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# Comparative experimental transmission of pancreas disease in Atlantic salmon, rainbow trout and brown trout

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ABSTRACT: The effects of experimental transmissions of pancreas disease (PD) were compared in Atlantic salmon *Salmo salar*, rainbow trout *Oncorhynchus mykiss* and brown trout *Salmo trutta* in fresh water. Only Atlantic salmon developed a total loss of exocrine pancreas acinar cells, which is used to diagnose PD. Brown trout were the least susceptible, exhibiting focal necrosis in an otherwise normal-appearing pancreas. The effect in rainbow trout was intermediate between that seen in salmon and brown trout.

KEY WORDS: Pancreas disease · Atlantic salmon · Rainbow trout · Brown trout

## INTRODUCTION

Pancreas disease (PD) is a condition of farmed Atlantic salmon *Salmo salar* which was first recorded in 1976 in Scotland (Munro et al. 1984). McVicar (1987, 1990) presented evidence for an infectious agent as the cause of PD and Raynard & Houghton (1993) described a method for the experimental transmission of PD disease which further supported an infectious agent as the cause.

In France, despite the low importance of salmonid farming at seawater sites, some PD outbreaks have occurred in Atlantic salmon, with diagnosis of PD by histological examination of the exocrine pancreas (Baudin Laurencin unpubl. data). In brown trout *Salmo trutta*, clinical signs attributed to PD do not appear so clearly as in Atlantic salmon. Affected fish are generally lethargic and display petechiae on pyloric caecae and adipose tissue, but histology reveals an inflammatory necrosis of exocrine pancreatic acinar cells which appears more similar to the histopathology of infectious pancreatic necrosis (IPN) than to that of PD. Currently, the only way of diagnosing PD in brown trout is by observing the pancreatic histopathology in the absence of detectable IPN virus. Since PD has not been observed in seawater-farmed rainbow trout *Oncorhynchus mykiss*, it would be of interest to determine whether or not rainbow trout are susceptible to PD.

The aim of this study was to compare the susceptibility of rainbow trout, brown trout and Atlantic salmon to PD.

## MATERIALS AND METHODS

**First transmission experiment.** ***Fish:*** Atlantic salmon *Salmo salar* (mean weight 23 g), rainbow trout *Oncorhynchus mykiss* (137 g) and brown trout *Salmo trutta* (42 g) were reared in fresh water at a controlled temperature of 13 ± 1°C in Ewos 200 l tanks supplied 5 l $min^{-1}$. Prior to all experimental procedures fish were anaesthetised using ethylene glycol mono-phenyl ether (0.02% bath). Species were held separately and fish injected with control

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homogenates were held in different tanks from fish injected with infective homogenates.

***Injection of fish:*** Kidney homogenates (origin: Marine Laboratory, Aberdeen, UK; Raynard & Houghton 1993) were used as infective and control material. Homogenates were diluted in phosphate buffered saline (PBS, pH 7.2; Biomérieux) and intraperitoneally injected (0.1 ml for salmon, 0.2 ml for brown trout and 0.5 ml for rainbow trout) at an approximate dose of 3 µg protein $g^{-1}$ wet body weight.

***Sample procedures and histological assessment:*** Fish were killed at the intervals shown in Table 2. Pyloric caeca with pancreas were fixed in Bouin's solution and sections (5 µm) of paraffin-wax-embedded tissue were stained with hematoxylin and eosin. Head kidney was taken for estimation of macrophage activity by chemiluminescence (Scott & Klesius 1981). Blood was drawn from the caudal vein into a heparinised vacutainer and plasma frozen (–70°C) until the following analyses were made using commercial kits (Boehringer) on an automated analyser (iEMS, Labsystems): lysozyme activity (Grinde 1989), and total protein, cholesterol and glucose concentration. Kidneys were pooled from 6 fish of each group at Day 20 post-injection and tested for IPN virus on *epithelioma papulosum Cyprini* (EPC) and rainbow trout gonad (RTG-2) cell lines (1 blind passage). Virulent and control material was also tested for IPN virus using classical methods.

Histological assessment of pancreas was made according to the following criteria using a semi-quantitative scale of 0 to 4.

(A) Abundance of exocrine pancreatic acinar cells which appear normal according to the criteria of Munro et al. (1984). Score 0 corresponds to a total absence of pancreatic acinar cells, scores 1 and 2 to a low or medium presence and scores 3 and 4 to a presumed normal abundance.

(B) Necrosis of the exocrine pancreas, characterised by shrunken cell volume, absence of zymogen granules, vacuolation, strongly stained bodies and pycnosis. Score 0 indicates a normal appearance; 1, focal necrosis; 2, multifocal necrosis; 3, extensive necrosis; 4, confluent lesions throughout the tissue section.

(C) Infiltration by lymphocytes and proliferation of tissue fibrocytes. Score 0 indicates normal appearance; 1, perivascular infiltration; 2, infiltration penetrating exocrine acinar tissue; 3, extensive infiltration; 4, general fibrosis.

**Second experiment.** Kidney homogenates were prepared from the 3 species and tested for virulence in the corresponding species. Briefly, 70 Atlantic salmon, 20 rainbow trout and 40 brown trout were injected with previously described Scottish kidney homogenates (20 fish of each species received control homogenates). Five days after injection, new kidney homogenates were prepared by the method of Raynard & Houghton (1993) and their infectivity tested by injecting 20 fish of each species with homogenates from the corresponding species. Histological assessments of the exocrine pancreas from subsamples of fish from each group were made at the intervals shown in Table 3.

Table 1 Mean values of different parameters evaluated in *Salmo salar, Oncorhynchus mykiss* and *Salmo trutta* from the first transmission experiment. Values are means with SD in parentheses; n = 10 for each group

| Time post-injection | Species | Inoculated or control | Chemiluminescence (mV) | Lysozyme (µg $l^{-1}$) | Total protein (g $l^{-1}$) | Cholesterol (g $l^{-1}$) | Glucose (g $l^{-1}$) |
|---|---|---|---|---|---|---|---|
| First week (Days 4 to 8) | Salmon | Inoculated | 29 (19) | 2.3 (0.1) | 61 (10) | 5.5 (1.5) | 1.0 (0.2) |
| | | Control | 24 (9) | 2.3 (0.3) | 61 (9) | 5.1 (1.8) | 1.2 (0.1) |
| | Rainbow trout | Inoculated | 41 (23) | 15.5 (1.4) | 59 (15) | 4.1 (1.3) | 1.3 (0.4) |
| | | Control | 64 (41) | 15.6 (0.4) | 64 (10) | 4.4 (1.1) | 1.8 (0.6) |
| | Brown trout | Inoculated | 13 (7) | 2.5 (0.2) | 52 (4) | 5.9 (1.2) | 1.0 (0.2) |
| | | Control | 21 (17) | 2.3 (0.3) | 53 (8) | 6.1 (1.7) | 0.9 (0.1) |
| Second week (Days 11 to 15) | Salmon | Inoculated | 38 (33) | 3.1 (0.2) | 64 (8) | 4.4 (1.2) | 0.9 (0.1) |
| | | Control | 46 (31) | 2.4 (0.4) | 62 (6) | 5.1 (1.2) | 1.0 (0.2) |
| | Rainbow trout | Inoculated | 68 (26) | 15.9 (0.7) | 58 (8) | 4.7 (0.8) | 1.9 (1.7) |
| | | Control | 39 (24) | 15.7 (0.6) | 63 (6) | 4.8 (0.8) | 1.2 (0.5) |
| | Brown trout | Inoculated | 12 (7) | 2.8 (0.5) | 57 (8) | 5.9 (1.0) | 0.8 (0.1) |
| | | Control | 22 (6) | 2.6 (0.2) | 53 (9) | 5.6 (1.5) | 0.8 (0.2) |
| Third week (Days 18 to 20) | Salmon | Inoculated | 39 (17) | 2.8 (0.3) | 65 (11) | 4.4 (1.3) | 1.2 (0.6) |
| | | Control | 69 (19) | 2.5 (0.6) | 63 (10) | 4.5 (1.0) | 1.5 (1.0) |
| | Rainbow trout | Inoculated | 29 (22) | 15.4 (0.7) | 63 (9) | 5.3 (1.0) | 1.3 (1.0) |
| | | Control | 27 (23) | 14.3 (1.9) | 68 (10) | 5.4 (1.0) | 1.7 (1.2) |
| | Brown trout | Inoculated | 20 (8) | 2.8 (0.3) | 56 (8) | 5.6 (1.0) | 1.3 (1.0) |
| | | Control | 22 (4) | 2.9 (0.5) | 55 (4) | 4.7 (0.6) | 1.4 (1.1) |

Table 2. Histological scores of *Salmo salar, Oncorhynchus mykiss* and *Salmo trutta* injected with infective kidney homogenates (first transmission experiment). PAN: abundance of exocrine pancreatic cells; score 0 corresponds to a total absence of pancreatic acinar cells, scores 1 and 2 to a low or medium presence and scores 3 and 4 to a normal abundance. NEC: necrosis of the exocrine pancreas; score 0 indicates normal appearance; 1, focal necrosis; 2, multifocal necrosis; 3, extensive necrosis; 4, confluent lesions throughout the tissue section. INF: infiltration by lymphocytes and proliferation of tissue fibrocytes; score 0 indicates no infiltration; 1, perivascular infiltration; 2, infiltration penetrating exocrine acinar tissue; 3, extensive infiltration; 4, general fibrosis

| Days post-injection | Fish no. | Atlantic salmon | | | Rainbow trout | | | Brown trout | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | PAN | NEC | INF | PAN | NEC | INF | PAN | NEC | INF |
| 4 | 1 | 4 | 1 | 1 | 4 | 0 | 0 | 3 | 0 | 0 |
| | 2 | 4 | 0 | 0 | 4 | 0 | 0 | 4 | 0 | 0 |
| 5 | 3 | 4 | 0 | 0 | 4 | 2 | 0 | 3 | 0 | 1 |
| | 4 | 3 | 0 | 0 | 4 | 2 | 0 | 3 | 0 | 1 |
| 6 | 5 | 3 | 1 | 1 | 3 | 3 | 1 | 4 | 0 | 2 |
| | 6 | 3 | 2 | 1 | 3 | 3 | 1 | 3 | 1 | 1 |
| 7 | 7 | 3 | 2 | 0 | 3 | 3 | 0 | 3 | 0 | 1 |
| | 8 | 4 | 2 | 0 | 3 | 3 | 1 | 3 | 0 | 0 |
| 8 | 9 | 3 | 2 | 1 | 4 | 0 | 0 | 4 | 0 | 0 |
| | 10 | 1 | 4 | 2 | 3 | 3 | 1 | 4 | 0 | 0 |
| 11 | 11 | 1 | 2 | 2 | 3 | 2 | 2 | 3 | 1 | 1 |
| | 12 | 3 | 0 | 1 | 3 | 3 | 2 | 3 | 1 | 2 |
| 12 | 13 | 0 | 0 | 2 | 3 | 2 | 2 | 2 | 1 | 1 |
| | 14 | 1 | 1 | 3 | 1 | 1 | 3 | 3 | 1 | 3 |
| 13 | 15 | 0 | 0 | 4 | 3 | 1 | 2 | 3 | 0 | 2 |
| | 16 | 1 | 0 | 4 | 3 | 1 | 1 | 3 | 0 | 1 |
| 14 | 17 | 0 | 0 | 4 | 2 | 0 | 3 | 3 | 0 | 2 |
| | 18 | 1 | 0 | 4 | 3 | 0 | 2 | 3 | 0 | 2 |
| 15 | 19 | 0 | 0 | 4 | 2 | 0 | 2 | 3 | 2 | 2 |
| | 20 | 0 | 0 | 4 | 3 | 0 | 1 | 3 | 0 | 0 |
| 18 | 21 | 2 | 0 | 4 | 3 | 0 | 3 | 3 | 0 | 0 |
| | 22 | 2 | 0 | 4 | 4 | 0 | 1 | 3 | 0 | 1 |
| 19 | 23 | 3 | 0 | 3 | 4 | 0 | 0 | 3 | 2 | 0 |
| | 24 | 3 | 0 | 1 | 3 | 0 | 1 | 3 | 0 | 0 |
| 20 | 25 | 0 | 0 | 3 | 3 | 0 | 2 | 3 | 0 | 0 |
| | 26 | 1 | 0 | 3 | 3 | 1 | 2 | 3 | 0 | 0 |
| | 27 | 0 | 0 | 2 | 3 | 1 | 0 | 3 | 2 | 0 |
| | 28 | 3 | 0 | 1 | 4 | 0 | 0 | 3 | 0 | 0 |
| | 29 | 2 | 0 | 2 | 3 | 0 | 2 | 3 | 1 | 0 |
| | 30 | 0 | 0 | 2 | 4 | 0 | 1 | 3 | 0 | 0 |

## RESULTS

### First transmission experiment

The original kidney homogenates and kidneys removed from fish 20 d post-injection tested negative for IPN virus as adjudged by the absence of cytopathic effects in EPC and RTG-2 cells. There were no significant differences in macrophage phagocytic activity, blood enzyme activities or blood biochemistry parameters between the infected and control groups (Table 1).

Results of the histological assessment are shown in Table 2. The pancreas tissues in the control fish appeared normal in all the 3 species.

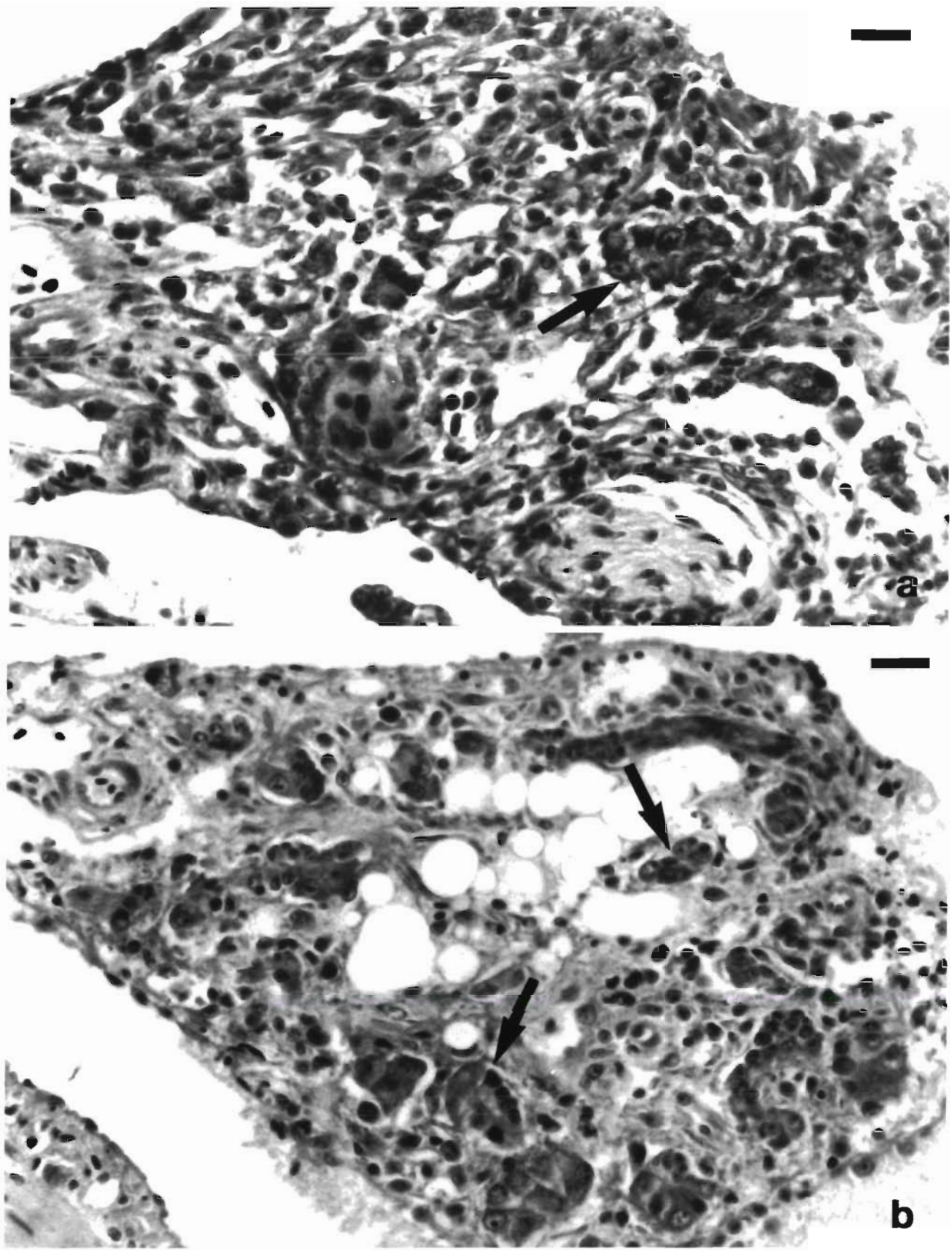
Of the 3 species, Atlantic salmon developed the most extensive exocrine pancreas pathology. The development of the disease was characterised by 3 stages. Necrosis developed by Day 4 post-injection and was frequently observed from Day 6 to Day 11 Between Days 8 and 15 a nearly total absence of acinar cells concomitant with fibrosis and collagen deposition was observed (Fig. 1a). From Day 18, acinar cells were more numerous in some fish but were apparently absent in others. Presumably, previously affected salmon regenerated pancreas tissue. In most cases the acinar cells associated with recovery were of the B-type described by Munro et al. (1984) and were arranged in small clusters or strips.

In rainbow trout, the pathological changes followed the same progression observed for salmon except that the lesions were more moderate in rainbow trout. Necrosis of the exocrine acinar cells occurred, with affected cells appearing similar to those seen in affected salmon. However, the loss of acinar cells from affected trout was not as extensive as that which occurred in salmon and only small areas of acinar cells disappeared (Fig. 1b).

In brown trout, necrosis was predominantly focal in an otherwise normal-appearing exocrine pancreas. Infiltrates were perivascular or just penetrating some areas of acini. No lysis or significant loss of pancreas tissue was observed (Fig. 1c).

### Second experiment: virulence of homogenates prepared from each species

Injection of the control homogenates did not cause changes in the histological appearance of the exocrine pancreas. The time course and the appearance of exocrine pancreas alteration following injection with the homogenates isolated from each species are shown in Table 3. The results were essentially similar to those obtained following the injection of the initial Atlantic salmon kidney homogenates. The severity of the pancreas histological alteration was greatest for salmon receiving the salmon homo-genate, compared with rainbow trout receiving the rainbow trout homogenate. The least alteration in pancreas histology


a
b

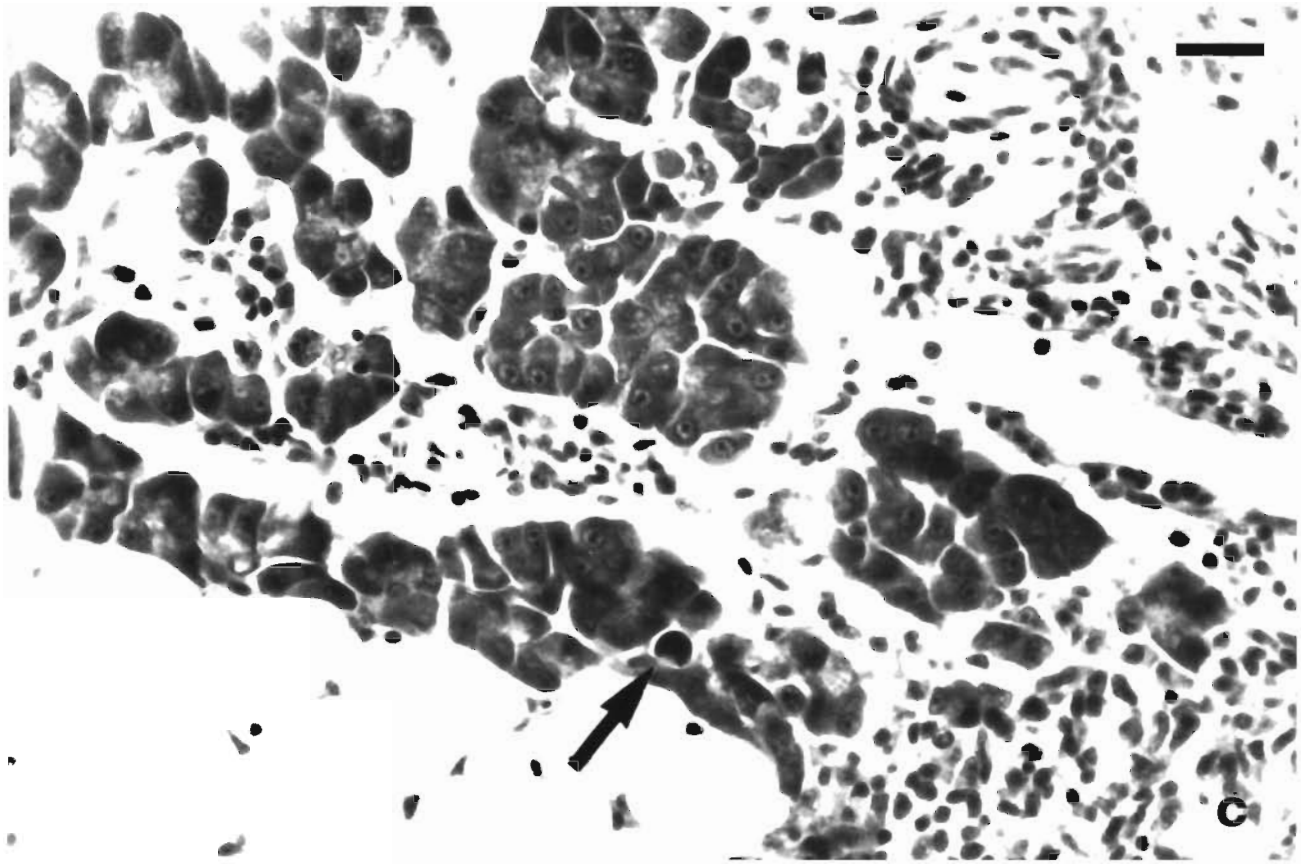



Fig. 1 (facing page and above). Histological appearance of the pancreas 12 d after the injection of virulent kidney homogenate into (a) Atlantic salmon *Salmo salar*: nearly total absence of acinar cells (arrow) and extensive fibrosis; (b) rainbow trout *Oncorhynchus mykiss*: extensive fibrosis but persistence of partially degenerated acini (arrow); (c) brown trout *Salmo trutta*: sparse focal necrosis (arrow) in an otherwise normal-appearing exocrine pancreas with a limited infiltrate. Scale bars = 20 µm

was recorded for brown trout receiving the brown trout homogenate.

## DISCUSSION

In order to reduce subjectivity in the diagnosis of PD, McVicar (1990) and Raynard & Houghton (1993) classified fish as affected when the normal acinar arrangement of the exocrine pancreas secretory cells became transformed to one of total apparent necrosis and when no zymogen cells could be observed. By this definition, in the present study, only the salmon developed complete PD. The kinetics of the development and recovery from PD in our study were essentially similar to those described by Raynard & Houghton (1993). The characteristics of experimental PD infections of salmon in fresh water were shown by Houghton (1994) to broadly mirror those of experimental infection in seawater. We would, therefore, expect the observations described with brown and rainbow trout in the present study to reflect what would happen to these species in seawater.

Although both trout species developed pancreatic changes, the extent of the pathology was such that PD could not be diagnosed by the criteria of Raynard & Houghton (1993). The differences in pathology between the species indicates differences in species susceptibility to PD, with brown trout more resistant than rainbow trout and rainbow trout more resistant than Atlantic salmon.

The apparently lower susceptibility of rainbow trout compared with salmon may explain why PD has not been diagnosed in farmed rainbow trout.

Pancreas pathology was not increased when kidney homogenates from each of the 3 species were injected back into fish of the same species. Therefore, no evidence was obtained that the PD agent is more virulent for the host from which it was isolated. The pancreas pathology described for IPN-negative seawater-farmed brown trout (Baudin Laurencin un-

Table 3. Histological scores of *Salmo salar, Oncorhynchus mykiss* and *Salmo trutta* injected with infective kidney homogenates isolated from each respective species (second transmission experiment). PAN: abundance of exocrine pancreatic cells; score 0 corresponds to a total absence of pancreatic acinar cells, scores 1 and 2 to a low or medium presence and scores 3 and 4 to a normal abundance. NEC: necrosis of the exocrine pancreas; score 0 indicates normal appearance; 1, focal necrosis; 2, multifocal necrosis; 3, extensive necrosis; 4, confluent lesions throughout the tissue section. INF: infiltration by lymphocytes and proliferation of tissue fibrocytes; score 0 indicates no infiltration; 1, perivascular infiltration; 2, infiltration penetrating exocrine acinar tissue; 3, extensive infiltration; 4, general fibrosis

| Days post-injection | Fish no. | Atlantic salmon PAN | NEC | INF | Days post-injection | Fish no. | Rainbow trout PAN | NEC | INF | Days post-injection | Fish no. | Brown trout PAN | NEC | INF |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 5 | 1 | 2 | 3 | 1 | 10 | 1 | 2 | 3 | 2 | 7 | 1 | 4 | 0 | 1 |
| | 2 | 1 | 3 | 0 | | 2 | 3 | 2 | 2 | | 2 | 4 | 0 | 1 |
| | 3 | 2 | 3 | 1 | | 3 | 3 | 2 | 2 | | 3 | 4 | 0 | 2 |
| | 4 | 2 | 2 | 0 | | 4 | 3 | 2 | 2 | | 4 | 4 | 0 | 1 |
| 12 | 5 | 0 | 0 | 4 | | 5 | 3 | 1 | 1 | | 5 | 4 | 0 | 1 |
| | 6 | 0 | 0 | 4 | 17 | 6 | 3 | 0 | 2 | 14 | 6 | 2 | 3 | 2 |
| | 7 | 0 | 0 | 4 | | 7 | 4 | 0 | 1 | | 7 | 3 | 0 | 1 |
| | 8 | 0 | 0 | 4 | | 8 | 4 | 0 | 0 | | 8 | 3 | 0 | 2 |
| 19 | 9 | 1 | 0 | 4 | | 9 | 3 | 0 | 2 | | 9 | 3 | 0 | 2 |
| | 10 | 0 | 0 | 4 | | 10 | 2 | 1 | 2 | | 10 | 3 | 1 | 3 |
| | 11 | 0 | 0 | 4 | 24 | 11 | 3 | 0 | 2 | 21 | 11 | 4 | 0 | 1 |
| | 12 | 0 | 0 | 4 | | 12 | 3 | 0 | 1 | | 12 | 3 | 0 | 2 |
| 24 | 13 | 0 | 0 | 4 | | 13 | 0 | 0 | 4 | | 13 | 2 | 1 | 2 |
| | 14 | 0 | 0 | 4 | | 14 | 4 | 0 | 1 | | 14 | 2 | 2 | 3 |
| | 15 | 0 | 0 | 4 | | 15 | 3 | 0 | 1 | | 15 | 3 | 0 | 2 |
| | 16 | 0 | 0 | 4 | 31 | 16 | 4 | 0 | 1 | 28 | 16 | 3 | 1 | 2 |
| 28 | 17 | 0 | 0 | 4 | | 17 | 4 | 0 | 1 | | 17 | 3 | 0 | 2 |
| | 18 | 0 | 0 | 4 | | 18 | 3 | 0 | 1 | | 18 | 3 | 0 | 1 |
| | 19 | 0 | 0 | 4 | | 19 | 4 | 0 | 0 | | 19 | 4 | 0 | 1 |
| | 20 | 0 | 0 | 2 | | | | | | | 20 | 3 | 0 | 2 |

publ. data) which was attributed to PD is similar to the experimentally induced pancreas pathology. Therefore, our experimental evidence supports the case that the pancreas pathologies previously observed in farmed brown trout were caused by the PD agent.

In summary, rainbow trout appear less susceptible to PD than salmon but more susceptible than brown trout, which appear little affected by the PD causative agent. Strictly speaking, the pathology which developed in rainbow and brown trout, while indicative of PD, could not be used to diagnose PD definitively as is seen in salmon. It remains to be demonstrated whether the more moderate changes in the exocrine pancreas of trout also mean that less physiological disturbance is caused by the PD agent in trout when compared with salmon.

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