

ACTIVE BIOMONITORING WITH BROWN TROUT (*SALMO TRUTTA*)
AND RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) IN DILUTED
SEWAGE PLANT EFFLUENTS

H. Schmidt*‡, D. Bernet*, T. Wahli*, W. Meier*+, and P. Burkhardt-Holm*†

*Centre for Fish and Wildlife Health, Institute of Animal Pathology, University of Berne,
Laenggass-Strasse 122, CH-3012 Berne / Switzerland and †Interdisciplinary Centre for General
Ecology, University of Berne, Falkenplatz 16, CH-3012 Berne / Switzerland.

running title: Effects of sewage plant effluents on trout

‡Author to whom correspondence should be addressed. Corresponding author: Tel.: ++41 31-
6312465; fax: ++41 31-6312611; e-mail: heike.schmidt@itpa.unibe.ch

+This paper is dedicated in memory to Prof. Dr. W. Meier who died unexpectedly by accident

Brown trout (*Salmo trutta*) populations of numerous Swiss rivers are declining. As a possible cause sewage plant effluents are discussed. To investigate the influence of sewage plant effluents brown trout as well as rainbow trout (*Oncorhynchus mykiss*) were exposed to diluted waste water (10% dilution ratio) over a period of 12 months. The effects were compared to those of trout kept in commercial tap water. The mortality rate was low and no pathogenic bacteria or viruses were recorded in exposed and tap water animals. Parasitological examination revealed a mild infestation with *Gyrodactylus* sp. in all groups. Macroscopically and histologically, only minor alterations in gills, skin and kidney of exposed animals were found when compared to fish kept in tap water. Degenerative and inflammatory reactions in the liver of exposed animals were the most prominent findings. For comparative reasons, several brown trout were caught in the river Langete. They showed marked proliferative, degenerative and inflammatory lesions of gills, liver and kidney. Our results do not suggest a harmful influence of the waste water on fish which would explain the decrease of fish populations. However, it is conceivable that the effluents in combination with other factors in the river enhance the development of alterations.

Key words: pollution; waste water; biomarker; trout; histopathology; parasitology.

INTRODUCTION

In Switzerland fish populations are declining (Pedroli *et al.*, 1991; Frick *et al.*, 1998). In the district of Berne populations of different fish species in several rivers are monitored by the Inspectorate of Fisheries of Berne. This includes the river Langete which is situated in the western midland of Switzerland. Although brown trout are reintroduced in the Langete every year, electrofishing revealed decreasing numbers along the course of the river (Fishing statistics 1990-1998, unpublished data). Agricultural impact as well as domestic and industrial effluents are discussed as possible causes. The level of organic pollution of this river measured by means of a saprobial- and algae-index system is regarded as moderate to critical (AquaPlus, 1994; VOKOS, 1997). Values of dissolved organic carbon (DOC) and occasionally of ammonia measured

downstream of sewage plant effluents exceed the quality aims for running water (AbwV, 1975) (Water and Soil Protection Laboratory of the district of Berne (GBL), unpublished data). Brown trout caught in the Langete as spot-checks in previous years showed degenerative and inflammatory reactions in liver and kidney as well as proliferative gill alterations (unpublished observations). Similar changes are reported in gills, skin, kidney, and liver in brown trout exposed to sewage plant effluents (Carline *et al.*, 1987; Bucher & Hofer, 1993; Burkhardt-Holm *et al.*, 1997). Therefore, we hypothesised that the findings in feral fish from the Langete are related to poor water quality due to sewage plant effluents.

The aim of the study was to investigate whether a long term exposure to effluents of a particular sewage plant causes alterations and, if so, whether these alterations are comparable to those found in feral fish of the river Langete. A further aspect of the study was to assess the suitability of brown trout and rainbow trout as bioindicators for environmental pollution particularly as the latter species is commonly used for experimental studies and recommended by law for toxicological tests.

MATERIAL AND METHODS

Our experiments were carried out during May 1996 to April 1997. The investigated sewage plant, located at Lotzwil, was constructed in 1968 and since 1996 phosphorus was removed by addition of chlorine-sulphate with 2% aluminium. The plant received waste water from an average of 7700 population equivalents with 33% originating from industry. The nitrogen elimination was 38% and the nitrification was 77%. Biochemical oxygen demand and temperature were measured daily, chemical oxygen demand, total ammonia, nitrite, nitrate, total phosphorus and total insoluble substances were measured every fifth day by the GBL (Table I).

For the active monitoring 30 brown trout (13-17 cm, 20-50 g) and 30 rainbow trout (19-22 cm, 70-110 g) from the stock culture at the Centre for Fish and Wildlife Health (CFWH) were randomly assigned to groups and transferred to a 2000-litre fibreglass tank at the sewage plant (sewage plant effluent (SPE) groups) separated according to species by a perforated steel sheet

(perforation diameter 1 cm). The tank received a constant flow-through (20 l/min) of aerated effluent water diluted with commercial tap water in a ratio of 1:10 which corresponds to the dilution of effluents directly downstream the site of discharge into the river. Water temperature ranged from 5°C to 17°C over the year. As a control 15 brown trout were held in commercial tap water (20 l/min) on the area of the sewage plant in a 100x60x50 cm tank. The remaining brown trout and rainbow trout were held in tap water (20 l/min) at the CFWH (reference groups) in 2000-litre fibreglass tanks (200 trout/tank). Here water temperature ranged from 13°C to 17°C. Oxygen concentration in all tanks was ≥ 8 mg/l during the experimental period. All fish were fed commercial trout pellets (HOKOVIT, Bützberg, Switzerland) with a daily food ration equal to 1-2% of body weight.

The first sampling of the SPE animals was performed in July, two months after stocking and thereafter every three months, in October, January, and April. Control animals were sampled twice, in October and April. Reference fish of the CFWH were sampled three times, in October, November, and February. At least five fish per species and sampling time were investigated. All fish were euthanized in buffered 3-aminobenzoic acid ethyl ester (Argent Chemical Laboratories, Redmont, USA). Trout were weighed and measured individually, and Fulton's condition factor was calculated ($100 \cdot \text{weight} / \text{length}^3$) (Bagenal, 1978). A standard necropsy was performed. For parasitological examination a skin scraping,, a gill sample and the intestinal content were investigated by light microscopy. Parasites of liver and kidney were examined by histology. The prevalence and the abundance (0= no, 1= mild, 2= moderate, 3= severe infestation) were assessed. On all animals a bacteriological examination of liver, spleen and kidney was performed using blood agar plates (Bio Merieux, Lausanne, Switzerland) and bromthymolblue-lactose-agar plates. For virological examination tissue from the head kidney and pyloric caeca of the five fish per trial were pooled and examined as described by Meier & Wahli (1988). Pieces of skin in front of the dorsal fin, gills, liver, kidney, and intestine from all animals were fixed in Bouin solution for 24 hours, routinely processed for histology and stained with haematoxylin and eosin (HE), periodic acid schiff (PAS), and if indicated with van Gieson (VG). Additionally, heart, spleen,

head kidney, thyroid gland, gonads, brain, mandible, and premaxilla of one fish of each group and, if present, of altered organs were processed as described above. Histopathological changes of all organs were described qualitatively and alterations of skin, gills, liver and kidney were evaluated according to an assessment tool (Bernet *et al.*, in press). The pathological changes were classified into five reaction patterns: circulatory, regressive, progressive, inflammatory, and neoplastic.

According to the degree and extent of lesions an organ index of each organ could be determined.

The sum of these indices resulted in a total index for the individual.

In July, feral brown trout were caught at four sampling sites along the course of the river.

Sampling site 1 was in the uppermost part of the river without any influence of sewage plant effluents. Sampling sites 2 to 4 were located downstream of the inflows of three sewage plants along the course of the river. Five fish (6-26 cm, 3-200g) per sampling site were transported to the CFWH and examined as described previously. Additionally, serological examinations for the presence of antibodies against viral haemorrhagic septicaemia (VHS) were performed as described by Olesen & Vestergaard Jørgensen (1986).

Differences of prevalence of parasitic infestation between groups were tested using a χ^2 - and a multiple χ^2 - test, respectively. Total indices of histopathological alterations of the SPE and the tap water groups were checked for assumption of normality. Differences were assessed by a multifactorial analysis of variance (MANOVA). Total index, exposure period, season, and species were included as variables. Pairwise comparisons between means were performed using LSD (Fisher's Least Significant Difference) adjustments. The level of significance of all statistical tests was set at 5%.

RESULTS

ACTIVE MONITORING (EXPOSED ANIMALS, CONTROL AND REFERENCES)

Water analysis

The results of the chemico-physical analysis of the treated waste water are shown in Table I.

Mean values of the measured compounds of treated waste water did not exceed the limits of the general parameters in the Swiss effluent regulative (AbwV, 1975). Nevertheless, there were occasional peak values of biological oxygen demand (BOD₅), nitrite, total phosphorus and total insoluble substances which were above the limits (Table I).

Mortality rate and condition factor

Apart from few dead fish in the sewage plant due to technical problems at the beginning of the experiment no mortality occurred. Control, reference and experimental fish were in a good nutritional state (condition factor ≥ 1). An exception were a few underweight brown trout at the last sampling in the SPE group (condition factor 0.8 ± 0.2).

Infectious agents

The parasitological examination revealed an infestation with *Gyrodactylus* sp. on the skin in all groups. The prevalences and abundances are shown in Table II. No significant group differences of the prevalence in brown trout were observed (multiple χ^2 -Test, $p > 0.05$). Nevertheless, in the SPE group the prevalence increased during the experimental period. In rainbow trout significant differences of the prevalence between the fish held in tap water and those in diluted waste water were evident (χ^2 -test, $\chi^2 = 4.7$, $p < 0.05$). The abundances showed no obvious differences neither for brown trout nor for rainbow trout. No viral nor bacterial pathogens were isolated.

Macroscopical examination

The SPE fish showed mild to moderate, occasionally severe erosions and ulcerations of the upper and lower jaw. Sporadically, erosions of the dorsal and caudal fins were seen. In the reference groups mild alterations of the dorsal and caudal fins were observed, predominantly in the rainbow trout. No gross lesions in the inner organs were observed in any group.

Histopathology

The analysis of variance indicated no significant group differences between total indices of histopathological lesions in the tap water and waste water ($p > 0.05$) (Fig. 1A,B). Nevertheless the exposure time and the species had a significant influence on the total index (MANOVA, $p = 0.021, 0.027$). The pairwise comparison showed significant differences between brown trout kept in diluted waste water for five months and those kept for eight or 11 months (Fig. 1A). The highest value was found in January. Rainbow trout showed no significant differences of the total index over the experimental period.

Obvious changes of organ indices in comparison to control and reference animals were found in the liver from fish from the SPE groups (Fig. 1). In trout from all groups blurred borders of hepatocytes and sporadically perivascular and pericholangiar fibrosis with lymphocytic infiltration were found. Additionally, in animals exposed to sewage plant effluents a mild to moderate, mainly perivascular and pericholangiar, lymphohistiocytic infiltration was present (Fig. 2A). A quantitative comparison between brown trout and the reference fish revealed a liver index increasing up to 328 % after the third sampling in January and decreasing again to 187 % in April. In rainbow trout the highest value was recorded in October (219 % increase compared to the reference). Controls had lower liver indices as well. Reference brown trout kept at the CFWH showed the most prominent gill and kidney alterations. In the gills they consisted of epithelial cell lifting, eosinophilic granular cytoplasm, epithelial hypertrophy, mild curvatures of the primary and secondary lamellae, and occasionally epithelial hyperplasia and leucocyte infiltration of gill epithelium. In comparison to the control group the waste water exposed brown trout also showed elevated gill indices (62 % to 205 %) (Fig. 1A). The highest value was in January. Alterations in the kidney were restricted mainly to the tubules. They consisted of granular cytoplasm, pycnotic nuclei and desquamating tubulus cells. Additionally rainbow trout showed mild fibroblast proliferation around tubules and glomerula. No obvious group differences were found in the skin. The skin lesions consisted of an irregular structured basal cell layer, erosions as well as sporadically ulcerations and leucocyte infiltration.

FERAL FISH

Several parasites (*Gyrodactylus* sp., sessile peritrichia, *Sphaerospora* sp., PKX cells, *Metechinorhynchus truttae*) were found. The most prominent findings were increasing prevalences and abundances of PKX cells in the examined organs of fish along the course of the river going along with kidney enlargement and splenomegaly. The histopathological examination revealed granulomatous inflammation in liver and kidney associated with Proliferative Kidney Disease (PKD). Additionally, proliferative and degenerative gill lesions and degenerative alterations in liver (Fig.2B) and kidney were present.

DISCUSSION

The aim of the study was to investigate the influence of a long term exposure to diluted sewage plant effluents on fish. The present results are not indicative for a direct effect of sewage plant effluents on fish as cause of the decline in the Langete population. Nevertheless, exposed animals showed mild morphological alterations probably associated with the poor water quality. However, the obtained results were not pathognomic for a specific substance. Histologically, degenerative and inflammatory lesions of the liver were obvious. In comparison to the control the exposed animals also showed structural alterations and proliferative lesions of the gills. It is shown that exposure to diluted waste water resulted mainly in lesions of gills, kidney, and liver (Carline *et al.*, 1987; Bucher & Hofer, 1990, 1993). The authors attributed these alterations to high levels of un-ionized ammonia (NH₃). In addition, sublethal concentrations of nitrite are known to induce gill damage as well as degenerative liver alterations (Ariello *et al.*, 1984; Michael *et al.*, 1987; Laurent & Perry, 1991; Mazik *et al.*, 1991). Uptake of nitrite is mediated over the chloride cells in the gill epithelium (Bath and Eddy, 1980; Williams and Eddy, 1986) and hepatocytes are responsible for the detoxification of nitrite (Doblander & Lackner, 1996). During the period of our study concentrations of nitrogenous compounds in the sewage plant effluents varied over a wide range, but only occasionally exceeded the limits (AbwV, 1975). High values could be attributed to the effluents of an abattoir. On the days of slaughtering the capacity of the sewage plant was not

sufficient. The high fluctuations and the sporadically high values in our study may have increased the effects of these substances. Liver lesions, such as perivascular and pericholangiar reactions as described here, are also reported in fish which were exposed to organic contaminants (Myers *et al.*, 1992; Johnson *et al.*, 1993; Schneeberger, 1995), to pesticides (Couch, 1975), or to heavy metals (Sastry & Gupta, 1978). The mechanism could be a reactivity of toxic metabolites with the bile duct epithelium (Hinton & Laurén, 1990). Some small factories and a wood processing industry drain into the sewage plant. Although values of different heavy metals in the sewage sludge do not exceed the limits of the sewage sludge regulative (StoV, 1986) (data not shown) there might be a chronic influence of these heavy metals on brown and rainbow trout since both species showed a significant increase in metallothionein-immunopositive chloride cells in the gills (Burkhardt-Holm *et al.*, unpublished observations).

Comparing the histopathological changes of the two tap water groups, brown trout in the CFWH showed a higher total index, mainly due to gill and to a lesser amount due to kidney alterations. The tap water in the CFWH was occasionally treated with chlordioxid (< 0.01 mg/l) which could have caused the observed lesions in the gill epithelium (Bass *et al.*, 1977; Mallat, 1985).

Exposed brown trout and rainbow trout showed alterations of the upper and lower jaw and of the fins. Fin erosions, jaw ulcers and other cutaneous lesions are reported in different fish species associated with environmental pollution (Munkittrik *et al.*, 1992; Sharples & Evans, 1996; Lair *et al.*, 1997). Fin ulcerations are also reported after acute confinement stress (Noga *et al.*, 1998).

Nevertheless, the etiology is still unknown. In our study especially the jaw ulcerations can rather originate from injuries at the middle steel sheet than from a direct effect of the sewage plant effluents. To confirm this hypothesis we kept brown and rainbow trout in the identical tank with the middle steel sheet in tap in the CFWH. These animals showed similar alterations (data not shown).

Our results revealed an infestation with *Gyrodactylus* sp. in control, reference and exposed animals. The SPE fish showed an increased prevalence over the experimental period. Poor water quality can promote increased parasitism in fish due to a decreased host immune response (Khan

& Thulin, 1991; Poulin, 1992). Nevertheless, in our study there was no effect on the abundance of the parasite. Because the infestation was only mild and restricted to the skin it is unlikely that differences in macroscopical and histopathological alterations between fish held in tap water and in diluted waste water can be attributed to the parasitic infestation.

The quantitative evaluation of the total index of histopathological alterations (MANOVA) revealed significant differences between brown trout and rainbow trout. Previous studies indicated that brown trout are more sensitive to environmental stress than rainbow trout (Pickering *et al.*, 1989; Schneeberger, 1995). In our study brown trout showed significant differences of the total index between the exposure time whereas there were no significant differences in rainbow trout.

However, changes observed in our study were only mild irrespective of the sampling time.

Comparing alterations of experimental and feral fish the most obvious differences were more prominent macroscopical and histopathological lesions and a higher incidence of parasitic infestations in the latter. Qualitatively, histopathological lesions in the active and passive monitoring were similar. But in feral fish these changes were much more pronounced. Many of the lesions, such as the granulomatous nephritis and hepatitis, can be attributed to a PKD-infection. A correlation between the increase in PKD infections along the course of the river and an increasing organic pollution in the Langete (VOKOS, 1997) appears likely. This is confirmed by additional observations of PKD in feral brown trout which were found frequently downstream the sewage plants during summer months but only occasionally upstream (data not shown). Poor water quality was shown to exert a predisposing effect on the occurrence of PKD (Hoffmann & Dangschat, 1981; Clifton-Hadley *et al.*, 1984). The involvement of the PKD prevents an unambiguous evaluation of the impact of treated waste water. However, degenerative alterations in the liver and gill lesions cannot directly be correlated to an infectious agent. These are non specific changes known to occur after exposure to unfavourable environmental conditions (Mallatt, 1985; Myers *et al.*, 1992; Haaparanta *et al.*, 1997).

From our results we conclude that treated waste water of the investigated sewage plant on its own provokes only mild effects in the organs of brown trout and rainbow trout. Nevertheless, it is

possible that the sewage plant effluents enhance the adverse conditions in the river. The pathological changes in feral fish were severe enough to lead to organ dysfunction and finally to death. To induce those pathological changes, there have to be additional stressors beside the influence of waste water, one of which may be the PKD infection. Further research is necessary to investigate whether river water on its own or only in combination with additional environmental factors is capable to induce those changes.

Thanks are due to Mr. Wächli from the sewage plant for taking care of fish, the Water and Soil Protection Laboratory of the district of Berne (GBL) for providing data of water analysis, the Inspectorate of Fisheries of Berne for carrying out the electrofishing, Mr. A. Busato for assistance in statistical analysis, Mrs. G. Lamche, L. Lagcher and E. Oldenberg for assistance during the samplings as well as to the laboratory of histology of the Institute of Animal Pathology. This work was supported by the Swiss Federal Agency for Environment, Forests and Landscape, the Inspectorate of Fisheries of Berne, the GBL and the Swiss National Science Foundation (3100-045894.95/1).

REFERENCES

- AbwV (1975). Verordnung über Abwassereinleitungen, 1975, Bern, SR 814.225.21, revised 1993, AS 1993 3022, Council of Ministers, Switzerland.
- AquaPlus (1994). Beurteilung der Gewässergüte in der Langete und Vergleich mit früheren Untersuchungen. Gewässerschutzamt des Kantons Bern, 77 pp. Berne.
- Arillo, A. E., Gaino, E., Margiocco, C., Mensi, P. & Schenone G. (1984). Biochemical and ultrastructural effects of nitrite in rainbow trout: liver hypoxia as the root of the acute toxicity mechanism. Environmental Research **34**,135-154.
- Bagenal, T. (1978). Methods for Assessment of Fish Production in Fresh Waters. Oxford: Blackwell.

- Bass, M. L., Berry, C. L. & Heath, A. G. (1977). Histopathological effects of intermittent chlorine exposure on bluegill (*Lepomis macrochirus*) and rainbow trout (*Salmo gairdneri*). Water Research **11**, 731-735.
- Bath, R. N. & Eddy, F. B. (1980). Transport of nitrite across fish gills. Journal of Experimental Zoology **214**, 119-121.
- Bernet, D., Schmidt, H., Meier, W., Burkhardt-Holm, P. & Wahli, T. Histopathology in Fish: Proposal for a method to assess aquatic pollution. Journal of Fish Diseases, in press.
- Bucher, F. & Hofer, R. (1990). Effects of domestic wastewater on serum enzyme activities of brown trout (*Salmo trutta*). Comparative Biochemistry and Physiology **97C**(2), 381-385.
- Bucher, F. & Hofer, R. (1993). The effects of treated domestic sewage on three organs (gills, kidney, liver) of brown trout (*Salmo trutta*). Water Research **27**(2), 255-261.
- Burkhardt-Holm, P., Escher, M. & Meier, W. (1997). Waste-water management plant effluents cause cellular alterations in the skin of brown trout. Journal of Fish Biology **49**, 744-758.
- Carline, R. F., Benson, A. J. & Rothenbacher, H. (1987). Long-term effects of treated domestic wastewater on brown trout. Water Research **21**(11), 1409-1415.
- Clifton-Hadley, R. S., Bucke, D. & Richards, R. H. (1984). Proliferative kidney disease of salmonid fish. A review. Journal of Fish Diseases **7**, 363-377.
- Couch, J.A. (1975). Histopathological effects of pesticides and related chemicals on the liver of fishes. In: Pathology of Fishes (Ribelin, W.E. & Migaki, G., eds), pp.559-584. Madison, Wisconsin: University of Wisconsin Press.
- Doblender, C. & Lackner, R. (1996). Metabolism and detoxification of nitrite by trout hepatocytes. Biochimica et Biophysica Acta **1289**(2), 270-274.
- Frick, E., Nowak, D., Reust, C. & Burkhardt-Holm, P. (1998). Der Fischrückgang in den schweizerischen Fließgewässern. Gas-Wasser-Abwasser (gwa) **4**, 261-264.
- Haaparanta, A., Valtonen, E. T. & Hoffmann, R. W. (1997). Gill anomalies of perch and roach from four lakes differing in water quality. Journal of Fish Biology **50**, 575-591.

- Hinton, D. E. & Laurén D. J. (1990). Liver structural alterations accompanying chronic toxicity in fishes: potential biomarkers of exposure. In: Biomarkers of environmental contaminations (McCarthy, J. F. & Shugart L. R., eds), pp. 17-57. Boca Raton: Lewis Publisher.
- Hoffmann R. & Dangschat H. (1981). A note on the occurrence of proliferative kidney disease in Germany. Bulletin of The European Association of Fish Pathologists **1**, 33.
- Johnson, L. L., Stehr, C. M., Olson, O. P., Myers, M. S., Pierce, S. M., Wigren, C. A., McCain, B. B. & Varanasi, U. (1993). Chemical contaminants and hepatic lesions in winter flounder (Pleuronectes americanus) from the northeast coast of the United States. Environmental Science and Technology **27**, 2759-2771.
- Khan, R.A. & Thulin, J. (1991). Influence of pollution on parasites of aquatic animals. Advances In Parasitology **30**, 201-238.
- Lair, S., Mailhot, Y., Higgins, R., Bélanger, D., Berthiaume, L., de Lafontaine, Y. & Martineau, D. (1997). Jaw ulcers in Atlantic tomcod, Microgadus tomcod (Walbaum), from the St Lawrence River. Journal of Fish Diseases **20** 11-17.
- Laurent P. & Perry S. F. (1991). Environmental effects on fish gill morphology. Physiological Zoology **64**, 4-25.
- Mallatt, J. (1985). Fish gill structural changes induced by toxicants and other irritants: A statistical review. Canadian Journal of Fisheries and Aquatic Sciences **42**, 630-648.
- Mazik, P. M., Hinman, M. L., Winkelmann, D. A., Klaine, S. J., Simco, B. A. & Parker N.C. (1991). Influence of nitrite and chloride concentrations on survival and hematological profiles of striped bass. Transactions of the American Fisheries Society **120**, 247-254.
- Meier W. & Wahli T. (1988). Viral haemorrhagic septicaemia (VHS) in grayling (Thymallus thymallus). Journal of Fish Diseases **11**, 481-487.
- Michael, M. I., Hilmy, A. M., El-Domiaty, N. A. & Wershana, K. (1987). Serum transaminases activity and histopathological changes in Clarias lazera chronically exposed to nitrite. Comparative Biochemistry and Physiology **86**, 255-262.

- Munkittrik, K. R., McMaster, M. E., Portt, C. B., Van Der Kraak, G. J., Smith, I. R. & Dixon, D. G. (1992). Changes in maturity, plasma sex steroid levels, hepatic mixed-function oxygenase activity, and the presence of external lesions in lake whitefish (Coregonus clupeaformis) exposed to bleached kraft mill effluent. Canadian Journal of Fisheries and Aquatic Sciences **49**, 1560-1569.
- Myers, M. S., Olson, O. P., Johnson, L. L., Stehr, C. S., Hom, T. & Varanasi, U. (1992). Hepatic lesions other than neoplasma in subadult flatfish from Puget Sound, Washington: Relationships with indices of contaminant exposure. Marine Environmental Research **34**, 45-51.
- Noga, E.J., Botts, S, Yang, M.-S. & Avtalion, R. (1998). Acute stress causes skin ulceration in striped bass and hybrid bass (Morone). Veterinary Pathology **35**, 102-107.
- Olesen, N. J. & Vestergaard Jørgensen, P. E. (1986). Detection of neutralizing antibody to Egtved virus in rainbow trout (Salmo gairdneri) by plaque neutralization test with complement addition. Journal of Applied Ichthyology **2**, 33-41.
- Pedroli, J. C., Zaugg, B. & Kirchhofer, A. (1991). Verbreitungsatlas der Fische und der Rundmäuler in der Schweiz. Schweizerisches Zentrum für die kartografische Erfassung der Fauna, pp. 207. Neuenburg, Switzerland.
- Pickering, A. D., Pottinger, T. G. & Carragher, J. F. (1989). Differences in the sensitivity of brown trout, Salmo trutta L., and rainbow trout, Salmo gairdneri Richardson, to physiological doses of cortisol. Journal of Fish Biology **34**, 757-768.
- Poulin, R. (1992). Toxic pollution and parasitism in freshwater fish. Parasitology Today **8**(2), 58-61.
- Sastry, K. V. & Gupta, P.K. (1978). Histopathological and enzymological studies on the effects of chronic lead nitrate intoxication in the digestive system of a freshwater teleost, Channa punctatus. Environmental Research **17**(3), 472-479.
- Schneeberger, U. (1995). Abklärungen zum Gesundheitszustand von Regenbogenforelle (Onchorhynchus mykiss), Bachforelle (Salmo trutta fario) und Groppe (Cottus gobio) im

- Lichtensteiner-, Werdenberger- und Rheintaler- Binnenkanal. Inaugural-Dissertation, 68 pp. Universität Bern, Bern, Switzerland.
- Sharples, A. D. & Evans, C. W. (1996). Pathology of fin erosion in goldfish Carassius auratus. Diseases of Aquatic Organisms **24**, 81-91.
- StoV (1986). Verordnung über umweltgefährdende Stoffe, 1986, Council of Ministers of Switzerland, Berne.
- VOKOS (ed.) (1997). Vollzugskonzept Siedlungsentwässerung. Bern, Bau-Verkehrs- und Energiedirektion.
- Williams, E. M. & Eddy, F. B. (1986). Chloride uptake in freshwater teleosts and its relationship to nitrite uptake and toxicity. Journal of Comparative Physiology **156B**, 867-872.

TABLE I: Results of the chemico-physical analysis of Lotzwil sewage plant effluents (Water and Soil Protection Laboratory of the district of Berne)

Parameter	BOD ₅ (mg/l)		COD (mg/l)		ammonia (mg/l)		nitrite (mg/l)		nitrate (mg/l)		total phosphorus (mg/l)		total insoluble substances (mg/l)		temperature (°C)	
	x	values	x	values	x	values	x	values	x	values	x	values	x	values	x	values
May	6.0	2.5-15.0	22.0	14.0-35.0	0.8	0.1-3.5	0.4	0.2-0.7	12.0	8.2-14.8	0.4	0.3-0.6	n.m.	13.0	11.2-13.4	
June	5.4	1.5-16.0	25.0	13.0-35.0	0.4	0.1-1.0	0.5	0.1- 1.5	11.0	5.2-14.4	0.6	0.4- 1.0	n.m.	14.0	13.2-15.2	
July	3.5	1.5-9.5	13.0	4.0-32.0	1.3	0.1-7.4	0.3	0.03-0.9	9.5	6.8-12.3	0.6	0.4- 0.8	n.m.	16.0	14.3-16.7	
August	3.1	1.5-6.5	20.0	11.0-46.0	1.5	0.1-5.6	0.4	0.03- 1.1	12.0	10.6-13.4	0.6	0.5-0.8	n.m.	17.0	16.2-17.6	
September	2.2	1.0-5.0	14.0	8.0-26.0	0.6	0.1-2.3	0.1	0.03-0.3	13.0	9.6-14.9	0.3	0.1-0.5	n.m.	15.0	14.3-16.2	
October	10.0	1.0- 64.5	37.0	21.0-56.0	3.5	1.2-7.0	0.7	0.3- 1.1	13.0	9.0-16.4	0.5	0.3- 0.9	n.m.	14.0	12.6-15.3	
November	5.9	2.0- 22.0	31.0	19.0-66.0	7.6	0.5-28.0	0.4	0.2-0.6	8.8	6.2-13.1	0.5	0.2- 0.9	8.9	7.6-10.3	11.0	7.2-12.9
December	5.1	2.0- 24.0	26.0	18.0-39.0	2.4	0.1-4.9	0.2	0.1-0.4	9.2	6.4-12.8	0.6	0.3-0.7	8.3	6.5-10.4	9.1	7.8-10.1
January	10.0	2.5- 57.0	44.0	20.0-63.0	3.4	0.8-5.6	0.5	0.3-0.7	12.0	9.5-15.2	0.5	0.3-0.8	11.0	6.9-15.1	7.9	7.2-8.9
February	9.5	2.5- 55.0	35.0	16.0-53.0	8.6	1.6-33.4	0.4	0.2-0.5	9.3	6.9-13.4	0.4	0.2-0.5	8.1	6.5-11.0	7.8	7.2-8.6
March	4.7	1.5-9.0	39.0	19.0-51.0	5.6	1.0-11.5	0.4	0.2-0.8	10.0	5.5-15.0	0.5	0.3-0.7	8.6	6.5-11.0	8.8	8.2-9.7
April	5.6	2.5- 20.0	53.0	37.0-79.0	2.3	0.2-9.2	0.6	0.2- 1.0	16.0	11.9-20.9	0.6	0.5- 1.1	11.0	5.5- 33.0	9.9	9.2-10.9

Limits

20

1

0.8

Values are presented as arithmetic means (\bar{x}) and ranges, values written in bold letters exceeded the limits (AbwV, 1975) for general parameters.

DOC = dissolved organic carbon; BOD₅ = biological oxygen demand; COD = chemical oxygen demand; n.m. = not measured

TABLE II: Prevalence (%) and abundance (mean values) of the infestation with *Gyrodactylus* sp. of fish included in the active monitoring

Treatment	Localisation	Eposure time (month of sampling)	number of animals examined	brown trout		rainbow trout	
				%	abundance	%	abundance
Tap water	Sewage plant		10	10	0.5	n.m	n.m.
Tap water	CFWH		18	17	0.6	15	0.6
Diluted sewage plant effluents	sewage plant	2 (July)	(bt: n=7; rt: n=6)	14	1	17	2
		5 (October)	5	20	0.5	60	1
		8 (January)	(bt: n=3; rt: n=4)	33	1	50	0.8
		11 (April)	5	60	0.5	50	1.3

n.m. = not measured; CFWH = Centre for Fish and Wildlife Health; bt = brown trout; rt = rainbow trout