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Impacts of Biomanipulation on Lake Ecosystem Structure Revealed by Stable Isotope Analysis

JYVÄSKYLÄN YLIOPISTO

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ABSTRACT

Eutrophication of aquatic ecosystems is an increasing problem, especially threatening small and shallow freshwater lakes. While the extreme inputs of nutrients (phosphorus and nitrogen) can nowadays be reduced and controlled rather efficiently, additional measures to reverse eutrophication may be needed. These often involve biomanipulation, typically mass removal of cyprinid fish. However, the success of biomanipulations has many times been limited or short lived. The reasons for this are not well known, perhaps because the more subtle impacts of biomanipulation on the ecosystem-wide processes of lakes have not been thoroughly studied. Natural abundance stable isotopes may provide a cost-effective tool to study such ecosystem level impacts of food web perturbations. In this thesis, I used stable isotope analysis (SIA) to study the impacts of biomanipulation on lake ecosystem properties in Jyväsjärvi, a lake recovering from severe eutrophication. Variability in stable isotope signatures within the studied lake was examined as a prerequisite for reliable SIA. Temporarily lower $\delta^{15}N$ values of fish in one area of Jyväsjärvi resulted from extensive fish migration into Jyväsjärvi from the connected lake Päijänne. Stable isotope data indicated that the migration was considerably reduced following biomanipulation and the immigrant fish from Päijänne did not quickly recolonise Jyväsjärvi. Isotopes also indicated significant changes in feeding niche widths of perch and roach after fish removals. From a wider perspective, contribution from pelagic energy sources to higher trophic levels in the Jyväsjärvi ecosystem increased after biomanipulation, in contrast to expectation. To enhance the potential of SIA in ecological studies, I also tested the possibility of using archived sample materials in retrospective SIA, which could allow for longer time perspectives in such ecosystem studies. I found that typical archived freshwater samples do provide valid materials for SIA.

Keywords: Biomanipulation; food chain; Jyväsjärvi; lake ecosystem; niche; restoration; stable isotopes.

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CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following five original papers, which will be referred to in the text by their Roman numerals I-V.

I did most of the planning for each of the studies and I was also responsible for collecting and analysing the data. I wrote all the papers and the co-authors have contributed to statistical analyses and revising the papers.

I   Syväranta, J., Hämäläinen, H. & Jones R. I. 2006. Within-lake variability in carbon and nitrogen stable isotope signatures. Freshwater Biology 51: 1090-1102.

II   Syväranta, J., Keskinen, T., Hämäläinen, H., Karjalainen, J. & Jones, R. I. 2008. Use of stable isotope analysis to evaluate the possible impact of fish migration on a lake biomanipulation. Aquatic Conservation: Marine and Freshwater Ecosystems. In press.

III   Syväranta, J. & Jones, R. I. 2008. Changes in feeding niche widths of perch and roach following biomanipulation, revealed by stable isotope analysis. Freshwater Biology. In press.

IV   Syväranta, J. & Jones, R. I. Altered energy flow pathways in a lake ecosystem following anthropogenic perturbation. Submitted manuscript.

V   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. In press.

1 INTRODUCTION

1.1 Eutrophication of lake ecosystems

Eutrophication of freshwater lakes, the enrichment of water by nutrients (mainly phosphorus and nitrogen), is an increasing problem threatening lakes throughout the world. Eutrophication is still a problem in Finland, for which the causes and consequences are many. Most severely eutrophicated Finnish lakes mainly suffered from point source pollution until 1970-80, when improvements in municipal and industrial waste water treatment processes reduced the external nutrient loading. These changes considerably improved the water quality of many polluted lakes, but over 2000 lakes in Finland are still estimated to be affected by eutrophication (Tammi et al. 1999, Mannio et al. 2000). Today freshwater lakes are mainly polluted from nonpoint sources, agriculture being the main source of anthropogenic phosphorus and nitrogen to lakes both in Finland and elsewhere (Rekolainen 1993, Carpenter et al. 1998). This means that although the most intense eutrophication from point source pollution is now effectively controlled, the less intense eutrophication from non-point sources is a more widespread problem leading to slow eutrophication of previously unaffected lakes.

The consequences of eutrophication are both environmental and socio-economical. Elevated nutrient concentrations increase algal biomass and water turbidity, decreasing the recreational value of a lake. Continued and more severe eutrophication may lead to an algal community dominated by cyanobacteria (blue green algae) and toxic cyanobacterial blooms may prevent most uses of the lake. Also the fish communities of these lakes change as cyprinids become more abundant with increasing eutrophication (e.g. Persson et al. 1991, Olin et al. 2002), leading to a decrease in biodiversity (Jeppesen et al. 2000). The increase in cyprinid abundance not only further enhances the eutrophication of lakes by increased planktivory and recycling of nutrients from the sediments (Hansson et al. 1998, Horppila et al. 1998), but also decreases the resource value of the lake. Tammi et al. (1999) estimated that in

10% of Finnish lakes the fishery values had decreased due to eutrophication. In severe cases of eutrophication, oxygen depletions caused by decomposing organic material may lead to large-scale fish kills and total disappearance of some fish species.

1.2   Lake restorations by biomanipulation

Considerable effort has been put into understanding the causes of eutrophication and developing strategies for controlling and reversing its impacts on water quality (Perrow et al. 1997, Hansson et al. 1998, Drenner & Hambright 1999, Benndorf et al. 2002, Mehner et al. 2002). The basic strategy to reduce loading of nutrients to lakes has generally proved successful, but additional measures are often required, typically involving "biomanipulation" (Shapiro & Wright 1984) of the lake fish community. The method is based on a theory of top-down control of pelagic food chains in lakes (Hairston et al. 1960, Carpenter et al. 1985), where the effects of consumer populations at the top of the food chain are expected to cascade down to the producer level of the food chain. The basic strategy is to reduce predation on herbivorous zooplankton by removing planktivorous fish or increasing the abundance of piscivores (or both), thereby promoting changes in zooplankton abundance and composition and hence increasing the grazing on phytoplankton. The ultimate aim of biomanipulation is to reduce algal biomass and increase water clarity. This seemingly rather straightforward strategy has sometimes been successful, but in many cases any success has been less obvious or short-lived (DeMelo et al. 1992, Hansson et al. 1998, Drenner & Hambright 1999, Benndorf et al. 2002, Søndergaard et al. 2007). The reasons for unsuccessful biomanipulations have not always been easy to identify, but many times the removal of planktivorous fish has not been sufficient to create a cascading effect to lower trophic levels and phytoplankton. Also, in some cases the manipulated fish community has returned to its original state soon after the biomanipulation has been stopped, even after intense and efficient fish removals. In part this could be due to considerable increases in the abundance of young-of-the-year (YOY) fish following removal of older fish and increased resources (Hansson et al. 1998).

The "top-down" control of aquatic food webs is challenged by an opposite theory of "bottom-up" control, in which the food chains are more strongly controlled from the bottom by resources and producers (Lindeman 1942, McQueen et al. 1986). This theory also suggests that top-down control is strongest at the top of the food chain but the effect weakens towards the bottom of the food chain (McQueen et al. 1986). In practice, probably both top-down and bottom-up forces concomitantly affect aquatic food chains, but it can be extremely difficult to assess which effect is stronger.

Generally biomanipulation as a restoration tool is considered more efficient in shallow lakes (e.g. Jeppesen et al. 1990, Benndorf et al. 2002), where extensive coverage of aquatic macrophytes can control excess algal growth and

create better opportunities for benthivorous feeding (e.g. Scheffer et al. 1993, Schriver et al. 1995, Jeppesen et al. 1997). Increased macrophyte coverage after biomanipulation may shift a turbid, phytoplankton-dominated lake into a clear water state as an alternate stable state (Scheffer 1989, Scheffer et al. 1993). Although Finnish lakes are typically rather shallow (e.g. Keto et al. 2004) and therefore potentially suitable for restoration by biomanipulation, many Finnish lakes also have relatively high humic content and coloured water, which naturally decreases water transparency. In this case, even successful biomanipulations (in terms of increased herbivore abundance and lower algal biomass) may not result in considerably increased water transparency. This would also mean that one of the desired results of biomanipulation, increased littoral production and macrophyte growth (Schriver et al. 1995, Moss et al. 1996a, 1996b, Hansson et al. 1998), is unlikely. Nevertheless, biomanipulation has also produced positive changes in deeper, stratifying lakes (Olin et al. 2006). For example, one of the largest successful biomanipulation experiments was done in Lake Vesijärvi, Finland, between 1989 and 1993, where positive results on water clarity were gained even in the absence of any significant impact on the zooplankton community (Horppila et al. 1998, Kairesalo et al. 1999). Although a lot of research has been done on lake restorations by biomanipulation, it still seems that the underlying impacts of biomanipulation on lake ecosystem structure and functioning are not fully understood. The wider implications to energy flow patterns and trophic structures in entire lake ecosystems have not been thoroughly investigated following mass fish removals. One obvious reason for the lack of knowledge on the effects of biomanipulation on these more subtle ecosystem properties is the sheer amount of work needed to study such ecosystem properties and processes by conventional methods. Stable isotope analysis (SIA) potentially provides more economical and integrative measures of these ecosystem properties and, combined with some basic research methods, could yield valuable data on ecosystem structure and functioning.

1.3   Stable isotope analysis

The use of SIA techniques has increased considerably in ecological studies over recent years. Almost all elements have different forms in which they can exist in nature, both stable and unstable (radioactive). These different forms are called *isotopes* and they differ only in the number of neutrons in their nucleus. For example, carbon can have several different isotopes, but only two stable isotopes (^{13}C and ^{12}C), which are used in SIA. The lighter isotopes are always present in greater abundance than the heavier ones in nature; for example, for carbon stable isotopes the abundances are ^{12}C=98.89% and ^{13}C=1.11%. Different number of neutrons in the atoms of the same element makes the isotopes differ in their size and atomic weight. This means that isotopes of the same element react at different rates in the same chemical or biochemical reactions. The result

is that the ratio of heavier to lighter isotopes changes in these reactions (*fractionation*). In other words, stable isotope methods are based on predictable changes in isotopic compositions as elements cycle through the biosphere (Peterson & Fry 1987).

The most often used elements in ecological stable isotope studies are carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$). Isotope values are typically expressed with a δ-notation (e.g. $δ^{13}C$, $δ^{15}N$), because the ratios analysed from samples are always compared to ratios in an international standard and reported in ppt (‰) differences. The fractionation of carbon isotopes is very little in animals (0-1‰ between an animal and its diet), meaning that the carbon isotope signature analysed, for example from fish, should reflect that of its prey (DeNiro & Epstein 1978, Peterson & Fry 1987). Thus carbon isotopes can be used as tracers in studies of the origins of energy in organisms. Nitrogen, on the other hand, fractionates more and so consumers are enriched in the heavier isotope relative to their diet (Minagawa & Wada 1984, Peterson & Fry 1987), and this can be used to estimate trophic positions. The advantage of using stable nitrogen isotopes to estimate trophic positions of consumers is that trophic positions can be quantified on a continuous scale instead of the traditional discrete trophic levels (e.g. Cabana & Rasmussen 1994, Vander Zanden et al. 1997, Vander Zanden & Rasmussen 1999, Vander Zanden et al. 1999, Vander Zanden et al. 2000, Grey et al. 2002). The enrichment of the heavier nitrogen isotope has been observed to be approximately 3-4‰ between each trophic transfer in several studies concerning animals (e.g. Minagawa & Wada 1984, Peterson & Fry 1987, Vander Zanden et al. 1997, Post 2002, McCutchan et al. 2003), and this value is now widely used to set trophic positions for individual organisms. The advantage of stable isotope analyses over more traditional diet analyses from gut contents is that stable isotopes, for example from fish muscle tissue, incorporate dietary information over a long time period, typically from weeks to months depending on how rapidly the fish is synthesising new tissue (mostly due to growth) (Hesslein et al. 1993, Vander Zanden et al. 1998, Maruyama et al. 2001, Zuanon et al. 2006). Typical gut content analyses are more like "snapshots" of what has been eaten during the past few hours and can sometimes be distorted by rarely eaten prey. Also, SIA provides information of *assimilated* diet as opposed to traditional gut contents analysis, which only tells what has been *ingested*. In the case of omnivores like roach (*Rutilus rutilus* (L.)) this may be particularly important, since roach may ingest a lot of material that passes through the gut without ever being assimilated. Therefore, SIA provides a better assessment of dietary proportions. However, stable isotopes provide only limited taxonomic information, which is clearly an advantage of more traditional methods. Therefore, it would be beneficial to combine isotope methods with more traditional ones, when possible.

1.4   Aims of the study

The purpose of this thesis work was to evaluate, using carbon and nitrogen stable isotope analyses, impacts of biomanipulation on a lake ecosystem. The aim was to take advantage of a recent biomanipulation project in Jyväsjärvi, where impacts on water properties, phytoplankton, zooplankton and fish communities were already monitored in a different project. Therefore, stable isotopes were included as an additional measure to investigate the impacts on energy flow patterns, trophic structures and species interactions in the Jyväsjärvi ecosystem.

However, to apply SIA with confidence in this kind of ecosystem-wide perturbation study, any possible biases that could affect the results must be carefully considered. One such potential bias could result from overlooked spatial and temporal variability of isotope signatures within a single lake ecosystem. The first paper (I) of this thesis concentrates on evaluating such variability within Jyväsjärvi. Not much is known of such variation in isotope signatures within a single ecosystem and these results will help in planning future sampling programs and potentially help to reduce the sampling work.

Another issue of concern was the close connection between Jyväsjärvi and the larger lake Päijänne, the second largest lake system in Finland. Concerns had been raised whether the removed fish from Jyväsjärvi would quickly be replaced by immigrant fish from Päijänne. A previous study by Lilja et al. (2003) reported considerable spring time upstream movement in a 700 m canal connecting Jyväsjärvi to Päijänne. Any biomanipulation of Jyväsjärvi could be seriously compromised if these fish would start to recolonise Jyväsjärvi immediately after fish removals. The second paper (II) of the thesis tries to evaluate the extent of this potential fish migration and its implications on the biomanipulation of Jyväsjärvi. SIA has been used successfully in different migration studies (Hansson et al. 1997, Doucett et al. 1999, Hobson 1999, Rubenstein & Hobson 2004, Harrod et al. 2005), since isotope signatures in different ecosystems can differ significantly, and organisms in these ecosystems ultimately become "imprinted" to their habitats. Immigrant fish or other organisms can then be differentiated from resident ones by their isotope signatures.

Papers III and IV concentrate more on the ecological impacts of biomanipulation. The main research objectives related to these papers can be categorised as follows:

1) To evaluate changes in trophic position and niche breadth of key species following changes in fish biomass.

2) To quantify changes in energy flow patterns within lake ecosystems under contrasting conditions of fish biomass.

Within these general objectives, more specific, testable hypotheses can be formulated based on ecological theory:

- Mass removal of roach will reduce interspecific competition pressure on other fish species leading to enlarged realised niche (increased niche breadth) of other fish, especially perch (*Perca fluviatilis* L.) (III).
- Mass removal of roach (a generalist feeder) will reduce the linkage between pelagic and littoral energy flows leading to more distinct trophic pathways originating from each habitat (IV).
- Mass removal of omnivorous fish will shorten food chains by reducing trophic linkages in the food web, thereby affecting the trophic positions of other fish species (IV).

One especially interesting issue in stable isotope ecology, that has recently received more attention, is the possibility to use preserved historical samples in stable isotope studies. SIA of archived sample materials offers the potential to reconstruct historical food webs and hence to investigate long-term changes in ecosystem properties. At the moment, there is not enough information available on how different preserving methods may change the isotope signatures of samples and whether such preserved samples provide information which can be reliably compared with data from freshly collected samples. The final paper (V) of this thesis evaluates the potential of using archived samples in stable isotope studies. Historical samples could provide a valuable source of information for many studies using SIA, one possibility being to compare successful biomanipulation experiments with unsuccessful ones using preserved samples, or to allow better evaluation of the impacts of biomanipulation by allowing reconstruction of longer time scale "pre-biomanipulation" conditions.

2   MATERIAL AND METHODS

2.1   Lake Jyväsjärvi

Jyväsjärvi (62° 14′ N, 25° 46′ E, Fig. 1) is an urban lake in central Finland, surrounded by the city of Jyväskylä with some 80 000 inhabitants. The lake has an area of 3.4 km^2, a maximum depth of 27 m and a mean depth of 7.2 m, and the theoretical hydraulic turnover time is 2.7 months (Meriläinen et al. 2003, Salonen et al. 2005). Jyväsjärvi is also characterised by rather steep and extensively reconstructed shorelines, and relatively humic and coloured water (colour 80-100 Pt mg l^{-1}). Jyväsjärvi has suffered from severe point source pollution, mainly from the municipal waste waters of the city of Jyväskylä and effluents from a local paper mill. The city waste waters were led untreated into the lake until the early 1970s and the paper mill released its effluents, containing for example sulphuric acid, directly into the lake. Jyväsjärvi became heavily polluted by the late 1960s, but today the lake receives no more municipal waste waters and the loading from the paper mill has decreased considerably (Meriläinen et al. 2003). The condition of the lake has therefore been gradually improving (Meriläinen et al. 2003, Salonen et al. 2005). Nevertheless, Jyväsjärvi is still moderately eutrophic and the total phosphorus concentrations have been typically around 35-40 µg l^{-1} and total nitrogen 850 µg l^{-1} (Keskinen et al. 2005). The fish community of Jyväsjärvi was also typical for eutrophic lakes, dominated by cyprinid fish species, such as roach and bream (*Abramis brama* (L.)), and small perch. Therefore further restoration of Jyväsjärvi, by biomanipulation involving mass removal of small cyprinid and percid fish species, was started in the spring of 2004.
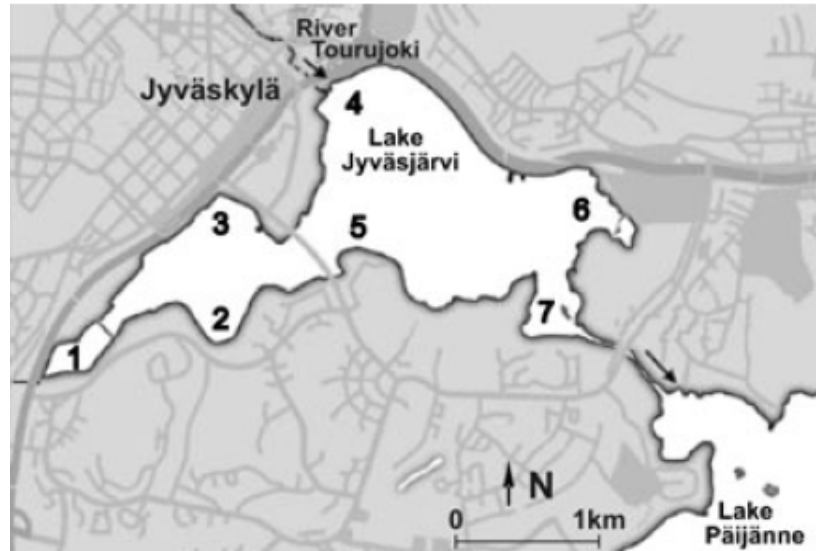
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FIGURE 1   A map of Jyväsjärvi showing the selected sampling areas (1-7), major inlet
            Tourujoki and the connecting Äijälänsalmi canal between Jyväsjärvi and
            Päijänne (modified from Meriläinen et al. 2003 and paper I).

During the biomanipulation fishing in 2004-2006, over 100 000 kg of fish was
removed from Jyväsjärvi (Table 1), using 8-20 large traps set around the lake
(more description of the biomanipulation fishing is given in section 2.2.3).
Bream, perch and roach were the most abundant fish species in the catches and
the biomasses for these fish species were considerably reduced following
biomanipulation. Using virtual population analyses (Peltonen et al. 1999a), the
perch population was evidently reduced by ~80% and the roach population by
~90%, while the bream population was reduced by ~70% based on catches
(researcher T. Keskinen, University of Jyväskylä, unpublished data). Also, the
population age structure was quickly skewed toward younger individuals in
both perch and roach populations.

TABLE 1   Fish catches from Jyväsjärvi during the biomanipulation fishing in 2004-2006
           (researcher T. Keskinen, University of Jyväskylä, unpublished data).

	Fish removed	
Year	kg	kg ha^{-1}
2004	53 381	158
2005	25 924	77
2006	21 795	65
Total	101 100	300

These changes in the fish community were quickly reflected in the zooplankton
community of Jyväsjärvi, which became dominated by larger bodied

individuals after biomanipulation. The most abundant cladocera before fish removals was the small *Daphnia cucullata* (Sars) but after fish removals, the larger bodied *D. cristata* (Sars) and *D. galeta* (Sars) became the most abundant cladocera (researcher P. Högmander, University of Jyväskylä, unpublished data). However, these apparent changes in the overlying food web have not cascaded down to the producer level and no changes have been evident in chlorophyll-a concentrations or water transparency in Jyväsjärvi after biomanipulation (P. Högmander, unpublished data). The characteristics of Jyväsjärvi (coloured water, relatively deep with steep and reconstructed shorelines) may effectively restrict the possibilities for a shift from greater pelagic primary production to increased littoral benthic production and macrophyte growth, and therefore to the establishment of any alternate stable state (Scheffer et al. 1993).

2.2   Sample collection and processing

All samples were collected during the open water periods (from May to October-November). In 2004 the sampling aimed to evaluate the extent of spatial and temporal variability in carbon and nitrogen isotope signatures within Jyväsjärvi. Therefore several sampling locations were used in 2004 (four locations for particulate organic matter (POM), zooplankton and fish, and seven locations for periphyton, macrophytes and benthic macroinvertebrates).

2.2.1 POM, periphyton and macrophytes

POM was collected from four different locations in Jyväsjärvi in 2004 (Fig.1; sampling sites 1, 2, 4, 7) and from one location (2) in 2005 and 2006. POM samples were collected bimonthly each year from the epilimnion using a Limnos water sampler and by filtering lake water through a 48 µm mesh into 1 litre washed plastic containers. These filtered water samples were later filtered onto precombusted (~500 °C, >5 h) glass-fibre filters, dried at 60 °C and stored for later SIA.

Samples of periphyton and macrophytes were collected from seven locations (Fig. 1). Periphyton was collected three times in 2004 and macrophytes twice. Depending on the substrates present at each site, periphyton (or more precisely biofilm) was collected from rocks, macrophytes and submersed wood by rubbing them in a plastic container filled with clean water. All extra items, such as larger pieces of debris or visible animals, were removed and samples were then filtered through a 100 µm mesh, followed by filtration onto precombusted glass-fibre filters and drying at 60 °C. Sampled macrophytes included typical aquatic plants of Finnish lakes (I). Healthy looking floating and submersed leaves were collected, gently wiped clean, cut into small pieces and dried at 60 °C. After drying, the samples were ground and stored in small glass vials for later isotope analysis.

2.2.2 Zooplankton and benthic macroinvertebrates

Zooplankton samples were collected bimonthly from the same four sampling locations as POM in 2004 and one site in 2005-2006. Zooplankton samples were collected by hauling zooplankton nets (mesh sizes 48 and 100 µm) vertically through the water column. Samples were then brought to the laboratory and left overnight in clean water to allow gut evacuation. The next day these samples were identified and sorted into three taxonomic groups: cladocerans, and cyclopoid and calanoid copepods. Samples were inspected while sorting and all other items and predatory cladocerans (predominantly *Leptodora kindtii* (Focke)) were removed. Samples were then filtered onto glass-fibre filters and dried at 60 °C. Lipids are depleted in ^{13}C (e.g. Parker 1964, Kling et al. 1992), and potentially variable lipid contents in zooplankton may confuse the interpretation of carbon isotope signatures. To evaluate this potential effect, some cladoceran samples were treated with 2:1 chloroform:methanol (volume ratios) solution to extract lipids (Bligh & Dyer 1959), and analysed for comparison with untreated samples.

Macroinvertebrate samples were collected from each of the seven sampling sites (Fig. 1) on five occasions in 2004 and 2-3 times in 2005-2006, from both littoral and deeper littoral/profundal depths. Littoral samples were collected by kick-netting (mesh size 500 µm) at depths less than 1 meter. Deeper samples were obtained with an Ekman grab from depths of 4-10 m, depending on the maximum depth at each sampling site, and sieved through a 500 µm mesh. Individuals were transferred into clean water in the laboratory to allow them to void their guts. The following day samples were identified and sorted for drying, and stored for later SIA. Additional samples of snails and mussels were collected from the connected lake Päijänne to represent baseline values for δ^{15}N in Päijänne (II). These samples were collected between June and August 2004 using an Ekman, scuba diving or directly hand-picking them from stones or macrophytes.

2.2.3 Fish

Analysed fish species included perch, roach, bream, smelt (*Osmerus eperlanus* (L.)), pike (*Esox lucius* L.), pike-perch (*Sander lucioperca* (L.)), and burbot (*Lota lota* (L.)), which were mainly collected from four areas (1, 2, 4, 7 in Fig. 1). Perch and roach were collected in greater numbers that other species, because these were the most abundant in Jyväsjärvi and also the target species for biomanipulation. Fish samples were obtained from the several large traps set around the lake as part of the biomanipulation mass removals. These traps (30-40 m in length and 10-15 m in width, mesh size 8 mm) were set close to shorelines in depths less than 5 m. Fish were also caught using monofilament nylon gill nets (1.5 × 30 m) and each net had 9 mesh sizes ranging from 10 to 55 mm (from knot to knot). Total length and weight were recorded from each fish and a small piece of white muscle tissue was dissected posterior to the dorsal fin (except for smaller individuals when a greater proportion of the dorsal

muscle tissue was dissected). Samples of perch and roach were also collected from Päijänne in 2004 and 2005, using gill nets, for comparison of isotope signatures in these fish between Jyväsjärvi and Päijänne (II). Muscle samples were either dried immediately at 60 °C or wrapped in aluminium foil and stored frozen for later drying. All dried fish samples were ground into fine powder using a mortar and pestle and stored in small glass vials for later SIA.

2.2.4 Archived samples

Zooplankton, macroinvertebrate and fish samples were also collected to evaluate the possible effects of preservatives on liquid preserved samples, and to test if scales and bones collected for fish aging could be used as a proxy for muscle tissue in SIA (V). These additional zooplankton and macroinvertebrate samples were collected from Jyväsjärvi, Päijänne and Äimäjärvi (the latter in southern Finland) using methods described above. Zooplankton samples were then divided into those dried and analysed immediately (control) and those preserved for 2 weeks, 2 months, 6 months or 12 months in ethanol (~80 %) or formalin (~10 %). For macroinvertebrates, two pooled samples of several individuals of the same genus or species were formed; one was analysed immediately as a control and the other preserved in ethanol (~80 %) in the same manner as zooplankton samples. After preservation, all samples were carefully rinsed several times in clean water and dried in an oven at 60° C over night.

Different sizes of perch and roach were fished from Äimäjärvi, Jyväsjärvi and the nearby Tuomionjärvi mainly using gill nets. Muscle tissue was dissected from each (and prepared for SIA as described above for fish) along with both operculum bones from perch and several scales from roach. Bones and scales were then divided into those being analysed without any treatment (except wiping off all extra materials) and those receiving acid treatment (1.2 normal HCl) to remove extraneous carbonates and careful rinsing with distilled water (Perga & Gerdeaux 2003). Only the outermost areas of both operculum bones and scales were sliced off for SIA to represent latest growth (and feeding).

2.2.5 Stable isotope analyses

All samples subject to SIA were dried and ground into fine powder using mortar and pestle (except scales and bones, which were cut into small pieces). Samples were then accurately weighed into small tin cups (0.6 mg for fish, 0.7 mg for zooplankton and macroinvertebrate samples, 0.6-0.8 mg for scales, 0.8-1.0 mg for bones. Variable amounts of sample materials were weighed for POM, periphyton and macrophyte samples due to differences in their carbon and nitrogen contents). Samples were analysed for their isotope composition at the Institute for Environmental Research, University of Jyväskylä, using a FlashEA 1112 elemental analyser coupled to a Thermo Finnigan DELTAplus Advantage mass spectrometer. Pulverised pike muscle tissue was used as internal laboratory working standard for animal tissues and citrus leaves as a standard

for plant tissue and POM. Replicate standards were run repeatedly in every analysis to assure accurate analysis and possible correction for linearity and drift. Internal precision for standards was usually less than 0.3 ‰ for both C and N in each run.

2.3   Data analyses

Analysis of variance (ANOVA) with Tukey's *post hoc*-tests or the non-parametric Kruskall-Wallis test were used to test for differences in isotope ratios of periphyton, macrophytes, macroinvertebrates and fish between sampling sites and sampling times (I). T-tests were used to compare perch and roach caught from Äijälä area in Jyväsjärvi (nearest to the canal connecting Jyväsjärvi to Päijänne) to those fish caught from other areas in Jyväsjärvi (I). Analysis of covariance (ANCOVA) was used to examine differences between pelagic samples (different zooplankton groups and POM). Taxonomic group and sampling site were set as fixed explaining factors, and sampling date as a covariate (I).

Fish used to estimate the extent of migration between Jyväsjärvi and Päijänne were grouped to represent three different locations: fish from Jyväsjärvi but outside the Äijälä area, fish from Äijälä area in Jyväsjärvi and fish from Päijänne (II). Canonical discriminant analysis (CDA) was used to build a model to classify perch and roach caught from the Äijälä area into fish originating from either Jyväsjärvi or Päijänne, based on their stable isotope ratios. Fish caught from Jyväsjärvi outside the Äijälä area in summers were used to define perch and roach resident in Jyväsjärvi, and fish caught from Päijänne to define perch and roach migrating from Päijänne.

Bearhop et al. (2004) recently proposed a novel approach to determine feeding niche width, using the variance associated with stable isotope values of individuals within a population. Levene's test for equality of variances was therefore used to test for differences in the degrees of variances (i.e. niche width) of perch and roach $\delta^{13}C$ and $\delta^{15}N$ values between 2003-2006 (III). Changes in the mean $\delta^{13}C$ and $\delta^{15}N$ values of perch and roach between 2003-2006 were tested using simple t-tests.

To estimate the contribution from littoral and pelagic primary production to higher trophic levels before and after the biomanipulation of Jyväsjärvi, a two-source mixing model was applied (IV). This model estimates the percentage contribution from littoral production using the equation:

littoral% = $(\delta^{13}C_c - \delta^{13}C_p) / (\delta^{13}C_l - \delta^{13}C_p)$,

where $\delta^{13}C_c$ is the $\delta^{13}C$ value of the target consumer, $\delta^{13}C_p$ is the value for pelagic end point and $\delta^{13}C_l$ littoral end point. The end point values for pelagic $\delta^{13}C$ values were calculated as a mean value extracted from zooplankton (cladocera and copepods) summer mean and mean for mussels collected in 2004

("before" end point) and 2006 ("after" end point). Similarly, littoral δ^{13}C end points were calculated from mean values of snails and most abundant littoral macroinvertebrates. Once the percentage reliance of fish for either energy source (or food chain) was estimated, these percentages were used in a different mixing model that calculates trophic positions for fish (Post 2002, IV). This model estimates the proportion of nitrogen acquired from each of the food chains using the equation:

$$TP = \lambda + (\delta^{15}N_c - (\delta^{15}N_{base1} \times \alpha + \delta^{15}N_{base2} \times (1-\alpha))) / \Delta,$$

where λ is the trophic position of the organism used to calculate $\delta^{15}N_{base}$ (primary consumers = 2), $\delta^{15}N_c$ is the analysed $\delta^{15}N$ value of the target organism, $\delta^{15}N_{base}$ are the end point values for the two food chains in question (e.g. pelagic and littoral), α is the proportional reliance on the other food chain (calculated as percentage littoral contribution with the previous equation) and Δ is the fractionation factor for $\delta^{15}N$ (most frequently 3.4). Baseline $\delta^{15}N$ values for pelagic and littoral food chains were calculated using the mean values of cladocera and mussels (pelagic) and snails, water slater (*Asellus aquaticus* L.) and mayfly larvae (littoral). Changes in food web structure were also evaluated by calculating some community-wide metrics from the stable isotope data. These metrics are described in more detail in paper IV and in (Layman et al. 2007).

The impact of preservation (ethanol- and formalin-preserved zooplankton and ethanol-preserved macroinvertebrates) was tested using ANOVA and repeated measures ANOVA (V). Comparisons between δ^{13}C and δ^{15}N values analysed from perch operculum bones and roach scales to those analysed from fish muscle tissue were done using regression analysis and t-tests.

3   RESULTS AND DISCUSSION

3.1   Spatial and temporal variability in stable isotope ratios

3.1.1 Littoral and profundal samples

Periphyton and macrophyte samples collected from the littoral areas of Jyväsjärvi showed considerable variation in their isotope signatures, not explained by spatial differences (I). Macrophyte $\delta^{15}N$ signatures were highly variable among species, which can potentially produce variable signatures in herbivores feeding upon these plants. Two major groups of plants could be differentiated by their $\delta^{13}C$ signatures. The most ^{13}C enriched had submerged leaves and therefore, like periphyton, obtain their CO_2 mainly from water, so that their $\delta^{13}C$ signatures are similar to those of periphyton. The plants in the other group have floating leaves and are therefore capable of acquiring CO_2 from the atmosphere, and their $\delta^{13}C$ values match those typically observed in C_3 terrestrial plants. Difference in growth form could also partly explain highly variable $\delta^{15}N$ signatures as plants with more developed root systems can acquire nitrogen from the sediments, whereas some plants acquire their nitrogen from nitrate (NO_3^-) or ammonium (NH_4^+) dissolved in the water.

Two of the seven sites sampled (3 and 4 in Fig. 1) had different macroinvertebrate $\delta^{13}C$ and $\delta^{15}N$ values (I). Especially nitrogen ratios seemed to vary within these sites and on average were significantly lower than in the rest of the lake. Site 3 is situated in a small harbour area, while site 4 is close to the main river inflow to Jyväsjärvi (Tourujoki) which may contribute to these variable isotope signatures. But more important was the clear separation of profundal and littoral macroinvertebrates by their isotope values. As expected, profundal macroinvertebrates had considerably more depleted $\delta^{13}C$ values (by ~4 ‰), but also slightly enriched $\delta^{15}N$ values compared to littoral macroinvertebrates (by ~0.5 ‰). Very few predatory invertebrate species were found from profundal depths, but those found from littoral depths had significantly elevated $\delta^{15}N$ values compared to non-predatory ones (F=82.3, p=0.001) but showed no difference in their $\delta^{13}C$ values.

Vander Zanden & Rassmussen (1999) reported similar differences between $\delta^{15}N$ values of produndal and littoral macroinvertebrates in deep oligotrophic Canadian Shield lakes, whereas Post (2002) found no such difference in another set of similar lakes. However, in Jyväsjärvi this relationship seems to be rather strong and may have considerable impacts on estimating trophic positions of fish using nitrogen isotope ratios. Especially fish occupying higher trophic positions in Jyväsjärvi may acquire nitrogen from both sources and potentially variable $\delta^{15}N$ baseline values in these food chains need to be acknowledged. This can be done by estimating source proportions using $\delta^{13}C$ values in simple two-source mixing models (e.g. Vander Zanden & Rasmussen 1999, Post 2002).

In the following years, further examination of individual profundal consumers in Jyväsjärvi revealed even greater differences in isotope ratios between profundal and littoral invertebrate consumers. Individual *Chironomus* sp. and *Propsilocerus jacuticus* (Zvereva) (Diptera: Chironomidae) reflected extremely high individual differences in both $\delta^{13}C$ and $\delta^{15}N$ values (Fig. 2). The variation within these chironomidae larvae was not related to any temporal or spatial differences in the profundal habitat.
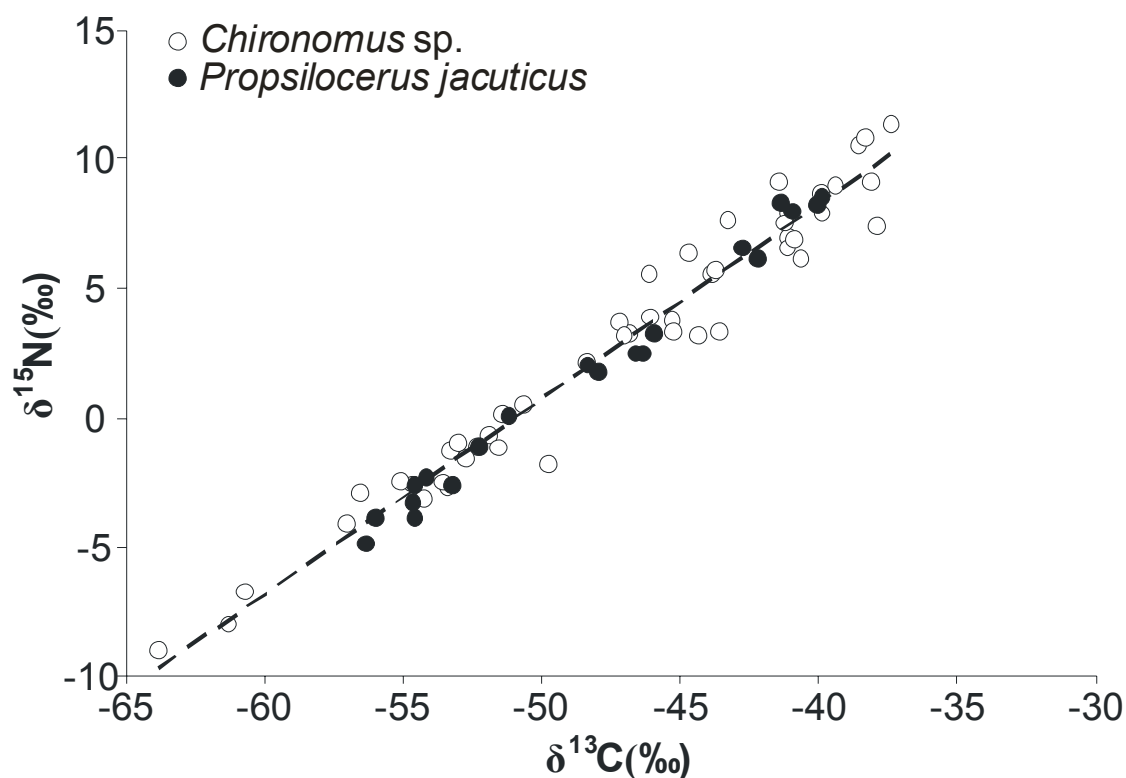
FIGURE 2   Relationship between $\delta^{13}C$ and $\delta^{15}N$ values of profundal *Chironomus* sp. and *Propsilocerus jacuticus*. Fitted regression is Y = 0.76X + 38.45, R^2 = 0.97.

Highly depleted $\delta^{13}C$ and $\delta^{15}N$ values have been reported before (Grey et al. 2004, Jones & Grey 2004, Kelly et al. 2004) and the depleted $\delta^{13}C$ values have been proposed to result from utilisation of methane-oxidizing bacteria (MOB) from the sediments since methane in lakes has very depleted $\delta^{13}C$ values. Highly correlated $\delta^{13}C$ and $\delta^{15}N$ values of chironomids (R^2 = 0.97 in Jyväsjärvi)

suggest that the depleted $\delta^{15}N$ values must also be related to utilisation of MOB but the mechanisms leading to these depleted $\delta^{15}N$ values are still uncertain. But the fact that these highly depleted isotope values in Jyväsjärvi chironomids were not observed in 2004, when each sample was a composite of several individuals, highlights the importance of analysing individuals when possible to detect individual level isotopic variation. Moreover, some individuals of ruffe (*Gymnocephalus cernuus* (L.)) analysed from Jyväsjärvi had considerably depleted carbon and nitrogen isotope values, indicating a diet specialisation on these chironomids (data not shown).

3.1.2 Pelagic samples

No differences were found between the carbon and nitrogen isotope signatures of samples of POM ($\delta^{13}C$ p=0.867 and $\delta^{15}N$ p=0.133) or zooplankton ($\delta^{13}C$ p=0.831 and $\delta^{15}N$ p=0.118) collected from four pelagic sites within Jyväsjärvi (sites 1, 2, 4, 7 in Fig. 1). This result is not surprising in a relatively small lake such as Jyväsjärvi. However, there was a clear seasonal trend in both POM and zooplankton nitrogen isotope signatures (Fig. 2 in I), and date as a covariate was statistically significant for both POM (F=40.0, p<0.001) and zooplankton (F=268.0, p<0.001). The $\delta^{15}N$ of POM increased by 5.7 ‰ from May to September, equivalent to the average increase in zooplankton $\delta^{15}N$ of 6.0 ‰. For carbon, date was a more significant covariate for POM (F=7.8, p=0.009), than for zooplankton $\delta^{13}C$ (F=3.9, p=0.051). The $\delta^{13}C$ values of zooplankton steadily increased until August when the values decreased rapidly, reaching their lowest in September. Increasing lipid storage of zooplankton could result in decreased $\delta^{13}C$ values, as lipids are ^{13}C depleted. However, the difference between untreated cladocera and lipid-extracted cladocera $\delta^{13}C$ values was minimal during summer, with only a slight increase towards autumn (Fig. 3 in I). Clearly lipids do not explain the patterns observed in zooplankton $\delta^{13}C$ values in Jyväsjärvi. A more likely explanation is that zooplankton $\delta^{13}C$ dynamics reflect steady ^{13}C enrichment of the dissolved inorganic carbon (DIC) pool by preferential uptake in photosynthetic carbon fixation of $DI^{12}C$ by phytoplankton (Jones et al. 2001). Quickly reduced $\delta^{13}C$ values could then simply indicate lake turnover or significant deepening of the epilimnion and hence introduce DIC with lower $\delta^{13}C$ signatures leading ultimately to lower $\delta^{13}C$ values in zooplankton. Although late August is rather early for lake turnover in Finland, in Jyväsjärvi this can happen due to artificial mixing of lake water (Salonen et al. 2005). Changes in the phytoplankton community composition could also partly explain the variations in zooplankton $\delta^{13}C$ values. Phytoplankton can show considerably high between-taxa variation in $\delta^{13}C$ values (Vuorio et al. 2006) and these changes could then be reflected further up the pelagic food chain.

The trend observed in $\delta^{15}N$ values in POM and zooplankton of Jyväsjärvi in 2004 reoccurred in 2005 and 2006. However, in 2005 and 2006 samples were also taken in October when an even more considerable peak in $\delta^{15}N$ values was evident (Fig. 3). The $\delta^{15}N$ of cladocerans increased ~9 ‰ from September to

October in 2005 and ~13.5 ‰ in 2006. The steady increase in $\delta^{15}N$ could be explained by preferential uptake of $^{14}NO_3^-$ by phytoplankton during the steady depletion of the epilimnetic nitrate. Lowering nitrate levels in the epilimnion could lead to progressive ^{15}N enrichment in the remaining nitrate pool, reduced isotopic fractionation and hence to steadily higher $\delta^{15}N$ signatures in phytoplankton, and ultimately in POM and zooplankton. However, it is unlikely that this would result in the extremely high values observed in October 2005 and 2006. This is more likely a result of increased NH_4^+ concentrations in the epilimnion after lake turnover, since NH_4^+ in Jyväsjärvi had elevated $\delta^{15}N$ value of 29.1 ‰ in autumn 2005 (researcher M. Tiirola, University of Jyväskylä, unpublished data), similar to those observed in POM and zooplankton. Ammonium-induced higher $\delta^{15}N$ values after lake turnover in late August-early September would then be first seen in POM (as in 2005) and ultimately in zooplankton.
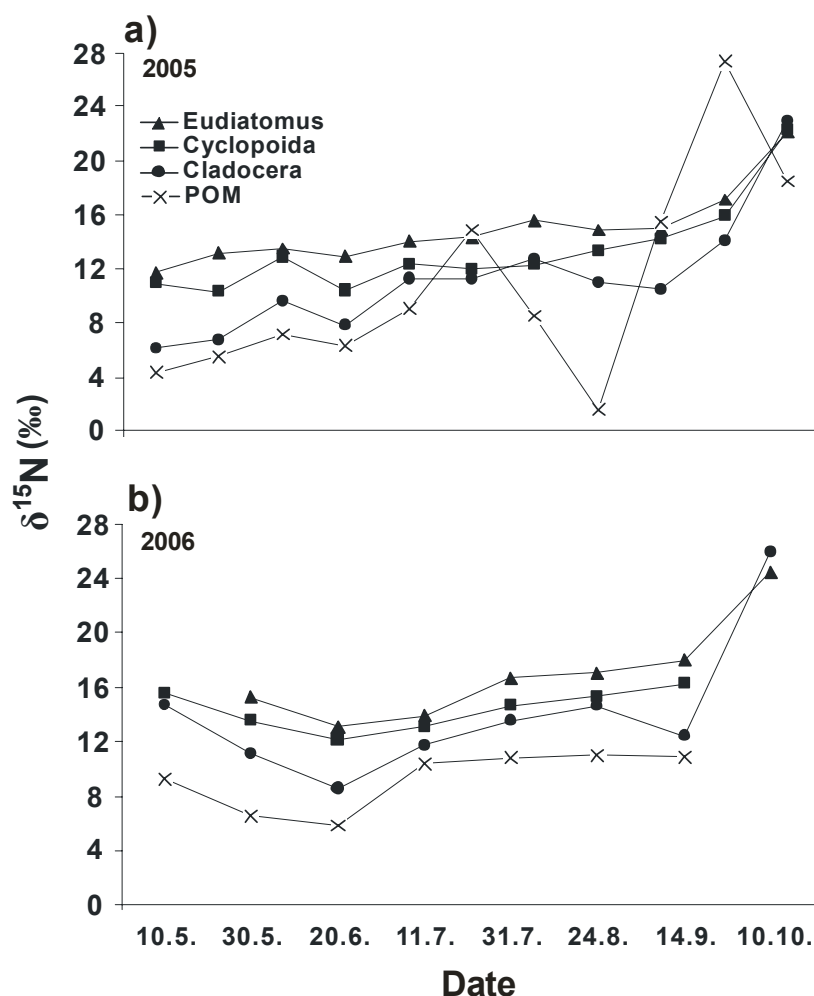

FIGURE 3   Nitrogen isotope ratios analysed from different zooplankton taxa and POM in Jyväsjärvi in a) 2005 and b) 2006.

It was important to sample the Jyväsjärvi pelagic community frequently to detect these temporal trends in isotope values. If samplings were done only a

few times during the summer, the trend could have been left unnoticed. Temporal variations in isotope values can strongly affect evaluation of trophic relationships and setting suitable isotopic baselines for lake food webs (Matthews & Mazumder 2003, Matthews & Mazumder 2005). Many times the isotope data are gathered from several lakes, which may limit the lake-specific sampling effort available and lead to unnoticed spatial or temporal isotopic variation. In fact, quantitative uses of stable isotopes (such as estimating trophic position, energy flow pathways and mixing models) require rather precise estimates of isotopic signatures in the baseline organisms or end members incorporated into these models, which are highly sensitive to any variation associated in their end member values.

3.1.3 Fish

Both perch and roach showed considerable spatial and temporal variation in their stable carbon and nitrogen isotope signatures. Perch and roach caught in spring (May) had depleted average nitrogen isotope signatures in the eastern part of Jyväsjärvi (site 7 in Fig. 1). Isotope signatures of fish caught from other sampling sites did not differ significantly and hence these fish were grouped to represent the rest of the lake. The difference in perch and roach nitrogen isotope signatures between site 7 and the rest of the lake was almost 2 ‰ for perch and almost 1‰ for roach. Towards the autumn the average nitrogen signatures for both perch and roach had elevated within site 7 and then matched those for the rest of the lake. The carbon isotope signatures of perch and roach also showed variation at site 7 but with an inverse pattern of being more ^{13}C enriched in the early part of the summer and becoming more ^{13}C depleted towards autumn. Excluding the variable isotope ratios observed in fish at site 7, isotope values of fish in general showed much less variance than those of organisms lower in the food chain, which is consistent with previous observations (e.g. Cabana & Rasmussen 1996) and explained by faster tissue turn over times in smaller organisms.

The pattern in fish isotope ratios can be explained by fish migrating to Jyväsjärvi from Päijänne for spring spawning, if fish in Päijänne have lower isotope ratios and considerable numbers of fish are migrating up the channel at spring time. Lilja et al. (2003) studied the migration in this channel using a horizontal split-beam echo sounder, and recorded over 100 000 individual cyprinids and percids migrating upstream in Äijälänsalmi channel. Most migration took place in May, when the surface waters in Jyväsjärvi warmed more quickly than in Päijänne and presumably attracted fish to spawn. Our stable isotope data are consistent with these earlier estimates of substantial migration of roach and perch, because a small number of migratory fish with depleted isotope signatures would not be sufficient to cause the observed depression of average signatures.

In summary, these results indicate that restricted sampling of ecosystem components from lakes may provide misleading single value end members to be used in isotope mixing models; hence studies of individual lakes or multiple

lake comparisons utilising stable isotope analyses should pay more attention to potential within lake spatial and temporal variability of isotope ratios. In addition, there was a clear relationship between $\delta^{13}C$ and $\delta^{15}N$ values in pelagic and littoral invertebrate consumers, with lower $\delta^{13}C$ values and higher $\delta^{15}N$ values found in the pelagic invertebrates. Such patterns are essential to notice before using stable isotope data to assess energy contribution from different sources or to calculate trophic positions of fish coupling both littoral and pelagic energy pathways.

3.2   Immigrant perch and roach from Päijänne

Mussels collected from Jyväsjärvi and Päijänne to represent baseline values for the pelagic food chain in these ecosystems (II) had considerably different mean nitrogen isotope ratios (mean ± SD of $\delta^{15}N$ for Jyväsjärvi 10.4 ± 1.1 ‰ and 7.6 ± 0.6 ‰ for Päijänne). Higher $\delta^{15}N$ values in mussels from Jyväsjärvi are consistent with earlier observations of increasing $\delta^{15}N$ values with anthropogenic impacts on catchments (e.g. Cabana & Rasmussen 1996). Carbon isotope ratios in mussels also differed, although slightly less ($\delta^{13}C$ for Jyväsjärvi -33.2 ± 2.6 ‰ and -30.9 ± 1.9 ‰ for Päijänne), consistent with the influence of lake area on $\delta^{13}C$ values (Post 2002). Snail $\delta^{15}N$ values were also higher in Jyväsjärvi than in Päijänne, but the differences in carbon or nitrogen isotope ratios were not so considerable as with mussels. Carbon isotope ratios in fish followed the pattern observed in mussels being generally more depleted in Jyväsjärvi, but showed considerably more variation than nitrogen isotope ratios in both Jyväsjärvi and Päijänne. When $\delta^{13}C$ and $\delta^{15}N$ of perch and roach were used as classifying factors in stepwise CDA, only $\delta^{15}N$ was left in the final model. Therefore, $\delta^{13}C$ signatures of perch and roach were not used to trace migrations of these fish.

CDA separated fish caught in Jyväsjärvi into those fish most likely originating either from Jyväsjärvi or from Päijänne, based on perch and roach $\delta^{15}N$ values (II). 93.1 % of perch and 76.8 % of roach grouped *a priori* and used in the model, were classified correctly according to the CDA. Mean probabilities (± SD) by the CDA for perch to belong to either Jyväsjärvi or Päijänne populations were 0.96 ± 0.09 for perch classified to Jyväsjärvi and 0.93 ± 0.13 for those classified to Päijänne. Mean probabilities for roach were 0.80 ± 0.13 and 0.76 ± 0.15, respectively. The numbers of immigrant fish were estimated to be highest in spring 2004 (71 % of perch caught in the Äijälä area and 56 % of roach). After this the numbers of immigrants were greatly reduced (only 24 % of perch and 14 % of roach in spring 2005) along with considerably reduced total fish catches from Jyväsjärvi in 2005 (II).

The reduced amount of immigrant fish caught from Jyväsjärvi in 2005 indicates that during the biomanipulation project the extent of migration decreased markedly, and that the immigrant fish had not significantly repopulated Jyväsjärvi following the fish removals. Therefore, the original

concerns that immigrant fish might quickly recolonise Jyväsjärvi and negate the efforts of the biomanipulation appear to have been unfounded. The reduced percentage of immigrant fish could also indicate that these migratory perch and roach are only a sub-population in Päijänne, and that the size of this population had been significantly reduced.

3.3   Feeding niche widths of perch and roach

SIA potentially provides an efficient method to track changes in feeding niche widths of consumers using the variance associated with population mean isotope values (Bearhop et al. 2004). However, some important issues need to be carefully considered before applying SIA to study feeding niche widths. First of all, the immigrant fish from Päijänne with distinct isotope signatures would of course have serious implications on the interpretation of niche widths from isotope variance. However, the isotope study to evaluate the extent of these migrations (II) also revealed that the immigrants were found only from very limited area in Jyväsjärvi (site 7 in Fig. 1). Therefore all fish caught close to that area were excluded from all further analyses (in papers III and IV). Another key issue is the isotopic range (especially in δ^{13}C) in prey categories (Matthews & Mazumder 2004). For example, in lake ecosystems δ^{13}C values can have a wide range from higher values in littoral to lower values in pelagic food sources and this range is likely to vary between different lakes. Since this range will affect the variance observed in consumers, the calculated variances for consumers in different ecosystems may not be directly comparable. However, this is of minor importance if comparisons are only made through time within the same population in a single ecosystem, as in this study, and no significant changes in the range (and variance) in isotopic signatures in prey categories are observed. Main prey categories in Jyväsjärvi (zooplankton and littoral macroinvertebrates) showed rather constant mean δ^{13}C and δ^{15}N values during the study period (III). Variances and ranges for all these values were consistent from year to year and there were no significant changes in the degrees of variances (all Levene's tests, $p>0.1$).

Both perch and roach populations showed significant changes in their mean δ^{13}C and δ^{15}N values, and in the variances around these mean values (III). These decreased δ^{13}C and increased δ^{15}N values most likely indicate a greater reliance of perch and roach on pelagic energy sources. Zooplankton in Jyväsjärvi has relatively low δ^{13}C and high δ^{15}N values and since this prey source became more profitable (more abundant and larger bodied individuals) after first fish removals, the remaining fish can be expected to have consumed this type of prey in greater proportions in 2005. The changes in perch mean isotope values in 2006 and the suddenly reduced variances around these mean values can only result from perch specialising on a certain, highly profitable, prey type. Lake biomanipulations are often followed by increases in YOY fish

(Hansson et al. 1998), which can be a valuable prey source for older perch (Svanbäck & Persson 2004). The YOY perch and roach analysed from Jyväsjärvi in 2006 reflected similar $\delta^{13}C$ values as adult perch but had 3.4 ‰ lower $\delta^{15}N$ values, which typically reported as one trophic level fractionation factor for $\delta^{15}N$. Adult perch specialising on YOY probably relaxed the competition between perch and roach for zooplankton prey and increased planktivory in roach population, reflected as a slight decrease in mean roach $\delta^{13}C$ values and increase in $\delta^{15}N$ values.

The hypothesis that feeding niche widths would expand following reduced inter- and intraspecific competition seemed to be valid, at least until summer 2006, when perch population specialised on feeding on a single energy source (YOY fish). This result is actually consistent with optimal foraging theory, since abundant YOY fish are extremely profitable prey for older perch. Also the hypothesis that these changes in feeding niche widths could be revealed as changes in variances around perch and roach mean $\delta^{13}C$ and $\delta^{15}N$ values was supported, especially for perch with increased and finally decreased variances. The perch population showed greater changes in its niche width compared to roach, which was expected because of their carnivorous and therefore more specialist feeding pattern (Persson, 1987). Roach, being able to utilise all kinds of prey including detritus and plant materials, was less affected by the relaxed competition. SIA also showed that both perch and roach population has individuals clearly specialising on either pelagic or littoral prey. This conclusion is consistent with recent studies on resource polymorphism on perch and roach (Svanbäck et al. 2008).

3.4   Changes in energy flow pathways and trophic structure

Before energy contributions from pelagic and littoral sources to higher trophic levels and to the whole ecosystems using SIA is possible, reliable baseline indicators and end member values for isotope mixing models need to be analysed. To do this, 127 samples of zooplankton and 141 macroinvertebrate samples were analysed. Zooplankton and littoral macroinvertebrates had distinct stable isotope signatures and the end points calculated from these samples were rather similar before and after biomanipulation (before: pelagic C=-34.6 ‰ N=11.2 ‰, littoral C=-27.5 ‰ N=9.2 ‰; after: pelagic C=-35.1 ‰ N=12.3 ‰, littoral C=-27.2 ‰ N=10.6 ‰).

A total of 532 fish were included for this study (260 before and 272 after biomanipulation). Taking all fish species together, the littoral production contributed 71 % to the whole Jyväsjärvi fish food web before and 51 % after fish removals using a trophic fractionation factor of 0‰ for $\delta^{13}C$. Using a fractionation factor of 1 ‰, the values were 57 % before and 38 % after removals. However, there were substantial species-specific differences in the %-littoral contribution (Fig. 4), and not all the fish species from the Jyväsjärvi

ecosystem were analysed. The choice of fractionation factor for $\delta^{13}C$ has a significant impact of the %-littoral contribution estimates. The most commonly used fractionation factors for carbon have been either 0 or 1 ‰ (Vander Zanden & Vadeboncoeur 2002), but recent studies have shown that values closer to 1 ‰ are perhaps more appropriate (e.g. Harrod & Grey 2006).

Both planktivorous/benthivorous (small perch, roach, bream and smelt) and piscivorous (larger perch, pike, pike-perch and burbot) fish showed greater reliance on pelagic production after biomanipulation (Fig. 4). The decrease in %-littoral contribution was 16 or 14 % for non-piscivores and 16 or 25 % for piscivores depending on the fractionation factor used for $\delta^{13}C$. Both these changes were highly significant (non-piscivores $t_{445}=7.8$, $p<0.001$ and $t_{83}=6.9$, $p<0.001$). However, there were clear species-specific differences within these changes. For example, there was no change in the littoral contribution for bream, 11-14 % change for smaller perch and 32-33 % change for smelt. The greatest estimated change was for burbot, but this result is unreliable, since unfortunately the sample size for burbot before the biomanipulation was particularly low (n=2, Fig. 4). Only small perch and smelt did not differ from each other in %-littoral contribution, but both differed from roach and bream (Kruskall-Wallis $\chi^2=118.0$, $p<0.001$), which also differed from each other (Mann-Whitney U=1055, p=0.006) before biomanipulation. After biomanipulation, all these fish differed significantly from each other in %-littoral contribution (Kruskall-Wallis $\chi^2=157.6$, $p<0.001$). Piscivorous fish did not differ from each other in terms of littoral reliance before the biomanipulation, but pike differed from the rest of piscivores after biomanipulation ($F_{3,49}=11.7$, $p<0.001$) being significantly more dependent on littoral production.
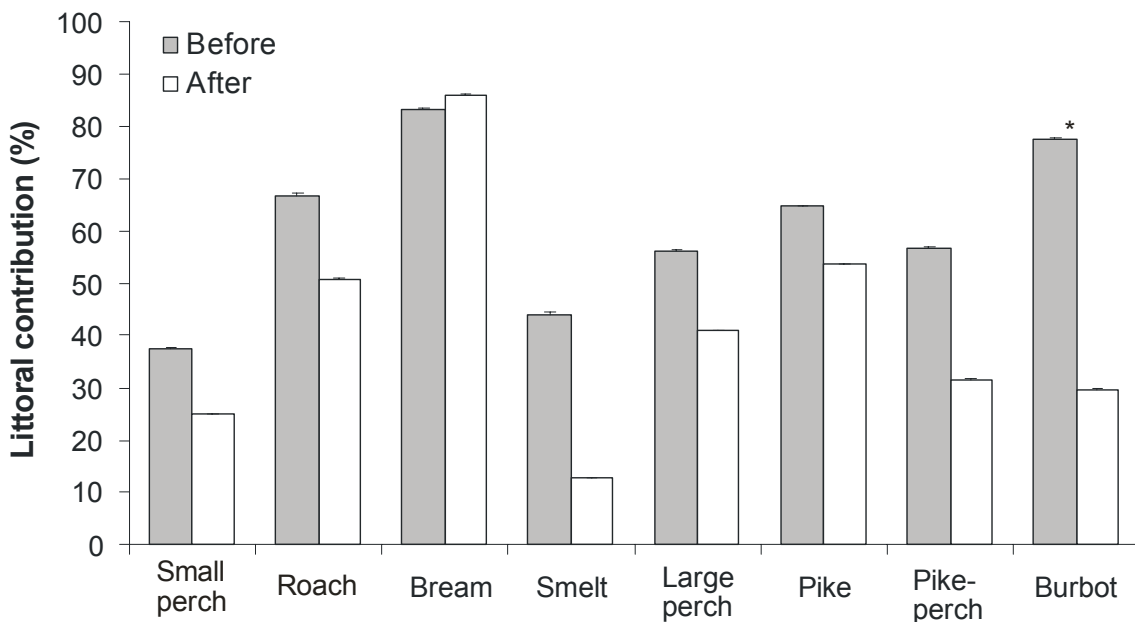

FIGURE 4   Changes in mean %-littoral energy contribution (±SD, calculated using fractionation factor of 1‰ for $\delta^{13}C$) to different fish species before and after the biomanipulation of Jyväsjärvi (*n=2).

According to the calculations based on $\delta^{15}N$ values, roach and bream occupied the lowest trophic positions and did not differ from each other, but did differ significantly from all other species both before and after biomanipulation (Kruskall-Wallis χ^2=121.5, p<0.001 and χ^2=177.9, p<0.001). Roach and bream had mean trophic positions of 3.1 before and 2.9 after the biomanipulation, whereas piscivores had a mean trophic position of 3.7 and 3.6, respectively (assuming trophic fractionation of 1‰ for $\delta^{13}C$ and 3.4‰ for $\delta^{15}N$). Small perch differed significantly in trophic position from both roach and bream and from piscivorous fish on both occasions (t_{114}=-7.3, p<0.001 and t_{142}=-5.5, p<0.001; mean trophic position for small perch was 3.4 both before and after biomanipulation). In fact, only roach and pike showed significant changes in mean trophic positions after fish removals; the mean trophic position for roach decreased from 3.1 to 2.9 (t_{161}=5.2, p<0.001) and that was probably reflected in trophic position of pike, which also decreased from 3.8 to 3.6 (t_{18}=2.3, p=0.034) after biomanipulation.

The community-wide metrics calculated from isotope data also indicated changes in the food web structure after biomanipulation (IV). Almost all metrics calculated for the whole food web showed increased values after biomanipulation; CD (indicating the degree of trophic diversity) increased by 47%, TA (total occupied niche area) by 11%, NND (average trophic redundancy, larger values indicate reduced redundancy) by 50% but SDNND (evenness of the distribution of trophic niches) decreased by 15%. In general, these values indicate increased total occupied niche area and greater trophic diversity within the Jyväsjärvi food web after biomanipulation. Especially roach and bream showed considerable increases in NND values, which indicates that these species show rather distinct feeding ecologies within Jyväsjärvi and are probably less affected by competition from other fish species. After fish removals, these two species were also less affected by interspecific competition from each other as well as by intraspecific competition (reflected as increased NND values).

The results of energy flow patterns in Jyväsjärvi presented here support recent arguments that littoral/benthic production in lakes is more important than perhaps previously thought to the whole lake energy budget (Vander Zanden & Vadeboncoeur 2002, Vander Zanden et al. 2006), even in relatively deep lakes. However, the results also illustrate how the food web in Jyväsjärvi became more dependent on pelagic production after the biomanipulation. This may be the opposite of what would typically be expected from a lake biomanipulation project. The ultimate goal of biomanipulation is to increase water clarity, subsequently leading to both increased littoral primary and secondary production. This, in turn, could lead to increased littoral energy contribution to higher trophic levels, especially considering the efficient transfer of benthic production up the food chain (Hecky & Hesslein 1995, Vander Zanden et al 2006). However, the characteristics of Jyväsjärvi may restrict the contribution from benthic energy sources. Jyväsjärvi is a relatively deep lake with steep and artificially modified shorelines, which effectively limit the

littoral area available for benthic production. Moreover, the water transparency did not increase as expected to allow greater benthic production after biomanipulation (Vadeboncoeur et al. 2003). This is partly due to naturally coloured and humic water in Jyväsjärvi. As the zooplankton prey became more profitable for fish after biomanipulation, it is no wonder that the Jyväsjärvi ecosystem shifted to be more dependent on pelagic primary production.

The increased pelagic energy contribution can be partly explained by an increase in smaller perch abundance. Smaller perch (< 15 cm) were highly dependent on pelagic energy sources before the biomanipulation, and became even more dependent after. At the same time, the abundance of these smaller perch increased (due to increases in YOY perch), and these planktivorous perch became the main prey item for most piscivores (burbot, larger perch and pike-perch). There were no significant changes in the energy contribution from littoral sources to pike, probably because pike is a sedentary piscivore in the littoral zone of lakes and preys on roach in greater proportions.

3.5 Retrospective SIA from archived sample materials

3.5.1 Preserved zooplankton and macroinvertebrates

Formalin preservation affected the zooplankton $\delta^{13}C$ values less than ethanol preservation throughout the 12 months preservation period (V). Formalin-preserved samples also showed less variation in their $\delta^{13}C$ values during the experiment. The impacts on $\delta^{15}N$ values were rather similar after preservation with both formalin and ethanol. Preserved samples did not differ significantly from control samples in their $\delta^{13}C$ or $\delta^{15}N$ values, but C:N ratios of ethanol preserved samples were significantly lower compared to those of control samples ($F_{2,42}$=19.7, p<0.001). There was no effect of formalin on C:N ratios. Ethanol had similar effects on benthic macroinvertebrates as on zooplankton, with $\delta^{13}C$ of preserved macroinvertebrates increasing slightly, and less impact on macroinvertebrate $\delta^{15}N$ values (V). Ethanol treated samples did not differ significantly from control samples in either $\delta^{13}C$ or $\delta^{15}N$. However, as with zooplankton samples, the effect of ethanol on C:N ratios was significant ($F_{1,48}$=6.17, p=0.017). These significant changes in C:N ratios in both zooplankton and macroinvertebrates after ethanol treatment accompanied with changes in $\delta^{13}C$ values, suggests that ethanol preservation alters the lipid content of the sample. Therefore, lipid rich samples preserved in ethanol may be more vulnerable to changes in their $\delta^{13}C$ values during preservation.

In general, the impacts of preservatives on zooplankton and macroinvertebrate carbon and nitrogen stable isotope ratios reported here are similar to those in some other published studies (e.g. Bosley & Wainright 1999, Edwards et al. 2002, Kaehler & Pakhomov 2001, Sarakinos et al. 2002), but our key finding was that the effects of preservation were lower than previously

reported. Also, preservation time (2 weeks, 2 months, 6 months or 12 months) did not seem to affect the isotope values, implying that any change took place very rapidly within the preservation medium.

3.5.2 Fish scales and operculae bones

Both δ^{13}C and δ^{15}N values of acid treated and untreated roach scales were strongly correlated with muscle δ^{13}C and δ^{15}N values (Fig. 4 in V). Strong correlation was also observed between stable isotope values in perch opercula bones and muscle tissue (Fig. 5 in V). Regression slopes were statistically not different from 1, except those for acid treated and untreated scale δ^{15}N versus muscle δ^{15}N regression slopes, which were significantly >1.

The impact of acid treatment may depend largely on the inorganic content of the scale which, in turn, is most likely dependent on the concentration of DIC in the water. As the fish in this study were collected from lakes with relatively low DIC concentrations, the impact of acid treatment might be stronger when done on scales and operculae bones of fish from waters with different DIC concentrations. Lipids may also influence the comparisons between δ^{13}C values of muscle and scales or operculae. Fish with lipid rich muscle tissues may have considerably lower δ^{13}C values in their muscle tissues, if lipids are not removed or values normalised (Kiljunen et al. 2006), leading to greater difference between muscle tissue and lipid free scale δ^{13}C values.

These results from preserved and archived freshwater sample materials show that fluid preserved zooplankton and macroinvertebrates can be analysed for their δ^{13}C and δ^{15}N values with only trivial uncertainty due to interpretation. These data could then be utilised in historical reconstructions of lake food webs, for example to assess ecosystem-specific baseline values for δ^{13}C and δ^{15}N to study changes in relative contributions from pelagic and littoral energy sources to higher levels in the lake food chain. In combination with archived scale and operculae samples of fish, it may then be possible to construct sufficiently comprehensive descriptions of historical food webs to allow for studies of long term ecosystem changes using SIA (Grey 2006, Vander Zanden et al. 2003). Such reconstructions could generate important information also on the impacts of invasive species, cultural eutrophication or even climate change.

For example, analyses of perch operculae from Äimäjärvi before and after an intense biomanipulation period (1997-2001) and winter fish kill (2002), revealed a considerable decrease in δ^{13}C values of perch operculae (Fig. 5). Such changes have been shown to correlate with total phosphorus concentrations (Perga & Gerdeaux 2003, Perga & Gerdeaux 2004, Gerdeaux & Perga 2006) and most likely reflect lake productivity. Higher productivity results in increased demand of DIC during phytoplankton photosynthesis and since ^{12}C is preferentially fixed in photosynthesis, the resulting DIC pool may become ^{13}C enriched. This would lead to progressively elevated δ^{13}C values observed in both producers and consumers (Perga & Gerdeaux 2003, Perga & Gerdeaux

2004). Äimäjärvi was a severely eutrophicated lake before the biomanipulation program and the lake primary production was undoubtedly high in 1996. The following slight decreases in perch operculae $\delta^{13}C$ values could therefore indicate a decrease in primary production. Severe oxygen depletion in winter 2002 caused a large-scale fish kill, which was followed by a considerable improvement in water transparency and a clear water phase for the next few years. This could explain the most depleted $\delta^{13}C$ values in perch operculae, which were analysed during the clear water phase (lower pelagic primary production) in 2004. Although the change in $\delta^{13}C$ values in Äimäjärvi is much greater than in Jyväsjärvi, the trend is similar. Therefore, greater contributions from pelagic energy sources could also partly explain the change in $\delta^{13}C$ values, especially after the fish kill.
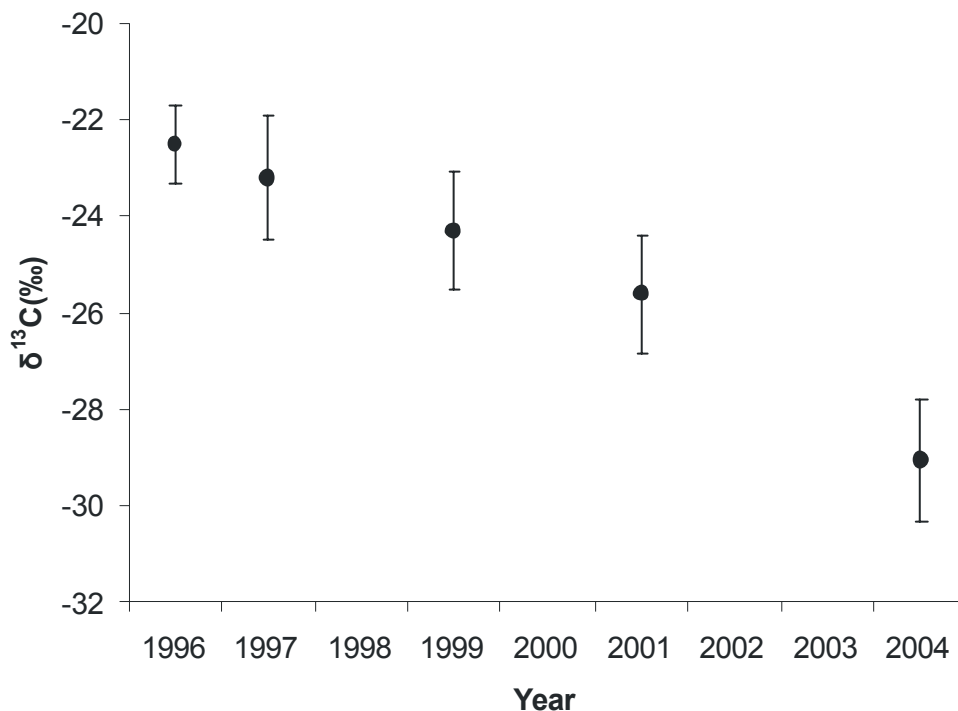
FIGURE 5   Changes in the $\delta^{13}C$ values (± SD) of perch operculum bones from Äimäjärvi during a biomanipulation project in 1997-2001, and after a large scale fish kill in 2002.

4   CONCLUSIONS

Results from the preliminary stable isotope studies, characterising spatial and temporal variations in isotope signatures within Jyväsjärvi biota, revealed important issues that are essential considering studies using stable isotope methods. Stable isotope ratios in biota can show considerable temporal and/or spatial variation, as shown in this thesis. If these issues are not being considered, stable isotope results may only represent 'snapshot' values and lead to considerable misinterpretations of food web data. The influence of immigrant fish from Päijänne to spatially variable isotope values in Jyväsjärvi fish was especially important for this study. The conclusion that these immigrant perch and roach from Päijänne did not significantly recolonise Jyväsjärvi after removal of resident fish, was also important considering the value of biomanipulation as a restoration tool in Jyväsjärvi. Thus these results also show that biomanipulation of fish stocks can be a worthwhile management option even in lakes that are not closed systems. Without SIA methods, it would have been extremely difficult to assess the influence of these migratory fish to the biomanipulation of Jyväsjärvi. Also, SIA proved to be an efficient method in migration studies even at such a small geographical scale.

Whether the fish removals were sufficient to allow for considerable improvements in water quality is difficult to judge here. When lake restorations are attempted by fish removals, these should be done extremely efficiently (Hansson et al. 1998, Sarvala et al. 2000). Moreover, sometimes improvements in water quality may take a long time to appear (Jeppesen et al. 2005) and perhaps we have not yet seen all the impacts of the biomanipulation. On the other hand, many times any success gained shortly after biomanipulation has been lost in the following years (Søndergaard et al. 2007). Nevertheless, these fish removals did have an impact on the zooplankton community of Jyväsjärvi, as larger bodied individuals became more abundant. The changes in zooplankton community were not sufficient to have an impact on the chlorophyll-a concentrations, which may suggest that bottom-up forces are stronger than top-down regulation at the lower levels in the Jyväsjärvi food web.

Although Jyväsjärvi has not responded to the biomanipulation quite as expected, the results presented in this thesis show that important ecosystem

level changes were nonetheless evident. Biomanipulation shaped the Jyväsjärvi ecosystem resulting in more discrete energy flow pathways from pelagic and littoral sources. However, these two energy sources (or food chains) were closely linked and especially predatory species at the top of the food chain effectively coupled both sources. Predatory fish other than pike were more efficient in preying on the most abundant planktivorous fish (small perch), especially after biomanipulation when the small perch were even more abundant. Therefore restorations by stocking of piscivorous fish should perhaps concentrate more on pike-perch stockings (as in the biomanipulation of Vesijärvi (Peltonen et al. 1999b)), which is also a more valuable fish species both commercially and recreationally. Pike does not seem to be efficient in controlling pelagic planktivorous fish and stocking of pike in lake restorations was also recently criticised by Søndergaard et al. (2007) and Skov & Nilsson (2007). Omnivorous fish (such as roach) can also directly utilise both benthic and pelagic energy sources, thereby linking these habitats and potentially transporting energy (nutrients) across habitat boundaries. SIA also revealed changes in feeding patterns and niche widths of two key fish species, perch and roach. Impacts of relaxed interspecific competition on perch niche width were evident, which may have also had an influence on the more abundant YOY perch classes after fish removals. Adult perch also shifted to piscivory at a much earlier stage after the fish removals than before, which may have a key role in controlling these abundant YOY fish. Overall, SIA was indeed a cost effective method to study the impacts of biomanipulation, and the value of SIA in ecosystem studies is further increased with the possibility of using archived sample materials, as shown in this thesis.

These SIA results provide new information on the more subtle effects of biomanipulation on lake ecosystem structure and functioning. The results improve our understanding that biomanipulated lakes may be subject to substantial ecosystem-wide changes, even if the expected changes (lower algal biomass, increased water clarity etc.) may be very limited. Lake restorations by biomanipulation are typically targeted towards individual species (e.g. roach) or feeding guild (planktivorous fish), whereas ecosystem-wide processes may not have been considered in detail. Yet these more subtle food web processes and complex interactions may be critical in structuring the ecosystems. SIA provides a cost effective tool to study these processes, which are difficult to characterise using traditional methods.

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YHTEENVETO (RÉSUMÉ IN FINNISH)

Biomanipulaation vaikutukset järviekosysteemin rakenteeseen vakaiden isotooppien avulla tarkasteltuna

Rehevöityneiden järvien kunnostaminen on yksi vaativimmista sisävesien hoitoon ja suojeluun liittyvistä haasteista. Huomattava määrä tutkimusta on tehty sekä rehevöitymisen syiden selvittämiseksi että jo rehevöityneiden järvien kunnostamiseksi. EU:n vesipuitedirektiivi (Direktiivi 2000/60/EY), jonka mukaan jäsenmaiden sisävesien tilan tulisi täyttää hyvän ekologisen tilan kriteerit vuoteen 2015 mennessä, on viime vuosina lisännyt painetta niin järvien kuin muidenkin sisävesien kunnostamiselle.

Vaikeimmin rehevöityneet suomalaiset järvet kärsivät pääasiassa voimakkaasta ravinteiden (lähinnä fosfori ja typpi) pistekuormituksesta etenkin 1950- ja 1970-luvuilla, jolloin teollisuuden sekä monien kaupunkien jätevedet johdettiin käsittelemättöminä suoraan vesistöihin. 1970-luvun lopulla rakennetut jätevedenpuhdistamot alensivat merkittävästi ravinnekuormitusta, ja monien rehevöityneiden järvien veden laatu alkoi hiljalleen parantua. Vaikka järviä rehevöittävät suuret jätevesipäästöt onkin nykyisin saatu suurelta osin hallintaan, kärsivät monet vesistöt edelleen merkittävästä hajakuormituksesta, jonka kontrolloiminen on huomattavasti pistekuormitusta vaikeampaa. Lisäksi etenkin maataloudesta ja ranta-asutuksesta peräisin oleva ravinnekuormitus on alkanut entistä voimakkaammin rehevöittää ennestään melko puhtaina säilyneitä järviä, jolloin rehevöitymisestä on tullut yhä laajemmalle levinnyt ongelma.

Kaikkien järvikunnostusten lähtökohtana on vähentää järveen kohdistuvaa ulkoisen ravinnekuormituksen määrää, mutta se ei monesti yksin riitä palauttamaan järveä lähellekään sen luonnollista tilaa. Etenkin 1990-luvulta lähtien järvien ravintoketjukunnostusta (biomanipulaatio) on käytetty varsin paljon sekä Suomessa että muualla rehevöityneiden järvien kunnostuksessa. Biomanipulaatiossa pyritään poistamaan järvestä pieniä eläinplanktonia syöviä kalalajeja (lähinnä eri särkikalat ja pienet ahvenet), jolloin eläinplanktonin määrä ja yksilökoko saadaan kasvamaan. Tällöin kasviplanktonia ravintonaan käyttävä eläinplankton pystyisi paremmin kontrolloimaan kasviplanktonin määrää järvessä. Biomanipulaation onnistumisen kriteerinä voidaan siis pitää kasviplanktonin määrän vähenemistä ja siitä aiheutuvaa veden kirkastumista. Varsin suoraviivaisesta teoriasta huolimatta saavutetut tulokset eivät läheskään aina ole täysin odotetun kaltaisia, ja monesti onnistuneidenkin biomanipulaatioiden tulokset ovat varsin lyhytkestoisia. Epäonnistumisten syitä ei aina tarkkaan tiedetä, koska biomanipulaation vaikutuksia koko järven ekosysteemiin (ravintoketjut, lajienväliset suhteet ja energian kierto) on varsin hankalaa ja työlästä tutkia perinteisin tutkimusmenetelmin. Luonnossa esiintyvien vakaiden isotooppien suhteiden määritykseen perustuva tekniikka tarjoaa kuitenkin erinomaisen työkalun nykyaikaiseen ekosysteemitutkimukseen, joten myös biomanipulaation vaikutuksista ekosysteemitasolla on mahdollista saada enemmän tietoa.

Tässä väitöskirjassa on tutkittu biomanipulaation vaikutuksia järviekosysteemin rakenteeseen ja toimintaan käyttämällä luonnossa esiintyviä hiilen (δ^{13}C) ja typen (δ^{15}N) vakaita isotooppeja. Tämän työn tarkoituksena oli täydentää Jyväskylän Jyväsjärven tutkimushanketta, jossa biomanipulaation vaikutuksia järven kasvi- ja eläinplanktonlajistoon sekä kalakantaan tutkittiin perinteisin menetelmin. Väitöskirja koostuu viidestä (I-V) osatyöstä, joista ensimmäisessä (I) luotiin pohja muille isotooppitutkimuksille Jyväsjärvessä. Kyseisessä osatyössä tutkittiin isotooppisuhteiden ajallista ja paikallista vaihtelua järven eri eliöissä. Työn päätuloksia olivat erittäin vähäinen paikallinen mutta huomattava ajallinen isotooppiarvojen vaihtelu järven ulappa-alueella, merkittävä paikallinen vaihtelu pohjaeläinyhteisön sisällä sekä tietyllä alueella havaittu ajallinen vaihtelu ahventen ja särkien isotooppiarvoissa. Kaikilla ensimmäisen osatyön tuloksilla oli suuri merkitys suunniteltaessa tulevien töiden näytteenottoja ja tulkittaessa tuloksia.

Ahventen ja särkien δ^{13}C- ja δ^{15}N-arvoissa havaittu vaihtelu ajoittui kevääseen ja vaihtelua esiintyi vain yhdessä paikassa järven itäisimmässä päässä. Jyväsjärvi on itäisimmästä päästään yhteydessä Päijänteeseen 700 m pituisen ruopatun joen välityksellä, ja vaihtelevia isotooppiarvoja kaloissa selitettiin Päijänteestä Jyväsjärveen kutemaan vaeltavilla kaloilla. Toinen osatyö (II) keskittyikin testaamaan vakaiden isotooppien menetelmää näiden vaeltavien kalojen osuuden arvioinnissa Jyväsjärven poistokalastussaaliissa. Yhtenä mahdollisena vaarana koko Jyväsjärven ravintoketjukunnostukselle oli poistettujen kalojen nopea korvautuminen Päijänteestä vaeltavilla kaloilla. Toisen osatyön tulosten mukaan keväällä 2004, jolloin tehokalastus Jyväsjärvessä alkoi, jopa 71 % järven itäisimmästä osasta poistetuista ahvenista ja 56 % särjistä olivat Päijänteestä vaeltaneita kaloja. Vastaavat osuudet järven muissa osissa olivat vain 7 ja 11 %, joten vaeltavia kaloja näytti olevan merkittävissä määrin ainoastaan Jyväsjärven itäpäässä. Seuraavana keväänä vaeltavien kalojen osuus poistokalastussaaliissa pieneni oleellisesti (24 ja 14 %) ja koekalastustulosten perusteella koko Jyväsjärven kalakanta oli selvästi pienentynyt. Tulosten mukaan Päijänteestä vaeltavat kalat eivät olleet välitön uhka Jyväsjärven kunnostushankkeelle ja poistokalastus oli tehokkaasti pienentänyt järven ahven- sekä särkikalakantoja.

Poistokalastuksen vaikutuksia pienentyneiden ahven- ja särkipopulaatioiden ravinnonkäyttöön tutkittiin käyttämällä populaatioiden δ^{13}C- ja δ^{15}N-keskiarvoihin liittyvää hajontaa ekolokeron koon mittarina ravinnon osalta (III). Koska δ^{13}C- ja δ^{15}N-arvot kuvaavat kalayksilöiden käyttämää ravintoa, voidaan populaation isotooppiarvoihin liittyvän hajonnan ajatella kuvaavan populaation käyttämän ravinnon vaihtelua. Kalakannan harventuessa sekä lajienvälinen että lajinsisäinen kilpailu ravinnosta vähenee, jolloin kalayksilöt voivat erikoistua käyttämään tiettyä ravintoa. Toisaalta harventunut kalakanta näkyy myös eri ravintokohteiden runsastumisena, mikä jo yksinäänkin voi vaikuttaa kalayksilöiden ravinnonvalintaan. Käyttäen populaatioiden δ^{13}C- ja δ^{15}N-arvojen varianssia hajonnan mittarina osoitettiin Jyväsjärven ahventen ja särkien ravintokohteiden määrän kasvaneen huomattavasti heti ensimmäisen tehokalastusvuoden jälkeen. Molemmissa populaatioissa osa yksilöistä erikoistui käyttämään lähinnä eläinplanktonravintoa ja osa puolestaan pohjaeläinravintoa. Kui-

tenkin jo vuonna 2006 ahvenpopulaation δ^{13}C- ja δ^{15}N-arvojen varianssit äkisti pienenivät kuvaten ahvenyksilöiden ravinnon suurta samankaltaisuutta. Särjillä vastaavaa ilmiötä ei havaittu, vaikka yksilöt erikoistuivatkin hieman aiempaa enemmän käyttämään eläinplanktonia. Ahventen ravinnon samankaltaisuuden selitti runsaan kalanpoikasravinnon syöminen, mikä on varsin tyypillistä poistokalastusten jälkeen. Työssä havaittiin luonnon vakaiden isotooppien suhteiden kuvastavan hyvin muutoksia populaatioiden ravinnonkäytössä, ja menetelmä tarjoaakin tehokkaan työkalun tämänkaltaisiin yhteisöekologisiin tutkimuksiin.

Biomanipulaation vaikutuksia koko järviekosysteemiin tutkittiin vertailemalla eri kalalajien asemaa ravintoketjussa ennen tehokalastusta ja sen jälkeen. Myös litoraalin ja pelagiaalin perustuotannon suhteellista osuutta energianlähteenä korkeammille ravintoketjutasoille vertailtiin ennen tehokalastusta ja sen jälkeen (IV). Ainoastaan särjellä ja hauella havaittiin muutoksia ravintoketjutasoissa biomanipulaation jälkeen, jolloin molemmilla lajeilla oli keskimäärin hieman alhaisempi ravintoketjutaso. Pelagiaalin perustuotannon osuus ravintoketjun energianlähteenä kasvoi biomanipulaation seurauksena lähes kaikilla lajeilla. Ainoastaan lahnalla litoraalin perustuotanto oli edelleen huomattavasti tärkeämpi energianlähde. Pelagiaalin tärkeyden energianlähteenä selittää eläinplanktonin muuttuminen tärkeämmäksi ravinnoksi pienemmille kaloille biomanipulaation jälkeen, mikä myös heijastui näitä kaloja syöviin suurempiin petokaloihin. Usein biomanipulaatioiden seurauksena myös litoraalin ravintokohteet voivat tulla entistä tärkeämmäksi mm. ahvenille, mutta Jyväsjärvessä näin ei juurikaan näyttänyt tapahtuvan. Syynä voisi olla esimerkiksi Jyväsjärven melko korkea veden humuspitoisuus, joka voi estää veden merkittävän kirkastumisen kasviplanktonin vähetessäkin. Tällöin näköaistin perusteella saalistavan ahvenen mahdollisuudet saalistaa pohjaeläimiä eivät oleellisesti parane. Pohjaeläinten saalistusta yleensä tehostava vesikasvillisuus ei myöskään lisääntynyt merkittävästi Jyväsjärvessä biomanipulaation jälkeen. Muutoinkaan Jyväsjärven erityispiirteet eivät näytä erityisesti suosivan tehokasta pohjaeläinten saalistusta.

Lisäinformaatiota biomanipulaation vaikutuksista voidaan vielä hankkia tutkimalla jo aiemmin tehtyjä biomanipulaatioita tai muunlaisia ravintoketjumuutoksia käyttäen säilöttyjä ja arkistoituja näytemateriaaleja. Viimeisessä osatyössä (V) testattiin kokeellisesti etanolin ja formaliinin vaikutuksia tyypillisimpien vesiympäristöistä kerättyjen näytemateriaalien, kuten eläinplanktonin ja pohjaeläimien isotooppiarvoihin. Samoin testattiin, voiko kalojen iänmäärityksiä varten kerättyjä suomu- ja luunäytteitä käyttää δ^{13}C- ja δ^{15}N-arvojen analysoinnissa lihaskudoksen tavoin ja kuinka hyvin analysoidut arvot vastaavat toisiaan. Tulosten perusteella säilöttyjä näytteitä voidaan käyttää isotooppitutkimuksissa historiallisten ekosysteemimuutosten arvioinneissa ilman kovin merkittävää epävarmuutta tulosten tarkkuudessa. Samoin suomu- ja luunäytteistä analysoituja δ^{13}C- ja δ^{15}N-arvoja voidaan käyttää lihaskudoksen tavoin isotooppitutkimuksissa tai muiden tutkimusten lisänä. Säilöttyjen ja arkistoitujen näytteiden analysoiminen mahdollistaa monien jo päättyneidenkin hankkeiden entistä tarkemman tutkimuksen sekä myös erittäin pitkien aikasarjojen

rakentamisen arvioitaessa ihmisten tai muuttuvan ilmaston aiheuttamia muutoksia ekosysteemeissä.

Tässä väitöskirjassa osoitettiin, että luonnon vakaiden isotooppien analysointiin perustuva tekniikka sopii mainiosti järviekosysteemien tutkimiseen ja ravintoketjukunnostusten vaikutusten selvittämiseen perinteisten menetelmien rinnalla. Isotooppimenetelmä toisaalta tukee ja antaa lisäinformaatiota perinteisillä tutkimusmenetelmillä saatuihin tuloksiin mutta myös vaatii perinteisiä menetelmiä omien tulostensa tulkintaan. Perinteisin menetelmin olisi erittäin vaikeata ja työlästä saada tietoa kalapopulaatioiden ekolokeromuutoksista, ravintoketjutason muutoksista eri lajeilla tai etenkin järven litoraalin ja pelagiaalin perustuotannon muuttuneesta merkityksestä ravintoketjun eri tasoille. Vakaiden isotooppien tutkiminen antaa myös mahdollisuuden analysoida historiallisia näytteitä, joiden tarjoama lisäinformaatio on perinteisten menetelmien saavuttamattomissa. Näin ekosysteemitason muutoksia voidaan tutkia vielä vuosien ja vuosikymmenten jälkeen.

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