

Nuclear alterations in hepatocytes of Arctic char *Salvelinus alpinus* from acidic high alpine lakes

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ABSTRACT: The hepatocyte morphology of Arctic char *Salvelinus alpinus* from 2 acidic, a circumneutral and 3 alkaline lakes in the Austrian Alps (Tyrol and Styria) was compared with respect to nuclear alterations and related to environmental parameters. Extensive glycogen storage of hepatocyte nuclei could regularly be found in Arctic char from the 2 acidic high mountain lakes. In contrast, in Arctic char from the circumneutral lake (slightly acidic during winter), nuclear glycogen storage could only be documented in a few specimens. In alkaline lakes, however, nuclear glycogen deposition was absent in 64 out of 65 specimens. Additional nuclear pathology in char of the most acidic lake was evident from a reorganization of nucleolar components. The number of nuclei involved in glycogen storage showed a distinct seasonal pattern which correlated with the intensity of feeding, but not with the seasonality of other environmental parameters (trace metals, temperature, pH).

KEY WORDS: High mountain lakes · Acidification · Arctic char · Liver pathology · Ultrastructure
Nucleus · Glycogen

INTRODUCTION

During the last few decades, fish populations of thousands of low alkalinity lakes in the northern United States, in eastern Canada and in Scandinavia have decreased or have been completely eradicated due to progressively increasing levels of acidity (Harvey et al. 1981, Hendriksen et al. 1989, Bulger et al. 1993). Industrial emissions of sulphuric and nitric acids have had an impact even on remote lakes, which may be unable to buffer the acid precipitation due to their geological background (Mason 1989). During this century, pH and alkalinity of sensitive lakes have decreased by approximately 0.8 units and $28 \mu\text{eq l}^{-1}$, respectively (Psenner 1994). However, acidification does not simply mean a decrease of pH, but frequently also includes progressive mobilization of metals (Hutchinson et al. 1987, Hutchinson & Sprague 1989, Reader & Dempsey 1989, Köck et al. 1995), particularly of aluminum (Backes & Tipping 1987), which has repeatedly

been shown to be extremely harmful to fish (Baker & Chofield 1982).

Acute effects of acidification on fish can be seen in running waters in which acid precipitation or acid runoff during snow melt leaches aluminum from the watershed. These episodic events result in severe damage to gills (Daye & Garside 1975, McDonald 1983, Chevalier et al. 1985, Mason 1989) or even fish kills (McDonald & Wood 1993). In lakes of low alkalinity, however, fish are chronically exposed to sublethal concentrations of acids and metals. At least adult fish have been shown to be capable of acclimatization to such extreme environmental conditions (McDonald & Wood 1993); their reproductive success, however, may be gradually impaired. Reduced egg production or declining spawning rates (Beamish et al. 1975, McCormick et al. 1989), decreased hatching rates of larvae (Daye & Garside 1979) and particularly increased lethality to swim-up fry as the most sensitive developmental stage (Sayer et al. 1993) are common effects of progressive atmospheric pollution on fish populations in acidic lakes.

Sublethal effects of acids and aluminum have primarily been studied with respect to gill structure and

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function (Leino & McCormick 1984, Karlsson-Norrgrén et al. 1986a, b, Leino et al. 1987a, b). Thus, increased mucus secretion (Fischer-Scherl & Hoffmann 1988) as well as hyperplasia, hypertrophy and necrosis of gill epithelia (Chevalier et al. 1985) have been described in addition to respiratory, haematological and ionoregulatory responses (Fromm 1980, McDonald 1983, Wood 1989). In contrast, little information is available about the influence of acidic environments on the morphology and physiology of other organs such as the liver, kidney, skin or pituitary gland of fish (Wendelaar-Bonga et al. 1986, Segner & Pechlaner 1987, Segner et al. 1987). Since the liver has repeatedly been demonstrated to react to numerous internal and external stimuli including sexual activity (Peute et al. 1985), season (Segner & Braunbeck 1990), temperature (Braunbeck et al. 1987), starvation and malnutrition (Segner & Braunbeck 1988), as well as various xenobiotics (Braunbeck 1994), the present study was designed to compare the histo- and cytopathology of hepatocellular nuclei in declining populations of Arctic char from 2 acidic high mountain lakes with those from well-reproducing populations from circumneutral or alkaline lakes.

MATERIAL AND METHODS

Sampling sites and dates. Arctic char of 6 oligotrophic alpine lakes in Austria (Table 1) were caught with gill nets or rod and line during summer and winter, respectively. Gill nets were checked every 30 to 40 min. Samples were taken from: Schwarzsee (SOS), Drachensee (DRS), Mittlerer Plenderlesee (MPL), and Oberer Plenderlesee (OPL), which are high mountain lakes located in the Ötztal Alps (Tyrol), from Achensee (ACH) near Jenbach (Inn valley, Tyrol) as well as from Grundlsee (GRS; Totes Gebirge, Styria). In DRS, ACH and GRS only 1 sample was taken (October); in MPL 2

samples (July and September), in SOS 6 (April to September) and in OPL 5 samples (March to September) were taken. During transport to the laboratory (1.5 to 2 h), water was aerated and maintained at the same temperature as the sampled lake. Fish from all sampling sites were treated in the same way.

Water analysis. Chemical and physical parameters of the water are listed in Table 1. For more detailed information on water characteristics of the lakes, see Köck et al. (1995). Measurements of pH in waters of low ionic strength raise methodical problems (Hoenicke et al. 1991) due to low conductivity, low buffering capacity and imbalanced CO₂ contents of the water. In fact, data taken immediately after sampling slightly differed from those obtained after transfer to the laboratory. For the present study, an Ingold pH electrode recommended for pH-measurements in low ionic strength water was equilibrated at the temperature conditions of the lake. The pH of the water was measured in the field immediately after sampling. *In situ* temperature of the lakes was measured with a temperature electrode.

For cadmium analysis, water samples of SOS were filtered in the field through 0.45 µm cellulose acetate membranes and acidified for preservation. Analyses were performed with a Hitachi Z-8200 graphite furnace atomic absorption spectrophotometry (GFAAS) with Zeeman background correction. Due to low cadmium concentrations, the 'sample concentration' option was used. Cadmium concentrations are given as volume-weighted means.

Morphometry. Fish were killed by a blow on the head. Weights of body, liver and stomach contents as well as total length were measured and opercula bones as well as otoliths were dissected for age determination.

Light microscopy. For light microscopical studies, pieces of liver were fixed in 5% formaldehyde (pH 7.4), dehydrated in a graded series of ethanol and embed-

Table 1 Physical and chemical parameters of the lakes investigated. Data from Köck et al. (1995). SOS: Schwarzsee; MPL: Mittlerer Plenderlesee; OPL: Oberer Plenderlesee; DRS: Drachensee; ACH: Achensee; GRS: Grundlsee. Data for depth of water samples from SOS, MPL, OPL, and DRS represent volume-weighted means (see Köck et al. 1995); for ACH and GRS, means at 30 to 50 m depth during autumn (specific environment of Arctic char in these lakes) are given

	SOS	MPL	OPL	DRS	ACH	GRS
Altitude (m)	2799	2317	2344	1874	929	712
Conductivity (µS cm ⁻¹)	12.7	11.2	30.2	138	275	235
Alkalinity (µeq l ⁻¹)	1.3	18.3	100	1357	2749	1900
pH	4.8–6.3	5.3–6.6	6.1–7.3	7.8–8.6	8.35	8.1
Ca ²⁺ (mg l ⁻¹)	1.02	1.1	3.1	25.2	35.0	44.0
Na ⁺ (mg l ⁻¹)	0.25	0.26	0.41	0.08	0.6	1.0
Cl ⁻ (mg l ⁻¹)	0.13	0.10	0.11	0.20	1.0	2.9
Al _{diss} (µg l ⁻¹)	40	<15	<15	<10	<10	–
Cd (µg l ⁻¹)	0.114	0.068	0.036	0.036	0.02	0.01

ded in methyl methacrylate. Sections 3 μm thick were stained with Giemsa, haematoxylin-eosin or periodic acid-Schiff (PAS). The numbers of hepatocellular nuclei with distinct glycogen vacuoles (occupying more than one-third of the nuclear area) were counted under the light microscope (Weibel 1979) and calculated as percentage of total number of nuclei; for each section, 400 nuclei were inspected. The number of hepatocytes with fatty degeneration was monitored semiquantitatively.

Electron microscopy. For electron microscopy, 6 individuals were taken each from SOS (July, September), GRS (September) and OPL (June). Fish were anaesthetized in 4-aminobenzoic acid ethyl ester (benzocaine; 50 mg l⁻¹). *In situ* cardiac perfusion fixation was achieved through the ventricular wall using a 3.5 mm I.D. Tygon™ tube (Ismatec, Switzerland) and a blunt 1.2 mm steel needle with a terminal opening of 0.8 to 1.0 mm (Microlance™, Becton & Dickinson, Dublin, Ireland). The vasculature was flushed with 4°C fish physiological saline (0.9%) containing 2% polyvinylpyrrolidone (PVP; Merck, Darmstadt, FRG) and 0.5% procaine hydrochloride (Serva, Heidelberg, FRG) for 30 s to remove blood cells. This was followed by 1.5% glutaraldehyde and 1.5% formaldehyde (freshly prepared from paraformaldehyde) in 0.1 M sodium phosphate buffer (pH 7.6) containing 2.5% PVP (4°C). Initial perfusion rate was adjusted to 12 to 15 ml min⁻¹. Livers were excised immediately after perfusion, immersed in perfusion fixative, and cut into slices of 60 to 70 μm using an Oxford™ vibratome. Fixation was continued in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.6) containing 4% PVP and 0.05% calcium chloride for 20 min.

For visualization of peroxisomes, catalase peroxidatic activity was demonstrated using alkaline 3,3'-diaminobenzidine (DAB; Le Hir et al. 1979): Tissue slices were incubated in 10 mM Teorell-Stenhagen buffer (pH 10.0) containing 5 mM DAB and 0.5% H₂O₂ for 60 min at 37°C in a shaking water bath. After repeated rinsing in Teorell-Stenhagen and cacodylate buffers, tissue slices were postfixed for 1 h with 1% osmium ferrocyanide (Karnovsky 1971).

After triple washing in 0.1 M cacodylate and 0.05 M maleate buffer (pH 5.2), tissues were stained *en bloc* with 1% uranyl acetate in maleate buffer for 1 h. Specimens were dehydrated in a graded series of ethanol and embedded in Spurr's medium. Ultrathin sections of 60 or 80 nm thickness were stained with alkaline lead citrate for 30 s or 1 min and examined in a Zeiss EM 9 or a Zeiss EM 10 electron microscope.

Semithin plastic sections of 0.5 to 0.75 μm were stained with methylene blue-Azur II and used for orientation. For visualization of glycogen, semithin sections were incubated in an alkaline 1% solution of

silver diamine for 1.5 h at 60°C. After rinsing in distilled water, sections were mounted in Entellan and examined in a Leitz Aristoplan photomicroscope.

Statistics. Data are presented either as means in normally distributed samples or as medians with 25% and 75% percentiles in non-normally distributed samples. Differences between groups were tested by means of the Student-Newman-Keuls test (pairwise comparison) or by Kruskal-Wallis 1-way analysis on ranks in non-normally distributed samples. Spearman rank order correlation was applied for testing relationships between 2 variables.

RESULTS

Water analyses

In winter, water pH of high alpine lakes is generally lower than in summer (Fig. 1). In extremely low alkalinity lakes (SOS and MPL), pH gradually decreased towards the ice-break. This may be the effect of both accumulating CO₂ and acid run-off occurring as episodic events up to 2 mo prior to ice break. In SOS, MPL, and DRS, water is exclusively supplied by the run-off from the catchment area. The circumneutral lake (OPL) reached minimum pH much earlier, which may be attributed to increasing volumes of the permanent water inflow during snow melt (April to June). In

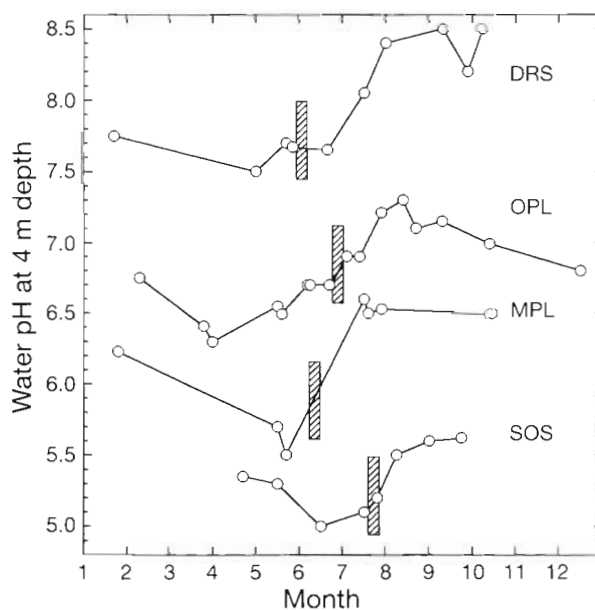


Fig. 1. Seasonal pattern of pH in 4 high mountain lakes of different alkalinities measured at 4 m depth. Columns represent the period of ice break. DRS: Drachensee; OPL: Oberer Plenderlesee; MPL: Mittlerer Plenderlesee; SOS: Schwarzsee

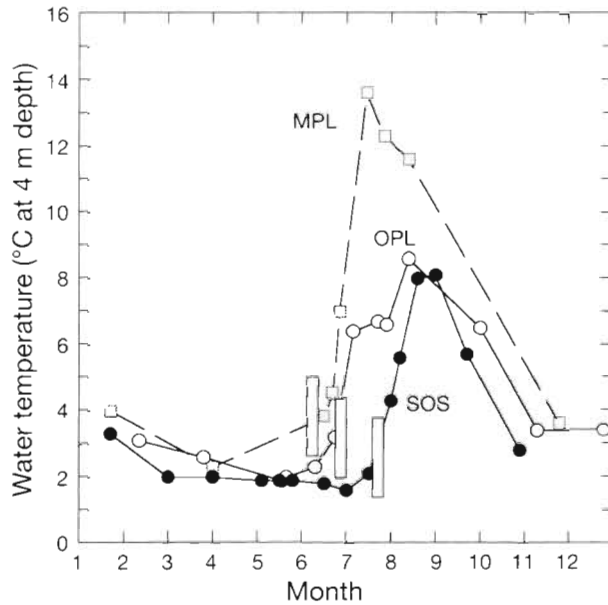


Fig. 2. Seasonal pattern of water temperature in 4 m depth. Columns represent period of ice break. MPL: Mittlerer Plenderlesee; OPL: Oberer Plenderlesee; SOS: Schwarzsee

the well-buffered low altitude lakes (ACH and GRS), however, pH remained almost constant throughout the year. SOS and MPL were acidic even in summer. Whereas temperature profiles were similar in OPL, SOS and DRS, maximum temperature of MPL in summer was considerably higher (Fig. 2). Surface temperatures of 20°C and more were reached in low altitude lakes. During summer, however, Arctic char prefer deeper water layers with temperatures below 17°C.

Cadmium concentrations in SOS displayed seasonal fluctuations with maxima in spring (Fig. 3).

Macroscopic observations

The status of fish populations is summarized in Table 2: the dwarfed and dense population of OPL

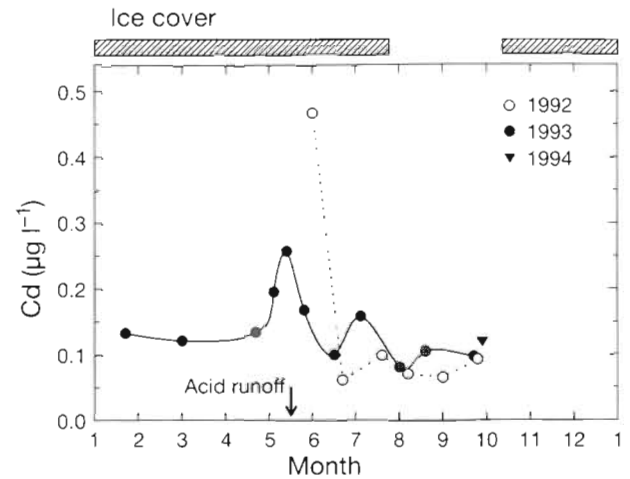


Fig. 3. Seasonal pattern of cadmium concentrations (0.45 µm filtered) in Schwarzsee. Data represent volume-weighted means

is typical of oligotrophic high mountain lakes. In acidic lakes (SOS and MPL), however, fish growth was significantly higher and condition better due to the failure of reproduction during the last few years which resulted in a decline of population densities (authors' unpubl. results). In DRS, the population density of Arctic char is controlled by piscivorous *Salvelinus namaykush*. In consequence, in DRS growth rate, but not condition factor, of Arctic char was higher than in OPL. Whereas condition factors of the commercially used Arctic char from ACH and GRS were similar to those of the acidic lakes, growth rates were higher.

In winter, stomach filling rate in Arctic char from SOS is significantly lower ($p < 0.05$) than that during ice break and summer (Fig. 4), when emerging aquatic and terrestrial insects (allochthonous diet) represent the main portion of the diet (authors' unpubl. data). A similar seasonal pattern can be demonstrated with the liver-somatic index (relative liver weight in % body weight) for both sexes being significantly increased ($p < 0.05$) during early summer.

Table 2. *Salvelinus alpinus*. Morphometric characterization of fish used for histological analysis of the liver. SOS: Schwarzsee; MPL: Mittlerer Plenderlesee; OPL: Oberer Plenderlesee; DRS: Drachensee; ACH: Achensee; GRS: Grundlsee

	SOS	MPL	OPL	DRS	ACH	GRS
Body length (<i>L</i> ; cm)	23.7–2.1	25.3–3.1	13.8–1.8	21.1–1.7	27.2–7.6	30.6–3.2
Body weight (<i>W</i> ; g)	107.5–28.2	132.9–49.2	18.0–7.3	62.9–14.5	210.0–167.0	249.7–65.9
Condition factor ($100 \times W/L^3$)	0.81–0.1	0.82–0.09	0.68–0.06	0.67–0.07	0.81–0.09	0.85–0.06
Age (yr)	5–16	4–15	3–9	4–8	4–9	4–6
Number of fish (n)	87	16	75	16	41	19
Reproduction	No	No	Yes	Yes	Yes	Yes

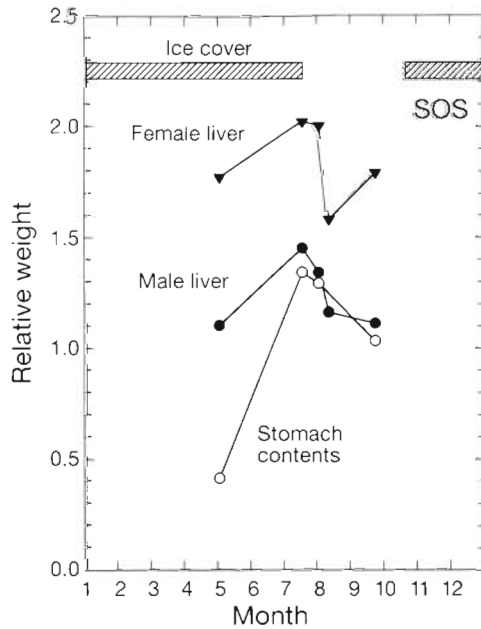


Fig. 4. *Salvelinus alpinus*. Relative weights of stomach contents and livers of male and female Arctic char from Schwarzsee. Data recorded from winter fish are significantly different from those in fish caught during and immediately after ice break. Data presented as means

Light microscopy

Glycogen accumulation in hepatocellular nuclei as indicated by positive PAS reaction was a characteristic feature of the liver of Arctic char in the 2 acidic lakes (SOS and MPL; Figs. 5 & 6). In SOS, the relative

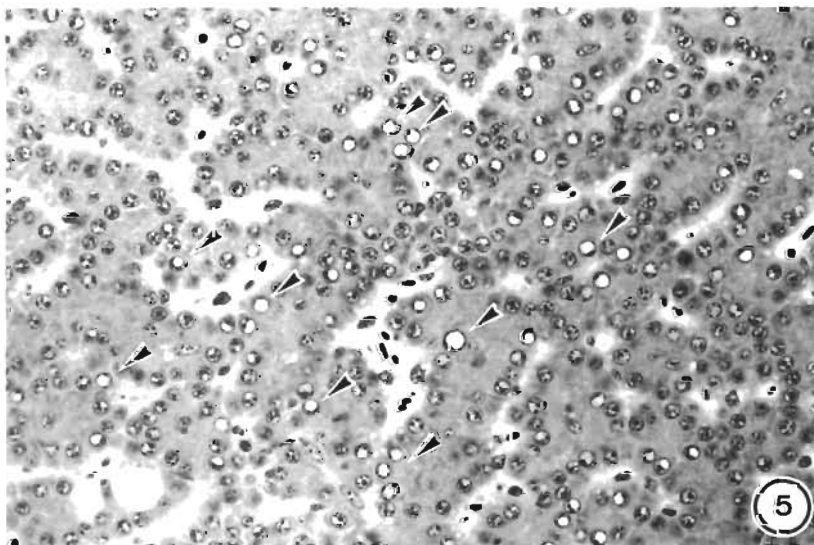


Fig. 5. *Salvelinus alpinus*. Hepatocyte nuclei with PAS-positive (glycogen) vacuoles (arrowheads) of Arctic char from Schwarzsee; $\times 400$

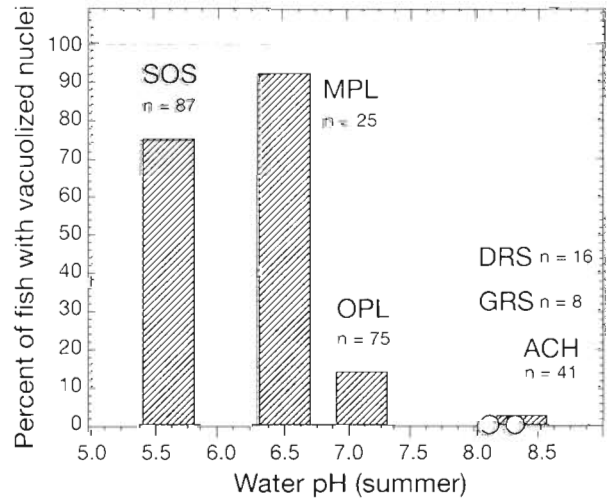


Fig. 6. *Salvelinus alpinus*. Percentage of Arctic char with vacuolized hepatocyte nuclei in 6 alpine lakes of different water pH (for means of water pH in summer, see Table 1). SOS: Schwarzsee; MPL: Mittlerer Plenderlesee; OPL: Oberer Plenderlesee; DRS: Drachensee; ACH: Achensee; GRS: Grundlsee

number of fish with nuclear vacuolization rates of more than 1% was almost constant throughout the year (58 to 87%) and did not correlate with age or sex. The degree of vacuolization, however, showed a distinct seasonal pattern (Fig. 7): Whereas occurrence was low during autumn and winter, there was a significant peak ($p < 0.05$) immediately after the ice break. In fish of the circumneutral OPL, however, only a few specimens (14% of the total population) displayed a relatively small number of vacuolized nuclei (<4%; Fig. 6). This alteration was absent in Arctic char from an alkaline high mountain lake (DRS) and 2 alkaline lakes at low altitudes (except in 1 out of 41 specimens from ACH).

Electron microscopy

The present communication is focused on variations in ultrastructure and glycogen storage of hepatocyte nuclei, since only these could be unambiguously correlated to ecological parameters, whereas differences in cytoplasmic appearance could also be attributed to different sex and age. A semiquantitative comparison of differences in the ultrastructural appearance of Arctic char hepatocytes be-

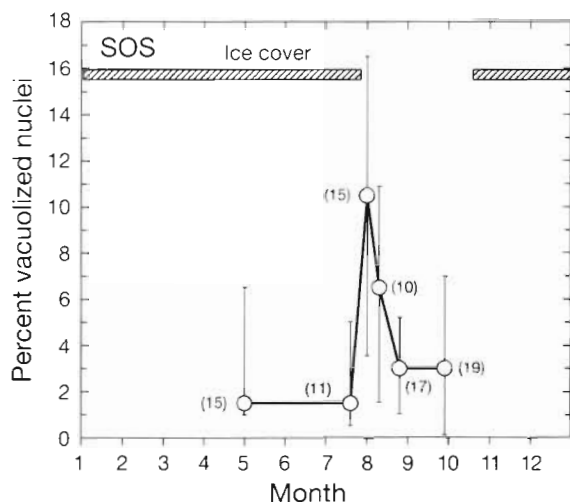


Fig. 7. *Salvelinus alpinus*. Seasonal pattern of vacuolization rate of hepatocellular nuclei in Arctic char from Schwarzsee. Medians, 25 and 75% percentiles as well as number of fish investigated (parentheses) are given

tween fish from Grundlsee (GRS), Schwarzsee (SOS) and Oberer Plenderlesee (OPL) is given in Table 3.

Arctic char from Grundlsee (GRS). Arctic char from GRS displayed little sexual dimorphism. Parenchymal

Table 3. *Salvelinus alpinus*. Semiquantitative evaluation of cytological characteristics of liver and hepatocyte organization in Arctic char from Grundlsee (GRS), Schwarzsee (SOS) and Oberer Plenderlesee (OPS). Data are given as means from inspection of at least 4 sections of 6 individuals from each lake. - = absent; (+) = only faintly developed; + = weakly developed; ++ = moderately developed; +++ = strongly developed; ++++ = very strongly developed. ER: endoplasmic reticulum

	GRS July	SOS July	SOS Sept	OPL June
Nucleus				
Amount of heterochromatin	(+)	++	++	+++
Irregular outline	-	-	-	(+)
Dilation of nuclear envelope	-	+	+	(+)
Nuclear glycogen inclusions	-	+++	+++	-
Nuclear lipid inclusions	(+)	-	-	-
Finely granular inclusions	-	(+)	(+)	-
Induction of binucleate cells	-	(+)	-	-
Nucleolus				
Size of nucleolus	++	+++	+++	+++
Electron density of nucleolus	+	+++	+++	+++
Honeycomb-like structure of nucleolus	-	+++	++	-
Induction of multiple nucleoli	-	++	-	-
Lipid inclusions				
Amount of lipid droplets	+++	++	++	+
Perisinusoidal accumulation of lipid	(+)	+	-	(+)
Irregular outline of lipid droplets	(+)	++	(+)	-
Glycogen				
Overall amount	++++	+	(+)	+
Association with ER	+	-	(+)	(+)
Formation of glycogenosomes	-	+	+	-

heterogeneity was low, and livers were free of necrotic areas. Hepatocytes were polygonal and showed a distinct cytoplasmic compartmentation (cf. Braunbeck et al. 1987) into an organelle-rich perinuclear region containing rough endoplasmic reticulum (RER), mitochondria and peroxisomes and a peripheral zone enriched in storage products such as glycogen and lipid (Fig. 8).

Centrally located, the single nucleus was almost spherical with a smooth outline. Each nucleus contained a slightly eccentric nucleolus with the *pars fibrosa* arranged in discrete patches of slightly higher electron density than the *pars granulosa*. Both nuclear and nucleolar diameters as well as the degree of separation of nucleolar *pars fibrosa* and *pars granulosa* were similar in males and females. Heterochromatin was sparse and restricted to the nuclear periphery and to very few randomly distributed patches of different size (Fig. 8). Binucleated hepatocytes and nuclear lipid inclusions were extremely rare.

Peripheral storage areas were occupied by small fields of glycogen rosettes (Fig. 8), which were occasionally associated with irregular profiles of smooth endoplasmic reticulum (SER), and lipid droplets. Glycogen contents were slightly lower in females versus males. The amount of lipid varied especially between individual male fish.

Arctic char from Schwarzsee (SOS). If compared to GRS char, parenchymal heterogeneity was conspicuously higher with respect to variations between both different individuals and within one specimen. Whereas cytoplasmic compartmentation was at least partly preserved in some SOS individuals (Fig. 9), hepatocytes of other SOS char completely lacked any separation of organelle-containing cytoplasm and storage areas. Independent of sex, about 50% of the SOS fish displayed extracellular spaces distended to variable degrees (Fig. 9).

Whereas light microscopically detectable PAS-positive areas within nuclei (Fig. 5) could be identified even at low electron microscopic magnifications as dense fields of monoparticulate glycogen (Fig. 9), the overall amount of cytoplasmic glycogen was slightly lower than in GRS fish (Fig. 8). Development of nuclear glycogen deposits from cytoplasmic invaginations into the nucleoplasm could be excluded, since glycogen areas were never enclosed within duplicate membranes (Figs. 8 to 10). Primarily due to glycogen reduction, but also due to comparatively low amounts of endoplasmic reticulum,

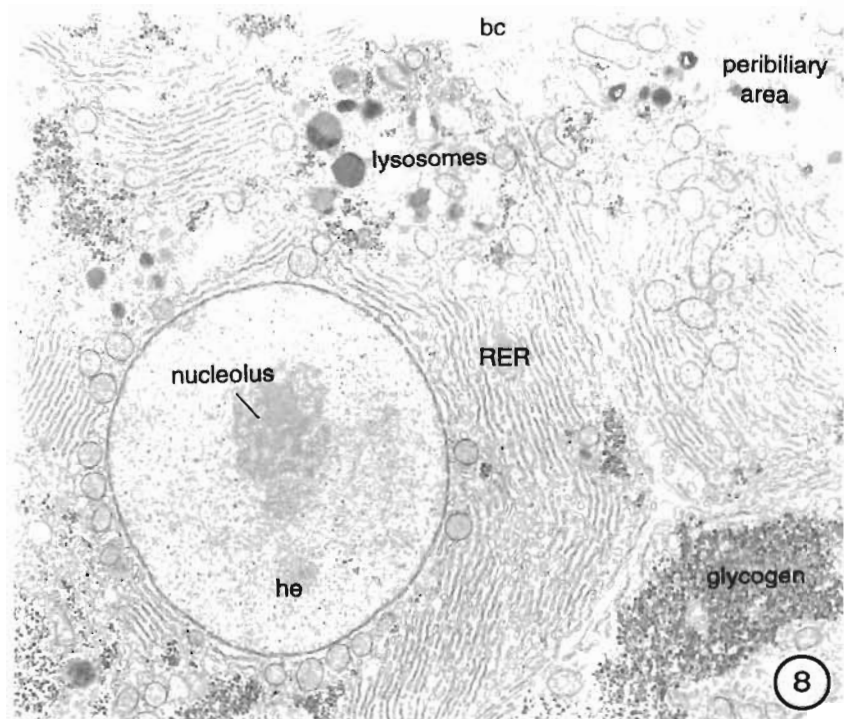


Fig. 8. *Salvelinus alpinus*. Hepatocytes of Arctic char from Grundlsee were polygonal and showed a distinct cytoplasmic compartmentation into an organelle-rich perinuclear region containing rough endoplasmic reticulum (RER), mitochondria and peroxisomes and a peripheral zone enriched in storage products such as glycogen and lipid. In the biliary area facing bile canaliculi (bc), the regular stacking of RER cisternae was regularly interrupted by Golgi fields and lysosomes. he: hepatocellular nucleus. Female; $\times 5900$

hepatocellular size was conspicuously reduced in SOS char.

Glycogen deposits in nuclei of SOS char mainly consisted of monoparticulate β -particles, the mean size of which (ca 40 nm) did not differ from that of single components of cytoplasmic α -glycogen rosettes (30 to 50 nm; Figs. 10 & 11). Due to their increased electron density, glycogen particles could easily be distinguished from granular components of heterochromatin (Fig. 11). In addition to fields of α -glycogen, nuclei of SOS char also displayed closely defined areas with fine granular inclusions with a mean diameter of 20 to 25 nm (Figs. 10 & 12) resembling interchromatin granules (Bouteille et al. 1974). Neither glycogen nor fine granular deposits showed any preferential localization within nuclei. Whereas nuclear size did not differ from GRS char, the number of binucleate cells was greater compared to SOS char.

Depending on the plane of section, hepatocellular nucleoli of 5 out of 6 SOS char sampled in July displayed a zebra- or honeycomb-like arrangement of nucleolar components (Figs. 13 & 14). The diameter of the spherical electron-lucent areas in cross-sections averaged 100 nm and regularly displayed a 50 to 55 nm filamentous core of medium electron density most likely representing chromatin clumps (Fig. 13, present study; cf. Bernhard & Granboulan 1968). In addition, in SOS char caught in July there was an elevated number of hepatocytes with multiple nucleoli.

Glycogen depletion in SOS char was accompanied by a decline in hepatocellular lipid deposits (compare Figs. 8 & 9). Increased levels of lipidolysis was also indicated by a considerably more heterogeneous outline of lipid droplets (Fig. 9) and a most intimate association between highly elongate mitochondria and lipid droplets.

Arctic char sampled in Oberer Plenderlesee (OPL). With respect to hepatocellular ultrastructure, Arctic char from the circumneutral Oberer Plenderlesee (OPL) took an intermediate position between GRS and SOS fish. Parenchymal heterogeneity was as low as in GRS char; cytoplasmic compartmentation into organelle-containing areas and glycogen fields, however, was not evident due to almost complete glycogen exhaustion. Except for a slight distension of the nuclear envelopes and mild glycogen storage within the nucleus of one individual, nuclei as well as nucleoli were free of pathological symptoms.

DISCUSSION

The present study clearly documents profound multiple modifications of structure and ultrastructure in hepatocyte nuclei of Arctic char from acidic lakes. Without doubt, the most conspicuous and unusual alteration is the accumulation of glycogen in the nucleoplasm. Since nuclear glycogen was consistently monoparticulate and never enclosed within separate

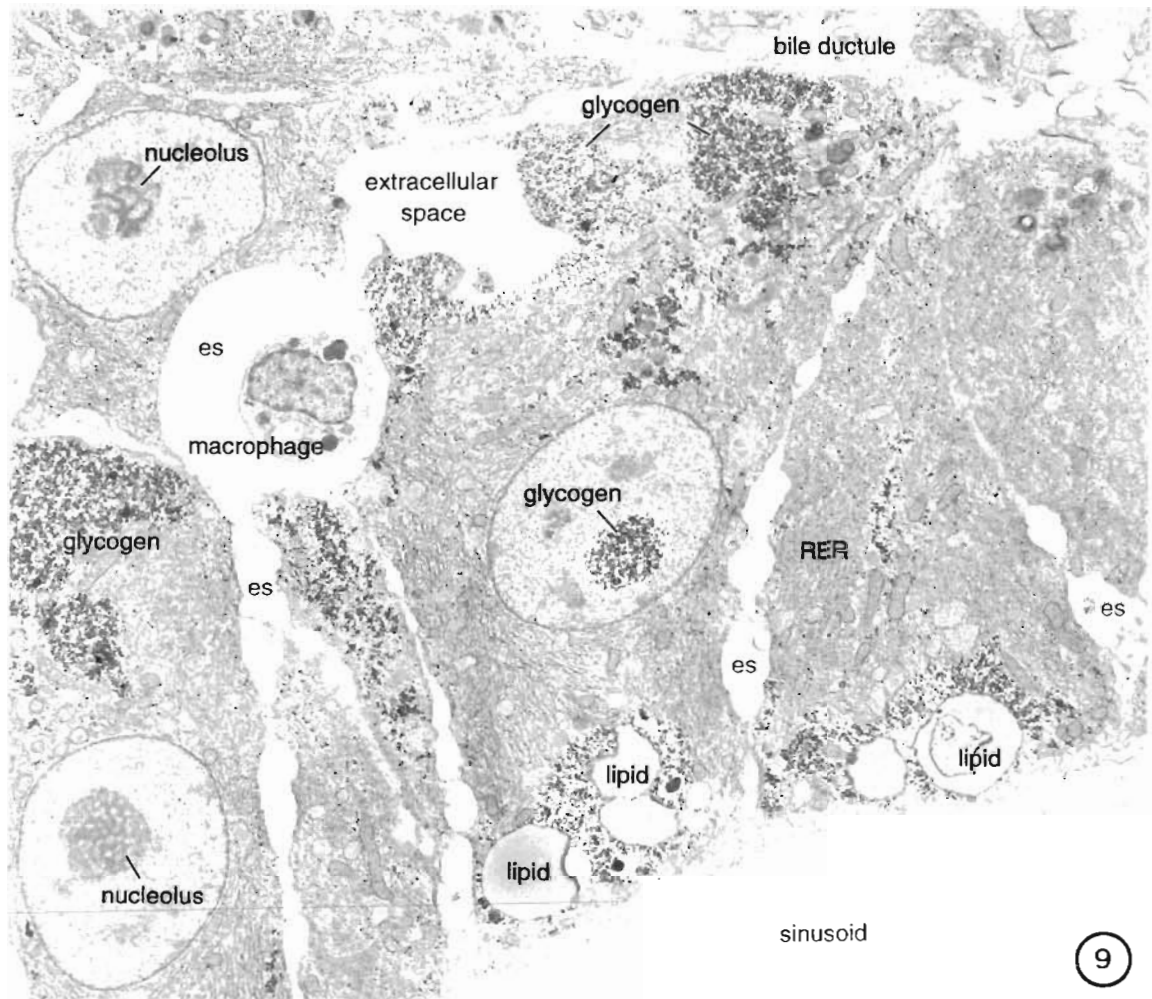
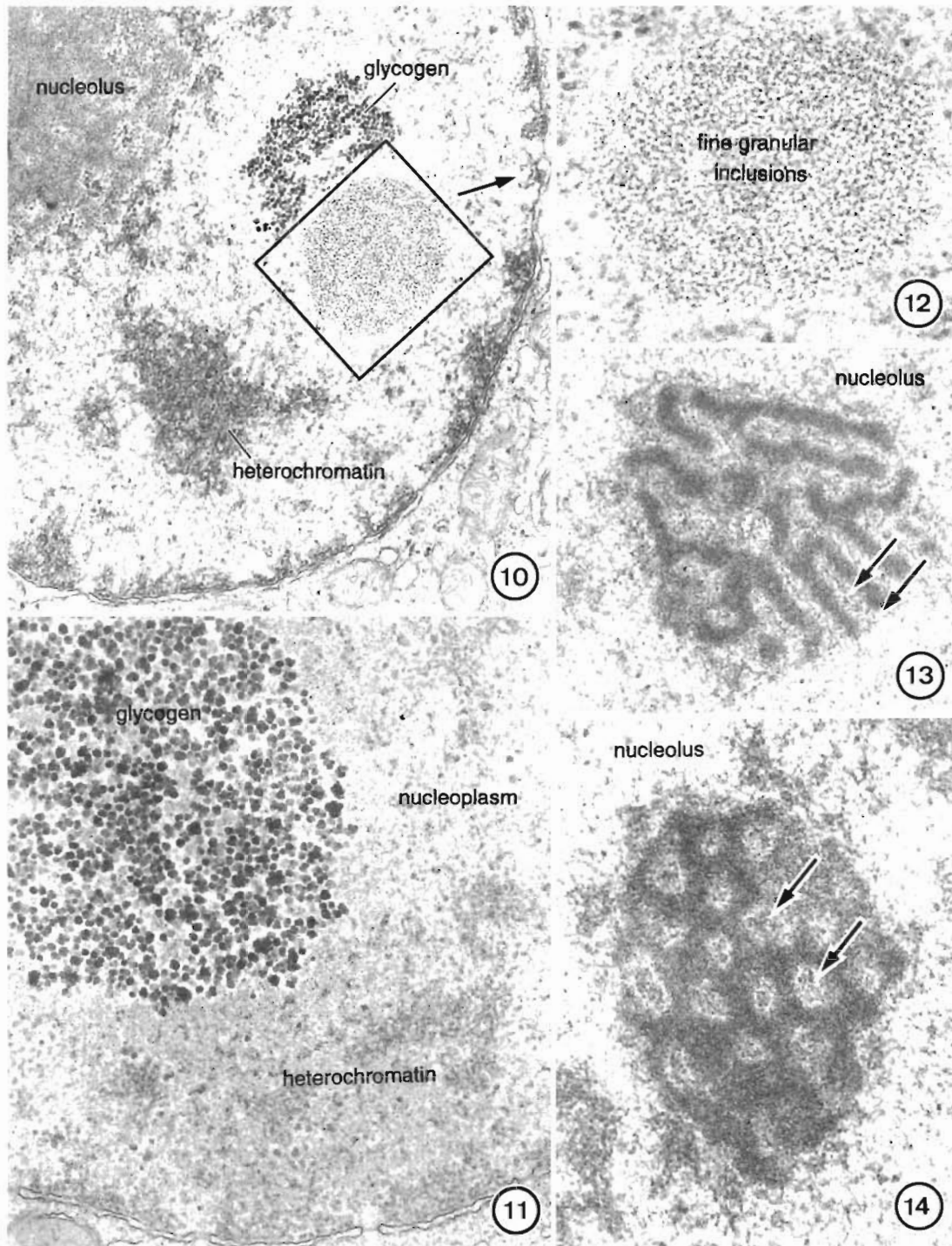


Fig 9 *Salvelinus alpinus* In Arctic char sampled in Schwarzsee in July, hepatocytes displayed conspicuous glycogen deposits as well as nucleoli with a typical honeycomb-like appearance. The cytoplasmic compartmentation into an organelle-containing areas and peripheral zones with storage products (glycogen, lipid) was inferior to that of Grundlsee char (cf. Fig. 8). The overall amount of cisternae of the rough endoplasmic reticulum (RER) was drastically reduced, if compared to Grundlsee char. Cytoplasmic glycogen depletion in Schwarzsee Arctic char was accompanied by a decline in hepatocellular lipid deposits. Extracellular spaces (es) between hepatocytes were severely distended and frequently contained invading macrophages. Female; $\times 3800$

membranes, the possible origin from cytoplasmic indentations, including glycogen rosettes (α -glycogen), into nuclei may be excluded. In contrast, Thiagarajah & Grizzle (1986) described α -glycogen in addition to predominant β -particles in hepatocyte nuclei of *Rivulus marmoratus*. Since in *R. marmoratus* glycogen has also been found within nuclear pores, Thiagarajah & Grizzle concluded that at least parts of glycogen might be imported into nuclei from the cytoplasm. As shown by Karasaki (1971), massive accumulation of glycogen can rapidly occur in nuclei of human hepatoma cells without the occurrence of similar deposits in the cytoplasm at periods of active DNA synthesis, provided the nuclear envelope is intact. In fact, during the period of ice break, the metabolism of Arctic char from high mountain lakes is highly activated, which most likely

also includes accelerated DNA synthesis. After a period of 9 mo with reduced feeding, ice break dramatically changes light intensity, temperature and food supply. However, nuclear vacuolization has also been observed in winter after spawning, when growth and metabolism are generally reduced. Moreover, the Arctic char population in OPL is exposed to identical seasonal changes without displaying the distinct pattern of nuclear vacuolization typical of SOS and MPL char.

On the other hand, the sudden rise in food consumption (approximately 5-fold increased relative stomach contents obtained for OPL fish; authors' unpubl. data) may result in an additional burden (due to catch of prey, active food uptake, enzyme production, etc.) for the fish from acidic lakes. Distinct ultrastructural changes of hepatocytes could also be shown for the



Figs. 10 to 14. *Salvelinus alpinus* Ultrastructural alterations in nuclei of Arctic char sampled in Schwarzsee comprised deposition of monoparticulate (β -)glycogen within the nucleoplasm (average diameter 40 nm. Figs. 10 & 11) as well as formation of fine granular deposits with a mean diameter of 15 to 20 nm (Fig. 10, box; Fig. 12). Depending on the plane of section, nucleoli showed a zebra- (Fig. 13) or honeycomb-like (Fig. 14) arrangement of nucleolar components with electron-lucent areas of 100 nm regularly displaying a 50 to 55 nm filament-like core of medium electron density (Figs. 13 & 14; arrows) between areas of increased electron density. Males sampled in July (Figs. 11 to 14) or September (Fig. 10). Fig. 10, $\times 23\,800$; Fig. 11, $\times 33\,300$; Fig. 12, $\times 45\,000$; Fig. 13, $\times 25\,700$; Fig. 14, $\times 30\,800$

resumption of active nutrition in early spring in field populations of golden ide (*Leuciscus idus melanotus*; Segner & Braunbeck 1990).

In the acidic lakes only (SOS, MPL), the majority of fish displayed distinct nuclear glycogen accumulation. In Arctic char of the circumneutral lake (OPL), however, nuclear alterations have only been seen occasionally and were even absent (with the exception of 1 individual) in fish from alkaline waters including those from a high altitude lake (DRS). Since environmental conditions except alkalinity-related parameters such as pH and calcium concentration of the water and, as a consequence, the increased accumulation of lead and cadmium in fish organs (Köck et al. 1995) are similar in the 4 high altitude lakes, one of these parameters or probably a combination of parameters may be made responsible for nuclear alterations. Aluminum, however, can be excluded as the primary inducer, since it reaches only slightly toxic concentrations in SOS, but not in MPL (Table 1). Furthermore, annual cycles of pH or cadmium concentration in SOS did not directly correlate with the seasonal pattern of the frequency of vacuolized nuclei.

Besides nuclear glycogen accumulation, livers of Arctic char from SOS and partly from MPL displayed a multitude of further pathological effects, which might at least partly be attributed to the acidic conditions of the lakes. The extraordinarily high accumulation of cadmium and lead in kidneys and livers of Arctic char (Köck et al. 1995), as well as the highly disturbed reproduction of these fishes, support this assumption. Furthermore, in SOS char muscle, concentrations of some organic pesticides (p'p-DDE, PCBs) are higher than in those of comparable remote European lakes (J. O. Grimalt pers. comm.).

Since hepatocytes are involved in exogenous vitellogenesis (Van Bohemen et al. 1981, 1982), impaired liver metabolism may well account for delayed or reduced spawning of fish in acidic lakes (Beamish et al. 1975, McCormik et al. 1989). In fact, Arctic char of OPL reproduce in October; the population of SOS, however, does not spawn before December. During the past few years, in fish populations from SOS and MPL, the absence of young individuals indicated complete failure of reproduction (authors' unpubl. results). While population size declined, nutritional status and growth of remaining fish improved. Probably due to reduced intraspecific competition, the maximum age of char is high (up to 16 yr). This, in turn, resulted in the high incidence of pathological livers predominantly seen in specimens older than 10 yr. Nuclear glycogen, however, was independent of age and also common in 5 yr old fish.

In natural or cultured populations of fish, glycogen accumulation in hepatocyte nuclei is generally un-

known except in studies on *Micropterus salmoides* (Hibiya 1982) and *Rivulus marmoratus* (Thiyagarajah & Grizzle 1985, 1986). However, none of these authors provided a useful interpretation of this phenomenon. In contrast, in mammalian pathology, nuclear glycogen accumulation has been described in association with a multitude of diseases including primary or secondary diabetes mellitus, type I glycogen storage disease, Wilson's, Grave's and Hodgkin's diseases, Lupus erythematosus, stomach carcinoma, hepatoma, infective hepatitis, total lipodystrophy, as well as Gilbert's and Rotor syndrome (for reviews, see Arias et al. 1988 as well as Phillips et al. 1987). In addition, with markedly different ultrastructural appearance, hepatocellular nuclear glycogenosis was found after administration of drugs such as amidodarone, cimetidine, cyanamid or methotrexate.

In mammalian liver, however, nuclear glycogen storage caused by metabolic diseases is usually associated with other specific pathological conditions such as steatosis or cirrhosis (Petersen & Christoffersen 1979, Phillips et al. 1987), which have not been observed in Arctic char. In fact, other symptoms of liver damage in Arctic char from SOS and MPL did not correlate with nuclear glycogen formation. As a consequence, nuclear vacuolization may be supposed to be an indication of an exceptional metabolic status induced by the specific water chemistry of acidic soft water lakes at high altitudes. Although this alteration might be pathological, there is no convincing evidence for a specific metabolic disease such as those described for mammals and man.

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