

III. Wissenschaftliches Programm Scientific Program

1. Ökomorphologische und ökophysiologische Anpassungen an den alpinen Raum Ecomorphological and ecophysiological adaptations to the alpine environment

**Adaptations to winter hypoxia in a shallow alpine lake.
Ecophysiological energetics of *Cyclops abyssorum* and rainbow
trout***

**Anpassungen an Winter-Hypoxie in einem kleinen alpinen See.
Ökophysiologische Energetik von *Cyclops abyssorum* und Regenbogen-
forellen***

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Summary

Aquatic hypoxia occurs naturally during winter in shallow alpine lakes at altitudes near the timber line. It is by far more severe than hypobaric hypoxia at extreme altitude. The cyclopoid copepod *Cyclops abyssorum* aggregated in the vertical oxygen gradient in the range of 0.5 to 2.5 kPa oxygen pressure during winter in lake Kalbelee (1650 m, Hochtannberg, Vorarlberg). Respiratory oxygen flux was a hyperbolic function of oxygen pressure, with a half maximal flux at 0.5 kPa and a critical oxygen pressure, p_c , of 1.6 kPa at which flux is 75% of maximum. By a combination of Twin-Flow respirometry and calorimetry it was shown that fluctuations of locomotor activity are maintained fully aerobic under hypoxia at <10% air saturation. The respiratory adaptation of *C. abyssorum* to hypoxia and low temperature ($Q_{10} = 2.3$), and the abundance of food near the anoxic boundary layer provide a basis for body

* Dedicated to Univ.-Prof. Dr. Roland Pechlaner at his 60th birthday

and reproductive growth, and the completion of a hypoxic winter generation. During spring stratification developing immediately after icebreak, the full range of anoxic and hyperoxic conditions was encountered up to 300% air saturation (46 kPa_{O₂}), indicating an active oxygen detoxification system even after months of hypoxic acclimation.

Complete winter kill of rainbow trout (*Oncorhynchus mykiss*) occurred and active *Daphnia* disappeared when maximum oxygen in the water column dropped to <3 kPa (ca. 60 μmol O₂ · dm⁻³). Positive growth and no mortality of rainbow trout were observed during less severely hypoxic winters at an average temperature of 1.7 °C. The fish behaved as a *diving trout*, periodically diving into depths of very low oxygen for scavenging food, as evidenced by stomach analyses.

Hypoxia in the environment and the cell

During half of the biological evolution, «normal» concentrations of molecular oxygen were less or much less than 10⁻³ the present atmospheric level, or <0.02 kPa (Kasting 1993), despite the occurrence of photoautotrophic prokaryotes as early as c. 3.5 billion years ago (Schopf 1993). When mitochondria associated with eukaryotic cells around 1.4 billion years ago (Margulis 1970, Schwartz and Dayhoff 1978), atmospheric O₂ pressure may still have been in this low range. The metazoan record starts when a rise in molecular oxygen in the earth's atmosphere is ascertained. However, the earliest benthic metazoan animals may have lived in an anoxic environment rich in H₂S (Giere 1992), although the energetic significance of very low levels of available oxygen (Gnaiger 1991, 1992) may have played a critical role in this period of evolution and mitochondrial function. Low oxygen continues to be important in many environments with extreme changes of oxygen in space and time (Boutilier 1990). Today, normoxia at sea level (*p*_o = 1 atmosphere = 101.3 kPa barometric pressure) corresponds to an oxygen pressure in water vapor saturated air of 19.9 kPa (at 37 °C) or 2.1 kPa (at 0 °C). The volume fraction of oxygen in dry air is 0.20946 and remains constant with altitude. Barometric pressure, *p*_b [kPa], however, changes with altitude above sea level, *h* [km], according to a power function (Forstner and Gnaiger 1983),

$$p_b = p_o (1 - h/44.3)^{5.25} \quad (1)$$

Concomitantly, the partial oxygen pressure, *p*_{O₂}, declines (Fig. 1A). Physiological performance of unacclimated humans may become impaired by hypoxia above 3000 m, corresponding to an O₂ pressure in the lungs of <13 kPa in air saturated with water vapor at 37 °C (Fig. 1A). No permanent human habitation is physiologically possible at altitudes above which oxygen is reduced to <50% of the concentration at sea level (Fig. 1A). Adaptations to hypobaric hypoxia in man are not genetically or developmentally fixed but are acquired (Hochachka 1993; Cerretelli 1993). The main feature is the avoidance of anaerobic lactate production and full exploitation of aerobic energy metabolism at maximum ATP stoichiometry. Finally, short-term survival in acclimated humans is limited to *p*_{O₂} levels of 25% of the oxygen pressure at sea level, at the risk of brain damage (*p*_{O₂} is 5.3 kPa at 8848 m; Fig. 1A).

Reduction of atmospheric oxygen pressure at extreme altitude is relatively mild when compared to severely hypoxic conditions in aquatic habitats (Fig. 1B and 1C). Moreover, oxygen concentrations in water at air saturation are less than 5% of the oxygen concentration in air. Therefore, it may be expected that adaptations to aquatic hypoxia in the alpine region are in general not different from those in lowland ponds and marine sediments, with environmental oxygen gradients spanning six orders of magnitude from the km (10³ m) to the mm range (10⁻³ m; Fig. 1A to 1C). Less is known about the oxygen gradients in the μm range (10⁻⁶ m) within cells towards the mitochondria, but the bioenergetic implications of the very low oxygen levels prevailing in the mitochondrial micro-environment may be profound (Gnaiger et al 1993). One of the most conspicuous adaptations described here for the planktonic copepod *Cyclops abyssorum* is the maintenance of fully aerobic functions under severe winter hypoxia.

The critical pO_2 is 1.6 kPa (see below), which may be compared to the critical pO_2 of 0.1 to 0.16 kPa in mitochondria isolated from rat liver (Gnaiger et al 1993) or cardiac myocytes (Rumsey et al 1990).

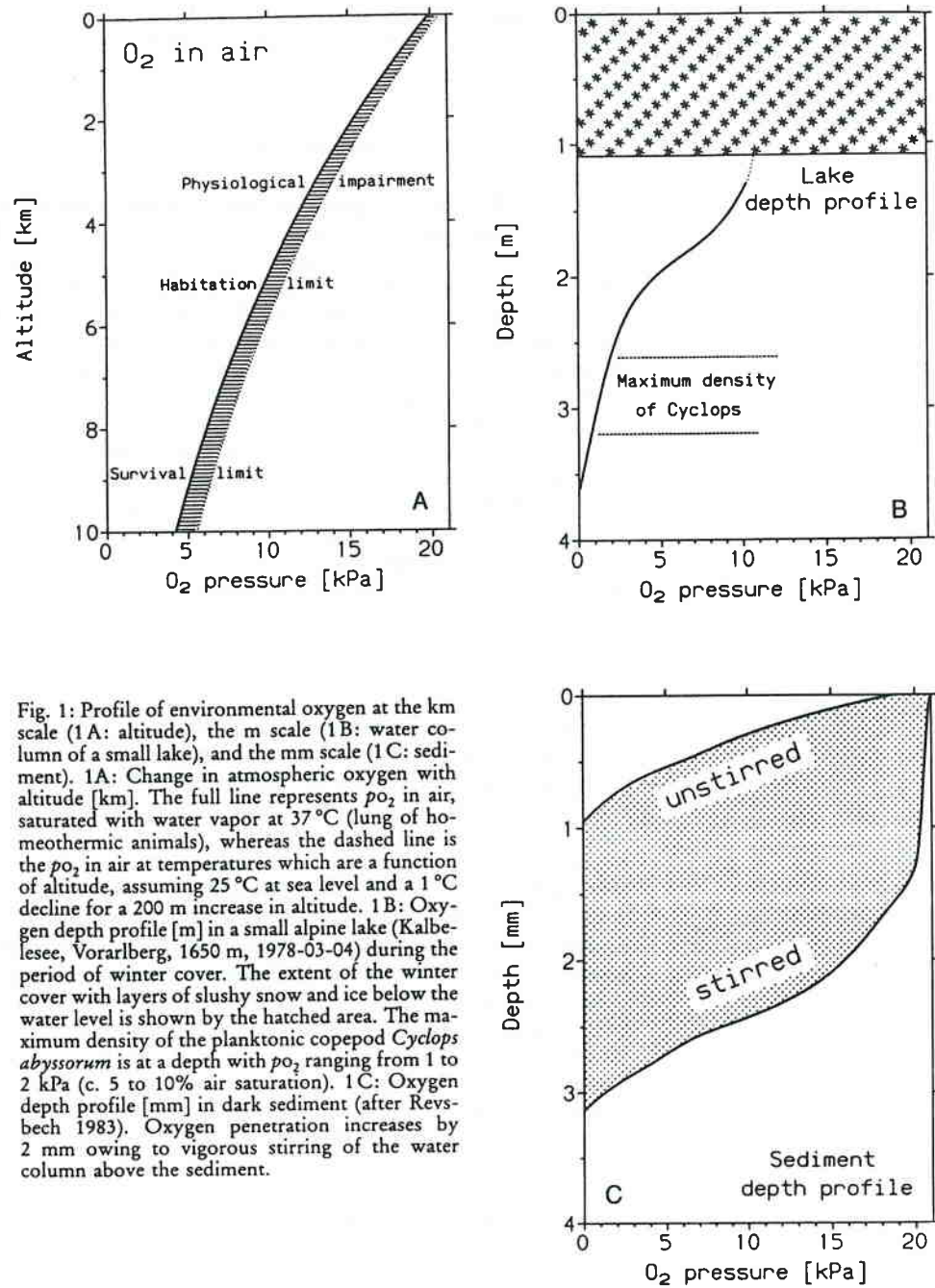


Fig. 1: Profile of environmental oxygen at the km scale (1A: altitude), the m scale (1B: water column of a small lake), and the mm scale (1C: sediment). 1A: Change in atmospheric oxygen with altitude [km]. The full line represents pO_2 in air, saturated with water vapor at 37°C (lung of homeothermic animals), whereas the dashed line is the pO_2 in air at temperatures which are a function of altitude, assuming 25°C at sea level and a 1°C decline for a 200 m increase in altitude. 1B: Oxygen depth profile [m] in a small alpine lake (Kalbelesee, Vorarlberg, 1650 m, 1978-03-04) during the period of winter cover. The extent of the winter cover with layers of slushy snow and ice below the water level is shown by the hatched area. The maximum density of the planktonic copepod *Cyclops abyssorum* is at a depth with pO_2 ranging from 1 to 2 kPa (c. 5 to 10% air saturation). 1C: Oxygen depth profile [mm] in dark sediment (after Revsbech 1983). Oxygen penetration increases by 2 mm owing to vigorous stirring of the water column above the sediment.

Winter anoxia in a small alpine lake

Hypoxic and anoxic conditions are common during summer stagnation in eutrophic ponds and lakes. Temperature stratification prevents vertical mixing and hence gas exchange below the thermocline (Finlay et al 1983). With increasing altitude of alpine lakes (alpine used in the sense of alpine region), winter stagnation is the more likely cause of hypoxia owing to the prolonged duration of the winter season and the massive winter cover which may convert the lake into a nearly isolated system (Fig. 1B; Greenbank, 1945; Gliwicz 1967; Pennak 1968; Schindler and Comita 1972; Ruttner-Kolisko 1975; Nagell and Brittain 1977). Exclusion of gas exchange between lake and atmosphere is practically complete. Nevertheless, 0.05 to 0.01% of the visible light penetrates through a winter cover of 1.5 m in high-alpine lakes (Pechlaner 1966, 1971). The low residual photosynthetic activity cannot compensate for the biological oxygen demand in the lake. However, prevalence of ultra-oligotrophic conditions in high-alpine lakes (above timberline) prevents large accumulation of degradable organic material and thus limits oxygen demand even during extended periods of winter enclosure in shallow lakes and ponds (Pechlaner 1966; Pechlaner et al 1972). Winter anoxia in lakes of the alpine region is intensified at moderate altitude near the timber line where winter cover is severe but organic production in the lake, and nutrient influx from the drainage area are not reduced to the high-alpine scarcity. Moreover, winter-anoxia is limited to shallow water bodies, illustrated by a comparison of two adjacent lakes with maximum depths of 4 and 7 m (Fig. 2).

Field and laboratory studies were carried out at lake Kalbelesee, a shallow productive lake encountering hypoxic and anoxic conditions during winter. The lake originated probably as a glacial ice-scour lake, situated at an altitude of 1650 m above sea level near the villages of Schröcken, Warth and Lech (Hochtannberg, Vorarlberg, Austria; Table 1). After deforestation in the 15th century, the drainage area of c. 1.5 km² consists of alpine meadows with a geologically predominant influence of calcareous sediments. The lake is separated into two basins

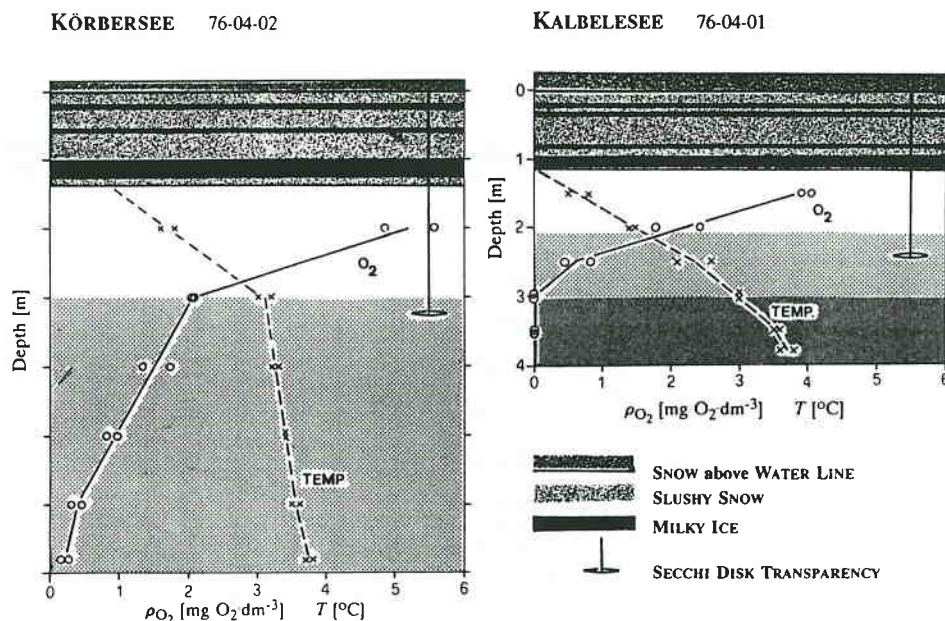


Fig. 2: Winter-hypoxia and anoxia in two adjacent lakes (Hochtannberg, Vorarlberg, Austria) of different maximum depth. Open circles: mass concentration of oxygen, ρ_{O_2} [mg · dm⁻³], determined by chemical Winkler analysis (Gnaiger 1983 a). Crosses: temperature measured in the 5 dm³ Schindler water sampler. The hatched area of the water column indicates the extent of the hypoxic zone at $\rho_{O_2} < 3$ kPa (63 μ mol O_2 · dm⁻³; 2 mg O_2 · dm⁻³). The anoxic zone is dark shaded. 1 mg O_2 · dm⁻³ = 31.215 μ mol · dm⁻³.

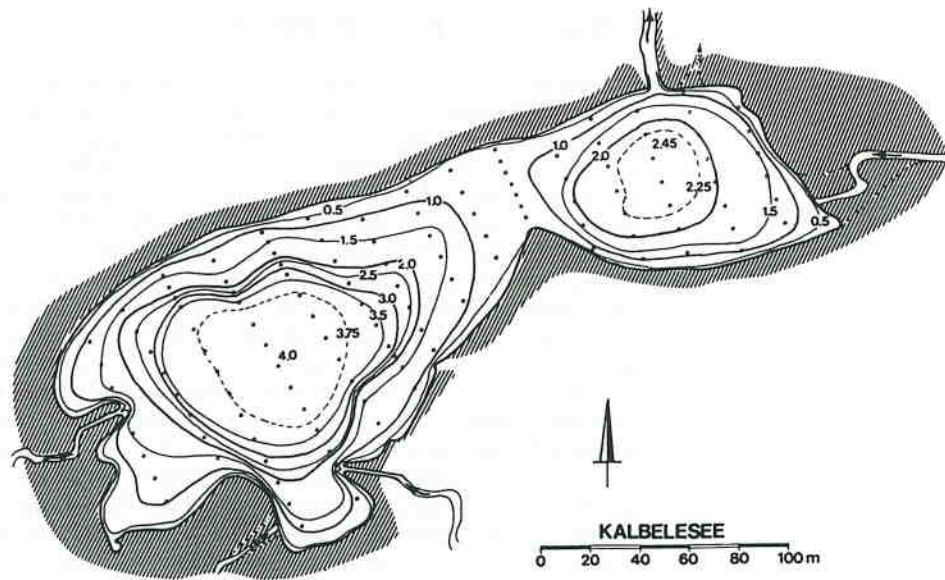


Fig. 3: Contour map of Kalbelesee with standard depths (numbers [m]). At the time of the record (August 1971), the water level was +0.3 m above standard zero, corresponding to a total maximum depth of 4.3 m. 155 depth soundings were taken (circles) in 15 horizontal profiles. The shore line within the *Carex* belt (shaded area) is at a standard water depth of 0.34 m.

Table 1. Morphometric parameters for the Western and Eastern basin of lake Kalbelesee (1650 m above sea level, latitude 47°16' N, longitude 10°08' E). After Gnaiger 1983 a and unpublished.

Parameter	Symbol	Western basin	Eastern basin
Maximum depth ^a	z_{\max}	4.0 m	2.45 m
Mean depth	$\bar{z} = V A^{-1}$	2.02 m	1.54 m
Relative depth	$z_r = 50 z_{\max} \sqrt{\pi} (\sqrt{A})^{-1}$	2.68%	2.63%
Length	l	210 m	130 m
Maximum breadth	b	130 m	70 m
Area ^b	A	$17.5 \cdot 10^3 \text{ m}^2$	$6.8 \cdot 10^3 \text{ m}^2$
Area at 1 m depth	A_1	$13.0 \cdot 10^3 \text{ m}^2$	$5.0 \cdot 10^3 \text{ m}^2$
Area at 2 m depth	A_2	$7.4 \cdot 10^3 \text{ m}^2$	$1.0 \cdot 10^3 \text{ m}^2$
Area at 3 m depth	A_3	$5.0 \cdot 10^3 \text{ m}^2$	
Volume ^a	V	$35.3 \cdot 10^3 \text{ m}^3$	$10.5 \cdot 10^3 \text{ m}^3$
Volume below 1 m	V_1	$19.0 \cdot 10^3 \text{ m}^3$	$4.3 \cdot 10^3 \text{ m}^3$
Volume below 2 m	V_2	$9.2 \cdot 10^3 \text{ m}^3$	$0.5 \cdot 10^3 \text{ m}^3$
Volume below 3 m	V_3	$3.2 \cdot 10^3 \text{ m}^3$	
Shore line ^b	L	660 m	340 m
Development of shore line	$D_L = L(2\sqrt{\pi A})^{-1}$	1.41 m^{-1}	1.16 m^{-1}

a All values are for a *standard* depth of 4.0 m (Fig. 3). The maximum *total* depth changes from 3.7 to 4.8 m during the year. For explanation of morphometric parameters see Hutchinson (1957).

b The shoreline is dominantly marked by *Carex* at a standard water depth of 0.34 ± 0.11 m when the maximum depth is 4.0 m. The area refers only to the open water not covered by stands of plants (Fig. 3 and 4).

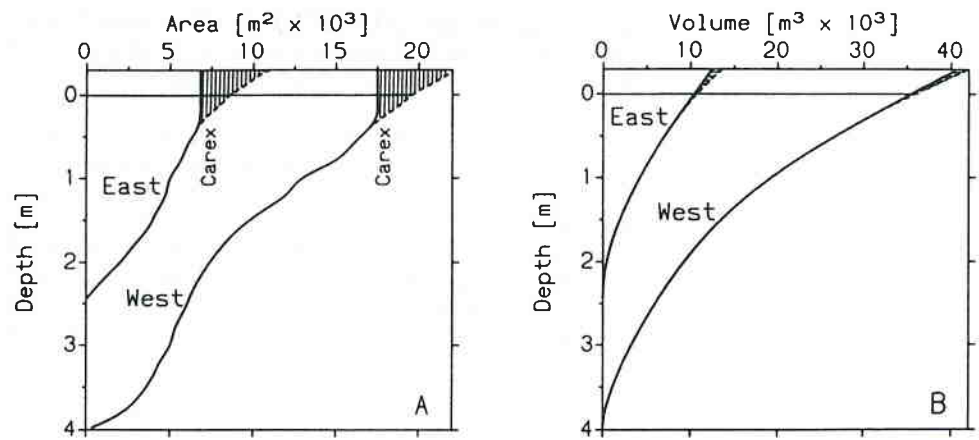


Fig. 4: Hypsographic relations of lake Kalbelesee, Eastern and Western basin. A: Area as a function of depth. B: Volume as a function of depth, obtained by integrating the area over depth (A). The area of the *Carex* shore belt is significant (A, hatched) but the corresponding volume is small (B). A winter cover extending to 1 m and 1.5 m depth reduces the free water volume to 30% and 50%, respectively.

by a shallow rim with a depth of 0.7 to 1.1 m, which is dominated by *Equisetum fluviatile* and *Carex sp.* as is the entire shoreline (Fig. 3 and 4A). The small inflow streams dry up particularly during winter. Light calcareous crusts, covering *Calliergon giganteum* in the South-eastern decline of the Western basin, indicate submerged inflows. In the region of maximum depth (>3.5 m; Fig. 3), the sediment is a homogenous mud of 70–76% water content; the carbonate content is 17–23% of the dry weight; the oxidizable carbon is 12–13 mg C.cm⁻³ (3–4% of the dry weight); and the grain size is 75–80% $<32 \mu\text{m}$, 15–17% 32–63 μm , and 3–10% 63 $>\mu\text{m}$ in the 0–10 cm layer (Gnaiger 1977). The pH of the water varies between 7.0 and 8.0 during winter.

The period of ice cover of up to 2.3 m thickness extends from October/November to mid May until early July (Amann and Gnaiger 1979). During this time the free water column at the maximum depth is regularly reduced to 2.5 m owing to the extent of the winter cover (Fig. 5). Then the available area is reduced to half (Fig. 4A) and the volume of the pelagial even to a third of the extent during summer, effectively concentrating the active zooplankton and fish population (Fig. 4B). In addition, a steep oxygen gradient develops during winter stagnation. The anoxic zone increases gradually 0.5 m (Fig. 5), up to 1.5 m from the bottom, thus reducing further the habitat volume of anoxia-intolerant species.

Mortality or growth of rainbow trout under winter hypoxia

Effect of hypoxia and low temperature

A complete winter kill of *Oncorhynchus mykiss* (previously *Salmo gairdneri*) was observed several times in Kalbelesee, such as in 1976 when oxygen concentrations under the ice dropped to $<30 \mu\text{mol O}_2 \cdot \text{dm}^{-3}$ ($p_{\text{O}_2} < 1.5 \text{ kPa}$) during March. Rainbow trout were restocked from a local fishery in May 1976 (II⁺; 340 g average fresh body mass). Winter hypoxia was less severe in 1977, when fish of an average mass of 500 g were sampled monthly in gill nets exposed under the ice. No dead fish were observed during this season nor after ice break, in agreement with the complete survival of rainbow trout in a Tyrolean high-alpine pond with $p_{\text{O}_2} < 5 \text{ kPa}$ over a period of 2.5 months (Pechlaner 1966). Moreover, a positive growth was recorded in terms of length and body mass (Fig. 6), despite the low oxygen in the lake and the low temperature, averaging 1.65 °C and not exceeding 2.9 °C in the euoxic zone (Fig. 5; un-

shaded area: $>60 \mu\text{mol O}_2 \cdot \text{dm}^{-3}$; $p\text{O}_2 > 3 \text{ kPa}$). Growth under winter hypoxia could be corroborated during the following year when ice-break occurred on the 10th of June and 4 days afterwards rainbow trout of 940 g average body mass were captured, compared to 880 g in November (Fig. 6) just prior to the formation of the winter cover (see Fig. 1C and 14 for oxygen conditions in March, May and June, 1978). This field observation of low oxygen requirements of rainbow trout agrees with the physiological indices of the critical $p\text{O}_2$ of 2.8 kPa (p_c , below which respiration declines steeply with ambient oxygen levels; Ott et al 1980), and blood O_2 affinity. The half-saturation pressure, p_{50} , is 2.6 and 3.1 kPa in rainbow trout acclimated to hypoxia and normoxia at 15°C (Bushnell et al 1984) and is independent of acclimation temperature (Weber et al 1976). As reviewed by Kramer (1987), there is evidence in general of reduced feeding of fish with reduced environmental oxygen availability. A correlation between angling success for rainbow trout and oxygen concentrations over the range $75\text{--}190 \mu\text{mol}$

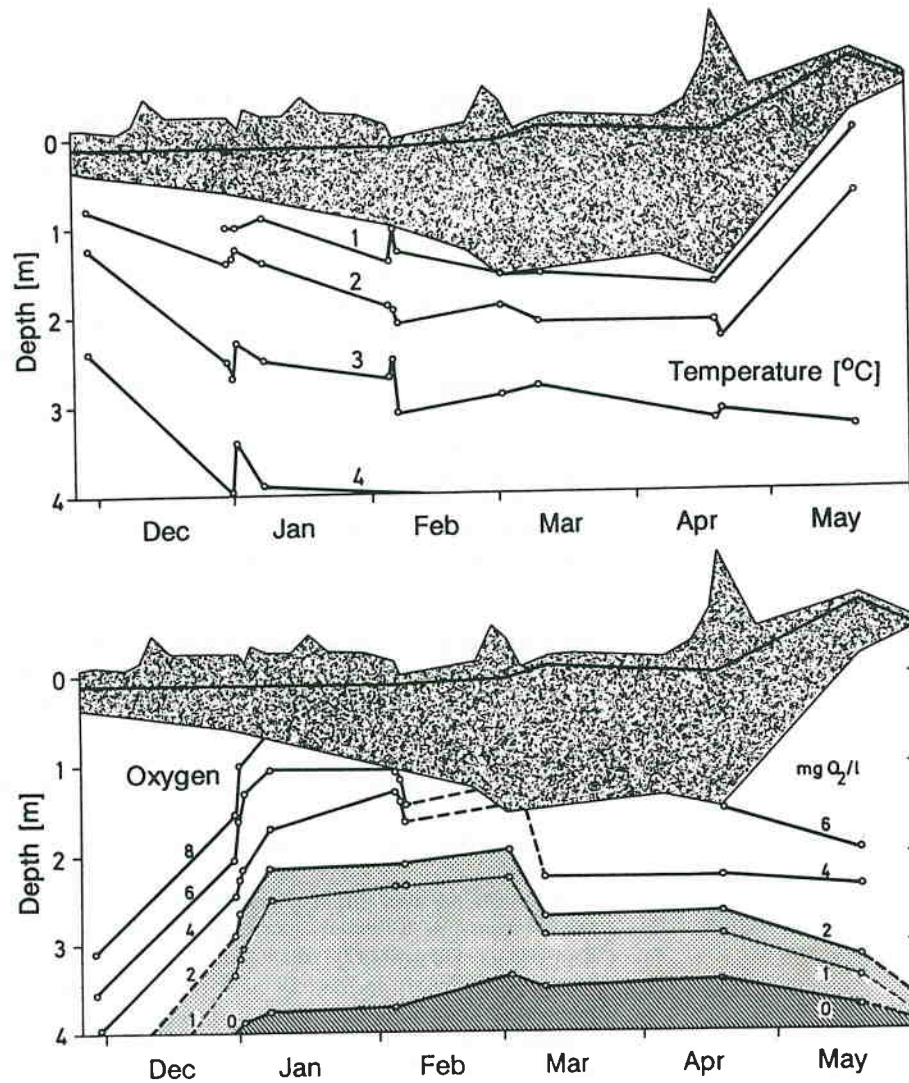


Fig. 5: Dynamics of temperature (top) and oxygen (bottom) during winter in lake Kalbelesee (1976/77; isopleths). The hatched area shows the extent of the winter cover, interpolated between periods of measurement. The shaded area indicates the zone of severe hypoxia ($<60 \mu\text{mol O}_2 \cdot \text{dm}^{-3}$; $<3 \text{ kPa } p\text{O}_2$).

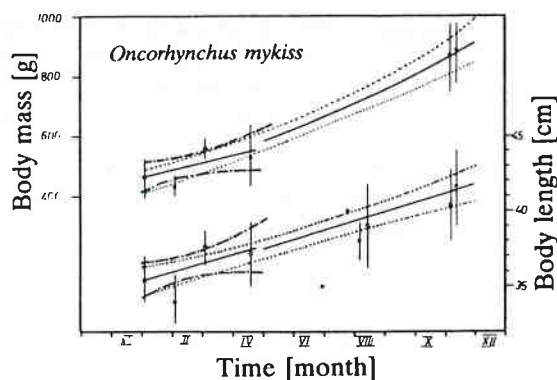


Fig. 6: Growth in terms of body mass [g fresh weight], and length [cm], of rainbow trout, *Oncorhynchus mykiss* (previously *Salmo gairdneri*), stocked into Kalbelese. The regression lines and their 95% confidence limits are shown for the winter catches (linear regressions; 25 individuals or 11% of the stocked population; $P < 0.05$) and for the entire year (exponential regressions; 49 individuals; $0.21 \pm 0.034\%$ body mass per day; 95% C.L.). The vertical bars are the standard deviations of the mean for each catch. The relation between mass and length was identical during winter and summer, $mass = 0.011 \cdot length^{3.01}$, despite a lower degree of gut filling during the period of hypoxia from February to April.

$O_2 \cdot dm^{-3}$ ($2.4-6.0 \text{ mg } O_2 \cdot dm^{-3}$) indicates reduced feeding at low oxygen which may be more pronounced at higher temperature, although the p_c of the standard metabolic rate is remarkably independent of temperature (Ott et al 1980). The low oxygen level sufficient for homeostasis and slow growth of rainbow trout (Fig. 6) contrasts with the incipient oxygen response thresholds of $>10 \text{ kPa}$ reviewed by Davis (1975).

Between January and April, 80 to 100% of the prey of rainbow trout consisted of benthic invertebrates (mainly trichoptera; Amann and Gnaiger 1979). Oxygen levels declined to zero towards the bottom of the lake during that period (Fig. 5). Therefore, *O. mykiss* appears to behave as a 'diving trout': For scavenging food, these fish dive temporarily into layers of very low oxygen levels which cannot be tolerated over extended periods of time. The swimming and feeding behavior under severe hypoxia is indicative of metabolic adaptations in skeletal muscle of rainbow trout (Johnston 1975; Boutilier et al 1988). The swimming performance at high speed of hypoxia-acclimated fish is not improved in comparison to normoxic controls (Bushnell et al 1984). Metabolic downregulation and reduction of heart rate under hypoxia, such as cardiac hypometabolism observed in diving turtles, does not seem to be invoked by fish (Driedzic 1992), although the remarkable survival of complete winter hypoxia up to six months in crucian carp involves adaptations to anoxia even at the level of glycolytic ethanol production (Holopainen and Hyvärinen 1985).

The growth potential of salmonids at low temperatures has generally been underestimated until recently. Rainbow trout is able to grow even at 0.3°C (Brännäs und Wiklund 1992). Therefore, models including the assumption that growth ceases during winter (Elliott 1975) may not be valid. Over the entire year, the average temperature in lake Kalbelese estimated for the trout population was 6°C (see also Fig. 16 and 17). The mass-specific exponential growth rate calculated from Fig. 6 was 0.21% per day (116% per year). When extrapolating the relationship between temperature, size and specific growth rate of 10 to 300 g brook trout fed on maximum ration (Elliott 1975) to rainbow trout of 500 to 880 g, a growth rate of 0.09% per day is obtained for 6°C (compared to $0.21\% \cdot d^{-1}$). This comparison is indicative of the larger potential for growth in rainbow trout and the optimum food conditions in the lake.

Fish stocking and cladoceran survival

During the ice-free period, 30% of the gut contents of rainbow trout consists of *Rivulogammarus lacustris*, which was stocked from another lake in the region in 1967 (Amann and

Gnaiger 1979). Zooplankton (mainly *Daphnia* sp.; some *Cyclops abyssorum*) amounts on average to 8% of the gut contents of the rainbow trout over the year (Amann and Gnaiger 1979). Up to 5,000 *Daphnia* are found in the gut of an individual fish. Nevertheless, the cladoceran population survived the stocking of the lake with rainbow trout as documented since 1967 (fingerlings, one and two year old fish), with scarce information from earlier years (Amann 1972). *Phoxinus phoxinus* is abundant in the lake but is partially decimated during winter kill conditions. The shallow depth, periodic winter kill, and the correlated abundance of benthic food explain the persistence of a strong population of *Daphnia* after fish stocking (compare Pennack 1968; Taylor and Gerking 1980), in contrast to the extinction of cladocerans in oligotrophic lakes after the introduction of planktivorous salmonids (Gliwicz and Rowan 1984; Gliwicz 1986).

Daphnia sp. reaches maximum abundance in Kalbelesee during August to October (Amann 1972; personal observations). There may be a higher abundance in the water column during the night than day. In September 1977, for instance, we observed in four vertical sample series an average of 324 individuals · dm⁻² during the day (± 65 S.D.; maximum density at 1.5 to 2.5 m depth) versus 1378 individuals · dm⁻² during the night (± 369 S.D.; even vertical distribution; 20 dm³ water samples at 7 depth positions, taken with a 5 dm³ Schindler sampler; Benzer 1978). During the day, near-bottom strata were over-proportionally favored by egg-carrying females. These amounted to $9 \pm 3\%$ of the population in the water column during the day but $22 \pm 3\%$ during the night ($N = 4$), possibly reflecting the effect of planktivorous fish. However, such behavior was not always apparent during summer and absent during winter. The cladocerans disappeared gradually from the water column during winter and survived winter kill conditions exclusively in the ephippial stage. Only when oxygen conditions supported survival of the trout (e.g. Fig. 5), then a proportion of active *Daphnia* remained in the water column throughout the winter season.

Adaptations of *Cyclops abyssorum* to environmental hypoxia

Population dynamics during winter hypoxia

In contrast to the cladocerans, *Cyclops abyssorum* (copepodites and adults but not nauplii) remained active under severe winter hypoxia, did not even select the more oxygenated water layers near the ice, and continued development with the completion of a hypoxic winter generation (Fig. 7). Whereas *Daphnia* was more abundant than the copepods during summer, the population maximum of *C. abyssorum* increased to >100 individuals per dm³ during January/February (Fig. 7). During the following winter months there was an exponential decline of the population, which was not due to planktivorous fish in this year of complete winter kill. Only 1% of the copepodite maximum early in February (mainly CIII and CIV) reached the adult stage at the beginning of May (Fig. 7). There was no diapause stage. Adults of the winter generation appeared in April and were significantly smaller than the adults found in January. The magnitude of this change resembles the long-term size-transition observed in populations of lakes which were originally oligotrophic (with small *C. abyssorum*) and became eutrophic (Einsle 1975; Nilssen 1979). Morphometric and genetic characteristics of the Kalbelesee population are described by Einsle (1969).

In Kalbelesee, the population dynamics is synchronized by the effect of the rapid temperature rise at the time of icebreak on the rate of egg development (Gnaiger 1980). *C. abyssorum* in high-alpine lakes is monocyclic without diapause (Eppacher 1968; Praptokardiyo 1979). In Northern lowland lakes, synchronization of population dynamics of this species is effected by periodic cessation of development in the copepodite V stage. The entire population may pass the winter in diapause in the bottom sediment under anoxia (Nilssen and Elgmork 1977). Aggregations of diapausing marine copepods are known at depths near the aerobic/anoxic boundary (Alldredge et al 1984). The physiological mechanisms in these resting stages are still