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Examination of an Oligocene lacustrine ecosystem using C and N stable isotopes

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Abstract

The Late Oligocene (25.8 Ma) Enspel Fossillagerstätte in Westerwald, Germany, contains a comprehensive fossil ecosystem preserved with specimens retaining morphological detail and a concentration of organic material. Stable carbon and nitrogen isotope analyses were used to examine the lacustrine ecosystem preserved in one stratigraphic horizon. These data suggest the presence of several trophic levels, including primary producers (diatoms and higher plants), primary consumers (e.g., tadpoles and insects), and secondary consumers (e.g., the fish species *Paleorutilus enspelensis*). Terrigenous and aquatic plants were associated with the lowest $\delta^{13}C$ and $\delta^{15}N$ values (mean plant = $-26.28‰ \pm 0.45$, $3.18‰ \pm 1.04$), primary consumers such as flies are one trophic level higher, and carnivores such as fish are yet another level higher. The $\delta^{15}N$ values for *P. enspelensis* also showed enrichment in ^{15}N with increasing body length, implying a shift in diet or feeding strategy with size. *P. enspelensis* and tadpole (*Pelobates decheni*) samples showed intraorganism fractionation between 'muscle' and 'bone' tissues. Stable carbon and nitrogen isotope data from the measurement of components (shale, leaves and seeds) common to a number of different stratigraphic horizons showed significant variation between horizons. A number of the features of the stable isotopic data are similar to those relationships seen in modern ecosystems and therefore suggest that stable isotope analyses can contribute to understanding ancient ecosystems.

Keywords: Enspel; Carbon; Nitrogen; Fossil; Trophic reconstruction; Europe; Germany; Fossillagerstätte

1. Introduction

With their immaculately preserved array of floral and faunal components (e.g., Storch et al., 1996; Köhler, 1997; Stankiewicz et al., 1997; Wedmann, 1998), the Upper Oligocene lake sediments at Enspel,

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Westerwald, Germany (Fig. 1) provide an attractive resource for examining a comprehensive fossil ecosystem. A number of techniques have been brought to bear on various fossil localities in hopes of understanding ancient trophic structures and functions. Paleontological ecosystem reconstructions traditionally rely on visual inspection of morphological characteristics such as gut contents and similarity to extant organisms (e.g., Baszio and Richter, 2001). Recently, chemical tracing, including stable isotope analysis, has been developed as a method for quantifying trophic relationships.

Stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) provide a tool for tracing nutrient sources in modern and prehistoric food webs. This approach is particularly useful in situations where alternative techniques for examining feeding strategies, trophic links, and elemental cycling in an ecosystem, such as behavioral studies, cannot be applied. From primary producers up the food chain to carnivores, each trophic level is enriched in the heavier isotopes of these elements, typically by 0–1‰ in $\delta^{13}C$ and by 2–5‰ in $\delta^{15}N$ (DeNiro and Epstein, 1978, 1981; Vander Zanden, 2001). The stable isotope signatures of organisms in a complex ecosystem therefore reflect their nutrient sources (e.g., MacFadden et al., 1999), and different components of the ecosystem have distinct and separate stable isotope signatures (e.g., Sealy et al., 1987; Cabana and Rasmussen, 1994; Vander Zanden and Rasmussen, 1999). Stable isotope analyses of ecosystem components can be used to trace multiple nutrient sources, yielding a food web instead of a food chain.

Reconstructions of ancient food webs have been difficult to create, because complete fossil ecosystems with numerous examples of component organisms are rarely preserved. Stable carbon isotope data have more commonly been used to reconstruct the diet of a single organism over time (e.g., MacFadden et al., 1994; Wang et al., 1994) and to study isolated predator–prey relationships (e.g., MacFadden et al., 1999; Sponheimer and Lee-Thorp, 1999). Stable isotope measurements in many of these fossil studies have been obtained from bones and teeth (e.g., Bocherens et al., 1995; Clementz and Koch, 2001), which contain far less carbon and nitrogen than soft tissue. A

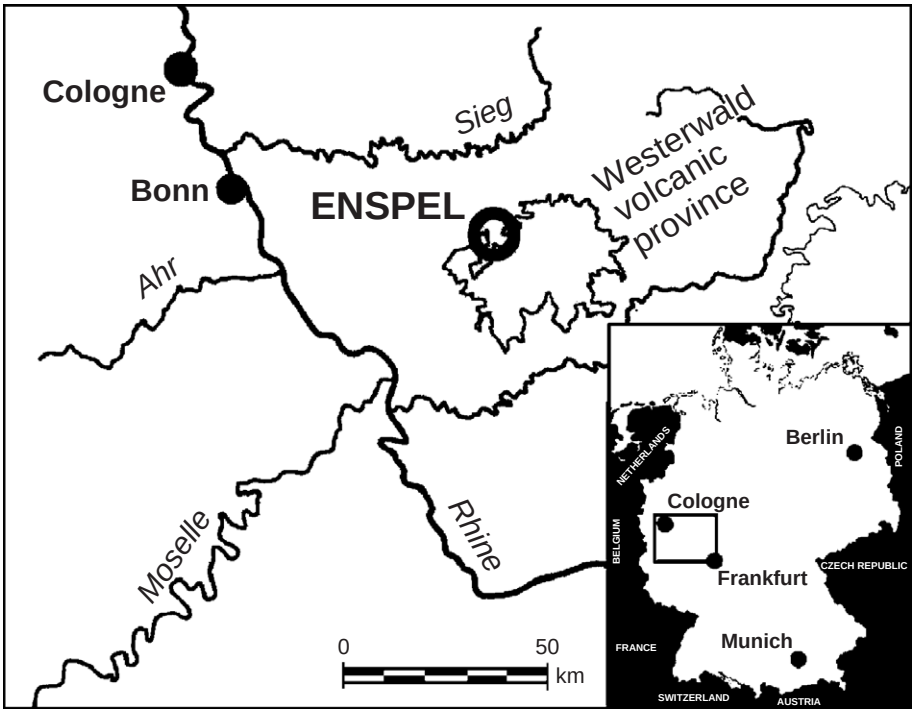

Fig. 1. Location of Enspel Fossillagerstätte in Westerwald, Germany (adapted from Lüniger and Schwark, 2002).

few prehistoric food webs have been studied using stable isotope analysis of preserved soft tissues (e.g., Bocherens et al., 1994; Schulting and Richards, 2002), but investigations into ancient, fossilized specimens have been foiled by insufficient preservation of carbon or nitrogen (e.g., Lécuyer et al., 2003). Pre-

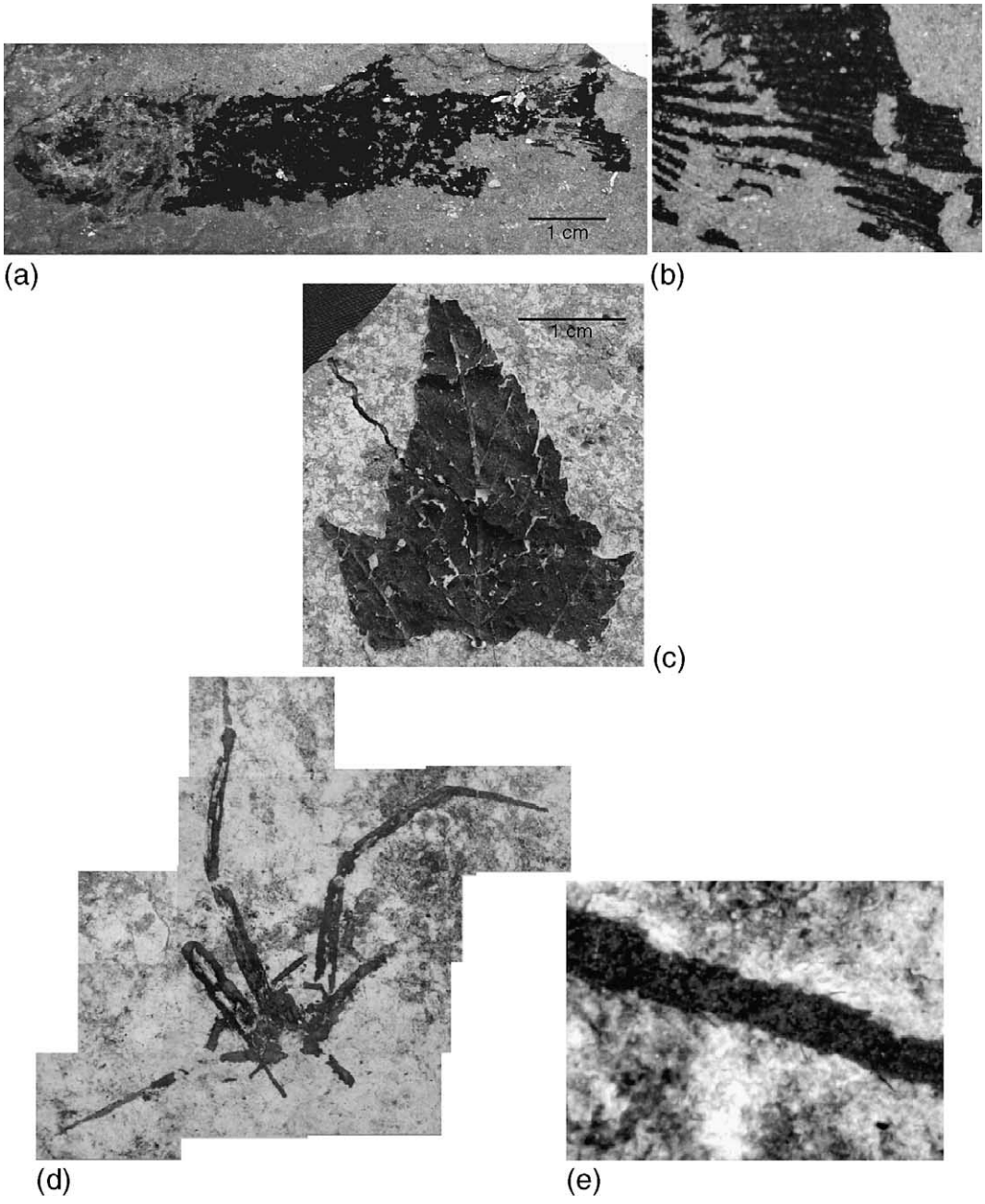

Fig. 2. Representative specimens of Enspel fossils indicating quality of preservation. (a) *P. enspelensis*; dark muscle tissue shadow is organic-rich autolithified bacterial biofilm. (b) At 30× magnification, fine bones in the tail of the fish are visible. (c) *Acer integrilobum* (maple) leaf. (d) Pyritized spider, species unknown, 30×. (e) Hairs on spider leg, 100×.

vious studies have also used calcium and strontium isotopic abundances (e.g., Hoppe et al., 1999), though carbon and nitrogen are most commonly used.

We have attempted to reconstruct the trophic structure of a lacustrine ecosystem at Enspel using stable isotope analysis. From primary producers to higher organisms, the Enspel Fossillagerstätte contains ample fossil specimens (Fig. 2) within stratigaphically similar units that permit examination of trophic relationships.

1.1. Site description

The Enspel Fossillagerstätte is a bituminous, partly volcanoclastic sediment with alternating layers of tuff and diatom-rich oil shale (Sieber et al., 1993). The fossiliferous shale (stratigraphic horizons S8, S10, S12, S14 and S16) has been dated to approximately 25.8 Ma, based on analysis of the floral assemblage (Köhler, 1997), characterization of mammal remains (Storch et al., 1996), and radiometric K–Ar dating of overlying basalt (Horn and Müller-Sohnius, 1988). Each fossiliferous horizon is tens of centimeters thick and these layers alternate with tuff. The shale is distinctly laminated with algae- and diatom-rich layers, suggesting annually controlled primary production (Clausing, 1998). The steep marginal bathymetry and closed hydrological system (Gaupp and Wilke, 1998; Clausing et al., 2000; Lüniger and Schwark, 2002) allowed the small, deep lake to serve as a trap for allochthonous flora and fauna.

Static, anoxic conditions prevailed in the epilimnion of Lake Enspel (Gaupp and Wilke, 1998), preventing bioturbation and inhibiting the decay of organic matter. The soft tissues of some macro-organisms have been preserved by bacterial biofilms that became autolithified in the process of degrading the organic matter (Wuttke, 1983; Toporski et al., 2002). These biofilms are preserved by means of calcium phosphate or an as yet unidentified mineralizing phase containing C, Ca, P, O, Si, S, Ti, and Fe (Toporski et al., 2002). One species of fish, *Paleorutilus enspelensis* Böhme, 1996, and one species of tadpole, *Pelobates decheni* Troschel, 1961, are abundantly represented in the fossiliferous layers, with soft tissues preserved by bacterial biofilms.

Fifty-seven taxa of insects have been recorded at Enspel (Wedmann, 1998), including bees, beetles, and flies. Beetle chitin (Stankiewicz et al., 1997) is often so well preserved that the original iridescent blues and greens can be distinguished for a few minutes following exposure, after which the optical interference layers dry out, contract, and appear deep brown. Some specimens are pyritized as a result of a partly oxic, partly anoxic process (Kott and Wuttke, 1987; Canfield and Raiswell, 1991) which may indicate Lake Enspel was occasionally oxygenated to the bottom.

Plant remains are exceptionally well preserved in the Enspel Fossillagerstätte, with 87 taxa (46 taxa of leaves and 41 taxa of fruits/seeds) recorded in stratigraphic layers S8 through S16 (Köhler, 1997). Previous stable isotope measurements of the shale at Enspel indicate that it is composed primarily of allochthonous plant matter, with a small contribution from phytoplankton and autochthonous plant matter (Lüniger and Schwark, 2002). The fossil flora is characteristic of a thick mesophytic forest (Storch et al., 1996) and a wet, temperate environment (Köhler, 1997).

2. Methods

2.1. Collection and preparation of fossils

Samples were collected from freshly exposed shale in the field during July and August 2002. The stratigraphic horizon S16 (Clausing, 1998) is particularly fossil-rich and was the focus of our investigation. Fossil organisms were identified when possible using catalogues published by Wedmann (1998), Köhler (1997), and Böhme (1996), and knowledge by experienced paleontologists at the site. Tables 1–3 summarize samples collected from Enspel Fossillagerstätte and analyzed for their stable carbon and nitrogen isotopic composition. Samples of features common to almost all stratigraphic horizons (e.g., shale, leaves and seeds) were also removed from stratigraphic units S8, S10, S12 and S14 to examine variation between horizons. A small amount (up to several milligrams) of macroscopically representative material was loosened with sterilized mounted needles and scalpels under a microscope and placed in aluminum-foil bags. The foil bags had previously been cleaned by heating them (500 °C) in a muffle furnace

Table 1
Stable isotope signatures, %C, %N, and C/N values of plants from stratigraphic horizon S16 (nd=no data)

Sample type	Species	δ^{13}C (‰)	δ^{15}N (‰)	C/N
Pure diatom crust		−26.66	1.20	29.83
Aquatic plant	*Tetraclinus articulata*	nd	3.92	nd
Aquatic plant	*Tetraclinus articulata*	−24.94	4.76	33.58
Aquatic plant	*Tetraclinus salicornioides*	−23.66	1.94	33.33
Aquatic plant	*Tetraclinus salicornioides*	−25.48	2.85	36.00
Reed	*Cyperaceae* sp.?	−25.05	2.86	35.54
Reed	*Cyperaceae* sp.?	−24.89	3.63	29.11
Terrigenous leaf	*Acer integrilobum*	−25.67	2.20	34.49
Terrigenous leaf	*Acer integrilobum*	−26.18	3.06	25.44
Terrigenous leaf	*Laurophyllum psuedopriceps*	−26.72	2.69	31.69
Terrigenous leaf	*Magnolia kristinae*	−26.91	4.94	26.24
Terrigenous leaf	*Quercus lonchitis*	−25.78	3.33	29.02
Terrigenous leaf	*Salix varians*	−26.67	1.63	30.29
Terrigenous leaf	*Salix varians*	−26.60	4.56	33.29
Terrigenous leaf	*Tilia irtyshensis*	−26.12	3.12	33.56
Terrigenous leaf		−25.44	2.11	23.31
Terrigenous leaf		−24.59	nd	38.13
Terrigenous seed	*Alangium enspelense*	−23.95	4.75	25.16
Terrigenous seed	*Alangium enspelense*	−23.87	2.35	35.33
Terrigenous seed	*Magnolia burseracea*	−23.89	nd	33.43
Terrigenous seed	*Magnolia burseracea*	−22.75	3.14	57.30
Terrigenous seed	*Magnolia burseracea*	−24.11	4.10	62.44
Terrigenous seed	*Magnolia burseracea*	−24.72	2.03	31.36
Terrigenous seed	*Meliosma wetteraviensis*	−23.32	3.75	60.90
Terrigenous seed	*Meliosma wetteraviensis*	−21.81	5.23	65.84
Terrigenous seed	*Meliosma wetteraviensis*	−23.30	3.49	65.13
Terrigenous seed	*Parthenocissus boveyana*	−23.11	4.96	28.57
Pyritized seed	*Platanus* sp.	−20.74	4.26	35.64
Terrigenous wood		−25.33	3.82	23.25
Terrigenous wood		−21.08	3.82	48.26
Terrigenous wood		−25.13	3.94	42.86
Terrigenous wood		−21.92	4.27	74.46
Terrigenous wood		−25.20	0.53	36.18

for one hour. Seeds were extracted whole, and foliar material could be peeled from the shale with little or no sediment contamination. Bacterial biofilms preserving soft tissues were scraped from the shale as a fine powder. The shale and diatoms surrounding the fossils were also sampled frequently. All samples were refrigerated to minimize alteration and microbial contamination of the organic-rich material.

The size of each *P. enspelensis* specimen was measured at the widest point, directly behind the head. The length of these fish was defined as the distance between the mouth and tailfins of the fish. Where only the head of a specimen was preserved, a common size-parameter was used to extrapolate the length of the fish based on its width (Fig. 3). The detailed preservation of the specimens allowed different tissue types to be distinguished and extracted. Sample material was classified as pure 'muscle', a 'mixture of muscle and bone' (termed here as mixed), pure 'bone', or occasionally as a specific morphological feature (e.g., an eye). Material from *P. enspelensis* is termed 'muscle', 'bone', or 'eye', though the original organic material of the soft tissues has been preserved by bacterial biofilms. The distinction was made based on anatomy, and for the purpose of sampling.

All fossiliferous horizons at Enspel contain plentiful coprolites from unknown organisms. Most coprolites are morphologically similar, measuring typically 1–2 by 0.75 cm. These are too small to be attributed to

Table 2
Stable isotope signatures, %C, %N, and C/N values of animals from stratigraphic horizon S16

Sample type	Species	δ^{13}C (‰)	δ^{15}N (‰)	%C	%N	C/N
Aquatic						
Beetle shell	*Coleoptera*	−25.03	7.04	23.62	1.60	14.77
Beetle shell	*Coleoptera*	−25.27	2.48	17.23	1.00	17.27
Beetle shell	*Coleoptera*	−26.08	nd	6.10	nd	nd
Beetle shell	*Coleoptera*	−25.57	3.40	15.83	0.69	23.02
Beetle shell	*Curculionoidea* (*Coleoptera*)	−23.68	4.86	32.96	2.18	15.13
Mosquito	*Zygoptera* (*Odonata*)?	−24.25	2.66	23.19	1.03	22.42
Tadpole bone	*Pelobates decheni*	−21.40	6.30	3.96	0.23	17.58
Tadpole bone	*Pelobates decheni*	−23.53	1.90	4.89	0.21	22.88
Tadpole bone	*Pelobates decheni*	−23.44	6.01	6.28	0.27	23.55
Tadpole bone	*Pelobates decheni*	−25.07	1.94	12.97	0.47	27.65
Tadpole bone	*Pelobates decheni*	−25.63	3.00	53.29	1.88	28.34
Tadpole bone	*Pelobates decheni*	−21.50	nd	5.49	nd	nd
Tadpole muscle	*Pelobates decheni*	−26.91	6.91	22.76	2.32	9.82
Tadpole muscle	*Pelobates decheni*	−20.42	nd	5.03	nd	nd
Tadpole muscle	*Pelobates decheni*	−26.90	4.00	31.80	3.22	9.89
Tadpole muscle	*Pelobates decheni*	−26.22	3.71	20.70	2.00	10.34
Tadpole muscle	*Pelobates decheni*	−28.08	5.49	41.74	4.29	9.74
Tadpole muscle	*Pelobates decheni*	−24.59	5.32	11.02	0.77	14.26
Tadpole gut contents	*Pelobates decheni*	−24.47	3.03	1.97	0.10	19.92
Terrestrial						
Earwig	*Forficula* sp.	−24.33	nd	15.46	nd	nd
Earwig	*Forficula* sp.	−24.00	6.37	34.83	1.37	25.42
Fly head	*Bibionidae*	−25.43	4.31	13.03	0.77	16.95
Pyritized fly	*Bibionidae*	−23.58	5.54	8.86	0.53	16.58
Fly abdomen	*Bibionidae*	−23.91	4.59	40.73	1.26	32.22
Fly abdomen	*Bibionidae*	−25.11	4.29	16.27	1.00	16.30
Fly abdomen	*Bibionidae*	−26.44	4.47	41.57	1.48	28.01
Fly abdomen	*Bibio* sp. (*Bibionidae*)	−25.77	3.89	21.53	1.09	19.80
Fly abdomen	*Bibionidae*	−25.14	6.76	9.98	0.38	26.00
Fly abdomen	*Tenthredinidae* (*Hymenoptera*)	−24.76	5.98	17.87	0.90	19.89
Wasp thorax	*Pompiliidae* sp.	−25.14	7.73	26.04	1.43	18.16
Fly wings	*Bibionidae*	−25.03	3.74	8.39	0.32	26.43
Pyritized spider	nd	−21.09	2.88	18.53	0.55	33.87
Pyritized spider	nd	−24.90	nd	2.32	nd	nd
Owl pellet	nd	−20.93	2.05	3.71	0.13	27.76
Rodent tooth*	*Eomys querci*?	−17.91	4.33	1.75	0.06	27.86

•Rodent tooth not acidified; no δ^{13} C measured.

a crocodile, but too large to have been produced by *P. enspelensis*. Occasionally, the coprolites contain macroscopic bone shards, leading workers at the site to assign them to a larger fish. Several coprolites were sampled and analyzed for comparison with *P. enspelensis* signatures.

Two teeth, one from a crocodile and the other from a rodent, were collected from unit S8 and an owl pellet in S16, respectively. The teeth were scraped clean and rinsed with deionized water, sonicated in deionized water for three minutes, and then crushed using a pestle and mortar. The crocodile tooth fragments were then treated with 10% HCl in excess in a 15 cc centrifuge tube to remove carbonate. After 30 min of reaction time, the tube was centrifuged to retrieve sample material for analysis. The pulverized tooth was rinsed repeatedly with deionized water to reach a neutral pH. The prepared rodent and crocodile teeth were then freeze-dried. The rodent tooth was not treated with acid due to insufficient sample mass, and accordingly only the δ^{15}N measurement is considered in this study.

Table 3
Summary of C and N preservation in material collected from units S8 through S16

	%C	%N	C/N (at)
Shale ($n=87$)	18.54 ± 5.17	0.64 ± 0.19	34.79 ± 3.59
Leaves ($n=53$)	30.20 ± 9.49	1.09 ± 0.40	33.33 ± 5.07
Seeds ($n=36$)	36.07 ± 11.81	1.18 ± 0.56	43.64 ± 20.02
Insects ($n=29$)	27.07 ± 16.56	1.59 ± 1.14	22.92 ± 6.10
Tadpole muscle ($n=16$)	29.18 ± 10.70	2.96 ± 0.98	12.47 ± 1.41
Tadpole bone ($n=18$)	7.50 ± 3.49	0.35 ± 0.15	26.20 ± 7.20
Fish muscle ($n=24$)	17.98 ± 8.08	1.06 ± 0.60	22.11 ± 5.91
Fish bone ($n=8$)	7.50 ± 2.74	0.32 ± 0.10	27.52 ± 3.27
Coprolites ($n=13$)	7.45 ± 5.85	0.29 ± 0.19	28.78 ± 6.29

2.2. Stable isotope analysis

An aliquot of each sample was weighed into a 3.5 × 5 mm tin capsule. Ideal quantities of sample material ranged from 300 to 2000 μg, and were reasonably easy to obtain from most fossil types. After weighing, the tin capsules were sealed and introduced via the EA carousel (Wooller et al., 2001) into the autosampler (A2100) of a CE Instruments, NA 2500 series, Elemental Analyzer (EA). Within the EA, each sample was combusted with ultra pure oxygen at 1020 °C in a quartz oxidation column containing Chromium (III) Oxide and Silvered Cobalt (II, III) Oxide. The resulting gases, mixed with zero-grade helium as the carrier gas, were passed through a quartz reduction column containing reduced copper wire grains, maintained at 650 °C. Purified combustion gases (CO_2 and N_2) were separated in a molecular sieve gas chromatographic column prior to entering a Finnigan Conflo II interface. Isotope ratios of the combustion gases were analyzed using continuous-flow, stable-isotope-ratio mass spectrometry (Finnigan MAT, Delta^{plus}XL). Both N_2 and CO_2 samples were analyzed relative to internal, working gas standards, using the following equation:

$$\delta^h X = \left(\frac{{}^hX_{sample}/{}^lX_{sample}}{{}^hX_{std}/{}^lX_{std}} - 1 \right) * 1000$$

where X is either carbon or nitrogen, h is the heavier isotope, and l the lighter isotope. The results are presented in standard notation. Acetanilide (C_8H_9NO) was analyzed to monitor the accuracy of isotopic ratios and elemental compositions measured by the EA ($\delta^{15}N \pm 0.35‰$, %N = ± 0.72 and $\delta^{13}C \pm 0.46‰$, %C = ± 5.45).

3. Results

3.1. Shale and plant remains

The stable carbon and nitrogen isotope compositions of all primary producers from stratigraphic horizon S16 are shown in Table 1. Diatomaceous

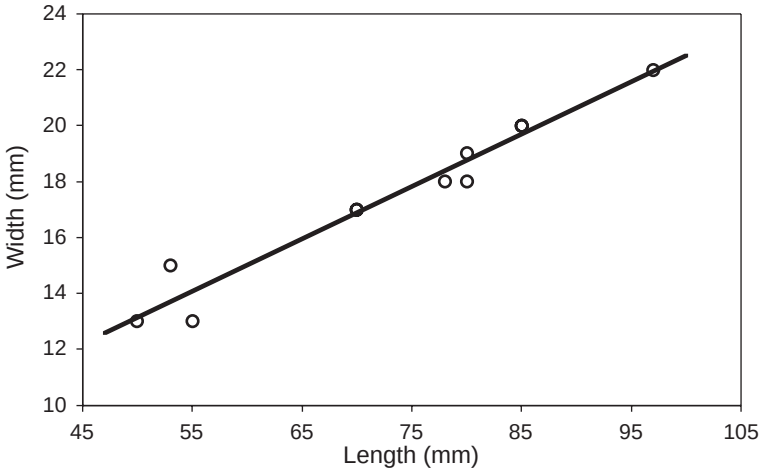

Fig. 3. Allometry of *P. enspelensis* specimens from Enspel ($R^2 = 0.95$; length = 5.35 × width − 20.4 mm).

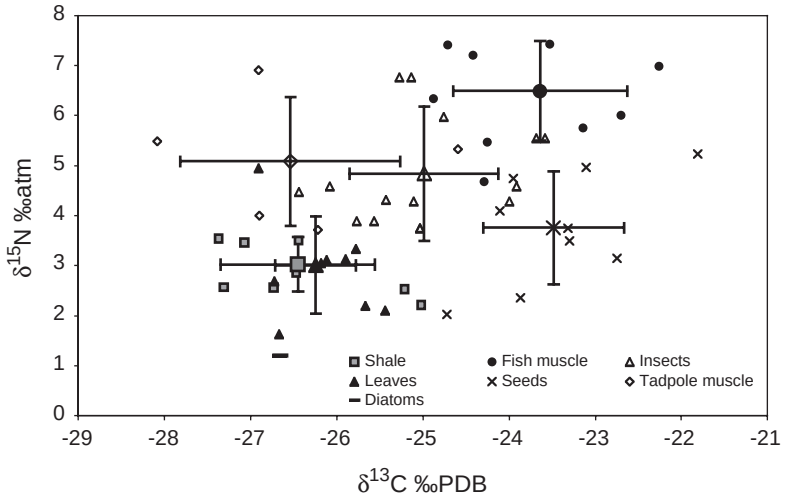

Fig. 4. Summary of $\delta^{13}C$ and $\delta^{15}N$ values of all ecosystem components from stratigraphic horizon S16. Data points with error bars represent mean values and standard deviations of symbol grouping.

shale and some aquatic plant specimens represent the lacustrine environment. Leaves, seeds, and wood represent the terrestrial surroundings. Leaves and shale from S16 are not statistically different from each other in terms of either their $\delta^{13}C$ or $\delta^{15}N$ values (t tests respectively 0.572 and 0.782, all t tests are two-tailed, heteroscedastic and assume normal distributions). Carbon and nitrogen stable isotopic values of pure diatom crust (Table 1) are similar to the average shale value in $\delta^{13}C$ (mean=

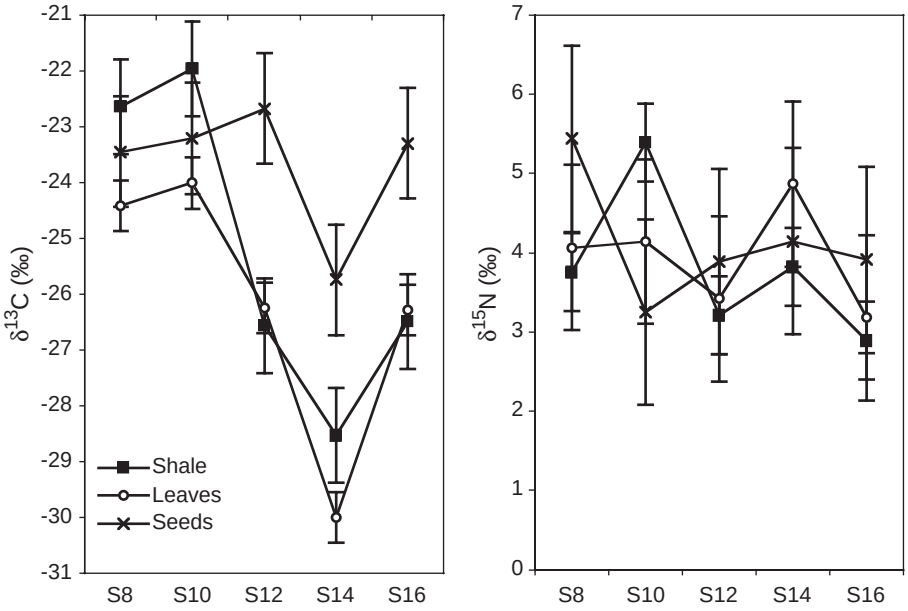

Fig. 5. Trends in (left) $\delta^{13}C$ and $\delta^{15}N$ (right) of shale, leaves, and seeds from different stratigraphic horizons at Enspel (S8, S10, S12, S14, and S16). Error bars represent one standard deviation from mean.

$-26.45‰ \pm 0.90$) and 1.8‰ lower than the average shale $\delta^{15}N$ value (avg. = 3.03‰ ± 0.54). Measurements of the diatom crust reflect a pure signal of lacustrine primary production, whereas the shale signal includes varying concentrations of diatoms, algae, and terrigenous leaf material (Lüniger and Schwark, 2002). The shale has lower %C and %N than the plant specimens (Table 3, *t* tests: 8.7×10^{-4} and 9.3×10^{-4}, respectively), though the C/N of shale and leaves are similar (*t* test: 0.51).

The $\delta^{15}N$ values of the plant leaves from S16 do not differ significantly from those of seeds from the same horizon (*t* test: 0.164) (Fig. 4) while the $\delta^{13}C$ values of these seeds and leaves are statistically distinct from each other (t test: 2.26×10^{-7}). The mean $\delta^{13}C$ value of leaf remains (−26.24‰) is 2.76‰ more negative than the mean value of seeds (−23.48‰). Seeds typically have higher C/N values (between 25.2 and 65.8) than either shale or leaves (Table 3).

The variations in C/N and $\delta^{13}C$ values of shale and terrigenous leaves between the five horizons are correlated (*p* values ≥ 0.8559 and 0.9190, respectively). All three primary producer components (leaves, seeds, and shale) are depleted in ^{13}C in hor-

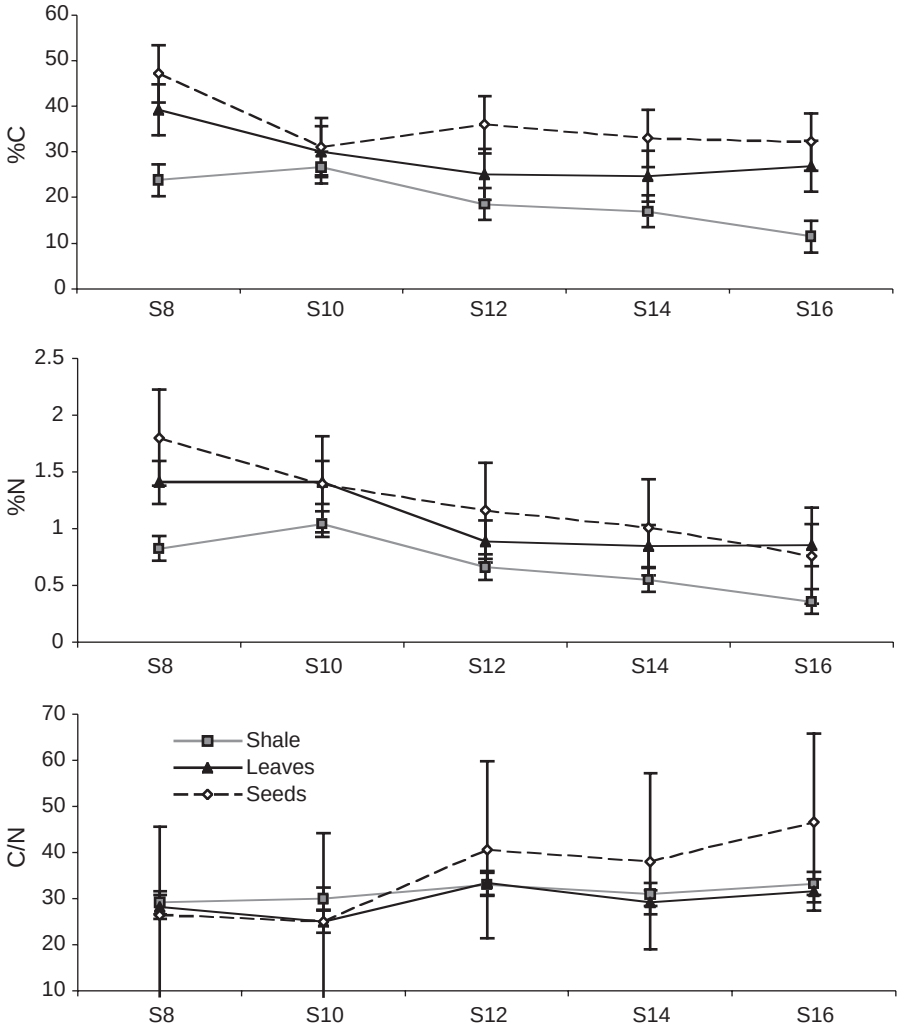

Fig. 6. Trends in weight %C (top), weight %N (center), and C/N (bottom) of shale, leaves, and seeds from different stratigraphic horizons at Enspel. Error bars represent one standard deviation from mean.

izon S14. The shale and leaves also showed a significant decrease in $\delta^{13}C$ in horizons S12 and S16 compared with horizons S8 and S10. (Fig. 5a). There was no significant correlation between the different horizons in terms of the $\delta^{15}N$ values of shale, leaves, and seeds (Fig. 5b, p values ≤ 0.5200 between shale and leaves, 0.1376 between leaves and seeds). The %C values of the shale and leaves remained relatively constant between horizons (Fig. 6a). The seeds from S8 had significantly higher %C compared with the other horizons analyzed (Fig. 6a). The %N of shale, leaves, and seeds generally decrease with increasing horizon depth (Fig. 6b). The C/N values of seeds appeared to increase with increasing stratigraphic depth (Fig. 6c).

3.2. Invertebrate remains

Insects such as beetles and flies fall in the center of the plot (Fig. 4). Most invertebrate fossils are enriched 1–2‰ in both ^{13}C and ^{15}N (mean $\delta^{13}C$ and $\delta^{15}N$ values 25.02‰ ± 0.86 and 4.84‰ ± 1.33, respectively) relative to the range of leaf and shale values (Fig. 4). Most of the beetles analyzed were aquatic, while flies and spiders represent terrestrial consumers. Spiders, which are generally preserved through pyritization, contain less organic carbon and nitrogen than bacterial biofilms, chitinous insect carapaces or plant matter, but their stable isotopic compositions fall within the range of chitinous insect values.

3.3. Vertebrate fossils

The mean C and N stable isotopic compositions of *P. enspelensis* (Table 3) are heavier relative to the mean values of plant and invertebrate remains (Fig. 4), with some of the highest $\delta^{13}C$ and $\delta^{15}N$ values from stratigraphic horizon S16. All *P. enspelensis* data shown in the $\delta^{13}C$ and $\delta^{15}N$ bi-plot (Fig. 4) are derived from fossilized 'muscle' samples. Replicate $\delta^{13}C$ and $\delta^{15}N$ analyses on the same tissue type from the same fish specimen yielded a reproducibility of 0.89‰ for both $\delta^{13}C$ and $\delta^{15}N$. 'Muscle' samples typically have higher $\delta^{15}N$ values (6.50‰ ± 1.00) relative to bone samples (2.55‰ ± 1.26) (Fig. 7). The $\delta^{15}N$ values of samples composed of a mixture of bone and 'soft tissues' (4.67‰ ± 0.84) fell between pure bone and pure 'muscle' values, and 'eye' samples are statistically indistinguishable from the other soft tissue, 'muscle' samples (t test: 0.743). The mean $\delta^{13}C$ value of 'muscle' (−23.64‰ ± 1.01) was isotopically lighter than that of 'mixed' (−22.76‰ ± 1.35) and bone (−22.89‰ ± 0.93) samples (t test: 0.044), though there was no significant difference between the latter two sample types (t test: 0.85).

A positive relationship between the length of each *P. enspelensis* specimen and its corresponding $\delta^{15}N$ value was observed (Fig. 8). Some data points were obtained from partial fossils, using the allometric equation obtained from whole specimens (Fig. 3, length = 5.35 × width − 20.4 mm, $R^2 = 0.95$) to calculate the length of the fish. Increasing length is positively corre-

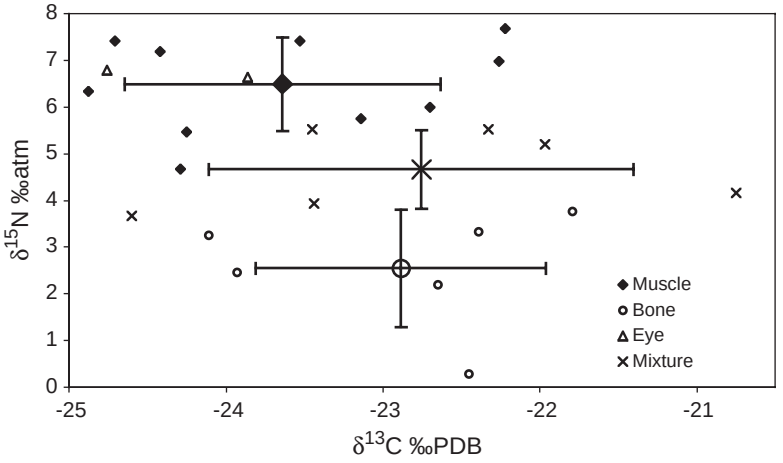

Fig. 7. Intra-organism isotope fractionation in *P. enspelensis*. Error bars represent one standard deviation from the mean.

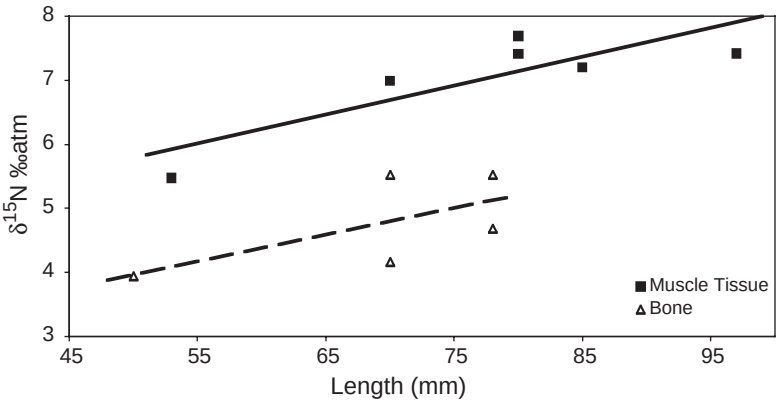

Fig. 8. Correlation between length (mm) and δ^{15}N for *P. enspelensis* ('muscle' $R^2=0.71$, bone $R^2=0.41$).

lated with fossilized muscle δ^{15}N signatures (0.45‰ enrichment per cm length, $R^2=0.71$). We found no significant relationship between the length of the fish specimens and δ^{15}N measurements of bone ($R^2=0.41$), or δ^{13}C measurements of "mixture" ($R^2=0.005$) or muscle tissue ($R^2<0.005$).

The δ^{13}C and δ^{15}N values from tadpole *(P. decheni)* remains range from −28‰ to −24‰ and 4‰ to 7‰, respectively (Table 3). The tadpole δ^{13}C values are not significantly different from the plants and shale samples (Fig. 4), although their mean δ^{15}N value is ~2‰ more positive than these baseline signatures. The mean δ^{13}C of *P. decheni* samples is ~3‰ more negative than *P. enspelensis* (Fig. 4). Also, the mean *P. decheni* δ^{15}N is ~1.5‰ less positive than *P. enspelensis*, though the range in δ^{15}N for these two vertebrates is not significantly different.

The δ^{15}N measurement for the rodent tooth (Table 2) is enriched 1.3‰ above the average for terrigenous leaves. The stable isotopic composition of the acidified crocodile tooth (δ^{13}C = −24.96‰, δ^{15}N = 4.66‰) falls within the range of insects from the S8 horizon. The eight coprolite samples analyzed (mean δ^{13}C = −21.88‰, δ^{15}N = 3.89‰) contain 18.6% to 28.8%C and 0.2% to 0.8%N.

4. Discussion

Stable isotopic signatures in modern ecosystems can be altered by environmental conditions (e.g., atmospheric CO_2, temperature, humidity). Some environmental parameters in Oligocene Europe were similar to today. Though global atmospheric CO_2 levels were comparable (Veizer et al., 1999; Berner and Kothavala, 2001) and the latitude of Enspel has remained nearly constant between the Oligocene and the present (Scotese et al., 1988), there was significant climatic variation over millions of years. Reptiles and other tropical animals diminished or disappeared during a cold period 28–29 Ma, to be renewed by Asian immigrants in the Upper Oligocene (25–26 Ma) (Antunes and Cahuzac, 1999; Blondel, 2001). The rejuvenation of crocodiles (Antunes and Cahuzac, 1999), certain ungulates (Blondel, 2001), and fish and aquatic invertebrates (Cahuzac and Poignant, 1992; Lauriat-Rage et al., 1993) in Western Europe suggests a warm, subtropical climate. This area was covered in temperate and coniferous forests, woodlands, and some open areas (Blondel, 2001). Insect and plant species found at Enspel also indicate a similar environment (Köhler, 1997; Wedmann, 1998). The mean δ^{13}C value of terrigenous plant specimens is consistent with a non-arid environment.

The δ^{15}N values of shale, leaves, and seeds are within range of a source derived from the fixation of atmospheric nitrogen (δ^{15}N ≡ 0‰) (Kohl and Shearer, 1980; Shearer and Kohl, 1989), and the δ^{13}C values fall well within the −36‰ to −22‰ range typical for C_3 vegetation (Farquhar et al., 1989). Variation among Enspel primary producer stable isotope compositions is similar to variation among modern primary producers from a single location (e.g., Smith and Epstein, 1971; O'Leary, 1981; Leavitt and Long, 1986). Seed signatures show higher standard deviation than either shale or leaf signatures, probably due to the heterogeneities of lipid and sugar distribution within seeds

(i.e., between the husk and meat). The similarity of C/N for shale and macroflora fossils supports a previous finding that the shale is composed of allochthonous terrestrial plant material and that lacustrine algae, which have characteristic C/N between 5 and 8, are only a minor component (Lüniger and Schwark, 2002).

The observed changes in isotope composition of primary producers between layers may be the result of a number of processes, including chemical modification of the shale by alternating layers of ash tuff and an environment changing over time. The organic carbon component of the shale was determined by algal content as well as terrigenous leaf input. Periodic ash falls enhanced the nutrient supply to the lake and resulted in algal blooms (Schwark et al., 1995). Similarly, irregular dinoflagellate distribution throughout the sediment column (Clausing et al., 2000) records longer-term environmental change. The $\delta^{13}C$ values of lacustrine algae are typically similar to terrigenous leaves from C_3 plants (avg. −27‰, Farquhar et al., 1989; Meyers, 1994). However, variation in terrestrial and aquatic carbon isotopic inputs might be caused by rainfall, oxygenation of the lake, and other factors (e.g., Meyers, 2003). The $\delta^{15}N$ record may indicate increased algal input in S12 and S16, since algal nitrogen is isotopically heavier than terrestrial plant nitrogen ($\delta^{15}N$ ~8‰ vs. ~1‰, respectively, Peterson et al., 1985). The negative excursion in $\delta^{13}C$ observed for all primary producer components from S14 may have been caused by increased input from isotopically light soil inorganic carbon, which is known to cause production of algae with $\delta^{13}C$ as low as −32‰ (Meyers, 2003).

The lower C/N ratio observed for S8 (Table 3) is also consistent with greater algal protein input, which typically has C/N between 5 and 8, compared to C_3 land plants which have C/N values of 16 and higher (Meyers, 1994). The C/N discrepancy between lacustrine algae and terrestrial plant material is sometimes caused by the abundance of carbon-rich cellulose in terrestrial plants and its absence in algae (Meyers, 1994); also, algae contain more proteins and thus more nitrogen than terrigenous plants. The higher C and N content in shale and macroflora fossils from the S8 horizon as compared to the S16 horizon (Tables 3 and 4), may be due to the shorter amount of time for which the younger S8 horizon was subject to bacterial modification before the entire section was covered by basalt. Because the shale is bituminous, these elements are able to diffuse through the sediment to a certain extent. Carbon and nitrogen signature changes in the Enspel stratigraphic record indicate that each horizon should be interpreted as an independent snapshot of an ecosystem.

Table 4
Stable carbon and nitrogen isotope signatures in *Paleorutilus enspelensis* specimens from stratigraphic horizon S16

Sample Material	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	%C	%N	C/N
Bone	−21.79	3.77	5.83	0.25	23.57
Bone	−23.93	2.46	6.26	0.22	29.12
Bone	−22.39	3.33	5.78	0.25	23.45
Bone	−24.11	3.25	11.02	0.46	23.86
Bone	−22.65	2.19	12.73	0.49	25.86
Eye	−25.50	nd	9.32	nd	nd
Eye	−23.86	6.65	30.65	1.55	19.84
Eye	−24.76	6.79	11.52	0.96	12.06
Muscle	−22.22	7.69	6.07	0.24	25.10
Muscle	−24.88	6.34	29.28	1.23	23.77
Muscle	−24.25	5.47	9.14	0.38	23.81
Muscle	−23.53	7.42	10.68	0.55	19.27
Muscle	−24.29	4.68	11.82	0.54	21.97
Muscle	−26.51	9.02	15.91	1.29	12.33
Muscle	−27.40	8.13	21.42	1.95	11.00
Muscle	−24.42	7.20	13.52	0.55	24.75
Muscle	−23.14	5.75	21.00	0.73	28.76
Muscle	−22.70	6.00	24.06	0.93	25.93
Muscle	−22.26	6.99	8.25	0.36	22.65
Muscle	−24.71	7.41	22.25	1.36	16.36
Muscle and bone	−21.97	5.20	6.21	0.39	16.09
Muscle and bone	−23.45	5.52	7.74	0.38	20.51
Muscle and bone	−22.33	5.52	3.96	0.19	20.75
Muscle and bone	−24.60	3.66	10.62	0.44	24.20
Muscle and bone	−23.44	3.94	7.60	0.27	27.81
Muscle and bone	−20.75	4.16	9.29	0.40	23.02
Gut contents	−23.88	3.42	8.37	0.29	28.43

4.1. Ecology of enspel

A number of ecologically sensible features emerge from the Enspel stable isotope data. Stable carbon and nitrogen isotope signatures of fossils from Enspel seem to record not only trophic levels of some organisms (Fig. 4) but also subtle fractionations associated with an evolving diet (Fig. 8). For example, a negative 2‰ $\delta^{15}N$ extrapolation from *P. enspelensis* isotopic signatures places points within the range of insect signatures (Fig. 4). This implies that *P. enspelensis* primarily consumed aquatic insects and terrestrial

insects that fell into the paleo-lake. Modern North American creek chubs, the closest living relatives of *P. enspelensis* (Böhme, 2000), consume mostly insects and insect larvae, and a smaller component of fruits and seeds, and inhabit warm, isolated pools (Moshenko and Gee, 1973), like Enspel in the Oligocene. *P. enspelensis* also seems to have become progressively carnivorous over its lifespan (Fig. 8), replacing any early consumption of primary producers with a purely insectivorous diet as it matured. The feeding habits, and thus $\delta^{15}N$ signatures, of many modern organisms change as the specimens reach maturity (e.g., Fry and Arnold, 1982). For example, some fish species tend to incorporate food from higher trophic levels as they grow, and some may even entirely shift their feeding strategy (e.g., from herbivore to carnivore). Also, at Enspel, the diets of entire species may have shifted over time to make use of available nutrients, including algal blooms, as is observed in modern organisms (e.g., Minagawa and Wada, 1984; Quade et al., 1992; Hecky and Hesslein, 1995; Johnson et al., 1999).

Variation in *P. decheni* stable isotope values is wider than in other components of the ecosystem (Fig. 4) and may reflect a widely varied, opportunistic diet. Modern tadpoles are typically plankton-feeders for the first week of life, after which they become progressively insectivorous and even cannibalistic (DeGraaf and Rudis, 1983). Lower $\delta^{15}N$ values for some Enspel tadpole specimens may reflect that these individuals were still in the grazing phase when they entered the fossil record. Some tadpoles analyzed have $\delta^{13}C$ signatures below those measured for any other fossil material. Tadpoles may have combined eating phytoplankton from near the surface with grazing benthic detritus from leaves. Primary producers in the deeper portions of a lake are typically depleted 2‰ to 10‰ (average 6.5‰, Post et al., 2000) in ^{13}C relative to primary producers in the littoral region, with a smooth isotopic gradient between the two extremes (Vander Zanden and Rasmussen, 1999; Post et al., 2000; Post, 2002). Several specimens also seem to have fed on invertebrates. Unfortunately, an allometric analysis of *P. decheni* was not feasible because, even in specimens with the best preservation, the dimensions of the organisms are not well represented by their skeletons. Hence, the possible dependence of feeding strategies on growth cannot be accounted for by this method.

The stable isotope data obtained from the treated crocodile tooth falls within the range of insect signatures. Modern adult crocodile diets typically consist of fish, birds, turtles, snakes, and small mammals; modern young crocodile diets include aquatic invertebrates and fish. The ratio between terrestrial and aquatic food sources varies with environment (Ross, 1989). One possible explanation for the low $\delta^{13}C$ and $\delta^{15}N$ measured for the tooth is that the crocodile ate primarily terrestrial organisms, such as birds or rodents. Alternatively, the specimen analyzed may have come from a juvenile that was still consuming invertebrates. Further analysis of more teeth clearly is required to determine crocodile feeding habits in this ecosystem. The rodent diet probably consisted primarily of plant material.

Enspel coprolites seem to be too heavy in ^{13}C and too light in ^{15}N for a diet of pure *P. enspelensis*, but inferences about the diet of animals based on the stable isotopic composition of coprolites can be complicated, as they include material that was not assimilated by an organism. Also, coprolite stable isotope signatures show wide scatter due to the heterogeneous nature of the sample material. Still, the abundance of coprolites begs the question: Why have no skeletons of this mystery predator been discovered? The Enspel coprolites may have come from a rare predator, such a crocodile, or a predator that was able to avoid being trapped in the lake, such as a bird.

4.2. Stable isotopes as a reconstructive tool

We have considered the stable isotope data from Enspel in terms of trophic and ecological relationships, but acknowledge that stable isotope signatures can be altered through taphonomic processes. Though individual compounds in these heterotrophic microorganisms may show significant isotopic fractionation relative to the substrate (e.g., Macko and Estep, 1988), the biomass of the microorganisms is often isotopically similar to the substrate (Boschker et al., 1999; Hullar et al., 1996; Coffin et al., 1990; Blair et al., 1985). Micro-heterotrophs are sometimes isotopically identical to their substrate, depending on the C/N of the substrate relative to the needs of the organisms. If C/N of the substrate is higher than that of the bacteria

(typically 3–5), they may not excrete nitrogen, and would therefore not cause any fractionation effect.

In experiments simulating anoxic lacustrine environments, δ^{15}N increased by 0.6‰ in the first 24 h of organic matter degradation, then gradually decreased by 3‰ over the next 50 days (Lehmann et al., 2002). This pattern may be caused by the counteracting processes of substrate degradation, which preferentially releases ^{14}N (Lehmann et al., 2002), and bacterial synthesis (Holmes et al., 1999), which preferentially incorporates ^{14}N. In the same experiment δ^{13}C decreased steadily by 1.6‰ over 50 days. Selective loss of degradation-prone proteins and carbohydrates (Harvey et al., 1995), the carbon of which is typically isotopically heavy (Degens, 1969; Deines, 1980), is likely responsible for this phenomenon (Lehmann et al., 2002). Though variation was observed in C/N ratios over the first 40 days of decomposition in Lehmann et al.'s simulation, there was negligible net effect. The isotope effects observed in these experiments may have been mitigated at Enspel due to the rapidity of silicification and the resulting isolation of remaining organic matter within silicified cells. Also, all of the above work applies to material left behind by degrading bacteria, while at Enspel the bacteria themselves, in addition to any underlying original organics, comprise the analyzed material.

The weight percent carbon and nitrogen in Enspel fossils is lower and more variable than in modern, fresh organisms (e.g., Gearing, 1991). This is to be expected, since typically only a few percent of initially biosynthesized organic matter consequently escapes biodegradation, hydrolysis, and mineralization and becomes buried in sediments (Meyers, 1994). However, carbon and nitrogen are unusually abundant in Enspel fossils due to the anoxic depositional environment, which inhibited decomposition. Variation among %C and %N values for Enspel fossils may result from inconsistent degrees of silicification between specimens. Mineralization of organic material is commonly associated with an increase in C/N ratios because of preferential degradation of nitrogen-rich compounds (Fenchel et al., 1998), though biomineralization may result in a smaller change in C/N ratios due to the input of low C/N bacterial biomass (Lehmann et al., 2002).

Despite some alteration, the relative amounts of carbon and nitrogen in different fossil materials are sensible compared to modern ecosystems. Similar to modern organisms, fossil fish- and tadpole-bone material contains less carbon and nitrogen than its associated soft tissue material. Animal muscle tissue C/N is also generally lower than vegetation ratios. Also, stable isotopic compositions of individual organs, bone, and other materials are characteristically different from the muscle signature, which is considered isotopically representative of the whole organism. Literature on ^{13}C and ^{15}N fractionation within a single organism (Gearing, 1991; Pinnegar and Polunin, 1999; Vander Zanden, 2001) describes values and shifts similar to those observed between soft tissues and bone in *P. enspelensis* (Fig. 7) and *P. decheni* (data not shown). Specifically, the nitrogen in bone is isotopically lighter than that in fossilized muscle tissue.

Stable isotope ratio analysis is particularly useful in situations where alternative techniques for examining feeding strategies, trophic links, and elemental cycling in an ecosystem, such as behavioral studies, cannot be applied. Fossilized ecosystems are examples of such situations. The prospect of reconstructing the trophic structure of an entire lacustrine ecosystem that is millions of years old is exceedingly novel and has been previously attempted using only paleontological arguments. Because the fractionation between bone and 'muscle' tissue sample materials is greater than typical trophic fractionation, sample purity is important. Further investigation is required to evaluate stable isotope signatures as representations of an entire complex organism at other paleontological sites. The Enspel data set provides a unique ecophysiological perspective, illustrating energy flow (carbon) and nutrient cycling (nitrogen) within an ancient ecosystem using analytical techniques applied in the study of modern ecosystems.

5. Conclusions

Organic carbon and nitrogen are sufficiently preserved in the Enspel Fossillagerstätte to allow stable isotope analyses. The δ^{13}C and δ^{15}N biosignatures of Enspel fossil organisms show a number of features that are ecologically sensible. For example, isotopic signatures are relatively distinct for different components of the ecosystem. Further, δ^{13}C and δ^{15}N values

appear to record the organisms' relative trophic positions within the paleo lake ecosystem. They indicate, for example, that *P. enspelensis* (fish) was insectivorous and that *P. decheni* (tadpole) was an opportunistic feeder. *P. enspelensis* stable isotopic signatures seem to record intraorganism fractionation and evolution of feeding habits with maturity. Additionally, Enspel fossil %C, %N, and C/N are broadly similar to those observed in modern organisms. Finally, although signatures fluctuate between stratigraphic layers, the trophic relationships remain approximately constant. The isotope data presented above provide an independent test of paleontological arguments on diets and feeding strategies of extinct organisms. Fossil lake environments such as Willershausen (3 Ma) and Messel (49 Ma), also in Germany, contain fossils preserved in similar quality and would allow this method to be tested on 46 Myr of ecological history.

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