

## Influence of Chronic Exposure to Treated Sewage Effluent on the Distribution of White Blood Cell Populations in Rainbow Trout (*Oncorhynchus mykiss*) Spleen

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Impairment of immune function in aquatic animals has been proposed as a possible consequence of low-level contamination of surface waters with anthropogenic substances such as through the discharge of wastewater into rivers, lakes, and oceans. The study at hand investigated the effects of chronic (32 weeks) exposure to sewage treatment plant (STP) effluent on the prevalence and distribution of different leucocyte populations in spleen samples of rainbow trout (*Oncorhynchus mykiss*). To simulate an infection, fish were injected intraperitoneally (ip) with inactivated *Aeromonas salmonicida salmonicida*, 6 weeks prior to the termination of the experiment. Immunohistological analysis revealed a marked decrease in thrombocyte numbers, an increase of monocytes, altered distribution of B-cells, and higher surface immunoglobulin expression, as well as activation of MHC class II expression in the spleen after exposure to 15% (v/v) effluent. The most prominent finding of the present study, however, was the occurrence of intraplasmatic deposits or inclusions with strong autofluorescence in spleen sections from effluent-exposed trout. In addition to effluent effects, injection of trout with *A. salmonicida* stimulated infiltration of monocytes, increased staining intensity on thrombocytes, and enhanced MHC class I expression in larger leucocytes surrounding melanomacrophage centres. In general, the current study demonstrates a marked, potentially adverse effect of STP effluent on spleen leucocytes and on the integrity of spleen tissue. The observed response suggests a constant unspecific stimulation of different leucocyte populations and is reminiscent of chronic inflammation.

**Key Words:** *Oncorhynchus mykiss*; fish immune system; fish immunology; *Aeromonas salmonicida*; trout leucocytes; trout specific monoclonal antibodies.

In the last few decades, the attention of scientists in the field of environmental toxicology has shifted from the observation of direct toxicity to the identification of more subtle effects of pollution. A variety of substances including pharmaceuticals,

industrial chemicals, and compounds contained in many household products, while not commonly leading to fish kills or other clearly visible effects, have recently been shown to affect endocrine as well as immunological mechanisms in vertebrates at relatively low concentrations (Daughton and Ternes, 1999). Contamination of surface waters with highly potent substances at low concentrations has also been related to altered reproductive performance, possibly leading to a reduction in fish populations (Matthiessen and Sumpter, 1998; Robinson *et al.*, 2003). The main source of such pollutant mixtures is the release of municipal, agricultural, and industrial wastewater into surface waters (Desbrow *et al.*, 1998; Kummerer, 2001).

Histological investigations within the field of fish toxicology have focused on general pathology (externally visible disease, skin structure and lesions, liver lesions, necrosis and apoptosis, as well as inflammatory reactions) and tumor incidence (Vethaak, 1992; Wahli *et al.*, 2002). Only in the last few years have environmental toxicologists started to consider effects of aquatic pollution on the immune competence of fish. With specific antibodies against fish immune cells becoming more readily available, it is now possible to track leucocyte populations in peripheral blood, as well as in organ samples. In the field of immunotoxicology, the occurrence and distribution of different white blood cell populations (e.g. in haematopoietic tissues) is of major relevance. Alterations in prevalence and activity of different types of leucocytes point to changes in a variety of immune reactions, as immunological activity is almost exclusively based on leucocyte integrity.

In the current study, rainbow trout (*Oncorhynchus mykiss*) were exposed to nominal concentrations of 1.5 or 15% (v/v) municipal sewage treatment plant (STP) effluent, reflecting concentrations known to commonly exist in the environment, over a period of 32 weeks (chronic exposure). Six weeks prior to the termination of the experiment, fish were injected (ip) with inactivated *A. salmonicida salmonicida* to stimulate the immune system or with phosphate balanced salt solution (PBS) as a control for the injection. Immunohistology with specific antibodies against rainbow trout leucocyte surface

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markers was used to investigate effects of sewage treatment effluent on occurrence and distribution of B-lymphocytes, monocytes, granulocytes, and thrombocytes, as well as the cell surface molecules MHC class I and MHC class II in spleen cryosections.

## MATERIAL AND METHODS

**Fish.** Two-year-old rainbow trout (*O. mykiss*), which were reared at New Zealand Forest Research Institute (Rotorua) were used for exposure experiments. Parent fish were purchased from the New Zealand Fish and Game Ngongataha hatchery (Rotorua, New Zealand). Fish were held in 12,000 l tanks, each containing 50 individuals, and were fed daily with commercial aquaculture feed pellets (Reliance stock food, Dunedin, New Zealand) at a ration of 0.7% of wet body weight, and the ration was increased by 25 g monthly as per the known growth trajectory with fish of this size. All trout were tagged with individually numbered T-bar type tags (HallPrint Pty Ltd, Holden Hill, SA, Australia), as well as weighed and measured at the start of the experiment.

**Sewage treatment plant effluent.** Final treated effluent was obtained from a sewage treatment plant located in Rotorua, New Zealand. This STP employs a pretreatment step with stop screens and a grit trap, a primary treatment step with sedimentation, and secondary activated sludge treatment (Bardenpho Reactor). Effluent holding tanks at the trout exposure facility were refilled with final treated effluent every second day.

### Experimental Set Up

**Trout exposure facility.** In the trout exposure experiment, activated carbon dechlorinated tap water was used as the diluent and as the reference treatment (aquifer source). Water flow was controlled by line pressure using stainless steel globe valves and spring-operated flowmeters. Effluent flow was controlled using a head tank to maintain constant pressure in combination with a PVC aperture calibrated for the nominal flow. Diluent flows were adjusted daily when necessary, and the effluent control apertures were cleaned daily to prevent reduction of flow due to biofouling. Two replicate tanks were used for each treatment. Trout exposure tanks were provided with a constant water flow of 10 l/min, which resulted in a 95% replacement time of approximately 45 h.

**Water parameters.** Dissolved oxygen, pH, and conductivity (Radiometer Pacific, Auckland, New Zealand) in the fish exposure tanks and in undiluted effluent were measured daily. Additional aeration was provided in the effluent holding tanks and the trout exposure ponds, maintaining dissolved oxygen above 90% saturation for the duration of the experiment. The average pH values with

standard deviation in the replicate exposure tanks were  $7.21 \pm 0.25$  and  $7.22 \pm 0.25$  in the 15% effluent tanks,  $7.15 \pm 0.28$  and  $7.20 \pm 0.28$  in the 1.5% effluent tanks and  $7.13 \pm 0.29$  and  $7.13 \pm 0.28$  in the control tanks. Conductivity in each tank, diluent conductivity, and effluent conductivity were used to calculate the actual effluent concentration in the fish tanks on a daily basis. The actual mean effluent concentration was  $13.48 \pm 3.25$  and  $12.91 \pm 3.51$  in the 15% effluent tanks and  $1.45 \pm 0.59$  and  $1.59 \pm 0.64$  in the 1.5% effluent tanks. Water temperature ranged between 12.7 and 16.7°C, 12.7 and 16.9°C, and 12.9 and 17.5°C in control water, 1.5% effluent, and 15% effluent, respectively.

**Exposure.** Trout were exposed to a nominal concentration of either 1.5 or 15% (v/v) effluent. Control fish were kept in dechlorinated tap water. The exposure was started on September 22, 2001, with immature fish and was terminated between May 6 and 14, 2002, when trout were close to spawning. After exposure for 26 weeks, trout were anesthetized with ethyl-3-aminobenzoate methanesulfonate (MS222) (Fluka, Switzerland), and 1 ml of blood was taken by syringe from the caudal vein. Fish were then either injected intraperitoneally with formaldehyde inactivated *A. salmonicida salmonicida*, strain MT 423 ( $1 \times 10^8$  cells in 250  $\mu$ l per 100 g body weight), or with a corresponding volume of phosphate balanced salt solution (PBS) as a control for the injection. Antigen preparation of *A. salmonicida* followed the description by Koellner and Kotterba (2002). Fish were exposed to effluent for a further 6 weeks until the experiment was terminated and the fish were sacrificed.

**Sampling.** Female trout were sampled first over two consecutive days by taking out three *A. salmonicida*-injected and three PBS-injected fish from each tank per day. Male fish were sampled 7 days later, following the same sampling scheme. Peripheral blood samples were taken from the caudal vein and used to obtain serum for analysis of *A. salmonicida*-specific antibodies. Representative samples of spleen were snap-frozen in liquid nitrogen and stored at  $-80^\circ\text{C}$  for histology.

**Immunohistology.** Spleen samples were cut using a cryostat microtome (Leica CM3050, Leica, Germany). Approximately 8- $\mu$ m-thick organ sections were placed on poly-L-lysine-coated glass slides (0.1% w/v in water; Sigma, Steinheim, Germany). After fixation in 100% acetone for 10 min at 4°C, the sections were air-dried. Dry sections were incubated with primary antibodies (list of antibodies used, see Table 1) for 1 h at room temperature. The slides were washed twice in Iscove's Modified Dulbecco's medium (Invitrogen, Karlsruhe, Germany) and subsequently incubated with secondary, fluorescence-labeled antibodies (see Table 1) for 1 h. After washing twice in medium, slides were mounted in PBS, containing 10% glycerin and 2.5% 1,4-diazobicyclooctan (Dabco) (Sigma, Steinheim, Germany), covered with cover slips, and examined for specific fluorescence using an LSM 510 confocal laser scanning microscope (Carl Zeiss, Hallbergmoos, Germany). The sections were scanned using a 40 $\times$  oil immersion objective. The 488 nm line of an argon laser was used for Alexa 488 and fluorescein-isothiocyanat (FITC) excitation, and the 543 nm line of a helium/

TABLE 1  
Primary and Secondary Antibodies Used for Immuno-Histochemical Staining

Cell Type Or Surface Marker	Primary Antibody	Secondary Antibody
Granulocytes	Monoclonal antibody (mab) Q4E (Kuroda <i>et al.</i> , 2000)	Goat-anti-mouse IgG <sub>3</sub> , FITC-conjugate (Medac, Hamburg, Germany)
Monocytes	mab 45 (Koellner <i>et al.</i> , 2001)	Goat-anti-mouse IgM ( $\mu$ ), R-PE-conjugate (Caltag, Hamburg, Germany)
B-cells	mab N2 (Fischer and Koellner, 1994)	Goat-anti-mouse IgG-Alexa Fluor 488 (MoBiTec, Goettingen, Germany)
Thrombocytes	mab 42 (Koellner <i>et al.</i> , in press)	Goat-anti-mouse-IgG <sub>1</sub> ( $\gamma$ ), FITC-conjugate (Caltag, Hamburg, Germany)
MHC I	mab H9 (Dijkstra <i>et al.</i> , 2003)	Goat-anti-mouse-IgG, Cy3-conjugate (Dianova, Hamburg, Germany)
MHC II	Oslo antiserum (Koppang <i>et al.</i> , 2003)	a-rabbit-FITC (Sigma, Steinheim, Germany)

neon laser for R-Phycoerythrin (R-PE) and indocarbocyanin (CY3) excitation. The fluorescence emission was recorded using a main beam splitter at 488/543/633 nm in combination with a second beam splitter at 505–550 nm and an emission filter for 560–615 nm. The obtained scans were analyzed using the LSM 510/2.1 software package (Carl Zeiss, Hallbergmoos, Germany).

Per treatment group spleen samples from four female fish were analyzed. Pictures of four representative areas of spleen tissue from each fish were taken. The 16 pictures per treatment group were evaluated as follows: of the four pictures per fish the one showing the average in staining intensity and amount of stained cells was chosen. From the resulting four pictures again the representative picture was chosen based on the same criteria. Using this “histomorphological average choice method,” the presented pictures show the true difference between each exposure group.

***A. salmonicida*-specific antibody ELISA.** The ELISA for the detection of *A. salmonicida*-specific antibodies in trout serum followed the description of Koellner and Kotterba (2002), except for sample dilution (1:4000) and detection, which was carried out using TMB (Sigma, St. Louis, U.S.A.). The color reaction was stopped by the addition of 1 M H<sub>2</sub>SO<sub>4</sub>, and absorption was measured at 450 nm in an SLT plate reader 340 ATTC (SLT Labinstruments, Groedig, Austria). As no standards were available for IgM determination, results are given as optical density. To enable comparison without a standard curve, all samples were measured in parallel in a single ELISA run, which was repeated once.

## RESULTS

### *Spleen*

Spleen tissue sections from fish exposed to 15% effluent appeared to have less structural integrity (the cell collective appeared less tight) as compared to tissue samples from control fish. Moreover, a high prevalence of an intense orange/red and patchily distributed autofluorescence was regularly observed after exposure to 15% effluent (Fig. 1). As shown in double staining using the monoclonal antibody (mab) 45 (anti-trout monocytes) and mab Q4E (anti-trout granulocytes), this autofluorescence seems to be concentrated in trout monocytes. In general, only marginal differences in the prevalence and distribution of different leucocyte populations between control fish and the 1.5% effluent group, as well as in surface expression of the markers, recognized by the mabs used were noted. Therefore, the following results concentrate on the differences between control fish and trout exposed to 15% effluent.

### *Monocytes and Granulocytes*

The patches with strong autofluorescence observed in trout were shown to coincide with the specific fluorescence staining of monocytes using mab 45 (Fig. 1). The exposure to 15% effluent induced an increased infiltration of monocytes into spleen tissue. However, the distribution of monocytes within spleen tissue was not observed to be altered by exposure to STP effluent. Injection of trout with *A. salmonicida* further enhanced the effect on monocyte numbers in spleen tissue.

Granulocytes were found to be distributed evenly within the sections (Fig. 1). All granulocytes in spleen sections from control fish showed an equally strong staining, and cell aggregates

were observed in some distance to melanomacrophage centers (MMCs). Whereas cell numbers in spleen from PBS-injected fish exposed to 15% effluent did not seem lower than in control fish, the immunostimulation by *A. salmonicida* injection led to a decreased number of granulocytes in spleen from fish exposed to 15% effluent compared to *A. salmonicida*-injected control fish. However in both groups (*A. salmonicida*-injected and sham-injected fish), granulocytes displayed diverse intensity of staining after exposure to 15% effluent, with some cells showing lower surface expression of the marker stained by mab Q4E.

### *Thrombocytes*

Spleen sections from control fish displayed an even distribution of thrombocytes (Fig. 2). In the spleens of trout exposed to 15% effluent, staining intensity and number of thrombocytes was markedly decreased compared to control fish. The intraperitoneal injection of *A. salmonicida* seemed to increase the staining intensity as well as the number of thrombocytes in spleen in nonexposed control fish. This was not found in the effluent group, where an injection of trout with *A. salmonicida* did not have any influence on thrombocyte numbers in spleen, compared to sham-injected fish.

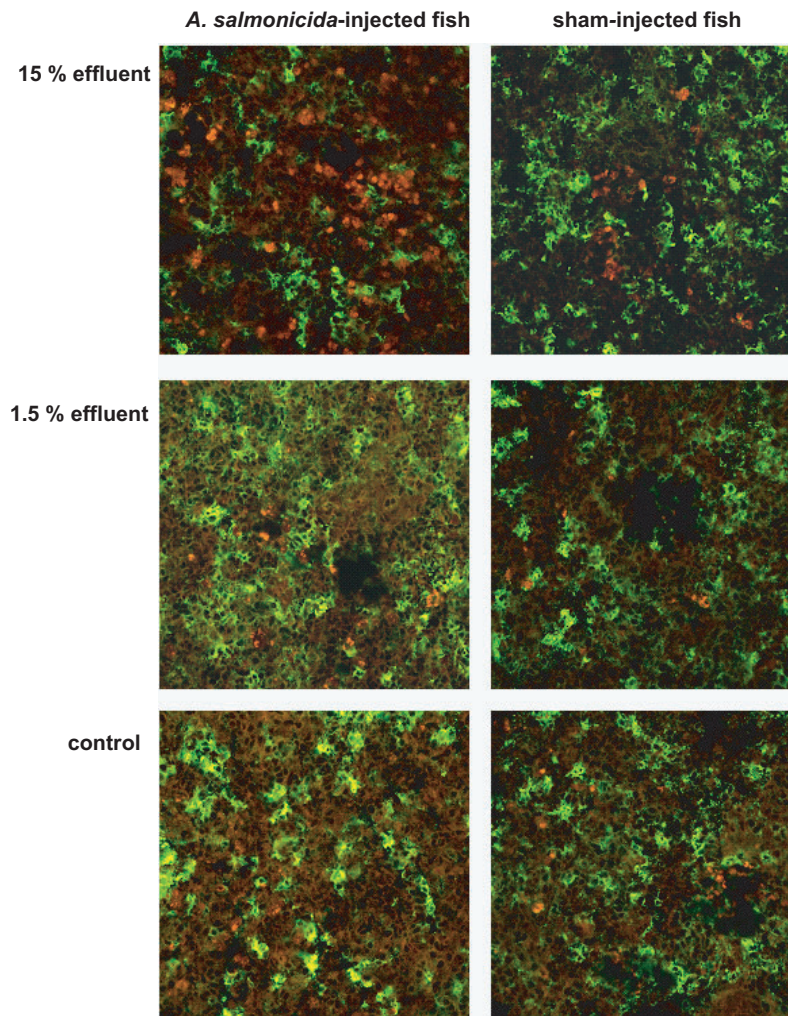
### *B-Lymphocytes*

In spleens from control trout and trout exposed to 1.5% effluent, B-lymphocytes showed a homogenous distribution and expression of surface IgM within the tissue. Exposure of trout to 15% effluent led to an uneven distribution of B-cells in spleen sections (Fig. 3) as well as a higher expression of surface IgM in larger cells assembled around MMCs. In other parts of the sections lower numbers of B-cells were stained, and stained cells furthermore displayed a lower fluorescence intensity, implying lower cell surface IgM expression. Injection of fish with *A. salmonicida* had no further effect, either on B-lymphocyte numbers, or on the expression of surface IgM, compared to sham-injected fish.

### *MHC Class I and MHC Class II*

The expression of MHC class I and MHC class II in spleen leucocytes seemed to be influenced both by the exposure to 15% STP effluent and by the injection with *A. salmonicida* particles (Fig. 4).

MHC II positive cells were unequally distributed within spleen tissue, with aggregates of MHC II expressing cells, as well as completely unstained areas. Spleen leucocytes in control fish and the 1.5% effluent group showed a very low expression of MHC class II. The exposure to 15% STP effluent resulted in a strong increase of MHC class II (green fluorescence) along with the above-described occurrence of autofluorescence (yellow/orange cytoplasmic fluorescence) in larger cells.



**FIG. 1.** Laser scanning microscopy of spleen cryosections from control fish and trout exposed to 1.5 or 15% effluent, labeled with mab 45 (anti-trout monocytes, red fluorescence) and mab Q4E (anti-trout granulocytes, green fluorescence). The exposure to effluent induced a strong increase in the number of monocytes, showing intracellular orange/red autofluorescence. In the *A. salmonicida*-injected fish the number of labeled monocytes was further increased compared to PBS-injected fish. Granulocytes seemed to be influenced by exposure to 15% effluent, as shown in altered distribution and slightly decreased fluorescence intensity.

In *A. salmonicida*-injected fish a stronger expression of MHC class I molecules (orange or orange/red fluorescence) in larger leucocytes surrounding the MMCs was found compared to PBS treated control trout (Fig. 4). It should be noted that in controls and in the 1.5% STP effluent group not all MHC class I positive cells also displayed an MHC II specific staining, while in the 15% effluent group, cells showed a specific MHC I/MHC II double staining.

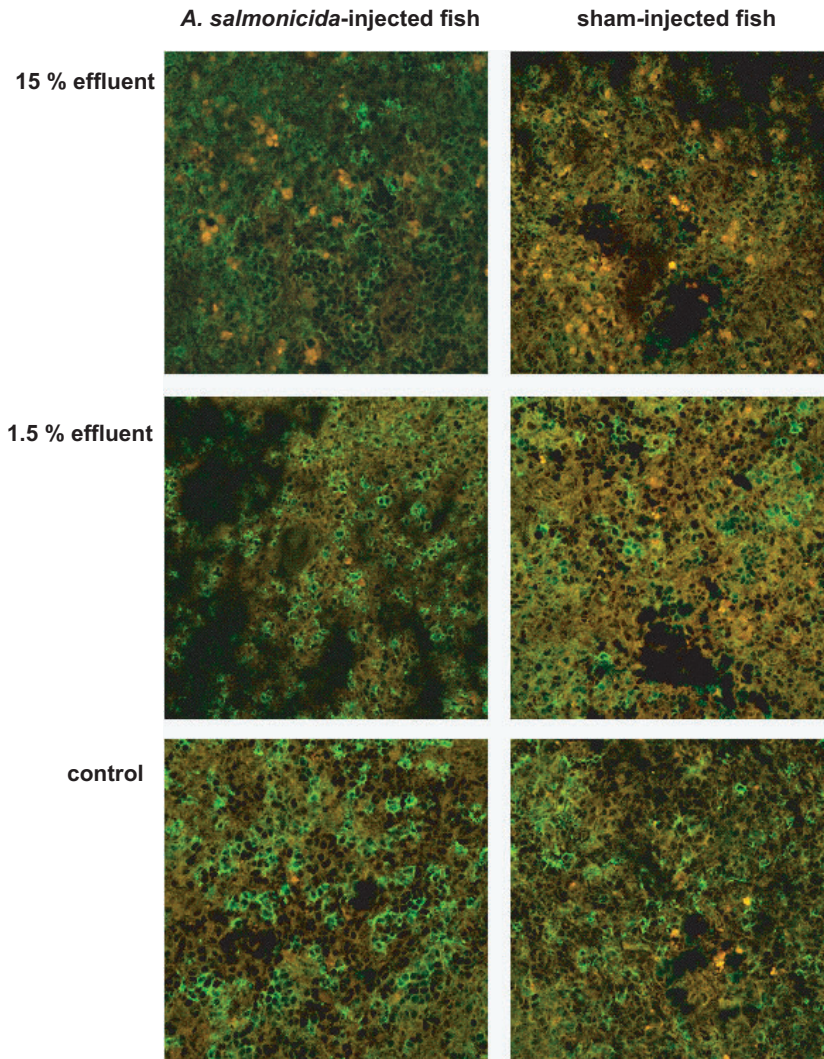
#### *Serum Level of anti-A. salmonicida salmonicida Immunoglobulin M*

Exposure of rainbow trout to STP effluent resulted in a decrease in serum antibody levels against inactivated *A. salmonicida*;

however, this decrease was only statistically significant in the 1.5% effluent group (Table 2).

## DISCUSSION

In this study, chronic exposure of rainbow trout to municipal STP effluent has been shown to alter the occurrence and distribution of different leucocyte populations in spleen tissue. The predominant finding was a high prevalence of a strong, intracytoplasmic red/orange autofluorescence, especially in large leucocytes in spleen sections from trout exposed to 15% effluent. These deposits or inclusions have been found to coincide with the specific antibody staining of monocytes and may therefore be



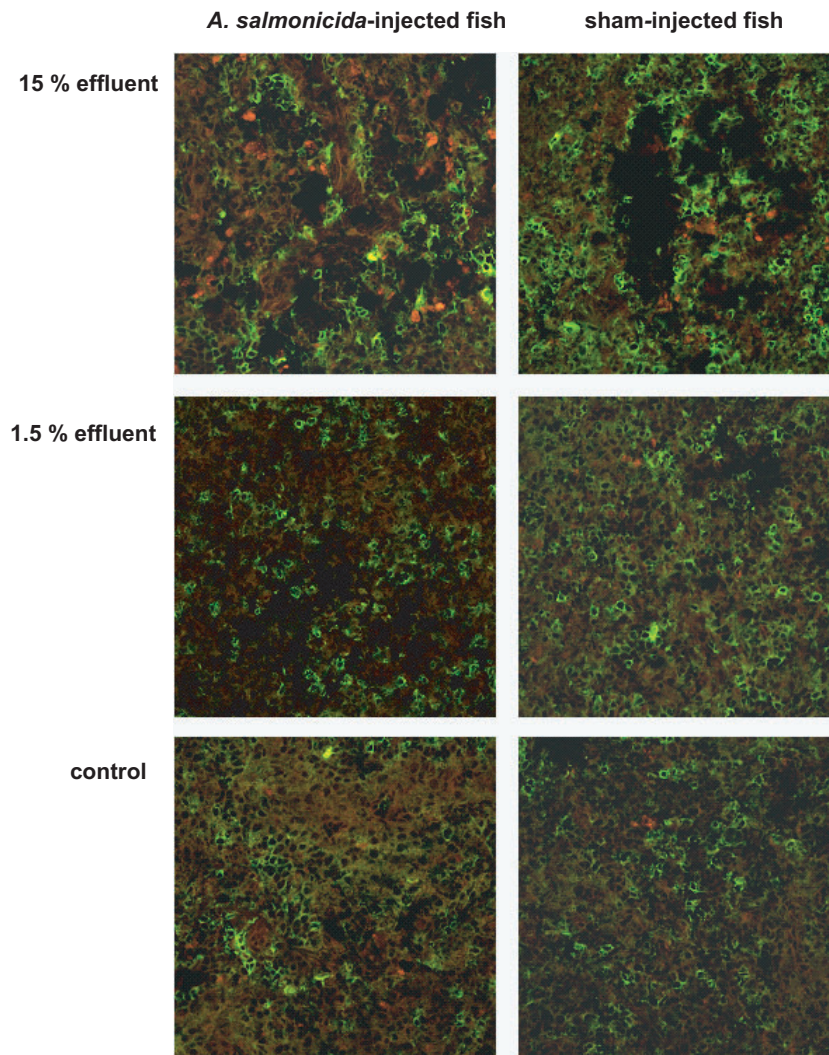
**FIG. 2.** Laser scanning microscopy of spleen cryosections from control fish and trout exposed to 1.5 or 15% effluent, after labeling with mab 42 (anti-trout thrombocytes, green fluorescence). Note the strong decrease in thrombocyte numbers and lower fluorescence intensity on cell surfaces in the 15% effluent group.

interpreted as depositions in macrophages. An example for an autofluorescent substance, which can usually be found in melanomacrophage centers is lipofuscin (Wolke, 1992). The identity of these deposits or inclusions could not be elucidated or satisfactorily explained. However, they may be considered to reflect an adverse effect of chronic exposure of trout to STP effluent. This impression is further supported by a loss of tissue integrity of the spleen tissue from effluent exposed trout compared to samples from control fish.

The increase of monocytes/macrophages, detected in spleen samples from trout injected with *A. salmonicida*, is in agreement with results from previous studies (Koellner and Kotterba, 2002). In addition to the effects of *A. salmonicida* injection, it appears that the exposure to 15% STP effluent had a “costimulatory-like” effect on monocytes, indicating the unspecific activation of cells involved in innate immune functions. This finding can be

interpreted as an indication of a possible chronic inflammation response to compounds found in STP effluent.

The marked decrease in thrombocyte numbers and lower staining intensity on these cells found in spleen samples from trout exposed to 15% effluent are further indications of effluent impacts on the immune system. The main function of thrombocytes, the phylogenetic precursors of platelets in lower vertebrates, is blood clotting (Rowley *et al.*, 1997). Recent findings, however, indicate that trout thrombocytes may also be involved in antigen presentation (Koellner *et al.*, in press). Lower thrombocyte numbers in spleen tissue after exposure to 15% effluent might be due to an efflux of these cells into the blood or other body compartments, possibly reflecting a chemotactic response of thrombocytes towards foreign material in STP effluent. Lower staining intensity may be due to a decreased expression of the CD42-like surface marker recognized by mab 42. As it has been



**FIG. 3.** Laser scanning microscopy of spleen cryosections from control fish and trout exposed to 1.5 or 15% effluent, after labeling with mab N2 (anti-trout IgM, green fluorescence). Note the altered distribution and increased staining intensity in trout exposed to 15% effluent compared to control fish.

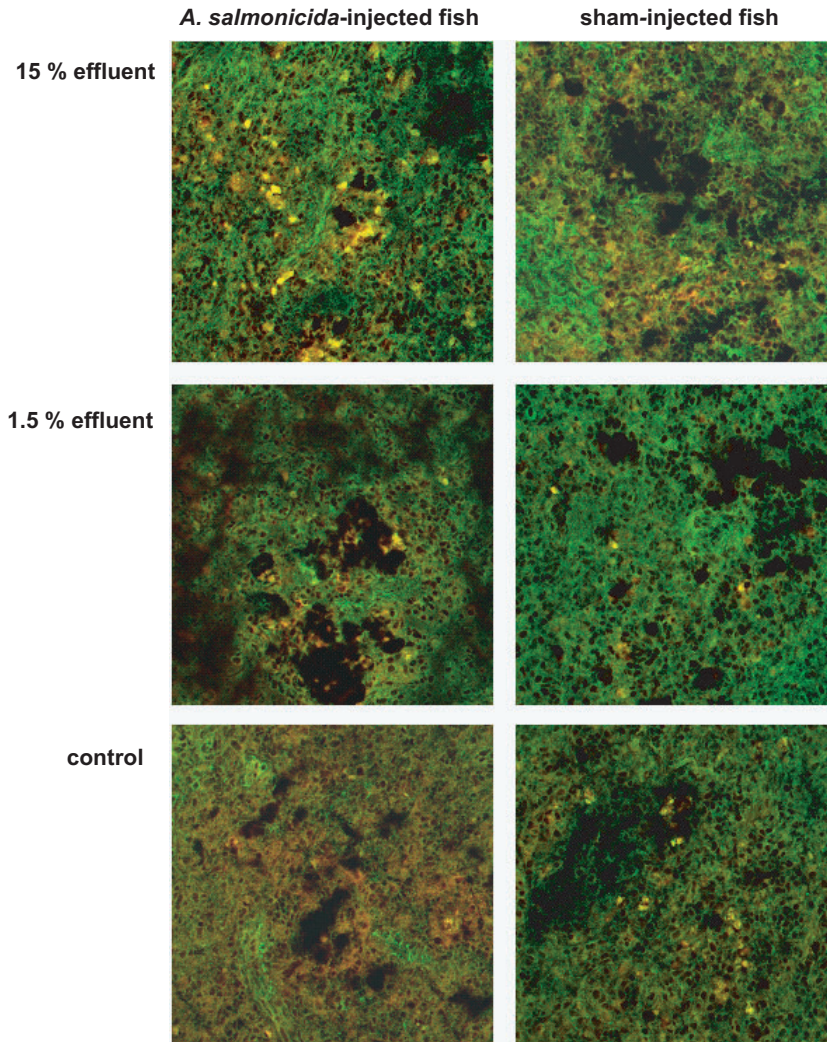
shown that this molecule is involved in thrombocyte aggregation, a decrease in surface expression could indicate a disturbance of aggregatory function after chronic exposure to 15% effluent (Koellner *et al.*, in press).

B-lymphocytes in spleen tissue from trout exposed to 15% effluent were observed to cluster near MMCs, in contrast to B-cells in spleen of control fish, which were evenly distributed in the tissue. Moreover, spleen B-cells in the 15%-effluent group displayed a markedly higher intensity of fluorescence staining. The B-cells were detected using a mab against trout IgM (Thuvander *et al.*, 1990). Therefore, not only the distribution of B-lymphocytes can be detected, but also an activation of these cells, leading to an increased expression of surface immunoglobulin (sIgM), reflected in a higher intensity of fluorescence staining on individual cells. Gathering of those B-cells with increased sIgM expression, around macrophage centers could be connected to antigen presentation in these areas of the spleen.

This is a further indication of a nonspecific activation of the immune system due to exposure to STP effluent.

The enhanced MHC class II specific staining in spleen sections observed after exposure to 15% effluent also suggests an effluent-induced stimulation of immune cells. It is well known, that activation and proliferation of immune cells after antigenic or mitogenic stimulation results in an increased expression of MHC II molecules on monocytes and activated B- and T-lymphocytes (Grusby and Glimcher, 1995; Rohn *et al.*, 1996). The higher MHC II specific staining found in our study may also be connected to activation of antigen-presenting cells (monocytes, B-cells). However, the functional relevance of such a finding would have to be proved using functional tests, such as phagocytosis assays or mixed leucocyte reaction (MLR), which could not be realized within the scope of this study.

The measurement of specific antibodies in serum of *A. salmonicida*-injected trout revealed lower levels in mature



**FIG. 4.** Laser scanning microscopy of spleen cryosections from control fish and trout exposed to 1.5 or 15% effluent, after labeling with mab H9 (anti-trout MHC class I, red fluorescence) and anti-salmon MHC II antiserum (green fluorescence). In the 15% effluent group, a strong increase in the number of MHC II positive cells was found. In spleen sections from *A. salmonicida*-injected trout MHC class I positive cells were found to surround the melanomacrophage centers.

**TABLE 2**  
**Serum Levels of *A. salmonicida*-Specific IgM**

Control	1.5% (v/v) effluent	15% (v/v) effluent
1.20; 0.846/1.553 (12)	0.595; 0.242/0.949 (12)*	0.767; 0.334/1.200 (8)

*Note.* Mean values; lower 95% confidence level / upper 95% confidence level for serum levels of *A. salmonicida*-specific IgM. Numbers in parentheses indicate the number of fish tested.

\* $p \leq 0.05$  (one-way ANOVA with Dunnett's post test).

female trout exposed to 1.5 and 15% effluent, compared to control fish. However, a direct association between putative antigen presentation and specific antibody levels in serum cannot be drawn, as suppression of antibody production could occur

at several other stages, following antigen-recognition (Sharma and Zeeman, 1980), and mere antigen presentation does not necessarily result in the production of antibodies by B-cells. Moreover, the nature of the putative antigen presented in this case is not known. Exposure to effluent may have resulted in exposure to several other antigens, and humoral immune reactions have been found to vary substantially with the antigen applied (Davis *et al.*, 2003; Sharma and Zeeman, 1980).

Although an effect of chronic exposure to 15% effluent on occurrence and distribution of leucocytes, as well as a possible deposition of degradation materials in the spleen of rainbow trout has been shown, a clear characterization of the (putative adverse) influence cannot be gained from the study at hand. However, our results can be regarded as an indicator for potential adverse effects of STP effluents on the

immune system of fish, reflected in an induction of a response, which appears similar to chronic inflammation and a constant unspecific stimulation of different leucocyte populations. Therefore, our findings further support the inclusion of immune parameters into monitoring of aquatic pollution, as has been suggested before by several scientists (Van Muiswinkel, 1992; Wester *et al.*, 1994). Analysis of the occurrence and distribution of white blood cell populations in hematopoietic tissue, with the help of specific antibodies, might specifically be regarded as a useful method to assess effects of environmental contamination on immune reactions. Adverse effects observed in hematopoietic tissues may reflect or subsequently lead to alterations in several critical immune reactions.

Given the complexity of immune responses, a single test method is not suitable for assessment of the overall immune competence of an organism. Therefore, investigations on immunotoxicity warrant the assessment of a range of immune parameters. Alterations in leucocyte numbers and expression of surface markers on different leucocyte populations, observed with the help of immunohistology, should be complemented by functional assays, in order to elucidate the implications of the histological effects. A final characterization of the overall immune competence has to be based on the investigation of a stimulated immune system, and immunosuppression, in its last consequence, can only be demonstrated with the help of challenge experiments. Further investigations on the effects of sewage treatment water on immune functions in fish are desirable. It will, however, be indispensable to complete such investigations with studies on mechanisms of immunotoxicity, in order to better understand how the immune competence of aquatic organisms can be influenced by different types of pollution.

In conclusion, the present study has clearly shown a (potentially adverse) effect of chronic exposure to a realistic concentration of STP effluent on rainbow trout spleen, reflected in an activation of different leucocyte populations, occurrence of inclusion bodies, and a decrease in tissue integrity. A negative influence of surface water contamination with effluent on the fish immune system and hence on immune competence may thus be expected. Consequently, these findings warrant a closer examination of the effects of anthropogenic pollution of the aquatic environment on immune function of aquatic organisms.

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