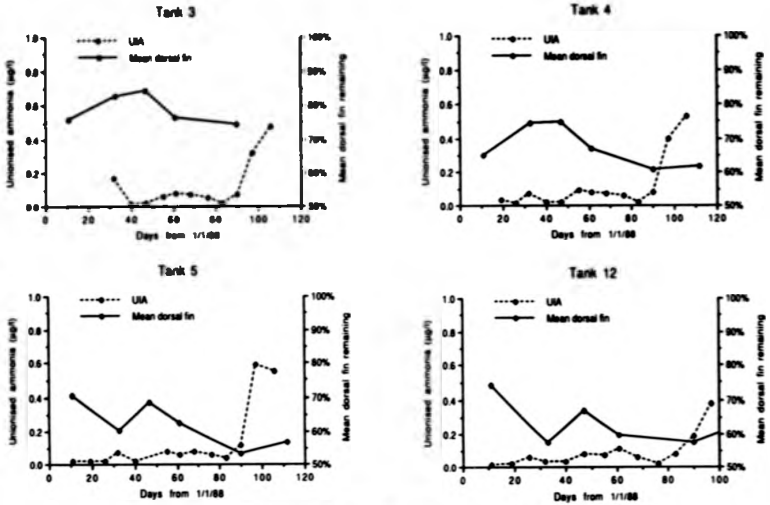


Figure 2.21. % unionised ammonia and mean dorsal fin remaining within the four populations under study for the duration of the survey.

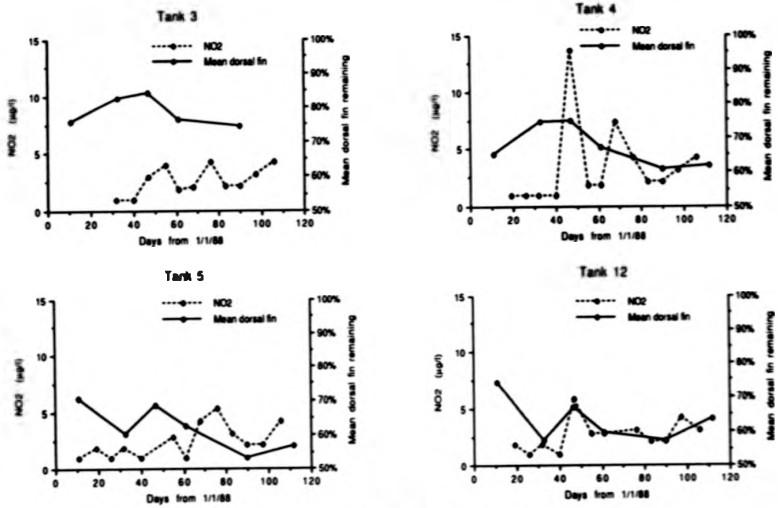


Figure 2.22. Nitrite and mean dorsal fin remaining within the four populations under study for the duration of the survey.

4. THE RELATIONSHIP BETWEEN DORSAL FIN ROT AND SIZE IN *Salmo salar* L. PARR

The Spearman rank correlation coefficients were calculated and are presented in Table 2.13.

Table 2.13.

Spearman rank correlation of % dorsal fin remaining against fork length (L), weight (W) and condition factor (KF).

Population	df(n-1)	Correlation of	r_s		p =0.10	p =0.02
2	9	DF & L	0.139		0.564	0.746
7	7	*DF & L	0.713		0.643	0.833
		*DF & W	0.665			
		DF & KF	0.218			
8	9	*DF & L	0.714		0.564	0.746
		*DF & W	0.736			
		DF & KF	-0.188			
9	7	DF & L	0.365		0.643	0.833
		DF & W	0.441			
		DF & KF	-0.050			
10	7	DF & L	0.423		0.0643	0.833
		DF & W	0.445			
		DF & KF	0.222			
12	12	*DF & L	0.443		0.426	0.601
		DF & W	0.297			
		DF & KF	-0.411			
					p=0.05	p=0.01
Total	85	DF & L	0.101		0.212	0.278
	70	*DF & W	0.360		0.232	0.300
	70	DF & KF	-0.069		0.232	0.300

DF = dorsal fin remaining, FL = fork length, W = weight in g.
KF = condition factor
* = significant at p = 0.10
† = significant at p = 0.01

II
(Results)

All the fish examined had a large amount of abdominal fat with no apparent relationship between the amount of fat and the dorsal fin damage.

CHAPTER II

DISCUSSION

1. METHODS FOR ASSESSING THE PREVALENCE OF DORSAL FIN ROT IN POPULATIONS OF *Salmo salar* L. PARR

A method for assessing the incidence and severity of fin rot was developed before a description of the condition had been completed. However, even at this early stage, it was apparent that fin rot consisted of at least two main features, that is thickening of the fin and loss of tissue. It was decided that it was more practical to quantify the size of the fin rather than the degree of thickening. Only at a later stage was it discovered that the loss of tissue was an indication of the severity of fin rot but not of the current activity of the lesion. It might be argued that the assessment of fin rot should have contained some indication of the degree of thickening. However later studies indicated that thickening of the fin was strongly temperature dependent (chapter IV). As a result,the degree of thickening would not have accurately indicated the presence of active disease without a model for the temperature dependent nature of the lesions. The criteria used (*ie* dorsal fin remaining) should have indicated deteriorations in the condition of the fins but would not have been able to detect the early stages of remission, before healing and re-growth of tissue. The results described in this chapter have been analysed taking this limitation into account.

II
(Discussion)

The first part of this study was the development of a technique to record the size and shape of the dorsal fin. Three techniques were investigated, the use of callipers, recording the details directly onto paper and a photographic technique.

Atlantic salmon are soft-rayed fish or malacopterygii, as opposed to the spiny-rayed fish or acanthopterygii. This means that the fins are flexible. Therefore the position (ie extended or held against the body) and any distortion affects many of the dimensions of the fin. For this reason the measurements were restricted to the constant dimensions, that is the height of the leading and trailing edges and the length of the base of the fin. Even these measurements were problematic, since the edge of the fin is extremely fine, easily distorted and also the precise position of the junction of the fin with the body is difficult to define.

Marking the measurements on to paper was attempted since it would have provided a permanent record for subsequent analysis. However it proved to be completely impractical. The other two techniques both suffered from the problems described above, that is it was difficult to precisely identify the points to be measured. The photographic technique had the advantage that it could be carried out more rapidly in the field, and so did not require the fish to be sacrificed. It also produced a permanent record that was available should additional information have been required at a later stage. Unfortunately there were also some technical problems with the camera which resulted in the loss of some of the information later in the study.

II
(Discussion)

Comparison between the photographic and calliper techniques indicated that they were both equally reproducible but produced slightly different results. The greatest discrepancy was over the trailing edge of the fin, which was also the least reproducible measurement. Therefore the 10% difference between the two trailing edge measurements may have been the result of a combined inaccuracy rather than a fault of the photographic method. Since the object of measuring the fins was to allow comparison between fish, precision was more important than accuracy. Since the photographic technique was equally precise, produced a permanent record and had the advantage of speed, it was selected for subsequent use.

In order to predict the amount of tissue lost due to fin rot it was necessary to predict the size of the undamaged dorsal fin. It had been suggested (Kindachi, 1967) that the height of the dorsal fin remained a constant proportion of the length of the fish within a species. This opinion contradicts the accepted view that phenotype is the result of both genetic and environmental factors. Such phenotypic variation has been used for identification of different Atlantic salmon stocks (MacCrimmon and Claytor, 1985 and 1986). In view of this evidence an attempt was made to investigate the relationship between the length of the fish and the size of the dorsal fin. The population selected was relatively homogeneous both phenotypically and genetically. Despite homogeneity of the population the regression analysis indicated that the length of the fish and the height of the fins were significantly but not strongly related. The strongest relationship was between the fork length of the fish and the height of

the leading edge of the dorsal fin. The R_2 value for this regression was 54.5%, implying that 54.5% of the variation in one variable was accounted for by variation in the other. Therefore the height of the dorsal fin varies as a proportion of the fork length even in a relatively homogenous population. Despite this variation the relationship was considered to be worthy of further study.

In order to examine the height of both normal and damaged fins the regression between the fork length of the fish and average height of the fin (R_2 = 49.2%) was selected. This marginally less related parameter was selected because loss of fin tissue was often uneven over the edge of the fin.

A very limited experiment was then conducted to examine the % fin factor (Kindachi, 1987) within different populations from different environments. The fish used for this experiment were also sampled for histology to obtain specimens of normal fin tissue from different populations (Chapter I). The populations selected included wild fish, a stock with the possibility of some farmed individuals, farm escapees and farmed fish. The fin factors from the farmed fish were obviously significantly different from all the others. Although the farm escapees were not necessarily from a genetic stock identical to the farmed fish they were of similar origin. Therefore there would appear to be an environmental effect on the fin factor. A Mann-Whitney U test indicated that there was a significant difference between the wild populations and the escapees. This may be due partially to the genetic effects on the dorsal fin size in populations from similar

wild environments. It would be unwise to draw profound conclusions from such a small study. It merely serves to corroborate the predicted differences in % fin factor between populations of Atlantic salmon and therefore its lack of value as a measure of fin damage.

Finally in this part of the study a comparison was made between the subjective visual assessment and the photographic technique discussed above. The categories used in the subjective technique were the most detailed that could be applied in practice. Every attempt was made to ensure that the results were reproducible. The photographic results were converted into the same categories and then both data sets were examined by X^2 analysis. This suggested that the subjective results only differed significantly in the 10-30% category. In view of the inherent inaccuracy of the photographic technique the subjective results would appear to be equally valid, with the reservation that the 10-30% category may have been overestimated.

A considerable and justified effort was directed into the development of an objective technique. The results indicate that subjective visual assessment by one worker may be just as valid as the other techniques investigated. This topic is discussed further in light of later findings in chapter VI.

II
(Discussion)

2. MONITORING DORSAL FIN ROT IN FOUR POPULATIONS OF FARMED *Salmo salar* L. PARR.

As previously discussed the results from the subjective monitoring were valid but may have overestimated the proportion of fish in the 10-30% category. In addition, since the factor measured was the average amount of fin remaining, the technique probably detected loss of fin tissue due to fin rot but was unlikely to demonstrate any decrease in active fin rot. Therefore the significant trends in the data could be summarised as a deterioration in the fin condition over time in all the tanks with less fin damage in tank 3. The statistic representing the average fin remaining was calculated to give a simple indication of the trends within the populations. It was useful for comparing the fin remaining with the other parameters examined in 3., when considered in conjunction with the significant differences (Tables 2.10. and 2.11.).

3. WATER QUALITY AND STOCKING PARAMETERS IN THE FOUR POPULATIONS OF FARMED *Salmo salar* L. PARR.

Any study of this type which tries to analyse a large number of uncontrolled parameters has severe limitations. The results at best demonstrate possible associations between parameters. Despite its limitations such a study can demonstrate controlling processes within a system (Hopke, 1978). There are many precedents for such a wide based study at the outset of an investigation (Phillips, 1988).

II
(Discussion)

The sampling frequency and the parameters analysed were adopted after consideration of theoretical importance and the practical constraints. Sampling frequency is a balance between the time available and the validity of the data. In an ideal situation the water quality should be analysed over a season with occasional 24 hour sampling periods to determine the variation, before the necessary frequency of sampling can be predicted. This requires the use of automatic sampling facilities and data loggers. However the sampling procedures used here were based empirically on the recommendations of Stirling, (1985).

The number of fish were monitored to obtain the average weight and to detect any significant mortalities. There were actually very few mortalities in the tanks under investigation during the study. The biomass was updated whenever a sample weighing was conducted and varied in a predictable manner.

The dissolved oxygen (DO) was monitored and the % saturation calculated from the DO and temperature using standard tables. The DO measurements did not drop below the recommended lower limit for salmonid hatcheries of 6mg/l (EEC, 1978). The water entering the farm was always saturated and therefore the % saturation measured at the out flow of the tank gave an indication of the oxygen consumption within the tank. The % saturation decreased significantly in all the tanks between 80 and 100 days into the sampling period. This is not easily explained as it is unlikely that the flow in all the tanks was reduced simultaneously, but was almost certainly due to un-controlled

external factors in the water supply. All the tanks were affected similarly and therefore the results remain comparable.

The water temperature was important as a variable in its own right but was also necessary for calculating the % oxygen saturation and the % unionised ammonia. The temperature was also used when referring to feeding tables. Although the average temperature was warmer than previous years the variation followed the normal seasonal pattern.

pH can affect the health of the fish in several ways. It has an important effect on the % unionised ammonia, can affect the solubility of toxic heavy metals and rapid changes or extremes can affect the ionic transport systems of the fish directly. Again in this study the pH remained within acceptable limits fluctuating within a small range.

The total alkalinity is an indication of the buffering capacity of the water. Very low alkalinity can result in rapid fluctuations in pH. The conductivity reflects the concentration of ions in the water. Although neither of these parameters have been reported as causes of damage to fish, they were included to provide a more complete picture of the nature of the water supply. Both parameters varied over the study period. The alkalinity increased at the time of the spate, whereas the variation in conductivity did not appear to be related to any obvious change in physical conditions.

II
(Discussion)

The harmful affects of suspended solids are more closely related to the nature of the material than the the total amount. Sharp particles may damage the surface of the fish especially the gills. Despite the high levels of suspended solids observed at the time of the spate there were no abnormalities detected on histological examination of the gills (Chapter I). It is therefore unlikely that the fish were adversely affected in any other way.

Ammonia is the end product of protein catabolism and nitrite is the product of ammonia nitrification. Both of these compounds can enter the system from external sources of decomposing organic material. Ammonia can also be produced within the tanks by bacterial breakdown of food and faeces. Nitrite is unlikely to be produced in large quantities within tanks due to the short residence time. The results reflect the origins of the compounds. Ammonia was present at a much higher concentration and increased steadily towards the end of the period as the biomass and level of feeding increased. Nitrite varied randomly over the period with a slight increase towards the end. Both substances are potentially toxic to fish. Ammonia is only severely toxic its unionised form and at the temperatures and pH encountered in this study the % unionised ammonia never exceeded 0.59%. Neither the concentration of unionised ammonia or nitrite approached their maximum recommended limit of tolerance *is* :

NH_3 = 5.0µg/l

NO_2 = 10.0µg/l

(EEC,1978)

II
(Discussion)

The data generated was not sufficiently detailed to produce a significant result by multivariate analysis, therefore it was examined by visual observation of the graphically displayed data. This basic procedure is recognised to be a powerful method of identifying relationships within complex data sets. Many of the observed trends in the environmental parameters were a function of the production system. For example, as the biomass and temperature rose the feeding rate was increased, this resulted in higher levels of NH_4. There were very few correlations between the loss of fin tissue and other parameters. Fin damage was less severe in tank 3 than the others and increased in tanks 5 and 12 during the period of seasonally low temperature. The fish with less fin damage were small triploids, however later findings suggested that fin rot tended to be more severe in smaller fish. It is possible that the lower level of fin damage may have been at least partially the result of a congenital or genetic component. The lower stocking density in tank 3 was unlikely to have affected the fin condition (Soderberg and Meade, 1987). The pattern of increasing fin damage during periods of low temperature reflects the inconsistent findings of studies by Schneider and Nicholson (1980) and Maheshkumar (1985).

4. RELATIONSHIP BETWEEN DORSAL FIN ROT AND SIZE IN *Salmo salar* L. PARR.

There appeared to be a correlation of low significance between the weight or length and the amount of fin remaining in some of the populations studied. However the only highly significant relationship

was between the amount of fin remaining and the weight of the fish, when all the samples were pooled. In this case the heavier fish tended to have less fin damage. It is not possible with this data to demonstrate any causal relationship. If such a relationship did exist, there are two possibilities - either smaller fish were more prone to fin rot or fish with fin rot did not grow as well as unaffected fish. This topic is discussed in greater detail in chapter VI. The condition factor or the length, weight relationship is not only effected by the nutritional status of the fish but also by the process of smoltification, when the fish become relatively more slender (Langdon, 1985). The effect of smoltification may have obscured any relationship between fin rot and nutritional status reflected in the condition factor.

The only two relationships worthy of further investigation were those between fin rot and temperature and fin rot and the size of the fish. These associations were investigated in greater depth in chapters IV and VI.

CHAPTER III

Bacteria associated with dorsal fin rot in farmed Atlantic salmon (*Salmo salar* L.) parr.

CHAPTER III

INTRODUCTION

As discussed earlier (chapter I) there are a number of conditions described as fin rot. Though many of these conditions may well have distinct aetiologies, the involvement of bacteria either in a primary or secondary role is a common thread through the literature (summarised in Table 3.1.).

In the majority of cases the significance of bacteria as aetiological agents is variously described. The possible scenarios include :

A. The bacteria are primary pathogens.

B. The bacteria are secondary pathogens but have a significant effect on the pathogenesis.

C. The bacteria are merely opportunists colonising existing damaged tissue without affecting the pathology.

A. Evidence for bacteria as primary pathogens is limited, although Oppenheimer (1958) did reproduce fin rot in a Norwegian cod fish (*Gadus callarius* now *G.morhua*) by injecting a pseudomonad isolated from a clinical case of fin rot. Bullock and Conroy (1971) also proposed a primary infection with aeromonads or pseudomonads as one of three possible causes of fin rot.

B. Bullock (1968) proposed that the bacteria might have a significant effect on the observed pathology through the secretion of

proteolytic enzymes, even if they were secondary to some other stress or damage.

C. The superficial bacteria on fish largely reflect the bacterial populations in the water, which in turn reflect the environmental conditions (Snow and Beard, 1939; Amend, 1970; Horsley, 1973). Superficial wounds are similarly prone to colonisation by water born bacteria (Ghittino, 1972). Therefore it is possible that the bacteria associated with fin rot may merely reflect the host's environment without having any significant effect on the pathology.

Although many workers report the recovery of aeromonads and pseudomonads, the majority of reports in salmonids describe Cytophaga-like bacteria (CLB) as the predominant organisms. The most commonly cited theory regarding the aetiology of fin rot suggests that the bacteria are secondary invaders following primary stress or damage (Bullock, 1968; Amend, 1970; Bullock and Conroy, 1971; Ghittino, 1972; Murchelano, 1975; Schneider and Nicholson, 1980). It is still unclear whether the CLB recovered from fin rot in salmonids play an active role in the pathology or are a reflection of CLB's general abundance in the aquatic environment (Reichenbach, 1989a) and their propensity for colonising the surface of fish (Pacha and Porter, 1968).

Table 3.1.

Bacteria associated with fin rot.

Host species	Bacteria	Reference
Salvelinus fontinalis (brook trout)	CLB	Bullock, 1968
Salmonids	CLB	Amend, 1970
Salmonids	CLB	Bullock and Snieszko, 1970
Salmo salar (Atlantic salmon)	CLB	Johansson, 1970
Salmonids	CLB, aeromonads & pseudomonads	Bullock and Conroy, 1971
S. salar	*Flexibacter* spp	Schneider and Nicholson, 1980
Carassius auratus (gold fish)	aeromonads & pseudomonads	Conroy, 1961, 1963 & 1964
Mollienisia sphenops (black molly)	aeromonads & pseudomonads	Schäperclaus, 1950
Clupea harengus (herring)	aeromonads & pseudomonads	Sindermann and Rosenfield, 1954
Gadus callarius or morhua (cod)	pseudomonads	Oppenheimer, 1958 Khan *et al*, 1981
Solea solea (sole)	pseudomonads	Fluchter, 1979
Pomatomus saetatrix (bluefish) *Paralichthys dentatus* (summer flounder) *Pseudopleuronectes americanus* (winter flounder) *Cynoscion regalis* (weakfish)	aeromonads, pseudomonads & vibrio	Mahony *et al*, 1973
P. dentatus *P. americanus*	un-specified	Murchelano, 1975 & Murchelano and Ziskovski, 1977
Un-specified	aeromonads, pseudomonads vibrio & CLB	Anderson and Conroy, 1969

Since CLB appear to have some relevance to dorsal fin rot it is useful to consider their role in other fish diseases. There are a number of CLB that have been associated with diseases in fish. In some cases these bacteria have yet to be definitively classified, and for many, Koch's postulates have not been fulfilled.

Perhaps the most significant CLB in terms of both pathogenicity and economic effect are *Cytophaga columnaris* and *Flexibacter psychrophilus*. Recent work has suggested that *Flavobacterium branchiophila* should also be considered an important pathogen (Ferguson, *et al* 1991).

COLUMNARIS or Saddleback disease

This is a serious condition affecting most species of fresh water and anadromous fish. In the UK it was considered sufficiently serious to be included in the 1937 Diseases of Fish Act as a notifiable disease, though it was subsequently excluded from the 1983 Act.

The organism responsible for columnaris has been moved between taxa on several occasions. It was initially described as *Bacillus columnaris* by Davis in 1922. In 1944, Ordal and Rucker suggested that it should be classified with the myxobacteria as *Chondrococcus columnaris* and in the following year Garnjobst (1945) re-classified it as *Cytophaga columnaris*. It was subsequently known as *Flexibacter columnaris* before returning to *Cytophaga columnaris* (Reichenbach 1989b).

The phenotypic characteristics of *C.columnaris* are summarised in Appendix IV.

C.columnaris, which can be found associated with the skin and mucus of both healthy and diseased fish, usually causes disease at temperatures in excess of 18°C to 20°C. There are strain differences in pathogenicity (Pacha and Ordal, 1967) and water quality can also affect the incidence (Fijan, 1968).

The disease often affects the skin on the dorsal surface or the gills. The skin lesions usually begin with a raised greyish plaque surrounded by a zone of hyperaemia. This grey plaque, often seen in the region of the dorsal fin in salmonids, gave rise to the name of 'saddle back' (Pacha and Ordal, 1967). The lesion rapidly progresses to produce haemorrhagic ulcers with a superficial area of bacterial cells and necrotic tissue. The bacterial cell-bound pigments can impart a yellow or orange colour to the lesions. The gross lesions are reflected by histological evidence of haemorrhage, oedema and necrosis extending into the dermis. When columnaris affects the fins its malignant 'centrifugal' nature distinguishes it from the benign 'centripetal' fin rot. Lesions on the gills tend to be necrotic and acutely fatal. The bacterium is also associated with a rapidly fatal systemic infection. *C.columnaris* may affect aquarium fish, especially in the oral region of *Coryodorus* spp., of catfish. In this region the cotton like appearance of the lesion has given rise to the name 'cotton mouth' or 'mouth fungus' (Van duijn, 1967).

Control is based on maintaining adequate water quality since chemotherapy is often unsuccessful.

BACTERIAL COLD WATER DISEASE or peduncle disease.

Flexibacter psychrophilus

The *in vivo* and *in vitro* propensity for growth at low temperatures of the bacterium (4-12°C) was responsible for the name psychrophilus(a). It causes a condition which was confined to north America until the mid 1980's when it was reported in Europe (Bernardet and Kerouault, 1989). Again the taxonomy of this organism has undergone many revisions. It was originally referred to as a myxobacterial fish pathogen (member of the order *Myxobacteriales*) (Borg, 1960; Pacha, 1968). Lewin, (1969) grouped *F.psychrophilus* with *Cytophaga aurantiaca* under the new name of *Flexibacter aurantiacus*. Both *C.psychrophila* and *F.aurantiacus* were considered as *species incertae sedis* but probably members of the *Flexibacter* (Leadbetter, 1974b). Subsequent serological studies (Holt, Rohovec and Fryer, In press) and DNA homology (Bernardet and Grimont, 1989) confirmed that *C.psychrophila* and *F.aurantiacus* were distinct. Christensen (1977) recommended that it should remain as *C.psychrophila* pending further analysis of its polysaccharidase activity. Subsequent publications (Richards and Roberts, 1978; Schneider and Nicholson, 1980) used the name *Flexibacter psychrophila*.

In 1989, Reichenbach, in Bergey's Manual of Systematic Bacteriology retained the name *C.psychrophila* (Reichenbach, 1989b). However in the

same year Bernardet and Grimont (1989) on the grounds of polysaccharide degradation proposed that *C.psychrophila* should be included in the *Flexibacter* as *Flexibacter psychrophilus* pending re-arrangement of the whole branch of the Eubacteria. This validly published name was supported by Holt *et al*, (In press).

The source of the organism is not known but is probably the natural aquatic environment. The bacterium typically induces profound epithelial hyperplasia with large numbers of mucous cells. The lesions progress from initial proliferation to necrosis extending deep into the subcutaneous tissue. Eventually the tissue sloughs and secondary bacterial invasion occurs. Cellular infiltration is often absent (Wood and Yasutake, 1957) or mild (Borg, 1960), in part due to the low temperatures (Roberts, 1989). Others, however, have reported a substantial cellular response (Wolke, 1975). When the caudal fin and surrounding tissue is involved, the entire area can be sloughed,leading to the description of 'peduncle disease'.

Several other syndromes have been identified in association with *F.psychrophilus*. In coho salmon (*Oncorhynchus kisutch*), the type and severity of the signs appear to be related to the stage of development of the fry (Wood, 1974). In young alevins high mortalities (30-50%) may be associated with erosion of the skin over the yolk sac as the only pathological change. In first feeding fry the the classical peduncle disease is more common and is usually associated with lower mortalities (<20%). In post-first feeding fry, lesions may occur in any region of the body surface. After an

epizootic a proportion of the fish may develop a chronic systemic infection with associated spinal deformities (Conrad and DeCew, 1967). Yearlings may also be affected by a systemic or a typical epidermal lesion, although other less common syndromes have been reported (Holt, 1972). The clinical signs appear to be similar in most species of salmonid.

Control of cold water disease is largely achieved by improving the environment since it does not respond well to chemotherapy.

In many respects, typical *F.psychrophilus* infections are similar to dorsal fin rot. They induce epithelial hyperplasia, loss of tissue, irregular cellular inflammatory response and occur at lower temperatures. An increase in mucous cells was not observed in dorsal fin rot but an increase in mucous cell numbers is a transient response to irritation (Roberts, 1989). In view of the similarities between the organisms and the syndrome mentioned above it was considered important to examine the isolates from dorsal fin rot to determine their relationship to *F.psychrophilus* (Discussion, Chapter III).

BACTERIAL GILL DISEASE (BGD)

(reviewed by Turnbull, In press)

This condition occurs in many countries world wide, affecting cultured fresh water fish (Snieszko, 1981; Farkas, 1985). It is typically associated with extensive colonisation of the gills by CLB. Other Eubacteria have been isolated from BGD but they are not thought to play a significant role (Wood and Yasutake, 1957). One bacterium has

been isolated from outbreaks in USA, Hungary and Japan and was published as a new species, *Flavobacterium branchiophila* by Wakabayashi, Huh and Kimura (1989). Individual outbreaks tend to be associated with a single isolates of bacteria and one isolates can be predominant on an individual site, however on other sites the isolates vary between outbreaks.

The phenotypic characteristics of the bacteria associated with BGD are summarised and compared to isolates from fin rot in the discussion of this chapter.

Early attempts at reproducing BGD were only successful when fish were exposed to a combination of adverse water quality and a bacterial challenge (Bullock, 1972). It was proposed at that time that the gills were damaged by the environmental conditions which increased their susceptibility to bacterial colonisation. Some more recent work has proposed a more primarily pathogenic role for some isolates of *F.branchiophila*. Despite the involvement of environmental conditions in some outbreaks, the bacteria still appeared to play a significant role since their removal results in resolution of the lesions. It was considered probable that BGD was the result of the complex interaction of adverse environmental conditions and bacteria of variable pathogenicity. Therefore similar if not identical pathology could result from either, extreme environmental conditions and the presence of a low grade pathogen, or marginal environmental conditions in the presence of a highly pathogenic bacterium.

The pathology associated with BGD is primarily hyperplasia of the epithelial tissue. There is some debate as to the mechanisms by which this occurs. It has been suggested that the hyperplasia may be in response to some extracellular hyperplasia-inducing factor (Kudo and Kimura, 1983c; Wakabayashi and Iwado, 1985). Alternatively other workers have observed substantial cellular necrosis in the lesions and propose that the hyperplasia is a simple response to the necrotic tissue (Ostland *et al*, 1989; Ostland, Ferguson, Prescott, Stevenson and Barker, 1990; Ferguson *et al*, 1991; Speare, Ferguson, Beamish, Yager and Yamashiro, 1991b). Resolution of the lesion occurs by sloughing of the hyperplastic tissue and can restore the normal structure of the gills provided the basement membrane is intact.

BGD is similar to fin rot in many respects, CLB are consistently isolated from the lesions and the predominant host response is hyperplasia. BGD was taken as a possible model for fin rot and an attempt was made to investigate the interaction between environment (Chapter II), bacteria (Chapter III) and the observed pathology (Chapter I).

The remaining CLB associated with fish disease are of doubtful taxonomic and pathogenic status.

Cytophaga succinicans

This bacterium was reported in association with fish disease by Anderson and Ordal (1981).

Cytophaga johnsonae and *C.rosea*

These were mentioned as fish pathogens by Rose and Smith, (1972).

Cytophaga aquatilis

Strohl and Tait (1978) recovered 13 isolates from the gills of diseased salmonids and suckers (*Carpoides cypranus*). These organisms were similar to those reported by Borg (1960), Pacha and Porter (1968) and Anderson and Conroy (1969).

Flexibacter maritimus

Originally named *F.marinus* by Hikida, Wakabayashi, Egusa and Masumura (1979), this organism was re-named by Wakabayashi, Hikida and Masumura (1986). It was isolated from juvenile red sea bream (*Pagrus major*) and black sea bream (*Acanthopagrus schagelli*) in Japanese mariculture sites. The clinical signs included eroded mouth and fins

Flavobacterium piscicida

Bein (1954) reported recovering this organism from a mass mortality associated with a 'red tide'.

Flavobacterium spp

Numerous authors have reported un-classified species of *Flavobacterium* (Briaou, Tyssat, and Vacher, 1964; Richards and Roberts, 1978; Acuigrup,1980; Farkas, 1985).

Sporocytophage spp

Organisms were recovered from a surface lesion of a marine salmonid by Wood (1968) and the presence of microcysts was recorded by Pacha and Ordal (1970).

In this study yellow pigmented Gram negative rods were referred to as CLB unless proved otherwise.

There were three main initial objectives in this area of the study. Firstly to examine the bacterial populations associated with dorsal fin rot. In order to obtain this information, normal and diseased fins were sampled from a number of sites and on a number of occasions from the commencement of the study in January 1988 until close to the time of smolt transfer in April 1988..

Secondly, the intention was to characterise any predominant strains to allow comparison with other bacteria including those associated with fish disease.

Thirdly to investigate the claim that fish with dorsal fin rot were more susceptible than fish with undamaged fins to *A.salmonicida* infections.

CHAPTER III

MATERIALS AND METHODS

1. BACTERIA ASSOCIATED WITH NATURALLY OCCURRING DORSAL FIN ROT IN *Salmo salar* L. PARR

1.1. Evaluation of three sampling methods.

Three methods were evaluated to find a suitable sampling protocol. The techniques were based on the experience of the bacteriology unit of the Institute of Aquaculture, University of Stirling. In all the techniques the epithelium and associated tissue was removed from the dorsal fin, affected with fin rot, using a sterile scalpel and observing standard aseptic technique. The material was suspended in 5ml of peptone water and agitated thoroughly for 60 seconds. The resulting solution was inoculated onto TSA using three different techniques (1.1.1. - 1.1.3.).

1.1.1. A 0.1ml aliquot was placed onto a TSA plate and spread out with a sterile spreader. A further 1ml of the solution was mixed with 1ml of sterile peptone water and 0.1ml plated out in a similar manner. This process was repeated resulting in three plates: one from the initial solution, one from a 1:1 dilution and one from a 1:2 dilution. These plates were incubated at 22°C.

1.1.2. One 0.1ml drop was placed onto a TSA plate and spread out with a sterile spreader, this spreader was then wiped over two further TSA plates in succession. The plates were incubated at 22°C.

1.1.3. Six 0.02ml drops of the original suspension were placed onto a TSA plate. This plate was also incubated at 22°C.

All the plates were examined after two and four days to determine the most suitable method for identifying the bacteria present on the dorsal fin.

1.2. Investigation into bacterial populations associated with dorsal fin rot.

Initially a study was undertaken to investigate the bacterial populations associated with both dorsal fin rot and undamaged dorsal fins.

From January to March 1988, samples were taken on six occasions from six farms (Table 3.2.). These samples totalled eighteen fish with dorsal fin rot and ten fish with undamaged fins.

For each sample two fish with similar dorsal fin condition were selected. Each fish in turn was killed by a blow to the head and then rinsed in the water from which it was removed. The epithelium and associated tissue was scraped off the fin with a sterile scalpel. The tissue from both fish was placed in a bijou containing 5ml of peptone water. The tissue was suspended by vigorous agitation of the bijou

for 60 seconds. From the suspension 0.1ml was transferred to each of six agar plates, and spread out with a sterile spreader. The six plates included two Tryptone Soya Agar (TSA Oxiod ltd), two Cytophaga Agar (CA) (Anacker and Ordal, 1959a), and two River Water Glucose Yeast Agar (RGY) (Alderman, 1982).

With the exception of FARM 1, 2/2/88 one of each type of plate was incubated at 10°C and the others at 22°C. The 10°C plates from FARM 1 2/2/88 were not used due to contamination.

Table 3.2.
Origin and nature of fins sampled 1.2.

Site	Date sampled	Number of fish: Undamaged fins 10°C	22°C		Damaged fins 10°C	22°C	
FARM 1 (tank 5)	2/2/88	2	-	+	2	-	+
FARM 3 (tank 17)	9/2/88	-	-	-	2	+	+
FARM 3 (tank 17)	10/2/88	-	-	-	2	+	+
FARM 1 (tank 12)	1/3/88	2	+	+	2	+	+
FARM 1 (tank 4)	1/3/88	2	+	+	2	+	+
FARM 2	10/3/88	-	-	-	2	+	+
FARM 5	10/3/88	-	-	-	2	+	+
FARM 6 (tank A21)	22/3/88	2	+	+	2	+	+
FARM 6 (tank B8)	22/3/88	2	+	+	2	+	+

All the farms were fresh water Atlantic salmon farms where the fish were reared in glass fibre tanks.

The plates, incubated at 22°C, were examined after five days and those at 10°C after ten days, both sets being examined again after fourteen days. The organisms were divided according to colony characteristics. The number of each colony type was counted on every plate. A representative of each type was sub-cultured onto the media from which it had been removed.

The cultures were re-plated until pure isolates were obtained, though in most cases the first sub-culture produced a pure isolate. These pure isolates were subjected to the following tests (Frericha, 1984) :

Grams stain

Oxidase production

Catalase production

Motility of a broth culture

Oxidative or fermentative attack of glucose (O-F)

All the isolates were retained on slopes of the media on which they were isolated.

1.3. The results from 1.2. allowed the bacterial types to be provisionally identified. There appeared to be a large number of Cytophaga-like bacteria (CLB) on all the fins with dorsal fin rot (Results 1.2.). To allow further analysis of these organisms at a later date, cultures of all the CLB were freeze dried.

Broth cultures were grown for 48h in Cytophaga broth (CB) and 10% meso-inositol was added to the cultures. The resulting suspension was divided into 0.1 ml aliquots and freeze dried in an Edwards EF 03 freeze drier. The cultures sealed within glass vials were stored at -4°C until required

1.4. Further characteristics of bacterial isolates.

These cultures were reconstituted with 0.5ml of CB. The resulting suspension was inoculated onto two CA plates which were incubated at 10°C. The cultures were subjected to the following tests : (over)

Grams stain

Oxidase production

Catalase production

H_2S production

Gelatinase production

Starch hydrolysis

Aesculin hydrolysis

Casein hydrolysis at 10%, 20% and 50% casein

Growth at 10°C, 15°C, 22°C, 27°C and 37°C

Ability to swarm on damp agar

Growth in 0%, 0.5%, 1%, 2% and 3% NaCl

Anaerobic growth

Production of acid or gas from sugars : glucose, galactose, sucrose, lactose and citrate

Antibiotic sensitivity was tested by the standard disc diffusion technique described in Difco, Technical Information (Difco Laboratories, Detroit, USA), with the following antibiotic sensitivity discs :

Oxytetracycline -	30µg	Oxolinic acid -	2µg
Nitrofurantoin -	100µg	Furazolidone -	50µg
Ampicillin -	10µg	Erythromycin -	5µg
Sulphafurazole -	100µg	Sulphamethazole & trimethoprim-	1.25µg
Chloramphenicol -	10µg	Sulphamethoxazole -	25µg
Cephaloridine -	5µg		

1.5. Results from other areas of the study (Chapter IV) suggested that the bacterial populations associated with dorsal fin rot in November 1989 were different to those observed in early 1988. To further investigate this discrepancy, fish with fin rot were sampled from one site between November 1989 and April 1990.

The object of this sampling was primarily to investigate the number and the proportion of bacterial types on dorsal fins affected by fin rot.

1.5.1. An experiment was conducted to compare the results obtained from six different methods of sampling the dorsal fin.

Five fish with active dorsal fin rot were randomly selected from farm 1 (tank 6). Each of these five fish was processed in an identical fashion (Figure 3.1.). The fish were killed by a blow to the head and then rinsed in the water from which they had been removed. The dorsal fins were sampled with a sterile loop onto three CA plates. The remaining epithelium and associated tissue was removed from the dorsal fin with a sterile scalpel. This tissue was suspended in 5ml of peptone water. The suspension was vigorously agitated for 30 seconds and three CA plates were inoculated with 0.01ml of this suspension. A further 1ml was removed and mixed with 1ml of peptone water and three 0.1ml aliquots of this dilution were taken from the top of the fluid and plated out onto CA plates. The same procedure was repeated from the bottom of the fluid. The remaining diluted suspension was retained and after one hour the container was agitated

and a further three 0.1ml aliquots plated out on CA plates. Prior to the one hour delay the remaining 4ml of the original suspension was homogenised, 1ml of this homogenate was diluted with 1ml of peptone water and three 0.1ml aliquots plated out onto CA plates.

With the exception of the samples taken by loop, all the plates were inoculated by dropping the suspension onto the plate with a sterile tip on a p 1000 Gilson pipette and spreading with a sterile spreader bar.

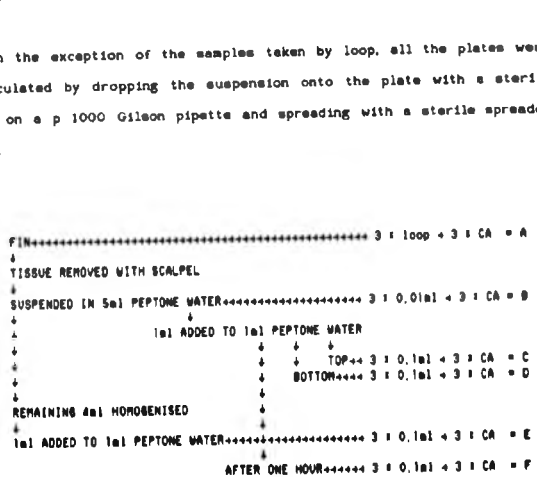


Figure 3.1. Diagrammatic representation of the techniques used in 1.5.1.

The results were analysed by estimating the mean of the % coefficient of variation and the 95% confidence limit of that mean. The mean and standard deviation of the observed bacterial counts, by each method, for each fish were also calculated. Finally a Wilcoxon's test for

matched pairs was used to compare the median number of cfu between sampling methods. A test for matched pairs was selected to take into account the difference between fish and still allow comparison between techniques.

1.5.2. The technique adopted for sampling was to kill the fish with a blow to the head and then rinse them in the source water. The epithelium and associated tissue was removed from the dorsal fin with a sterile scalpel. This tissue was suspended in 5ml of peptone water. The suspension was vigorously agitated for 30 seconds and then 1ml was removed and mixed with a further 1ml of peptone water. Three 0.1ml aliquots of this suspension were taken from the container and spread out on CA plates with a sterile spreader bar.

On each of the following seven occasions five fish with evidence of dorsal fin rot were randomly selected from farm 1 (tank 6)

Sample dates 9/11/89
12/11/89
17/11/89
7/12/89
12/2/90
14/3/90
19/4/90

On each occasion the source of the fish , the temperature of the water and the condition of the dorsal fins was recorded. The results were analysed by the Spearman rank correlation coefficient.

2. EXPERIMENTS TO INVESTIGATE THE BACTERIA ASSOCIATED WITH THE DORSAL FIN FOLLOWING CONTROLLED DAMAGE

Work in other areas of this study had suggested that physical damage was an important factor in the aetiology of dorsal fin rot (Chapter IV). The following experiments were conducted to further investigate the relationship between damage to the dorsal fin and the associated bacteria.

2.1. Sixty Atlantic salmon parr with undamaged dorsal fins were randomly selected from a production tank (Farm 1). These fish were returned to aquarium facilities at the Institute of Aquaculture and placed in a 50l container with an internal power sponge filter (Aquaclear 2401) and additional aeration for two days acclimatisation.

The water was de-chlorinated mains supply. At the end of the period of acclimatisation pure oxytetracycline was added to produce a concentration in the container of 150mg/l. After 1h the fish were removed and placed in an identical system with fresh water, and subsequently left in this system for a further three days.

The fish were then individually removed and aneasthetised. Their dorsal fins were excoriated by first gripping the fin with a bulldog clip and then dragging it off the fin distally. To produce moderate erosion of the fin it was necessary to repeat this procedure twice for each fish. Once excoriated the fish were randomly divided between two 30l tanks with small airlift filters.

Concurrently with the acclimatisation of the fish, a broth culture of a CLB was prepared. The dorsal fin of a fish suffering from severe fin rot was sampled by the method described in 1.2. An isolate of the predominant CLB was grown in 500ml of CB for 84h. At the end of this period a sample was take from this culture for serial dilution in peptone water (Figure 3.2.).

Initial culture →1:100→1:100→1:10→1:10→1:10
↓ A ↓ B ↓ C

Figure 3.2. Diagrammatic representation of the dilution method used in 2.1.

0.1ml of A, B & C were each plated out onto three replicate CA plates and the resulting colonies counted after five days incubation at 15°C.

[1]
(Materials & Methods)

The sample for serial dilution was taken as soon as all the fish had recovered from the anaesthetic, the rest of the culture was then added to one of the 30l tanks. The dilutions and plating was performed without delay.

On the day after the excoriation and every second day thereafter three fish were removed from each tank. One fish from each tank was sampled for bacteriology and the dorsal fins of the other two were removed and fixed in 10% neutral buffered formalin for histology. The bacteriological sampling involved removing the epithelium and associated tissue from the fin with a sterile scalpel and suspending it in 2.5ml of peptone water. This suspension was vigorously agitated for 30 seconds and then 0.1ml was spread over each of two CA plates. These plates were incubated at 15°C and the resulting colonies counted after seven to ten days, depending on growth. Both the total number of cfu and the number of CLB cfu were recorded. The CLB cfu were identified by colony characteristics. The fixed fins were processed for histology in the standard fashion and the resulting sections stained with H & E.

The fish were not fed during the experiment and the daily maximum and minimum temperatures were recorded.

The results from this experiment suggested that further investigation into this area might be useful and so the following experiments were conducted.

2.2. Two identical 1 × 0.5m glass fibre flow-through tanks were set up. 140 fish with apparently undamaged fins were randomly selected from farm 1, tank 6, and divided equally between the tanks. The fish were fed twice daily by hand throughout the experiment with the exception of the day before and the day after excoriation. Following three days acclimatisation the fish were netted out in pairs and anaesthetised. The fish from one tank had their dorsal fins excoriated with a fixed parr's head (Chapter IV) to simulate fin nipping. The fish from the other tank were handled in a similar fashion but the dorsal fins were not damaged. The daily water temperatures were recorded with a maximum-minimum thermometer.

Five fish were sampled from each tank on the day prior to excoriation, 24h after excoriation and on alternate days thereafter. Sampling involved removing five fish from the tank and placing them in a clean and rinsed plastic bag filled with the water from the tank. The fish were transported to the laboratory in this bag. Once in the laboratory the fish were removed and killed with a blow to the head. They were rinsed in the water from the bag and then the epithelium and associated tissue was removed from the dorsal fin with a sterile scalpel. This tissue was suspended in 5ml of sterile peptone water and agitated vigorously for 30 seconds. From this suspension, 1ml was removed and mixed with a further 1ml of peptone water. Three 0.1ml aliquots were removed and spread onto three replicate CA plates which were incubated at 15°C (technique C from 1.5.1.). The plates were incubated until the colonies appeared to be in optimum condition for counting, this time varying depending on the number of colonies

present, but usually between 7-10 days. The number of CLB and total colonies were recorded. The condition of the dorsal fins of the fish was also recorded prior to sampling.

The results were tested for normality by the Shapiro-Wilks test (Zar, 1984) and for homogeneity of variance by the Bartlets test. Since only some of the data sets were normally distributed and there were highly significant differences between the variances, non-parametric tests were employed. The differences between the non-CLB cfu (*i.e.* total cfu-CLB cfu) from the excoriated and control fish were compared by the Mann-Whitney U test. The CLB cfu from the excoriated fish and the non-CLB cfu from both the excoriated and control fish were analysed by the Kurskal-Wallis test. When a statistically significant difference ($p < 0.05$) was detected by the Kurskal-Wallis test the difference between pairs was tested by Dunn's multiple comparisons procedure (Zar, 1984).

2.3. A similar experiment was conducted to obtain further information. The experiment was the same in every respect except the fish were sampled every 24h. The same data was recorded in an identical manner.

3. SUSCEPTIBILITY OF *Salmo salar* L. PARR WITH DORSAL FIN ROT TO AN *Aeromonas salmonicida* BATH CHALLENGE

An experiment was conducted to investigate the susceptibility of fish with undamaged fins and fish with dorsal fin rot to a bath challenge with *Aeromonas salmonicida*. The strain of *A.salmonicida* and information regarding its nature and pathogenicity was provided by Dr V.B.Inglis. The bacteria (identified as FCC) was taken as a primary isolate from a diseased fish. This isolate was grown for 24h in 12.5ml of brain heart infusion broth (BHIB Oxoid Ltd). The resulting culture was divided into twenty-five aliquots, to each of which two drops of glycerol were added. They were frozen at -20°C for 24h and then placed in liquid nitrogen. Thus a store of identical cultures was produced.

3.1. Nine black polythene bins with netting covers were prepared. Each bin was supplied with a standard aquarium heater-thermostat, aeration via an air stone and a power sponge filter (Aquaclear 2401) fitted with a spray bar.

In order to test the system the bins were filled with 50l of water and the temperature adjusted to 18°C (± 0.5°C). Once the temperature had stabilised the electricity was turned off and the bins emptied.

The fish were obtained from farm 1, where a stringent disease monitoring policy had produced no evidence of *A.salmonicida* in the salmon parr. Fish were selected by hand grading and consisted of 180

with apparently undamaged fins and 180 with severe dorsal fin rot. On return to the laboratory the bins were re-filled with de-chlorinated mains water and the aeration turned on. The fish were randomly divided among the bins until each bin contained twenty fish with undamaged dorsal fins and twenty fish with dorsal fin rot. The temperature of the farm water had been 5.5°C and the temperature of the water in the aquarium 6.5°C. After 24h, the filters were switched on causing a rise in temperature. After a further 24h the heaters were switched on taking the temperature up to 18°C. The broth cultures were added the following day.

To prepare the broth culture one of the frozen 0.5ml aliquots was thawed and added to 10ml of BHIB. This was incubated at 22°C for 24h in a shaking incubator and then added to 400ml of BHIB. This second culture was incubated for a further 24h.

After agitation, a sample was taken from the final culture and the optical density determined at 610nm in a LKB Biochrome Ultraspec II. With the aid of this measurement and a standard curve obtained from the Aquatic Vaccine Unit, University of Stirling (Appendix II), the approximate number of cfu/ml was estimated. Thus the volume of culture required to produce 5×10^2, 5×10^3 and 5×10^4 cfu/ml in the 50l bins was calculated. At the same time as the sample was removed for measurement of the optical density another sample was taken for serial dilution and plate counts. This sample was diluted as described in Figure 3.3. The bins were randomly assigned a concentration to produce three replicates of each level of challenge.

Figure 3.3. Three replicate TSA plates were inoculated from each of A, B and C. These plates were incubated for 48h and then the colonies were counted. Thus an indication of the initial concentration of viable *A.salmonicida* within the bins was obtained.

The average daily temperatures were recorded in the bins. The mortalities were removed twice daily where necessary and each was sampled from the kidney and the dorsal fin onto TSA. The kidney was sampled with a sterile loop. The dorsal fin was sampled by first searing the surface with a red hot scalpel and then introducing a sterile loop into the tissue immediately adjacent to the base of the fin. The resulting plates were retained for two weeks and any evidence of *A.salmonicida* recorded. *A.salmonicida* was identified by the colony characteristics and the presence of diffusible brown pigment.

In an attempt to maintain the water quality in the bins a 50% water change was carried out every two days after introduction of the culture and the filters were cleaned in running water (approximately 60°C) during the second water change.

The results from 3.1. produced different rates of specific mortalities at the different levels of challenge. However in the tanks exposed to the lowest challenge there was a high proportion of mortalities from which *A.salmonicida* was not recovered. To further investigate this area of the challenge, another experiment was conducted.

3.2. This experiment was conducted in a similar manner to 3.1. On this occasion three bins were used, each containing twenty fish with normal fins and twenty with severe dorsal fin rot. The fish were obtained from the same source and introduced and acclimatised in a similar manner. A broth culture was set up and the concentration estimated by the optical density. Sufficient broth was added to the bins to produce an estimated challenge of 5×10^{4} cfu/ml and a plate count was conducted to estimate the challenge dose more accurately. The mortalities were sampled in an identical fashion. In an attempt to prevent deterioration in the water quality 50% water changes were conducted every day and the filters were cleaned every second day.

CHAPTER III

RESULTS

1. BACTERIA ASSOCIATED WITH NATURALLY OCCURRING DORSAL FIN ROT IN *Salmo salar* L. PARR

1.1. Evaluation of three sampling methods.

The only technique that produced an acceptable result was the direct plating out of 0.1ml of the original solution (1.1.1.). The spreader dilutions did not transfer many bacteria and with the direct drop technique the slower growing bacteria were overgrown before they could be easily counted. A refinement of the first technique was used for 1.2.

1.2. Investigation into bacterial populations associated with dorsal fin rot.

The bacteria recovered from the six sampling dates were preliminarily identified according to the results of Gram, oxidase, catalase, motility and O-F tests (Table 3.3.). The number of colonies from each bacterial group was calculated for each plate. The results were added to produce the number and type of bacteria grown at 10°C and 22°C for each sample. These results are displayed graphically in Figures 3.4. to 3.7. The undamaged fins sampled produced no more than 40 colonies from any of the groups, with a mean of 7.58 colonies per plate.

Table 3.3.
Preliminary identification of bacterial isolates.

Bacteria grouping	Oxidase	Motility	O/F	Catalase	Gram reaction
1	+/-	-	-	+/-	-ve' bacilli
2	-	-	O	+	"
3	-	+/-	F/-	+/-	"
4	+	+	O/-	+	"
5	+	-	O	+	"
6	+	-	F	+	"
7	+	+	F	+	"
8	-	-	-	+/-	-ve' cocci
9	+	-	-	-	"
10	-	+/-	O/-	+	+ve' cocci
11	-	-	F	-	"
12	+/-	-	F	+	"
13		-	F/-	+	+ve' bacilli
14		+	O/-	+	"
15		+	F	+	"
16		-	F	-	"

Bacterial grouping	Preliminary identification
1	Cytophaga-like bacteria (CLB)
2	*Acinetobacter*
3	*Enterobacter, Streptobacillus, Shigella dysenteriae Haemophilus, Bacteroides* & CLB.
4	*Pseudomonas, Alteromonas* & *Alcaligenes*
5	*Flavobacterium, Moraxella* & *Bordetella*
6	*Aeromonas salmonicida, Pasteurella, Necromonas, Actinobacillus* & *Cardiobacterium*
7	*Vibrio, Aeromonas, Benekea* & *Plesiomonas*
8	*Veillonella*
9	*Branhamella*
10	*Micrococcus* & *Arthrobacter*
11	*Streptococcus, Aerococcus* & *Gamella*
12	*Staphylococcus*
13	*Corynebacterium, Rothia* & *Bacillus*
14	*Bacillus* & *Kurthia*
15	*Bacillus* & *Listeria*
17	YEASTS.
18	FUNGI.
19	UNIDENTIFIABLE ON PRELIMINARY TESTS.

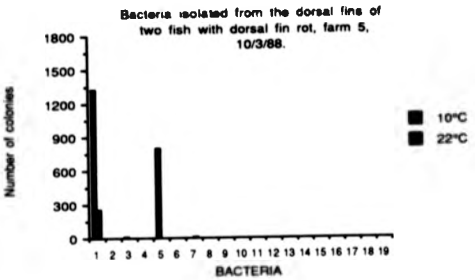


Figure 3.4. Provisional identification of bacteria and number of cfu from fish with dorsal fin rot from farm 5. The bacterial number refers to the preliminary identification detailed in Table 3.3.

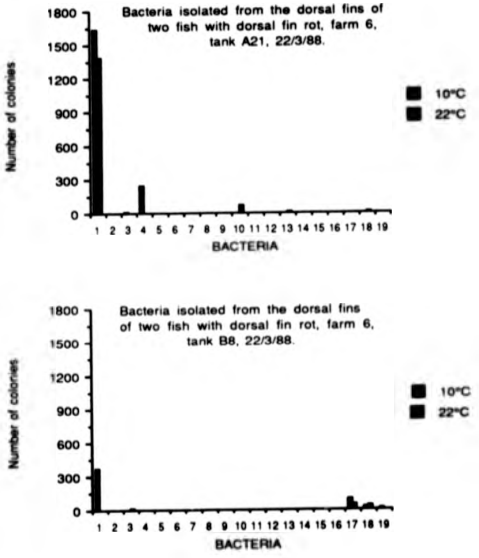


Figure 3.5. Provisional identification of bacteria and number of cfu from fish with dorsal fin rot from farm 6. The bacterial number refers to the preliminary identification detailed in Table 3.3.

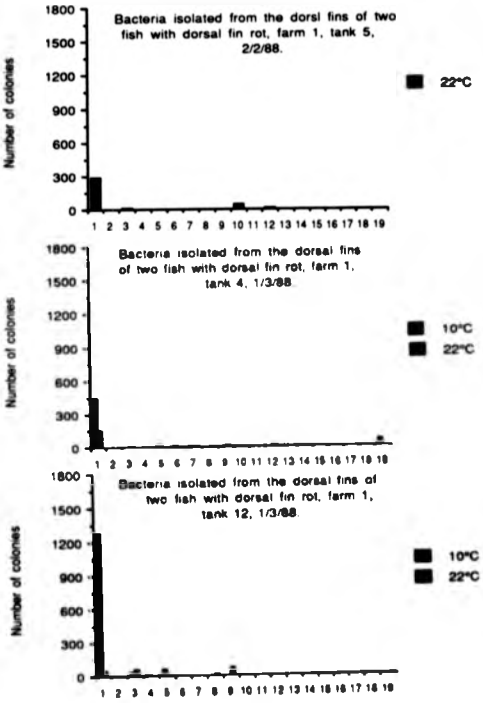


Figure 3.8. Provisional identification of bacteria and number of cfu from fish with dorsal fin rot from farm 1. The bacterial number refers to the preliminary identification detailed in Table 3.3.

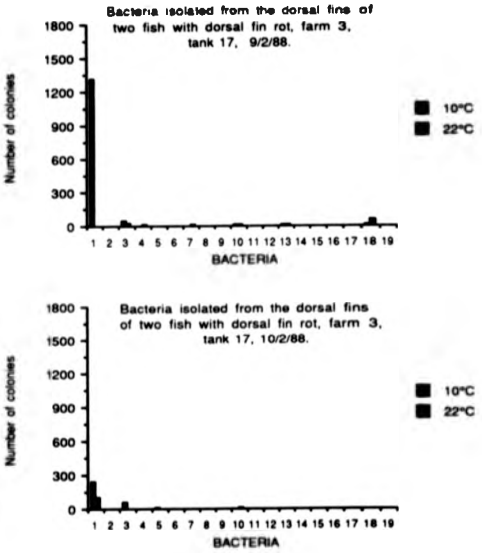


Figure 3.7. Provisional identification of bacteria and number of cfu from fish with dorsal fin rot from farm 3. The bacterial number refers to the preliminary identification detailed in Table 3.8.

1.4. Further characterisation of bacterial isolates.

Of the twenty two cultures freeze dried twenty were successfully revived. Information regarding the origin of the revived cultures is contained in Table 3.4.

Table 3.4.

Identity of CLB isolates.

ID N°	Origin of Sample Site/tank	Sample date	Fin condition	Initial cultivation media	Temp	N° of colonies
1	1/12	1/3/88	fin rot	CA	10°C	1276
2	1/4	"	"	RBY	10°C	246
3	1/4	"	"	CA	10°C	280
4	1/4	"	"	CA	10°C	150
5	1/4	"	normal	CA	10°C	48
6	1/12	"	fin rot	CA	22°C	20
7	1/4	"	"	RBY	22°C	150
8	2/-	12/4/88	"	TSA	10°C	201
9	2/-	"	"	CA	10°C	150
10	2/-	"	"	CA	10°C	9
11	2/-	"	"	CA	22°C	67
12	2/-	"	"	CA	22°C	49
13	3/17	9/2/88	"	CA	10°C	1120
14	3/17	10/2/88	"	CA	10°C	210
15	5/-	10/3/88	"	CA	10°C	1326
16	6/A21	22/3/88	"	CA	10°C	1498
17	6/A21	"	"	CA	10°C	86
18	6/A21	"	"	TSA	10°C	86
19	6/88	"	"	CA	10°C	10
20	Iceland*	30/3/88	"	CA	22°C	-

* = An isolate obtained from a severe outbreak of dorsal fin rot on an Icelandic Atlantic salmon farm.

III
(Results)

The results from the phenotypic study of the bacterial isolates are contained within Tables 3.5. - 3.8.

Table 3.5.

Morphology observed under light microscope following grams stain.

Isolate ID N°	Approximate average length
1	long rods ± 1,5-7µm
2	short cocco-bacilli ± 1µm
3	long rods ± 1,5-7µm
4	long rods ± 1,5-7µm
5	long rods ± 1,5-7µm
6	short rods ± 1,5µm
7	short cocco-bacilli ± 1µm
8	long rods ± 1,5-7µm
9	short rods ± 1,5µm
10	short rods ± 1,5µm
11	short rods ± 1,5µm
12	long rods ± 1,5-7µm
13	short rods ± 1,5-3µm
14	short rods ± 1,5µm
15	long rods ± 1,5-7µm
16	short rods ± 1,5µm
17	short rods ± 1,5µm
18	long rods ± 1,5-7µm
19	short rods ± 1,5µm
20	long rods ± 1,5-7µm

All the isolates were thin Gram negative rods of varying length.

Table 3.6.

Isolate ID N°	1	2	3	4	5	6	7	8	9	10
Oxidase production	-	-	-	-	-	-	-	+	+	+
Catalase production	+	+	+	+	+	+	+	-	+	+
NO_3 reduction	-	-	-	-	-	-	-	-	-	-
H_2S production	-	-	-	-	-	-	-	-	-	-
Gelatinase production	-	+	-	-	-	-	±	-	-	-
Starch hydrolysis	+	+	+	+	+	+	±	+	+	+
Aesculin hydrolysis	+	+	+	+	+	+	+	-	-	-
Casein hydrolysis at 1 casein : -										
10%	±	+	±	±	±	+	±	-	-	-
20%	±	+	-	-	-	+	±	-	-	-
50%	±	±	-	-	-	-	±	-	-	-
Ability to swarm on deep agar at 18°C	-	±	±	±	±	±	-	+	+	+
Growth at : -										
5°C	±	±	±	±	±	+	±	-	±	-
10°C	±	±	±	±	±	+	±	-	±	±
15°C	+	+	+	+	+	+	-	+	+	+
22°C	+	+	+	+	+	+	-	+	+	+
27°C	+	+	+	+	+	+	-	+	+	+
37°C	-	-	-	-	-	-	-	-	-	-
Growth in NaCl : -										
0%	+	+	+	+	+	+	±	+	+	+
0.5%	+	+	+	+	+	+	±	+	+	+
1%	+	+	+	+	+	+	±	+	+	+
2%	+	-	+	+	+	+	±	+	-	-
3%	+	-	±	-	±	±	±	+	-	-
Anaerobic growth	+	-	+	±	-	-	-	-	-	+
Utilisation of : -										
Glucose- Acid	-	+	-	-	-	-	-	-	-	-
Gas	-	-	-	-	-	-	-	-	-	-
Galactose- Acid	-	+	-	-	+	+	-	-	-	+
Gas	-	-	-	-	-	-	-	-	-	-
Sucrose- Acid	-	-	-	-	-	-	-	-	-	-
Gas	+	-	-	-	-	-	-	-	-	+
Lactose- Acid	+	-	-	+	+	+	-	+	+	+
Gas	-	-	-	-	-	-	-	+	-	+
Citrate	+	-	-	-	-	-	-	-	-	-

(+ = positive, - = negative, ± = marginally positive reaction, ± = No growth)

Table 3.7.

Isolate ID N°	11	12	13	14	15	16	17	18	19	20
Oxidase production	-	+	+	+	-	+	+	-	+	+
Catalase production	+	+	+	+	+	+	+	+	+	+
NO_3 reduction	-	-	-	-	-	-	-	-	-	-
H_2S production	-	-	±	-	-	-	-	-	-	-
Gelatinase production	-	I	+	-	-	-	-	-	-	+
Starch hydrolysis	+	+	+	+	+	I	+	+	+	+
Aesculin hydrolysis	+	-	+	+	+	+	+	-	+	+
Casein hydrolysis at										
% casein : -										
10%	+	-	+	+	-	-	+	+	-	-
20%	+	-	+	+	-	-	+	+	-	-
50%	±	-	+	+	-	-	+	±	-	-
Ability to swarm on								θ22°C		
damp agar at 15°C	±	+	+	+	±	+	±	+	-	+
Growth at : -										
5°C	+	±	+	+	±	±	±	±	±	±
10°C	+	±	+	+	+	±	±	±	+	+
15°C	+	+	+	+	+	+	+	+	+	+
22°C	+	+	+	+	+	+	+	+	+	+
27°C	+	+	+	+	+	+	+	+	+	+
37°C	-	-	±	±	-	-	-	-	-	-
Growth in NaCl : -										
0%	+	+	+	+	+	+	+	+	+	+
0,5%	+	+	+	+	+	+	+	+	+	+
1%	+	+	+	+	+	+	+	+	+	+
2%	+	+	+	+	+	+	+	+	+	-
3%	+	+	+	+	-	+	+	+	+	-
Anaerobic growth	-	-	-	-	-	±	+	±	-	-
Utilisation of : -										
Glucose - Acid	-	-	-	-	-	+	-	-	-	-
Gas	-	-	-	-	-	-	-	-	-	-
Galactose -Acid	+	-	+	+	-	+	+	-	+	-
Gas	-	-	-	-	-	-	-	-	-	-
Sucrose - Acid	-	-	-	-	-	-	-	-	-	-
Gas	+	-	+	+	-	+	+	+	+	-
Lactose - Acid	+	-	+	+	+	+	+	-	+	-
Gas	-	-	-	-	-	-	-	-	+	-
Citrate -	-	-	+	+	-	+	+	-	-	-

(+ = positive, - = negative, ± = marginally positive reaction, I = No growth)

Table 3.8.
Antibiotic sensitivity of CLB isolates.

Isolate ID N°	1	2	3	4	5	6	7	8	9	10
Sensitivity to ; -										
Oxytetracycline	+++	+++	+++	+++	+++	+++	‡	+++	+++	+++
Oxolinic acid	++	+++	+++	+++	+++	+++	‡	+++	+++	++
Nitrofurantoin	-	+++	+++	+++	+++	+++	‡	+++	+++	-
Furazolidone	+	+++	+++	+++	+++	+++	‡	+++	+++	±
Ampicillin	-	+++	+++	+++	+++	+++	‡	+++	+++	++
Erythromycin	+++	+++	+++	+++	+++	+++	‡	+++	+++	++
Sulphafurazole	+	-	-	-	+	-	‡	+++	-	‡
Sulphamethazole & trimethoprim	-	+	-	-	++	-	‡	+++	-	‡
Trimethoprim	-	-	-	-	-	-	‡	-	-	‡
Chloramphenicol	+++	++	-	+++	+++	+++	‡	+++	+	‡
Sulphamethoxazole	-	-	-	-	+	-	‡	+++	-	‡
Cephaloridine	-	-	-	++	+	+	‡	+++	-	‡

Isolate ID N°	11	12	13	14	15	16	17	18	19	20
Sensitivity to ; -										
Oxytetracycline	++	+++	+++	++	+++	++	++	++	++	+++
Oxolinic acid	++	+++	++	+	+++	++	++	++	+	+++
Nitrofurantoin	++	++	‡	-	+++	-	-	++	-	+++
Furazolidone	+++	+++	-	±	+++	±	±	+++	±	+++
Ampicillin	+++	++	-	-	+++	-	-	++	+	+++
Erythromycin	+++	+++	+++	++	+++	++	+++	+++	++	+++
Sulphafurazole	+	+	-	-	-	-	-	-	-	-
Sulphamethazole & trimethoprim	+	+	-	-	-	-	-	-	-	-
Trimethoprim	-	-	-	-	-	-	-	-	-	-
Chloramphenicol	+++	+++	+++	+++	+++	++	++	+++	+	+++
Sulphamethoxazole	+	+	-	-	-	-	-	-	-	-
Cephaloridine	++	++	-	-	++	-	-	+++	-	++

Zones of inhibition :
+++ =) 25mm
++ =21 - 25mm
+ =16 - 20mm
± =11 - 15mm
- =< 11mm
‡ =No growth

1.5.1.

A trial was set up to compare the results obtained from six different methods of sampling the dorsal fin (represented graphically in Figure 3.1., reproduced below). The results are detailed in Table 3.9. and Appendix III.

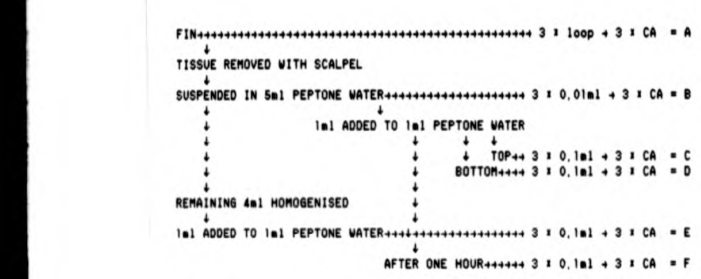


Figure 3.1. Diagrammatic representation of techniques used in 1.5.1.

The mean of the % coefficients of variation were calculated and the 95% confidence limits of that mean, these are presented in Table 3.9.

Table 3.9.

Mean (% CV $\bar{x}$), standard deviation n-1 (% CV s) and 95% confidence interval $\bar{x} \pm (t \times SE)$, 4 d.f., P = 0.05, t = 2.776,(95% CI) for the % coefficient of variation from Appendix III.

sample	% CV $\bar{x}$	% CV s	95% CI
A	52.6	20.9	25.9
B	73.1	46.5	57.7
C	40.4	30.6	38.0
D	49.8	41.1	51.0
E	30.6	33.0	41.0
F	73.9	49.8	61.8

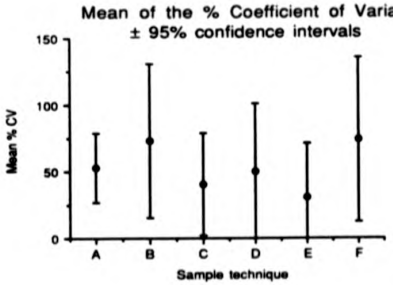


Figure 3.8. Mean of the % coefficient of variation from Table 3.9. ± 95% confidence limits.

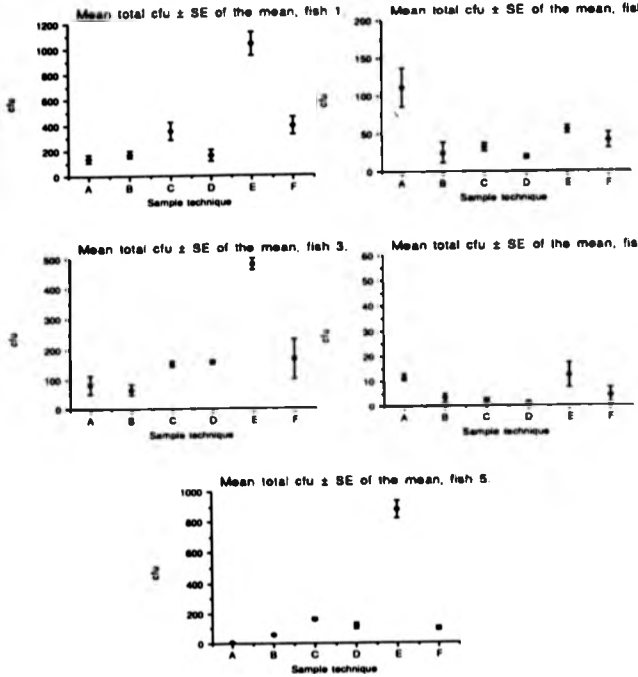


Figure 3.9. The means and standard errors of the mean total bacterial cfu for the five fish (1-5) by the six sampling techniques (A-F).

In Figure 3.9. the means and standard errors of the mean total bacterial counts are represented graphically. There would appear to be significant differences between some of the sampling methods. In order to investigate this further, a Wilcoxon's test for matched pairs was performed on these results. B, C, D, and F were not significantly different, however there was a highly significant difference (T = 1 ; P < 0.002) between the median of E and the others..

1.5.2. The bacteria from naturally occurring dorsal fin rot were monitored over a period of approximately five months. The number of CLB cfu, total cfu, water temperature and fin condition are recorded in Tables 3.10 and appendix III (Tables III.3. and III.4.) and displayed graphically in Figures 3.10., 3.11., 3.12. and 3.13.

The % fin remaining was converted to the mean fin loss in order to carry out a statistical analysis.

0%-10%	=	0.95
10%-30%	=	0.8
30%-60%	=	0.55
60%-90%	=	0.25
90%-100%	=	0.05

Spearmann's rank correlation for the data in Appendix III revealed only one correlation of low significance (0) (Table 3.10.).

Table 3.10.
The Spearmann's rank correlation for the data from Appendix III.

	r_s Fin remaining	r_s Temp'
Mean CLB	-0.089	+0.090
Mean Total	+0.113	-0.138
% CLB	-0.200	+0.3238
Temp'	+0.239	

n = 35
p = 0.05; r_s = 0.335 p = 0.1; r_s = 0.283

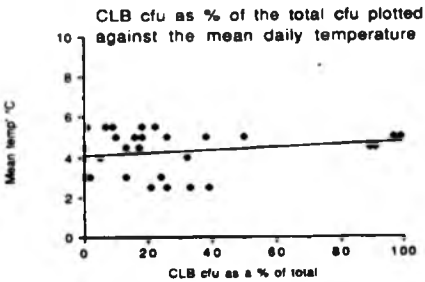


Figure 3.10. CLB cfu as a percentage of the total cfu plotted against the mean daily temperature. Spearman rank correlation coefficient, significant at P = 0.1. The line represents the the best fit regression by the least squares method.

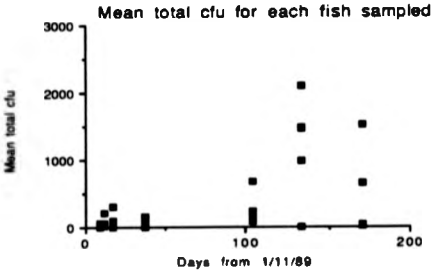


Figure 3.11. The mean total cfu, for each fish sampled.

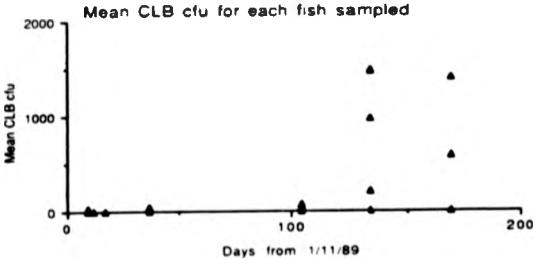


Figure 3.12. The mean CLB cfu, for each fish sampled.

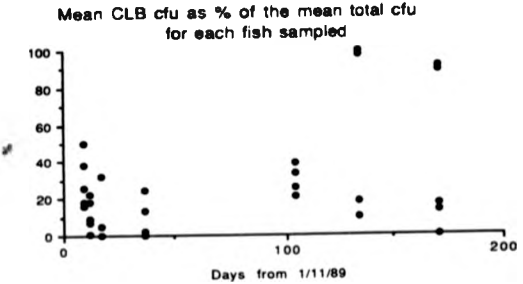


Figure 3.13. The mean CLB cfu as a % of the total cfu, for each fish sampled.

2. EXPERIMENTS TO INVESTIGATE THE BACTERIA ASSOCIATED WITH THE DORSAL FIN FOLLOWING CONTROLLED DAMAGE

2.1. The plate dilution method estimated that there were 2.04×10^7 cfu/ml in the broth culture. 500ml of this culture were added to the 35l aquarium, therefore the fish were exposed to 2.91×10^6 cfu/ml. The average daily temperature for the duration of the experiment was 13°C.

Gross and histological examination of the fins revealed that epithelial integrity was mostly re-established within two days after the excoriation, but the fins did not appear grossly normal until fourteen days after excoriation. There was no detectable difference between the fins of the fish challenged with CLB and those in the control tank *i.e.* :

DAY 0 : (Day of excoriation) Gross - The fins had irregular rough gaps between the rays, with tags of loose tissue. Some of the rays were exposed.

Histo' - There was extensive epithelial damage with complete absence of epithelium over the majority of the sections.

DAY 2 : Gross - There were still exposed fin rays with gaps in the tissue between them, however the damaged tissue now appeared more smooth and rounded than rough.

Histo' - The epithelium was only absent from a few areas, but appeared thin and attenuated in may areas.

DAY 4 : Gross - There was no evidence of exposed fin rays and all

the tissue now appeared smooth and rounded.

Histo' - The epithelium and other fin tissues were apparently normal.

DAY 6 : Gross - The fins were similar to DAY 4 however there was some thickening at the edges of the tissue defects.

Histo' - As DAY 4.

DAY 8 : The tissue defects were smaller and there was less thickening of the surrounding tissue, but some of the fin rays were slightly distorted.

Histo' - One of the fish from the challenged tank had an area of fin with no epithelium, this may have been a processing artifact.

DAY 10 & 12 : Gross - No significant changes from DAY 8 were detected.

Histo' - No abnormalities were detected on histological examination of any of the fins after DAY 8.

DAY 14 : Gross - All the fins appeared normal at this stage.

The mean CLB cfu, non-CLB cfu and the total cfu are presented graphically in Figure 3.14.

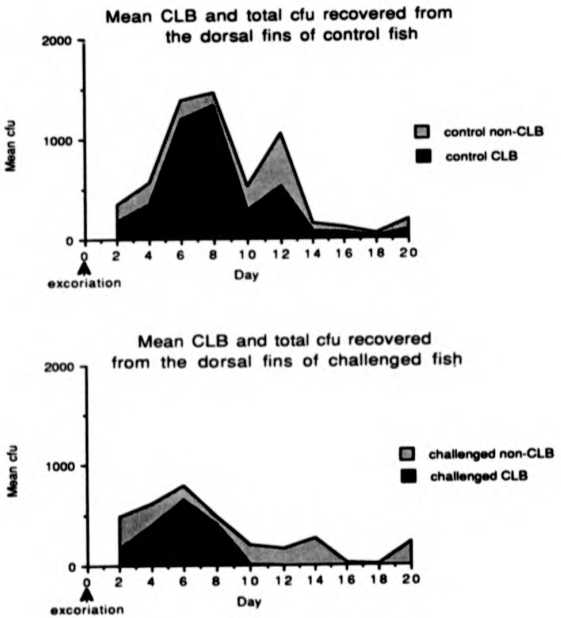


Figure 3.14. The mean CLB, total and non-CLB cfu recovered from the dorsal fins of the control and challenged fish.

2.2. Gross examination of the fins revealed that the erosion of the excoriated fins was most noticeable by the third day after excoriation. There was some thickening of the fin tissue noted from day 5 which became more obvious on day 7 and subsequently subsided. By day 11 the only detectable damage was limited to minor lesions on one fish. On the control fish the only damage noted was some mild erosion affecting two fish on day 7, otherwise no abnormalities were detected on the fins of the control fish.

The mean CLB cfu and the non-CLB cfu recovered during experiment 2.2. are displayed graphically in Figures 3.15. 3.16. and 3.17.

One excoriated fish on day 11 produced a mean of 23 CLB cfu and 121.33 non-CLB cfu. This was a higher number of both types of colony than recovered from any other fish on day 7, 9, or 11.

The temperature during the experiment ranged from 6-10.5°C, mean 8.46°C.

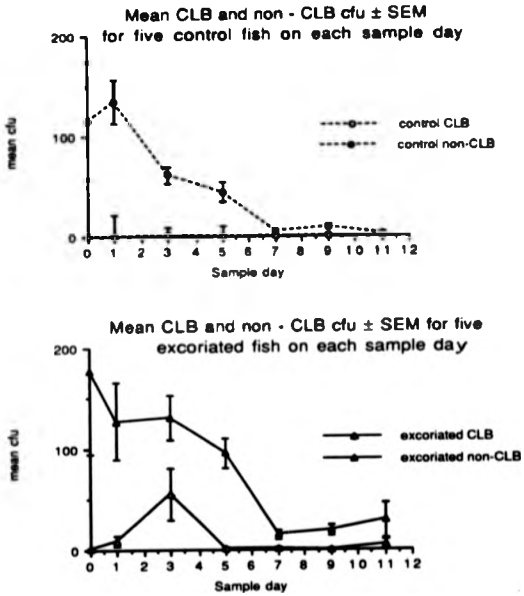


Figure 3.15. The mean ± SEM CLB cfu and non-CLB cfu, from the five control and five excoriated fish sampled on each occasion (2.2.). The first sample (DAY 0) was taken immediately prior to excoriation.

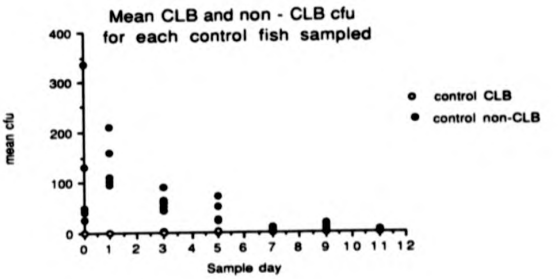


Figure 3.16. The mean CLB cfu and non-CLB cfu for each control fish sampled.

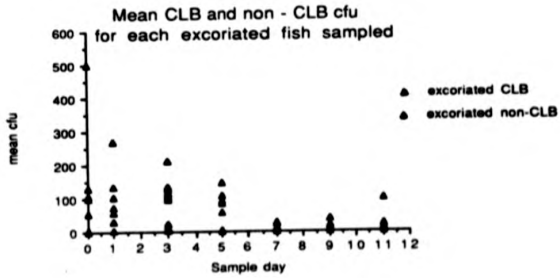


Figure 3.17. The mean CLB and non-CLB cfu for each excoriated fish sampled.

It would appear that the non-CLB cfu of the excoriated fins was different from the controls prior to excoriation and apart from the first day after excoriation remained higher until the end of the experiment.

The CLB cfu for the control fish showed little variation. There was an apparent rise in the CLB cfu on the excoriated fins peaking at day 3. Both the control and excoriated total - CLB cfu were higher for the first five days of the experiment and thereafter were lower.

2.3. Both the gross abnormalities and the bacterial populations produced a less obvious pattern in this experiment compared to 2.2. There were three identifiable forms of damage. Erosion was typified by generalised roughening and loss of peripheral tissue. Thickening was obvious but confined to the damaged areas. The third form of damage was splitting, this was the separation or splitting of the tissue between the fin rays not necessarily associated with obvious tissue loss. This was similar to the changes described as some erosion and ray splitting in chapter I. The gross pathology in the control fins was confined to the intermittent and irregular observation of mild erosion from the second day after excoriation until the end of the experiment. In the excoriated fish, erosion appeared to be at its most severe around 5 to 6 days after excoriation and some thickening was apparent on day 3 and 4. Splitting was first observed in 3 fish on the day 7 and was seen in all the fish sampled on days 8 and 9. There was no obvious

correlation between the bacteriological results and the observed gross pathology for individual fish.

The mean CLB cfu and the non-CLB cfu recovered during experiment 2.3. are displayed graphically in Figures 3.18. 3.19. and 3.23.

The temperature during the experiment ranged from 4-6°C, mean 5.4.°C.

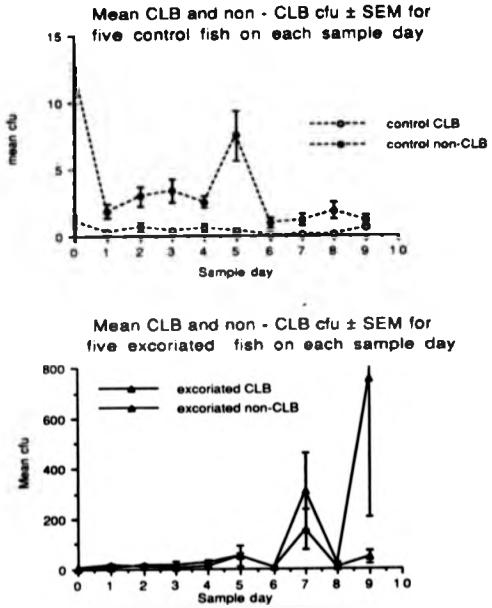


Figure 3.18. The mean ± SEM CLB cfu and non-CLB cfu, from the five control and five excoriated fish sampled on each occasion (2.3.). The first sample (DAY 0) was taken immediately prior to excoriation.

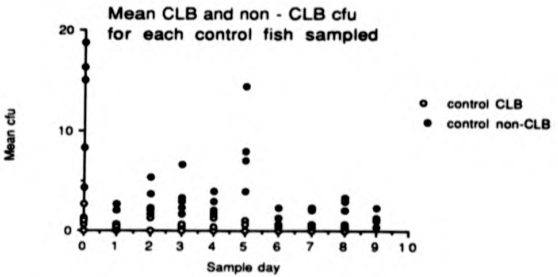


Figure 3.19. The mean CLB cfu and non-CLB cfu for each control fish sampled.

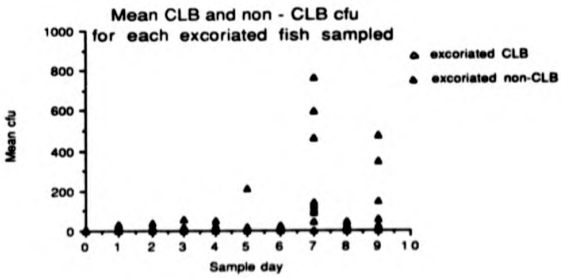


Figure 3.20. The mean CLB and non-CLB cfu for each excoriated fish sampled.

III
(Results)

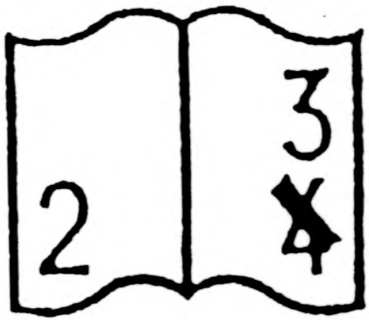
In this experiment the control fish appeared to have more non-CLB cfu on their fins before excoriation, though thereafter the excoriated fish had more until the end of the experiment. On days 5, 7 and 9 there was a large variation between the numbers of cfu recovered from individual excoriated fish.

3. SUSCEPTIBILITY OF *Salmo salar* L. PARR WITH DORSAL FIN ROT TO AN *Aeromonas salmonicida* BATH CHALLENGE

3.1. The results from this experiment are detailed in Appendix III. The majority of the fish with undamaged fin suffered mild peripheral erosion during the experiment but were still easily differentiated from the fish suffering from severe dorsal fin rot.

The data from challenges which produced sufficient specific cumulative mortalities were analysed by the Wilcoxon's test for matched pairs. However two challenges (*i.e.* 4.7×10^{3}, bin 5 and 4.7×10^{4}, bin 2) only produced 8 pairs of results and were therefore analysed with the Mann - Whitney U test. No significant differences were detected. The specific cumulative mortalities are presented graphically in Figures 3.21., 3.22. and 3.26.

PAGINATION ERROR



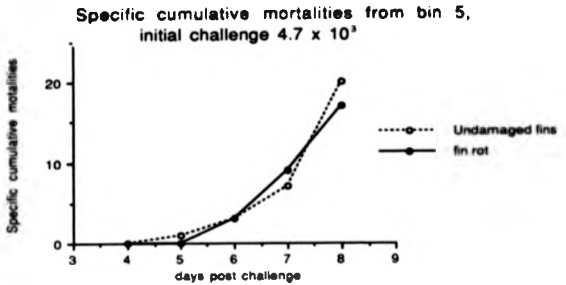


Figure 3.21. Specific cumulative mortalities from bin 5, initial challenge dose 4.7×10^3. The days refer to time from the initial challenge.

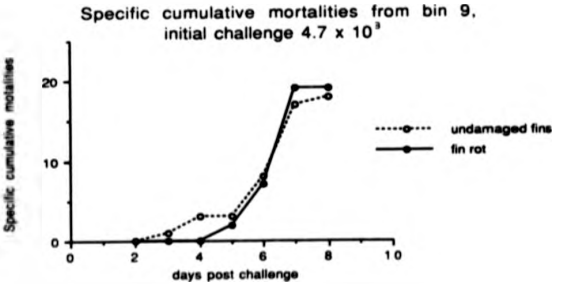


Figure 3.22. Specific cumulative mortalities from bin 9, initial challenge dose 4.7×10^3. The days refer to timefrom the initial challenge.

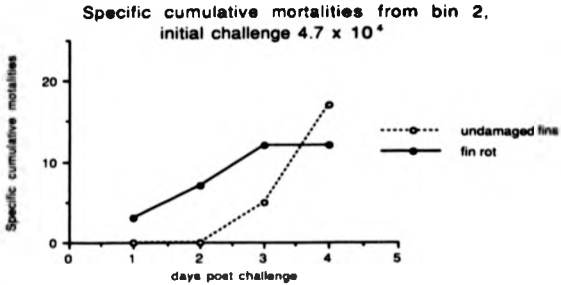


Figure 3.23. Specific cumulative mortalities from bin 2, initial challenge dose 4.7×10^4. The days refer to time from the initial challenge.

3.2. The results are detailed in Appendix III. The specific cumulative mortalities were analysed by the Wilcoxon's test for matched pairs. In the first replicate (bin 1) there was no significant difference. However in bin 2, there was a difference of low significance between the means of the two data sets ($P < 0.1$), the fish with undamaged fins suffering a higher specific cumulative mortality. In bin 3 the fish with dorsal fin rot suffered a significantly higher specific cumulative mortality ($P < 0.002$).

The results are displayed graphically in Figures 3.24., 3.25. and 3.26.

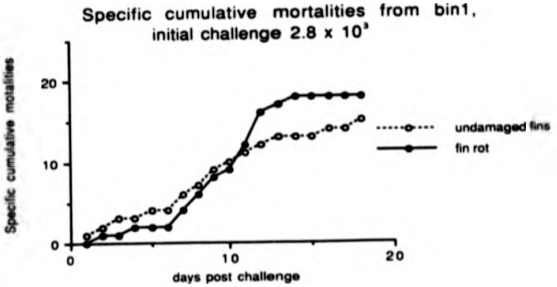


Figure 3.24. Specific cumulative mortalities from bin 1, initial challenge dose 2.8 x 10^3. The days refer to time from the initial challenge.

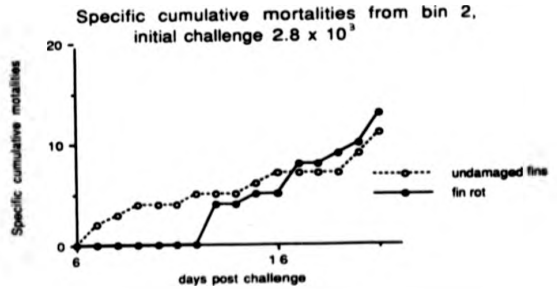


Figure 3.25. Specific cumulative mortalities from bin 2, initial challenge dose 2.8 x 10^3. The days refer to time from the initial challenge.

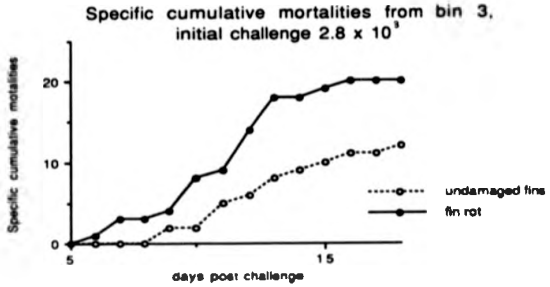


Figure 3.26. Specific cumulative mortalities from bin 3, initial challenge dose 2.8 × 10^3. The days refer to time from the initial challenge.

CHAPTER III

DISCUSSION

1. BACTERIA ASSOCIATED WITH NATURALLY OCCURRING DORSAL FIN ROT IN *Salmo salar* L. PARR

At the start of this study bacteriology was considered to be one of the more important areas of the investigation, since the literature repeatedly made reference to the presence of bacteria in association with fin rot (Introduction). The objective in this area was to examine the organisms present on dorsal fins suffering from fin rot (1.2.). It was important to obtain information on as wide a range of organisms as possible. Hence the selection of three media and two incubation temperatures. Tryptone Soya Agar (TSA) is a widely used media for the isolation of fresh water fish pathogens (Frericha, 1984). Cytophaga Agar (CA) (Anacker and Ordal, 1959) is suitable for the culture of CLB and other organisms requiring dilute media. River water Glucose Yeast (RGY) has been found suitable for the isolation of aquatic fungi (Alderman, 1982). The two temperatures (22°C and 10°C) were selected to for the growth of organisms suited to a range of temperatures. 10°C was selected with regard to the potential importance of psychrophilic organisms, in view of both the low temperature on the farms (0°C to approximately 18°C) and the similarity between *F.psychrophilus* infections and dorsal fin rot (Introduction). The results from the three media were pooled in order

to provide a qualitative indication of the bacterial populations present.

Numerous other cultural options (*e.g.* anaerobic culture) were excluded at this stage for practical reasons. However their inclusion at a later stage, if appropriate, was not ruled out.

It was obviously important to sample not only diseased but also healthy fins in order to differentiate between normal commensals and organisms specifically associated with diseased fins. The resultant bacterial isolates had to be analysed within a finite time or they became overgrown or inactive. Therefore the breadth of the study was limited by practical constraints. Within these constraints it was considered necessary to sample more than one healthy and damaged fish, where possible, thus providing an indication of the bacteria associated with the population rather than individual fish. The technique selected analysed two pools of fin tissue, one from two undamaged fish and one from two with dorsal fin rot.

The ROY failed to produce consistent fungal growth from the samples. This is consistent with the opinion of Bullock and Conroy (1971) that fungi may be secondary invaders of fin rot lesions but do not play a primarily pathogenic role (Chapter IV).

There were very few bacteria isolated from the undamaged fins. Those that were isolated followed no detectable pattern and were probably a

reflection of the bacterial microflora of the hosts environment (Snow and Beard, 1939; Horsley, 1973; Amend, 1983).

The predominant bacterial type cultured from the diseased fins were CLB, in every case. These were more numerous on the CA (Table 3.4.) plates and at lower temperatures. The predominance of CLB reflects the published opinion of many authors (Introduction, Table 3.1.).

A phenotypic examination of the CLB isolated was conducted to determine their relationship to recognised organisms or the presence of any other pattern. Since the bacteria were isolated over a three month period and it was not possible to analyse them immediately, they were freeze dried for storage (1.3.). Reichenbach (1989a) reviewed the storage of CLB and suggests several methods. Freeze drying was selected since it provided convenient long term storage and was the method used by the National Collection of Marine Bacteria (NCMB) and the American Type Culture Collection (ATCC).

The phenotypic tests (1.3.) were selected to allow comparison with studies on other CLB. They were not primarily chosen to allow classification of the organisms, since the taxonomy of this group was the subject of considerable debate during the study. The publication of Volume III of Bergey's Systematic Bacteriology in 1989 clarified this area but further work is still required.

It is necessary to consider the current taxonomic structure in order to examine the position of the CLB isolated in this study. Figure

3.27. represents the current taxonomic status of the CLB which have been associated with fish diseases. The *Cytophagales*, some *Fusobacterium* and probably both *Capnocytophaga* and *Sphingobacterium* constitute one of the ten main branches of the Eubacterial phylogenetic tree. As such they should probably be regarded as a separate phylum (Woese, Stackebrandt, Weisburg, Paster, Madigan, Fowler, Hahn, Blanz, Gupta, Nealson and Fox, 1984a; Woese, Weisburg, Paster, Tanner, Krieg, Koops, Harms and Stackebrandt, 1984b) This group of CLB are not related to *Lysobacter* or to the *Myxobacters*. Formerly the terms 'myxobacteria' or 'myxobacterial pathogens' were widely used to describe the CLB isolated from fish (Roberts 1989). M^Curdy (1974) defined the *Myxobacteriales* as containing only bacteria producing fruiting bodies and having a G+C ratio of 67-71 mol%. These organisms are predominantly if not exclusively terrestrial. Therefore the terms 'myxobacteria' or 'myxobacterial' are no longer appropriate description of bacteria associated with fish disease (Holt *et al*, In press).

The CLB are abundant and widely distributed in the natural environment. Despite their significance, their taxonomy is still largely un-resolved. Although recent molecular taxonomic studies have elucidated some aspects (Reichenbach, 1989a) a more comprehensive study is still necessary. It has been suggested that classification based on molecular taxonomy and further studies to identify distinguishing phenotypic characteristics are required. The present taxonomy is not necessarily based on the optimum or even suitable phenotypic criteria.

Current taxonomy of Cytophaga-like Bacteria

ORDER	*Cytophagales*	
FAMILY	*Cytophagaceae*	
GENUS	*Cytophaga*[*]	
SPECIES	*C. aquatilis*[*]	
	C. columnaris[*]	
	C. johnsonae[*]	
	C. marina[*]	
	C. rosea[*]	
GENUS	*Capnocytophaga*	
GENUS	*Flexibacter*[*+]	
SPECIES	*F. maritimus*[*+]	
	F. psychrophilus[*+]	
GENUS	*Microcilla* [+]	
GENUS	*Flexithrix* [+]	
GENUS	*Sporocytophaga*[*]	
GENUS	*Chitinophaga*	
FAMILY	*Flavobacteriaceae*	
GENUS	*Flavobacterium*[*]	
SPECIES	*F. balustinum*[*]	
	F. branchiophila[*]	
	F. piscicida[*]	

[+] = Uncertain affiliation
[*] = Implicated as a fish pathogen

Current taxonomy of bacteria related to CLB

FAMILY	*Bacteriodaceae*	-	Anaerobic only in the human gut
GENUS	*Saprospira*	-	Screw shaped
	Taxeobacter	-	Brick red colonies (not yet officially proposed)
	Haliscomenobacter	-	Sheathed
	PL-12fs	-	Anaerobic (not yet officially proposed)
ORDER	*Lysobacterales*	-	Colonies cream, pink or yellow brown often producing a diffusible pigment
	Beggiatoales	-	Typically multicelled filaments

Figure 3.27. Current taxonomy of Cytophaga-like Bacteria (CLB) and related bacteria (From Bergey's Systematic Bacteriology, Vol I, 1984 and Vol III, 1989)..

III
(Discussion)

The distinguishing characteristics of the taxa listed in Figure 3.27. are as follows (from Reichenbach 1989a & b) :

ORDER - *Cytophagales*

These bacteria are rods or filaments producing yellow, orange or red cell bound pigment. They are aerobic or facultative anaerobic chemo-organotrophs with a G+C ratio of 30-66mol%. Any motility is confined to gliding which only occurs under certain environmental conditions and when the bacteria are in contact with a solid surface.

The *Cytophagales* are considered to contain two closely related families *Cytophagaceae* and *Flavobacteriaceae*.

FAMILY - *Cytophagaceae*

These rods which may be long and flexible are found free living in many terrestrial and aquatic environments. Many have the ability to degrade bio-macromolecules like gelatin and starch. Their G+C ratio is mostly in the range 30-45mol%

GENUS - *Cytophaga*

Members of this heterogeneous genus have a G+C content over the full *Cytophagaceae* range of 30-45mol%. Some of them contain flexirubin pigments. These are a variety of pigments found irregularly distributed through the CLB group of organisms. They can be identified by their characteristic reversible change from yellow-orange to violet-red or purple-brown in the presence of KOH.

The ability of *Cytophaga* to degrade bio-macromolecules has been used to differentiate them from the *Flexibacter* (Leadbetter 1974a & b). Other criteria have been suggested *e.g.* Reichenbach, Behrens and Hirsch (1981) suggested a strategy based on bio-macromolecule degradation, morphology and G+C ratios. Bernardet and Grimont (1989) and Reichenbach (1989a) have suggested reverting to the earlier taxonomic tool of cellulose and agar degradation, although these phenotypic characteristics result in a largely artificial classification.

Differentiation of *Cytophaga* and *Flavobacterium* is also based on a dubious taxonomic criteria as the Cytophaga-Flexibacter complex are said to be motile by gliding and the *Flavobacterium* non-motile. Problems arise because gliding motility is influenced by many environmental parameters, including nutrient concentration and the age of the colony. Apparently reliable techniques for observing this form of motility have been reported (Lautrop, 1965; Perry, 1973; Reichenbach and Dworking 1981) but errors have still occurred (Reichenbach, 1989a).

GENUS - *Capnocytophaga*

These organisms have only been found in the oral cavity of humans.

GENUS - *Flexibacter*

This isolated genus is probably not affiliated to the *Cytophagaceae* and may justify a family of its own. Earlier studies used the shape of *Flexibacter* as a major taxonomic characteristic, but have since been found to be unreliable (Reichenbach, 1989a). Typically this genus undergoes a cyclical change in morphology during the development of

the colony. They often begin as long very agile cells eventually becoming short non-motile coccoid rods in old cultures.

They may contain flexiruben pigments and have a G+C ratio of 37-47mol%. Although cellulose and agar are not decomposed chitin and starch often are.

GENERA - *Microscilla*
Flexithrix
Sporocytophaga
Chitinophaga

Microscilla, *Flexithrix* and *Chitinophaga* are also genera of uncertain affiliation. Both *Microscilla* and *Flexithrix* are very similar to *Flexibacter* but are marine organisms requiring at least half strength sea water for growth. *Flexithrix* is distinguished by its production of sheaths. *Sporocytophaga* and *Chitinophaga* which both have a G+C ratio of 36mol%, produce microcyst resting stages. These two organisms are differentiated by *Chitinophaga*'s ability to digest chitin.

FAMILY - *Flavobacteriaceae* (Holmes, Owen and M^c^Meekin, 1984)
These aerobic rods of variable length produce yellow/orange or pale colonies. Most are free living terrestrial or aquatic bacteria which are often incapable of digesting starch, agar or other high molecular weight compounds. As already discussed they are primarily differentiated from the *Cytophagaceae* by their lack of gliding motility. Some are very similar to the *Cytophagaceae* in all respects

except motility, while others are dissimilar and are probably not part of the CLB. There is at present considerable overlap between the *Flavobacterium*, *Flexibacter* and *Microscilla*. This may have resulted from the use of gliding motility as a major taxonomic characteristic, however there is insufficient information to consider re-classification. Subsequent work may divide the bacteria along different lines, for example, fish pathogens do not appear to be closely related to saprophytic bacteria found in the same environment and flexiruben pigments are common in terrestrial bacteria but not in aquatic varieties.

The bacteria isolated in this study, if classified according to gliding motility and degradation of bio-macromolecules may be considered to fall into the groups listed in Table 3.19.

Table 3.19.
Provisional identification based on motility and bio-macromolecules degradation.

Isolate ID N°	Probable taxonomic position
1	? *Flavobacterium*
2	*Cytophaga*
3	? *Cytophaga*
4	? *Cytophaga*
5	? *Cytophaga*
6	*Cytophaga*
7	? *Flavobacterium*
8	*Flexibacter*
9	*Flexibacter*
10	*Flexibacter*
11	*Cytophaga*
12	*Flexibacter*
13	*Cytophaga*, poss' *C. johnsonae*
14	*Cytophaga*
15	? *Cytophaga*
16	? *Cytophaga*
17	*Cytophaga*
18	? *Cytophaga*
19	? *Flavobacterium*
20	? *Cytophaga*

Swarming on agar was taken as an indication of gliding motility. This is not an infallible method since a few CLB are capable of gliding but not swarming (Perry, 1973). The non-motile *Flavobacterium* are often incapable of degrading bio-macromolecules, therefore there must be some doubt about classifying isolate numbers 1, 7 and 19 as *Flavobacterium* since they were non-motile but able to degrade bio-macromolecules.

With one exception the CLB in this study do not fit the published description of any recognised or proposed species (Appendix IV). The exception is that isolate number 13 is very similar to *C.johnsonae*, an organism mentioned in association with fish disease by Christensen (1977). The other CLB from this study differ from *F.branchiophilus* and *C.columnaris* on the basis of bio-macromolecules degradation. They differ from *Flexibacter maritimus* and *Flavobacterium balustinum* on the basis of nitrate reduction and motility.

At this stage, work in other areas suggested that CLB might not play a significant role in the aetiology of the condition. Therefore the freeze dried isolates were retained but no further work to classify the isolates was undertaken.

Work carried out concurrently on the effects of damage on dorsal fins (1 & 2 - 9/89 - Chapter IV, 2.B.) indicated that CLB might not always be predominant in dorsal fin rot. To study this area further a technique was required which would result in a reproducible indication of the number and proportion of bacteria present on the fins (1.B.1.).

[1]
(Discussion)

In addition to examining the suitability of the techniques, this experiment also confirmed that CLB were not invariably associated with dorsal fin rot in large numbers. In fact insufficient CLB were recovered for statistical analysis.

Technique A (direct loop sample), subjectively, appeared to produce a variable result compared to the other five, sometimes higher, sometimes lower (Table 3.12.). Technique E involved homogenisation of the tissue and predictably produced a significantly higher number of cfu than the other techniques. This technique was, however, not practical for multiple samples under field conditions. The number of cfu recovered was not significantly different for B, C, D or F. In addition, analysis of the % coefficient of variation and 95% confidence intervals suggested that there was no justification for selecting any of the techniques on these grounds.

Technique B was not selected since it was difficult to spread 0.01ml of inoculum evenly over a plate. Technique C was selected as the practical method of choice. The lack of significant difference between C, B, D and F, suggest that the subsequent results obtained should not have been significantly altered by the portion of the sample plated out or delays of up to an hour prior to plating.

Experiment 1.5.2. was conducted to further investigate the differing numbers of CLB recovered in other parts of the study (1.5.1. and Chapter IV). The initial samples, in November 1989, produced low numbers and proportions of CLB. Since this conflicted with the

evidence from 1.2., several samples were taken over a relatively short period of time, to obtain further information. The initial four samples all had low numbers and proportions of CLB. It was only in samples 6 and 7 that CLB were detected in large numbers representing the majority of the bacteria present. Even at this stage this was only evident in some of the fish. The possible reasons for the discrepancies between 1.2. and 1.5.1., 1.5.2. and Chapter IV, 2.5. could be summarised as follows:

A. The discrepancy may have resulted from sampling error.

B. The bacteria recovered could have been related to the time of year.

C. The bacteria recovered could have been related to the degree of fin damage or some other aspect of the condition.

D. The bacteria recovered could have been related to temperature.

A. The results from 1.5.1. would tend to suggest that significant sampling error is unlikely. However the samples from 1.2. were pooled, the results are therefore not representative of individual fish. The pooled results may have given the impression that CLB were present in high numbers and proportion uniformly through the population, when this was not the case.

B. Both experiments produced high numbers of CLB at the same time of the year. It is possible the counts obtained may reflect the bacteria in the environment at that particular time.

C. Analysis of the results indicated that there was no obvious correlation between the bacterial counts and the degree of gross fin damage in individual fish. At this stage of the study the working hypothesis was that dorsal fin rot was associated with biting by other fish. If this were the case, the number and proportion of bacteria on the fin may have been related to the time since the last active damage to the fin. This would assume that the external appearance of the fins was not closely related to the proximity to the last injury. Also that the bacterial populations increased following injury but dropped before the gross appearance of the fin returned to normal.

D. There is the further possibility that bacterial colonisation and pathological changes were related to temperature. The evidence obtained to determine the effect of temperature on bacterial colonisation of the fins was limited (chapter III, 2.2. and 2.3.), however the correlation between % CLB cfu and temperature was not highly significant in this experiment ($P = 0.1$). Other areas of the study (chapter III, 2.2., 2.3. and chapter, IV) suggested that healing was strongly related to temperature. In lower temperatures the lesions heal slowly so they are apparent for longer after the damage. In higher temperatures they heal more quickly and therefore any visible lesions are likely to be more recent. If the bacteria decline rapidly after injury, regardless of temperature, there is a greater possibility of obtaining bacteria from lesions at higher temperatures since they will be more recent and therefore have more bacteria associated with them. More information was required to further investigate this hypothesis. Experiments 2.1., 2.2. and 2.3. were

conducted in an attempted to provide more information regarding the changing bacterial populations on fins following damage.

2. EXPERIMENTS TO INVESTIGATE THE BACTERIA ASSOCIATED WITH THE DORSAL FIN FOLLOWING CONTROLLED DAMAGE.

The first experiment to investigate the bacterial populations on the dorsal fins following excoriation (2.1.) was a small pilot study. It was conducted as a challenge to obtain some information regarding the pathogenic effects of CLB combined with damage. There had been no particular pattern to the isolates obtained from natural outbreaks therefore there was no reason to select any one isolate as the challenge strain (1.4.). It is preferable to use a recently isolated strain, since in common with other bacteria (Adams, Leschen, Wilson and Horn, 1987), *in vitro* storage can affect the pathogenicity of CLB (Ferguson et al, 1991). The fish were prophylactically treated with oxytetracycline in the hope that this would prevent infection in the control fish, thus allowing a comparison between 'sterile' and challenged damage. A period of three days was allowed for the antibiotic to be excreted from the fish, this time being selected empirically since there was very little information on which to base a decision. The non-CLB cfu fluctuated with no particular pattern during the experiment. The CLB however increased rapidly in both populations following excoriation. The fish exposed to the bath challenge started with a higher number of CLB cfu but the number on the controls exceeded the challenged fish by the sixth day after excoriation. In both populations the number of CLB cfu subsequently

dropped to a background level observed in undamaged fins (1.2.). Histology revealed that the epithelial continuity on the fin was re-established soon after day 2 in both populations; around the same time that the CLB cfu started to decrease. There was some very slight gross thickening of the fins in both populations but this disappeared before the end of the experiment. None of the fish developed anything resembling natural dorsal fin rot.

As already stated this was a small pilot study of limited significance. Therefore the results were considered as an indication, rather than proof of trends.

Under these circumstances the combination of a single injury and the presence of CLB were not able to induce fin rot and therefore the CLB used was unable to cause fin rot. Despite the prophylactic antibiotic treatment the control fish developed a larger population of CLB than the challenged fish. Contamination was predictable due to the ubiquitous presence of CLB in the environment (Reichenbach, 1989a). This might also imply that residual antibiotic did not prevent the establishment of bacterial populations.

The principle findings from the following experiment (2.2.) were that the CLB increased for a short time after excoriation and the non-CLB were higher in the first five days in both control and excoriated fish.

The number of CLB was highest at the same time as the erosion of the fin was most noticeable in the population with no correlation between

bacterial numbers and the gross pathology in individual fish. There was also some minor thickening of the fins again not significantly related to the bacterial numbers. The increased severity of the gross erosion for 3 days after excoriation was probably due to the necrosis and sloughing of tissue damaged, but not removed, during the excoriation. The relationship between CLB and the observed damage agrees with the findings from 2.1. *i.e.* the bacterial numbers appeared to decrease once epithelial continuity was re-established. It would appear that, under these conditions, the act of excoriation only briefly increased the number of CLB on the fins.

Although the excoriated fins had a higher number of bacteria on them from day 3 onwards they also had a higher number of bacteria prior to excoriation. Therefore it is not possible to state, with any confidence, that the higher bacterial numbers were due to excoriation. In addition, since the numbers of non-CLB were higher in both controls and excoriated fish from before the time of excoriation until five days after, it is possible that the pattern observed was due to some other factor and not the excoriation. For example the stress of handling may have been responsible for the initial higher bacterial numbers or some aspects of the tanks environment.

One final observation was that one excoriated fish on day 11 produced more CLB and non-CLB cfu colonies than any other fish sampled on days 7, 9 and 11. It was thought possible that this fish either represented a persistent infection or subsequent damage to the fin.

It was considered appropriate to conduct a further similar experiment. The objectives were to obtain more information about the rapid peak of CLB, the inconsistent result on day 11 and the relationship between excoriation and non-CLB.

Experiment 2.3. repeated 2.2. with more frequent sampling. In this case the non-CLB were higher in the control fish prior to excoriation but were lower for the rest of the experiment. It is therefore probable that the higher numbers of non-CLB were associated with the excoriation.

In this experiment the excoriated fish had three peaks of bacterial colonisation with increases in CLB and non-CLB. However there were large differences between the mean results from individual fish. This may be a further example of the phenomenon observed on day 11 of 2.2., resulting from a more severe and persistent infection, subsequent damage or other unidentified factors. The excoriation in 2.3. may have differed in some way between fish, resulting in a different pattern of infection *i.e.* there may have been a threshold of damage necessary for the establishment of severe persistent infections.

If it were due to subsequent damage then there must have been some cumulative effect, associated with repeated damage, resulting in increasing bacterial numbers. The third possibility is that there was some additional factor affecting the fish. Such a factor would have to affect only a proportion of the excoriated fish to produce the

observed results. Perhaps there was some difference in the resistance of individual fish to infection.

The most likely explanation at the time was thought to be that the fish from which high numbers of bacteria were recovered had been subjected to repeated injury, from other fish. The subsequent SEM study (chapter V) suggested that the variation in bacterial counts may have resulted from the extent to which the fin rays were exposed by the injury.

In this experiment the wounds were not completely healed by day 9 and severe erosion persisted until day 6 and 7 in two fish. In addition splitting of the rays became obvious by day 7. Again there was no significant correlation between the bacterial counts and the gross appearance of the fins, in individual fish.

Despite similar initial damage, the delay between excoriation and maximum gross erosion varied between 2.2. and 2.3. (Table 3.20.).

Table 3.20.
Mean temperature and time until maximum erosion in experiments 2.2. and 2.3.

Experiment	Mean temperature (°C)	Max' gross erosion (Days post excoriation)
2.2.	8.46	3
2.3.	5.4.	5-6

The association between pathology and temperature is discussed further in chapter IV.

III
(Discussion)

Experiments 2.2. and 2.3. suggested that bacterial populations (CLB and non-CLB) increased following damage to the dorsal fin. The results of 2.3. were complicated possibly by variation between the individual injuries. An investigation into the relationship between exposure of the fin rays, bacterial colonisation and persistance might provide additional useful information.

3. SUSCEPTIBILITY OF *Salmo salar* L. PARR WITH DORSAL FIN ROT TO AN *Aeromonas salmonicida* BATH CHALLENGE.

The bath challenge experiments were conducted to investigate the unpublished opinion that fish suffering from dorsal fin rot are more susceptible to *A.salmonicida* infection. The bath challenge was developed from the technique published by Adams *et al* (1987). A bath challenge cannot hope to reproduce all the complex interactions of natural infection, it is however an acceptable model for natural infection. There were many aspects of this experiment that could not be adequately controlled. The fish with dorsal fin rot were selected on gross appearance, as demonstrated by 2.1., 2.2. and 2.3. the gross appearance is not a reliable indication of the time since the last episode of damage. In addition the dynamic bacterial populations vary between tanks (Hossain and Turnbull, 1990). For these reasons a number of replicates were performed. The high number of non-specific mortalities in the first nine replicates probably indicated an unacceptable deterioration in the water quality. These experiments provided no evidence for significant difference between the fish with damaged and undamaged fins. The most protracted and numerous

specific mortalities were obtained from the initial challenge of 4.7 * 10^5cfu/ml. The second set of replicates was conducted with a similar initial challenge. The frequency of water changes was also increased in an attempt to maintain water quality within the systems. This second set of replicates produced one result with no significant difference between fish with damaged and undamaged fins, one which indicated higher cumulative mortalities in the fish with undamaged fins (this was not a highly significant result, $P < 0.1$); in the third replicate, the cumulative mortalities were higher in the fish with damaged fins, (this result was highly significant, $P < 0.002$). In view of the nature of these experiments, one highly significant result is important, implying that under some circumstances fish with dorsal fin rot may suffer significantly higher mortalities than fish with undamaged fins. Although it may be speculated that recent injuries to the dorsal fins provided a route of entry for the bacteria, the route of entry of the *A.salmonicida* has yet to be fully investigated (Adams *et al*, 1987; Rose, Ellis and Munro, 1989). Alternatively fin rot may be associatedwith a higher level of stress, reducing the resistance of the fish to infections (Pickering and Duston, 1983).

In six fish with dorsal fin rot, *A.salmonicida* was recovered from the fin but not the kidney. A similar pattern of bacterial recovery has been found in samples for routine bacteriology (February 1988, Diagnostic Unit, Institute of Aquaculture, University of Stirling, pers' comm'). *A.salmonicida* is a systemic infection which spreads via the circulation, it is therefore probable that the bacteria were present in both organs but only recovered from the fin. There is however the

possibility that there was a higher concentration of bacteria in the fins, due to some effect of the fin rot. For example, either increased blood supply associated with the inflammatory response and/or sequestration of bacteria within the lesion. Alternatively it may be related to the route of entry of the bacteria, implying that the bacteria entered in the region of the fin and remained there in larger numbers. The failure to detect *A.salmonicida* from the kidney emphasises the need for multiple samples to maximise recovery of the organisms from clinical cases.

In view of the economic significance of *A.salmonicida* for the European salmon industry, the pathogenesis of this condition is extremely important and would justify further investigation.

Finally some of the fish with fin rot were observed to have haemorrhagic lesions around the area of the dorsal fin (chapter I). Although these were often associated with the recovery of *A.salmonicida* from the fins, this was not invariably the case and there was insufficient data to demonstrate any significant relationship.

CHAPTER IV

Experiments to study the effects of damage to the dorsal fin and healing from dorsal fin rot in farmed Atlantic salmon (*Salmo salar* L.) parr.

CHAPTER IV

INTRODUCTION

At the time the experiments described in this chapter were started dorsal fin rot was thought to be associated with some form of physical damage. This conclusion was reached following examination of the pathological lesions and investigations into other possible causes.

The pathology described in chapter I suggested that fins affected with fin rot may have been subjected to some form of excoriation, whilst observations reported in chapter II implied that dorsal fin rot was not directly related to water quality or stock origin. The bacteriological studies conducted up to this stage although not ruling out a secondary role, demonstrated that bacteria were not the primary cause of the condition. Consideration of these findings and published evidence suggested that physical damage to the fins was the most likely cause of dorsal fin rot. Several possible types of damage had been proposed in the literature including handling (Snieszko, 1958), rough tank surfaces (Bullock and Conroy, 1971) and biting or nipping by other fish (Abbott and Dill, 1985). There is very little evidence to support these proposed sources of damage although several authors have expressed support for the opinions of Abbott and Dill (Holm and Møller, 1988; Holm, 1989; Kindschi, Shaw and Bruhn, 1991).

Although there is relatively little published information regarding the role of damage in dorsal fin rot there is a considerable body of work

dealing with injuries to and repair of the fins of teleosts. This literature falls into two main areas *ie* studies at a cellular level of the healing process and reports of fin damage used as a means of identifying fish.

The cellular response to partial or complete amputation of the fins has been studied in considerable depth (Nabrit, 1929; Goss and Stagg, 1957; Geraudie and Singer, 1985; Fukaya, Fugii and Inaba, 1986; Marí-Beffa, Carmona and Becerra, 1989; Santamaría and Becerra, 1991). Most detailed reports describe the complete regeneration and restoration of the fin or epimorphosis. This is the process by which lower vertebrates can regenerate limbs. Epimorphosis involves initial healing of the wound, formation of a blastema and differentiation to produce the cells that regenerate the different tissue elements.

Although complete regeneration can occur following damage to the fins this is not invariably the result. Damage can result in abnormal regeneration of the fin (Nabrit, 1929; Geraudie and Singer, 1985). Lindeejöö and Thulin (1990) claimed to be able to to detect abnormally regenerated fins after several years after the injury. Removal of fins or 'fin clipping' is a well established method of identifying fish following release. Most studies in this area have not concentrated on the regeneration of the fins but Nicola and Cordone (1975) carried out a limited investigation and recommended that the fins should be removed at the base to avoid regeneration. Slater (1947) produced evidence that complete regeneration following total amputation rarely if ever occurred. Absence of or abnormal fins have also been used to

differentiate wild and farmed stocks of fish (Craik *et al*, 1987). However, fin condition alone is a not particularly reliable method of differentiation since not all farmed fish have damaged fins.

It is probable that many factors can affect the regeneration of damaged fins though the published information is limited to descriptions of inadequate innervation (Geraudie and Singer, 1985) and the effects of toxic chemicals or pollutants (Weis and Weis, 1976; Fujii and Inaba, 1985).

The healing process can also be effected by temperature (Roberts, 1975) and stress (Roubal and Bullock,1983), although not all aspects of healing are affected equally. In addition, epithelial hyperplasia is more common at lower temperatures (Roberts, 1975). However, there is insufficient information to determine the exact effects of temperature and stress on fin regeneration.

In view of the lack of relevant information, the initial objectives of this part of the study were to examine the response to injury and the healing processes of the dorsal fin. It was hoped that the information obtained would clarify the role of damage in the aetiology of dorsal fin rot. As the work progressed three lines of investigation developed, these were :

- Response to a single surgical injury,
- response to repeated excoriation and
- healing from natural fin rot at different temperatures.

(Introduction)

These experiments are grouped in these three categories, though not necessarily in chronological order.

CHAPTER IV

MATERIALS AND METHODS

1. HEALING FROM SURGICAL DAMAGE TO THE DORSAL FIN

Three experiments were conducted to investigate the healing response of the dorsal fin following surgical incisions. All the fish were obtained from farm 1 and initially had undamaged dorsal fins. In all three experiments 301 aquaria were used with power sponge filters and aeration, water quality being maintained by twice weekly 50% water changes. After two days acclimatisation, the fish were individually removed, anaesthetised with 2-phenoxyethanol and the fins incised with a scalpel. The fish were offered food to appetite twice a day from 48 hours after recovery from the anaesthetic. The maximum and minimum temperatures and the condition of the dorsal fins were recorded daily.

1.1 Healing following excision of a portion of the dorsal fin.

A single fish was obtained, anaesthetised and a section was removed from the distal dorsal fin (Figure 4.1). The fish was retained in the system for 60 days. At the end of this period it was removed, killed and the dorsal fin fixed in 10% neutral buffered formalin.

Figure 4.1. A diagrammatic representation of the portion of the dorsal fin removed in 1.1.

1.2. Healing following vertical incision in the dorsal fin.

Twenty parr with undamaged dorsal fins were obtained. After acclimatisation the fish were individually removed, anaesthetised and a vertical incision made in the dorsal fin (Figure 4.2). Two fish were killed immediately after incision and their dorsal fins removed for histological processing. A further two fish were sampled every 24 hours. Both the gross and histological appearance of the fins were recorded.

Figure 4.2. Diagrammatic representation of the vertical incision made in dorsal fins of the fish in 1.2.

1.3. Healing following horizontal cut across the dorsal fin.

A further ten parr were obtained. After acclimatisation the fish were removed, anaesthatised and the distal dorsal fin removed parallel to the back of the fish (Figure 4.3.). In this experiment the fish were retained in the tank for the duration of the experiment and the gross appearance of the fins was recorded daily. Eight of the fish were killed and sampled for histology after 14 days and the other two were retained in isolation, by dividing the tank, for a further 76 days to observe long term healing.

Figure 4.3. Diagrammatic representation of the horizontal incision made in the dorsal fin of the fish in 1.3.

2. RESPONSE OF THE DORSAL FIN TO REPEATED EXCORIATION

Five experiments were conducted to investigate the response of the dorsal fin to repeated injury. The aquaria used and details recorded were similar to those in 1.

2.1. Repeated excoriation of the dorsal fins with and without prior injection of corticosteroid.

Forty parr with undamaged dorsal fins were obtained from farm 1 and randomly divided between two aquaria. After acclimatisation the fish were individually removed form the tanks and anaesthetised. The fish from the first tank were injected intra-abdominally with 20mg/kg of prednisolone PhEur (Predsnavet, Willows Francis Veterinary). The dorsal fins of all the fish were excoriated by grasping them with a piece of folded sand paper and whilst still applying pressure, pulling the sand paper off the fin. The fish were re-anaesthetised and their fins excoriated on four occasions at seven day intervals. The experiment was concluded after 30 days.

2.2. Repeated excoriation of the dorsal fin.

A further twenty fish were kept in a similar system and excoriated as described in 2.1. In this case the fish were excoriated on four occasions at 48 hour intervals, and an attempt was made to avoid removing the accumulating tissue at each subsequent excoriation.

2.3. Repeated excoriation of the dorsal fin by simulated bites.

A total of 80 fish with undamaged dorsal fins were randomly divided between four aquaria. On four occasions at five day intervals the

fish were individually removed from the tanks and anaesthetised with benzocaine (ethyl P-aminobenzoate, Sigma Chemical Co). The fish from two of the tanks had their dorsal fins excoriated. The fish from the other two tanks were allowed to recover from the anaesthetic and then placed back in the tank without further injury.

The excoriation was carried out with a fixed parr's head. A batch of parr were killed and fixed whole in 10% neutral buffered formalin. The excoriation was conducted by removing the head from one of the fixed parr. The head was then attached to a pair of forceps by three pieces of wire allowing the mouth to be opened and closed (Figure 4.4.). This device was used to simulate a bite wound by grasping the dorsal fin between the fixed jaws and pulling the clasped jaws off the fin. The jaws were rinsed in de-chlorinated mains water after every excoriation, to remove debris. Each head was used to excoriate ten fish and then replaced.

The maximum and minimum water temperatures were recorded daily. Five days after the last excoriation the fish were individually removed from the tanks and the physical appearance of the dorsal fin recorded. The dorsal fins were removed and fixed in 10% neutral buffered formalin.

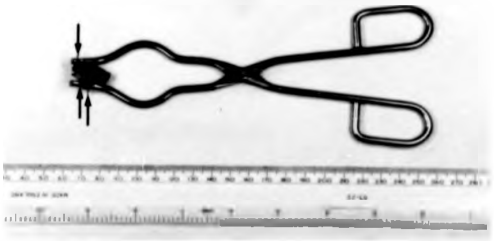


Figure 4.4. Fixed parr's head attached to forceps at three points (<) with wire.

The damage was classified under three headings *ie* :

-Loss of fin tissue

1 = 90 - 100% of the fin remaining

2 = 60 - 90% of the fin remaining

3 = 30 - 60% of the fin remaining

4 = 10 - 30% of the fin remaining

-Splitting of the fin

1 = No splits

2 = Minimal splitting *ie* less than six splits all less than 5mm in length

3 = Some splitting *ie* any fish between 2 and 4

4 = Severe splitting *ie* majority of the distal edge of the fin damaged or more than six splits deeper than 1cm

-Thickening of the fin

1 = No detectable thickening

2 = Minimal thickening, detectable only on close examination

3 = Some thickening, easily detectable but not noticeably nodular

4 = Severely thickened and nodular, similar to a severe natural fin rot.

The physical classification of the fins was found to have heterogeneous variance by the F_{max} test and the data was therefore analysed by the Games and Howell method for comparison between means with heterogeneous variance.

2.4. Comparison between the effects of simulated biting and rubbing.

One of the few alternative sources of damage to the dorsal fin, apart

from biting of the fin, was thought to be damage from other fish during feeding. An attempt was made to simulate both biting and this hypothetical feeding damage. Two groups each of ten parr with undamaged fins were placed in separate aquaria. After acclimatisation they were removed and anaesthetised with 2-phenoxyethanol and the dorsal fin damaged. One population was damaged by simulated bites (as described in 2.3.) the fish in other population had the open fixed mouth rubbed over the dorsal edge of the fin from trailing to leading edge. The fins were excoriated on six occasions at 48 hour intervals. Both the simulated bites and the rubbing were repeated three times on every occasion. After the last excoriation the fish were retained in the system and examined daily and the changes in the lesions recorded.

2.5. Comparison between the effects of simulated biting under two different anaesthetics.

Two groups each of ten parr with undamaged dorsal fins were placed in separate aquaria. Both groups of fish had their dorsal fins excoriated with a fixed parr's head. The simulated bites were repeated every 48 hours on eight occasions. An attempt was made to damage the fins without removing the hyperplastic tissue. This was achieved by clasping the jaws on the fins but not drawing the closed mouth off the distal edge. One of the populations was anaesthetised with 2-phenoxyethanol and the other with benzocaine. The thickening of the dorsal fins was classified by the method described in 2.3. before every excoriation. The results were analysed by the Mann-Whitney U test. The surviving fish were sampled for bacteriology by the method described in chapter III 1.5.2. Approximately half of the

fish were sampled two days after the last excoriation and the remaining fish six days after the last excoriation. The bacteria isolated from the fins were classified as CLB or non-CLB on colony characteristics and Grams stain.

3. HEALING FROM NATURALLY OCCURRING DORSAL FIN ROT AT A RANGE OF TEMPERATURES

Previous results had indicated that temperature may have had a significant effect on the development of dorsal fin rot. In order to obtain more information on the relationship between dorsal fin rot and temperature a study was undertaken to investigate the process of healing at different temperatures.

3.1. Healing from naturally occurring dorsal fin rot.

One fish with severe dorsal fin rot was obtained from farm 1, 30-60% of the dorsal fin remained with severe distal nodularity. The fish was placed in a 30l aquarium with a power sponge filter and aeration. The water quality was maintained by partial water changes once a week. The tank was maintained in the laboratory at ambient temperature which was recorded twice daily. The fish started to accept food after ten days and was thereafter fed to appetite twice daily, six days a week. The condition of the dorsal fin was recorded by line diagrams, daily for the first two weeks and thereafter once a week. The fish was retained in the system for six months. Unsuccessful attempts were made to record the condition of the dorsal fin photographically. At the end of the period of observation the fish

was killed and the dorsal fin fixed in 10% neutral buffered formalin and processed for histology.

3.2. Short term healing from dorsal fin rot at two different temperatures, a pilot study.

This preliminary study was conducted on two occasions. In both cases two fish with similar severe dorsal fin rot (severe nodularity, 30-60% of the fin remaining) were obtained from farm 3. The fish were placed in a divided 50l aquarium, with a power filter and aeration. On the first occasion the temperature within the system was increased with an aquarium heater-thermostat. On the second occasion the tank was maintained at ambient temperature. The fish were offered food but did not accept it. Water quality was maintained by twice weekly water changes. In both experiments the maximum and minimum temperatures and the gross appearance of the dorsal fins were recorded daily. The condition of the fins was recorded in the form of line diagrams. Again an unsuccessful attempt was made to record the condition of the fins photographically.

3.3. Short term healing from dorsal fin rot at two different temperatures.

A glass tank (127 × 46 × 46cm) was constructed (Figure 4.5.). The tank was divided by perspex sheets into ten similar compartments. The tank was covered with a close fitting mesh. Each compartment was supplied with aeration, an individual water inflow and a syphoned outflow. The inlets were individually controlled by valves, the outflows were all interconnected and the level in the tank controlled

with two stand pipes. All the perspex dividers had five 1cm diameter holes in them. A continuous flow through system was used with dechlorinated mains water. Before the start of the experiment a bolus of rhodamine-B dye (Hopkin and William Ltd, Essex) was added to the inflow and its distribution through the system observed to evaluate the water flow in the system. After two days of acclimatisation the fish were offered pelleted food to appetite twice daily. The fish ate only a few pellets each day. The accumulated food and faeces were syphoned from the tank on alternate days. The maximum and minimum temperatures and the gross appearance of the dorsal fins were recorded daily

3.3.1. In the first experiment ten fish with similar severe dorsal fin rot (severe nodularity, 30-60% of the fin remaining) were obtained from farm 1. On this occasion the temperature of the farm water and that in the experimental system were 3.5°C and 4°C respectively. Therefore the fish were placed directly into individual compartments. The experiment was concluded after 58 days.

3.3.2. In the second experiment the temperature in the tank was raised by the addition of aquarium heater-thermostats to each of the compartments. Again ten fish with a similar degree of severe fin rot were obtained from farm 1. The water temperature on the farm was 6°C and the temperature in the system before the heaters were turned on was 6°C. The fish were allowed two hours to acclimatise to 6°C and then placed in the individual compartments. The heaters were switched on after 24 hours and the temperature subsequently rose by a further 5°C. The experiment was concluded after 18 days.

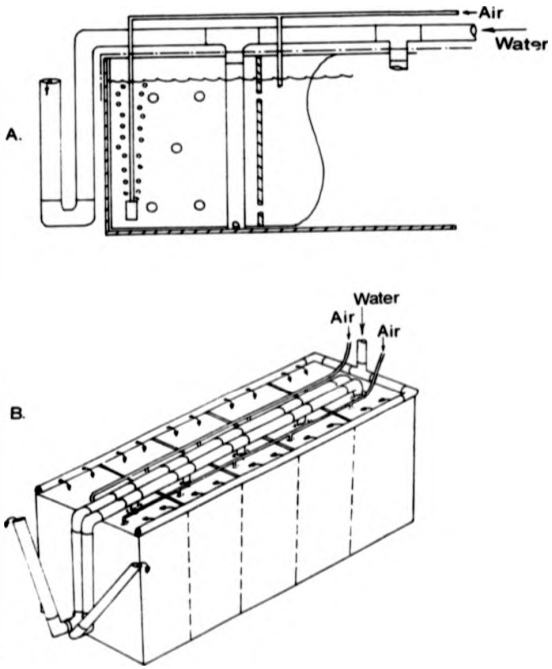


Figure 4.5. A diagrammatic representation of a single compartment (A.), and the divided tank (B.) used in 4.3.

3.4. Healing from dorsal fin rot in two populations held under controlled conditions at two different temperatures.

A further experiment was conducted with additional controls. Eight 20l tanks were used, four at ambient temperature and four at a controlled lower temperature. The system is represented diagrammatically in Figure 4.6. Every tank was divided into two compartments with a fish in each. The tanks were surrounded with polystyrene for insulation. Sixteen fish with similar fin rot (severe nodularity 30-80% of the fin remaining) were obtained from farm 3. The fish were randomly divided between the ambient and controlled temperature tanks. They were acclimatised to the two temperatures gradually over 24 hours. The flow was adjusted until it was similar in all eight tanks. The fish were fed to appetite twice a day from the third day of the experiment. Uneaten food and faeces were removed every second day. The temperature in each tank was measured twice a day and the condition of the dorsal fin recorded every morning. The time taken for all thickening to disappear was recorded. The fish were always examined from the same distance above the tank and with similar illumination. The experiment ran for 43 days after which the fish were killed weighed, measured and the dorsal fins preserved in 10% neutral buffered formalin.

The temperature control unit was obtained from C Exley (Institute of Aquaculture, University of Stirling). The unit which was manufactured by Cryotechnics (Medical and Environmental equipment, Edinburgh) consisted of a central microprocessor controlling both heating and cooling units. The water entered the unit via a float controlled

solenoid allowing the reservoir tank to be maintained at a constant level. The temperature within this tank was monitored and adjusted constantly with an immersion heater and cooler. The water was pumped from the reservoir tank at a constant rate. A proportion of the water was removed for use in the experimental tanks the rest was returned to the reservoir tank.

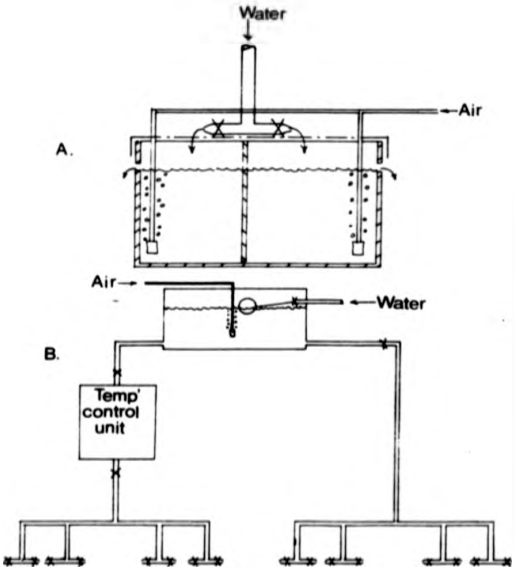


Figure 4.6. A diagrammatic representation of a single tank (A.) and the supply system (B.) used in 3.4.

CHAPTER IV

RESULTS

1. HEALING FROM SURGICAL DAMAGE TO THE DORSAL FIN

1.1. Healing following excision of a portion of the dorsal fin.

The average temperature for the duration of the experiment was 17°C (range = 13 to 21°C). The fish did not start to feed until ten days after the excision. There was no sign of thickening or nodularity, however the horizontal damaged surface of the fin developed an opaque appearance within two days of the fin being damaged. This opacity persisted for approximately seven days, although it was difficult to determine exactly when the fin regained its normal translucent appearance. The appearance of the fin during healing is represented diagrammatically in Figure 4.7. By nine days after the injury a thin membrane was developing within the defect. Fourteen days after the initial injury the membrane had filled more than half of the defect and there was an apparent extension of the damaged fin rays into the new membrane. Over the next 14 days the fin continued to repair the defect. The site of the injury was still detectable on the fin rays as they continued to extend into the new tissue. Twenty one days after the initial injury the developing fin rays started to fork. Thirty seven days after the injury the profile of the fin and the fin rays appeared normal. The regenerated rays forked more distally than the normal rays and there was a change in the thickness at the site

of the initial injury. The appearance of the fin did not change between 37 and 60 days after the excision.

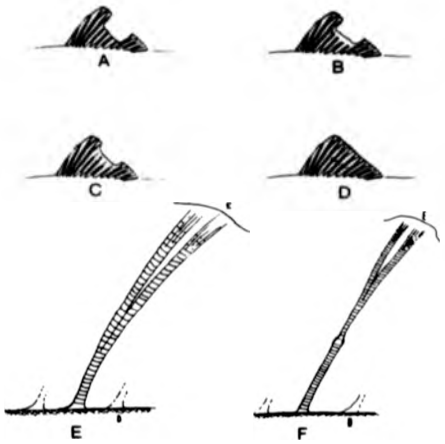


Figure 4.7. A diagrammatic representation of the healing process observed in 1.2. A = The condition of the fin immediately after the incision. B = After nine days the defect was partially filled by a thin membrane. C = After 14 days the fin rays were growing in to the new membrane. D = 37 days after the excision the only evidence of damage was a slight swelling at the point where the fin rays had been cut. E = normal fin ray. F = healed fin ray.

1.2. Healing following a vertical incision in the dorsal fin.

The average temperature during this experiment was 15±1°C. On gross examination there was very little evidence of thickening or reaction round the wounds. During the course of the experiment some of the fins developed other minor splits but it was always possible to differentiate these from the scalpel wound. The wounds on the remaining fish had healed between five and seven days. Histologically within 24 hours the epithelium had re-established continuity. The regenerated tissue initially had thin epithelium, convoluted stratum compactum and very little dermal tissue. In later samples the damaged area returned to a more normal appearance from the base of the wound distally. Grossly the defect in the fin then repaired from the apex of the wound distally. There remained an area free of melanocytes which subsequently contracted (Figure 4.8.).

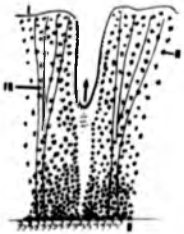


Figure 4.8. Diagrammatic representation of one of the fins in the process of healing from a vertical wound. ↑ = direction of healing. M = Melanocyte. E = Distal edge of the fin. FR = Fin ray. D = Dorsal surface of the fish.

1.3. Healing following a horizontal cut across the dorsal fin.

The average temperature during the experiment was 14°C±2°C. The only obvious thickening of the dorsal fin occurred at the anterior distal edge of the fin. Minor thickening was first observed on one fish within one day after the incision. All of the fish showed limited anterior distal thickening by two days after incision. The thickening rapidly decreased on all the fish over the following 24 hours and could not be detected by five days after excoriation. By nine days after excoriation the distal edge of the fins developed an opaque appearance without any significant thickening. Histologically the distal edge of the fin consisted of thickened epithelium containing necrotic cellular debris. The basal layer of epithelial cells in the region had a distinct cuboidal shape. The dermis underlying this epithelium contained cells with large homogenous basophilic nuclei. These were probably the differentiating cells of the blastema.

The remaining two fish were killed 90 days after their fins had been damaged. These fish had re-grown a distorted distal fin. The re-grown fin was twisted laterally and supported by thickened, distorted fin rays, some of which had failed to branch (Figure 4.9.). These fins had a similar appearance to those described as healed from naturally occurring fin rot (chapter I, 1.2., F).

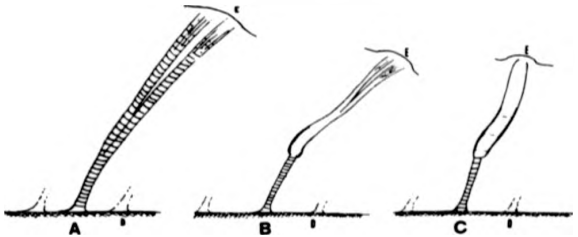


Figure 4.9. Diagrammatic representation of the re-grown fin rays following horizontal incision of the dorsal fin. A = Normal fin ray. B = Branched healed fin ray. C = Healed fin ray that had failed to branch. E = Distal edge of the fin. D = Dorsal surface of the fish.

2. RESPONSE OF THE DORSAL FIN TO REPEATED EXCORIATION

2.1. Repeated excoriation of the dorsal fins with and without prior injection with corticosteroid.

The average temperature for the duration of the experiment was 6±2°C. Twelve of the fish injected with prednisolone died between 12 and 16 days after the initial excoriation. The first eight had severe fungal infection on the dorsal fin, there was no sign of fungal infection or other gross pathology on the four fish that died on day 16. All the

fish that died were sampled for bacteriology but no significant pathogens were isolated. The remaining fish that had been injected were killed on day 18, due to a general deterioration in their health. These fish were sampled for histology and bacteriology but no significant pathogens or pathology were detected. There was no apparent difference between the changes observed in the dorsal fins of the fish not affected by fungus. All the fish suffered from loss of tissue and exposure of fin rays. There was some thickening of the tissue but the excess tissue was removed at each subsequent excoriation. The thickened tissue was very friable and easily detached from the fin. The thickening was at a maximum around three to five days after each excoriation. None of the fish developed the thickened nodular appearance of naturally occurring fin rot.

2.2. Repeated excoriation of the dorsal fin.

The average temperature was 8±1°C. Ten of the fish died with fungal infections within the first eight days. To avoid further suffering the remaining fish were killed. The pattern of fin loss was similar to that seen in natural fin rot, however the thickening of the fin was limited. The majority of the hyperplastic epithelium was removed by subsequent excoriation even thought an attempt was made to keep the damage caused to a minimum.

2.3. Repeated excoriation by simulated bites.

The average temperature was 7±2°C. Six of the original 80 fish died before the end of the experiment. No significant pathogens were isolated from any of the mortalities. The physical condition of the

dorsal fins at the end of the experiment is recorded graphically in Figures 4.10. and 4.11. The data was analysed by the Games and Howell method and the results are recorded in Table 4.1. Histological appearance of the resulting lesions was indistinguishable from natural fin rot described in chapter I as 'peripheral erosion with some nodularity'.

Table 4.1.
Statistically significant ($p = 0,05$) differences in the mean damage to the dorsal fins in the control (C1 & C2) and excoriated (E1 & E2) populations, according to the Games and Howell method.

Populations	Fin loss	Splitting	Thickening
C_1 & C_2	-	-	-
E_1 & E_2	-	-	-
C_1 & E_1	+	-	+
C_1 & E_2	+	-	+
C_2 & E_1	+	-	+
C_2 & E_2	+	-	+

- = not significant, + = significant.

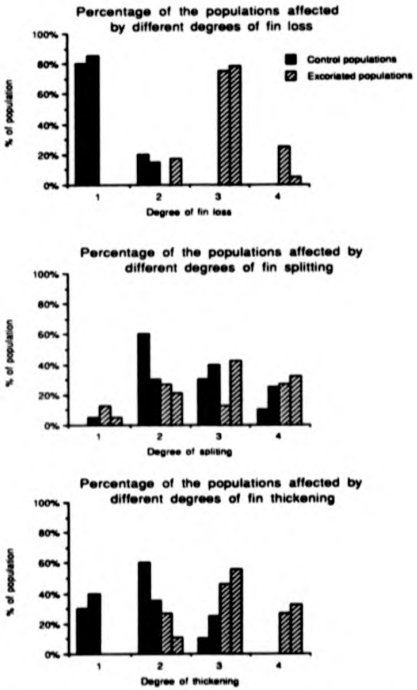


Figure 4.10. The percentage of the two control and two excoriated populations affected by different degrees of loss of fin tissue, splitting and thickening of the fin.

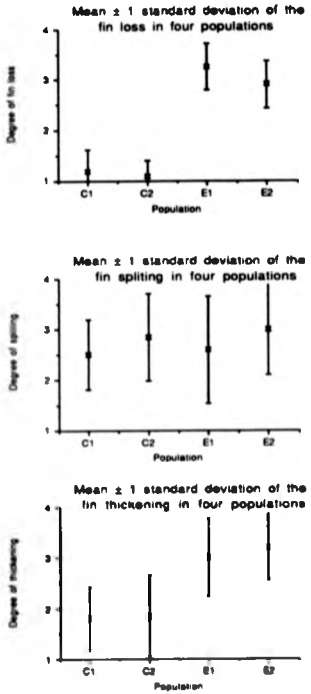


Figure 4.11. The mean ± 1 standard deviation of the loss of fin tissue, splitting and thickening of the fin, for the two control (C1 and C2) and two excoriated populations (E1 endE2).

2.4. Comparison between the effects of simulated biting and rubbing.

The average temperature during this experiment was 15±2°C. The lesions produced by rubbing the fin from trailing to leading edge did not resemble natural dorsal fin rot. They consisted of two to five deep splits in the fin extending down from the distal edge to the base of the fin. There was very little loss of tissue or thickening. Following the last episode of damage the fins started to heal with no apparent increase in thickening or nodularity. The fish that were subjected to the simulated biting rapidly developed the characteristic loss of tissue but did not develop much thickening of the fins. The limited thickening of the fins was most noticeable two days after the last excoriation and thereafter decreased. There were a number of non-specific mortalities in both systems after the last excoriation. The remaining fish subjected to the rubbing were killed nine days after the last excoriation due to deteriorating health. The remaining fish subjected to the simulated biting were killed after 14 days.

2.5. Comparison between the effects of simulated biting under two different anaesthetics.

The temperature for the duration of the experiment was 14±1.5°C. The fish in both populations developed similar patterns of fin loss and nodularity. The thickening of the fins in the two populations 2, 8 and 13 days after the first excoriation are displayed graphically in Figure 4.12. The median thickening of the fins in the two populations were not found to be significantly different by the Mann-Whitney U test on any of the three occasions.

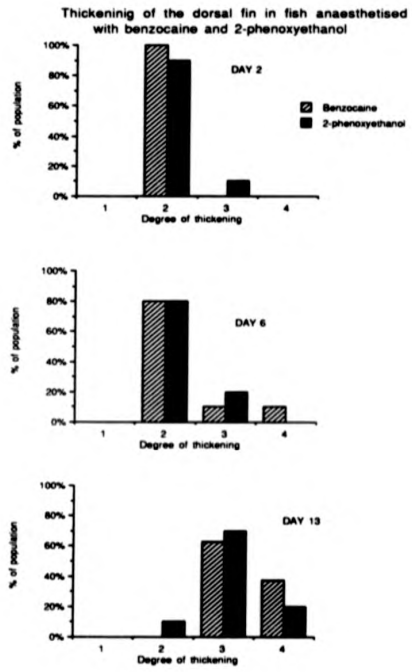


Figure 4.12. The percentage of the two populations affected by thickening of the dorsal fin to different degrees, 2, 6 and 13 days after the initial excoriation.

IV
(Results)

The mean number of CLB and total colonies from the two populations are recorded in Table 4.2. and graphically in Figure 4.13. The results of analyses by the Mann-Whitney U test are recorded in Table 4.3.

Table 4.2.
The mean number of colonies isolated from fish anaesthetised with benzocaine and 2-phenoxyethenol two and six days after the last excoriation.

Population	Fish number	Number of colonies			
		CLB	x̄	Total	x̄
Benz' 2 days post excoriation	1	0		49	
	2	1		49	
	3	0	0	19	39
Benz' 6 days post excoriation	4	2		80	
	5	3		94	
	6	3		146	
	7	7		113	
	8	5	4	160	119
Phenoxy' 2 days post excoriation	1	0		29	
	2	1		5	
	3	0		27	
	4	0		32	
	5	1	0	18	22
Phenoxy' 6 days post excoriation	6	10		278	
	7	25		318	
	8	6		315	
	9	8		278	
	10	18	7,9	189	276

The number of colonies represents the mean of three replicate plates for each sample.

Table 4.3.
Statistically significant differences (U = 2; P < 0,05, Mann-Whitney U test) between the medians of the cfu recovered from the fish anaesthetised by 2-phenoxyethanol and benzocaine, 2 and 6 days after the last excoriation.

	Total cfu	CLB cfu
Benz' day 2 & 6	+	+
Phenoxy' day 2 & 6	+	+
Day 2 benz' & phen'	-	-
Day 6 benz' & phen'	+	+

- = not significant, + = significant.

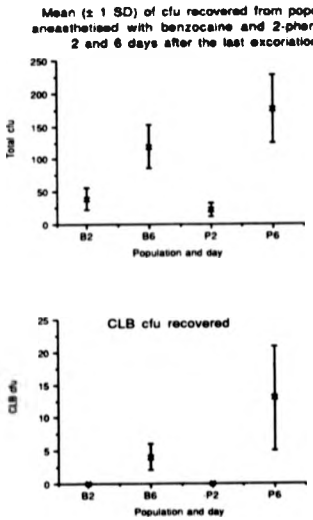


Figure 4.13. Mean ± 1 standard deviation of the CLB and total cfu recovered from the two populations on the second and sixth day after the last excoriation.

3. HEALING FROM NATURALLY OCCURRING DORSAL FIN ROT AT A RANGE OF TEMPERATURES

One of the main criteria for healing from dorsal fin rot was taken to be the return of the dorsal fin to normal thickness. In some cases it was difficult to identify exactly when this occurred. Occasionally there were three days when the fin may or may not have lost all thickening. In these instances the middle day was recorded as the date when thickening resolved.

3.1. Healing from naturally occurring fin rot

Several unsuccessful attempts were made before a fish was maintained in the system long enough to observe long term healing from naturally occurring dorsal fin rot.

The range of temperatures for the duration of the experiment was 17-21°C. The dorsal fin lost its nodular appearance within five days. Thereafter the distal edge of the dorsal fin retained an opaque appearance for approximately thirty days. A thin membrane extended from the distal edge of the dorsal fin and subsequently distorted fin rays grew into the new tissue. By four months there was a sharp division between the normal fin and the regenerated tissue. The new tissue consisted of a thin membrane supported by abnormal fin rays which were twisted and had a distinct swelling at the junction of new and old tissue (Figure 4.14.). This appearance was similar to that seen in fish described as having healed from fin rot under farmed conditions, although the fish from the farms tended to have less distinct swelling at the site of re-growth, with only limited branching

distally. The fish appeared incapable of fully extending the new tissue which was slightly folded laterally. There was no detectable change in the condition of the dorsal fin between four and six months when the experiment was concluded. Histologically the regenerated portion of the fin was indistinguishable from the distal portion of a normal fin.

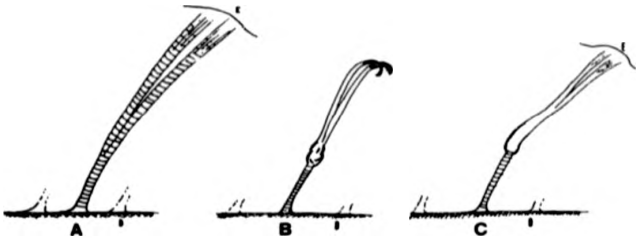


Figure 4.14. Diagrammatic representation of the dorsal fin rays from : A = Normal undamaged fin. B = The fish which recovered from dorsal fin rot in the laboratory. C = A fish thought to have recovered from dorsal fin rot on a farm (Figure 1.13.). E = Distal edge of the fin. D = Dorsal surface of the fish.

3.2. Short term healing from dorsal fin rot at two different temperatures, a pilot study.

In the first part of the experiment the average temperature was 13±1°C. There was no detectable difference between the two fish. When the fish were placed into the system they held their dorsal fins folded close to their back. This increased the apparent nodularity of the fin lesions. Within 18 hours they held the dorsal fin erect resulting in a distinct reduction in the nodularity of the fins. By 72 hours after introduction to the system there was no thickening detectable on the fins, though the distal edge of the fin retained an opaque appearance. The rough edge of the fin became smooth and less indented by 96 hours. An attempt was made to maintain the fish for a prolonged period but the fish died seven and ten days after introduction to the system.

In the second experiment the average temperature was 8.5±1.5°C. One of the fish died after 48 hours, up to this time there was no detectable difference between the fish. Initially both fish held their fins folded but were observed to extend them after 22 hours. In this case obvious nodularity was detectable over the distal edge of the dorsal fin for 72 hours. Some thickening was detectable on the posterior distal edge of the fin for ten days after the fish was introduced to the system. The surviving fish was retained for a further 5 days, by the end of which period the distal edge of the fin was smooth but still slightly opaque.

3.3. Short term healing from dorsal fin rot at two different temperatures.

The rhodamine-B dispersed rapidly through the system and was subsequently removed at a uniform rate, suggesting similar water flow in all the compartments. The pattern of healing was similar to that observed in the earlier experiments (3.3.). The dates that the dorsal fins returned to their normal thickness here recorded. This data is presented graphically in Figure 4.15.

3.3.1. The average temperature in the system was 5±1.5°C. Three of the fish died during the experiment before their dorsal fin had returned to its normal thickness. One of these fish died on the 55th, day one day after developing a fungal infection on the dorsal fin. The experiment was terminated on the 60th day due to deteriorating health in all the remaining fish.

3.3.2. The average temperature was 13±2°C and all the fish survived for the duration of the experiment.

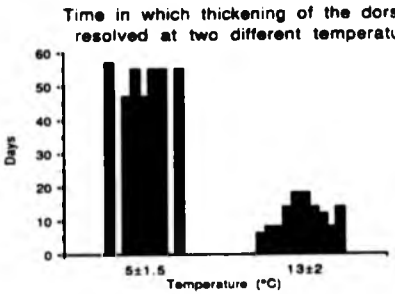


Figure 4.15. The time taken for the dorsal fin to return to normal thickness at two temperatures. Each bar represents one fish.

3.4. Healing from dorsal fin rot in two populations held under controlled conditions at different temperatures.

The average temperatures within the two sets of tanks were 17±0.5°C and 5.5±1°C respectively until 31 days into the experiment. At this time a technical fault in the temperature control unit allowed the cooled system to rise to 12°C. Unsuccessful attempts were made to repair the unit and the experiment was concluded on the 43rd day. When analysed by the t test no significant difference was found between the weights and fork lengths of the fish in the two populations at the end of the experiment.

Three fish in the cooled water died before the 43rd day, one on the second day of the experiment with no obvious cause of death and two which were killed at a later stage due to severe fungal infections on their dorsal fins.

The time taken for the dorsal fin to return to normal thickness was recorded and is illustrated graphically in Figure 4.16. All the surviving fish in the cooled water still had some thickening of the dorsal fin at the end of the experiment.

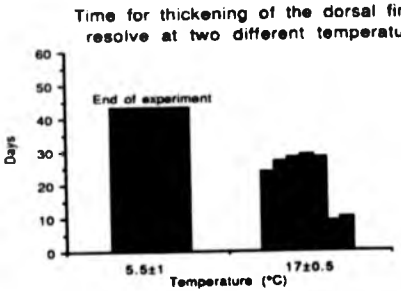


Figure 4.16. The time taken for the dorsal fin to return to normal thickness at two temperatures. Each bar represents one fish. The fish held at 5.5±1°C all still had signs of thickening of the dorsal fin when the experiment was concluded on the 43rd day.

CHAPTER IV

DISCUSSION

The object of the work described in this chapter was to to investigate the response of the dorsal fins to injury and their capacity to heal. By this stage in the study the most likely cause of dorsal fin rot appeared to be some form of physical damage. This hypothesis had been arrived at following observation of the lesions and elimination of other causes. Possible sources of damage included handling (Snieszko, 1958), rough tank surfaces (Bullock and Conroy, 1971; Schneider and Nicholson, 1980; Gibson, 1981; Moring, 1982) or biting by other fish (Abbott and Dill, 1985; Needham,E.A. Pers' comm')

1. HEALING FORM SURGICAL DAMAGE TO THE DORSAL FIN

In order to investigate healing from 'simple' injuries three, small experiments were conducted. These involved damaging the dorsal fin with a scalpel and observing the subsequent response.

1.1. Healing following excision of a portion of the dorsal fin.

The first experiment involved only one fish which was maintained for 60 days. Under these conditions it was only possible to observe the gross appearance of healing. However this was consistent with the published description of epimorphosis and the descriptions of Nabrit (1929). The initial wound appeared to heal then an area of opaque tissue formed, presumably the blastema, from this region membranous and subsequently fin ray tissue developed. The only remaining

evidence of damage at the end of the experiment was a slight reduction in the thickness of the fin rays where they had been sectioned.

1.2. Healing following a vertical incision in the dorsal fin.

In the second experiment the vertical incisions in the fins healed very rapidly. There was an area left that lacked melanophores but this subsequently contracted and became difficult to detect. Otherwise the fins returned to their normal appearance.

1.3. Healing following a horizontal cut across the dorsal fin.

In the third experiment the initial wound appeared to heal rapidly but regeneration of the fin was slower than observed in 1.1. The resulting new tissue was abnormal and resembled the fins described as 'healed fin rot lesions' in chapter I (1.2. F). Although the initial stages of healing followed the pattern of epimorphosis the subsequent regeneration was abnormal. The literature dealing with healing of fins is discussed in the introduction to this chapter. The limited observations described here suggested that the fin may require some lateral support from intact fin rays to regenerate normally. However there are many other factors that could have affected the observed regeneration, for example temperature, the nature of the damage, the nutritional status of the fish and bacterial populations present on the fin. These experiments demonstrated that the dorsal fin can regenerate following substantial damage but the fin does not always regain its normal gross appearance. More information is required to explain the role of factors affecting the regeneration of fins.

The surgical injuries inflicted did not reproduce any of the major characteristics of dorsal fin rot. Evidance from chapter III (2.1.) suggested that a single incidence of excoriation also failed to reproduce fin rot. Therefore if damage is important in the aetiology of dorsal fin rot it must either be repeated or of a different nature

2. RESPONSE OF THE DORSAL FIN TO REPEATED EXCORIATION

The rough eroded nature of dorsal fin rot lesions and the clefts observed histologically suggested that the nature of the hypothetical damage was a form of excoriation or abrasion and the evidence discussed above suggested that the injury was probably repeated. These experiments (2.1. - 2.5.) were conducted to investigate the effects of repeated excoriation in an attempt to provide more information about the precise aetiology of dorsal fin rot.

2.1. Repeated excoriation of the dorsal fin with and without prior injection of corticosteroids.

This experiment was an attempt to reproduce dorsal fin rot artificially and examine the effects of steroids on that process. There was no evidence with which to predict the frequency or number of episodes of damage required to reproduce the condition. It was assumed that the pathology would be cumulative provided the repeated injury occurred before the previous response resolved. At this stage there was no indication that the nature of the excoriation was essential to the development of the lesion, therefore sandpaper was selected as a convenient way of causing a relatively mild but extensive excoriation.

Since dorsal fin rot is associated with farmed populations of fish it seemed reasonable that the condition may have been associated with a combination of repeated injury and stress. Pickering and Pottinger (1988) reported a high incidence of fin rot in populations of chronically stressed Atlantic salmon parr. Stress results in stimulation of the hypothalamic-pituitary-interrenal axis, thereby elevating plasma cortisol levels. In order to mimic this effect, half of the fish were injected with a corticosteroid prior to the excoriations. The wide ranging effects of corticosteroids were thought to have two main implications for dorsal fin rot.

First, corticosteroids cause an increase in susceptibility to infectious diseases (Pickering and Duston, 1983). This is thought to be mediated through a suppression of lymphoid tissue and leucocyte activity (Pickering, 1984; Stave and Roberson, 1985; Maule, Schreck and Kaattari, 1986; Pickering and Pottinger, 1988). This reduction in immune cellular response has been shown to increase mortalities due to disease in brown trout (Pickering and Duston, 1983; Pickering and Pottinger, 1985 and 1987a; Pickering, 1986) and is thought to have a similar effect in Atlantic salmon (Pickering and Pottinger, 1988). Corticosteroids including prednisolone have also been used in the so called 'stress test' to detect carriers of the bacterial pathogen *A salmonicida*. This test was first described by Bullock and Stuckey (1975) using triamcinolone. M°Carthy (1977) subsequently suggested that the test was improved by substituting prednisolone for triamcinolone.

Secondly corticosteroids affect the healing process. When Roubal and Bullock (1988) described wound repair in juvenile Atlantic salmon following injection with hydrocortisone, they observed that epithelial migration was not affected but dermal repair and wound contraction were inhibited.

The results indicated that the pattern of tissue loss was similar to that observed in naturally occurring dorsal fin rot, there was little evidence of the typical thickened hyperplastic response. It appeared that the excoriation was too severe and removed superficial tissue produced in response to the previous injury. The limited thickening that did occur was at a maximum three to five days after injury.

The steroid injected population had to be killed due to deteriorating health. Some of the fish had signs of superficial fungal infections. Although the fungus was not specifically identified it was probably a member of the *Saprolegnia* genus. The taxonomy of these organisms has yet to be resolved. They commonly affect superficial wounds on the surface of salmonids and other temperate species. Although Pickering and Duston (1983) did report increased susceptibility to fungus in stressed fish, the fungus infections observed in this experiment were not necessarily directly related to the steroid injection. Once one fish in the tank was infected the number of infective zoospores would have increased. Therefore fungal infections may have been self perpetuating within the closed system. Some of the fish showed no signs of specific pathology or pathogens. It is possible that these mortalities were the result of stress and deteriorating environmental

conditions. Stress in this context is used according to the definition of Brett (1958) "stress is a stage produced by environmental or other factors which extends the adaptive responses of an animal beyond the normal range, or which disturbs the normal functioning to such an extent that the chances of survival are significantly reduced". Pickering and Pottinger (1987b) and others have reported that sub-optimal environmental conditions frequently produces signs of chronic stress. More specifically Donaldson (1981) reported that fish held under adverse conditions in an aquarium suffered from endocrine imbalance, cellular hyperplasia and lymphoid involution. It would appear that fish can die as a direct result of stress via loss of homoeostasis or indirectly as a result of secondary infections.

There is an added complication within small closed systems, *ie* once a fish dies in the system there is rapid deterioration in the water quality associated with an increase in the bacterial population (Hossain and Turnbull, 1990). Therefore the death of one fish may lead to further mortalities within the tank.

In retrospect, the fish were probably sufficiently stressed by their confinement in a small aquarium and therefore the injection of corticosteroid may have been unnecessary.

2.2. Repeated excoriation of the dorsal fin.

In view of the results from 2.1. the experiment was repeated with more frequent, less severe damage and without the steroids. This was an attempt to produce a response more characteristic of dorsal fin rot.

Deteriorating health necessitated premature termination of this experiment and therefore little useful information was obtained.

2.3. Repeated excoriation of the dorsal fin by simulated bites.

This experiment was designed to allow comparison between control populations and fish subjected to simulated bite wounds on the dorsal fin. It was postulated that the response to injury might be increased by altering the nature and increasing the frequency of the injury. Simulated biting was selected as being the most likely cause of damage to the dorsal fin (Abbott and Dill, 1985).

Loss of fin tissue and thickening of the fin were seen in both control and excoriated populations but were significantly more severe in the excoriated fish. Splitting of the fin rays was not significantly different between the control and excoriated populations. The damage to the control fish may either have resulted from injury occurring in the tank or during netting. Damage in the tank could have been caused by contact with structures in the tank and or damage from other fish. Sources of injury within the tank were investigated in greater depth in chapter VI.

A proportion of the excoriated populations (27% and 33%) developed lesions similar to naturally occurring dorsal fin rot with nodular thickening. Although none of the lesions were as severe as the worst natural cases, these results strongly suggest that dorsal fin rot may result from repeated bite wounds. The variation between fish may

have resulted from individual susceptibility, variation in the severity of the damage or additional concurrent damage within the tank.

No definitive diagnosis was obtained for the mortalities that occurred in the first control tank. If these non-specific mortalities were associated with cumulative stress either directly by a loss of homoeostasis or indirectly as a result of secondary infections , there are significant implications for the interpretation of the results reported in this chapter. Elevated plasma corticosteroid levels can increased susceptibility to infections (Peters, Faisal, Lang and Ahmed, 1988) and inhibit dermal healing (Roubal and Bullock, 1988). Both of these effects could have influenced the observed response to injury and healing in these experiments. In effect, if the fish that died were severely stressed then the responses of the surviving animals may not have been normal. This limitation has to be taken into account when analysing the implications of this work.

2.4. Comparison between the effects of simulated biting and rubbing. Since even the worst lesions produced in 2.3. were not as severe as the worst field cases it was necessary to examine other factors that could influence the pathology. Possible areas for further investigation included the type of injury, the frequency of the injuries, possible bacterial involvement and environmental conditions including temperature. The effect of bacteria and temperature were studied in greater depth in later experiments.

IV
(Discussion)

It was assumed that an increased frequency of injury was likely to produce more reaction unless it removed more of the tissue. Most processes in poikilothermic animals occur more rapidly at higher temperatures therefore it is possible that more frequent injuries would be necessary to stimulate the same response at higher temperatures. In accordance with this theory the frequency of injury was increased to once every 48 hours, since the temperature was higher during this experiment.

An alternative type of injury was also investigated. Previously it had been assumed that the lesion would be similar for any form of widespread mild superficial injury to the fin. Studying the parr in numerous culture systems allowed analysis of the potential sources of damage. Only two sources of repeated injury to the distal dorsal fin were identified, both were due to other fish. Biting has already been mentioned and was suggested by Abbott and Dill (1985) as a possible cause of dorsal fin rot. The other possibility was that damage occurred during feeding. Fish could damage the fins of those below them as they accelerated past to take pellets.

Most other sources of damage within the tanks were thought unlikely to cause repeated injury. These sources include netting and abrasion against rough surfaces in the tank. Netting only occurs infrequently and there is no evidence that fin rot only occurs after handling. Abrasion against rough surfaces in the tank has been suggested as a cause of fin damage (Sniezko, 1956). The ventral and caudal fins could quite easily come into contact with the surface of the tank and

screens. However for the distal edge of the dorsal fin to be damaged the fish would have to rotate laterally and be washed back over the surface. It was therefore assumed that repeated damage from the surfaces within the tanks is unlikely. No other potential source of repeated injury were identified.

To simulate the hypothetical injury occurring during feeding, the dorsal fins of some of the fish were rubbed by the open mouth of a fixed parr, from trailing to leading edge of the fin. This form of injury should have been similar to any damage caused by the dorsal fin being dragged over a rough surface.

The pattern of damage caused by the two forms of injury were distinctly different. The loss of tissue caused by the simulated biting again resembled natural fin rot whereas rubbing resulted in a small number of deep splits in the fin. It was concluded from this that rubbing was unlikely to be the cause of dorsal fin rot. However the severe thickening observed in the previous experiment did not occur. It was necessary to investigate why the simulated biting failed to produce thickening on this occasion. The differences between this experiment and 2.3. were the temperature, the frequency of injury, the anaesthetic and possibly some other parameters that were not measured *eg* bacterial populations on the fin.

Temperature could have affected the results in several ways. These experiments were conducted at ambient temperature. Although it is relatively easy to maintain a constantly elevated temperature, it is

difficult to maintain a constant artificially low temperature. This is dimcussed in greater detail later below. It has been reported that epithelial hyperplasia is more common at lower temperatures (Roberts, 1975). However this effect has neither been quantified nor has the mechanism been explained. Schneider and Nicholson (1980) also reported more thickening in cases of fin rot at lower temperature and more erosion at higher temperature. Temperature also effects other aspects of the healing response. At lower temperatures fibroblast activity is delayed (Finn and Nielson, 1971) as is the macrophage response and clearing of bacteria and necrotic tissue. Many other aspects of the immune and inflammatory response are also inhibited at lower temperatures (Anderson and Roberts, 1975). Since hyperplasia is only one component of dorsal fin rot (chapter I) it was not possible to predict the the effect of temperature on the development of the lesion. Further work was required to investigate the effects of temperature since it was only one of the variables in the previous experiments.

In 2.4. the frequency of the injuries was higher than in 2.3. As already discussed it would seem unlikely that a higher frequency of injuries would have produced less reaction unless the accumulating tissue was removed on subsequent excoriations.

Up to this point there was no evidence that the type of anaesthetic affected the development of dorsal fin rot. Benzocaine was used in 2.3. because there was no 2-phenoxyethanol available. It is possible that the anaesthetics may have influenced the development of the

lesions. 2-phenoxyethanol is said to have some anti-bacterial and anti-fungal activity whereas benzocaine does not (Rose and Rose, 1984) therefore the 2-phenoxyethanol may have influenced the development of the lesion via an effect on the microflora associated with the lesion. At this stage in the study the role of bacteria following injury to the fin had not been studied in any depth since these experiments were conducted before those described in chapter III, 2.2. and 2.3.

2.5. Comparison between the effects of simulated biting under two different anaesthetics.

Two populations of fish were anaesthetised with either benzocaine or 2-phenoxyethanol and the method of simulating the bite was altered to avoid removing tissue from the fins. The gross physical appearance of the fins and the bacteria present were recorded two and six days after the last excoriation.

There was a significant increase in the number of cfu recovered between the second and sixth day, on both occasions the CLB cfu were in the minority. There were also significantly more CLB and total cfu recovered on the sixth day from the fish anaesthetised with 2-phenoxyethanol compared to those anaesthetised with benzocaine. The bacteria associated with the dorsal fin following damage was studied in greater depth in chapter III (2.2. and 2.3).

If the 2-phenoxyethanol had inhibited bacteria which were important in the aetiology of the condition, there should have been less bacteria on the fins exposed to 2-phenoxyethanol, in fact the opposite was

true. In addition there was no significant difference between the populations after two days, the difference only developed after six days. Therefore it is more likely that the differences were related to the changing environmental conditions within the tanks rather than the effects of the anaesthetics.

The degree of thickening of the fins in the two populations was similar to that seen in 2.3. despite the increased frequency of excoriation and the attempt to damage the fins without removing tissue. It is possible that there was an interaction between the amount of damage and the temperature, with more frequent damage being required to produce similar lesions at higher temperature. This is however supposition since the experiments were not designed to investigate this relationship.

3. HEALING FROM NATURALLY OCCURRING DORSAL FIN ROT AT A RANGE OF TEMPERATURES

Maintaining Atlantic salmon parr affected by dorsal fin rot in isolation and under conditions where they could be closely observed proved difficult. Despite several attempts only one fish was maintained for more than one month. It is generally agreed by workers in the field that Atlantic salmon are not suited to confinement under laboratory conditions. Many of the studies into healing and response to injury have used species more suited to confinement in aquaria. It has been the experience of workers at the Institute of Aquaculture, University of Stirling, that Atlantic salmon show more evidence of stress than other salmonids when confined in small aquaria. Similar

opinions have been expressed by other workers (Christiansen,J.S. Universiteteti Tromsö, Norway, Pers' comm').

Prolonged maintenance of parr was also complicated by the smoltification process. Most of the parr available from commercial farms were potential Si's. These fish smoltify after their first winter in fresh water. Since serious fin rot usually occurs from late Autumn onwards there is a relatively short period between obtaining the fish with severe fin rot and their smoltification. The transformation from parr to smolts involves many physical and physiological changes. These include more active behaviour, increased fragility of the skin and increased susceptibility to stress and infections (Langdon, 1985).

It was found that if the tank were screened such that no external movement was visible the fish appeared less stressed. However feeding the fish in the screened tank proved difficult. If the fish could detect movement outside the tank they would not feed. It was possible to feed the fish by employing a piece of pipe to deliver the pellets. However the tanks still had to be cleaned out regularly and fish in screened tanks became very stressed during this process. They would refuse food for up to one week after such disturbances. It was found that fish within un-screened tanks eventually became accustomed to external movement and would start feeding within two days after the tank was cleaned. Schreck (1981) proposed that the stress response could be reduced by habituating fish to stressful stimuli.

He proposed that this effectively changed the fishes perception of what constituted a threat.

3.1. Healing from naturally occurring dorsal fin rot.

There were several interesting observations made during this experiment. The fin rot lesion started to heal as soon as the fish was placed in isolation. This would suggest that something essential to the maintenance of dorsal fin rot was removed or changed. Several factors may have been altered when the fish was placed in isolation including sources of damage, nutrition, environment including water quality and microbial populations.

On introduction to the aquarium facilities most of the sources of damage including bites from other fish were removed.

The fish was offered the same food it had been receiving on the farm but did not accept it for 10 days by which time there had been substantial healing.

Several factors in the fishes environment changed when it was introduced to the aquarium. The fish was probably subjected to more stress during and immediately following transfer. The water temperature was higher in the laboratory, although fin rot was found to increase during periods of low water temperature (chapter II) there was no direct correlation. Other water quality parameters would also have altered but it is reasonable to assume that water quality was less suitable for the fish in the confined re-circulating system than

on the farm. For example ammonia and nitrite would have increased following the introduction of the fish and the higher water temperature would result in lower dissolved oxygen. There is one exception in that the water from the farm may have contained higher levels of suspended solids. However suspended solids do not appear to be directly related to dorsal fin rot. The microbial populations on the fin may have been affected. However, even if the environmental conditions within the aquarium were less suitable for any infective agent it would seem unlikely that they would be removed immediately.

These arguments would appear to further support the hypothesis that dorsal fin rot is caused and maintained by repeated injury. This supports the assertion of Kindschi *et al* (1991) that isolation prevented rainbow trout from developing fin rot.

Previous experiments have suggested that the thickening and nodularity of the fin is at a maximum two to five days after the last injury. Since the thickening started to resolve immediately it would suggest that the fin had not been injured for at least two days. Analysis of such results might allow the frequency of injuries under farmed conditions to be estimated.

This experiment demonstrated that the dorsal fin has the capacity to heal from severe fin rot but it does not necessarily regain it's normal appearance. The healed dorsal fin strongly resembled both the fins of the fish that had the distal edge of their fins removed (1.3.) and the fish presumptively described as having healed from natural fin

rot (chapter I 1.2. F). The appearance of this fin would support the assumption that the fins described in chapter I resulted from healed fin rot lesions. Nabrit (1929) demonstrated that fins can regenerate abnormal fin rays following serious injury. Therefore any workers wishing to use the appearance of the dorsal fin to identify farmed stock of Atlantic salmon (Nicola and Cordone, 1973) would have to ensure they could differentiate between undamaged and healed fins.

There are several reasons why the a fin might not regain its normal appearance after injury. Some of the causes are well documented, for example inadequate innervation (Goss and Stagg, 1957; Geraudie and Singer,1985) or the presence of toxic pollutants (Weis and Weis, 1976; Fujii and Inaba, 1985). Presumebly there could also be some species differences and the nature of the damage could affect the subsequent recovery. In this study the fins that regained their normal appearance had some intact fin rays remaining, it is possible that this is also a requirement for rapid complete regeneration in Atlantic salmon.

3.2. Short term healing from dorsal fin rot at two different temperatures, a pilot study.

The subsequent experiments concentrated on the short term healing from the thickened lesions, since maintaining the fish in the system for prolonged periods had proved difficult. Thickening of the fin was selected since it is the most obvious clinical sign of the condition. The method employed for monitoring the degree of thickening on the fins was subjective visual assessment. This was considered to be the

most suitable method. As already mentioned attempts at recording the thickening of the fin photographically had been unsuccessful. Any physical method of measuring the thickness of the fin would have necessitated removing the fish from the water and possibly anaesthetising them. It was demonstrated in 2.3. that such handling may result in further damage to the fin and would also have stressed the fish. In order to minimise variation as a result of personal interpretation the fish were observed from a fixed distance above the tank with consistent illumination. However it was sometimes difficult to determine exactly when the fin had returned to normal. In most cases it was possible to identify three days when the fin may or may not have regained its normal appearance and in such cases the middle day was recorded.

This small pilot study was not well controlled or replicated but suggested dorsal fin rot healed more rapidly at the higher temperature.

3.3. Short term healing from dorsal fin rot at two different temperatures.

This experiment involved more fish and the temperature difference was greater. Again the fish were not held simultaneously at different temperatures and the method of assessment was subjective visual assessment. The second part of the experiment was started 17 days after the conclusion of the first part in March 1990.

Although the fish held at the higher temperature appeared to heal significantly faster there are several uncontrolled effects that could have influence the result. From the winter equinox onwards, Atlantic salmon parr have a tendency to undergo smoltification, a process which is usually completed in April or May. In farmed stocks most of the population will either smolt as S1's in the first year after hatching or as S2's in the second. Parr undergo some of the changes associated with smoltification whether they smolt that year or not (Langdon, 1985). The process of smoltification involves a number of complex physical and physiological changes. It is possible that the different stages of smoltification in the two populations could have affected the healing process. There may also have been other differences between the two parts of the experiment for example water quality, external disturbances *etc.* Therefore the results are of limited significance.

3.4. Healing from dorsal fin rot in two populations held under controlled conditions at two different temperatures.

In order to control as many aspects of the experiment as possible it was necessary to maintain two groups, randomly selected from a population, simultaneously under similar conditions with temperature as the only variable. A number of alternatives were examined but found to be unsuitable. For example if the fish had been held in separate locations at different temperatures they may have been subjected to different water quality and environmental disturbance. Maintaining closed recirculation systems at different temperatures would have been relatively easy, however it would have been difficult

if not impossible to maintain identical water conditions in two systems running at different temperatures. These problems were overcome by employing a piece of apparatus obtained from C.Exley (Institute of Aquaculture, University of Stirling). There were considerable technical difficulties involved with supplying a flow of water at a constant temperature. The development of the temperature control unit is described by Exley (1989).

With the experimental design employed the fish held at the lower temperature ate less food. Since there was no significant difference between the weights or the fork lengths of the two populations at the end of the experiment, it would appear that the fish maintained at the lower temperature did not suffer excessively from their lack of appetite. However the result may have been affected by the lack of some essential nutrients.

Despite the premature conclusion of the experiment due to a technical problem with the water cooler, there was a significant difference between the time taken to recover from thickening.

In conclusion the work described in this chapter provides more evidence that damage to the fins, probably from bite wounds, is essential to the development and maintenance of dorsal fin rot and that the healing process is significantly affected by temperature. Although the response of the fin to damage at different temperatures was not studied, it is probable that there is a relationship between the frequency of the injuries, the temperature and the severity of the

resulting lesion. For example if a fin is damaged at regular intervals at a low temperature it may not have time to heal between injuries resulting in an accumulation of pathological changes. Whereas at a higher temperature the fin is more likely to recover before the next injury. In addition it has been reported that the epithelial hyperplastic response is greater at lower temperatures (Roberts, 1975). Therefore there may be a combined effect at lower temperatures. That is there may be more reaction to the injury and that reaction takes longer to heal. This theory requires further investigation. However if it were accurate then it would explain the higher incidence of dorsal fin rot associated with low water temperatures (chapter II). The implication that dorsal fin rot results from fewer bites at low temperatures means that control would also be more important at lower temperatures.

CHAPTER V

A scanning electron microscopic study of dorsal fin rot in farmed Atlantic salmon (*Salmo salar* L.) parr.

CHAPTER V

INTRODUCTION

Work in other areas of the study had suggested that bite wounds were an important aspect of the aetiology of dorsal fin rot. Light microscopy had only been of limited value for studying the earlier stages of superficial damage, therefore scanning electron microscopy (SEM) was considered as a source of additional information. SEM has been extensively used in many species to study changes in superficial epithelium. In teleosts it has been employed for analysis of the digestive (Ezeasor, 1984; Diaz, Connes and Trudelamoreau, 1987), respiratory (Lakshmikantham, 1990) and olfactory systems (Doroshenko and Motavkin, 1986). Of more relevance to this study, it has been used to examine the structure of fish skin (Hawkes, 1974), the normal fin in blennies (Brandstätter, Misof, Pazmandi and Wagner, 1990) and bacterial gill disease (reviewed by Turnbull, In press). As mentioned previously bacterial gill disease is a condition which is similar in many ways to dorsal fin rot. Despite the large volume of literature concerning the use of SEM in epithelial studies, reports concerned with superficial damage in teleosts largely deal with parasitic damage (*eg* Kabata, 1974; Robertson ,1979). No references were found dealing specifically with SEM examination of superficial physical injuries in fish.

In the light microscopic study, superficial clefts had been observed in the epithelium. However, it was not clear if these represented

genuine lesions or artifact. It was hoped that SEM would provide evidence for the nature of the initial injuries and their relationship to the hyperplastic response. The SEM study involved adapting a suitable technique for processing fin tissue, the examination of a range of specimens and the production of photographic records.

CHAPTER V

MATERIALS AND METHODS

1. THE DEVELOPMENT OF A TECHNIQUE FOR PROCESSING AND EXAMINATION OF DORSAL FIN TISSUE BY SCANNING ELECTRON MICROSCOPY.

A series of trials were conducted to investigate the suitability of several methods of sampling and processing dorsal fin tissue. The fixation and dehydration protocols (Olsuert, 1981) were identical in all these methods. The tissues were :

A. Fixed in 2.5% glutaraldehyde in 0.2M sodium cacodylate for 2h.

B. Washed twice, each for 1h, in 0.1M sodium cacodylate.

C. Post-fixed in 1% OsO_4 in 0.1M sodium cacodylate for 2h.

D. Washed twice, each for 1h, in 0.1M sodium cacodylate.

E. Retained overnight in 70% acetone.

F. Placed into 100% acetone for 30 minutes and then into fresh 100% acetone for a further 30 minutes.

G. Critical point dried with 2.5h impregnation in a Bio-Rad Critical Point drier.

H. Mounted onto SEM stubs with EMScope A860 silver conducting paint.

I. Sputter coated with gold in an Edwards 5150B sputter coater.

J. The coated preparations were all examined in a Phillips PSEM 500.

K. The photographs were taken with Pan-f black and white 35mm film (Ilford), in a SLR camera body.

1.1. Preliminary samples.

Six fish were sampled from each of two production tanks on farm 1. The fish included individuals with undamaged, eroded, and nodular fins. The fish were killed individually by a blow to the head and the dorsal fin with surrounding tissue removed. A portion from the centre of the fin was taken and placed into the fixative (Figure 5.1). The tissues were processed as described above. The pieces of fin were all attached to the stubs by the proximal muscle of the fin in such a way that one lateral aspect of the fin lay parallel to the surface of the stub, or formed a 45° or 90° angle with the surface of the stub.

1.2. Evaluation of alternative processing methods.

A further twelve fish with similar fin condition to those in 1.1 were selected from two tanks on farm 1. They were killed in a similar manner but the fins were prepared for fixation by three different methods. The dorsal fin and surrounding tissue was removed from four fish (Figure 5.1) and all the excised tissue was placed directly into the fixative. The dorsal fins of the remaining fish were removed by severing the proximal fin (Figure 5.2). Four of the fins were placed directly into the fixative, the remaining fin were attached to a small sheet of dental wax (Anûtex, Associated Dental Products Ltd, Swindon, England) by placing tissue paper over the fin and stapling the paper to the wax, avoiding the fin tissue. These fins remained attached to the wax until after critical point drying. The other eight fins were attached to wax after the second 100% acetone bath. The process of attaching the fins to the wax sheets was carried out in a shallow bath of acetone to avoid the tissues drying out. All the fins were

removed from the wax and tissue paper after critical point drying and before being mounted on the SEM stubs.

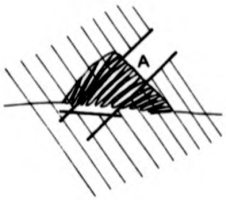


Figure 5.1. A = diagrammatic representation of the tissue removed for examination in 1.1. The cross hatched areas were discarded.

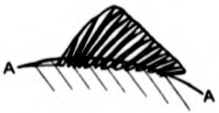


Figure 5.2. A-A = diagrammatic representation of the incision made to remove the distal fin for examination in 1.2. and 1.3. The cross hatched areas were discarded.

1.3. Processing and examination of a range of dorsal fin rot lesions.

A third group of fish was sampled from three production tanks on farm 1. These fish included individuals with undamaged dorsal fins, mild erosion, severe nodularity and some eroded but smooth fins thought to be in the process of healing. The dorsal fins were processed by a combination of the most successful techniques from 1.1. and 1.2. Appropriate fish were selected from the production tank and taken to an on site laboratory, in a 50l bin. The fish were individually netted out and killed by removing their head, which served to bleed the fish. They were briefly dipped into the water from which they had been removed, to wash off the blood. The dorsal fin and surrounding tissue was immediately removed (Figure 5.1.) and placed into fixative. The tissue were processed as described above (1.) until they were in the second 100% bath. They then had the excess muscle removed and were attached to small wax sheets. They remained attached to the wax until after critical point drying when they were removed and fixed to the SEM stubs so that the fins formed a 45° angle with the surface of the stub.

CHAPTER V

RESULTS

1. THE DEVELOPMENT OF A TECHNIQUE FOR PROCESSING AND EXAMINATION OF DORSAL FIN TISSUE BY SCANNING ELECTRON MICROSCOPY.

1.1. Preliminary samples.

The distal edge of the fins examined had curled and twisted. The nodular fins showed large numbers of cracks that had occurred during processing. The fins attached to the stubs at 45° were most easily examined. Figure 5.3. demonstrates some of the artifacts observed.

1.2. Evaluation of alternative processing techniques.

The distal fin tissue placed directly into fixative and then later attached to the wax showed contraction and distortion of the tissue between the fin rays. Some of their superficial features of the fins attached to the wax from the outset were obscured by retained mucus. The areas of these fins which had been directly in contact with the wax were inadequately fixed and the epithelium in contact with the tissue paper had acquired a pattern of marks from the tissue paper fibres. The fins with the dorsal muscles retained which were subsequently attached to the wax, appeared to produce the best specimens, however there were a large number of red blood cells on the surface of these fins.

Figure 5.3. (Fish 1 from 1.1., x 27) The lateral surface of a fin with severe nodularity. The fin is orientated with the dorsal edge on the right and the anterior at the top. This fin demonstrates some of the artifacts observed on the more severely hyperplastic fins. There are areas of hyperplastic epithelium (A) and areas with no epithelium (B). There are also cracks due to artifacts (C) and portions of epithelium folded back from the underlying tissue (D). The artifacts were identified by the lack of reaction in the surrounding cells. This fin was sampled as part of 1.1. but a few similar artifacts were observed in the fins sampled later in the study.

1.3. Processing and examination of a range of dorsal fin rot lesions.

All the fins processed in this section were suitable for examination. Most of the features observed in these preparations were also observed in earlier specimens from all the tanks. However all the photographs were taken from the latter preparations since they were relatively free from artifacts. The photographs represent the best examples of the observed features and the number ascribed to the fish are only intended to differentiate between individuals. Unfortunately no fins with substantial healing were available for examination. All the photographs are enlarged four times from the 35mm negatives, which were 0.67 × the size of the original screen. The magnification of the specimen represented by the photographs are recorded in the legends.

All the information pertaining to each photograph has been combined with the figure legend to make reference between the photographs and the text easier.

Figure 5.4. (Fish 1, × 54) The dorsal edge of an undamaged dorsal fin of a farmed parr. The smooth areas (A) overlay the fin rays, it is the corrugated areas between the fin rays (B) which are capable of expansion.

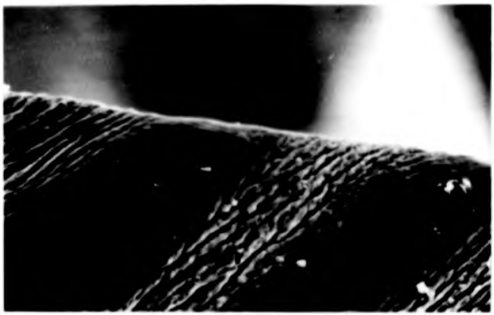
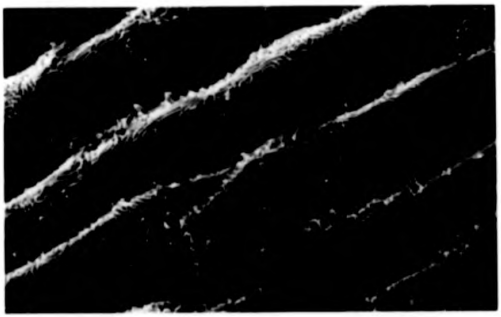


Figure 5.5. (Fish 1, × 3357) An enlargement of an area of smooth epithelium over a distal fin ray. The microridge pattern of the superficial epithelial cells is displayed. The nodular extensions from the microridges in the bottom right (A) may be small accumulations of mucus.

Figure 5.6. (Fish 1, × 1679) An area of epithelium between the distal fin rays. Strands and small globules of mucus have been retained on the superficial epithelium.



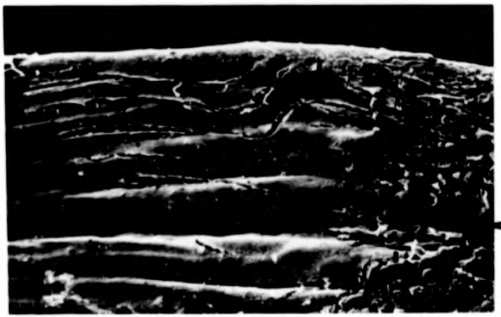


Figure 5.7. (Fish 2, × 13) Leading edge of a dorsal fin with some erosion and nodularity. The dorsal edge of the fin is at the right and the anterior edge at the top of the photograph. The distal part of the fin has a rough disorganised appearance with clefts (A) and nodules (B). One large split in the fin (C) extends in from the right. There are several deep parallel clefts (D) in the otherwise normal lateral tissue of the fin.

Figure 5.8. (Fish 3, × 27) The lateral surface of a fin with some slight peripheral erosion. There are a number of deep clefts in the epithelium (A), surrounded by a small areas of raised tissue. The majority of the remaining epithelium is apparently undamaged.

Figure 5.9. (Fish 3, × 215) An enlargement of one of the clefts from Figure 5.8. Some of the epithelial cells on the edge of the wound appear rounded, smooth and enlarged (A). One of these cells is spherical and may be a leucocyte (B). The area of the cleft is surrounded by apparently normal epithelial cells and mucous cell pores (C).

Figure 5.10. (Fish 3, × 839) A crack in the epithelium at the base of the fin. This defect which is thought to be a processing artifact has a very different appearance from the clefts in Figures 5.6., 5.7. and 5.8. This split in the tissue follows the edge of the superficial epithelial cells and in some areas superficial cells have been removed leaving a clear outline of their shape (A). None of the surrounding cells have undergone hypertrophy or other changes despite some obvious damage (B).

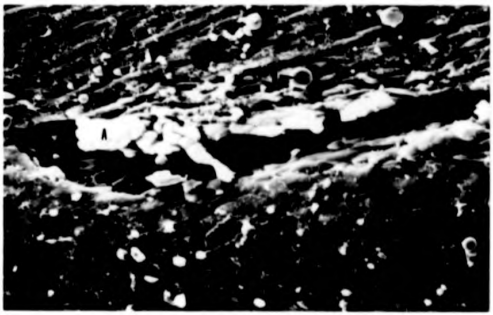
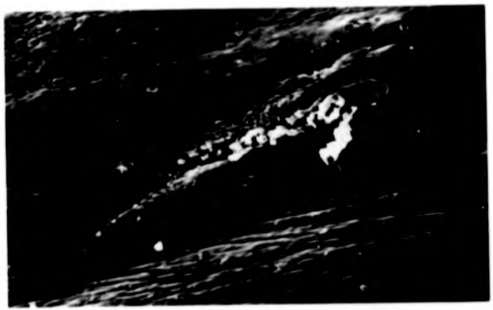


Figure 5.11. (Fish 4, × 320) A cleft on the lateral surface of a fin with some erosion. This lesion is on the junction between the corrugated and smooth epithelium associated with the fin rays. Again some of the surrounding epithelial cells appear rounded and enlarged (A). However on the lower side of the cleft there is an area where the superficial cells appear to form a smooth edge to the lesion. There are also some more spherical cells, possibly leucocytes (B).

Figure 5.12. (Fish 5, × 54) A cleft on the lateral surface of a fin with some erosion. The edges of the cleft are mostly smooth, with the superficial cells apparently extending into the lesion. Within the cleft there are a large number of rounded cells which protrude in a disorganised manner. Most of the surrounding epithelial cells are normal but there is a small area where the cells have a roughened appearance (A).



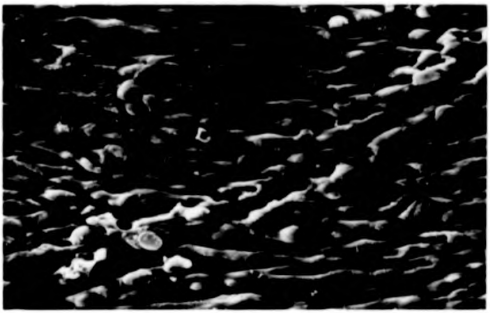


Figure 5.13. (Fish 6, × 215) A corrugated area on the lateral surface of a slightly nodular fin. There is a depression in the epithelial cells running from the bottom left to the top right of the photograph (A). Some of the superficial cells are rounded and a few may be in the process of sloughing (B).

Figure 5.14. (Fish 7, # 13) The dorsal edge of a severely nodular and eroded fin with some of the fin rays protruding (A) from the hyperplastic tissue. Most of the epithelium in this area is thickened and rough. There are numerous clefts in the superficial tissue. On closer examination only a few of these appeared to be processing artifacts.

Figure 5.15. (Fish 8, × 54) The dorsal edge of a fin with a similar gross appearance to fish 7. The fin rays are protruding from roughened nodular hyperplastic tissue. When examined at higher magnification the irregular surface on some of the fin rays (A), consisted largely of bacteria (Figures 5.16. and 5.17.). In one area there was no epithelial cover (B). This was thought to be a processing artifact.

Figure 5.16. (Fish 9, × 107) A broken fin ray extending from the dorsal surface of a severely thickened nodular fin. An enlargement of the material adhering to the fin ray (A) is shown in Figure 5.17.

Figure 5.17. (Fish 9, * 3357) An enlargement of the material adhering to the fin ray in Figure 5.16. The material is largely composed of bacteria, which are mostly rods of various length. There is one chain of long rods (A), possibly cytophage-like bacteria. There is also a large amount of granular (B) and fibrous (C) material. This is thought to be fish mucus or bacterial glycocalyx. Bacterial plaques were only seen on the fin rays.

Figure 5.18. (Fish 9, × 107) An area of severe epithelial hyperplasia. Many of the superficial cells are enlarged and globular, however the mucous cell pores are still detectable (A).

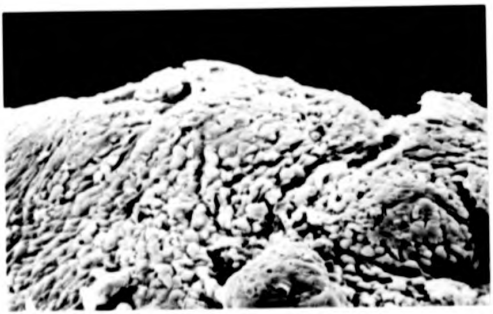
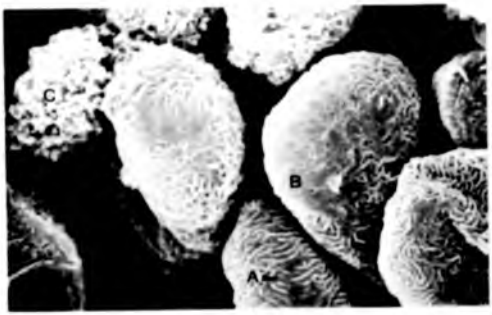


Figure 5.19. (Fish 9, × 430) A magnification of a central area from Figure 5.18. There is a depression running vertically through the area (A) possibly a healed wound. There appeared to be at least two forms of pores, some with the typical appearance of mucous cell pores (B) and some within individual cells which were less typical (C). On closer examination they all appeared to be mucous cell pores. The abnormal appearance may have been caused by distortion of the neighbouring cells.

Figure 5.20. (Fish 9, × 1679) An area of globular epithelial cells from Figure 5.18. In some areas the microridges appear to be coalescing and losing definition (A). In other areas it is difficult to determine whether the cells have lost their microridges or if they have been covered by some homogenous material, possibly mucus (B). Other cells are shrunken with nodular microridges (C), these cells were probably in the process of sloughing.



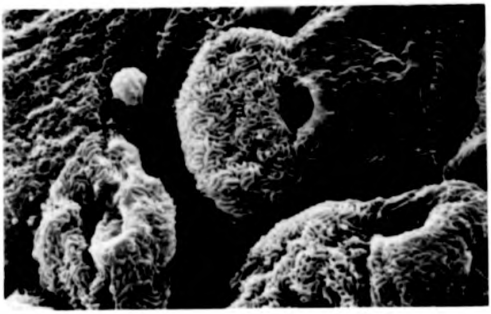
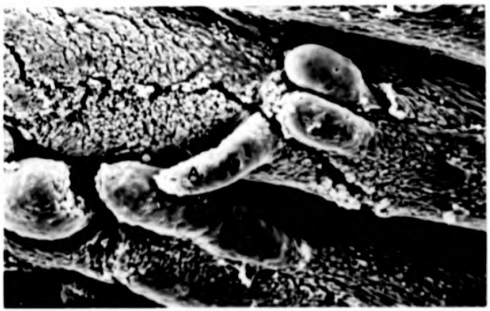


Figure 5.21. (Fish 9, × 1679) An area from Figure 5.18., which demonstrates areas of abnormal microridges, both flattening (A) and excessive nodular convolutions (B). Mucous cell pores are also present in this area (C).

Figure 5.22. (Fish 11, × 54) The lateral surface near the dorsal edge of a fin with severe nodular hyperplasia. Outgrowths of hyperplastic tissue (A) were commonly observed on the surface of such fins.



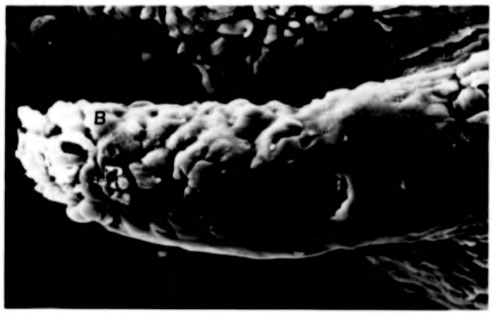


Figure 5.23. (Fish 11, × 215) An enlargement from Figure 5.22., in this case the outgrowth was covered both by areas of smooth relatively normal epithelium (A) and roughened nodular epithelium (B). In other cases outgrowths were observed entirely covered by either normal or abnormal epithelial tissue.

Figure 5.24. (Fish 12, × 27) The dorsal edge of a fin with severe tissue loss. The majority of the epithelium is relatively normal with limited areas of slight roughening of the surface (A). Some epithelial outgrowths can be seen near the edge (B). There is also a cleft extending through the soft tissue of the fin (C).

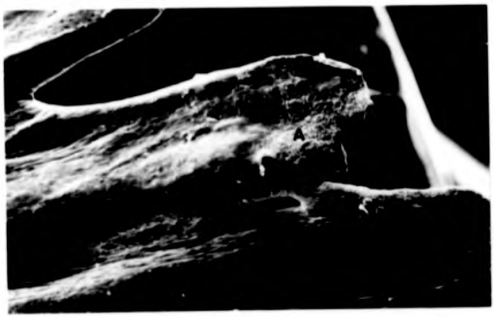


Figure 5.25. (Fish 12, × 107) An enlargement of the cleft in the fin shown in Figure 5.24. The defect is surrounded by both areas of normal smooth (A) and rough (B) epithelium.

CHAPTER V

DISCUSSION

A period of development was undertake to reduce the artefacts produced during processing (Figure 5.3.). Although the artefacts were reduced by the latter methods of processing they were not eliminated. The cracks and loss of tissue, due to artefact, were most frequently observed in the areas of severe hyperplasia, presumably this tissue was more susceptible to damage.

The epithelium of the undamaged fins (Figures 5.4., 5.5. and 5.6.) was similar to that in other areas of the Atlantic salmon (Jóhnsdóttir, Wootten, Bron and Turnbull, In press). Microridges are typical of teleost epithelium but their structure varies between species, in Atlantic salmon they are thicker and more densely packed than those of the coho salmon (*Oncorhynchus kisutch*) or the steelhead trout (*Oncorhynchus.mykiss*) (Hawkes, 1974). The microridges are thought to serve several functions, they provide resistance to mechanical trauma, may improve gas exchange by increasing the surface area up to 2.5 times and help to retain the protective layer of mucus (Randall, 1970; Hawkes, 1974). Among the cell surfaces examined in other animals there are few examples of surface microridges, the cells that do have them are usually associated with sites where retaining a mucus layer is advantageous, such as squamous cells of the female reproductive tract (Williams, Jordan, Murphy and Allen, 1973; Parakkal, 1974).

The SEM study produced strong evidence to support the theory that dorsal fin rot is associated with bite wounds on the fin. Figure 5.7. shows wounds apparently caused by a number of small sharp regularly spaced points being dragged across the fin. The wounds on the fins (Figures 5.7. - 5.12.) were all consistent with damage resulting from the teeth of other parr. Indeed, there were no other objects in the production tanks which could have caused such lesions. The wounds on the fin were also easily differentiated from cracks due to processing (Figure 5.10.) since these artifacts showed no evidence of changes in the surrounding cells.

The response of teleost epithelium to injury has been reported by several authors, although the SEM appearance has not formed a large part of these studies. Healing occurs initially by migration of epithelial cells and not by the burst of mitotic activity typical of mammals (Bereiter-Hann, 1986). The migration of teleost epithelial cells bears some resemblance to the healing of mammalian serous membranes. The time taken to seal an epithelial wound is largely unaffected by temperature and more rapid than in mammals. Anderson and Roberts (1975) reported that it took, depending on temperature, between 3 to 24 hours to develop a 2 to 3 cell thick cover over the wound. Work by Roubal and Bullock (1988) agreed with these findings and suggested that the closure of the wound was not affected by the presence of corticosteroids. The rapid closure of superficial wounds serves to protect fish from pathogens and adverse osmotic effects of either fresh or salt water.

Damage to the epithelium frequently results in a hyperplastic response especially at lower temperatures (Roberts and Bullock, 1976). Stimuli for epithelial hyperplasia include chemicals, hormones and putative viral agents (Roberts, 1989). It has also been suggested that bacterial extracellular products can induce epithelial hyperplasia. Kudo and Kimura (1983c) claimed that a bacterial product was responsible for the hyperplasia associated with bacterial gill disease. However other workers (Speare *et al*, 1991b) have claimed that the epithelial hyperplasia in fish is a direct response to necrotic cells, similar to that seen in the mammalian bronchus (Meyers and Katzenstein, 1988), which is presumed to be mediated by the release of substances from the damaged cells. In mammals epidermal hyperplasia is usually associated with either the stratum spinosum (acanthosis) or the stratum corneum (hyperkeratosis) (Jones and Hunt, 1983), whereas hyperplasia in teleost epithelium occurs at all levels since cells are mostly viable and not well stratified.

The wounds depicted in Figures 5.7., 5.8., 5.9. and 5.11. were all recent. Since they appeared to be almost surgical in nature and not yet sealed it must be assumed that they were less than 24 hours old. Some of the cells surrounding the wounds appeared to be swollen with loss of surface structure, probably due to a combination of the initial injury and exposure to osmotic stress. In addition there were some smooth spherical cells with an appearance typical of lymphocytes (Blaxhall, 1983). However positive identification of the cell type is difficult under SEM. These cells may either have been the only blood cells at the site or they may have been more firmly attached and

therefore remained *in situ* while others were removed. In Figures 5.9., 5.11. and 5.12. the superficial epithelial cells at the edge of the wound appear to have extended into the wound. The processes of repair and sloughing of damaged tissue appear to have been proceeding concurrently in Figure 5.12. The areas of rough epithelium indicating swelling, possibly hypertrophy and hyperplasia were present near wounds which were surrounded by otherwise normal epithelium (Figures 5.12. and 5.13.). The proposed sequence of events within a wound is represented diagrammatically in Figure 5.26. (see over).

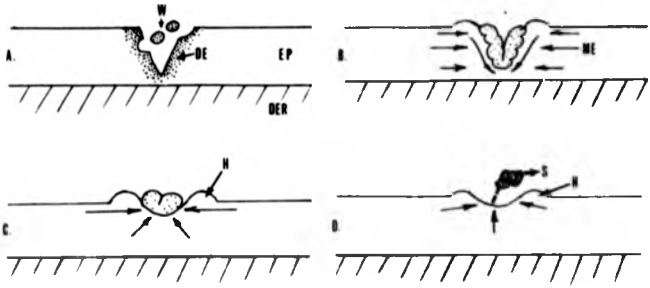


Figure 5.26 Proposed sequence of events from initial injury (A), to sloughing of the necrotic tissue (D). W = wound. S = sloughing tissue. DE = damaged epithelium. EP = epithelium. DER = dermis. ME = migrating epithelium. H = hyperplastic epithelium. Following injury the damaged cells may be lost immediately, undergo more gradual degeneration and eventual sloughing or may recover. The migrating epithelial cells would rapidly smooth the edges of the wound and soon result in the protrusion of the necrotic tissue from the wound. Concurrently, in response to the damage, the surrounding epithelium may become hyperplastic. Repeated injury in an area may result in an accumulation of hyperplastic tissue.

Wounds were more common in hyperplastic fins and especially on the areas of abnormal epithelium (Figure 5.14.). It is not possible to accurately age the wounds on the hyperplastic tissue since there is little if any information regarding the healing response of such

tissue. Therefore the hyperplastic tissue may either have been more frequently damaged or may have taken longer to heal, once damaged.

Bacteria were only observed on the exposed fin rays. In these sites the bacteria were present in thick plaques including material that could have been either fish mucus or bacterial glycocalyx (Figure 5.17.). There are two main reasons for suspecting that the fin rays were the main area of firm bacterial attachment. First bacteria were not observed in other sites, even on the fins with retained mucus and red blood cells (1.2.). Secondly the bacteria associated with bacterial gill disease (Ostland, Ferguson, Prescott, Stevenson and Barker, 1990) can be readily detected by light microscopy, but the tissue examined in chapter I failed to demonstrate any other area of significant bacterial colonisation.

It is necessary to examine previous results in light of these findings. In chapter III, experiments 2.1. and 2.2. produced an unexpected difference in bacterial populations between individual excoriated fish. It was postulated that the observed higher bacterial populations might have been due to additional damage. The observation of large bacterial colonies on the exposed fin rays provides an alternative explanation. It is possible that the fish with higher bacterial populations later in the experiments had exposed fin rays with the associated bacterial colonisation. Although every attempt was made to standardise the damage, the nature of the excoriation was crude and variations between fish were inevitable. There still remains some discrepancy between the SEM and bacteriological findings. Wounds of

various ages were examined by SEM, however no evidence of the temporary rise in bacteria following damage was observed (chapter III, 2.1. and 2.2.). It can only be assumed that the bacteria responsible for the initial temporary colonisation were not firmly attached and consequently were removed during processing for SEM.

It is impossible to accurately identify bacterial types by SEM but the findings were consistent with a mixed population containing some cytophage-like bacteria. The large dense plaque of bacteria also serves to emphasise the potential differences between the number of viable cells and the number of colony forming units.

The distal accumulation of hyperplastic tissue typical of dorsal fin rot was present in large mounds (Figure 5.18.) and also in small finger-like extensions (Figure 5.22. and 5.23.). In cases of grossly thickened fins the surface of the epithelium was hyperplastic with a rough nodular surface (Figure 5.18.). Individual cells showed a range of changes, from gross swelling possibly hypertrophy to severe shrinking (Figures 5.20. and 5.21.). Many of the superficial cells appeared to be in the process of sloughing and all of the cells had abnormal microridge patterns with excessive convolutions or increased nodularity. These changes were very similar to those reported by Kudo and Kimura (1984) and Speare *et al*, (1991) associated with bacterial gill disease. Occasionally hypertrophic cells appeared to be losing microridge definition though this was difficult to differentiate from the covering of microridges by mucus (Figure 5.20.). During processing most of the mucus was removed intentionally to allow examination of

the superficial epithelium. Retaining the mucus might have retained more superficial bacteria. However previously workers have encountered difficulties in either completely removing or fixing teleost epithelial mucus *in situ* (Handy and Eddy,1990).

The origin of the outgrowths depicted in Figures 5.22. and 5.23. is debatable. The outgrowths were seen partially or completely covered in normal epithelium and were present on the healed fins (Figures 5.24. and 5.25.). Therefore these structures were apparently relatively stable once formed, suggesting the dermal support which was observed in similar outgrowths examined by light microscopy (chapter I). They may have either resulted from partially detached pieces of tissue including some dermis or from the abnormal development of both the dermis and epidermis.

Since the hyperplastic tissue associated with dorsal fin rot was similar to that seen in bacterial gill disease it is reasonable to assume that healing progresses in a similar fashion, that is, primarily by necrosis and sloughing of the superficial cells (Kudo and Kimura, 1983a). Healing was discussed in greater detail in chapter IV.

As already mentioned no fins with substantial amounts of regenerated tissue were obtained for SEM examination. There was however no reason to suspect that the epithelium on such fins would have been abnormal. Most of the epithelium of the healed fins in Figures 5.24. and 5.25. was normal although there were still some small rough areas.

These could either have been the result of protracted recovery or subsequent damage.

In most of the diseased specimens the pathological changes varied over the surface of the fins. As observed in the gross specimens the distal edge tended to be most severely but not necessarily uniformly affected. On a smaller scale there were areas of normal, hyperplastic and sloughing tissue all interspersed (eg Figure 5.22.). Therefore the gross appearance of the fin was a product of damage, the response to damage and the healing, all proceeding concurrently.

This SEM study proved to be more informative than anticipated. In retrospect SEM could have been useful in many of the earlier studies. A great deal of useful information might still be obtained by SEM studies of controlled damage and healing of normal and hyperplastic epithelium.

CHAPTER VI

A behavioural study into patterns of aggressive attack and the resultant damage in Atlantic salmon (*Salmo salar* L.) parr.

CHAPTER VI

INTRODUCTION

A this stage in the study the most likely cause of dorsal fin rot was thought to be physical trauma arising from fish bites. This proposed aetiology was strongly supported by evidence from experiments conducted to artificially induce fin rot (chapter IV) and the SEM study (chapter V). In addition, very little evidence had been found to support some of the alternative aetiologies that have been proposed, for example bacterial infections (chapter III) and water quality (chapter II).

Although there are published reports of damage to the dorsal fin caused by other fish, there is only one paper that provides evidence to relate this damage to fin rot (Abbott and Dill, 1985). In a study of rainbow trout they found that a considerable amount of damage was caused to the dorsal fin as the result of bites, they proposed that "aggressive interactions may be the major cause of fin damage in hatchery salmonids". This theory has been adopted by subsequent authors without additional corroboration (Holm and Møller, 1988, Holm; 1989; Kindschi, Shaw and Bruhn, 1991). It was considered important to investigate this assumption further using Atlantic salmon.

There is a considerable body of work describing the behaviour of Atlantic salmon, a proportion of which is not directly relevant as it was conducted on wild fish and in simulated natural conditions.

However there is evidence that the behaviour of salmon is different from other species of salmonid and that the dorsal fin is not necessarily a prime target in aggressive interactions. Gibson (1978) found that charges and chases were more common than displays in Atlantic salmon when compared to brown trout. Kalleberg (1958) claimed that sham fights were more common than actual contact in Atlantic salmon especially between larger parr. Keenleyside and Yamamoto published a comprehensive paper on the behaviour of Atlantic salmon in 1962, in which they reported that most bites are directed towards the caudal fin with only occasional attacks on the other fins.

The aim of this study was to analyse agonistic interactions and the resulting damage in groups of Atlantic salmon, to determine whether the dorsal fin was damaged by bites. Agonistic behaviour was defined by Scott and Fredericson (1951) as all activities directly associated with fighting.

The experiments were based on those conducted by Abbott and Dill (1985) since the objectives were similar. This involved the use of video-recordings to analyse behaviour. Although such recordings have the disadvantage that they present three dimensional events in two dimensions, there should be no consistent directional bias (Abbott and Dill, 1985). Since little information was available about any diurnal variation in behaviour full photoperiods were video-taped and then randomly sampled.

VI
(Introduction)

Agonistic behaviour in Atlantic salmon consists primarily of charging, chasing, biting (or nipping), fleeing, frontal and lateral displays (Kalleberg, 1958, keenleyside and Yamamoto, 1962). Other behaviour patterns have also been reported in other species of salmonids, for example mouth fighting in rainbow trout, Abbott and Dill, (1985). Initially, it was necessary for the author to become familiar with these behaviours and develop a method for recording interactions. Therefore the initial experiments were conducted with pairs of fish to facilitate detailed observations. The subsequent experiments involved larger numbers of fish to maximise agonistic behaviour.

CHAPTER VI

MATERIALS AND METHODS

1. PRELIMINARY BEHAVIOURAL STUDY

In order to become familiar with aspects of salmonid behaviour two small preliminary experiments were conducted.

1.1. Interactions between two parr following removal of a partition.

Two parr from farm 4 were introduced to a divided 30l glass aquarium. The aquarium was screened from the rest of the laboratory such that the only visual access to the tank was through a small opening for the video camera lens. The tank was supplied with an artificial light source with an eight hour photoperiod. The fish were fed through a small opening in the top of the tank. The fish did not start to eat regularly for one week. After two weeks the tank divider was removed and the fish allowed to come into contact. The behaviour of the fish was then recorded on video tape over the following eight hours.

1.2. Interaction between two parr introduced to a small aquarium.

A further two parr from farm 4 were introduced to the same system without the tank divider. On this occasion their behaviour was recorded over the first two, eight hour, photoperiods.

2. INTERACTIONS BETWEEN GROUPS OF FISH

At this stage it was considered appropriate to use larger groups of fish. The fish used were taken from farm 1 and kept in a 1m circular

tank at the Institute of Aquaculture, University of Stirling, for at least two weeks before introduction to the experiments. Some of the fish used had up to three splits on individual fins, none exceeding 3mm prior to introduction to the system. Each fish was examined briefly and those with excessive damage were rejected. However a detailed description was not recorded to avoid further stressing or damaging the fish.

A 100 l glass aquarium shielded from external light and visual interference was used in all the experiments. The tank was supplied with an internal power filter, aeration and an artificial nine hour photoperiod. The light was provided by two tungsten lights and produced 10.67lux at the surface of the water. In addition a 30l divided aquaria was situated next to the main tank. This smaller tank was supplied with similar filtration, aeration and photoperiod. When the fish were introduced to the main aquarium a further two fish were placed in isolation in the 30l tank. At the end of each experiment all the fish were individually inspected and the damage to the fins recorded. Since most of the damage consisted of splits, the number and size of the splits on each fin were recorded.

The fish in the 100 l tank were video-taped for the whole nine hour photoperiod on two occasions. The time was recorded with the aid of a digital clock which was included in the field of view. Fifty random numbers were produced between 1 and 540, each number corresponded to a time during the nine hours (540 minutes) of video tape. The video-tapes were analysed by examining and recording the first aggressive

interaction after the 50 random points within each nine hour period. The details recorded from each interaction included whether the interaction was reciprocal, the site of any aims and nips, the position of both fish before the confrontation and a brief description of the behaviour patterns displayed.

The interaction was described as reciprocal if both fish charged or bit their opponent. Aims and bites were described according to the site on the target fish (Figure 6.1.) according to the method described by Abbott and Dill (1985). Aims were estimated from a straight line through the tail and the eye of the attacking fish. In some cases it was not possible to determine the site of an aim or bite due to the direction of the attack or the action being obscured by the body of fish. Such interactions were not included in the statistical analysis.

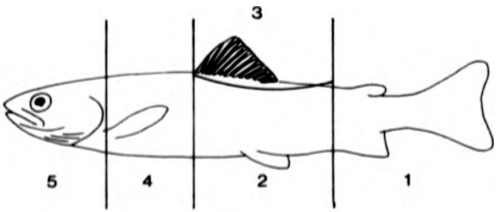


Figure 6.1. Numbers ascribed to the areas of the victim's body.

The position of the fish were recorded as follows :

1 = resting on the bottom of the tank

2 = tail still in contact with the bottom but the rest of the body at 45°

3 = in mid water

The behavioural patterns included a brief description of the sequence of events including recognisable behaviour patterns *eg* frontal display, lateral display, charge, chase and bite.

Most of the interactions occurred very rapidly and it was therefore essential to examine them with the aid of slow motion, freeze frame and frame advance facilities.

The first experiment in this section was a brief pilot study. The subsequent three experiments, despite slight differences, were considered to be replicates and some of the resulting data was combined for analysis.

2.1. Individual marking of fish.

Ten parr with undamaged fins were anaesthetised with 2-phenoxyethanol and marked with the Panjet system (Wright Dental Group, Dundee) using alcian blue (Sigma Chemical Co). Each fish was marked in the same position on both sides of its body. A combination of three sites below and three above the lateral line allowed all the fish to be individually identified (Figure 6.2.). The fish were allowed one hour to recuperate and then placed in the aquarium. After a delay of three hours the fish were video-taped for a further three hours. The

resulting tape was examined to determine the suitability of the system for studying agonistic behaviour and the value of the alcian blue marks.

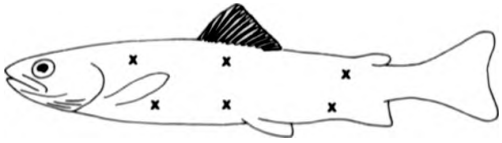


Figure 6.2. Position of marks (X) used to identify the fish in 2.1.

2.2. Behavioural study on the day of introduction and after three days.

Thirteen fish were randomly selected and placed into the system. Their mean fork length was 9.85cm (standard deviation 0.59). The interactions of the group of 11 fish were recorded on video-tape for nine hours on the day of introduction and a further nine hours three days after introduction. The information described above was recorded.

2.3. Behavioural study on the third and fourth day after introduction.

A further eight experimental and two control fish were selected.

Their mean fork length was 10.73cm (standard deviation 0.58). In an attempt to increase the number of interactions recorded, the fish were video-taped on the third and fourth day after introduction to the system.

2.4. Behavioural study of a group containing one fish with severe dorsal fin rot, three and four days after introduction to the system.

On this occasion one fish with severe fin rot and seven with undamaged fins were placed in the main aquarium. Their mean fork length was 9.33cm (standard deviation 0.85) The fork length of the fish with fin rot was 9.33cm and there were three smaller fish in the main aquarium. Again the interactions were recorded three and four days after introduction to the system.

CHAPTER VI

RESULTS

1. PRELIMINARY BEHAVIOURAL STUDIES

In both experiments differences in colouration of the fish allowed them to be individually identified throughout.

1.1. Interactions between two parr following removal of a partition.

The fish tended to stay in the side of the tank to which they had been confined prior to removal of the partition. Only six agonistic episodes were observed. The main incidents that occurred followed a classical pattern for Atlantic salmon parr, with examples of frontal and lateral displays, charging, fleeing, chasing and biting. No evidence of specific attacks on the dorsal fin were observed.

1.2. Interactions between two parr introduced to a small aquarium.

With the exception of one brief charge no agonistic behaviour was observed during this experiment.

2. INTERACTIONS BETWEEN GROUPS OF FISH

The results from this work have been divided into two parts. First there is a brief description of information pertaining to individual experiments and then the bulk of the data has been combined for presentation and analyses. The temperature during these experiments was in the range of 13 to 20 °C.

VI
(Results)

2.1. Behavioural interactions between a group of marked fish.

The video picture was of insufficient quality to allow identification of the individual fish. Although the agonistic interactions were not recorded in detail they occurred more than once every ten minutes.

2.2. Behavioural study on the day of introduction and after three days.

Only four agonistic interactions were observed on the video-tape from the nine hours immediately following introduction. These interactions were included in the subsequent analysis. On the second occasion after three days, 50 agonistic interactions were recorded.

2.3. Behavioural study on the third and fourth day after introduction.

Fifty agonistic interactions were examined from each nine hour period.

2.4. Behavioural study of a group containing one fish with severe dorsal fin rot, three and four days after introduction.

No agonistic incidents were recorded after the 37th random point on the first day and the 45th point on the second day

It was only possible to identify the fish with dorsal fin rot during a small proportion of the agonistic interactions. Therefore it was not possible to compare the role of this fish in agonistic interactions. Since the dorsal fin of this fish was damaged at the start it was excluded from the analysis of fin damage at the end of the experiment.

COMBINED RESULTS OF 2.2., 2.3. and 2.4.

In some cases there was no agonistic behaviour in the period between random points. In these cases the behaviour observed was ascribed to the random points in turn and not the nearest random point. In some of the experiments less than fifty agonistic incidents were recorded in each day.

Of the 234 agonistic incidents analysed only seven were reciprocal. There was very little evidence of frontal or lateral displays with most of the agonistic incidents involving one brief charge with or without a resulting bite. In some cases the aggressor appeared to charge rapidly at a moving fish and in other cases, especially when both fish were stationary, the approach was slower and resulted in the grasping of a fin. The latter behaviour was observed in all three replicates (2.2., 2.3. and 2.4.) and the target fins included the caudal, dorsal, pectorals and pelvic fins. It was the authors impression that this behaviour was more common in attacks directed at the dorsal fin. However this proved difficult to quantify. Repeated bouts of stereotypic swimming were observed in all the experiments (2.1., 2.2., 2.3. and 2.4.)

The percentage of aimed attacks that resulted in an observed bite for the different areas of the body are presented graphically in Figure 6.3. and the percentage of observed bites on the different areas of the body are recorded in Figure 6.4.

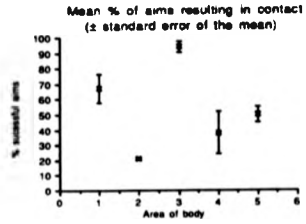


Figure 6.3. The mean (± standard error of the mean) percentage of aims at the different areas of the body resulting in an observed bite, from 2.2., 2.3. and 2.4.

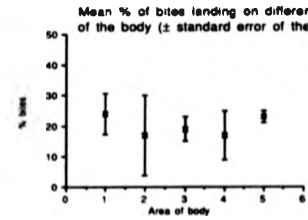


Figure 6.4. The mean (± standard error of the mean) percentage of bites landing on the different areas of the body, from 2.2., 2.3. and 2.4.

VI
(Results)

The damage to the fins consisted of splits in the tissue these were measured and the total damage to each fin calculated. These results were then divided into the following three categories :

1 = 0 to 15mm of splits in the fin

2 = 16 to 30mm of splits

3 = 31 to 45mm of splits

The results from the three replicate experiments are displayed graphically in Figure 6.5.

There was very little damage to the fins of the control fish. One control from 2.3. had a 2mm split in the right pectoral fin and the controls from 2.4. one had a 2 and one a 4mm split in their left pectorals.

Regression analyses were performed on the extent of damage to the fins and the fork length of the fish but no significant relationships were found.

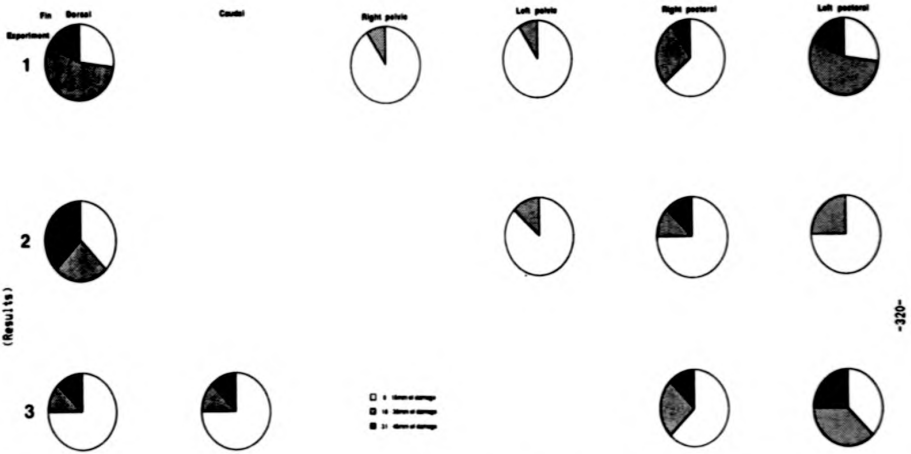


Figure 6.5. The percentage of fins falling into each of the categories of damage (1, 2 or 3). The fins which were all in category 1 (0 - 16mm) have been omitted.

VI
(Results)

The percentage of the bites to the areas of the body containing fins were calculated and compared to the percentage of the total damage occurring on the fins (Table 6.1.)

Table 6.1.
Percentage of the total bites occurring in areas of the body with fins and the percentage of damage to those fins.

Experiment number	1		2		3	
	B%	D%	B%	D%	B%	D%
Area of body						
1 Inc' caudal and anal	23	15	22	12	50	21
2 Inc' pelvics	54	15	4	14	7	9
3 Inc' dorsal	15	26	35	34	23	12
4 Inc' pectorals	8	44	39	40	20	58

B% = percentage of bites and D% = percentage of the damage.

The positions of the aggressor and subordinate fish were recorded before each agonistic event. The positions of the fish were defined as described in the Materials and Methods.

The percentage of the aggressors and victims in each position is recorded in Table 6.2. The numbers of fish were analysed by the X^2 method. There was no significant difference in the position of the aggressors. When the data from the subordinate fish was analysed more than one fifth of the expected frequencies were less than five.

Table 6.2.

Percentage of aggressors and victim fish in positions 1, 2, and 3 prior to agonistic interactions.

	Aggressor			Victim		
Position in tank	1	2	3	1	2	3
Site of attack						
Dorsal fin	50%	32%	18%	45%	23%	32%
Caudal fin	58%	5%	37%	42%	0%	58%
Other	38%	29%	33%	13%	16%	71%

CHAPTER VI

DISCUSSION

1. PRELIMINARY BEHAVIOURAL STUDY

The rationale behind these experiments was to record behaviour under the least complex circumstances, to allow detailed observation of individually identifiable fish. The first experiment attempted to encourage the fish to establish small territories and then induce aggression by removing the partition. The second experiment was conducted in the hope of inducing aggression without the initial delay.

Although these experiments did not produce much detailed information they did serve to familiarise the author with aspects of salmonid behaviour. They also provided an opportunity to formulate a method for recording data. The use of video recordings facilitated the analysis of the interactions, since most could only be analysed with the aid of slow motion and frame advance.

2.1. INTERACTIONS BETWEEN GROUPS OF FISH

One of the main objectives of the second series of experiments was to try to maximise the number of agonistic interactions, to produce as much data as possible from the observations. A number of factors had to be taken into account to achieve this aim. Keenleyside and Yamamoto (1962) made several suggestions for maximising agonistic interactions within small aquarium populations. They recommended the use of freshly caught wild fish, that the fish should not be kept in

overcrowded conditions for prolonged periods prior to the experiments and that the fish should be allowed at least 24 hours to acclimatise before being observed. It has also been reported that in three sizes of tank, most aggression was seen in groups of six to fourteen fish (Keenleyside and Yamamoto, 1962) or in groups of eight to fourteen (Fenderson and Carpenter, 1971).

The use of wild fish was not appropriate in this study since dorsal fin rot exclusively effects farmed fish. Studies since 1962 have suggested that farmed salmonids are more aggressive than wild fish, especially at high stocking densities. This increased aggression has been shown to have both a genetic (Swain and Riddell, 1990) and environmental (Fenderson and Carpenter, 1971) component.

An attempt was made to keep the fish for at least two weeks under reduced stocking levels. The effect of overcrowding in wild fish is thought to be a suppression of territorial behaviour, therefore maintaining fish at a lower stocking density increases territoriality and aggression (Kalleberg, 1958). It is not clear if the same is true for farmed fish.

The fish in 2.2. were observed on the first day since experiment 2.1. had suggested that there was a large number of agonistic interactions soon after introduction to the system. Keenleyside and Yamamoto (1962) reported that the fish did not start to actively defend territories until at least 12 hours after introduction. However the fish did engage in some agonistic behaviour within the first 12 hours.

VI
(Discussion)

In was considered necessary to examine all aspects of behaviour including the pre-territorial interactions and the establishment of territories. However in the second experiment there was very little agonistic behaviour observed on the first day. There is insufficient information to speculate on the reasons for different levels of agonistic behaviour in 2.1. and 2.2.

Keenleyside and Yamamoto (1962) suggested that there was less tendency for the fish to establish territories at higher stocking densities. There is very little scope for fish to establish territories in commercial farms since the stocking density can exceed one fish per litre. Therefore more than eight fish were placed in the tank in an attempt to reduce the territoriality whilst maintaining a high level of agonistic behaviour. Following analysis of the video-tape from 2.2. it became obvious that with 11 fish in the tank some interactions took a considerable length of time to analyse, so the number of fish was reduced to eight for the subsequent experiment.

The fish were not fed for the four days of the experiment. Although Keenleyside and Yamamoto (1962) and Fenderson and Carpenter (1971) described an increase in aggression after feeding, they only observed the fish for 30 minutes. Symons (1971) demonstrated that the increase in aggression following feeding was limited to the first 45 minutes and in fact agonistic behaviour increased during at least the first three days of starvation, although starvation for more than one week resulted in decreased aggression (Keenleyside and Yamamoto, 1962; Symons, 1971).

VI
(Discussion)

The analysis of the video recording produced a variety of relevant information. The majority of agonistic interactions observed were very simple, consisting of a charge with or without a bite, examples of frontal or lateral displays were infrequent. Gibson (1978) also found that charges and chases were more common than displays in Atlantic salmon compared to brown trout (*Salmo trutta*). Displays are accepted to be due to a conflict of tendencies. Keenleyside and Yamamoto (1962) suggested that frontal displays occur when the fish is more inclined to fight and the lateral when it is more inclined to flee. Therefore frontal displays are more common in territory holding fish towards invaders or subordinate fish, whereas lateral displays are more common when two aggressive fish meet on the boundary of their respective territories. It is possible that under the conditions within this present study the fish did not establish territories and therefore there was less conflict between tendencies and fewer displays.

There were very few reciprocal attacks observed and there were no episodes of repeated displays and attacks described as typical by Keenleyside and Yamamoto (1962). In this study less than 3% of agonistic interactions were reciprocal compared to 20% observed by Abbott and Dill (1985) in rainbow trout (*Oncorhynchus mykiss*)kept under similar conditions.

Many of the bites resulted from a charge culminating in a bite. However many of the attacks on the dorsal fin consisted of a slower approach and grasp. This form of attack was also occasionally

directed at the caudal, pectoral and pelvic fins. It proved difficult to quantify the difference between the two forms of attack, since they were extremes of a continuum rather than distinct behaviours. However the nature of the attacks may be reflected in the significantly higher proportion of attacks on the dorsal fins resulting in bites (Figure 6.3.). It was the subjective opinion of the author that the slower attacks on the dorsal and other fins often resembled a displaced feeding response rather than an aggressive attack. The relatively slow orientation, approach and grasp was similar to the behaviour associated with picking food off the floor of a tank or the displacement feeding response described by Kalleberg (1958). Hoar (1954) also reported that aggressive behaviour in schooling fish frequently appeared to be a displacement reaction. It is possible that the dorsal fin acts as a releaser for a feeding response.

In some farmed populations a small proportion of the fish can be affected with very severe dorsal fin rot whilst the majority have relatively undamaged fins (Personal observation). McDonald, Heimstra and Damkot (1968) proposed that damaged fins in the green sunfish (*Lepomis cyanellus*) may elicit attacks. It is therefore possible that once a parr has a damaged fin the nodular lesion acts as an enhanced releaser. An attempt was made to examine the response of fish to severe dorsal fin rot in 2.4., but unfortunately it was unsuccessful.

Under the artificial conditions in these experiments, bites directed at the dorsal fin constituted a significant proportion of the total bites *ie* 12%, 26% and 17%. The evidence presented here does not support

the assertion of Keenleyside and Yamamoto (1962) that most bites (or nips) were directed to the caudal fin with only occasional attacks directed at the dorsal, pectoral and pelvic fins. Extrapolation from the data produced by Abbott and Dill (1985) studying rainbow trout also indicated a low proportion of bites landed on the dorsal fin (less than 10%) in non-reciprocal aggressive bouts. However they did find that a higher proportion of bites were directed at the dorsal fin in reciprocal bouts and that the dorsal fin was the most damaged of all the fins. The difference between the present study and Keenleyside's and Yamamoto's may have been due to a large number of factors including the environment and the method of observation. At the time their paper was published the authors did not have access to video technology.

In addition to the number of bites there was also substantial damage to the dorsal fins. The dorsal fins and pectoral fins sustained the greatest damage, with minor damage to the pelvic fins on two occasions and substantial damage to the caudal fins in one experiment. It appeared that the dorsal fins actually sustained the most damage but unfortunately the data was not suitable for statistical analysis. The gross appearance of the damage was identical to the first sign of damage in the simulated bite experiments (chapter IV) and the naturally occurring lesions described as ray splitting (chapter I).

Kalleberg (1958), studying Atlantic salmon in a simulated natural environment, claimed that sham fights were more common than actual contact in Atlantic salmon especially between larger parr. In the

present study between 21% and 94% of attacks appeared to result in contact. Analysis of the percentage bites compared to the damage indicated that the pectorals may have suffered relatively more damage per bite. It is possible that the fish were able to grasp and damage the pectorals more easily than the other fins, assuming that the observed bites reflected accurately the total bites occurring during the experiment.

With three minor exceptions, the fins of the control fish appeared undamaged at the end of the experiments. Since all the fish with one exception (fish with dorsal fin rot 2.4.) had very minor damage to the fins at the start of the experiments, it seems reasonable to assume that the deterioration in the condition of the fins was caused by the other fish. It is not possible to say if the small splits observed in the control fish were present before the fish were introduced to the system. However the splits observed in the controls were small enough to be considered insignificant.

Some of the fish in the experimental aquarium were observed to perform periods of stereotypic or escape swimming. This behaviour which consists of one or more of the fish swimming repeatedly up and down the side of the aquaria is common among many fish held in rectangular aquaria (Keenleyside, 1955; Fenderson and Carpenter,1971). Although it was not quantified it is possible that such behaviour was more common among the experimental compared to the control fish. It is however unlikely that this behaviour contributed significantly to

the fin damage, since with the exception of the air stone there were no rough or abrasive surfaces in the tank.

No significant relationship was demonstrated between the size of the fish and the damage sustained. Abbott, Dunbrack and Orr (1985) demonstrated that a 5% weight advantage was sufficient to ensure dominance and an associated lower level of fin damage when the fish were held in pairs. Although the length and not weight was measured in these experiments the lack of any correlation may reflect the failure of fish to establish territories during the experiments and reduced perception of dominance and subordinance in farmed fish (Fenderson and Carpenter,1971; Abbott *et al* 1985). Within schools of fish there may be so-called dominant individuals which are more frequently aggressive, but it is not possible to predict which fish will be dominant in any given interaction (Stringer and Hoar, 1955). Under any circumstances salmon respond to each other as territory holder, invader, dominant or subordinate. They do not develop hierarchical groups, or pecking orders as seen in some other fish, birds or mammals (Keenleyside and Yamamoto, 1962).

The work described in this chapter demonstrates that Atlantic salmon parr will attack and damage each other's dorsal fins. The conclusion from the whole study is that dorsal fin rot in Atlantic salmon parr is the result of bites from other fish. In these experiments the biting resulted in damage to the fin but no nodular thickening. This may have been due to the short duration of the experiments and the relatively high temperatures (chapter IV). In addition, as discussed

earlier, the frequency of injury required to produce fin rot at different temperatures has not been defined.

Behavioural experiments such as this are of value in investigating aspects of fish behaviour under controlled conditions. However it is important to emphasise that the conditions during these experiments were very different to those experienced by fish on farms. Therefore further, laboratory-based, behavioural studies would be of limited value. At present there have been very few behavioural studies under farmed conditions probably due to the technical difficulties involved. Alternative methods for investigating aggression have been suggested, Christiansen and Jobling (1990) counted the number of bites on the fins of arctic charr. However Christiansen,J.S. (Pers' comm' and unpublished photographs) confirmed that individual bite wounds are more obvious in Arctic charr than Atlantic salmon. Therefore this technique is unlikely to provide an accurate indication of aggression in Atlantic salmon. Attempts earlier in this study (chapter II) to objectively quantify dorsal fin rot were also unsuccessful. It is suggested that observation of attacks under farmed conditions is the approach most likely to facilitate further study of dorsal fin rot.

GENERAL DISCUSSION

GENERAL DISCUSSION

SUMMARY OF WORK AND RESULTS

Chapter I

The gross and histological appearance of dorsal fin rot were described. The main evidence of damage to the fin were clefts in the superficial epithelium and the host response was dominated by epithelial hyperplasia. In the samples analysed the dorsal fin was often the only or most severely damaged fin.

Chapter II

An unsuccessful attempt was made to develop an objective technique with which to assess the incidence and severity of dorsal fin rot. A range of parameters was examined for the presence of correlations with dorsal fin rot. The only significant relationships demonstrated were the prevalence of dorsal fin rot in smaller fish and at lower temperatures in some populations.

Chapter III

The bacterial populations associated with naturally occurring dorsal fin rot were examined. Initially in association with the lesions, there appeared to be a high number and proportion of CLB (Cytophaga-like bacteria), with no consistent phenotypic characteristics. Subsequent findings suggested that CLB were only associated with a proportion of the affected fish at certain times of the year.

(General discussion)

An investigation into the bacterial populations on dorsal fins following controlled injury demonstrated that, in the majority of cases, there was a transient rise in bacterial numbers which subsided within one week. However some individuals developed a larger and more persistent population of bacteria.

In a bath challenge study, some limited evidence was found to suggest that fish with dorsal fin rot were more susceptible to *A.salmonicida*.

Chapter IV

In experiments to study the response of the dorsal fin to injury, the recovery from surgical wounds was similar to published descriptions. Dorsal fin rot lesions were successfully reproduced by repeated simulated bites. There appeared to be a greater tendency for epithelial hyperplasia at lower temperatures but this was not demonstrated conclusively.

Fish held in isolation began to recover from naturally occurring dorsal fin rot immediately and the length of time for the fin to lose its hyperplastic nodularity was significantly shorter at higher temperatures.

Chapter V

Scanning electron microscopy (SEM) was used to examine superficial pathology. Bite wounds were regularly found on the fin and many other aspects of the pathological processes were demonstrated. Bacteria were only observed on the exposed fin rays.

Chapter VI

In the experiments to examine the agonistic behaviour of parr the pattern most commonly observed was a simple non-reciprocal attack. The fish were shown to both attack and damage each others dorsal fins.

CONCLUSIONS AND HYPOTHESES

Substantial evidence was obtained to suggest that dorsal fin rot in farmed Atlantic salmon parr was caused and maintained by repeated biting. Both the histological and SEM lesions were consistent with bite wounds and repeated simulated biting reproduced typical lesions, while recovery started immediately after affected fish were placed in isolation. Kindachi *et al* (1991) also demonstrated that dorsal fin rot did not occur in isolated rainbow trout (*O.mykiss*). In addition the parr were shown to both bite and damage each other's dorsal fins under laboratory conditions.

Bacteria were not able to either initiate or maintain the lesions. The higher and more persistent populations of bacteria may have been associated with the exposed fin rays which provided a stable substrate for bacterial attachment (Costerton, Geesey and Cheng, 1978). Otherwise there was no strong relationship between the lesion and the bacteria present. The lesions also started to heal when the source of injury was removed regardless of the presence of bacteria. This would suggest that the common practice of using disinfectant compound such as Chloramine T is unlikely to have any effect on the condition.

Dorsal fin rot may be more common at lower temperatures due to a combination of slower healing and increased epithelial hyperplasia (Roberts, 1975). The observed pathology was not closely relatedto the incidence of damage, therefore any method to monitor the condition would have to address the cause *ie* biting directly.

Smaller fish appeared to be more susceptible to dorsal fin rot, although it is not possible to determine if they were small because they were repeatedly attacked (Symons, 1971) or if they were attacked because they were small (Abbott *et al* 1985).

The evidence for a relationship between dorsal fin rot and infection with *A.salmonicida* was not conclusive. However it is possible to speculate that susceptibility to *A.salmonicida* might be affected by two aspects of dorsal fin rot. Either the lesions may provide a route of entry for the bacteria or the stress associated with repeated attacks may predispose to infections. Although the route by which *A.salmonicida* enters the fish is still far from clear (Rose *et al* 1989), there is substantial evidence that stress predisposes to infection (Pickering and Duston, 1983).

In the behavioural study the dorsal fins were only one of the fins attacked and damaged, whereas on the farms, damage to the dorsal fin was frequently seen in the absence of damage to the other fins. This may suggest that the environment on the farm induced the fish to attack the dorsal fin preferentially. Therefore it may be possible to reduce the incidence of attacks by manipulating the environment.

Biting of the dorsal fin appears to be similar to intraspecific aggression in other intensively reared animals, especially feather pecking in chickens. Feather pecking more closely resembles feeding than aggression, which is usually directed at the head. Under feral conditions chickens may spend 50% of their time searching for food (Savory, Wood-Gush and Duncan, 1978). Under farmed conditions this tendency to explore is largely frustrated, it has therefore been suggested that feather pecking may be more common in chickens that spend less time feeding (ADAS, 1976; Appleby and Hughes, 1991). It is possible that the biting of the dorsal fin may have a similar cause. In the wild, parr would spend a considerable amount of their time investigating sources of food, whereas under farmed conditions their food requirement is supplied in a relatively small number of identical, concentrated packages. In pigs it has been suggested that stereotypic behaviour, ear and tail biting may be due to frustrated behaviours or expectations and that a more stimulating environment may prevent such behaviour (Radostits and Blood, 1985; Appleby, 1991). It has also been reported that tail biting may be used to obtain nutrients, by pigs maintained on a mineral deficient diet (Fraser, 1987). Although it is unlikely that the fish obtain any nutrients from the dorsal fin, their behaviour might be affected by nutritional deficiencies.

FUTURE WORK

The initiative for this study came from the perception of dorsal fin rot as a significant problem in farmed Atlantic salmon parr. Therefore it was hoped that the study might lead to a method of control. The work conducted so far has not produced any practical techniques for control but it has suggested some promising areas for further investigation.

As mentioned above, it would be necessary to develop a technique for monitoring the incidence of attacks on the dorsal fin before any method of control could be developed. Since behaviour is influenced by the environment it would be necessary to conduct trials under farm conditions. It would also be helpful to obtain more information about the relationship between the amount of reaction to damage at different temperatures. SEM proved to be a useful technique for examining superficial pathology and could be used to study the response of the fin epithelium to controlled injury and the associated hyperplastic response at different temperatures.

The problem is caused by a form of behaviour influenced by environmental conditions, therefore manipulation of these conditions may provide a means of control. Flow rate in tanks may be an example of an environmental parameter that might be manipulated to reduce biting. It has been reported that under natural conditions parr will hide in the substrate during periods of high flow (Kallenberg, 1958). However the lack of substrate in production tanks combined with a positive rheotactic nature, ensures that the fish actively swim into

the current. Christiansen and Jobling (1990) have demonstrated that increasing the flow decreases damage due to aggression in arctic charr and can also improve feed conversion since at higher flow rates the fish waste less energy on aggression.

Similarities between dorsal fin biting and intraspecific aggression in other species might provide areas for investigation, *eg* feather pecking is less prevalent at lower light levels and it is also easier to prevent feather pecking than stop it once it has started (ADAS, 1976).

If dorsal fin biting is related to frustrated feeding behaviour, alternative or additional methods of supplying food might be investigated, in order to occupy more of the fish's time. For example the frequency, distribution and concentration of feed might be altered as well as the structure of the feed to supply a neutral density feed which would stay in suspension longer.

It may also be possible to select for strains that are less prone to dorsal fin rot, whether as a result of biting less or being bitten less. Such an approach has been useful in poultry where some strains have less tendency to feather peck. However this approach has to be viewed with some caution. Noakes (1986) advised that "responses to artificial selection almost always involve unpredictable twists, especially if behavioural characters are among those being selected.". Doyal and Tarbot (1988) using game theory predicted that aggression in domesticated stock should decrease over time. However Swain and

Riddle (1990) found a genetically related increase in aggression among farmed coho salmon (*Oncorhynchus kisutch*) and postulated that this was due to selection for the largest and consequently the most aggressive individuals. Unfortunately if the whole population becomes more aggressive they expend more energy on competition and less on growth, reducing the beneficial effects of selection for weight gain (Christiansen and Jobling, 1990; Swain and Riddell, 1990).

Since behaviour is not closely related to individual genes, a quantitative genetic approach would be most appropriate. This would require a suitable measurement for the characteristic, sufficient variation in the population and sufficient heritability. It would also be necessary to define all the other characteristics thought to be desirable in the population to avoid detrimental effects from the selection programme. In view of the behavioural nature of the problem, a three generation offspring-parent regression might be the most suitable design (Falconer, 1981). However it is unlikely that such a large and complex program would be practical at present.

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APPENDICES

APPENDIX I

Statistical methods used in this thesis were derived from Sokal and Rohlf (1981) and Snedecor and Cochran, (1980). This appendix contains some of the frequently used statistical terms and methods.

Mean

The arithmetic or sample mean, $\bar{x}$, provides the best estimate of the population mean, μ.

$$\bar{x} = \Sigma x \div n$$

n = number of observations

Σx = sum of the observations

Standard deviation

provides an indication of the variation about the mean, 68.26% of the observations should be contained within the mean ± 1 standard deviation.

$$SD = \sqrt{\sum(x - \bar{x})^2 \div n-1}$$

n = number of observations

SD = standard deviation of the sample mean

Standard error of the mean

Estimates of the sample mean may be presented ± 1 standard error of the mean (± SEM).

$$SEM = s \div \sqrt{n}$$

n = number of observations

s = standard deviation

$$s = \sqrt{[\Sigma x^2 - (\Sigma x)^2 \div n] \div n - 1}$$

Median

The median represents the middle of a ranked series of observations or the average of the two scores which constitute the middle pair.

% Coefficient of variation

The coefficient of variation (CV) is a measure of the relative variability and therefore allows the comparison of variations between populations with different means.

$$\% CV = (s \times 100) \div \bar{x}$$

% Confidence limit of the mean

The confidence limit of the mean (% CL) gives a measure of confidence with which the position of the mean can be predicted *e.g.*

$$95\% CL = \bar{x} \pm t \times SEM$$

$$p = 0.05$$

Wilcoxon's test for matched pairs.

This test involves ranking the absolute differences between the pairs $\neq 0$. The ranks were attributed positive or negative signs corresponding to the the signs of the initial differences. The positive and negative ranks are then summed separately. The lesser sum was the test statistic T. The null hypothesis is 'the medians of the two data sets are not significantly different'. When T is compared to the tabulated values, if T < the tabulated values the null hypothesis is rejected *i.e.*

If T < tabulated value the two data sets are significantly different.

Spearman rank correlation coefficient r_s. The the two data sets were ranked and the sum of the squares of differences between ranks calculated

$$r_s = 1 - 6\Sigma d^2 \div n^3 - n$$

n = number of observations

Σd^2 = sum of squares of differences between ranks

r_s is compared to the tabulated probability distribution, if the calculated value exceeds the tabulated value there is a significant correlation at the relevant significance level.

The Mann-Whitney U test involves computing two statistics U_1 and U_2, the smaller of the two is the test statistic U.

$$U_1 = n_1 \cdot n_2 + [n_2(n_2 + 1) \div 2] - R_2$$

$$U_2 = n_1 \cdot n_2 + [n_1(n_1 + 1) \div 2] - R_1$$

n_1 and n_2 = number of observations in each data set

R_1 and R_2 = Sum of ranks for each population

The null hypothesis is that there is no difference between the medians of the data sets. When U > than the tabulated value the null hypothesis is rejected, indicating a significant difference between the medians of the data sets.

When a statistically significant difference was detected by the Kurskal-Wallis test the difference between pairs was tested by Dunn's multiple comparison procedure (Zar, 1984).

$$Q_{0.05,k} = \bar{R}_2 - \bar{R}_1 \div SE$$

$\bar{R}_2$ and $\bar{R}_1$ = mean ranks of the two data sets *i.e.*

($\bar{R}_1$ = rank sum, $R_1 \div n_1$)

SE = Standard error =

$$\sqrt{[(N(N+1) \div 12 - \Sigma(t^3 - t) \div 12(N-1))(1 \div n_1 + 1 \div n_2)]}$$

N = total number of observations in all (k) groups

t = number of ties for a tied value

n_1 and n_2 = number of observations in each of the two data sets

If the calculated value for Q was greater than the tabulated value for Q at P = 0.05 for the number of groups k, the groups were concluded to be significantly different.

APPENDIX II

Standard curve for optical density of *Aeromonas salmonicida* cultures, provided by N. Auchinachie, Aquatic Vaccine Unit, Institute of Aquaculture, University of Stirling.

APPENDIX III

Data from experiments conducted in chapter III.

The detailed results from chapter III, 1.5.1. are contained in Tables III.1. and III.2., the results from 1.5.2. in Tables III.3. and III.4., the results from 3.1. in Table III.5. and 3.2. in Table III.6.

Table III.1.

The CLB and total cfu from 1.5.1. All the fins were estimated to have 30% - 60% of the fin remaining with severe nodularity, (chapter III, 1.5.1.).

Fish N°	Sample	Plate a CLB	Plate a Total	Plate b CLB	Plate b Total	Plate c CLB	Plate c Total
1	A	0	176	3	177	0	59
	B	1	112	2	174	10	233
	C	7	511	8	342	8	203
	D	4	126	2	83	5	276
	E	11	1035	8	1242	5	832
	F	16	422	12	533	26	228
2	A	0	76	4	82	10	177
	B	1	60	2	8	0	9
	C	1	19	1	31	6	46
	D	3	13	3	22	2	23
	E	0	53	4	67	4	44
	F	1	22	1	36	5	66
3	A	3	18	3	72	0	166
	B	6	43	10	45	17	108
	C	34	129	38	148	37	171
	D	28	142	46	188	37	168
	E	111	480	104	521	90	440
	F	20	61	26	101	74	334
4	A	3	16	0	11	1	9
	B	0	8	1	1	0	1
	C	2	4	1	3	0	0
	D	0	3	0	0	0	1
	E	4	26	3	6	0	6
	F	0	0	1	1	9	12
5	A	2	10	1	9	1	3
	B	0	66	0	67	0	38
	C	0	138	1	184	1	168
	D	1	142	0	70	1	137
	E	3	972	2	731	2	930
	F	1	120	1	68	6	88

Table III,2.

Means (T $\bar{x}$), standard error (T SE), standard deviations n-1, (T s) and % coefficient of variation = s x 100 / $\bar{x}$, (% CV) for the total bacterial counts, (chapter III, 1,5,1,).

Fish N°	Isample	T $\bar{x}$	T SE	T s	T % CV
1	A	137,3	30,3	67,8	49,4
	B	173,0	27,0	60,5	35
	C	352,0	69,0	154,2	43,8
	D	161,7	45,3	101,3	62,7
	E	1036,3	91,7	205	19,8
	F	394,3	69,0	154,4	39,1
2	A	111,3	25,5	57	51,2
	B	24,7	13,7	30,7	124,3
	C	31,7	5,0	13	41,1
	D	19,3	2,5	5,5	28,5
	E	54,7	5,2	11,6	21,2
	F	41,0	10,1	22,6	55,1
3	A	82,0	31,1	69,5	84,8
	B	65,3	16,5	37	56,6
	C	149,3	9,4	21	14
	D	156,0	5,9	13,1	8,4
	E	480,3	18,1	40,5	8,4
	F	165,3	65,9	147,4	89,2
4	A	11,7	1,3	3	26,2
	B	3,3	1,8	4	121,2
	C	2,3	0,9	2,1	89,2
	D	1,3	0,7	1,5	114,6
	E	12,3	4,9	11	89
	F	4,3	3,0	6,7	153,7
5	A	7,3	1,7	3,8	51,6
	B	56,7	7,2	16,2	28,6
	C	163,3	10,4	23,3	14,3
	D	116,3	18,0	40,2	34,6
	E	877,7	57,2	128,7	14,7
	F	94,7	13,4	30,6	32,3

Table III.3.
Condition of fins sampled for bacteriology, (chapter III, 1.5.2.).

Sample date	Fish N°	Fin condition	% fin remaining
9/11/89	1	Severe nodularity	30% - 60%
	2	Severe nodularity	30% - 60%
Mean temp'	3	Severe nodularity	30% - 60%
5°C	4	Severe nodularity	30% - 60%
	5	Severe nodularity	30% - 60%
12/11/89	1	Severe nodularity	30% - 60%
	2	Severe nodularity	30% - 60%
	3	Severe nodularity	10% - 30%
5,5°C	4	Severe erosion	30% - 60%
	5	Severe erosion	30% - 60%
17/11/89	1	Severe nodularity	30% - 60%
	2	Nodularity	60% - 90%
	3	Severe nodularity	30% - 60%
4°C	4	Nodularity	60% - 90%
	5	Nodularity	60% - 90%
7/12/89	1	Severe nodularity	30% - 60%
	2	Severe nodularity	60% - 90%
	3	Severe nodularity	30% - 60%
3°C	4	Severe erosion	30% - 60%
	5	Severe nodularity	30% - 60%
12/2/90	1	Severe nodularity	30% - 60%
	2	Severe nodularity	30% - 60%
	3	Severe nodularity	10% - 30%
2,5°C	4	Severe nodularity	30% - 60%
	5	Severe nodularity	60% - 90%
14/3/90	1	Severe nodularity	30% - 60%
	2	Severe nodularity	30% - 60%
	3	Severe nodularity	30% - 60%
5°C	4	Severe nodularity	30% - 60%
	5	Severe nodularity	30% - 60%
19/4/90	1	Severe nodularity	30% - 60%
	2	Nodularity	60% - 90%
	3	Severe nodularity	30% - 60%
4,5°C	4	Nodularity	60% - 90%
	5	Nodularity	60% - 90%

Table III.4..

CLB cfu, total cfu, mean CLB (x̄ CLB), mean total (x̄ T) and mean CLB as a % of the mean total (% CLB), (from chapter III, 1.5.2.).

Sample date	Fish N°	Plate a CLB	Plate a Total	Plate b CLB	Plate b Total	Plate c CLB	Plate c Total	x̄ CLB	x̄ T	% CLB
9/11/89	1	1	2	1	2	1	2	1,0	2,0	50,0
	2	22	54	17	63	27	57	22,0	58	37,9
	3	1	7	2	5	0	5	1,0	5,7	17,5
	4	8	30	5	19	6	24	6,3	24,3	25,9
	5	1	8	0	5	4	11	1,7	8,0	21,3
12/11/89	1	5	16	1	10	2	8	2,7	11,3	23,9
	2	4	253	2	167	3	271	3,0	203,3	1,3
	3	6	67	3	57	4	33	4,3	52,3	8,2
	4	1	26	5	25	9	30	5,0	27,0	10,5
	5	1	47	5	56	5	44	3,7	49,0	7,6
17/11/89	1	2	351	2	425	0	164	1,3	313,3	0,4
	2	0	31	0	26	2	49	0,7	35,3	2,0
	3	6	127	5	56	0	82	3,7	88,3	4,2
	4	5	14	6	22	8	24	6,3	20,0	31,5
	5	0	10	1	4	0	1	0,3	5,0	6,0
7/12/89	1	4	126	2	83	5	276	3,7	161,7	2,3
	2	3	13	3	22	2	23	2,7	19,3	14,0
	3	28	142	46	158	37	168	37,0	156,0	23,7
	4	0	3	0	0	0	1	0,0	1,3	0,0
	5	1	142	0	70	1	137	0,7	116,3	0,6
12/2/90	1	11	44	12	152	5	21	9,3	72,3	12,9
	2	45	1185	56	765	28	42	39,7	664,0	5,8
	3	0	259	0	195	0	10	0,0	154,7	0,0
	4	47	106	74	147	95	414	72,0	222,3	32,4
	5	17	26	25	156	35	189	25,7	123,7	20,8
14/3/90	1	212	2248	198	1808	198	2220	202,7	2092,0	9,7
	2	2168	2176	1024	1032	1247	1256	1479,7	1488,0	99,4
	3	1	7	3	12	1	7	1,7	8,7	19,0
	4	1516	1520	1340	1356	1500	1504	1452,0	1460,0	99,5
	5	876	902	936	961	1088	1103	966,7	988,7	97,7
19/4/90	1	0	38	1	41	contaminated		0,5	39,5	1,3
	2	630	712	575	633	556	622	587	655,7	89,5
	3	2	40	6	37	11	37	6,3	38,0	16,6
	4	1363	1503	1431	1540	overgrown		1397,0	1521,5	91,8
	5	3	18	4	24	1	16	2,7	19,3	14,0

Table III,5.

Day 1 represents 24h post challenge, day 2 48h *etc*, (chapter III, 3,1,).

Initial challenge 4,7*10²

Bin N° 3

Day	1	2	3	4	5	6
Temperature °C	18	18	18	19	19	19
Good fins						
Total mortalities	0	0	0	0	20	
Total *A. sal* recovered					5	
Recovered kidney					4	
Recover fin					2	
Fin rot						
Total mortalities	0	1	0	1	16	3
Total *A. sal* recovered		0		0	4	0
Recovered kidney					4	
Recovered fin					2	

Initial challenge 4,7*10²

Bin N°4

Day	1	2	3	4	5	6	7
Temperature °C	18	18	18	18,5	17,5	18	18
Good fins							
Total mortalities	0	0	0	0	1	19	
Total *A. sal* recovered					1	3	
Recovered kidney					1	2	
Recovered fin					1	1	
Fin rot							
Total mortalities	0	0	0	0	1	16	3
Total *A. sal* recovered					1	2‡	3‡
Recovered kidney					1	1	1
Recovered fin					0	2	2

‡ = *A. salmonicida* recovered from the fin but not the kidney.

Table III.5. cont'

Initial challenge $4,7 \times 10^2$

Bin N° 7						
Day	1	2	3	4	5	6
Temperature °C	17,5	17,5	18	17,5	18	17,5
Good fins						
Total mortalities	0	0	0	1	12	7
Total *A. sal* recovered				0	2	1
Recovered kidney					2	1
Recovered fin					0	0
Fin rot						
Total mortalities	0	0	0	0	20	
Total *A. sal* recovered					7	
Recovered kidney					7	
Recovered fin					0	

Initial Challenge $4,7 \times 10^2$

Bin N° 1						
Day	1	2	3	4	5	
Temperature °C	18,5	16,5	17	18	17,5	
Good fins						
Total mortalities	0	0	0	0	19	
Total *A. sal* recovered					11	
Recovered kidney					11	
Recovered fin					0	
Fin rot						
Total mortalities	0	0	0	2	18	
Total *A. sal* recovered				2	10	
Recovered kidney				1	6	
Recovered fin				1	6	

Initial challenge $4,7 \times 10^2$

Bin N° 5										
Day	1	2	3	4	5	6	7	8	9	
Temperature °C	16,5	16,5	17,5	17	17	17,5	17,5	17,5	17,5	
Good fins										
Total mortalities	0	0	0	0	1	0	2	4	13	
Total *A. sal* recovered					1		2	4	13	
Recovered kidney					1		24	13		
Recovered fin					1		2	2	0	
Fin rot										
Total mortalities	0	0	0	0	0	0	3	9	8	
Total *A. sal* recovered							3	6	8	
Recovered kidney							3	6	8	
Recovered fin							2	3	1	

Table III,5, cont'

Initial challenge 4,7x10^{3}

Bin N° 9								
Day	1	2	3	4	5	6	7	8
Temperature °C	18	17,5	18	17,5	18,5	17,5	18	18
Good fins								
Total mortalities	0	0	1	2	0	5	11	1
Total *A. sal* recovered			1	2		5	9	1
Recovered kidney			1	2		5	9	1
Recovered fin			1	2		3	3	1
Fin rot								
Total mortalities	0	0	0	0	2	5	13	
Total *A. sal* recovered					2	5	12	
Recovered kidney					2	5	12	
Recovered fin					2	0	3	

Initial challenge 4,7x10^{4}

Bin N° 2				
Day	1	2	3	4
Temperature °C	17,5	17,5	17	17
Good fins				
Total mortalities	0	0	5	15
Total *A. sal* recovered			5	12
Recovered kidney			3	12
Recovered fin			2	0
Fin rot				
Total mortalities	8	5	7	
Total *A. sal* recovered	3‡	4	5	
Recovered kidney	2	3	5	
Recovered fin	3	1	0	

‡ = *A. salmonicida* recovered from the fin but not the kidney.

Initial challenge 4,7x10^{4}

Bin N° 6					
Day	1	2	3	4	5
Temperature °C	16,5	17,5	17,5	16,5	18
Good fins					
Total mortalities	0	0	0	5	16
Total *A. sal* recovered				5	16
Recovered kidney				5	16
Recovered fin				1	6
Fin rot					
Total mortalities	0	0	0	1	19
Total *A. sal* recovered				1	15
Recovered kidney				1	15
Recovered fin				1	9

Table III,5, cont'

Initial challenge 4,7x10^4

Bin N° 8						
Day	1	2	3	4	5
Temperature °C	17,5	19,5	18	19	18
Good fins					
Total mortalities	0	0	0	16	6
Total *A. sal* recovered				12	6
Recovered kidney				12	6
Recovered fin				4	1
Fin rot					
Total mortalities	0	0	0	18	2
Total *A. sal* recovered				17	2
Recovered kidney				16	2
Recovered fin				5	0

Table III.6.

Day 1 represents 24h post challenge. day 2, 48h *etc* (chapter III, 3.2.).

Initial challenge 2.8×10^8

Bin N° 1

Day	1	2	3	4	5	6	7	8	9	10	11
Temperature °C	17.5	17.5	18	18	18	18.5	18	18	18	18	17.5
Good fins											
Total mortalities	0	0	0	1	1	1	0	1	0	2	1
Total *A. sal* recovered				1	1	1		1		2	1
Recovered kidney				1	1	1		1		2	1
Recovered fin				1	1			1		1	1
Fin rot											
Total mortalities	0	0	0	0	1	0	1	0	0	2	2
Total *A. sal* recovered					1		1			2	2
Recovered kidney					1		1			2	2
Recovered fin							1			2	2

Initial challenge 2.8×10^8

Bin N° 1 (cont')

Day	12	13	14	15	16	17	18	19	20	21	killed
Temperature °C	18	18	18	18	18	18	18	18	18	18	
Good fins											
Total mortalities	2	1	1	1	1	0	0	1			1
Total *A. sal* recovered	2	1	1	1	1			1			1
Recovered kidney	2	1	1	1	1			1			1
Recovered fin	1	1	1	1	1						
Fin rot											
Total mortalities	2	1	3	4	1	1					
Total *A. sal* recovered	2	1	3	4	1	1					
Recovered kidney	1	1	3	4	1	1					
Recovered fin	1	1	2	3							

Table III,6. cont'

Initial challenge 2.8×10^{3}

Bin N° 2 Day	1	2	3	4	5	6	7	8	9	10	11
Temperature °C	17	18	18,5	18,5	18	19	16,5	16	16,5	17	19,5
Good fins											
Total mortalities	0	0	0	0	0	0	2	1	1	0	0
Total *A. sal* recovered							2	1	1		
Recovered kidney							2	1	1		
Recovered fin							2		1		
Fin rot											
Total mortalities	0	0	0	0	0	0	0	0	0	0	0
Total *A. sal* recovered											
Recovered kidney											
Recovered fin											

Initial challenge 2.8×10^{3}

Bin N° 2 (cont') Day	12	13	14	15	16	17	18	19	20	21killed
Temperature °C	17	17,5	17,5	16,5	16,5	19	17,5	18	18,5	18
Good fins										
Total mortalities	1	0	0	1	1	0	0	0	2	0	4
Total *A. sal* recovered	1			1	1				2		2
Recovered kidney	1			1	1				2		2
Recovered fin				1	1				2		2
Fin rot											
Total mortalities	0	4	0	1	0	3	0	1	1	3	0
Total *A. sal* recovered		4		1		3		1	1	3	
Recovered kidney		4		1		3		1	1	3	
Recovered fin		3				3		1	1	3	

Table III.6. cont'?

Initial challenge 2,8x10⁵
Bin N° 3

Day	1	2	3	4	5	6	7	8	9	10	11
Temperature °C	17,5	17,5	18	19	18,5	17,5	17,5	18	18	18	18
Good fins											
Total mortalities	0	0	0	0	0	0	1	0	3	0	4
Total *A. sal* recovered							0		2		3
Recovered kidney									2		3
Recovered fin									1		2
Fin rot											
Total mortalities	0	0	0	0	0	1	2	0	1	4	1
Total *A. sal* recovered						1	2		1	4‡	1
Recovered kidney						1	2		1	3	1
Recovered fin						1	1		1	4	1

‡ = *A. salmonicida* recovered from the fin but not the kidney.

Initial challenge 2,8x10⁵
Bin N° 3 (cont')

Day	12	13	14	15	16	17	18
Temperature °C	18	18	18,5	17,5	17	18	18
Good fins							
Total mortalities	2	3	1	1	1	0	1
Total *A. sal* recovered	2	3	1	1	1		1
Recovered kidney	2	3	1	1	1		1
Recovered fin		3	1	1	1		1
Fin rot							
Total mortalities	6	4	0	1	1		
Total *A. sal* recovered	6	4‡		1	1		
Recovered kidney	6	3		1	1		
Recovered fin	6	4		1	1		

‡ = *A. salmonicida* recovered from the fin but not the kidney.

APPENDIX IV

Characteristics of recognised species of CLB relevant to fish disease.

Table IV 1.

Reference	A	B	C	D	E	F
BACTERIA Size - Diameter Length	.4-.6 2-6	.4-.5 1.5-3.5	.4 2-12	.3-.4 2-5		
Flexirubin pigment	+	+	+	+		
Gliding motility	+	+	+	+	+	-
CULTURE Temperature (°C)	> 30	> 25	< 37	< 37	>35	+ 35
Growth in > 0.1% NaCl	+	+		+		
Anaerobic growth	FAC	-	-	-		
BIOCHEMISTRY Cytochrome oxidase	-	+	+	+	+	+
Catalase	+	+/-	+	+	+	+
Voges-Proskauer reaction					-	
Indole production	-	-	-	-	-	+
H^2S production	-	-	+	-	-	-
Nitrate reduction	+	-	-	-	+	+
O/F reaction					o	o
Hydrolysis/degredation Aesculin					-	+
Casein	+	+	+	+	+	+
Cellulose	=			-		
Chitin				+	-	
Gelatin	+	+	+	+	+	+
Starch	-	-	-	+	-	-
Tributyrin		+			+	+
Tyrosine		+	d+		+	-
ACID FROM Arabinose	+	-	-	+	-	
G+C mol%	(A)32.9-35.9 (B)32-34	(A)32-(B)34.3	30	33	31.3-32.5	33.1

Legend for Table IV.1. on previous page :

A = *C.aquatilis* (Reichenbach, 1986b)

B = *F.psychrophilus* (Holt *et al*, In press)

C = *C.columnaris* (Reichenbach, 1989b)

D = *C.johnsonae* (Reichenbach, 1989b)

E = *F.maritinus* (Hikida *et al*, 1979)

F = *F.balustinum* (Holmes *et al*, 1984)

\+ = Positive

\- = Negative

d = Strain differences

S = Sensitive

M = Microcysts

n/n = Proportion of multiple isolates

Table IV.2.

Reported characteristics of *F.branchiophila*.

Reference	G	H	I	J	K	L	M	N	O	P	Q
BACTERIA Morphology	M										
Size - Diameter Length	.5 X 5-8	.6-.8x 4-7									
Flexirubin pigment	-		+ 6/11								
Gliding motility	-	+ 7/10	+ 6/11		+ 16/16						
CULTURE Spreading	-		+ 3/11								
Temperature (°C) 5-30 37	+ -		+ 9/11				25+	+	+	+	25+
Growth in > 0.1% NaCl	d		+				d	d	d	d	d
Anaerobic growth	FAC	FAC	-				-	+	-	-	-
BIOCHEMISTRY Cytochrome oxidase	+	+	+	+	+		+	-	d	d	-
Catalase	+	+	+		+		+	+	+	+	+
Voges-Proskauer reaction				+							
Indole production	-		-	-			-	-	-	-	-
H^2S production	+		-	-	+ 12/16	+ 3/4	-	d	d	-	-
Nitrate reduction	-		+ 6/11				-	d	d	d	d
O/F reaction	O	O	O				O/-	+	-	-	
Hydrolysis/degredation Aesculin	-						d	+	+	+	
Casein	+		+				+	+	+	+	+
Cellulose	-		-				-	-	-	-	-
Chitin	-						d	+	+	+	
Gelatin	+		+	+	+8/16	+3/4	+	+	+	+	+
Starch	+		+				+	+	+	+	+
Tributyrin							d	+	+	d	+
Tyrosine							+	d	d	d	d

Table IV.2. (cont')

	G	H	I	J	K	L	M	N	O	P	Q
ACID FROM Arabinose	-			+	+6/16						
Cellobiose	+										
Fructose	+										
Galactose	-										
Glucose	+			-	d	+1/4	-	d	-	-	d
Inositol	-										
Inulin	+										
Lactose	-			-			-	d	-	-	
Maltose	+										
Mannitol	-			+	+1/16						
Melibiose	+				+6/16						
Raffinose	+										
Rhaminose	-										
Salicin	-										
Sucrose	+				+6/16						
Sorbitol	-										
Trehelose	+										
Xylose	-										
SENSITIVITY TO Penicillin	S	Sa/18	Sz/11								
Nifurprinol			S4/11								

Legend for Table IV.2. on previous two pages :

G - Q = *F.branchiophila*

G = (Wakabayashi, Egusa and Fryer, 1980; Farkas, 1985; Ototake and Wakabayashi, 1985; Austin and Austin, 1987; Huh and Wakabayashi, 1987 and 1989; Wakabayashi *et al*, 1989; Ostland *et al*, 1989; Heo ,Kasi and Wakabayashi, 1990; Heo, Wakabayashi and Watabe, 1990b; Ferguson *et al*, 1991)

H = (Ostland *et al*, 1990)

I = (Ostland *et al*, 1991)

J = (Acuigroup, 1980)

K = (Pyle and Shotts, 1980)

L = (Borge, 1960)

M - P = (Pache and Porter, 1968)

Q = (Bullock, 1972)

\+ = Positive

\- = Negative

d = Strain differences

S = Sensitive

M = Microcysts

n/n = Proportion of multiple isolates