Freshwater Biology (2009) **54,** 1663–1670 doi:10.1111/j.1365-2427.2009.02215.x

Stable isotope analysis of archived roach (*Rutilus rutilus*) scales for retrospective study of shallow lake responses to nutrient reduction

JONATHAN GREY*,†, CONOR T. GRAHAM‡, J. ROBERT BRITTON§ AND CHRIS HARROD*
*Department of Physiological Ecology, Max Planck Institute for Limnology, Plön, Germany
†School of Biological & Chemical Sciences, Queen Mary University of London, London, U.K.
‡Department of Zoology, Ecology and Plant Science, University College Cork, Cork, Ireland
§National Fisheries Laboratory, Environment Agency, Cambridgeshire, U.K.

SUMMARY

1. There is increasing interest in the use of stable isotope analysis of archived materials to study the long-term impacts of lake perturbations, including nutrient manipulation or species invasion. We tested the utility of this approach in a shallow productive lake using the zooplanktivorous early life stages of roach (*Rutilus rutilus*), a fish species that is widespread throughout Eurasian lakes.
2. Barton Broad is a shallow lake with a well-documented history of earlier eutrophication followed by nutrient reduction, including sediment removal from 1997 to 2000. Using scale samples collected pre- and post-sediment removal, we demonstrated a strong, positive relationship between roach scale $\delta^{13}C$ and total phosphorus. We argue that this reflects a decrease in the phytoplankton production which had dominated dissolved inorganic carbon dynamics, and a relative increase in the contribution of respired carbon in the food web.
3. We also derived a scale : muscle isotope relationship for roach which allowed us to model changes in fish muscle against putative prey. Concomitant isotopic shifts in preserved zooplankton samples indicated that the phosphorus reduction measures had an ecosystem-wide impact and that changes in roach scale isotope values were not a result of fish switching diet.
4. Roach scale $\delta^{15}N$ increased after sediment removal. Since this was not due to a switch in fish diet, we suggest that it probably reflects the loss of nitrogen-fixing, heterocystous cyanobacteria from the plankton.

Keywords: carbon, ecosystem perturbation, fish, nitrogen, zooplankton

Introduction

There is increasing interest in using stable isotope analysis of archived materials to examine changes in ecosystem food web structure or energy sources. Museum specimens have provided samples for reconstructions of food webs or specific trophic linkages for comparison with contemporary data. Samples such as feathers or bones are typically stored without preservatives and can be used for stable isotope analysis with confidence (e.g. Thompson, Furness & Lewis, 1995). However, other materials are preserved in a

Correspondence: Dr Jonathan Grey, School of Biological and Chemical Sciences, Queen Mary University of London, London, E1 4NS, U.K. E-mail: j.grey@qmul.ac.uk
Present address: J. Robert Britton, School of Conservation Sciences, Bournemouth University, Talbot Campus, Dorset BH12 5BB, U.K.
Present address: Chris Harrod, School of Biological Sciences, Queen's University Belfast, 97 Lisburn Road, Belfast BT9 7BL, U.K.

way which may alter isotope integrity (Feuchtmayr & Grey, 2003; Syväranta *et al*., 2008). Nevertheless, provided there are no temporal effects of the preservative and that any effects of tissue preservation on isotope values can be characterised robustly and accounted for, then analysis of preserved specimens can still be valuable. Thus, Vander Zanden *et al.* (2003) quantified food web changes in Lake Tahoe over the last century based upon stable isotope analysis of muscle tissue of formalin-preserved, archived fish and aquatic invertebrates. Similarly, Maguire & Grey (2006) analysed temporal trends in archived and preserved crustacean zooplankton samples to assess the impact of invading zebra mussels (*Dreissena polymorpha* Z.) in Lough Erne.

Although archives of preserved zooplankton exist for some well-studied lakes, they are relatively rare and may not have been consistently preserved or well maintained. In addition, to examine ecosystem-wide processes operating over seasonal, annual or interannual timescales, it is necessary to analyse many zooplankton samples to account for short-term fluctuations in isotope values driven by their phytoplankton or microbial food resource (see Matthews & Mazumder, 2005; Harrod & Grey, 2006). A simple and effective substitute is to use tissues from longer-lived, integrator organisms (*sensu* Cabana & Rasmussen, 1996). Many aquatic research institutes and fisheries departments hold extensive collections of archived fish materials such as scales, opercula or otoliths, but their potential for 'hindcasting' in aquatic systems using stable isotope analysis has yet to be explored fully (Grey, 2006). In fact, interpretation of isotope data can be uncertain in hindcasting studies unless a sound ecological basis exists regarding selection of both taxa and archived tissue for the particular scientific question. For example, fish scale stable carbon isotope values might change after an ecosystem perturbation due to an ecosystem-wide shift in the basal inorganic carbon resource assimilated by primary producers affecting the whole food web, but with no change in the food web structure *per se*. Alternatively, fish diet may have altered and hence the resultant scale isotope values reflect a different basal resource and/or trophic level of feeding. Perga & Gerdeaux (2004) demonstrated a significant correlation between whitefish (*Coregonus lavaretus* L.) scale $\delta^{13}C$ and phosphorus concentration during a period of re-oligotrophication in Lake Geneva, which they attributed to decreasing input of ^{13}C-enriched atmospheric CO_2 compensating for the dissolved inorganic carbon (DIC) fixed by photosynthesis (Schindler *et al*., 1997). Two studies of whitefish diet by conventional gut content analysis, one during the peak of eutrophication and the other at the end of the re-oligotrophication period indicated that the fish were still reliant upon the same cladoceran prey and thus any changes in isotope ratios were not due to a diet switch over time (Perga & Gerdeaux, 2004). These authors also suggested that the observed correlation between $\delta^{13}C$ and total phosphorus (TP) might only be valid for large lakes where eutrophication leads to a greater contribution of atmospheric carbon relative to endogenous carbon. The relationship may be more complex in small lakes where a greater littoral to pelagic ratio probably increases allochthonous contributions (Post, 2002) and benthic-derived DIC contributes significantly to pelagic production (Jonsson, Karlsson & Jansson, 2003), but this has yet to be tested. Furthermore, whitefish may be a good integrator organism for Lake Geneva, but is a species with a restricted distribution.

The purpose of our current study was to test the relationship of $\delta^{13}C$ and TP in a relatively small lake using roach (*Rutilus rutilus* L.) as the integrator species. Roach are widespread throughout Europe and are representative of the fish communities of a great diversity of lake systems (Lappalainen, Tarkan & Harrod, 2008). Although roach are ecological generalists, they are predominantly zooplanktivorous when small (Winfield & Nelson, 1991). Thus, by targeting the early life stages when the roach are gape-limited and should exhibit dietary fidelity to zooplankton, we were confident that any isotopic shift in roach scales observed would not be due to a dietary switch. Our study site, Barton Broad (52°44′N; 1°29′E), has a surface area of 60 ha but a mean depth of only 1.4 m and can be considered representative of small, shallow lakes. The lake was highly eutrophic by the mid-20th century, phosphorus removal was implemented at a sewage treatment plant upstream of the lake in 1977, and by the end of 1980 all significant effluent discharges upstream were either diverted or treated (Phillips *et al*., 1999). The lake was then left to recover naturally, but suction dredging of the surface sediments was undertaken from 1997 to 2000 to facilitate recovery and increase water depth. Therefore, Barton Broad has a clearly defined phosphorus

history and the lake recovery has been summarised by Phillips *et al.* (2005). If the arguments of Schindler *et al*. (1997) hold for small lakes, we would expect that, following phosphorus removal, there should have been a concomitant shift in Barton Broad toward lower $\delta^{13}C$ values in those food web components reliant upon autochthonous resources, from phytoplankton through zooplankton to zooplanktivorous 0+ roach.

Methods

To provide a contemporary comparison between roach muscle and scale stable isotope values, 1+ roach were gill-netted from a number of northern European lakes (J. Grey & C. Harrod, unpubl. data). Carbonates were removed from scales using a modification of the protocol developed for whitefish scales by Perga & Gerdeaux (2003). Briefly, scales were immersed in 1.2N HCl for 2 min, and subsequently passed through three distilled water baths prior to oven-drying at 60 °C. Muscle tissue was excised from above the lateral line, and below the dorsal fin to standardise between fish. Lipids were removed from muscle tissue by chloroform–methanol extraction (Bligh & Dyer, 1959) which does not alter $\delta^{15}N$ values (allowing dual isotope analyses from single samples, Ingram *et al*., 2007), the tissues were oven-dried at 60 °C, pulverised in an agate pestle and mortar and stored in glass vials.

Archived roach scales, which had been collected from Barton Broad by the Environment Agency and its predecessor organisations, and stored dried in paper envelopes, were available in sufficient quantities (>5 per individual fish) from 1985, 1988, 1996, 2000, 2001 and 2002; 3 years pre- and post-sediment removal. Five scales from eight to 10 individuals were age-confirmed at 1+ using a microfiche and acid-treated, as above. However, since the fish had been collected in late spring, the scale material actually reflected the previous summer growth when the fish were 0+. Zooplankton was collected on a monthly or bi-monthly basis by the same organisations for determination of community composition and subsequently preserved with 70% ethanol. Ethanol did not alter stable isotope composition of zooplankton significantly in previous studies (Feuchtmayr & Grey, 2003; Syväranta *et al*., 2008) and so we did not compensate for any effect of preservative. Summer monthly samples, probably corresponding to the main period of fish feeding and growth (Harrod & Grey, 2006), were examined using a microscope to determine suitability (sufficient quantity, amount of contaminant matter) for stable isotope analysis. Samples were too small to be able to separate taxa and so zooplankton was analysed in bulk from July and August (range: 2–5 samples). Long-term monitoring data of nutrient dynamics in Barton Broad were similarly sourced (see Phillips *et al*., 2005).

Samples for stable carbon and nitrogen analyses were weighed into tin capsules and combusted in a Eurovector elemental analyser interfaced to a Micromass Isoprime isotope ratio mass spectrometer. Isotope ratios are expressed conventionally in per mille (‰), relative to a secondary standard of known relation to Vienna PDB or atmospheric nitrogen for $\delta^{13}C$ and $\delta^{15}N$ respectively. Repeat analysis of internal fish muscle standards inserted after every five samples, indicated measurements were precise to <0.2‰.

Comparisons were drawn between the stable isotopes of acidified scales and defatted muscle tissue using linear regression. To determine any shift in stable isotope values over time, one-way ANOVA was used to compare mean scale or zooplankton stable isotope values from the six separate year classes in conjunction with Bonferonni *post hoc* comparisons. Prior to analysis by ANOVA data were checked for normality (Anderson-Darling test) and homogeneity of variance (Levene's test). All variables apart from scale $\delta^{13}C$ ($P < 0.01$) were normally distributed, and all displayed homogeneity of variance. As ANOVA is largely resistant to deviations from normality (Tabachnick & Fidell, 2001), we retained its use for all comparisons of variation in mean isotope values. Pearson's correlation analysis was used to determine relationships between annual mean monthly TP concentrations and mean annual scale stable isotope values.

Results

The stable carbon and nitrogen isotope values of acidified roach scales from contemporary samples both exhibited a linear relationship with defatted muscle (Fig. 1) and the deviation of either slope (calculated by linear regression) from 1 was not significant.

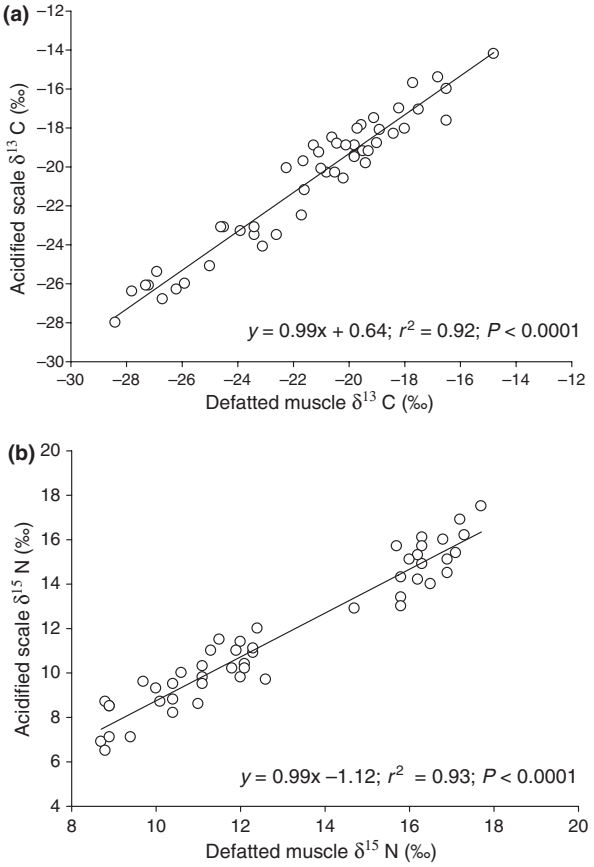

Fig. 1 Comparison between roach dorsal muscle (defatted) and acidified scales for (a) δ^{13}C and (b) δ^{15}N; linear regressions are fitted, $n = 49$.

Individual roach collected from the same year exhibited archived scale δ^{13}C variability spanning up to 2‰ in 1984, and the least variability in 2001 (SD = 0.3‰, $n = 9$, Table 1); scale δ^{15}N was most variable in 1999 (SD = 0.8‰, $n = 9$, Table 1). Among years, Barton Broad individual roach scale δ^{13}C spanned almost 5‰ from −31.1‰ to −26.2‰ and δ^{15}N ranged from 15.3‰ to 19.8‰. Results of a one-way ANOVA comparing mean scale stable isotope values from the six separate year classes showed significant changes over time (δ^{13}C: $F_{5,50} = 78.5$; $P < 0.001$; δ^{15}N: $F_{5,50} = 18.8$; $P < 0.001$). Bonferonni *post hoc* comparisons showed that scale δ^{13}C was relatively consistent pre-manipulation, and values from the 3 years post-manipulation were not significantly different from each other (Fig. 2). Temporal patterns in δ^{15}N were not so clear (Table 1); there was a gradual increase from 1984 to 1999, and although immediately post-manipulation (1999) was significantly different from pre-manipulation (1996), there was no significant difference between 1996 and 2000–01. Annual mean monthly TP declined from *c.* 0.3 mg L^{-1} in the late-1970s to *c.* 0.06mg L^{-1} in 2002 (Fig. 2). Scale δ^{13}C and δ^{15}N values were both

Table 1 Summary of roach scale and zooplankton stable isotope values

Years	1984	1987	1995	1999	2000	2001
Scale δ^{13}C (‰)						
n	8	10	10	9	10	9
Minimum	−28.4	−28.7	−28.7	−30.9	−31.1	−31.1
Maximum	−26.2	−27.3	−26.6	−29.0	−29.9	−30.2
Mean	−27.0	−28.0	−27.3	−30.1	−30.3	−30.6
SD	0.7	0.5	0.7	0.6	0.4	0.3
Zooplankton δ^{13}C (‰)						
n	3	3	2	4	5	3
Minimum	−28.3	−27.0	−27.1	−29.6	−31.2	−32.3
Maximum	−24.1	−25.6	−25.1	−28.8	−29.2	−29.8
Mean	−25.8	−26.4	−26.1	−29.4	−30.4	−31.0
SD	2.3	0.7	1.4	0.4	1.0	1.3
Scale δ^{15}N (‰)						
n	8	10	10	9	10	9
Minimum	15.3	16.4	17.1	17.5	17.5	17.2
Maximum	17.1	17.7	18.6	19.8	19.3	18.8
Mean	16.5	17.0	17.9	18.9	18.1	18.0
SD	0.7	0.4	0.6	0.8	0.7	0.5
Zooplankton δ^{15}N (‰)						
n	3	3	2	4	5	3
Minimum	11.9	14.7	13.6	16.2	15.7	15.8
Maximum	16.2	15.1	14. 9	17. 6	16.9	17.7
Mean	13.4	14.9	14.2	16.8	16.1	16.7
SD	2.4	0.2	0.9	0.6	0.4	1.0

Years relate to roach year class; scales were taken in the following year when fish were 1+.

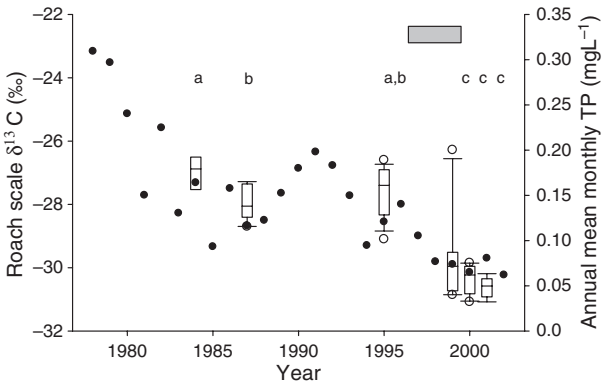

Fig. 2 Box and whisker plots for roach scale δ^{13}C overlaying annual mean monthly total phosphorus (TP) concentration (closed circles) in Barton Broad. Whiskers represent 10th and 90th percentiles; open circles indicate outliers; lower-case letters above box-plots relate to values shown not to be significantly different after Bonferonni *post hoc* comparisons. The shaded bar represents the period of sediment removal from the lake.

significantly correlated with TP (δ^{13}C: $r = 0.922$; $P = 0.009$; δ^{15}N: $r = -0.864$; $P = 0.026$).

Bulk zooplankton stable isotope values exhibited most variability prior to sediment removal (±2‰ SD for δ^{13}C and δ^{15}N) and among the years of study spanned 8.2‰ (δ^{13}C: −32.3‰ to −24.1‰) and 5.8‰ (δ^{15}N: 11.9–17.7‰); Table 1. Zooplankton also exhibited significantly different mean stable isotope values pre- and post-sediment removal (one-way ANOVA; δ^{13}C: $F_{5,13} = 11.18$; $P < 0.001$; δ^{15}N: $F_{5,13} = 4.61$; $P = 0.012$; Table 1, Fig. 3). We derived stable isotope values for roach muscle from the relationships between contemporary muscle and scale tissues (Fig. 1) and plotted them with bulk zooplankton (Fig. 3). The general direction of the isotopic shift for muscle was similar to that in zooplankton (as we predicted) but the magnitude of change was not as great in the fish (Fig. 3). Mean δ^{13}C values for roach were 2.0‰ lower than their assumed prey prior to sediment removal and only 0.8‰ lower afterwards; mean roach δ^{15}N values were 4.2‰ higher than zooplankton prior to sediment removal and 3.1‰ higher afterwards. There was a strong correlation between scale and zooplankton mean δ^{13}C values ($r = 0.987$, $P = 0.0002$).

Discussion

If the purpose of the study simply had been to determine any isotopic shift over time, then there would be no need to determine any relationship between archived scale tissue and fish muscle. However, because we wanted to compare the magnitude of any temporal isotopic shift in both fish and their putative prey, in this instance zooplankton, it was necessary to relate scale isotope values to those of muscle. This is because muscle is generally considered the tissue of choice for stable isotope dietary studies since it most closely resembles the dietary source (Pinnegar & Polunin, 1999). The significant and strong, positive linear relationships between stable isotope values of acidified scale and defatted dorsal muscle from roach collected from a variety of European lakes of differing size and productivity clearly demonstrate that archived scale material can be used as a proxy for muscle in hindcasting studies such as this. This supports the appraisal by Syväranta *et al.* (2008) of the use of stable isotope analysis of archived materials. Although the general relationship they reported for roach tissues from a suite of Finnish lakes was essentially the same, the mean isotopic difference between scale and muscle differed from our results. Their roach were sourced from relatively nutrient poor, low DIC lakes; the scale material used was cut from the outer ring of growth and material pooled from several scales for single analyses; and a relationship was drawn between scale and muscle on the premise that their roach were lipid poor. As outlined in the methods, we used whole scales sourced from a wide range of systems of varying size and productivity and compared the acidified scale to defatted muscle tissue. Hence any of the above methodological variations may have contributed to the slight differences in results between the two studies.

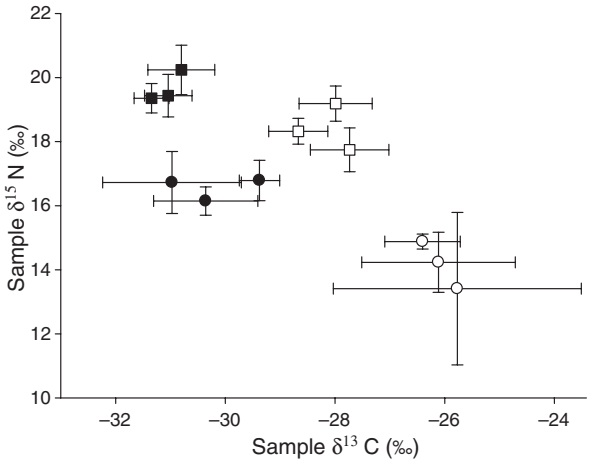

Fig. 3 Stable isotope bi-plot showing mean ± 1 SD of bulk zooplankton (circles) and theoretical roach muscle (squares) calculated using the relationships depicted in Fig. 1. Open and closed symbols represent samples collected from pre- and post the sediment removal manipulation of Barton Broad, respectively.

Roach scales were markedly ^{13}C-depleted after the sediment removal period in Barton Broad. We rule out the possibility of this change being induced by a dietary switch for the following reasons. We analysed scales from 1+ roach which would primarily reflect the diet assimilated by those fish in their first summer of growth and thus avoid any issues arising from ontogeny. Fish of such a size are gape-limited and restricted in their choice of diet; roach in Broadland lakes are predominantly zooplanktivorous, particularly in algal-dominated waters (Cryer, Peirson & Townsend, 1986). Furthermore, Phillips *et al.* (2005) were surprised to note that there were no substantial changes in zooplankton community composition in

Barton Broad spanning the manipulation period. This contrasts with findings from other shallow lakes (e.g. Jeppesen, Jensen & Søndergaard, 2002). Total zooplankton biomass was reduced suggesting that the fish population remained zooplanktivorous, but there was no reduction in individual *Daphnia hyalina* (L.) body size. A decrease in larger zooplankter body size might have increased the number of prey species available to small roach and potentially allowed them to source their carbon differently, but this does not appear to have been the case.

Moreover, unlike Perga & Gerdeaux (2003), we analysed the stable isotopes of the putative prey of the roach from the corresponding years under study and the zooplankton showed a similar temporal trend to the fish of ^{13}C-depletion and ^{15}N-enrichment after the sediment removal had occurred. The fish were not 'classically' related to their prey by the oft quoted +0–1‰ for δ^{13}C and +3‰ for δ^{15}N but nevertheless, were closer to the zooplankton values than to benthic macroinvertebrates, which in these shallow broads typically have δ^{13}C values *c.* 2–4‰ lower and δ^{15}N values 4–7‰ lower than zooplankton (J. Grey & J. Laws, unpubl. data). Roach scale or muscle is a temporal integrator of the diet over a considerable, and varying, period with scale tissue being a more permanent record because the carbon and nitrogen laid down is less likely to be remobilised and metabolised, especially at the 1+ age that we sampled (Mugiya & Watabe, 1977). This will occur when energy is apportioned to reproductive output in later life (Lappalainen *et al.*, 2008). The zooplankton samples we analysed as representative of roach diet (i.e. between two and five summer samples), would be sufficient to give an estimate of dietary isotope value but would not have captured the full range of temporal isotopic variability that such small organisms can express over a growing season (e.g. Matthews & Mazumder, 2005; Harrod & Grey, 2006). Hence, we would not expect a 'perfect' isotopic shift from prey to consumer but we are confident that there was no change in diet because assimilation from alternative macroinvertebrate prey would have caused a stable carbon and nitrogen isotopic shift in the fish scales of much greater magnitude.

It is more likely that the changes in roach scale δ^{13}C simply reflected changes in the water column DIC as a result of a nutrient reduction which, we suggest, is why there was such a strong correlation between annual mean monthly TP and scale δ^{13}C (r = 0.922). When TP and consequently primary productivity within the water column were high, demand for DIC also would have been high resulting in reduced isotopic fractionation and a characteristically ^{13}C-enriched phytoplankton dominated by cyanobacteria. Reduced DIC concentrations within the epilimnion would be balanced by influx of atmospheric CO_2 (relatively ^{13}C-enriched). A reduction in primary productivity induced by sediment removal would result in a shift toward proportionately more respiration and subsequent ^{13}C-depletion of the DIC due to recycling (France, del Giorgio & Westcott, 1997). Furthermore, Phillips *et al.* (2005) noted greater water clarity in Barton Broad post-manipulation leading to a gradual shift from planktonic to benthic algal production and associated respiration (DeNicola *et al.*, 2003).

Although there was a significant increase in roach scale δ^{15}N over the total period of lake manipulation, and a significant negative correlation with annual mean monthly TP (P = 0.026), the magnitude of the shift was less than that observed for carbon with only *c.* 1‰ difference between mean scale δ^{15}N values immediately pre- and post-sediment removal. Baseline δ^{15}N is already high in the Norfolk Broads which are surrounded by agricultural land and hence subject to ^{15}N-enriched nutrient loading (Cabana & Rasmussen, 1996). The gradual increase in δ^{15}N over time is unlikely to be due to an increase in trophic level because we are assuming there was no dietary shift. Even if roach switched to feeding on different cladoceran prey (*Bosmina* versus *Daphnia*) there would have been no discernible shift in δ^{15}N as these planktonic organisms are isotopically indistinct in such polymictic systems (J. Grey, unpubl. data). Instead, we hypothesise that the trend reflects the observed change in phytoplankton community; heterocystous species such as *Anabaena* and *Aphanizomenon flos-aquae* (L) were slow to decline but these species are able to fix atmospheric nitrogen (at a metabolic cost) which is relatively ^{15}N-depleted. Thus, the reduction of cyanobacteria reported by Phillips *et al.* (2005) actually may have contributed to an overall increase in δ^{15}N throughout the food web. Interestingly, within-year isotopic variability decreased for both zooplankton and fish after the sediment removal. There is some suggestion that the magnitude of temporal variation in zooplankton isotope values may increase with increasing productivity

(e.g. oligotrophic Loch Ness, annual $\delta^{13}C$ range = 5‰, Grey, Jones & Sleep, 2001; highly eutrophic Plußsee, annual $\delta^{13}C$ range = 25.6‰, Harrod & Grey, 2006), in which case the reduction in intra-annual isotopic variability in Barton Broad is consistent with the reduced nutrient status of the lake. It might then be predicted as a consequence of the reduction of cyanobacteria species in Barton Broad because, although they are not preferred choice of food for zooplankton, many taxa can still sustain themselves upon cyanobacterial carbon if there is little else available (Tillmanns *et al*., 2008). Prior to sediment removal, the span of nitrogen isotope values in the basal resources would likely have been greater because of the greater proportion of heterocystous species contributing to the phytoplankton community.

Like Perga & Gerdeaux (2003), we have shown a correlation between fish scale stable carbon isotope values and phosphorus loading in a lake system that has undergone anthropogenic manipulation to reduce nutrient loading. Thus, we provide further evidence that fish scales can be used as indicators of long-term variation in aquatic productivity. Importantly, we have shown that such a relationship is still clear even in a small, shallow lake with a greater potential contribution of allochthony to food web resources (Post, 2002), or benthic DIC contribution to pelagic production (Jonsson *et al*., 2003). Finally, it appears that, despite the close proximity of benthic and pelagic compartments in small, shallow lakes, our chosen indicator species (the 0+ zooplanktivorous life stages of an ecological generalist, the roach) still allowed detection of a change in lake productivity. Thus, the actual species selected appears less important than selecting for a life stage that exhibits strong dietary fidelity that is unlikely to be affected by environmental perturbations.

Acknowledgments

We wish to thank G. Phillips & J. Johnson (Environment Agency) for access to data and a manuscript in preparation and for tracking down some of the archived zooplankton samples. J. Bays and N. Ings (QMUL) and H. Buhtz and A. Möller (MPIL) are thanked for assistance with sample preparation and isotopic analyses. The manuscript was improved with inputs from the editor R.I. Jones, M.-E. Perga and another referee.

References

Bligh E.G. & Dyer W.J. (1959) A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, **37**, 911–917.

Cabana G. & Rasmussen J.B. (1996) Comparison of aquatic food chains using nitrogen isotopes. *Proceedings of the Natural Academy of Science of the United States of America*, **93**, 10844–10847.

Cryer M., Peirson G. & Townsend C.R. (1986) Reciprocal interactions between roach, *Rutlius rutilus*, and zooplankton in a small lake: prey dynamics and fish growth and recruitment. *Limnology and Oceanography*, **31**, 1022–1038.

DeNicola D.M., de Eyto E., Wemaere A. & Irvine K. (2003) Production and respiration of epilithic algal communities in Irish lakes of different trophic status. *Archiv für Hydrobiologie*, **157**, 67–87.

Feuchtmayr H. & Grey J. (2003) Effect of preparation and preservation procedures on carbon and nitrogen stable isotope determinations from zooplankton. *Rapid Communications in Mass Spectrometry*, **17**, 2605–2610.

France R.L., del Giorgio P.A. & Westcott K.A. (1997) Productivity and heterotrophy influences on zooplankton $\delta^{13}C$ in northern temperate lakes. *Aquatic Microbial Ecology*, **12**, 85–93.

Grey J. (2006) Stable isotopes in freshwater ecology: current awareness. *Polish Journal of Zoology*, **54**, 563–584.

Grey J., Jones R.I. & Sleep D. (2001) Seasonal changes in the importance of allochthonous organic matter to the diet of zooplankton in Loch Ness indicated by stable isotope analysis. *Limnology and Oceanography*, **46**, 505–513.

Harrod C. & Grey J. (2006) Isotopic variation complicates analysis of trophic relations within the fish community of Plußsee: a small, deep, stratifying lake. *Archiv für Hydrobiologie*, **16**, 281–299.

Ingram T., Matthews B., Harrod C., Stephens T., Grey J., Markel R. & Mazumder A. (2007) Lipid extraction has little effect on the $\delta^{15}N$ of aquatic consumers. *Limnology Oceanography Methods*, **5**, 338–343.

Jeppesen E., Jensen J.P. & Søndergaard M. (2002) Response of phytoplankton, zooplankton, and fish to re-oligotrophication: an 11 year study of 23 Danish lakes. *Aquatic Ecosystem Health and Management*, **5**, 31–43.

Jonsson A., Karlsson J. & Jansson M. (2003) Sources of carbon dioxide supersaturation in clearwater and humic lakes in northern Sweden. *Ecosystems*, **6**, 224–235.

Lappalainen J., Tarkan A.S. & Harrod C. (2008) A meta-analysis of latitudinal variations in life-history traits of roach, *Rutilus rutilus*, over its geographical range: linear or non-linear relationships? *Freshwater Biology*, **53**, 1491–1501.

Maguire C. & Grey J. (2006) Determination of zooplankton dietary shift following a zebra mussel invasion, as indicated by stable isotope analysis. *Freshwater Biology*, **51**, 1310–1319.

Matthews B. & Mazumder A. (2005) Consequences of large temporal variability of zooplankton δ^{15}N for modeling fish. *Limnology and Oceanography*, **50**, 1404–1414.

Mugiya Y. & Watabe N. (1977) Studies on fish scale formation and resorption – II. Effect of estradiol on calcium homeostasis and skeletal tissue resorption in the goldfish, *Carassius auratus*, and the killifish, *Fundulus heteroclitus*. *Comparative Biochemistry and Physiology Part A: Physiology*, **57**, 197–202.

Perga M.-E. & Gerdeaux D. (2003) Using the δ^{13}C and δ^{15}N of whitefish scales for retrospective ecological studies: changes in isotope signatures during the restoration of Lake Geneva, 1980–2001. *Journal of Fish Biology*, **63**, 1197–1207.

Perga M.-E. & Gerdeaux D. (2004) Changes in the δ^{13}C of pelagic food webs: the influence of lake area and trophic status on the isotopic signature of whitefish (*Coregonus lavaretus*). *Canadian Journal of Fisheries and Aquatic Sciences*, **61**, 1485–1492.

Phillips G.L., Bramwell A., Pitt J., Stansfield J. & Perrow M. (1999) Practical application of 25 years' research in the management of shallow lakes. *Hydrobiologia*, **395**/396, 61–76.

Phillips G., Kelly A., Pitt J., Sanderson R. & Taylor E. (2005) The recovery of a very shallow eutrophic lake, 20 years after the control of effluent derived phosphorus. *Freshwater Biology*, **50**, 1628–1638.

Pinnegar J.K. & Polunin N.V.C. (1999) Differential fractionation of δ^{13}C and δ^{15}N among fish tissues: implications for the study of trophic interactions. *Functional Ecology*, **13**, 225–231.

Post D.M. (2002) The long and short of food chain length. *TREE*, **17**, 269–277.

Schindler D.W., Carpenter S.R., Cole J.J., Kitchell J.F. & Pace M.L. (1997) Influence of food web structure on carbon exchange between lakes and the atmosphere. *Science*, **277**, 248–251.

Syväranta J., Vesala S., Rask M., Ruuhijärvi J. & Jones R.I. (2008) Evaluating the utility of stable isotope analyses of archived freshwater sample materials. *Hydrobiologia*, **600**, 121–130.

Tabachnick B.G. & Fidell L.S. (2001) *Computer-Assisted Research Design and Analysis*. Allyn and Bacon, Boston, MA.

Thompson D.R., Furness R.W. & Lewis S.A. (1995) Diets and long-term changes in δ^{15}N and δ^{13}C values in northern fulmars *Fulmarus glacialis* from two northeast Atlantic colonies. *Marine Ecology Progress Series*, **125**, 3–11.

Tillmanns A.R., Wilson A.E., Pick F.R. & Sarnelle O. (2008) Meta-analysis of cyanobacterial effects on zooplankton population growth rate: species-specific responses. *Fundamental and Applied Limnology*, **171**, 285–295.

Vander Zanden M.J., Chandra S., Allen B.C., Reuter J.E. & Goldman C.R. (2003) Historical food web structure and restoration of native aquatic communities in the Lake Tahoe (California–Nevada) basin. *Ecosystems*, **6**, 274–288.

Winfield I.J. & Nelson J.S. (1991) *Cyprinid Fishes (Systematics, Biology and Exploitation)*. Chapman & Hall, London.

(Manuscript accepted 26 February 2009)