Carbon and nitrogen isotopic ratios in archaeological and modern Swiss fish as possible markers for diachronic anthropogenic activity in freshwater ecosystems

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Abstract

The aim of this study is to investigate isotopic variability in archaeological (n=85) and modern (n=29) freshwater fish specimens from Switzerland. Here, carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope ratio analysis was performed on bone collagen samples of pike (*Esox lucius*), perch (*Perca fluviatilis*), barbel (*Barbus barbus*), roach (*Rutilus rutilus*) and carp (*Cyprinus carpio*) from eleven archaeological (11th to 18/19th centuries) and modern sites. The archaeological vs. modern fish data revealed significant isotopic differences for pike, perch and barbel ($\delta^{13}\text{C}$ p $\leq$ 0.03; $\delta^{15}\text{N}$ p $\leq$ 0.008), and provides possible evidence for a temporal change in Swiss aquatic ecosystems from Medieval to modern times. In comparison to archaeological fish ($\delta^{13}\text{C}$ mean $\pm$ SD; $-23.3 \pm 1.6\text{‰}$; $\delta^{15}\text{N}$ mean $\pm$ SD; $8.3 \pm 1.8\text{‰}$), the modern fish samples show decreased $\delta^{13}\text{C}$ and increased $\delta^{15}\text{N}$ values ($\delta^{13}\text{C}$ mean $\pm$ SD; $-27.4 \pm 2.3\text{‰}$; $\delta^{15}\text{N}$ mean $\pm$ SD; $12.5 \pm 4.1\text{‰}$) that could be associated with anthropogenic effects: fossil fuel combustion, deforestation and organic waste in the form of sewage and fertilizers. The isotopic signatures of archaeological fish remains indicate a local fishery practice, but also the exploitation of distant fishing grounds and freshwater fish transportation. Furthermore, a diachronic isotopic trend is observed in young perch from sites in Basel, dating between the 12th and 15/16th centuries, and the isotopic data from the Rhine freshwater fish (18/19th century) suggests that a significant shift in the river's trophic state was possibly caused by organic pollution from urban and industrial wastewater. This retrospective research illustrates possible natural processes and human activities which can cause differences in fish stable isotope data and highlights the ability to elucidate changes in past bodies of water. Furthermore, this study provides an interpretative framework for additional palaeoenvironmental studies and modern restoration projects focused on freshwater ecosystems.

Keywords

Freshwater fish, Middle Ages, stable isotope analysis, archaeoichthyology, water condition, pollution, human impact

1. Introduction

Stable isotope ratio analysis has become an important tool to examine ecological processes and anthropogenic impact on modern freshwater ecosystems and fish stocks in lakes and rivers (e.g. Macko and Ostrom, 1994; Kendall et al., 2007; Gladyshev, 2009). Past studies have found that freshwater fish carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope values of muscle tissue, bone and scales can act as important bio-indicators that reflect the ecological water conditions and the input of contaminants (e.g. Harvey and Kitchell, 2000; Lake et al., 2001; Perga and Gerdeaux, 2003; Miller et al., 2010). While it is clear that isotopic studies provide invaluable information about modern ecological processes, only a handful of studies have addressed these topics in prehistoric or historic freshwater ecosystems (e.g. Dufour et al., 1999; Van Neer et al., 2009; Miller et al., 2010; Fuller et al., 2012). Thus, it is still unclear to what extent humans have influenced freshwater fish stocks and aquatic environments in the past. This is especially true for early modern, Medieval and Roman periods, where possible human impact on aquatic ecosystems is assumed to have occurred (e.g. Van Neer et al., 2009).

One approach to evaluate past aquatic environments with archaeozoological material is to reconstruct the species composition of the fish stocks populating a body of water at a specified time. A shift from sensitive to more tolerant fish species may indicate a change in water condition (Van Neer et al., 2009; Van Neer and Ervynck, 2010). Indeed, such a decrease in the sensitive freshwater fish species spectra has been detected at numerous archaeological sites of the Rhine drainage basin in Switzerland (Basel, Stein am Rhein, Schaffhausen, Winterthur; c.f. Fig. 1; Häberle et al., 2015). This decline in indicator species including several salmonids (Salmonidae), barbel (*Barbus barbus*) and bullhead (*Cottus gobio*) occurred at minimum, from the 14th to 19th centuries and suggests the possibility that there was increased anthropogenic impact on fish and water systems during this time. Although Medieval written records reveal evidence of coeval human interference in Swiss water bodies (Amacher 1996; Simon-Muscheid 2006), a further methodological approach, the analysis of stable isotope signatures of freshwater fish, was undertaken here in order to better understand the archaeozoological data. This study was performed on archaeological pike (*Esox lucius*), perch (*Perca fluviatilis*), barbel (*Barbus barbus*), roach (*Rutilus rutilus*) and carp (*Cyprinus carpio*) from sites of the Rhine drainage basin, dating between the 11th-19th centuries AD (Fig. 1). Due to the fact that modern fish isotopic values can detect alterations in water systems (Macko and Ostrom, 1994; Brenner et al., 1999; Lake et al., 2001; Schlachter et al., 2005), a comparison of the archaeological fish isotope signatures with those obtained from modern freshwater fish species from the same region was performed to provide more information about the possible changes in aquatic environments from the Medieval period onwards.

2. Isotopic analysis of archaeological and modern freshwater fish: opportunities and limitations

Many factors can account for the different isotopic signatures of modern and archaeological freshwater fish, and these will be briefly addressed in this section. Variations are caused by spatial (e.g. benthic-pelagic gradients) or temporal differences (e.g. seasonal shifts) in aquatic ecosystem as well as by differences in fish feeding habits and fish habitats, a change in primary production levels or even by organic pollution (c.f. Cabana and Rasmussen, 1996; McClelland et al., 1997; Lake et al., 1999; Vander Zanden and Rasmussen, 1999; Grey et al., 2000; Perga and Gerdeaux, 2003; Schlachter et al., 2005; Gu et al., 2011; Miller et al., 2010; Morrissey et al., 2012; Thibodeau et al., 2013). Several studies on modern fish confirm the relationship between

¹⁵N-enriched values and the input of organic pollutants like sewage and fertilizers that change the trophic state or condition of the water (Macko and Ostrom, 1994; Brenner et al., 1999; Lake et al., 2001; Schlachter et al., 2005). For archaeological fish, the exploration of anthropogenic pollution in past aquatic ecosystems with stable isotope analysis is a promising avenue of research, when combined with available historical records, archaeological and archaeoichthyological information (e.g. Van Neer et al., 2009; Miller et al., 2010; Fuller et al., 2012).

Other factors influencing $\delta^{15}\text{N}$ values in both modern and archaeological fish, are age and size related trophic level effects (e.g. DeNiro and Epstein, 1978; Schoeninger and DeNiro, 1984; Hansson et al., 1997; Szpak et al., 2012; Häberle et al., 2016). These effects can result in higher mean $\delta^{15}\text{N}$ values of the larger and older fish specimens. Furthermore, the comparison of specimens with similar or identical niches from different water bodies can be complicated by the fact that water systems have specific isotopic signatures due to differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ inputs (c.f. Fry and Sherr, 1984; Finlay and Kendall, 2007). Disentangling these various factors presents a major challenge for modern and even more so for archaeological data. These problems are enhanced by the fact that studies of archaeological samples are limited by the availability of the archaeological record. Degradation and contamination is another common problem with archaeological fish remains (e.g. Szpak, 2011; Fuller et al., 2012, Häberle et al. 2016). In addition, even if the dating and location of the presented archaeological sites are well defined, determining freshwater fish provenance at archaeological sites is more difficult in comparison to modern fish, because specimens could originate from either local waters or have been transported from distant fishing grounds. For example, a historical source from the 12th century describes the transportation of whitefish from Lake Lucerne to Basel (about 110 km) by the order of the Provost of Basel (Müller, 1989). Additionally, some species could even come from fish ponds (especially carp, other cyprinids and pike) due to the rise of freshwater fish cultivation in the Middle Ages (Aston, 1988). All of these circumstances can increase the variability of the isotopic values of freshwater fish from both modern and archaeological sites. Therefore, it is important to study the detailed context of the specimens and extra care is needed in the interpretation of the isotopic results of freshwater fish.

3. Material and Methods

Fig. 1 Map of Switzerland with sites sampled in this study. Archaeological fish samples come from the following sites: 1 = Basel, Canton Basel-Stadt (5 sites); 2 = Füllinsdorf, Canton Basel-Landschaft (1 site); 3 = Schaffhausen; Canton Schaffhausen (1 site); 4 = Stein am Rhein, Canton Schaffhausen (1 site); 5 = Zürich, Canton Zürich (1 site); 6 = Winterthur, Canton Zürich (2 sites); 7 = Weesen, Canton St. Gallen (1 site). Modern fish samples come from the following locations: 1 = Rhine, Basel, Canton Basel-Stadt; 4 = Rhine, Stein am Rhein; Canton Schaffhausen; 5 = Lake Zürich, Zürich, Canton Zürich; 7 = Lake Walen, Weesen, Canton St. Gallen; 8 = Lake Constance; Altenrhein, Canton St. Gallen; 9 = Lake Greifen, Maur and Schwerzenbach, Canton Zürich; 10 = Lake Nussbaum, Canton Thurgau; 11 = Two artificial fish ponds in Pfaffenau, Canton Luzern and in Brittnau, Canton Aargau.

3.1. Archaeological fish

Archaeological fish bones ($n=54$) from 12 sites in northern and eastern Switzerland were selected for study. Sample and site information are presented in Figure 1 and Table 1. Fish remains were obtained by wet sieving sediment over a column of 4, 1 and 0.35 mm mesh size. Sieving, sorting, archaeozoological analysis and species identification was carried out at the Institute of Prehistory and Archaeological Science (IPAS), University of Basel, Switzerland (Hosch and Zibulski, 2003; Hüster Plogmann, 2006a). The species targeted for study included low trophic level feeders, i.e. barbel, roach and carp, and high trophic level feeders, i.e. pike and perch. The fish remains represent individuals of different age and size. Different skeletal elements, mostly vertebrae and head bones were used. To obtain sufficient bone collagen from small and young specimens, samples of several skeletal elements from the same closed stratigraphic context were compiled - a commonly applied method (Szpak et al., 2009; Fuller et al., 2012). Most fish bones were recovered from cess pits; only three assemblages come from refuse or other cultural layers (Burg Altenberg, Kleinhüningen Fischereihaus, Weesen). The selected sites date between the 11th to 19th centuries and the dating was made by typology (pottery, coins and artefacts) and/or radiocarbon measurement. In addition, to the samples presented here, the previously published isotopic data of 50 fish samples from ten of these sites (Häberle et al., 2016) is integrated into this study to produce a more complete dataset for analysis.

3.2 Modern Fish

Modern freshwater fish (n=29) were analysed from five Lakes: Constance, Zürich, Walen, Greifen and Nussbaum in northern and eastern Switzerland and from two different fishing locations in the River Rhine (Fig.1, Table 2). These locations were selected due to their close proximity to the archaeological sites (<20 km). While Lakes Constance and Walen are oligo-mesotrophic, Lake Zürich is mesotrophic (pers. comm. M. Kunz, Swiss Federal Office for the Environment, FOEN), and Lake Greifen and Nussbaum is thought to reflect a eutrophic environment (pers. comm. A. Scholits, Amt für Umwelt, Kanton Thurgau). In addition, the Rhine water quality (FOEN monitoring stations at Weil am Rhein and Stein am Rhein) was estimated as moderate to good by DOC (dissolved organic carbon), ammonium, nitrite, nitrate, phosphorous and phosphate concentrations. The same modern fish species as in the archaeological data set were chosen for analysis, using vertebrae and fin ray bone samples. Due to the established minimum legal size of modern fish, specimens were only obtained from individuals with a size >25 cm, except two samples obtained from 5 cm carp yearlings from an extensive cultivation pond (no addition of food, fertilizer or medication).

Some authors recommend a correction of the measured $\delta^{13}\text{C}$ values because of the Suess effect (Suess, 1955; Keeling, 1997; Schloesser et al., 2009), and there exist many diverse approaches to do this (Friedli et al., 1986; Schelske and Hodell, 1995; Verburg, 2007). The Suess effect describes a relative decrease of atmospheric ^{13}C levels caused by the increased input of lighter carbon (^{12}C) from the burning of fossil fuels and the reduction of forest and soil carbon reservoirs over the last 150 years. In regard to inland freshwater ecosystems, the need to correct for the Suess effect is controversial (e.g. O'Reilly et al., 2005; Verburg, 2007). It is unknown, to what extent single samples are affected by varying factors (e.g. size and depth of the lake/river, variations in surface fluctuations, lake inflow/outflow rate or riverine flow rate, vertical water mixing, organic matter inputs), and it is not inferable how strongly the Suess effect influences $\delta^{13}\text{C}$ values of freshwater fish. Furthermore, it can be assumed that the Suess effect has accelerated in the last few decades. Therefore, an adjustment of the modern fish isotope values was not pursued in this study.

3.3 Fish length determination

A length estimation of the archaeological fish was undertaken in order to determine life stage, feeding habits and habitat of a fish species (e.g. Barrett et al., 2011; Fuller et al., 2012; Robson et al., 2012). Therefore, the selected archaeological fish remains were compared with fish skeletons of known size from the reference collection (IPAS, University of Basel) in order to estimate the total length (TL) in centimeters: length from the tip of the snout to the tip of the longer lobe of the caudal fin. Furthermore, this method allows comparisons between the archaeological and modern fish, which were taken from individuals of known size. Archaeological and modern fish were assigned to one of five defined length classes: TL 1 = 5-10 cm, TL 2 = 10-20 cm, TL 3 = 20-30 cm, TL 4 = 30-50 cm, TL 5 = > 50 cm.

3.4 Isotopic analysis

Archaeological collagen extraction and isotopic analysis was conducted at the Department of Human Evolution, Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany, using the protocol outlined in Richards and Hedges (1999) and complemented by an additional ultrafiltration (cut-off >30 kDa) step (Brown et al., 1988). Previous work found a strong decline in

collagen yield in samples with a starting mass <20mg (Häberle et al., 2016), therefore a starting mass >20mg was preferred. The extracted collagen was weighed into tin capsules and combusted in a Flash Elemental Analyser (Flash EA 2212, Thermo-Finnigan® Bremen, Germany) coupled to a continuous-flow isotope ratio-monitoring mass-spectrometer (Thermo-Finnigan® Delta V Bremen, Germany).

Modern collagen preparation and isotopic measurement was performed at the Imprint-Analytics GmbH, Austria using the same collagen extraction method as described above, adding a lipid removal step due to the high lipid content of fresh fish bone (2:1 methanol/chloroform, e.g. Szpak, 2011; Fuller et al., 2012). ~0.5 mg of collagen was combusted online using a Eurovector 3000 elemental analyzer (HekaTech, Wegberg, Germany). The resulting CO₂ and N₂ was separated by Gas Chromatography (GC) and introduced into a Nu Horizon (Nu Instruments, Wrexham, UK) isotope-ratio mass-spectrometer via an open split and compared to pure CO₂ or N₂ reference gas.

The archaeological and modern results were obtained with a standard deviation of better than 0.2 per mil on the duplicates and long term monitoring with international standards (for more information on the standards see supplementary Table A.1 and A.2). Results are scaled against VPBD and air for d13C and d15N, respectively.

3.5 Statistics

It was tested if there were significant differences between stable isotope signatures of modern and archaeological fish bone samples, using a variance analysis (one-way ANOVA). To detect differences of stable isotope signatures from the different sites, a non-parametric analysis (Kruskal-Wallis and Mann-Whitney-U), and a linear regression analysis was applied. Statistical analysis was performed with PAST (Paleontological Statistics Software Package for Education and Data Analysis; Hammer et al., 2001) and Excel.

4. Results

Site/Locality/Canton	Code	Site number in Fig. 1	Time period (cen. AD)	Species	Total length class (TL)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	C:N	For more site information see references:	
Allerheiligen, Schaffhausen, Canton Schaffhausen	SHA	3	11 th /12 th	Pike	<i>Esox lucius</i>	3	no collagen			Rehazek, Brombacher 1999; Bünteli et al.,1999
				Perch	<i>Perca fluviatilis</i>	2	-22.1	10.8	3.7	
				Perch	<i>Perca fluviatilis</i>	3	-20.9	8.9	3.2	
				Perch	<i>Perca fluviatilis</i>	4	-24.9	9.4	3.1	
				Perch	<i>Perca fluviatilis</i>	4	-21.4	9.6	3.2	
				Barbel	<i>Barbus barbus</i>	3	-23.3	7.9	3.5	
				Barbel	<i>Barbus barbus</i>	3	-23.2	8.3	3.4	
Burg Altenberg, Füllinsdorf, Canton Basel-Land	AB	2	11 th	Pike	<i>Esox lucius</i>	5	-23.4	9.6	3.4	Marti-Grädel 2008; Marti et al., 2013
				Pike	<i>Esox lucius</i>	5	-25.0	14.2	3.3	
				Pike	<i>Esox lucius</i>	3	-23.2	9.0	3.5	
				Pike	<i>Esox lucius</i>	5	-24.7	13.9	3.2	
				Pike	<i>Esox lucius</i>	5	-25.1	14.2	3.4	
				Pike	<i>Esox lucius</i>	4	-23.3	9.3	3.5	
				Pike	<i>Esox lucius</i>	4	-21.6	9.1	3.5	

					Pike	<i>Esox lucius</i>	4	-24.5	8.9	3.5	
					Barbel	<i>Barbus barbus</i>	3	-23.9	8.3	3.5	
					Barbel	<i>Barbus barbus</i>	3	-22.0	8.6	3.4	
					Barbel	<i>Barbus barbus</i>	4	-21.7	9.5	3.4	
					Barbel	<i>Barbus barbus</i>	4	-21.7	9.5	3.4	
					Barbel	<i>Barbus barbus</i>	5	-21.7	9.4	3.4	
					Perch	<i>Perca fluviatilis</i>	4	-23.9	9.8	3.3	
					Perch	<i>Perca fluviatilis</i>	4	-24.0	9.9	3.5	
Kleinhüningen Fischereihaus, Basel, Canton Basel-Stadt	KLFH	1	18 th /19 th	Pike	<i>Esox lucius</i>	5	-21.7	7.9	3.3	Hüster Plogmann 2005; Springer 2005	
				Barbel	<i>Barbus barbus</i>	5	-20.4	11.5	3.3		
				Barbel	<i>Barbus barbus</i>	3	-19.6	11.5	3.5		
				Perch	<i>Perca fluviatilis</i>	3	-23.4	10.7	3.3		
				Perch	<i>Perca fluviatilis</i>	3	-23.9	7.8	3.4		
Museum der Kulturen, Basel, Canton Basel-Stadt	MKB	1	15 th /16 th	Pike	<i>Esox lucius</i>	5	-15.4	11.1	3.3	Straumann 2009	
				Pike	<i>Esox lucius</i>	5	-20.5	9.1	4.4		
				Perch	<i>Perca fluviatilis</i>	1	-23.8	6.4	3.5		
				Perch	<i>Perca fluviatilis</i>	1	-23.8	5.7	3.3		
				Perch	<i>Perca fluviatilis</i>	1	-24.3	6.5	3.3		
				Perch	<i>Perca fluviatilis</i>	1	-24.5	9.4	3.6		
				Perch	<i>Perca fluviatilis</i>	1	-24.6	8.4	3.5		
				Perch	<i>Perca fluviatilis</i>	3	-24.1	8.8	3.6		
				Barbel	<i>Barbus barbus</i>	3	-24.0	8.1	3.7		
				Roach	<i>Rutilus rutilus</i>	3	-25.6	7.5	3.5		
Reischacherhof, Basel, Canton Basel- Stadt	REI	1	17 th	Pike	<i>Esox lucius</i>	3	-21.3	8.7	3.3	Schibler, Hüster Plogmann 1996	
				Perch	<i>Perca fluviatilis</i>	1	-25.2	9.0	3.7		
				Perch	<i>Perca fluviatilis</i>	2	-21.1	10.3	3.2		
				Perch	<i>Perca fluviatilis</i>	2	-22.2	7.1	3.2		
				Roach	<i>Rutilus rutilus</i>	1	-24.2	8.7	3.6		
				Carp	<i>Cyprinus carpio</i>	3	-25.4	7.9	3.2		
				Carp	<i>Cyprinus carpio</i>	4	-26.7	6.8	3.4		
				Carp	<i>Cyprinus carpio</i>	4	-25.7	7.6	3.4		
Schnabelgasse, Basel, Canton Basel- Stadt	SG	1	12 th	Pike	<i>Esox lucius</i>	4	-24.3	7.1	3.8	Häberle 2010, Rodel 2003	
				Pike	<i>Esox lucius</i>	3-5	-22.5	8.5	3.4		
				Perch	<i>Perca fluviatilis</i>	1	-23.6	6.5	3.3		
				Perch	<i>Perca fluviatilis</i>	1	-23.3	6.3	3.2		
				Perch	<i>Perca fluviatilis</i>	1	-23.8	7.0	3.4		
				Perch	<i>Perca fluviatilis</i>	1	-24.0	7.2	3.4		
				Perch	<i>Perca fluviatilis</i>	1	-23.0	7.6	3.2		
				Perch	<i>Perca fluviatilis</i>	1	-24.4	7.0	3.3		
				Perch	<i>Perca fluviatilis</i>	1	-23.9	5.9	3.3		
				Perch	<i>Perca fluviatilis</i>	1	-23.6	6.6	3.3		
				Perch	<i>Perca fluviatilis</i>	1	-24.2	7.8	4.1		
				Perch	<i>Perca fluviatilis</i>	1-2	-24.2	6.5	3.3		

					Perch	<i>Perca fluviatilis</i>	1	-27.3	13.0	3.5		
					Barbel	<i>Barbus barbus</i>	4	-25.5	9.9	3.2		
					Barbel	<i>Barbus barbus</i>	4	-24.0	9.1	3.3		
Oberгasse, Winterthur, Canton Zürich					WOG	6	13 th					
					Perch	<i>Perca fluviatilis</i>	1	no collagen			Hüster Plogmann et al., 2002; Hüster Plogmann and Kühn 2013	

Table 1 Summary information and isotopic results from archaeological freshwater fish. Samples with sufficient collagen content and acceptable C:N are in bold type and previous published results from Häberle et al. (2016) are shaded in grey. Individual sample details listed in Supplementary Table A.1.

Fig. 2 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ results for 85 archaeological (solid) and 29 modern (open) fish species with acceptable C:N.

4.1 Archaeological fish isotopic signatures

A total of 35 out of 54 (65%) archaeological freshwater fish remains produced sufficient collagen with acceptable C:N between 2.9 and 3.6 as defined by DeNiro (1985). These 35 results were then combined with the previously published isotope values (n=50) of Häberle et al. (2016) so that 85 specimens were available for interpretation (Table 1, Fig. 2). The $\delta^{13}\text{C}$ values ranged between -27.3‰ and -15.4‰ (mean $\pm$ SD; -23.3 $\pm$ 1.6‰), and the $\delta^{15}\text{N}$ values ranged between 5.3‰ and 14.2‰ (mean $\pm$ SD; 8.3 $\pm$ 1.8‰) (Fig. 3 and Supplementary Table A.3). The range of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values is similar to other archaeological freshwater isotopic signatures (e.g. Grupe et al., 2009; Fuller et al., 2012). For both the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, no significant diachronic trend was visible (Supplementary Fig. A.4.1). Furthermore, a Kruskal-Wallis-test revealed significant differences between the $\delta^{13}\text{C}$ values (Kruskal-Wallis p = 0.010), and between the $\delta^{15}\text{N}$ values of the different archaeological sites (Kruskal-Wallis p = 0.000). An additional pairwise Mann-

Whitney-test revealed differences between the pairwise compared sites, in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (Supplementary Table A.5).

Fig. 3 a) Mean $\pm$ SD $\delta^{13}\text{C}$ values and b) Mean $\pm$ SD $\delta^{15}\text{N}$ values of archaeological (solid) and modern (open) samples of the five sampled freshwater fish species classified by their length (TL 1-TL 5). Standard deviations are calculated for $n > 2$.

4.2 Modern fish isotopic signatures

Catching site/nearby locality/Canton	Code	Site number in Fig. 1	River length km / Lake surface area km ²	max. lake depth m	Species	Total length class (TL)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	C:N
(High-) Rhine, Stein am Rhein, Canton Schaffhausen	RSR	4	145		Barbel <i>Barbus barbus</i>	5	-26.0	12.2	3.0
					Barbel <i>Barbus barbus</i>	5	-26.1	11.7	3.1
(Upper-) Rhine, Basel, Canton Basel-Stadt	RBS	1	350		Barbel <i>Barbus barbus</i>	5	-23.8	14.3	3.6
					Barbel <i>Barbus barbus</i>	5	-22.9	11.1	3.2
Lake Constance, Altenrhein, Canton St. Gallen	LC	8	536	254	Pike <i>Esox lucius</i>	5	-24.7	13.2	3.3
					Pike <i>Esox lucius</i>	5	-26.2	13.3	3.5
					Roach <i>Rutilus rutilus</i>	4	-24.8	10.1	3.2
Lake Zürich, Zürich, Canton Zürich	LZ	5	88	136	Pike <i>Esox lucius</i>	5	-26.6	14.4	3.5
					Pike <i>Esox lucius</i>	5	-27.3	14.4	3.0
					Perch <i>Perca fluviatilis</i>	3	-27.8	14.1	3.6
					Perch <i>Perca fluviatilis</i>	3	-26.7	14.4	3.6
					Roach <i>Rutilus rutilus</i>	3	-27.8	11.8	3.5
					Roach <i>Rutilus rutilus</i>	4	-28.1	11.5	3.6
Lake Walen, Weesen, Canton St. Gallen	LW	7	24	150	Pike <i>Esox lucius</i>	5	-26.3	8.1	3.1
					Pike <i>Esox lucius</i>	5	-24.9	8.5	3.3
					Perch <i>Perca fluviatilis</i>	3	-27.5	6.7	3.1
					Perch <i>Perca fluviatilis</i>	3	-26.5	8.2	3.5
					Roach <i>Rutilus rutilus</i>	4	-23.4	4.0	3.6
					Roach <i>Rutilus rutilus</i>	4	-22.7	4.5	3.3
Lake Greifen, Maur, Canton Zürich					Pike <i>Esox lucius</i>	4	-30.2	20.3	3.5
					Pike <i>Esox lucius</i>	4	-29.7	20.1	3.3
Lake Greifen, Schwerzenbach, Canton Zürich	LG	9	8	34	Perch <i>Perca fluviatilis</i>	4	-30.2	18.3	3.4
					Perch <i>Perca fluviatilis</i>	4	-29.9	20.2	3.5
					Roach <i>Rutilus rutilus</i>	4	-25.1	13.5	3.6
					Roach <i>Rutilus rutilus</i>	3	-27.0	12.9	3.4
Lake Nussbaum, Canton Thurgau	LN	10	0.3	3.9	Pike <i>Esox lucius</i>	5	-27.7	15.5	3.0
					Roach <i>Rutilus rutilus</i>	3	-32.0	11.2	3.1
Pond 1, Pfaffenau, Canton Luzern	POND 1	11	0.002	1	Carp <i>Cyprinus carpio</i>	1	-24.4	12.3	3.5
Pond 2, Brittnau, Canton Aargau	POND 2		0.005	1		1	-20.2	6.8	3.4

Table 2 Summary information and isotopic results of modern freshwater fish. Individual sample details are listed in Supplementary Table A.2.

All modern freshwater fish remains (n=29) produced sufficient collagen with acceptable C:N as defined by DeNiro (1985) (Table 2; Fig. 4). The $\delta^{13}\text{C}$ values range between -32.0‰ and -20.2‰ (mean $\pm$ SD; -27.4 $\pm$ 2.3‰; pike mean $\pm$ SD of -27.1 $\pm$ 1.8‰; perch mean $\pm$ SD of -28.1 $\pm$ 1.5‰; barbel mean $\pm$ SD of -24.7 $\pm$ 1.4‰; roach mean $\pm$ SD -26.4 $\pm$ 2.8‰; carp mean $\pm$ SD of -22.3 $\pm$ 2.1‰) and do not show high heterogeneity. In contrast, the $\delta^{15}\text{N}$ values reveal a high variability, which is visible in the wide standard deviation. The $\delta^{15}\text{N}$ values range between 4.0‰

and 20.3‰ (mean $\pm$ SD; 12.5 $\pm$ 4.1‰). A similar heterogeneity in carbon and nitrogen isotope values was detected in whitefish scales (*Coregonus spec.*) from 14 Swiss lakes ($\delta^{13}\text{C}$ mean $\pm$ SD of -27.0 $\pm$ 2.8‰ and $\delta^{15}\text{N}$ mean $\pm$ SD of 12.3 $\pm$ 4.0‰; Perga and Gerdeaux, 2003). A Kruskal-Wallis-test confirms that both the $\delta^{13}\text{C}$ ($p = 0.004$) and $\delta^{15}\text{N}$ ($p = 0.005$) results from the different water bodies were significantly different, and this could be attributed to the different size, basin morphology and possible trophic state of the selected modern water systems. In addition, the modern fish samples are lower in $\delta^{13}\text{C}$ and higher in $\delta^{15}\text{N}$ values compared to the archaeological fish (Fig. 3).

Fig. 4: $\delta^{13}\text{C}$ (a) and $\delta^{15}\text{N}$ (b) results of modern pike, perch, barbel, roach and carp samples (n=29) with acceptable C:N. Modern samples are arranged as follows: River Rhine (RSR; RBS) – lakes (LC; LW; LZ; LG; LN) – ponds 1 and 2. Additionally, lakes and ponds are arranged according to trophic state.

5. Discussion

5.1 Interpretation of isotopic signatures of archaeological fish

The archaeological freshwater fish show a large isotopic variability, possibly due to the above mentioned influencing factors. Thus, no temporal pattern was observed between the fish samples from the different archaeological sites (Supplementary Table A.4.1). Due to the age and size related trophic level effects, a separate analysis of the isotopic data from adult fish (TL 3-5) and young fish (TL1) was performed (note: fish samples from the TL 2 category were excluded because they are in a transition stage). Most of the young fish in the archaeological dataset are perch which come from sites in Basel from different time periods, between the 12th and the 15/16th centuries (Fig. 5). These young specimens are assumed to be caught near Basel since they are less likely to migrate (c.f. Wang and Eckmann, 1994) and due to the fact that young freshwater fish would not have been transported over long distances by humans because of their fast perishability (e.g. Hüster Plogmann, 2006; Häberle et al., 2015). Furthermore, catching small freshwater fish was not part of professional fishing operations but a casual activity by individual households to add foods to the daily diet (e.g. Amacher 1992; Häberle et al. 2010). This non-professional preservation of small amounts of fish was too costly and time consuming and not commercially lucrative, because of the small amount of flesh relatively to the amount of bone or other non-eatable body parts. Thus, it is reasonable to conclude that these smaller fish were eaten immediately after being caught and were not preserved for transportation. In contrast, for larger fresh specimens, an overland transport by horse within 150 km was possible (Hoffmann, 2001), and even greater distances were likely for large fish that were preserved. Thus, young fish can act as baseline indicators of the local isotopic environment as they have isotopic signals that correspond to approximately a year of life, a uniform diet and to a limited inhabited biotope whereas older specimens represent the average diet, habitat and even migration over the entire lifespan of an individual (Hesslein et al., 1993; Harrod et al., 2005).

In this data set (Fig. 5), young perch from the oldest site of Schnabelgasse (SG) show significantly decreased $\delta^{13}\text{C}$ values (mean $\pm$ SD; $-23.8 \pm 0.4\text{‰}$) compared to the $\delta^{13}\text{C}$ values of perch from Wildensteinerhof (WI) (mean $\pm$ SD; $-24.7 \pm 0.4\text{‰}$) and Museum der Kulturen (MKB) (mean $\pm$ SD; $-24.2 \pm 0.3\text{‰}$) (Kruskal-Wallis $p = 0.034$), and this could indicate different catching grounds along the Rhine or one of its tributaries for these fish. In contrast, the $\delta^{15}\text{N}$ values from SG (mean $\pm$ SD; $6.7 \pm 0.5\text{‰}$) cluster together, and perch from the younger site of WI (mean $\pm$ SD; $-8.1 \pm 0.9\text{‰}$) and MKB are more scattered (mean $\pm$ SD; $7.3 \pm 1.4\text{‰}$). Even though there are no significant differences in the $\delta^{15}\text{N}$ values ($p = 0.138$) between these sites, it can be speculated that the nitrogen distribution pattern of the visible outliers from WI and MKB (individuals 10, 11, 13, 14) may indicate a change in water condition/quality near Basel. However, it is important to remember that variations in the $\delta^{15}\text{N}$ values can also represent different feeding strategies or spatial variability in small biotopes.

Fig. 5: $\delta^{13}\text{C}$ (a) and $\delta^{15}\text{N}$ (b) results of archaeological TL 1 perch from three sites in Basel: Schnabelgasse (SG), 12th century (individuals 1-9), Wildensteinerhof (WI), 14th century (individuals 10-12), and Museum der Kulturen (MKB), 15/16th century (individuals 13-17).

A separate Kruskal-Wallis-test of the TL 3-5 pike, perch and barbel from different sites (only sites with $n \geq 2$ fish samples were analysed: Allerheiligen (SHA), Burg Altenberg (AB), Kleinhüningen Fischereihaus (KLFH), Weesen (WEE), Obere Kirchgasse (WKG)) shows no differences in $\delta^{13}\text{C}$ values ($p = 0.253$), while the $\delta^{15}\text{N}$ values are significantly different ($p = 0.0495$). This is possibly due to the different feeding habits of the three different fish species: adult pike and perch are piscivorous, whereas adult barbel feed mainly on molluscs and insects. When the three species are analysed separately, the statistical differences are no longer visible in the $\delta^{13}\text{C}$ values for pike ($p = 0.06$) and perch ($p = 0.451$) and in the $\delta^{15}\text{N}$ values of pike ($p = 0.693$), perch ($p = 0.127$) and barbel ($p = 0.061$) even though it is assumed they were from different catching localities. This is possibly the result of the low number of samples studied. Only the $\delta^{13}\text{C}$ values of the barbel show a difference ($p = 0.044$), due to the two ^{13}C -enriched KLFH individuals. Nevertheless, some archaeological TL 3-5 fish specimens with exceptional isotopic signatures exist (Supplementary Fig. A.4.2), and possible explanations for these outliers will be described below.

Compared to archaeological pike of the same size, three pike (TL 5) from AB (a castle of the 11th century AD) show increased $\delta^{15}\text{N}$ and decreased $\delta^{13}\text{C}$ values. In addition, these pike differ from the other pike at the same site (Fig. 6). Therefore, a provenance from a waterbody other than the Rhine, or even from a fish pond or an oxbow lake, and the transportation of these specimens to the site is possible (maybe as a part of the tribute system to the castle's inhabitants) (c.f. Häberle et al., 2015). Of exceptional interest is the discovery of a ^{13}C -enriched (-15.4‰) pike from the MKB site (15th/16th century AD). Freshwater can become ^{13}C -depleted by changes in primary production of the carbon cycle or as a result of a gradient in ^{13}C between marine and freshwater systems (Fry and Sherr, 1984; Fry, 2002; Harrod et al., 2005). Pike are flexible species in terms of habitat, and are able to live in brackish water zones (Muus and Dahlström, 1990). This could suggest that this individual originated much further downstream, possibly from the marine influenced Rhine estuary area. As pike do not migrate long distances (Muus and Dahlström, 1990), the human transport of this individual (likely preserved) from the 1000 km distant Rhine estuary area to Basel is a possible scenario (Pulsiano and Wolf 1993). Furthermore, for predatory species such as pike a specific prey specialisation (fish, invertebrates, small mammals or waterfowl) and a shift from pelagic to littoral feeding habits can also cause changes in $\delta^{13}\text{C}$ values (Maitland and Campbell, 1992; Beaudoin et al., 1999).

Samples from two sites along the Rhine show special $\delta^{13}\text{C}$ values. Two perch (TL 3: -20.9 and TL 4: -21.4) from SHA (11th/12th century AD) have slightly increased $\delta^{13}\text{C}$ values like those of two barbel (TL 3: -19.6‰ and TL 5: -20.4‰) and like a single pike (TL 5: -21.7‰) of KLFH (18th/19th century AD). Possible explanations for these different values include a variation in primary

production levels (Wainright et al., 1993; Kiriluk et al., 1999; Perga and Gerdeaux, 2003), a provenance from different fishing grounds or a specific prey specialisation as mentioned above.

The two KLFH barbel (TL 3 and TL 5) from the 18th/19th centuries AD also exhibit increased $\delta^{15}\text{N}$ values (Fig. 6c). Since this species is a low trophic level feeder that lives in fast running and oxygenated water (Muus and Dahlström, 1990), a provenance from a pond is not likely. Barbel show large individual movement patterns and can migrate over 300 km during their spawning season (c.f. Steinmann et al., 1937, Lucas and Batley, 1996). Therefore, a distant catching ground or ecological niche can be assumed but also a migrating specimen that was locally caught could be possible (cf. Häberle et al., 2016). Nevertheless, short-term spatial changes should not influence the stable isotope signatures, and since this site dates from the era of industrialisation, a rise in organic pollution in the Rhine could also be possible, as was shown recently in other streams and rivers (cf. Diebel and Vander Zanden, 2009; Morrissey et al., 2012). During the 18th and 19th centuries the textile and chemical industries became prominent in Basel and the surrounding area, causing increased economic activity and a rise in population density (Degen and Sarasin, 2002). Under these circumstances high amounts of organic waste, sewage, and industrial wastewater was introduced into the Rhine and its tributaries. All these inputs may have altered the natural isotopic signatures of these waterways, but additional large scale studies are needed to confirm this possibility.

5.2 Comparison of the archaeological and modern fish

The archaeological and modern fish were mainly compared using the larger specimens (TL 3 – TL 5). However, in the case of carp there are only two modern specimens with lengths of TL 1 that needed to be compared with the archaeological carp (TL 3 – TL 4); keeping the possible size and age related trophic level effects in mind. The modern specimens of the investigated fish species have significantly decreased $\delta^{13}\text{C}$ and increased $\delta^{15}\text{N}$ values compared to their archaeological counterparts (Fig. 6). A statistical analysis of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of archaeological and modern fish with lengths between TL 3 – TL 5 shows a significant difference in pike ($\delta^{13}\text{C}$ $p=0.00$; $\delta^{15}\text{N}$ $p=0.008$), perch ($\delta^{13}\text{C}$ $p=0.00$; $\delta^{15}\text{N}$ $p=0.006$) and barbel ($\delta^{13}\text{C}$ $p=0.03$; $\delta^{15}\text{N}$ $p=0.003$) (Supplementary Table A.6). This prominent isotopic shift between the archaeological and modern specimens suggests a temporal change in the aquatic ecosystems (Fig. 6). Thus, the significantly ^{13}C -depleted values of these three species are likely caused by a change in the sources of carbon over time, possibly as a result of the human pollution by fossil fuel combustion (Peterson and Fry, 1987; Macko and Ostrom, 1994; Brenner et al., 1999; Lake et al., 2001; Schlachter et al., 2005). Furthermore, the ^{15}N -enriched values of the modern samples suggest an input of anthropogenic organic substances like sewage and fertilizers that are known to raise the trophic state and condition of the water (Macko and Ostrom, 1994; Brenner et al., 1999; Lake et al., 2001; Schlachter et al., 2005).

Fig. 6: $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ results of archaeological and modern pike (a), perch (b), barbel (c) samples (TL 3 – TL 5) diachronically plotted by archaeological site/catching site.

While direct comparisons between archaeological and modern fish specimens from different lakes can be complicated by possible isotopic differences between water systems, our results show some interesting patterns. The $\delta^{15}\text{N}$ values of the archaeological pike and perch are most similar to the modern pike and perch from meso-oligotroph Lake Walen (Fig. 6a and b). These results could suggest constant feeding habits at similar trophic levels for these species over time or a re-oligotrophication phase in Lake Walen after a phase of eutrophication in the last centuries because of different water protection actions (FOEN, status reports 2016). In contrast, pike from SG, Reischacherhof (REI) and KLFH and one perch from KLFH show decreased $\delta^{15}\text{N}$ values. These results are remarkable for high trophic level feeders, and could suggest low nutrient concentrations in the aquatic biotope or modifications in their feeding ecology due to a change in food supply. As the pike and perch of KLFH date to the era of industrialisation, a reduction of prey fish species due to water pollution and therefore a shift to a diet consisting of lower trophic organisms can be suggested (cf. Häberle et al., 2015).

In contrast, the modern pike and perch $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of eutrophic Lake Nussbaum and Lake Greifen differ the most from their archaeological counterparts likely due to the high content of organic matter as well as agricultural runoff and sewage input over the last 100 years (Liechti, 1994). In addition, deforestation in the areas around these lakes may account for the reduction of allochthonous (terrestrial) organic matter leading to decreased carbon isotope values. Such effects can be observed in forest streams for example, where various organisms show increased $\delta^{13}\text{C}$ values caused by terrestrial input, from sources such as leaf litter and terrestrial invertebrates (c.f. Hicks, 1997; Finlay, 2001).

A comparison of the archaeological and modern barbel (TL 3 - TL 5) shows that they have a wide range of $\delta^{13}\text{C}$ values during both periods, even though all four modern specimens were caught in the Rhine (Fig. 6c). The $\delta^{13}\text{C}$ differences between the modern barbel samples from the two Rhine catching sites could be caused by differing water velocity in those parts of the Rhine (cf. Trudeau and Rasmussen, 2003). Basel is situated at the slow-moving Upper-Rhine (between Basel, Switzerland and Bingen, Germany) and Stein am Rhein is located at the fast-running High Rhine, the part of the Rhine from Lake Constance to Basel. One archaeological perch and two barbel from KLFH, dating from the era of industrialisation (18th/19th centuries), show the most ^{15}N -enriched signatures and cluster with the modern samples mirroring a change in the ecology of the Rhine water system from this time to the present; likely due to the input of urban waste (Brenner et al., 1999; Lake et al., 2001; Degen and Sarasin, 2002; Morrissey et al., 2012). As there were no sewage treatment plants until 1977, sewage flowed untreated into the Rhine and its tributaries until this time (Degen, 2002). Industrial wastewater from the chemical industry, existing in Basel since 1859, was also deposited untreated into the river (Degen und Sarasin, 2002). It is possible that these anthropogenic inputs caused the increased $\delta^{15}\text{N}$ values of the KLFH fish. However, many factors can influence $\delta^{15}\text{N}$ values (e.g. Diebel and Vander Zanden, 2009), and additional studies are required in the future.

Although the comparison between archaeological and modern roach ($\delta^{13}\text{C}$ $p=0.329$; $\delta^{15}\text{N}$ $p=0.385$) and carp ($\delta^{13}\text{C}$ $p=0.114$; $\delta^{15}\text{N}$ $p=0.386$) revealed no significant differences, the high intra-species variability in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of these cyprinid species is remarkable (Fig. 3). This variability is possibly caused by the high flexibility of carp and roach in their diet and habitat preference (Muus and Dahlström, 1990) or by their provenance from biotopes with different trophic states and water quality. Distinctly apparent are the decreased $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the roach from Lake Walen (Table 2), likely due to the lakes re-oligotrophication.

Furthermore, archaeological carp from the REI site revealed different isotopic signatures in comparison to the modern carp from the two artificial ponds in Pfaffenau and Brittnau (Fig. 2). The two archaeological carp from the early modern site of REI (16th/17th centuries AD) have relatively low $\delta^{15}\text{N}$ values (6.8‰ and 7.9‰) and do not, as expected, reflect a nutrient-rich artificial pond environment, even though this non-endemic species was an important pond fish in late Medieval times (e.g. Hoffmann, 1995; Benoit et al., 2004). These results may be related to the multitude of possible carp cultivation systems (closed ponds, mill races, moats or open ponds with fresh water supply through a connection to a river or lake; c.f. Amacher 1996; Benoit et al., 2004) that can lead to different isotopic signatures in cultured carp. In addition, the variable diet of cultured carp is an important factor to consider (Muus and Dahlström, 1990). Carp feed on benthic invertebrates and plants but can become primarily herbivorous (Muus and Dahlström, 1990). Therefore, the low $\delta^{15}\text{N}$ results suggest the archaeological carp fed mainly on plant material. The two modern and smaller carp (TL 1) do not show significantly lower isotopic values, likely due to agricultural runoff from the surrounding area. Furthermore, these two individuals have very different $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, even though they originate from two neighbouring fish ponds (7.2 km apart). In both ponds, no food or fertilizer was added that could have changed the carp isotopic signatures. Thus, a possible explanation might be that for pond 1, a stronger accumulation of organic mud and detritus is documented that might account for the ^{15}N -enriched values (pers. comm. Manfred Steffen, Swiss association KarpfenPurNatur).

6. Conclusions

The detection and control of anthropogenic pollution in aquatic environments is a major challenge for ecological projects focused on the environmental protection of modern waterways. Currently, it is not exactly known how strong the impact of pollution was on aquatic ecosystems in Switzerland during different historical periods. The results presented here illustrate that comparative isotopic studies of archaeological and modern freshwater fish can make substantial contributions to understanding changes in modern and past aquatic ecosystems.

We observed significant differences between archaeological and modern fish (TL 3 - TL 5) isotope signatures, which are most likely a result of human impact by urban and agricultural activities (e.g. input of sewage, industrial wastewater, fertilizer, fossil fuel combustion and deforestation). So far, the limited archaeological data from the Rhine water systems allows the conclusion that noticeable human water pollution can be traced back to at least the era of industrialisation in 18th/19th centuries AD. In addition, differences in isotopic values of small perch (TL 1) from three sites in Basel and a decrease in abundance of sensitive fish species (salmonids, barbel, bullhead) from the 14th century AD onwards in the fish spectra of several studied sites in Basel (Häberle et al. 2015) suggests that a change in water condition possibly started much earlier. However, caution is needed in the interpretation of these results as many factors can cause the high isotopic variability observed between the Medieval and modern periods. Therefore, careful consideration of all the available evidence: historical records, archaeological information, archaeoichthyological and isotopic results are needed to fully understand the past ecology of fish species and their paleo-environment. The development of larger and more detailed diachronic isotopic studies comparing archaeological and modern fish remains from single sites, and the incorporation of multiple species at various life stages will build upon the work presented here and provide important information for palaeo-environmental and conservation projects.

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