

## Changes in whitefish scales $\delta^{13}\text{C}$ during eutrophication and reoligotrophication of subalpine lakes

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### Abstract

We measured the isotope composition of whitefish (*Coregonus lavaretus*) scales from 50-yr collections to determine the pattern of change of  $\delta^{13}\text{C}$  during the eutrophication and reoligotrophication of two subalpine lakes: Lakes Constance and Geneva. The isotope composition of scales from Lake Annecy, a subalpine lake that has been protected from eutrophication, was also determined, to provide a control value for modifications in the values of whitefish scale  $\delta^{13}\text{C}$  in the absence of any significant changes in the trophic status of the lake. In Lakes Constance and Geneva, changes in whitefish scales  $\delta^{13}\text{C}$  were closely correlated with those in the mean annual phosphorus concentrations and, to a lesser extent, also reflected the efficiency of winter mixing in Lake Geneva. In contrast, in Lake Annecy, whitefish  $\delta^{13}\text{C}$  exhibited only minor changes during the survey. Changes in whitefish scale  $\delta^{13}\text{C}$  with the trophic status of Lakes Geneva and Constance were not linked to either a shift in whitefish feeding behavior or to alterations in the length of the pelagic trophic chain. Hence, the pattern of variation of whitefish  $\delta^{13}\text{C}$  during the last decades mirrored the lakes' trophic history. The responses of the Lakes Geneva and Constance to changes in  $P_{\text{tot}}$  were strictly synchronous and parallel, with a response during restoration that may have been the result of the antagonism between the effects of the falling phosphorus concentration and increasing temperature.

Stable isotopic analysis (SIA) has been extensively used to study trophic relationships in lakes worldwide (Kling et al. 1992; Cabana and Rasmussen 1994; Post 2002). As the carbon isotopic composition ( $\delta^{13}\text{C}$ ) of the consumer reflects that of its prey with nearly no enrichment (+0.39‰ [Post 2002]),  $\delta^{13}\text{C}$  can be used to track the primary producer at the base of the trophic chain. In addition, any isotope modification at the bottom of the lake food web can be transmitted up to the top predators, such as fish, which can therefore potentially be used as integrators (Vander Zanden and Vadeboncoeur 2002). It has been shown that the  $\delta^{13}\text{C}$  of pelagic components depends on the primary production of the lake (Schelske and Hodell 1991; Gu et al. 1996; Schindler et al. 1997). When the lake's primary production increases, the pelagic components get enriched in  $^{13}\text{C}$ . Many processes and mechanisms combine to produce this pattern, but the end result is that the  $\delta^{13}\text{C}$  of the pelagic components could provide a reliable indicator of the primary production of the lake (Lehmann et al. 2004). Eutrophication and reoligotrophication have affected many lakes worldwide, and may have thus altered the  $\delta^{13}\text{C}$  of pelagic components. So far,  $\delta^{13}\text{C}$  changes in response to eutrophication and reoligotrophication of lakes have been studied from the organic and

inorganic carbon in sediments, but there have been few or no studies of the changes in the  $\delta^{13}\text{C}$  changes of pelagic consumers during eutrophication and reoligotrophication. One reason for this may be that SIA is usually performed on fish dorsal muscle. Hence, observing isotopic changes with trophic status implies that fish dorsal muscle samples have been frozen and preserved for a long time, which is not usually the case.

In contrast, many laboratories keep long-term fish scale collections, but using fish scales to perform SIA raises some questions. Scales are composed of both organic and calcified fractions. The organic constituents, mainly proteins, come from food constituents but the source of the carbonates in fish scales is very variable, and can involve both food and dissolved inorganic carbon (DIC) (Hoie et al. 2003). Estep and Vigg (1985) found that the  $\delta^{13}\text{C}$  values of muscle and scale were not closely correlated. Wainright et al. (1993) used scale  $\delta^{13}\text{C}$  values to investigate the relationship between the changes in trophic structure and a series of environmental and population variables associated with the Georges Bank food web, but did not address how scale and muscle  $\delta^{13}\text{C}$  values were interrelated. In these two studies (Estep and Vigg 1985; Wainright et al. 1993), either the fish scales were not decalcified at all, or the decalcification protocol was not reported, which may account for the apparent lack of any correlation between scale and muscle  $\delta^{13}\text{C}$  values. In a previous study, we demonstrated that the  $\delta^{13}\text{C}$  values of decalcified whitefish scale mirrored those of muscle tissue and diet, with a  $^{13}\text{C}$  enrichment of 4‰ (SD = 1.3) (Perga and Gerdeaux 2003). Now that this link has been established, it is possible to use stable isotope measures of whitefish  $\delta^{13}\text{C}$  as an indicator of the potential carbon sources or pathways in the food web. In our previous study (Perga and Gerdeaux 2003), the decrease in  $P_{\text{tot}}$  concentrations during the restoration phase of Lake Geneva (1980–2001) was shown to be mirrored by an impoverishment in  $^{13}\text{C}$  of whitefish scales by about 3‰. This study concerns a longer period of time, cov-

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ering both the pre- and posteutrophication periods (up to 50 yr), and, furthermore, three alpine lakes are compared, which had never been done previously.

Lakes Geneva and Constance underwent a phase of eutrophication beginning in the 1950s, due to increasing anthropogenic pressure on the catchment areas. Phosphorus concentrations in lakes Constance and Geneva increased eightfold by the end of the 1970s. From the early 1980s, sewage diversion and wastewater treatment were undertaken, leading to a reduction of the phosphorus concentration. In contrast, Lake Annecy was sheltered from eutrophication. Lakes Geneva and Constance are now engaged in a reoligotrophication process, with phosphorus concentrations tending to return to their original values. Changes in the trophic status of these lakes have influenced the algal community structure (Gaedke 1998; Kümmerlin 1998; Anneville et al. 2002); the zooplankton crustacean biomass (Straile and Geller 1998); and the production, growth, and fecundity of fish (Hartmann and Quoss 1993; Eckmann and Rösch 1998). Eutrophication and reoligotrophication thus affect various food web compartments, and so may have a great effect on both the food web structure and carbon flows. The objective of this study was therefore to provide evidence that stable isotope composition of fish scales provides a record of the effects of the change on the trophic dynamics of two European subalpine lakes during a eutrophication stage followed by reoligotrophication.

Whitefish scales were used for this purpose. Whitefish, *Coregonus lavaretus*, is a preferentially zooplanktivorous fish, living in the pelagic area of many deep subalpine lakes. This fish is the main resource of lake fisheries, and fisheries scientists have long used scales for ageing fish. They have stored scales since 1946 for Lake Constance (Germany/Switzerland/Austria), since 1958 for Lake Geneva (Switzerland/France), and since 1971 for Lake Annecy (France). The scale collections cover the phases of eutrophication and reoligotrophication of Lakes Geneva and Constance, and the phase of stable phosphorus concentration of Lake Annecy. We intend to monitor the change in whitefish scales  $\delta^{13}\text{C}$  during the period of survey in all three lakes. Our purpose is to find out whether the  $\delta^{13}\text{C}$  of whitefish scales has changed according to the hypothesis that  $\delta^{13}\text{C}$  in the food webs may increase during eutrophication and then decrease during reoligotrophication. Lake Annecy did not undergo any significant changes in its trophic status during the period of the survey, and so whitefish  $\delta^{13}\text{C}$  changes in this lake were expected to be relatively minor compared to those in Lakes Geneva and Constance.

## Methods

**Study sites**—Located on the Swiss–French border, Lake Geneva is the biggest lake in Western Europe (area 582 km<sup>2</sup>, max depth 309 m), and has a catchment area of 7,400 km<sup>2</sup>. Winter mixing in Lake Geneva, the depth of which is calculated from the winter O<sub>2</sub> profiles, rarely reaches the bottom of the lake. Increasing human pressure during the economic development that started in the late 1950s and continued in the 1960s and 1970s led to a dramatic increase in the phos-

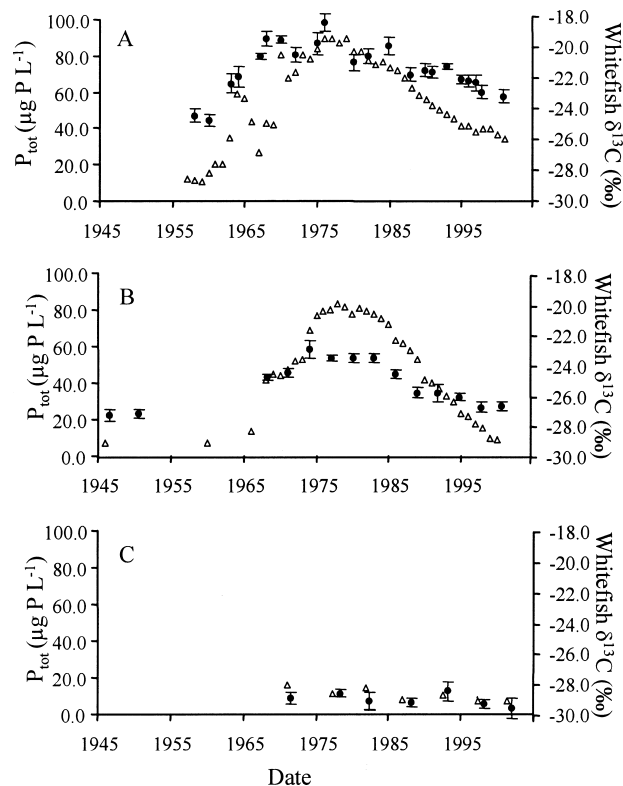


Fig. 1. Changes in mean annual phosphorus concentrations (open triangles, left axis) and mean annual whitefish scales  $\delta^{13}\text{C}$  ( $\pm$ SD) (closed circles, right axis) in all three lakes. (A) Lake Geneva. (B) Lake Constance. (C) Lake Annecy.

phorus load.  $P_{\text{tot}}$  initial concentrations of 12.4  $\mu\text{g L}^{-1}$  in 1957 increased eightfold over 20 yr, to reach 89.6  $\mu\text{g L}^{-1}$  in 1978. The lake, which was initially classified as oligotrophic, had become eutrophic by the end of the 1970s. Measures intended to reduce P inputs were taken in the early 1980s. The mean phosphorus concentrations fell between 1980 and 2001, to reach 36.5  $\mu\text{g L}^{-1}$  (Fig. 1). The mean annual concentration of chlorophyll *a* (Chl *a*) in the 0–10-m layer was 7  $\text{mg m}^{-3}$  in 2001. No acidification of the lake waters could be observed, and the pH in the epilimnion was typical of alkaline lakes, and exhibited values between 7.27 and 8.99 during 2001. The phytoplankton was typical of mesotrophic lakes, and included many pigmentary groups, but cyanobacteria were always poorly represented, even during the eutrophication stage. Nutrient concentrations and mixing depth data were obtained from the lake monitoring, by the International Commission for the Protection of the Water of Lake Geneva ([www.cipel.org](http://www.cipel.org)).

Lake Annecy is situated in the French Alps, 60 km south of Lake Geneva. It has a catchment area of 302 km<sup>2</sup>, a surface area of 28 km<sup>2</sup>, and a maximum depth of 81 m. It was ultraoligotrophic before 1970, with phosphorus concentrations of <2  $\mu\text{g L}^{-1}$ , and became oligomesotrophic, with 5–7  $\mu\text{g L}^{-1}$  by the end of the 1970s. Current phosphorus concentrations are 1 to 3  $\mu\text{g L}^{-1}$  (Fig. 1). Winter mixing is always complete. The mean annual concentration of Chl *a* in the 0–10-m layer was <3  $\text{mg m}^{-3}$  (1998–2002), and the current pH, typical of alkaline lakes, ranged between 7.46

and 8.33 during 2002. The phytoplankton community is dominated by diatoms. Nutrient concentrations were obtained from the lake-monitoring data provided by the Syndicat Intercommunal du Lac d'Annecy (Balvay et al. 2003).

In Lakes Geneva and Annecy, total phosphorus concentrations were determined throughout the study period, using the acid molybdate method (AFNOR) after digesting unfiltered samples with potassium peroxodisulfate.

Lake Constance is the second biggest lake in Western Europe, with an area of 534 km<sup>2</sup> and a maximum depth of 253 m. Winter mixing is always complete. Lake Constance underwent a pronounced eutrophication, mainly due to domestic phosphorus loading. Mean annual P concentrations had risen from 5–8 µg L<sup>-1</sup> in the 1950s to 80 µg L<sup>-1</sup> in 1980. The lake became mesoeutrophic. A reoligotrophication process then began as a result of sewage treatment. The mean annual phosphorus concentrations had decreased to 9 µg L<sup>-1</sup> in 2001 (Fig. 1). Mean annual concentrations of Chl *a* in the 0–10-m layer were about 4 mg m<sup>-3</sup> in 2001. The pH varied within a range of 7.9 to 8.8 throughout the year. As in Lake Geneva, the phytoplankton community is typical of mesotrophic lakes, and cyanobacteria are poorly represented (Kümmerlin 1998). Data were obtained from the monitoring program of Lake Constance from the Internationale Gewässerschutzkommission für den Bodensee (<http://www.hydra-institute.com/igkb/inhalt.html>). In Lake Constance, the total phosphorus concentration has been measured since the 1970s using an automated molybdenum-blue method with ascorbic acid (DIN 1189) after unfiltered samples have been digested with potassium peroxodisulfate (E. Ivanyi pers. comm.).

**Fish sampling**—Whitefish is predominantly zooplanktivorous in alpine lakes (Mookerji et al. 1998; Heikinheimo et al. 2000; Gerdeaux et al. 2002). Whitefish may rely on benthic preys (such as chironomids) in late winter or early spring, but somatic growth is restricted during this period, and the isotope composition of the diet is not mirrored in that of whitefish muscle or scales (Perga and Gerdeaux in press). In our group of lakes, whitefish was assumed to have remained zooplanktivorous in spite of changes in the trophic status of the lakes.

Female whitefish usually mature at the end of the third year of life, when the fish reaches a length of 35–40 cm. Special catches are made every year in December during the spawning season to collect eggs for restocking Lakes Geneva, Annecy, and Constance. A survey has been conducted regularly of the spawning fish and this was the source of the scale collection. Scales were used to age the fish, and then dry-preserved, stored in scales packets, and archived. Collections were started in 1946 for Lake Constance (Swiss collection from Amt für Jagd and Fischerei, St Gallen), in 1958 for Lake Geneva, and in 1971 for Lake Annecy (French collections from Station d'Hydrobiologie Lacustre, INRA, Thonon les Bains). Seven to 11 fish (depending on scale availability) were selected per year, and one scale from each fish was used for stable isotope analysis. Sex had previously been shown to have no effect on the isotope composition of whitefish (Dufour and Gerdeaux 2001) but, as age may affect fish δ<sup>13</sup>C, the fish selected were all of the same age (first or second year of maturity).

**Scale decalcification**—Fish scale is a mixed-origin tissue, with an organic fraction and an inorganic fraction. The organic constituents are essentially proteins such as collagen and keratin (ichtylepidin), and the inorganic constituents are essentially carbonates and apatite (Wainright et al. 1993). The origin of the inorganic carbon is unknown, and it can have a δ<sup>13</sup>C value that differs from that of the organic constituents. To measure only the δ<sup>13</sup>C of the proteins, the scales were decalcified for 2 min in 1.2 mol L<sup>-1</sup> HCl and rinsed in distilled water before isotopic composition analysis according to the protocol established by Perga and Gerdeaux (2003).

**Isotopic analyses**—Samples (1.5 mg) of decalcified and dried scales were weighed into 6 × 4 mm<sup>2</sup> tin cups for continuous flow isotope-ratio mass spectrometry (CF-IRMS) analysis using a Europa Scientific ANCA-NT 20-20 stable isotope analyzer with a NCA-NT solid/liquid preparation module (PDZ Europa). The analytical precision (SD) was 0.2‰ estimated from five standards analyzed along with the samples. The working standards used were 1.5 mg of leucine prepared by freeze-drying 50 ml of a 20 mg ml<sup>-1</sup> stock solution in the tin cups, and calibrated against “Europa flour” and International Atomic Energy Agency (IAEA) standards N1 and N2. Measures were performed by C. Scrimgeour at the Scottish Crop Research Institute (Dundee, UK). Isotope ratios are expressed as parts per thousand (‰) differences from a standard reference, which is PeeDee Belmrite.

$$\delta^{13}\text{C} = \left[ \frac{(^{13}\text{C}_{\text{sample}}/^{12}\text{C}_{\text{sample}})}{(^{13}\text{C}_{\text{standard}}/^{12}\text{C}_{\text{standard}})} - 1 \right] \times 1,000$$

**Statistical analysis**—Statistical analysis were performed on S-PLUS 6.0 (Insightful 2001). Relations between isotopic signatures and factors (date, P – P<sub>tot</sub>, mixing depth) were analyzed by least-squares regression and one-way analysis of variance (ANOVA). Homoscedasticity was ensured by Levene tests. The absence of autocorrelation between residuals was checked using the Durbin-Watson test. Interannual variability in whitefish δ<sup>13</sup>C were compared between lakes by *F*-test. As the fish were 3 yr old, and winter mixing efficiency can vary considerably from one year to the other in Lake Geneva, we used the mean of the previous 3 yr to study the effects of winter mixing on δ<sup>13</sup>C values.

The eutrophication period lasted from 1956 to 1979 for Lake Geneva, and from 1946 to 1980 for Lake Constance. The reoligotrophication period covered 1980–2001 for Lake Geneva and 1981–2001 for Lake Constance. To find out whether adding winter mixing as a supplementary factor would significantly improve the variability explained in Lake Geneva, regression models without and with the winter mixing factor were compared by one-way ANOVA.

## Results

The δ<sup>13</sup>C of whitefish scales exhibited significant changes in all three lakes (ANOVA Constance,  $F_{13,124} = 144.06$ ,  $p < 10^{-5}$ ; Geneva,  $F_{21,184} = 102.03$ ,  $p < 10^{-5}$ ; Annecy,  $F_{6,52} =$

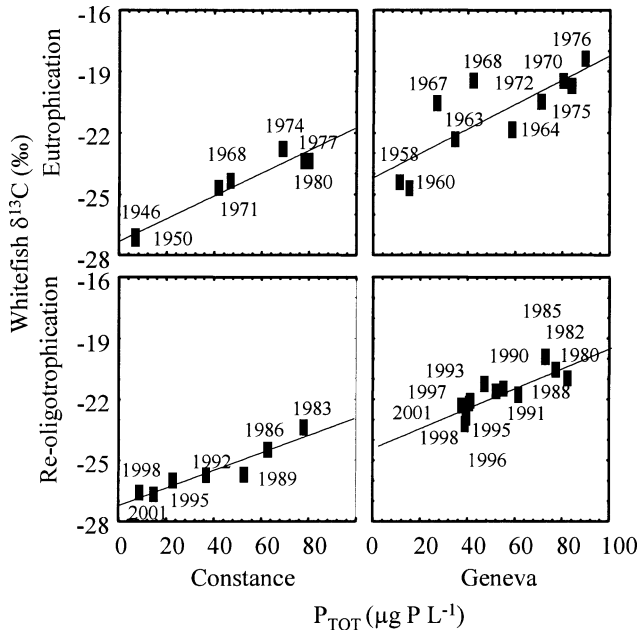


Fig. 2. Relationships between mean annual  $\delta^{13}\text{C}$  of whitefish scales and  $P_{\text{tot}}$  during eutrophication and reoligotrophication of lakes Constance and Geneva.

6.615,  $p = 2 \times 10^{-5}$ ). However, as we expected, the inter-annual variability of whitefish scales  $\delta^{13}\text{C}$  in Lake Anney was significantly lower than that in Lake Geneva ( $F$ -test  $F_{21,6} = 16.17$ ,  $p = 10^{-3}$ ) and that in Lake Constance ( $F$ -test  $F_{13,6} = 14.51$ ,  $p = 10^{-3}$ ). In Lakes Geneva and Constance, whitefish  $\delta^{13}\text{C}$  changes were significantly correlated with those of the annual mean phosphorus concentrations (Constance,  $r^2 = 0.884$ ,  $p < 10^{-5}$ ; Geneva,  $r^2 = 0.604$ ;  $p = 10^{-5}$ ).

Changes in  $P_{\text{tot}}$  were therefore considered separately for the pre- and posteutrophication phases of Lakes Geneva and Constance (Fig. 2). For each lake, the slope of the regression line fitted to the eutrophication data was significantly higher than the one fitted to those of reoligotrophication (Table 1). However, the intercepts were not significantly different. Moreover, the slopes of the regression lines corresponding to eutrophication were not significantly different for Lakes Geneva and Constance, nor were the reoligotrophication slopes (Table 1).

In these regression models, the variability of  $\delta^{13}\text{C}$  ex-

plained by  $P_{\text{tot}}$  was greater for Lake Constance than for Lake Geneva (Table 1). The years 1967 and 1968 contributed 65% of the residual variability of  $\delta^{13}\text{C}$  during the eutrophication phase in Lake Geneva. The lakes differed with regard to the depth and duration of winter mixing. Lake Constance undergoes a complete turnover every winter, whereas winter mixing is rarely complete in Lake Geneva. The mean winter mixing depth was then introduced as a supplementary factor into the above regression models. Mixing depth was negatively correlated with  $\delta^{13}\text{C}$  in Lake Geneva (Table 2). The variability explained by the multiple regression taking  $P_{\text{tot}}$  and mixing depth into account was significantly improved during eutrophication (ANOVA,  $F_{11,12} = 7.4$ ,  $p = 0.029$ ).

## Discussion

The patterns of variations in whitefish scale  $\delta^{13}\text{C}$  are the same in Lakes Geneva and Constance, yet the planktonic and fish communities in these lakes do not have exactly the same dynamics. In contrast, in Lake Anney, which remained oligotrophic throughout the survey, there were only minor changes in whitefish scale  $\delta^{13}\text{C}$  values. Changes in  $P$  concentrations therefore seemed to be a major cause of the changes in whitefish scale  $\delta^{13}\text{C}$ , although the innermost process underlying these changes could be discussed. As the slope of the regression line fitted to the restoration data was significantly lower than that of the regression line fitted to the eutrophication data, the response of lakes to decreasing  $P_{\text{tot}}$  seems to be slowed. The change in whitefish scales  $\delta^{13}\text{C}$  may be related to (1) a change in whitefish diet, (2) a change in the pelagic food web length, and (3) a change in the signature at the base of the food web.

**Whitefish diet**—In alpine lakes, whitefish are zooplanktivorous, at least during their growth period, regardless of the trophic status of the lake (Giussani and De Bernardi 1977; Mookerji et al. 1998; Gerdeaux et al. 2002). Results of feeding studies based on stomach contents revealed no major change in whitefish diet between the 1980s and 2001 (Ponton 1986; Gerdeaux and Hamelet 2001) in Lake Geneva. In Lake Constance, there have been fewer gut-content studies, but whitefish show a preference for great cladocerans (*Daphnia* spp.) and predator species of zooplankton (*Bythotrephes longimanus*, *Leptodora kindtii*) (Becker and Eckmann 1992). Whitefish may occasionally consume benthic preys in winter

Table 1. Coefficients of regression between whitefish  $\delta^{13}\text{C}$  and  $P_{\text{tot}}$ , considering pre- and posteutrophication phases for Lakes Geneva and Constance. Coefficients of the different regressions were compared by a  $t$ -test.

N	Regression	$r^2$	$p$	Slope	Intercept	$t$ -test	$p$ Slope comparison	$p$ Intercept comparison
1	Geneva Eutrophication	0.661	0.0043	0.0598	-24.23	1 vs. 2	0.480	0.000
2	Constance Eutrophication	0.923	0.0004	0.0554	-27.33	2 vs. 4	0.004	0.291
3	Geneva Reoligotrophication	0.692	0.0080	0.0488	-24.33	1 vs. 3	0.037	0.762
4	Constance Reoligotrophication	0.861	0.0016	0.0430	-27.22	3 vs. 4	0.216	

Table 2. Coefficients of regression between whitefish  $\delta^{13}\text{C}$ ,  $P_{\text{tot}}$ , and winter mixing depth in Lake Geneva during the eutrophication and reoligotrophication phases.

	$P_{\text{tot}}$	$p$	Mixing depth	$p$	$r^2$	$F$	Degrees of freedom	$p$
Eutrophication	0.0486	0.050	-0.0128	0.0295	0.836	17.79	2,7	0.02
Reoligotrophication	0.0525	0.026	-0.0024	0.6087	0.701	10.55	2,9	0.004

or early spring, but as somatic growth does not occur for this time, the isotopic composition of the diet is not mirrored in the tissues affected by somatic growth, such as muscle and scales (Perga and Gerdeaux in press). Whitefish diet is not likely to be the main reason for whitefish  $\delta^{13}\text{C}$  changes that occurred in Lakes Geneva and Constance during the survey.

*Pelagic trophic chain length*—Although whitefish have not substantially changed diet during the survey in Lakes Geneva and Constance, eutrophication and reoligotrophication may affect the food web structure at the lower trophic levels, i.e., within the zooplankton community. Changes in the zooplankton trophic links may alter the pelagic food chain length and then modify the isotope composition of the

top predator. Modifications in trophic chain length are usually assessed from  $\delta^{15}\text{N}$ , and  $\delta^{15}\text{N}$  measurements on fish scales are well correlated with that found for the dorsal muscles (Perga and Gerdeaux 2003). As  $\delta^{15}\text{N}$  increases from prey to predator with an isotopic fractionation at each trophic step of 3.4‰ (SD = 1‰ [Post 2002]), the  $\delta^{15}\text{N}$  difference between two organisms belonging to the same trophic chain gives an assessment of the number of trophic levels separating them. The  $\delta^{15}\text{N}$  value of an organism therefore depends on its trophic level, and on the  $\delta^{15}\text{N}$  at the base of the food web. Whitefish scales  $\delta^{15}\text{N}$  exhibited significant changes over time in all three lakes (ANOVA Annecy,  $F_{6,57} = 8.59$ ;  $p < 10^{-5}$ ; Constance,  $F_{13,124} = 35.9$ ,  $p < 10^{-5}$ ; Geneva,  $F_{21,184} = 9.40$ ,  $p < 10^{-5}$ ). Although the patterns of variation of whitefish  $\delta^{13}\text{C}$  were very similar between Lakes Geneva and Constance, the pattern of change of  $\delta^{15}\text{N}$  differed markedly in the different lakes. Whitefish scales got  $^{15}\text{N}$ -enriched during the study period in Lake Constance, whereas patterns of  $\delta^{15}\text{N}$  variations were much more complex in Lakes Geneva and Annecy (Fig. 3). None of these patterns of  $\delta^{15}\text{N}$  variations could be related to changes in environmental variables, such as the concentrations of nitrates, ammonium, and phosphorus, or populational data, such as the numbers of fish caught per year (results not communicated).

These changes in whitefish  $\delta^{15}\text{N}$  may not ensue solely from structural changes within the pelagic food web, and various other processes could also have affected the phytoplankton N-isotope composition.

Differences in phytoplankton  $\delta^{15}\text{N}$  have often been considered to result essentially from changes in the N-isotope fractionation rate during nutrient uptake (Altabet and Deuser 1985). However, Lehmann et al. (2004) recently produced evidence showing that this process makes little contribution to the changes in suspended organic matter  $\delta^{15}\text{N}$  in Lake Lugano. In contrast, the isotope composition of the source of inorganic nitrogen taken up by phytoplankton, changes in algal populations, and N-cycling reactions within the microbial loop may be the main factors controlling the phytoplankton  $\delta^{15}\text{N}$  value.

The anthropogenic sources of nitrogen varied over the study time. Sewage and wastewater discharges were not controlled before the end of the 1970s, and they may account for a  $^{15}\text{N}$ -enriched contribution to the lake nitrogen stock (McKinney et al. 1999). From the early 1980s, sewage diversion and wastewater treatment measures were taken, but nonpoint agricultural inputs may have continued. Industrial fertilizers have a low  $\delta^{15}\text{N}$ , but denitrification processes in tributaries can also affect the  $\delta^{15}\text{N}$  of nitrogen inputs into the lake (Lehmann et al. 2004). No data for the  $\delta^{15}\text{N}$  of the nitrate stock were available for the period surveyed.

Communities of algal species also change as a result of

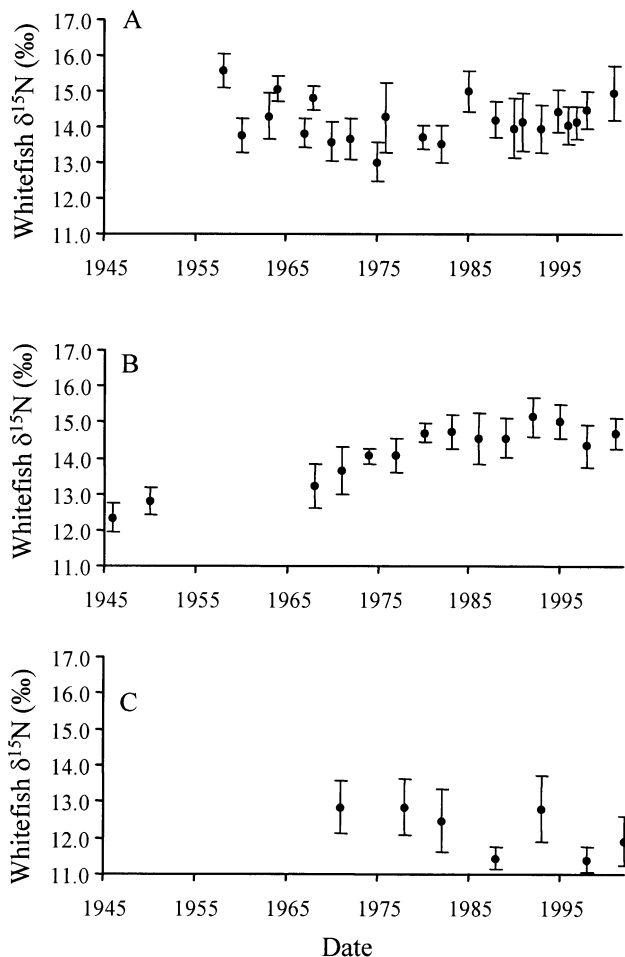


Fig. 3. Changes in whitefish  $\delta^{15}\text{N}$  ( $\pm$ SD) in all three lakes. (A) Lake Geneva. (B) Lake Constance. (C) Lake Annecy.

eutrophication and reoligotrophication. During the restoration of Lakes Geneva (Anneville et al. 2002) and Constance (Anneville pers. comm.), the phytoplanktonic community structure has evolved toward species typical of the oligotrophication process: *Dynobryon sociale* and *Cyclotella* spp. appeared in the seasonal succession. *Dynobryon* is a mixotrophic species. As it may rely upon heterotrophic nitrogen, it may display higher  $^{15}\text{N}$  values than the rest of the phytoplanktonic community. The same pattern can be expected to be seen for *Ceratium hirunditella* and *Gynodinium* spp., which have also appeared in Lake Constance.

The microbial loop can also affect the phytoplankton  $\delta^{15}\text{N}$  value. The microbial loop processes all have the net effect of concentrating  $^{15}\text{N}$  in the seston (Lehmann et al. 2004). The  $^{15}\text{N}$ -enrichment of the phytoplankton may vary, depending on the relative efficiency of the microbial processes within and among lakes.

Moreover,  $\delta^{15}\text{N}$  is more sensitive to changes in the food web structure than  $\delta^{13}\text{C}$ . Changes in the zooplanktonic community structure can influence the predatory pressure on herbivorous species from carnivorous zooplanktonic species. All these changes may strongly influence whitefish  $\delta^{15}\text{N}$  at the top of the food chain.

The  $\delta^{15}\text{N}$  values of long-living primary consumers, such as bivalves, can be used to distinguish between the effects that result from changes in the  $\delta^{15}\text{N}$  of phytoplankton and those due to changes in the pelagic trophic structure (Cabana and Rasmussen 1994; Post 2002; Vadeboncoeur et al. 2003). Such data were lacking for the time corresponding to the study. Patterns of variations of whitefish scales  $\delta^{15}\text{N}$  may ensue from multiple N-cycling processes, and could not therefore be addressed for our set of lakes. Whitefish  $\delta^{15}\text{N}$  values could not provide any help in finding out whether the food chain length changed during the eutrophication and reoligotrophication periods.

The range of interyear  $\delta^{13}\text{C}$  variations was 4–6‰ for Lakes Geneva and Constance. According to the latest estimations (Post 2002; McCutchan et al. 2003), the typical enrichment in  $^{13}\text{C}$  from one trophic level to the next up is about +0.4‰. A change in the lake trophic status can influence the zooplanktonic community structure (Straile and Geller 1998). Depending on the ratio of herbivorous zooplankton (*Daphnia* spp.) to predator zooplankton (*Bythotrephes* spp., *Leptodora* spp.), the trophic position of whitefish could actually change, although not by more than one trophic level. Consequently, a change in the zooplanktonic food web structure could only account for a fraction of the  $\delta^{13}\text{C}$  range. This means that changes in whitefish  $\delta^{13}\text{C}$  can be assumed to be mainly influenced by changes in the signature of the phytoplankton at the base of the food web.

*Relationships between  $\delta^{13}\text{C}$  at the base of the food web and  $P_{\text{tot}}$* —According to our data, *C. lavaretus* scales became increasingly enriched with  $^{13}\text{C}$  as the lakes became eutrophic, and increasingly  $^{13}\text{C}$ -depleted as the lakes recovered. The same pattern was observed in Lake Erie using archived wall-eye muscle  $\delta^{13}\text{C}$  during the decrease in phosphorus loading (Kiriluk et al. 1999). As whitefish scales  $\delta^{13}\text{C}$  provides an integrated image of the primary producer's signatures, our findings would support the hypothesis that the mean annual

phytoplanktonic  $\delta^{13}\text{C}$  depends on the trophic status of the lake.

Various processes combine to produce this pattern of  $\delta^{13}\text{C}$  changes related to phosphorus concentrations (Gu et al. 1996; Schindler et al. 1997; Lehmann et al. 2004).

The  $\delta^{13}\text{C}$  of phytoplankton depends on both the fractionation rate between DIC and organic matter that occurs during photosynthesis, and on the  $\delta^{13}\text{C}$  of the inorganic carbon source (Raven et al. 1994).

The fractionation rate, imposed by a carboxylation reaction and a kinetic mode of carbon fixing during photosynthesis, depends on the concentration of inorganic carbon available. For terrestrial C3 plants, the concentration of atmospheric  $\text{CO}_2$  is quite constant, and the fractionation rate is around  $-20\text{‰}$ . In contrast, in oceans and lakes, DIC diffusion is slowed by water viscosity, and consequently DIC concentration is always a limiting factor. In the context of high primary production, algal demand for DIC is high, and this depletes the epilimnetic DIC. Since the fractionation rate decreases as DIC concentration decreases, phytoplankton tends to exhibit more positive  $\delta^{13}\text{C}$  values when primary production increases (Takahashi et al. 1990; Goericke et al. 1994). However, Lehmann et al. (2004) provided evidence that this process cannot account for the entire seasonal variability of particulate organic carbon  $\delta^{13}\text{C}$  in Lake Lugano, and evoked the Rayleigh distillation processes. The DIC pool gets as well enriched in  $^{13}\text{C}$  when primary production increased, as phytoplankton preferentially fixes  $^{12}\text{C}$ -DIC. Hence,  $^{13}\text{C}$ -DIC accumulates in the epilimnion and also contributes to an increase in phytoplankton  $\delta^{13}\text{C}$ .

Moreover, DIC can originate from three different sources: the geology of the catchment area, the atmosphere, and the mineralization of organic matter. DIC from geology of the catchment area can be assumed to have a constant  $\delta^{13}\text{C}$  throughout the study period. Atmospheric and endogenous DIC have differing carbon isotopic signatures. The  $\delta^{13}\text{C}$  of DIC is higher when it originates from the dissolution of atmospheric  $\text{CO}_2$  (Schindler et al. 1997), and lower when the contribution of respired carbon is high (Post 2002).

In a context of a high carbon demand, atmospheric  $\text{CO}_2$  invasion increases, leading to a  $^{13}\text{C}$ -enriched DIC pool (Emerson 1975; Schindler et al. 1997). Once fixed, this enriched DIC leads to  $^{13}\text{C}$ -enriched organic matter in the pelagic food web (Schindler et al. 1997). Moreover, Lakes Geneva and Constance are located on an alkaline watershed and undergo considerable whitening due to calcium carbonate precipitation during the summer peaks of phytoplanktonic production. Carbonate precipitation contributes to enhancing the burial of atmospheric carbon in alkaline lake waters (Waninkof and Knox 1996; Einsele et al. 2001).

Phytoplankton species effects may also be implicated in producing this pattern of  $\delta^{13}\text{C}$  variation with P concentrations. First, as  $[\text{CO}_{2,\text{aq}}]$  decreases and pH increases in conditions of high primary production,  $\text{HCO}_3^-$  assimilation may be promoted. When  $\text{HCO}_3^-$  is used as an inorganic carbon source for phytoplankters, the apparent C-isotope fractionation during photosynthesis is lower, as  $\text{HCO}_3^-$  is about 9‰ heavier than dissolved  $\text{CO}_2$  (Goericke et al. 1994). Second, phytoplanktonic species succession changed through eutrophication and reoligotrophication of Lakes Geneva and Con-

stance, as already pointed out. The C-isotope fractionation rate depends on the algae taxonomy and cell size (Burkhardt et al. 1999). Species succession changes may then contribute to change the phytoplankton  $\delta^{13}\text{C}$  during the changes in trophic status in Lakes Geneva and Constance.

Subsequently, as the lakes underwent eutrophication, the increase of the  $\text{CO}_2$  demand led to a decrease in the photosynthetic fractionation rate and an increase in the DIC pool  $\delta^{13}\text{C}$  by Rayleigh distillation kinetics and invasion of atmospheric  $\text{CO}_2$ , amplified by the alkaline nature of the waters. When the lakes eutrophied, these phenomena contributed in the same way to producing more positive values of the phytoplanktonic  $\delta^{13}\text{C}$ , which was transmitted up to whitefish  $\delta^{13}\text{C}$ , at the top of the pelagic food web.

As the lakes were restored, endogenous carbon became increasingly able to meet the declining phytoplanktonic demand for DIC. The contribution of the atmospheric carbon decreased, and at the same time, the fractionation rate tended to rise again. Both these processes led to more negative values for the pelagic  $\delta^{13}\text{C}$  during the lake restoration.

*Winter mixing effect*—The relations between  $\delta^{13}\text{C}$  and  $P_{\text{tot}}$  are very similar in Lakes Geneva and Constance, but the residual variability of the  $P_{\text{tot}}$  model is greater for Lake Geneva. A great part of the residual variability can be related to the efficiency of winter mixing during the eutrophication phase of Lake Geneva. In contrast, during the restoration phase,  $\delta^{13}\text{C}$  was not influenced by winter mixing.

In Lake Geneva, mixing reaches depths of 50 to 309 m, depending on the mean winter temperature and wind force. During eutrophication, winter mixing depth and  $\delta^{13}\text{C}$  were negatively correlated. The years 1967 and 1968, which contributed to the greater residual variability, corresponded to very warm winter periods with a mixing depth of only around 50 to 75 m (from 1965 to 1969). During this period of superficial mixing,  $P_{\text{tot}}$  concentrations decreased from  $60 \mu\text{g L}^{-1}$  in 1964 to  $27 \mu\text{g L}^{-1}$  in 1967, because of the repeated absence of rehomogenization of the water column and of the remobilization of deep P. When the next complete mixing occurred in 1970 the  $P_{\text{tot}}$  concentration rose to  $80 \mu\text{g L}^{-1}$ . Whereas  $P_{\text{tot}}$  concentrations were very low in 1967 and 1968, whitefish  $\delta^{13}\text{C}$  remained very high, illustrating a lower-than-expected contribution of endogenous inorganic carbon. A large proportion of mineralization takes place in the hypolimnion, leading to a very negative DIC  $\delta^{13}\text{C}$  (Quay et al. 1986). Hypolimnetic DIC is made available to the primary production by the winter mixing. If the winter mixing is less efficient, the amount of hypolimnetic DIC remobilized may decrease. In Lake Constance, the entire quantity is available each spring to the primary production. In contrast, in Lake Geneva, the proportion of the DIC stock remobilized depends on the depth of the mixed layer, which limits the potential contribution of endogenous DIC to the epilimnetic primary production.

*Patterns of  $\delta^{13}\text{C}$  variations in eutrophication and restoration stages*—Slopes of the regression lines fitted exhibited, for both lakes, lower values during the restoration stage than during the eutrophication stage. This could mean that the lake responds more slowly to restoration than to eutrophication,

and that the state of the lake during restoration is probably not the same as during eutrophication. Besides  $P_{\text{tot}}$ , many other environmental factors have changed. These include the fish community structure and fisheries management, nitrates, and also temperature regimes.

As for many other lakes worldwide, the period when restoration efforts occurred coincided with a period of positive North Atlantic Oscillation (Scheffer et al. 2001; Van Donk et al. 2003). Indeed, since 1988, the water temperature in both lakes has been higher in the first half of the year (Anneville pers. comm.) than before 1988 (over the period 1974–1988). These two factors (a decrease in  $P_{\text{tot}}$  and an increase in temperature) have been shown to act in synergy to produce synchronic changes in the phytoplanktonic community in the two lakes (Anneville pers. comm.). Spring temperatures are getting warmer, and phytoplankton is developing earlier in the season. The clear water phase is also occurring earlier. Consequently, the phosphorus in the euphotic layer is depleted by mid-summer, whereas this did not happen until the autumn before 1988 (Anneville et al. 2002). Phytoplanktonic species adapted to low phosphorus concentrations are typically autumnal, but now emerge earlier in the year. These modifications in algal phenology and succession are synchronic and similar in Lakes Geneva and Constance (Anneville pers. comm.). The decreasing  $P_{\text{tot}}$  concentration limits the potential productivity, whereas higher temperatures stimulate productivity and the emergence of a new assemblage. These antagonistic effects of temperature and  $P_{\text{tot}}$  may explain that the pattern of  $\delta^{13}\text{C}$  variation in restoration is not parallel to the one in eutrophication.

In summary, the effect of eutrophication and reoligotrophication on carbon flows have been transmitted along the pelagic food chain, and have been recorded by whitefish scales. Lehmann et al. (2004) provided evidence that the  $\delta^{13}\text{C}$  of organic matter is a reliable indicator of the seasonality of the primary production and stable isotope ratios are reliable tools for tracing C-cycling in lacustrine systems. Our findings enlarge this, suggesting that whitefish scale  $\delta^{13}\text{C}$  values record the trophic evolution of lakes. Using fish scales to trace the isotopic evolution of lacustrine systems offers the prospect of many different ways of detecting historic C-cycling changes following anthropogenic disturbances, such as introductions of invasive species (Vander Zanden et al. 2003) or global warming.

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