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ABSTRACT

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Yhteenveto: Solu- ja kudosmuutokset ahvenella (*Perca fluviatilis*) ja särjellä (*Rutilus rutilus*) suhteessa veden laatuun
Diss.

Cell and tissue changes were studied in perch, *Perca fluviatilis*, and roach, *Rutilus rutilus*, over five seasons from four lakes in central Finland differing both in parasite composition and water quality. Fish gills displayed a variety of abnormalities including cell proliferations, fused lamellae and parasitic nodules. Among the four lakes the occurrence of gill alterations was greatest in the natural Lake Peurunka, the reference area. Prevalence of infection with *Henneguya creplini* (Protozoa, Myxosporea) on the gills of perch varied among lakes between 26.5 % and 39.6 % with no apparent relationship with the level of pollution. Infection with *Raphidascaris acus* (Nematoda, Ascaridoidea) larvae in the intestine, liver and pancreatic tissue of roach showed seasonal and size-related patterns. The most heavily infected fish were recorded from the polluted Lake Vatia whilst abundance was lowest in Lake Peurunka. Histology revealed that most worms in the liver and pancreatic tissue were dead. The typical host response to the parasite was a granulomatous inflammatory reaction. There was no difference in the type of response when fish from the different lakes were compared. Special attention was paid to the occurrence of macrophage centres (MC) in fish. MCs were initially studied in the fish heart in association with tissue inflammation. Subsequently the study focussed on the fish liver, spleen and the hematopoietic part of the kidney. The MC parameters displayed variability among individual fish, species, organs, and lakes. However, none of the observed patterns correlated with water quality and MCs are concluded to have limited value as indicators of pollution. The future role of histopathology in fisheries research is also discussed.

Key words: gills; hemosiderin; macrophage centre; spleen; water quality.

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CONTENTS

List of original publications

This thesis is based on the following original articles, which will be referred to by their Roman numerals I - V. Some unpublished data are also presented. I have performed most of the work in the papers I, II, IV and V, and a significant proportion of it in the work III.

I. Haaparanta, A., Valtonen, E. T. & Hoffmann, R. W. 1997. Gill anomalies of perch and roach from four lakes differing in water quality. - J. Fish Biol. 50, 575-591.

II. Haaparanta, A., Valtonen, E. T. & Hoffmann, R. 1994. Pathogenicity and seasonal occurrence of *Henneguya creplini* (Protozoa, Myxosporea) on the gills of perch *Perca fluviatilis* in central Finland. - Dis. Aquat. Org. 20, 15-22.

III. Valtonen, E. T., Haaparanta, A. & Hoffmann, R. W. 1994. Occurrence and histological response of *Raphidascaris acus* (Nematoda, Ascaridoidea) in roach from four lakes differing in water quality. - Int. J. Parasit. 24, 197-206.

IV. Haaparanta, A., Valtonen, E. T., Hoffmann, R. & Holmes, J. 1996. Do macrophage centres in freshwater fishes reflect the differences in water quality? - Aquat. Toxicol. 34, 253-272.

V. Haaparanta, A., Valtonen, E. T. & Hoffmann, R. W. 1993. Heart inflammation in perch *Perca fluviatilis* and roach *Rutilus rutilus* from central Finland. - Dis. Aquat. Org. 17, 25-32.

1 INTRODUCTION

Urbanization and industrialization over the past 400 - 500 years has resulted in widespread aquatic pollution (Bucke 1993). The possibility that pollution may be a factor contributing to the aetiology of disease in aquatic organisms has been of interest to scientist and non-scientist alike for some time (Vethaak & ap Rheinallt 1990, 1992) the debate being frequently emotive. However, despite considerable effort and many costly investigations, there are only a few instances of a clear statistical association between anomalies in fish and pollution (Bucke 1993).

There is voluminous literature on fish kills and fish pathology caused by various pollutants, but much less information is available on the effects of toxic substances in the environment and the incidence of infectious diseases of fishes (Snieszko 1974). One reason for this is the fact that there are many natural stress factors that vary in the absence of human influence. For example, in eutrophic waters there are frequent and wide fluctuations in dissolved oxygen and pH and numerous fish pathogens are present (Snieszko 1974).

Environmental changes may also influence, directly or indirectly, the prevalence and intensity of parasites in fish. Pollution might, for example, promote an increase in parasitism by impairing the host's immune response or by favouring the survival and reproduction of the intermediate hosts. A decrease in parasitism might result from pollutant toxicity to the free-living stages of parasites and to intermediate hosts or by alteration of the host's physiology (Khan & Thulin 1991, Valtonen et al. 1997). Currently, due to the inevitable rise in fish disease levels and the increased economic value of fishing and fish cultivation, research has increasingy focussed on parasitic fish diseases (Kennedy 1994a, 1994b).

1.1 Overview of fish defence mechanisms

The Latin "immunis" means "exempt from", and the term "immunology" is used to mean the study of defence mechanisms against infectious disease (Ellis 1989). Defence mechanisms can be categorized into nonspecific, natural immunity, which is an innate defence mechanism rendering the host resistant to an infection, or acquired, specific processes induced in response to a foreign agent (Ingram 1980). These two systems *in vivo* act in concert with each other, being interdependent in many ways (Ellis 1989).

The integrity of the immune system is fundamental to successful defence against a variety of pathogenic agents in the environment. The major immune cells, lymphocytes and macrophages, are regulated by a variety of multi-step control processes of cellular cooperation and interaction (Dunier & Siwicki 1993). In fish the cellular immunity may be more critical for host survival than the role played by humoral immunity (Evans & Gratzek 1989) and the protection given by antibodies against parasite infections may be less important in fish than in higher vertebrates. For example, many workers have failed to correlate protection with the specific antibody titre in fish immunized against bacteria (Whyte et al. 1990); in these cases macrophages are suggested to play the major role in immunity to the infective agent (e.g. Olivier et al. 1985). Additionally, Whyte et al. (1990), who studied the protection of rainbow trout against *Diplostomum spathaceum* (Digenea), concluded that specific circulating antibody alone could have a role in diplostomulicidal activity but that activated macrophages in combination with specific antibodies are required to kill diplostomules.

The most typical defence mechanism in fish is a nonspecific, granulomatous inflammatory reaction. This may cause massive tissue destruction but may not be so hazardous for fish as for mammals since most fish tissues have a remarkable capacity for regeneration (Ellis 1986). In fish the inflammatory responses may occur soon after an original stimulus, and the inflammatory focus may resolve after the causative agent has been destroyed or the reaction may chroniciate. Phagocytes are attracted to a site of infection by both pathogen- and host-derived chemoattractants, the latter including complement components and eicosanoids (Secombes & Fletcher 1992). Both granulocytes and macrophages are phagocytic in fish, although the phagocytic activity of granulocytes varies between and even within fish species (Moritomo et al. 1988).

1.2 The role of macrophage centres in fish

Macrophages are prominent cells in the lymphoid organs of all teleosts (Lamers & De Haas 1985). In higher teleosts macrophages are organized into discrete centres, which occur primarily in the hemopoietic tissues, but also in other sites (Roberts 1975, Agius 1980). These centres are called melano-macrophage centres

(MMCs) (e.g. Roberts 1975, Ferguson 1976, Agius 1980), macrophage centres (e.g. Haensly et al. 1982), pigment nodules (e.g. Fulop & McMillan 1984), macrophage aggregates (MA) (e.g. Wolke et al. 1985a, Brown & George 1985, Blazer et al. 1987) or macrophage accumulations (e.g. Wolke et al. 1985b). They have been recognized as an integral part of the fishes reticuloendothelial system (Agius 1985).

The number, size and pigment distribution of macrophage centres (MCs) varies according to the fish species (e.g. Roberts 1975), organs (Agius 1979, Kranz & Peters 1984, Ziegenfuss & Wolke 1991), age (Agius 1981a, Brown & George 1985, Blazer et al. 1987, Pulsford et al. 1992) or nutritional status and health of the fish (Agius 1981a, 1981b, Agius & Roberts 1981, Kranz 1989).

The formation of a new MC may be originated by macrophages, which have phagocytosed foreign material or cell debris elsewhere or, for example, in the sinusoidal wall, after which single macrophages migrate to the parenchyma of the organ and aggregate (see, e.g. Ferguson 1976, Wolke et al. 1985b, Vogelbein et al. 1987, Tsujii & Seno 1990). New macrophages reaching existing MCs are not simply attached to the surface of them but are mixed within the other cells, so that *these centres can act as a dynamic moving body* (Wolke et al. 1985b, Ziegenfuss & Wolke 1991). The macrophages apparently form new aggregates and migrate to existing aggregates simultaneously (Ziegenfuss & Wolke 1991). The aggregates may become replete or "mature" and exclude further macrophages (Ziegenfuss & Wolke 1991). The final stage of a macrophage centre formation is a nodular structure with a delicate argyrophilic capsule (Roberts 1975, Agius 1985). The descriptions of degeneration of MCs are not entirely consistent. For example, Herraez and Zapata (1986) interpreted the process as a regeneration mechanism. Furthermore, it may be possible that macrophages leave these centres via blood vessels (Kranz & Gercken 1987).

In addition, many studies suggest that the general function of these aggregates is the centralization of destruction, detoxification or recycling of endogenous and exogenous materials (Vogelbein et al. 1987, Wolke 1992, see e.g. Ferguson 1976 & Ellis et al. 1976) and these cells not only have the ability to phagocytose but are also able to degrade organic material after ingestion (Braun-Nesje et al. 1982). Macrophage centres with ingested, processed material in them can also act as germinal centres for the induction of immune reactions (Ellis & de Sousa 1974, Ferguson 1976, Zapata 1982, Lamers & De Haas 1985) and they have an important role in the responses of fish to foreign material, including infectious agents (Ellis et al. 1976, Wishkovsky et al. 1989).

Three pigment types, melanin, lipofuscin and hemosiderin, are normally observed in fish macrophages; all three pigment types can be present in one and the same macrophage (Agius 1985). Very often melanins and lipofuscins are called "melanin", according to the definition by Edelstein (1971) (see also Sealy et al. 1980, Agius 1985). It has been suggested that in both healthy and diseased fish only splenic centres are normally involved in handling hemosiderin (Agius 1979, 1981b, 1985). Nevertheless, authors have typically not taken samples from all the organs where iron accumulation may occur. Herraez and Zapata (1986) found a positive Perls' reaction in the spleen, pro- and mesonephros of goldfish, which were the only organs they studied. They used phenylhydrazine to induce a fast and massive anaemia, and as a consequence the number and size of the MCs increased rapidly.

These centres degenerated and fragmentated from the 5th day, coincident with the appearance of young red blood cells, suggesting a relationship between these two factors (see Yu et al. 1971). Wolke et al. (1985a) studied the spleen and liver of winter flounder (*Pseudopleuronectes americanus*, the present name *Pleuronectes americanus*) and found iron in both organs. Brown and George (1985) found pigments in the anterior kidney of 136 specimens of yellow perch (*Perca flavenscens*), melanin, lipofuscin and hemosiderin being present in all samples. Furthermore, Blazer et al. (1987), Bucke et al. (1984) and Bucke and Feist (1993), also observed positive Perls' reactions from fish livers, in addition to the spleen. In certain pathological states, e.g. vibriosis (Richards 1980), and in experimental conditions (Agius 1981b), iron has also been shown to accumulate in other tissues. Exceptionally, Yu et al. 1971 studied the spleen, liver and kidney of blue gourami (*Trichogaster trichopterus*) and found hemosiderin bodies to occur in all of the organs.

The amount of hemosiderin in the spleen is usually small, and excessive accumulations of the pigment or accumulations in other organs is known as hemosiderosis and reflects a pathological process (Wolke et al. 1985b). Hemosiderosis is caused by an increase in the rate of destruction of red blood cells in the spleen (Takashima 1982, van de Graaff 1985). There are some observations that fish from an impacted environment may have increased iron in MCs (e.g. Bucke et al. 1984, 1992, Wolke et al. 1985a, Langdon 1986, Blazer et al. 1987).

In the fish kidney MCs are thought to be randomly distributed throughout the lymphohemopoietic tissue (Brown & George 1985, Herraez & Zapata 1986), whereas in the spleen the MCs appear to have a tendency to be associated with blood vessels (Fulop & McMillan 1984, Herraez & Zapata 1986, Quesada et al. 1990) and in the liver with portal tracts (Roberts 1975, Agius 1980). Macrophage centres form in greater number and more rapidly in the spleen and kidney than in the liver (Ziegenfuss & Wolke 1991). In the spleens of some fish species including roach, *Rutilus rutilus*, macrophages form clusters with lymphocytes and plasma cells. These complexes may represent primitive germinal centres (Ellis & de Sousa 1974, Roberts 1975, Ferguson 1976, Ellis et al. 1976, Agius 1980, Zapata 1982).

More detailed studies are required for the characterization of these cells which perform a variety of functions in fish such as immunological responses (Lamers & De Haas 1985).

1.3 Stress and xenobiotics as predisposing factor

Disease conditions are diverse in nature and may elicit very varied response patterns in fish (Bruno & Poppe 1996). In the past most diseases were regarded as having single causes, i.e. infectious agents would cause disease in a host under appropriate environment conditions. However, the multifactorial aetiology of diseases is now generally appreciated (Vethaak & ap Rheinallt 1990) (Fig. 1).

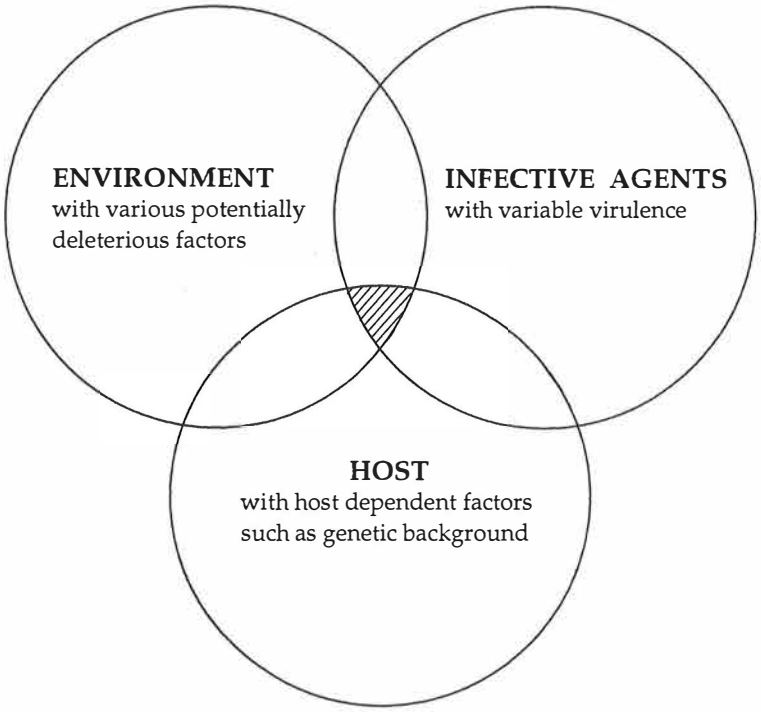

FIGURE 1 An overt infection disease occurs when a susceptible host is exposed to a virulent pathogen under proper environmental conditions, ▨ = disease outbreaks (modified from Snieszko 1974).

Natural environmental changes include physical effects caused by climatic conditions and variations and extremes in salinity, pH, temperature, oxygen levels and currents (Bucke 1993). Biological consequences of these changes include starvation, increased predation, reduced spawning, alterations in population density and the appearance of genetic disorders in fish and induction of plant toxins (Bucke 1993). A common belief is that inland waters may be more easily affected by these events. Furthermore, the influences of human activities such as waste disposal and agriculture on water quality, and hence fish, are generally more obvious in inland waters, estuaries and coastal areas (Snieszko 1974, Bucke 1993). Consequently, fishes in these areas are frequently exposed to stress (Snieszko 1974).

In general, stress is a significant but elusive concept in biology (Sindermann 1983). Following the work of Selye (1950) dealing with "stress and the general adaptation syndrome", stress has been interpreted to represent all the mechanisms by which an organism attempts to maintain equilibrium in the face of environmental change. With subsequent extrapolations and expansions of that definition, much of the study of pollution effects on aquatic organisms can be

considered a study of stress (Sindermann 1983).

Chronic exposure to sublethal concentrations of xenobiotics such as pesticides or PAH is suspected of predisposing fish to diseases because of their immunodepressive effects (Dunier & Siwicki 1993). In addition to a high organic loading, effluent discharged from pulp and paper mills contain a variety of toxic organic compounds (Sandström et al.1991). The two most common resident species normally occurring in downstream areas, roach and perch, react similarly to the toxic substances (Sandström et al. 1991), although the roach is far more sensitive to pulp effluent (Höglund 1961). Physiological variables of perch (Andersson et al. 1988) and whitefish, *Coregonus lavaretus* L. *s.l.*, (Soimasuo et al. 1995) living in the receiving water body of a pulp bleaching plant are strongly affected by the pollutants.

In the field, pinpointing the exact culprit is often difficult when a problem arises because the contaminants may be diluted before biologists are alerted. Furthermore, the effects of individual contaminants may be worsened because of their interactions with each other or with normally inert factors in the environment (Anderson et al. 1989). In the environment many toxic agents may be present, and an unknown number are available for uptake anytime. In a natural fish population the culmination of a disease outbreak is the result of a multifactorial complexity of interrelated variables (Bucke 1991). In epidemiological studies it is also essential to distinguish between the effects of pollution and the natural variability of disease occurrence in the populations (Vethaak & ap Rheinallt 1990). In biological monitoring, the most useful indicator disease is likely to be one whose effects are chronic without either recovery or mortality (Vethaak & ap Rheinallt 1990).

Overall, there are several specific ways in which an organism may respond to stress (Sindermann 1983, 1984):

1) Physiological, biochemical, and behavioural changes;
2) Changes in resistance to infection, usually a reduction in immunocompetence;
3) The classical cell and tissue changes, such as inflammation (acute and chronic), degeneration (necrosis, edema, metaplasia), repair and regeneration (proliferation, hyperplasia, and scar formation), neoplasia, and genetic derangement (including chromosomal changes, and some skeletal abnormalities).

The involvements of MCs in various disease processes and the changes caused in them by factors such as starvation suggest that these centres could provide sensitive indicators of stressful conditions in the aquatic environment (Agius 1985, Wolke et al. 1985a, 1985b, Blazer et al. 1987, Bucke 1991, Pulsford et al. 1989). Indeed, there are data showing changes in the number of MCs and the activity of macrophages caused by environmental changes (Weeks & Warinner 1986, Weeks et al. 1986, Kranz & Gercken 1987, Secombes et al. 1991). In addition, Peters & Schwarzer (1985) suggested that stress itself may induce cellular changes in the fish tissues, the main effects including both increased macrophage-like cells and enhanced red blood cell degradation.

Much of the knowledge of interaction between xenobiotics and associated effects on aquatic life is derived from acute toxicity studies or from farmed fish.

Farmed fish are usually held under conditions favouring invasions by microbes and parasites (see Peters et al. 1984, Ostland et al. 1989 and Valtonen & Koskivaara 1994), whereas laboratory studies often use concentrations of irritants orders of magnitude higher than those that occur in nature (Malins 1982, see Malins & Collier 1981). Furthermore, in the laboratory the acclimation periods have often been too short. Both factors may cause even a complete failure of fish to acclimate (McWilliams 1980). In addition, the numbers of fish or cells studied has been sometimes too small for definite and correct conclusions (Finco-Kent & Thune 1987, Speare et al. 1997).

The responses of these fish may be exaggerated. Studies of responses of natural populations of wild freshwater fish species would be desirable.

1.4 Aims of the study

In a preliminary histological investigation in 1986-1988 approximately 20 feral perch (*Perca fluviatilis*) and similar numbers of roach (*Rutilus rutilus*) were studied from four lakes near Jyväskylä. Some indications were found of pathological changes in the gills and inner organs. These changes included proliferative disorders and fusion of lamellae in the gills, various types of heart inflammations, and granulomas in different organs.

The present study compared histopathological changes in the two fish species focussing on gill alterations and the macrophage centres. Roach and perch were sampled over 5 seasons from four lakes differing both in parasite species composition and water quality. The aims were to:

1. Describe the tissue changes noted in the preliminary study in greater detail.

2. Clarify, whether the changes are of natural etiology or induced primarily by parasites, pollution or a combination of these factors.

3. Compare the cell and tissue reactions between the two fish species.

4. Assess the validity of histopathology as a tool in fisheries research.

2 THE STUDY AREA, MATERIAL AND METHODS

The four lakes studied, Peurunka, Vatia, Saravesi and Leppävesi, are located in central Finland close to the city of Jyväskylä. Although they are interconnected fish migration between the lakes has been prevented by rapids, a dam and a hydroelectric power station. A map of the study area is provided in paper V.

Water flows from Lake Vatia to Lake Saravesi and then to Lake Leppävesi. These lakes are eutrophic and during this study Vatia was also polluted by the effluent from a paper and pulp mill located 15 km upstream in the city of Äänekoski. The mill used an organochlorine pulp bleaching process between the end of the 1950s and 1992. Traces of these pollutants can also be detected in Saravesi and Leppävesi. The reference area, Lake Peurunka, is an oligotrophic and unpolluted lake connected via a small stream to Lake Vatia. In the eutrophic and polluted Lake Vatia changes have been recorded in both phytoplankton and zooplankton composition (Granberg et al. 1987), in benthic animal composition (Hynynen 1987, Meriläinen 1987), in fish physiology (Oikari & Soivio 1976), immunology (Jokinen et al. 1995) and in the form of fish red blood cells (Jeney et al. 1996). The lakes are covered by ice between the second half of November and the beginning of May. At least 14 fish species occur in all of the lakes, of which roach and perch are among the most common.

The majority of this work was performed using histological methods. For histological examination (I - V) feral perch and roach were caught from each of the four lakes during the autumn of 1989 and the winter, spring, summer and autumn of 1990 by angling or ice-fishing. Fish were transported to the laboratory in lake water. The size-distribution of each fish species sampled from each lake is given in paper IV (Fig. 1).

In the laboratory each fish was killed by severing the medulla spinalis, after which the second and third gill arches from right side (i.e. when the head of the fish is on the right) and inner organs (liver, a piece of intestine with interconnected fat and pancreas tissues, head and trunk kidney, spleen and heart) of each fish were immediately removed, fixed in buffered formalin and embedded in paraffin

(melting point 56-58 °C). Serial, longitudinal 5-8 µm thick sections were routinely stained with Harris' haematoxylin and eosin (H&E). Giemsa's solution for protozoans, Milligan's and Goldner's trichrome stains for connective tissue, Gomori's silver stain for reticulin, PAS and AB-PAS for cell differentiation and for carbohydrates demonstration in liver, and Ziehl-Neelsen for lipids and Prussian Blue (Luna 1968) for iron were also used.

For transmission electron microscopy (TEM), small pieces of tissues were fixed in phosphate-buffered 6.25% glutaraldehyde (4°C, 3 to 4 h, pH 7.4), post-fixed in 1% OsO4 for 2 h, dehydrated in a graded acetone series and embedded in LX-112 (Ladd) (I) or EPON 812 (II). Semi-thin sections (~1.5 µm) were stained with toluidine blue and safranine red. Ultrathin sections were contrasted with uranyl acetate and lead citrate and examined with a Zeiss EM 109 transmission electron microscope at 80 kV. When it was desirable to check paraffin embedded tissues at high magnifications, small pieces of tissue were deparafinized and handled as earlier (I, II).

In addition to the basic material, *Raphidascaris acus* larvae from the inner organs and intestine of 513 adult roach were studied from the same 4 lakes between September 1985 and November 1986. Monthly or bimonthly samples of 15 fish specimens were collected 7-10 times from each lake. The inner organs of the fish were studied by pressing them organ by organ between two glass sheets using 12x magnification and transmitted light. The intestinal contents were studied using the same method. Nematodes found were fixed in glacial acetic acid and stored in 70 % ethanol. Prior to examination the worms were cleared in lactophenol (III).

Additional material for studying *Henneguya* were collected as monthly or bimonthly samples of about 15 perch in 1986 - 1987, totaling 711 fish (II). *Henneguya* plasmodia were divided into "early developmental stages", "developing spores" and plasmodia in which at least some spores were fully developed and had a fully-developed tail. Identification of *Henneguya* to the species level was possible only when mature spores were present.

Entire gill arches were examined under low magnification for gill alterations (I), proliferations and fusion of secondary lamellae, whilst higher magnification was used to confirm details such as the presence of protozoans or bacteria. To enable a comparison of tissue alterations between samples the intensity of changes was quantificated according to a scale of 0 to 3 subdivided into 12 classes, where 0.25 showed a change that was just notable and 3.0 represented serious pathology occurring throughout the gills. In work IV the mean number of MCs per mm^2 was counted from 5-10 randomly chosen fields under a magnification of 156X in the spleen and liver and from 10-25 sites (313X) in the hematopoietic part of the kidney. An estimate of the area covered by macrophage centres ($\mu m^2/mm^2$) was also calculated planimetrically as an average value from the same fields. The maturity of MCs (IV) was evaluated using the scale from 0-2.00 with increments of 0.25. The smallest value of 0.25 indicates the first noticeable signs of capsule formation, where a few fibroblasts were seen around an MC; 1.00 in the scale was a MC surrounded by one layer of fibroblasts, or by a thin collagen rim, and 2.00 indicates an MC surrounded by two or more fibroblast layers. The data were recorded as mean values for each organ in each fish. MCs with a value

greater than 1.00 were classified as mature.

Statistical analyses were performed using the SYSTAT statistical package (Wilkinson 1990). The methods used were Analysis of Variance (ANOVA) and a test of independence. Non-parametric Kruskal-Wallis Analyses of Variance were used when the assumptions of ANOVA were not fulfilled. In article IV the influence of fish species, lake and size-group of fish on the numbers of MCs in liver, spleen and kidney was studied by three-way Manova. For likelihood ratio X^2 statistics, see Sokal & Rohlf (1981).

3 RESULTS

3.1 Gill alterations (I and II)

Gill anomalies were generally more common and abundant in roach than in perch. Chloride cell proliferation was the most frequent histological change in roach but was that least often found in perch. Most of the changes were systemic and light in severity. There was a marked pattern in the severity of histological changes in perch when comparing the natural Lake Peurunka with three "altered" lakes. Gills of perch from Lake Peurunka possessed more alterations in all the recorded parameters. In roach a significant difference was detected in chloride cell and epithelial proliferation that were greater in Lake Peurunka and "polluted" Lake Vatia. In perch all histological changes were significantly more frequent at the end of autumn 1989 when compared with other seasons. In roach increased frequencies of all gill changes were noted in winter and spring samples.

In perch an additional type of lesion was also observed consisting of eosinophilic inclusions without any cell organelles in an enlarged epithelium, especially at the base of secondary lamellae and on the tips of the primary lamellae (I: Fig. 6). The phenomenon occurred most commonly in the winter and spring and was least common in the summer and autumn. These eosinophilic inclusions occurred only in male fish, most often in the oligotrophic Lake Peurunka (prevalence 39.0 %) and least often in the polluted Lake Vatia (4.4 %) (I: Fig. 8).

In general, tissue reactions to parasites were localised, and neighbouring gill tissue remained unaffected (I, II). A remarkable exception were the copepods, which severely irritated the primary and adjacent lamellae (I: Fig. 4). The dominant *Henneguya* species was *H. creplini* representing more than 95 % of all *Henneguya* cysts. The remaining cysts belonged to *H. psorospermica*. Both the prevalence of infection and development of *H. creplini* displayed a seasonal pattern in all lakes (II: Fig. 6). No relationship was found between the pollution level in the lakes and the prevalence of infection. Interestingly, large differences in the prevalences were

found in the polluted Lake Vatia and eutrophic Lake Saravesi between the years 1986 and 1987 (II: Fig. 7). *Henneguya creplini* infections were recorded in all age groups of fish, but there was a definite tendency for younger perch to be more heavily infected in all of the lakes. The infection type was normally multifocal and interlamellar (II: Figs. 1 and 5).

3.2 Parasites of the inner organs: *Raphidascaris acus* larvae in roach (III)

The inner organs of roach were most heavily infected with *R. acus* in the polluted Lake Vatia, where 63 % of fish carried an average of 4.0 nematodes per fish. In natural Lake Peurunka the values were 23 % and 0.84 worms. In the other two lakes the values were intermediate between these. Similar results were also found in the occurrence of *R. acus* in the intestine. However, the overdispersion index (variance-to-mean ratio) was highest in Lake Leppävesi where the highest intensity of infection was recorded both in the inner organs and intestine. The prevalence of infection had significantly higher values in autumn in most cases, and larvae accumulated in the inner organs and intestine of older roach (III).

Of the 236 worms found in during the histological examination of 141 roach, 60 % were in the pancreas (including fat tissue) and 40 % in the liver parenchyma. Some individual worms were found in other areas such as inside blood vessels or in the submucosa of the intestinal wall. Only 20.6 % of the worms in the pancreas were alive, which is a significantly smaller proportion than in the liver (36.8 %).

The host response to *R. acus* larvae was a typical chronic granulomatous inflammatory reaction. Macrophage centres were often found between the granulomatous capsule and host parenchyma tissue, although they also were found from other areas both in liver and pancreas. No obvious difference in the histological response to *R. acus* was noted among the lakes.

3.3 Macrophage centres and heart inflammations in fish (III, IV and V)

3.3.1 Description, formation and localisation of macrophage centres

In haematoxylin and eosin stained sections macrophage centres in both fish species were yellowish, spherical aggregates surrounded by a rim of fibrocytes and fibres, whose thickness was dependent on the age of the MC (IV). The diameter of the centres was variable. The normal range was 35 - 140 μm in all organs of both species, except in the perch kidney, where the diameter was smaller, typically about 25 μm. In the kidneys, especially in roach, the intensity of the yellow colour

was generally very light, while in the heart, spleen and liver the colour was typically yellow-brown. Most MCs also contained a small amount of melanin, but always in less than one third of its area. Melanin was found most abundantly in the kidney MCs (IV). Those in the spleen were often and in the heart occasionally surrounded by a lymphocyte rim, which was sometimes very thick, consisting of 5 or even more cell layers (V: Fig. 4). MCs in the kidney and liver were also occasionally surrounded by a single interrupted layer of lymphocytes. Most centres contained macrophages that were Ziehl - Neelsen and to various degrees PAS positive (IV).

The first stage in the formation of an MC is an irregular aggregation of macrophages, occasionally associated with single neighbouring macrophages (IV: Fig. 3). Early MCs consisted of an aggregation of macrophages arranged in more or less spherical areas, and often containing phagocytosed material. These early stages of MCs were often in close contact with the sheaths of arteriolar capillaries without any signs of surrounding connective tissue (IV: Fig. 4). In the spleen MCs favoured subcapsular sinuses and periarteriolar sheaths. In the kidney MCs were irregularly distributed in the hematopoietic tissue, whereas in the liver MCs were found predominately in periportal areas. These aggregates were found to occur near parasitic invasions and between the parasite-induced granulomas and the parenchymal tissue of the host, but not inside developing capsules (III, IV). Macrophage centres were also found in 16.0 % and 24.1 % respectively of the epicardium and myocardium samples from perch and 8.7 and 7.8 % respectively from roach. In the bulbus arteriosus 8.0 % and 15.7 % and in the atrium 19.3 % and 6.6 % of perch and roach, respectively, contained macrophage centres (V).

The further development of a young MC is characterized by the accumulation of more macrophages and the appearance of single fibroblasts in the periphery (IV: Fig 5), and finally, the formation of an enveloping network of fine argyrophilic fibres (IV: Fig 6). The mature MC is characterized by a thicker fibroblastic wall, consisting of two or more layers of fibrocytes. At this stage many epithelioid cells containing cell debris in or between them are found inside the MC.

3.3.2 Quantitative analyses (IV)

Data from the two fish species and the three organs were analysed separately. There were no significant differences between male and female fish, so data from the two sexes were combined. Both fish species had individuals with an irregularly formed spleen, predominantly spleens consisting almost entirely of red pulp. In these cases, lymphocytes and MCs in the spleen were scarce or almost absent. Otherwise, MCs were regularly found in the spleen of both fish species. MCs were also found in almost all kidneys of roach, and only a few roach in each lake lacked MCs in the liver. Somewhat fewer perch had MCs in the kidneys or liver. However, there were no significant differences in the occurrence of MCs between lakes.

There were significant differences in the number of MCs / mm^2 between fish species, lakes, and fish size groups. In perch the kidney was the site of the highest number of MCs, whereas in roach the highest numbers were found in the spleen

and kidney (IV: Fig. 7a). There were no significant interactions between species and size of the fishes or between lake and size. Larger fish have more MCs in both fish species in all lakes. However, a significant interaction was found between fish species and lake, which means that the two fish species have different trends of MC numbers in different lakes. The interaction was due to differences in MC numbers in kidneys in Lake Peurunka, where perch had the lowest average number (7.2 MCs/mm^2) of all lakes, whereas numbers in roach (17.3) were similar to those in other lakes. There was also a significant interaction between lake, fish species and size group. Again, this interaction was due to differences in MC numbers in the kidney. As seen in Fig. 8 (IV), the greatest differences were in perch. In Lakes Saravesi and Leppävesi the highest numbers occurred in the largest fish, but in Lakes Peurunka and Vatia in medium sized fish. In addition, the medium-sized group in Lake Saravesi had the lowest number of MCs. In the roach kidney the occurrence of MCs was more similar to that found in the other organs of both fish species.

The areas covered by MCs were found to differ significantly between the fish species, lakes (IV Fig. 7b) and size groups, the latter so that larger fish had a greater proportion of tissue covered by MCs. The difference between fish species in the area covered by MCs in livers was nearly significant.

3.3.3 Differences in maturity of MC:s (IV)

Differences between the lakes in relation to the maturity of MCs were significant only for those in perch spleen, roach kidney and roach liver. In all these cases the most advanced levels of maturity were in fish from Lake Vatia and most immature forms were found in the kidney of roach in Lake Peurunka. The degree of maturity of the MCs was not influenced by season or fish size.

3.3.4 The occurrence of iron in the samples (IV)

Perls' positive material was found from the MCs in spleen and liver, interstitial tissue of the gonads and in the lumen of alimentary tract of both fish species. At a higher magnification, kidney and pancreatic MCs and liver cells were also occasionally seen to have a very light, diffuse, positive Perls' reaction. This was considered to be within the normal range of variation for iron. Differences in Perls' reaction in the MCs were therefore measured only in the spleen and liver.

Iron was significantly more frequent in the spleen of roach than that of perch (IV: Fig. 9). In the liver the difference between fish species was also significant, although opposite and less pronounced. There were no differences in the occurrence of iron between size groups of fishes. Comparing lakes, significantly more perch had iron positive material in the liver and spleen in Lakes Vatia and Leppävesi, respectively (IV: Fig 9). In contrast, the spleens and livers of roach from polluted Lake Vatia had the lowest iron values. The differences in iron levels between lakes were significant for roach livers and almost significant for roach spleens.

To study seasonal variation in the number of MCs and the area covered by them, fish from all four lakes were pooled. Most often the lowest numbers of both parameters were recorded during the winter from all of the organs of both fish species. During the spring the values increased in perch and the highest values were recorded most often during the summer or autumn, the differences between samples being statistically significant, except for the MCs in the spleen (IV: Fig. 10a, b). In roach the highest values of both parameters were found in the autumn samples, the differences also being significant, except in the area covered in the kidney (Fig. 11a, b). The differences in the area of individual MCs were statistically significant only in the case of roach kidneys. The size of an MC in a roach spleen was equal in all lakes and throughout the year (see IV Figs. 10c and 11c). There was no seasonality in hemosiderosis or differences between sexes in MC parameters between different seasons.

3.3.5 Heart inflammations in fish (V)

Before tackling other aims in the study of fish hearts it was necessary to classify the types of heart inflammation. This then permitted an examination of the relationship between heart inflammations and other variables such as water quality among the study lakes or differences between species. Consequently, the heart inflammations were subdivided according to the relative age (acute to chronic) and area occupied (myocarditis versus epicarditis).

Epicarditis was found more often than myocarditis in both fish species from all seasons and lakes, 22.0 % and 14.8 % of the perch and roach being affected, respectively, while the corresponding values for myocarditis were 16.1 % and 5.4 %. Most of the inflammation was chronic in perch, but in roach its proposition was only 23.7 %. No relationship was found between the occurrence or severity of heart inflammations (or macrophage centres) and the level of pollution in the lake. Furthermore, all stages of inflammation were found in all seasons with no significant seasonal trend in their prevalence, nor was any relationship found between the prevalence of inflammation and the size of the fish.

3.3.6 Etiological background of the disorders (I, IV and V)

No causative agents, such as parasites or bacteria, were ever seen to be associated with either the gill proliferations or in heart inflammations (I, V). Nevertheless, myxosporean spores, their polar capsules or valvular cells were frequently seen in MCs of *roach* spleen and kidney, but less regularly in liver MCs (IV). The myxosporean material was occasionally intact, but more often at various stages of a breakdown. Most of those rare cases in which the material was possible to identify represented *Myxobolus pseudodispar* but other species were also present. The situation was different in *perch*, where only single spores of *Henneguya* spp. were occasionally found in the spleen (IV). MCs were also regularly seen near granulomas surrounding parasites, such as the cestode *Triaenophorus nodulosus* in perch liver and larvae of the nematode *Raphidascaris acus* in roach inner organs (IV).

4 DISCUSSION

4.1 Cell and tissue disorders

In this work two species of fish were studied from a system of four lakes with graded water quality and differing parasite composition. This allowed an examination of the effect of water quality on many different factors and the consistency of the response between the two fish species to be compared. The present study focussed particularly on the host response against certain parasites, the occurrence of heart inflammations, the responses of macrophage centres and the variety of gill changes. All the pathological features found in the preliminary study were still present.

4.1.1 Gill alterations (I, II)

The proliferative gill changes (I) noted mainly in roach, *Rutilus rutilus*, may be an adaptive response to diluted fresh water rather than a pathological state. This is consistent with Laurent et al. (1985), who suggested that fish living in very soft water, i.e. where [NaCl] < 0.1 meq/l, have chloride cell proliferation as an adaptive response. There were insufficient valid [NaCl] measurements performed from all of the present study lakes, but on average the [Na] normally reaches 0.035 meq/l in Finnish waters (Forsius 1992). The fusion of perch, *Perca fluviatilis*, lamellae in the autumn sample 1989 may be caused by a peak occurrence of one or various chemicals in the water. Acid rain, either directly or via snow melting, could have caused a rapid change in pH and may have been the sole causative factor. The *differing response of perch and roach* may also be explained by their different behaviour and feeding habits. On the other hand, the two fish species may differ in their degree of response or even react completely differently to induction. The fundamental biology of these economically and ecologically important fish species should be better known.

The prevalence of infection and the development of *Henneguya creplini* plasmodia (II) displayed a seasonal pattern in all lakes. The younger fish were frequently more heavily infected. This may reflect a parasite-induced reduction in growth of the infected fish or the parasites may more easily infect weaker fish, e.g. those which have not been successful in competition of food and have subsequently been among the slow growing stock and poor immunological responders.

The growth of *Henneguya* plasmodia in fish gills may be extremely fast. The mechanisms which are used to transport nutrients from the host must therefore be very effective. This, especially when combined to the co-occurrence of plasmodia of different stages, leads to the idea that the location of plasmodia in relation to fish blood vessels is very important in determining the developmental success of the parasite.

4.1.2 Aspects of the study of changes in inner organs

The non-mammalian *blood cell* nomenclature is based on the framework in higher vertebrates, being applied with varying degrees of success (Hine 1992). In fact the term "blood cell" is partially misleading since a major proportion of the white blood cells may congregate/function in other fish tissues, and a proportion of the red blood cells may be stored in the spleen. The literature concerning fish blood cells is confusing and conflicting. Often the methods used differ from the standard procedures or terms are used without prior reference. One of the most frustrating issues is the habit of referring to all cells with a reddish-yellowish cytoplasm as "eosinophilic", regardless of which staining method or pH has been used. Ellis (1976, 1977) criticised the fact that few workers have made it clear whether heparin was used, since anticoagulants may markedly alter the staining affinity of blood cells.

Thrombocytes are responsible for blood clotting and are important in preventing the loss of tissue fluids from a surface injury. Typically they are elongated cells, often being termed "spindle cells" (Ellis et al. 1989). The preparation of the blood smear must be done carefully or else the thrombocytes may cast off most of their cytoplasm and appear as small, densely staining nuclei, surrounded by a minute amount of cytoplasm. This "spent" thrombocyte has been frequently confused with the lymphocyte and has lead to many unreliable differential counts of these two cell types (Ellis 1977, Ellis et al. 1989). The teleost *erythrocyte* is similar in size and ultrastructure to that of the other vertebrates but, like avian and reptilian erythrocytes, it is nucleated (Ellis et al. 1989). The fact that fish red blood cells and thrombocytes are nucleated makes the histopathological examination of fish tissues very slow and complicated even accounting for the existence of possible artefacts. For example, estimating even the gross numbers of lymphocytes in different spleen or blood samples is difficult.

The idea that perch might represent a higher phylogenetic level than roach is widely accepted in ichthyology. In fact, Perciformes is one of the youngest branches in vertebrate development (Siewing 1985). Quesada et al. (1990) suggested that the ratio of red and white pulp reflects the different capacities of the

fish spleen. If this ratio also reflects differences between fish species, there may be a significant difference in the immunological capacity between perch and roach, since perch have greater quantities of white pulp. Furthermore, the kidneys of perch, with a large and highly differentiated head kidney, give the impression of a higher level of organization when compared to roach. The present work IV supports the results of Press et al. (1994), who stated that there are great differences between individual fish of the same species in relation to the development of the spleen. Unfortunately this phenomenon could not be studied in detail, but there appeared to be no obvious trend in relation to, for example, seasonality.

4.1.3 MCs in the organs of perch and roach

Marked differences between the fish species under study, the roach and perch, were found in many of the variables measured. The mean size of a single MC was relatively constant between the organs of roach, whereas in perch the liver contained large MCs whilst the spleen, and especially the kidney, very small ones (IV). Macrophages do execute a multiplicity of functions, and MCs in the perch kidney may represent a different functional population of macrophages in comparison to those in the roach kidney, where a clear defensive nature against myxosporeans was apparent for most MCs. Furthermore, MCs in the spleen, kidney and liver, although morphologically similar, have been suggested as having important functional differences even within an individual fish (Agius 1985).

4.1.4 The role of MCs in parasitic infections

It has been suggested that neither the level of parasitic infection nor the presence of gross lesions are significantly related to MC parameters (Brown & George 1985, Wolke et al. 1985a). In metazoan parasites there is too much material to be removed and destroyed by macrophages and a nonspecific inflammatory reaction with the formation of a granuloma is typical (III). In these cases some single MCs are often noted, although it is not known whether the parasite itself or the cell and tissue damage causes the formation of these aggregates (see Vogelbein et al. 1987, Wolke 1992). In the present material, MC responses to larvae of the nematode *Raphidascaris acus*, especially in the liver and pancreas of roach (III, IV) and to cestode *Triaenophorus nodulosus* (IV) in perch liver, were common. With small parasites such as protozoans the reaction may be different. Many histozoic species have a tough wall of plasmodia around the spores, which is possibly not detectable by macrophages as an antigen (II). *M. pseudodispar* was also frequently found in roach of the present study area, its prevalence being lower in the polluted lake when compared to others (Brummer-Korvenkontio et al. 1991). The spores of *Myxobolus pseudodispar* could also be identified in MCs in this study (see Roberts 1975). The myxosporean load could account for the large number and area covered

by MCs in roach when compared to perch, in which myxosporeans are mainly located in the gills (see Brummer-Korvenkontio et al. 1991).

4.1.5 The role of MC capsule (IV)

The turnover rate of MCs and their surrounding capsule, where present, may be affected by a variety of factors such as the inducing agent and temperature. No differences were recorded in the 'maturity' of MCs in relation to the season or fish size groups, although significantly more MCs were recorded in older fish (see Brown & George 1985, Blazer et al. 1987). Kranz (1989) observed only small differences in the occurrence of the capsule between healthy, ulcerous and scarred dab, *Limanda limanda*. However, it is notable that the scarred dab had less encapsulated MCs than the ulcerous fish. One could assume that there may also exist differences in capsule thickness between fish living in polluted and reference areas. Some differences were indeed found between lakes in the present study. The fact that the highest values were found from polluted Lake Vatia appears to be promising. However, there were no obvious trends between the lakes and the differences may reflect a differing inducing agent or time of induction rather than pollution. Furthermore, the use of mean values may have masked any existing differences.

4.1.6 Hemosiderin in MCs (IV)

In the present study all of the inner organs of free-living perch and roach were studied and Perls' positive material in MCs was found to occur in the spleen and liver of both fish species. At times a very light, diffuse, positive Perls' reaction was also observed in kidney and pancreatic MCs and within liver cells. This was noted to be within the normal wide range of iron. The phenomenon has also been noted by Bucke and Feist (1993), who diagnosed it as siderosis when it existed with iron overloaded MCs. Trace amounts of hemosiderin have also noted on the hepatocytes of splenectomised trout, *Salmo gairdneri* Richardson (the present name *Oncorhynchus mykiss* Walbaum), after prolonged starvation (Agius 1981b), in the splenic red pulp and ellipsoids, as a stippling of pigment in the liver and occasionally also as small deposits in the kidney in *Platycephalys bassensis* Cuvier from Tasmania (Langdon 1986).

There have been several reports that fish from an impacted environment may have increased iron in MCs (e.g. Bucke et al. 1984, Wolke et al. 1985a, Langdon 1986, Blazer et al. 1987). Marked differences in hemosiderosos were apparent between the present study lakes but different patterns were recorded for the two fish species under investigation, the perch and roach. Perch in the polluted Lake Vatia had the highest iron values, whereas the roach had the lowest. In Lake Leppävesi both species had high iron values. Hemosiderosis in the clean, oligotrophic Lake Peurunka, although with a tendency to have relatively low values, was generally similar to the other lakes. The differences between the two fish species, especially in the polluted Lake Vatia for hemosiderosis in the liver,

show that more than one fish species should be used for this type of investigation. Apparently, if only one fish species and two study lakes had been selected, the "clean" (Peurunka) and "polluted" (Vatia) ones, this study would have been more likely to draw "*conclusions*" about the effects of pollution. The increased iron values in perch of polluted Lake Vatia also suggest that perch have more prominent alterations in the iron content of MCs than roach in relation to decreasing water quality, and it might be a better indicator of water pollution. In any case, further investigations of hemosiderosis are needed to confirm these findings. If fishes from other clean waters than Lake Peurunka also show signs of systemic hemosiderosis, it may not be appropriate as an indicator of water pollution (see Peters et al. 1987).

How can the present findings on hemosiderosis be explained? Some possibilities are as follows:
(1) The existence of hemosiderosis in wild fish populations may have been ignored by histopathologists, and hemosiderosis in the spleen and other sites may in fact normally exist and form a natural part of fish biology (see Roberts 1975, Nounou et al. 1981, Langdon 1986). In general, hemosiderosis is thought to be a pathological state reflecting a severe hemolytic process. It may be possible that in Finland, where roach and perch are close the northern limit of their distribution, this phenomenon is common and no other special inducing factors are needed. Anyway, no annual cycles in hemosiderosis were observed, and this suggests that iron accumulation be caused by other factors. (2) In the present study area one or more unknown factors may exist in both fish species which cause deformatation or destruction of red blood cells, or iron demobilization as a defensive mechanism (see Bullen 1981, Griffiths 1987, Kranz 1989). Hemosiderosis has been suggested to be caused by the short longevity of red blood cells in polluted waterbodies (van de Graaff 1985). In any case, it is difficult to find pollutants which would be present in all of the present study lakes, with exception of agricultural ones. However, land surrounding the study lakes is not intensively farmed. Larvae of the nematode *Raphidascaris acus*, especially in the liver and pancreas of roach (II), and the cestode *Triaenophorus nodulosus* in perch liver (Valtonen et al. 1997), are common. Nevertheless, they cannot be held responsible for systemic hemosiderosis. Neither can other parasites, except perhaps the protozoans mentioned above. There is still the possibility that both roach and perch have one or more undetected parasites, such as blood protozoans, which might prove to be the causative agent. 3) Dietary iron sources may be more prominent or iron could accumulate more in fish MCs than elsewhere. These hypotheses could be confirmed or rejected only by experimental work (see Salte et al. 1994).

It is difficult to avoid the idea that hemosiderosis is present not only in some pathological conditions, but also when more iron is present than the fish can utilize; i.e. hemosiderosis in different sites may be analogous to fat and glycogen storage. It would, therefore, be interesting to know the quantity of iron in fish MCs showing differing degrees of Perls' positive material, as compared with other sites in fish organs.

4.1.7 Why did most of the noted tissue changes not increase with decreased water quality?

In Scandinavia the bedrock consists mainly of slowly weathering granites, gneisses and porphyries with low buffering capacity. The fresh waters therefore have only minor amounts of calcium, magnesium and bicarbonate, which are of great importance for the buffering capacity (Karsson-Norrgren et al. 1986) and fish maintenance. In the present study the explanation for some contradictory findings may be that the pollution has increased the buffering capacity of the affected lakes, so minimizing the peaks caused by chemical imbalances, Lake Peurunka alone showing the peak effects. This also means that the level of pollution even in the most polluted Lake Vatia has not on average been detrimental for fish health, the enhancing effects being also present. It is obvious that those works which use only two study sites, one clean and one polluted, may give simple but misleading information caused by accidental or unknown factors. Replication, gradients, or preferably both, seem required.

4.2 The future role of histopathology in fisheries research

Histology is a branch of biology associated with the microscopic examination of thin, stained tissue sections in order that the nature of the material and ways in which components are structurally and functionally related can be studied (Bruno & Poppe 1996). Cytology, electron microscopy and histochemistry are commonly also counted as branches of histology.

Histology is often now considered an old-fashioned technique in biology and omitted from many courses in favour other techniques or targets of interest (Kime 1995). Instead, there are several immunological (Weeks et al. 1989), biochemical and other novel techniques such as molecular biology which are increasingly used in fish research (Kime 1995). As a result there is a generation of fish biologists who have received little formal background in histology and its use in interpreting the structure or pathology of animal tissues (Kime 1995). This inadequate knowledge may be impossible to rectify later, which may cause severe diagnostical difficulties. This is especially the case in the study of fish diseases, because the symptoms and gross pathology of many fish diseases are frustratingly similar. Distinguishing between the different pathological conditions may be difficult for inexperienced personnel, notably where two or more diseases are present (Bruno & Poppe 1996).

However, histology has adopted many new techniques in the past 20 years, including immunological, molecular biological and biochemical techniques. The histological procedures themselves have also developed. For example, there have been advances in all aspects of histological preparation, such as methods to produce better paraffin (e.g. Barlow 1993) and electron microscopy sections (e.g. Mesy Jensen & Sant'Agnese 1992), new reagents (such as LR gold) or different ways to handle tissues, such as microwave stabilization (e.g. van de Kant 1990).

Also the methods that permit the acquisition of quantitative data from the microscopic image or from micrographs now provide new possibilities. In fact, the entry of small computers into most laboratories and the ready availability of software programs for morphometry have made the technique feasible for integrative toxicological investigations. Not only have the quantitative data been obtained from these investigations, but a systematic approach to structural investigation at progressive levels of organization (organ, tissue, cell, and organelle) has also arisen (Hinton et al. 1987).

What about the future? First and foremost there is likely to be a continuing need for the initial morphological stain (such as Papanicolaou) to act as a springboard for the special stains (Cook 1991) or for other purposes, such as screening the fish for background information about the population of fish taken for laboratory experiments. Data on unusual tissue changes, for example, may be essential for selecting (or avoiding) the population for laboratory experiments. The use of conventional staining techniques is also supported by their low costs as compared with other methods.

Secondly, with recent concern about the effects of environmental pollutants on fish, and the more extensive use of aquaculture, fisheries scientists increasingly require histological methods to examine fish for malformations or pathological signs of disease (Kime 1995). Indeed, the histopathological biomarker approach has recently been found a valid one for field investigations, especially because of its ability to answer to the question "what price does a fish pay for residence at a given location?" (The et al. 1997). This is in contrast to PCR technology in particular, which only indicates that somewhere in the sample there is a gene of interest, meaning that PCR does not answer the crucial question:"in what cell is it located" (Brigadi 1991). Standard histopathology may also be a valuable technique for detecting environmental changes, especially if it is used to examine a specific system sensitive to a wide range of factors (Wolke et al. 1985b).

Thirdly, the special techniques now being developed will eventually form part of the standard procedures in many laboratories. For example, immunocytochemistry and its avant garde constituents, *in situ* hybridization and the polymerase chain reaction will, without doubt, continue to develop and expand (Cook 1991). In fact, *in situ* hybridization is a method every histopathology laboratory should master because it increases the power of a standard light microscope (Brigadi 1991). In addition, molecular biology as a technique will be more closely connected with histology and will be used routinely at least in larger laboratories. Furthermore, it has been stated that a histopathological laboratory must have both immunopathological and molecular pathological branches in future, or else it will have a very limited role by the turn of the century (Brigadi 1991, Coates 1991).

Additionally, new possibilities in histopatological laboratories arise from the fact that recent modifications in molecular biological techniques for DNA and RNA analysis can be performed using formalin-fixed and paraffin-processed tissues (see review by Coates 1991). This means that enormous collections of medical and biological materials will be available for future studies.

5 SUMMARY AND CONCLUSIONS

In this work information was provided on the cell and tissue responses in the gills and inner organs of two common freshwater fish, perch and roach. Samples were collected over 5 seasons from a system of four lakes with graded water quality. Some cell and tissue changes were of parasitic origin and some are suggested to be connected with water quality, although often the most prominent changes were found from the clean Lake Peurunka. Although many answers were found, many new questions were also raised. Further investigations are needed, for example, to quantify and understand the depression of the fish spleen, and to determine whether this phenomenon is correlated with water quality and could it be used as a new biomarker for aquatic pollution.

Many differences in responses between the two common and ecologically important fish species were recorded. For example, chloride cell proliferation was the most frequent gill alteration in roach but least often found in perch. The species also showed markedly different patterns in MC parameters. The use of more than one fish species as an indicator is therefore highly desirable. Furthermore, there is an obvious need to clarify the basic biology of even the most important fish species.

Usually the patterns observed in fish tissues were not directly related to either season or aquatic pollution. This may be explained by the normal multifactorial background of fish stress and disease, meaning that there are normally numerous stress factors present in aquatic environment. They appear to cause complex responses, determined in part by differences in inducing agents, time of induction or the capacity of the fish to respond. Most bioindicators, such as MCs in the present study, may only reflect the balance between enhancing and depressing responses. All this supports the idea that a battery of tests is needed to evaluate the different responses of fishes (see Dunier & Siwiski 1993).

Finally, the future role of histopathology in fisheries research was considered. Overall, there will be a continuing need for the histopathological

laboratory with some of its present methods. However, the future of such laboratories depends on their willingness to learn and adopt new techniques, especially *in situ* hybridization.

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YHTEENVETO

Solu- ja kudosmuutokset ahvenella (*Perca fluviatilis*) ja särjellä (*Rutilus rutilus*) suhteessa veden laatuun

Tutkimuksessa on selvitetty kudosmuutosten esiintymistä ahvenella (*Perca fluviatilis*) ja särjellä (*Rutilus rutilus*) neljällä likaantuneisuudeltaan erilaisella järvellä Keski-Suomessa. Yksi järvistä on lähes luonnontilainen Peurunkajärvi, josta saatua kalojen kudosmateriaalia verrattiin likaantuneen Vatian vastaaviin näytteisiin. Kaksi muuta järveä olivat Saravesi ja Leppävesi, jotka sijaitsevat Vatian alapuolisessa vesistössä ja tarjosivat siten likaantumisen suhteen gradientin. Tutkimusmateriaali kerättiin vuosina 1989 - 1990 eli aikaan jolloin puunjalostusteollisuuden klooripäästöt vielä rasittivat Vatiaa ja sen alapuolista vesistöä.

Tutkimus perustuu vuonna 1986 - 1988 suoritettuun esikokeeseen, jossa kaksikymmentä särkeä ja ahventa käsiteltiin histologisesti ja tutkittiin valomikroskoopilla. Esitutkimuskaloista löydettiin viitteitä sydäntulehduksista sekä runsaasti eriasteisia granuloomia. Lisäksi kalojen kiduksilla havaittiin kiduslamellien yhteenkasvua sekä huomattavasti tavanomaista suurempi määrä muita kuin hengitykseen liittyviä soluja.

Varsinaisessa tutkimuksessa selvitettiin histologisin tekniikoin esikokeessa havaittujen muutosten yleisyys ja voimakkuus. Lisäksi tutkittiin makrofaagien esiintymistä ja ominaisuuksia ahvenen ja särjen elimissä ja kalalajien eroavaisuuksia kudosvasteissa, sekä pyrittiin selvittämään kudosmuutoksia aiheuttaneet tekijät. Näyte-erät kerättiin kaikilta vuodenajoilta, jolloin saatiin selvitettyä myös kudosmuutosten ja eräiden loisten mahdolliset vuodenaikaisvaihtelut. Kiduksissa havaitut muutokset olivat yleensä lieviä, mutta niitä esiintyi laajoilla alueilla kiduksissa. Kidusmuutoksia esiintyi särjellä enemmän kuin ahvenella. Yleisin särjeltä tavattu muutos oli kloridisolujen proliferaatio eli kloridisolujen lukumäärän lisääntyminen. Kloridisolujen lisääntymisen syy särjen kiduksilla voi

olla sopeutuminen suomalaisten vesien suhteellisen alhaiseen ionimäärään tai ionien poikkeaviin keskinäisiin suhteisiin. Ahvenella kloridisolujen proliferaatio oli harvinainen.

Luonnontilaisen Peurunkajärven ahvenelta tavattiin runsaasti vakaviksi luonnehdittavia kidusmuutoksia. Muutokset keskittyivät vuoden 1989 syksyn näyte-erään, mikä viittää kemiallisen ärsykkeen aiheuttamaan lyhytaikaiseen altistumiseen. Todennäköisin selitys on nopeasti ohimennyt happaman sateen aiheuttama alenema veden pH:ssa. Lisäksi kaikkien järvien koiraspuolisilta ahvenilta löydettiin etiologiselta taustaltaan selvittämätön kidusinkluusio. Talvinäytteissä inkluusioita oli jopa yli puolella koirasahvenia, kun taas kesällä sitä tavattiin vain satunnaisesti. Ilmiö korreloi suoraan vedenlaatuun, kuitenkin siten, että Peurungan kaloissa sitä esiintyi runsaimmin ja Vatian kaloissa vähiten.

Ahvenen kiduksissa loisivan *Henneguya* -suvun (Protozoa, Myxosporea) alkueläinloisen todettiin olevan *H. creplini*, joskin vähäisiä määriä tavattiin myös *H. psorospermica*a. *Henneguya*n kystit katosivat ahvenen kiduksilta loppukeväästä tai alkukesästä. Eritoten keväällä loisella esiintyi runsaasti erivaiheisia plasmodioita yhtäaikaisesti, mikä poikkeaa eräistä muista *Henneguya* -lajeista. Plasmodioiden koon vaihteluun esitettiin mahdolliseksi syyksi loisen sijoittuminen suhteessa isännän verisuoniin. Puolustusreaktiota kyseistä loista vastaan ei yleensä esiintynyt.

Särjessä esiintyvän *Raphidascaris acus* -lieriömadon (Nematoda, Ascaridoidea) todettiin esiintyvän runsaimmin vanhemmissa kaloissa, ja onkin todennäköistä, että infektio kumuloituu kalojen kudoksiin koko isännän eliniän. Runsaimmin loista tavattiin syksyn näytteissä. Luonnontilaisessa Peurunkajärvessä loista oli vähiten, ja eniten likaantuneessa Vatian järvessä. Histologiassa todettiin suurimman osan elimissä olevista loisista olevan kuolleita, kuolleiden määrä haimassa oli suurempi kuin maksassa. Isäntä reagoi loista tai sen aiheuttamaa kudostuhoa vastaan epäspesifisellä puolustusreaktiolla, granulooman muodostuksella.

Sydäntulehduksia löydettiin noin 20 %:lla sekä särjistä että ahvenista. Tyypillisin tulehdustyyppi oli epicarditis, joka ahvenella oli useimmiten krooninen, kun taas särjellä vain noin neljännes epicarditiksista oli luokiteltavissa kroonisiksi. Myocarditikset olivat molemmilla lajeilla lähes kaikki kroonisia. Tulehdusten esiintyminen ei korreloinut veden laadun eikä kalojen koon kanssa. Myöskään mitään vuodenaikaisvaihtelua ei havaittu.

Kehittyneillä luukaloilla makrofagit keräytyvät, ns. makrofagikeskuksiksi erityisesti pernaan ja munuaisiin. Näitä keskuksia on pidetty lupaavina veden laadun sekä kalan kunnon indikaattoreina. Tässä työssä tutkittiin makrofagikeskusten esiintymistä ja ominaisuuksia lähinnä sydämessä, maksassa, pernassa sekä munuaisen vertamuodostavassa osassa. Keskusten määrän sekä niiden peittämän alan todettiin vaihtelevan yksilöiden, lajien, elinten ja järvien välillä. Lisäksi todettiin raudan kertyvän kalan elimistä lähinnä vain pernaan ja maksaan hemosideriininä; jälleen todettiin selvät erot yksilöiden, elinten, lajien ja järvien välillä. Kuitenkaan mikään edellä mainituista vaihteluista ei korreloinut veden laadun kanssa. Mahdollisesti kyseiset keskukset ovat toiminnoiltaan liian moninaisia, että niiden käyttö sellaisenaan vedenlaadun- tai kalan kunnon indikaattorina olisi perusteltua.

Töiden yhteenvedossa otettiin myöskantaa histopatologian asemaan tieteen kentässä. Onko histopatologialla tulevaisuutta modernien tekniikoiden joukossa? Asiaa voidaan tarkastella monesta näkökulmasta. Histologia tekniikkana vaatii hyvin harjaantuneen henkilökunnan ja on suhteellisen suuritöinen. Toisaaltaan menetelmät ovat hyvin standardoituja ja halpoja. Varmaa ainakin on, että perusvärjäyksiä, kuten esimerkiksi Papanikolaou, tullaan jatkossakin käyttämään yleisesti rutiinidiagnostiikkassa sekä esikokeissa esim. valittaessa materiaalia muita tekniikoita varten. Uudet tekniikat tuovat histopatologian laboratorioille myös mahdollisuuksia. Erityisesti tulee hyödyntää immunosytokemian ja *in situ* hybridisaation tuomat mahdollisuudet, koska ne vahvistavat histopatologian vahvimman aseen, valomikroskoopin, asemaa. Mikäli histopatologian alalla työskentelevät eivät ole valmiita omaksumaan ja soveltamaan uusia tekniikoita, tulee histopatologian laboratorioiden rooli tulevaisuudessa olemaan hyvin rajoittunut.

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ORIGINAL PAPERS

I

Gill anomalies of perch and roach from four lakes differing in water quality

by

Haaparanta, A., Valtonen, E. T. & Hoffmann, R. W.

J. Fish Biol. 50: 575-591, 1997

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II

Pathogenicity and seasonal occurrence of *Henneguya creplini* (Protozoa, Myxosporea) on the gills of perch *Perca fluviatilis* in central Finland

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III

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IV

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V

Heart inflammation in perch *Perca fluviatilis* and roach *Rutilus rutilus* from central Finland

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