BOREAL ENVIRONMENT RESEARCH 3: 387–393
Helsinki 19 January 1999

ISSN 1239-6095

Smolting of the brown trout (*Salmo trutta* L.) in Lestijoki water

Antti Soivio[1,2], Kari Myllynen[2,3], Jukka Pakkala[3] and Sinikka Jokela[3]

[1] *Finnish Game and Fisheries Research Institute P.O. Box 6, FIN-00721 Helsinki, Finland.*
[2] *Department of Biosciences, Division of Animal Physiology, P.O. Box 17, FIN-00014 University of Helsinki, Finland.*
[3] *Central Ostrobothnia Regional Environmental Centre, Torikatu 40 B, FIN-67100 Kokkola, Finland.*

Soivio. A., Myllynen, K., Pakkala, J. & Jokela, S. 1998. Smolting of the brown trout (*Salmo trutta* L.) in Lestijoki water. *Boreal Env. Res.* 3: 387–393. ISSN 1239-6095

The brown trout (*Salmo trutta* L.) of the Lestijoki strain, reared to the pre-smolt stage in fish farm conditions, were tested for their smolting ability in water from Lestijoki, a river in western Finland, during final winter before smolting. Reference fish were reared in local ground water. During the pre-smolt winter the fish were analysed for their gill ATPase activity, condition factor (CF) and osmoregulatory ability after exposure in 30% balanced sea water solution. The osmoregulatory ability of the gills was severely altered by the water quality of Lestijoki water. The decrease of condition factor (CF) and changes in gill Na^+, K^+-ATPase activity revealed an attempt of smolting in fish reared in the river water. The results of this investigation questions the ability to give Lestijoki a reproductive brown trout stock by re-establishing the species.

Introduction

In well-drained soils, organic material is transported from soil down to river waters with metals, mainly iron and aluminium (Lundin 1991). Organic matter is of great importance to aluminium mobility. The high concentrations of iron in natural waters is bound to the abundant humic acids (Stumm and Lee 1960) capable of binding metal ions effectively. Acidic, aluminium rich drainage is common in Finland especially in agricultured areas. However, the river water usually contains humic compounds and insoluble particles which are known to be effective chelators (Peuranen et al. 1994). In the situation of slight acidification, humic compounds can prevent the toxicity of metal ions, especially iron and aluminium (Peuranen *et al.* 1994, Myllynen *et al.* 1997). In Finnish humidic lakes, the reproduction of ruffe (*Gymnocephalus cernua*) and roach (*Rutilus rutilus*) is affected below the pH of 5.0–5.5 (Rask and Tuunainen 1990). Aluminium and low pH is a very toxic combination (McDonald and Wood 1993) and acidification has been reported to increase slime excretion in the gills (McDonald, 1983) causing physiological disturbances and even large fish kills.

Increased concentration of iron caused gill damages in brown trout (*Salmo trutta*) (Peuranen *et al.* 1994). Both functional and histological damages were similar to those caused by aluminium (*see* Tietge *et al.* 1988). A lowered capacity of ion regulation and gas transfer in the damaged gills may cause serious problems to smolt entering the sea water.

For a smolting salmonid a decrease of the condition factor is typical because of its intense growth and utilization of metabolic reserves (Boeuf 1993). Simultaneously the gill Na$^+$, K$^+$-ATPase activity and their osmoregulatory capacity increases and the parr are prepared to regulate their internal sodium and potassium concentrations even in fully marine waters (Boeuf 1993). The osmoregulation of magnesium in the intestine and kidney is usually the last osmotic component to mature during smolting. If the smolt does reach the sea these changes are restored (Boeuf 1993). Because of the active metabolism of the smolt, fully functional gills are of advantage in the warming water after the down-stream migration.

In the present study, cultivated trout, originating from the local brown trout strain, were tested using common smolting indices for their osmoregulatory capacity and smolting ability. This was to estimate the possibilities to rehabilitate their native river, Lestijoki, a river in western Finland, from where trout has almost totally disappeared during the years of draining the flow area of the river.

Material and methods

Two-year-old trout (*Salmo trutta* L.) were transported from Laukaa Fisheries Reseach and Aquaculture (62°27´N, 25°35´E) on 26 November 1993 to the laboratory of Central Ostrobothnia Regional Environmental Centre (63°40´N, 25°10´E). The starting temperature of the experiment was 2.0 °C. Two groups, 150 fish each, were introduced to 3 000 l pools, one filled with ground water and the other with water from Lestijoki. During the experiment 70% of the water was renewed twice a week. At the beginning of the experiment the water was cooled to the level of river temperatures (ca. 0.2 °C) and regulated to this temperature until 30 March 1994, when water temperature was slowly elevated, in accordance with the water temperature in the river. From 15 November onwards the pools were covered to total darkness until 14 March 1994 after which the period of light was increased from 0.5 h day^{-1} to reach 12 h day^{-1} in three weeks.The experiment ended in June 1994 at a temperature of 12 °C. The quality of the river and ground waters are presented in Table 1.

The stage of the smolting process was checked three times during the spring. Prior to each sampling 10 fish from both groups were exposed to 30% balanced sea water (Aqua Life®) for 96 hours (Virtanen 1988). As controls fish from the same groups were sampled similarly, but they were not exposed to sea water.

The ability to control the osmoregulation in sea water was estimated by Na$^+$, K$^+$-ATPase activity of the gills. The ability of the fish to regulate the ion concentrations (Na$^+$, K$^+$, Mg$^+$ and Cl$^-$ of plasma) and the water content of white muscle after the 30% salt exposure were measured.

For sampling, the fish were immobilized with a sharp blow on the head and the blood samples were collected from the caudal vessels, centrifuged (3 min., 10 000 × G) and the plasma Na$^+$, K$^+$ and Mg^{2+}, Cl$^-$, lactate and glucose were analysed according to Soivio *et al.* (1989). Muscle tissue samples were taken from the lateral white muscle, dorsal to the lateral line. The gill filaments from the first arch were analysed for gill Na$^+$, K$^+$-ATPase (Silva *et al.* 1977). The condition factor was calculated individually for each fish by using the equation: CF = $wl^{-3} \times 100$, where CF = condition factor, w = weight (g), l = length (cm). The condition of

Table 1. The quality of test waters during the experiment (mean ± SD).

	Fe µg l^{-1}	Al µg l^{-1}	Cu µg l^{-1}	Zn µg l^{-1}	Ni µg l^{-1}	Pb µg l^{-1}	NH4-N µg l^{-1}
Ground water	29 ± 6.15	180.31 ± 114.5	2.0 ± 0.3	9.24 ± 1.8	0.673 ± 0.15	0.329 ± 0.072	1 114
River water	1 196.67 ± 49.06	513.69 ± 216.7	2.653 ± 0.3	7.4 ± 1.1	1.921 ± 0.64	1.277 ± 0.446	1 282

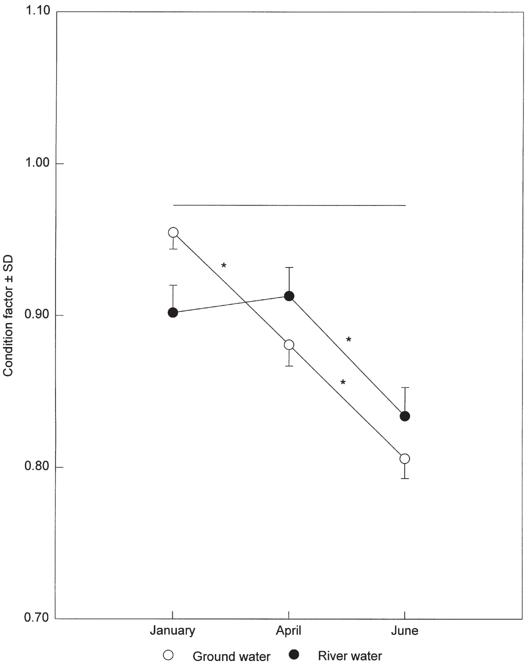

Fig. 1. The condition factor of the ground water and river water smolt groups of *Salmo trutta*. The horisontal line indicate the CF value estimated in September for the same stock and fish group of trout from which the experimental fish originate in Laukaa Fisheries and aquaculture. * = $p < 0.05$.

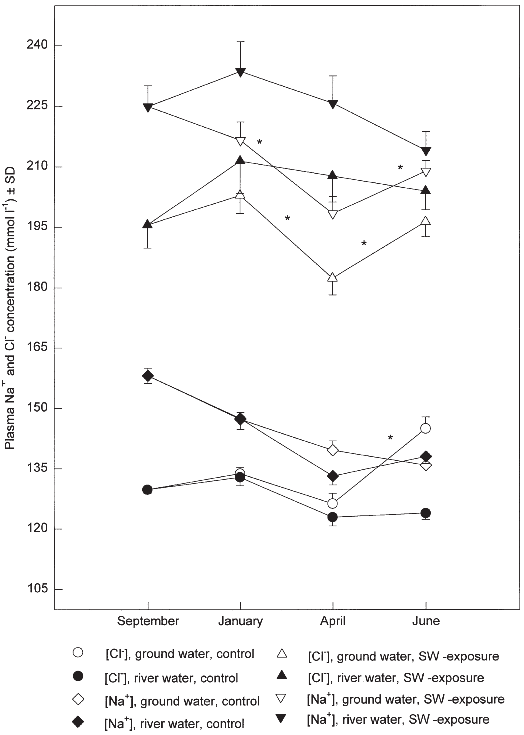

Fig. 2. The plasma sodium and chloride concentrations prior and after a sea-water (30%) exposure test of the ground water control fish and river water test fish. * = $p < 0.05$.

the finns was visually observed (scale 0–5).

One way factorial analysis of variance (ANOVA) was used to test the results.

Results

The fish were healthy and in good condition during the experiment. The amount of damages on the dorsal fin increased at the end of the year 1993. However, the fins recovered during the spring, when the water temperature increased. In both groups the condition factor of the fish decreased during the spring; the ground water group showing a significant decrease through January–June, the river water group, however, first in June (Fig. 1.).

The salt water test for osmoregulatory capacity did not show any clear adaptation in either group. In the groundwater control groups there was a slight (non significant) overall decrease of plasma sodium (Fig. 2) from the reasonably high September value till April–June. In the salt water test the decreasing trend of plasma Na^+ concentration was very similar to the controls, but on a 60 mmol l^{-1} higher level. However, the river water group exposed to salt water showed a slight but continuous decrease in plasma Na^+ concentration through January–April. The ground water group, having a lower Na^+ concentration in January, showed a continued increase in Na^+ concentration from April onwards. The plasma Cl^- concentration in the salt water exposed ground water group varied in accordance with the sodium concentration. During the experiment the Na^+ and Cl^- concentrations in both control groups corresponded, except for the June values for Cl^- showing higher value for ground water controls. The ATPase activities measured (Fig. 3) were high through the whole winter, but without any significant differences between groups. The plasma Mg^{2+} regulation during salt water exposure indicate an activation of the ground water group from April onwards (Fig. 4), while the regulation in

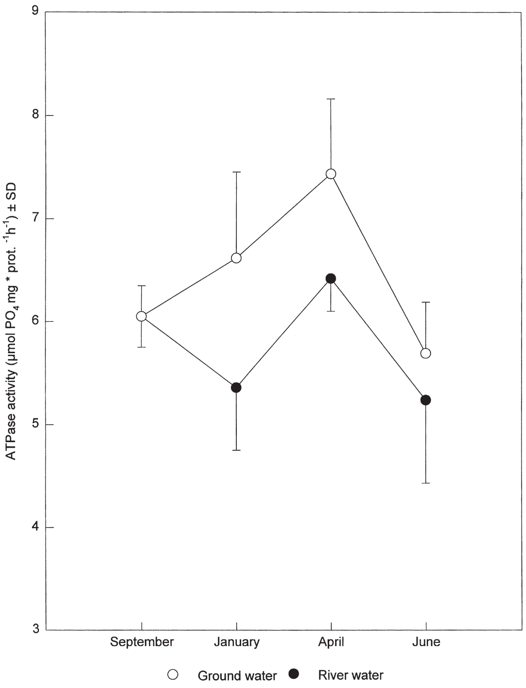

Fig. 3. The ATPase activity of the gills of the fish of ground water and river water groups.

the river water group was not activated until June.

Muscle tissue water content decreased with 5% units in all salt exposures.

From April onwards the plasma glucose concentration in both groups showed a decreasing trend ($p < 0.05$) and the lactate concentrations (Fig. 5) of the ground water fish were increasing ($p < 0.05$).

It is evident that the development of the osmoregulation was delayed in the river water group when compared to the ground water group.

Discussion

The ammonium concentrations in the test waters during the experiment were high because they were analysed from samples taken prior to the twice a week water change. The pH-value near neutral, however, support ammonia turnover to ammonium and in well oxygenated water ammonia is in ionic (NH_4^+) state which is not poisonous to fish (Thruston *et al.* 1981, Thruston and Russo 1983).

Virtanen *et al.* (1983) noticed that reared and wild smolts do not always smoltify to the same extent. The increase of the gill Na^+, K^+-ATPase activity was more clear in the wild smolt than in the reared one. The reared smolts often have difficulties in osmoregulation while exposed to full strength sea water (Virtanen 1988). This can be seen in the increased plasma electrolyte concentrations and lowered muscle water content during the seawater exposure. In this study the fish exposed to fully marine water presented a slow development of osmoregulatory components indicating a low stage of osmoregulatory capacity. This poor success in smolting is apparently not merely due to the rearing process. The experimental fish used in this investigation originated from a reared group of brown trout in Laukaa Fisheries Recearch and Aquaqulture. The original group was expected to smolt during the experiment as seen in earlier experiments (Muona *et al.* 1988, Söderholm-Tana and Soivio 1990). Our results, however, reveal a disturbed development of the osmoregulatory capacity even in the control groups.

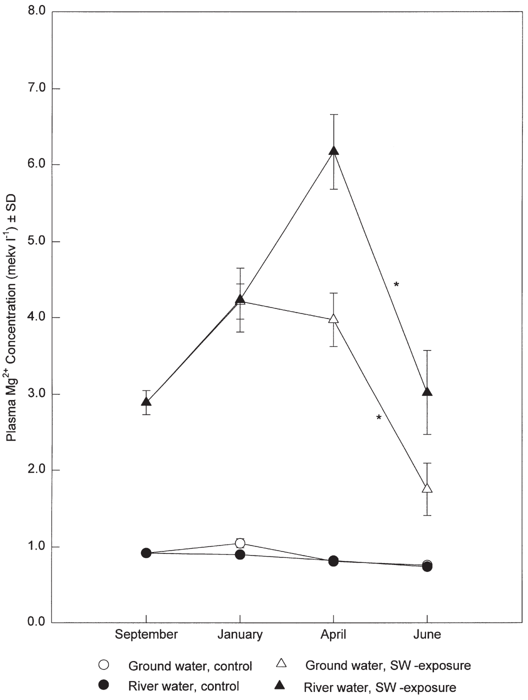

Fig. 4. The plasma Mg^{2+} concentration of ground water control fish prior and after to the sea-water exposure (30%) test. * = $p < 0.05$.

Water quality may affect the results when testing smolting in sea water. In springtime the water quality decreases especially in the agricultural areas. The water pH may decrease to near 5 and the aluminium and iron concentrations are high (ca. 7 310 mg l^{-1} and 1 400 mg l^{-1} respectively; Jokela and Saastamoinen 1988). Both metals are known to be toxic when pH decreases below 6 (McDonald and Wood 1993). Peuranen *et al.* (1994) demonstrated gill damages and difficulties in the osmoregulation of brown trout caused by iron in lowered pH in humic water. Myllynen *et al.* (1994) noticed that iron is more toxic than aluminium in slightly acidic, humic waters. In this investigation the iron concentration of the river water was about 50 times that of the ground water used (Table 1.) This could explain the failure of osmoregulation in the river water group.

During the winter, when water temperature decreases, the gill epithelium grows thicker preventing ion loss through the gills (Leino and McCormic 1993). Thicker epithelium, however, decreases the oxygen passage through the gill. In cold water this is not a problem as oxygen affinity of haemoglobin (Wyman 1948) increases in low temperatures and the oxygen consumption of the fish decreases (Johnston and Dunn 1987). No differences were found in the epithelial thickness of secundary lamellae of rainbow trout kept in temperatures between 10 °C and 2 °C in good quality of water (H. Tuurala, pers. comm.).

In the Lestijoki district, upstream from the experimental site, one-year-old brown trout, (seatrout and brooktrout strains) have suffered from hyperplasia of the chloride cells and heavy hypertrophy of the epithelium leading to massive deaths in March–May at the water temperature of 0.2 °C and pH-values varying from 5.3 to 6. The changes were more serious in the seatrout (Koski, pers. comm.)

The ion transport of gills is very sensitive to heavy metal poisoning. The common response to chronic exposures of zinc and aluminium is an increase of gill Na^+, K^+-ATPase activity (McDonald and Wood 1993). The morphological basis for this syndrom is to be found from the hyperplasia of chloride cells in the gill epithelium during chronical sublethal exposures to zinc (Mathiassen and Bradfield 1973) and aluminium (Karlsson-Norrgren *et al.* 1986a, 1986b, Evans *et al.* 1988, Tietge *et al.* 1988, Mueller *et al.* 1991).

The ATPase activation in the fish gills is capable in compensating the changes in the ion transport activity due to the toxic effects of heavy metals or low temperature. For that reason the high ATPase activity of gills found in this investigation cannot be taken as an indicator of smolting. The elevated ATPase activity is needed to compensate for the osmoregulatory disturbance caused by the hypertrophy of the gill epithelium possibly due to high aluminium and iron concentrations, or low temperature (Leino and McCormic, 1993) of the test water. According to the functional measurements concerning the effects of Zn on the fish gill by Lappivaara *et al.* (1995) the low zinc concentration of the test water in our experiment, obviously had no significant effect on the lamellar epithelium. Our results revealed impaired osmoregulatory capacity of the fish. The elevated ionic concentrations of plasma and 'drying' of the muscle tissue of fish exposed to sea water, indicate that the gill ATPase primarily has been on an elevated level due to other (environmental) reasons, i.e. stressors.

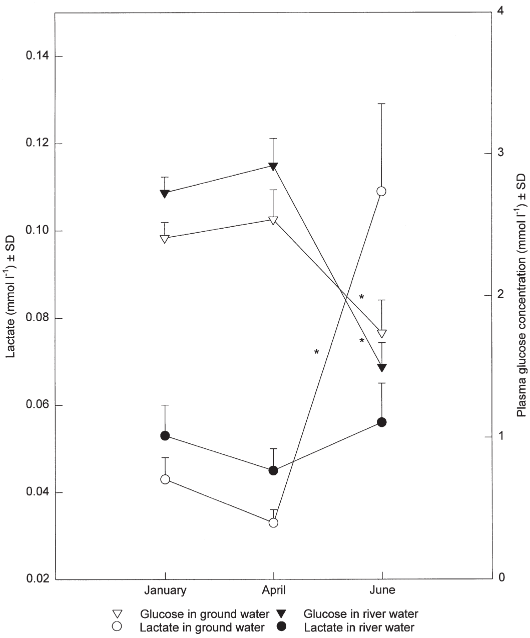

Fig. 5. The plasma glucose and lactate concentrations for trout in ground water and river water. * = $p <$ 0.05.

The water in the natural habitats of brown trout and salmon parr is clean, with very low electric conductivity and low or no concentration of solved solids, ammonia, humus and metals. Any of these addititives may affect the physiological development of smolting in cultured brown trout, which are not even capable of chosing their environmental temperature.

However, the slight trend to higher activities of ATPase in the ground water group from January to June give some support to the plasma sodium results, in some extent typical for smolting (Figs. 2 and 3). A clear indices of a good trial for smolting is also the decrease in Mg^{2+}, showing the increased osmoregulatory capacity of the gut. The decrease of plasme glucose and the increase in lactate concentrations are good indices of the parr turning its lipid metabolism to a carbohydrate mode typical for smolt (Virtanen 1988).

In the light of the present results, it is obvious that the smolting process of brown trout in Lestijoki is altered. The migratory behaviour is not developing sufficiently, and the trout stay in the river or nearby the rivermouth, not being able to carry out a real feeding migration. In addition to the lack of spawning grounds in the river, the disturbed osmoregulation during parr-smolt transformation will be difficult to overcome when trying to restore the brown trout to a natural population in Lestijoki.

Acknowledgements: We express our gratitude to the skillful and effective team in the Laboratory of Central Ostrobothnia Regional Environmental Centre, which made this investigation possible.

References

Boeuf G. 1993. Salmonid smolting: a pre adaptation to the oceanic environment. In: Rankin J.C. & Jensen F.B. (eds.), *Fish ecophysiology*. Chapman & Hall. pp. 105–135.

Evans R.E., Brown S.B. & Hara T.J. 1988. The effects of aluminium and acid on gill morphology in rainbow trout, Salmo gairdneri. *Env. Biol. Fishes* 22: 299–311.

Johnston I. A. & Dunn J. 1987. Temperature acclimation and metabolism in ectotherms with particular reference to teleost fish. In: Bowlwr K. & Fuller B.J. (eds.), *Temperature and animal cells,* Symp. of the Soc for Exp. Biol. XXXXI.

Jokela S. & Saastamoinen V.-L. 1988. Lestijoen luonnontaloudellinen suunnitelma, veden laatu, tutkimuksen tila ja tarpeet. *Vesi- ja ympäristöhallituksen monistesarja* 83. 80 + 30 pp.

Karlsson-Norggren L., Dickson W., Ljungberg O. & Runn P. 1986a. Acid water and aluminium exposure: gill lesions and aluminium accumulations in farmed brown trout, Salmo trutta. L. *J. Fish Dis*. 9: 1–8.

Karlsson-Norggren L., Björklund I., Ljungberg O. & Runn P. 1986b. Acid water and aluminium exposure: experimentally induced gill lesions in brown trout, Salmo trutta. L. *J. Fish Dis*. 9: 11–25.

Lappivaara, J., Nikinmaa, M. & Tuurala, H. 1995. Arterial oxygen tension and the structure of the secondary lamellae of the gills in rainbow trout (Oncorhyncus mykiss) after acute exposure to zinc and during recovery. *Aquat. Toxicol*. 32: 321–331.

Leino R.L. & McCormic J. H. 1993. Responses of juveline largemouth bass to different pH and aluminium levels at owerwintering temperatures: effects on gill morphology, electrolyte balance, scale calcium, liver glycogen and depot fat. *Can. J. Zool*. 71: 531–543.

Lundin L. 1991. Influence of silviculture on content of organic matter and metals in water. In: *Humusuutiset*. The third international nordic symposium on humic substances, August 21–23, 1991. Turku, Finland. Vol 3, pp. 21–26.

Mathiessen P. & Brafield A.E. 1973. The effects of dissolved zinc on the gills of the stickleback, Gasterosteus aculeatus (L.). *J. Fish Biol*. 5: 607–613.

McDonald, D. G. 1983. The effect of H^+ upon the gills of freshwater fish. *Can. J. Zool*. 61: 691–703.

McDonald D.G. & Wood C.M. 1993. Branchial mechanisms of acclimation to metals in freswater fish. In: Ranking J.C. & Jensen F.B. (eds.), *Fish ecophysiology*, Chapman & Hall, London, pp. 297–321.

Mueller M.E., Sanchez D.A., Gergman H.L., McDonald D.G., Rhem R.G & Wood C.M. 1991. Nature and time course of acclimation to aluminium in juveline brook trout (Salvelinus fontinalis). II. Histology. *Can. J. Fish aquat. Sci*. 48: 2016–2027

Muona M., Soivio A. & Virtanen E. 1988. Viljellyn meritaimenen ja järvitaimenen vaellusvalmiuden kehittyminen. *Hanka-Taimen Oy 1986–1987*. 34 pp.

Myllynen K., Nikinmaa M. & Ojutkangas O. 1997. River water with high iron concentration and low pH causes mortality of lampray roe and newly hatched larvae. *Ecotox. and env. safety* 36: 43–48.

Peuranen S., Vuorinen P. J. & Hollander A. 1994. The effects of iron, humic acids and low pH on the gills and physiology of brown trout (S. trutta). *Ann. Zool. Fennici* 31: 389–396.

Rask M. & Tuunainen P. 1990. Acid-induced changes in fish populations of small Finnish lakes. In Kauppi P., Anttila P. & Kenttämies K. (eds.), *Acidification in Finland*, Springer, Berlin, pp. 911–928.

Silva P., Solomon R., Spokes K. & Epstein F.H. 1977. Ouabain of gill Na-K-ATPase: relationship to active chloride transport. *J. Exp. Zool*. 199: 419–426.

Soivio A., Muona M. & Virtanen E. 1989. Smolting of two populations of Salmo trutta. *Aquaculture* 82: 147–153.

Stumm W. & Lee G.F. 1960. The chemistry of aquenous iron. *Schweiz. Z. Hydrol*. 22: 295–319.

Söderholm-Tana L. & Soivio A. 1990. Physiological condition and smoltification in young Baltic salmon (stock of the river Neva), land-locked salmon and sea trout (stock of the river Lestijoki and Isojoki) reared at several fish farms in 1990. *Finnish Game and Fisheries Research Institute*, Helsinki, Finland. 29 pp.

Tietge J.E., Johanson R. D. & Bergman H. L. 1988.

Morphometric changes in gill secondary lamellae of brook trout (Salvelinus fontinalis) after long-term exposure to acid and aluminium. *Can. J. Fish. Aquat. Sci.* 45: 1643–1648.

Thruston R.V. & Russo R. C. 1983. Acute toxicity of ammonia to rainbow trout. *Trans. Am. fish. Soc.* 1983. 112: 696–704.

Thruston, R. V., Russo, R. C. & Vinogradov, G. A. 1986. Ammonia toxicity to fishes, effect of pH on the toxicity of the un-ionized ammonia species. *Environ. sci.Technol. 1981*, 15: 837–840.

Virtanen E. 1988 Smolting and osmoregulation of Baltic salmon Salmo salar L., in fresh water and brackish water. *Finnish Fish. Res.* 7: 38–65.

Virtanen E., Westman K. & Soivio A. 1983. Condition and smoltification in wild and hatchery-reared young Atlantic salmon, Salmo salar. *Suomen kalatalous* 49: 57–73. [In Finnish with English abstract].

Wyman J. 1948. Hemeproteins. *Adv. Protein Chem.* 4: 407–531.

Received 24 June 1997, accepted 24 November 1998