

Stable isotope evidence of trophic interactions between introduced brook trout *Salvelinus fontinalis* and native brown trout *Salmo trutta* in a mountain stream of south-west France

J. CUCHEROUSSET*, J. C. AYMES†, F. SANTOUL AND R. CÉRÉGHINO

*EcoLab, Laboratoire d'écologie fonctionnelle, UMR 5245 (CNRS-UPS-INPT),
Université Paul Sabatier, bât 4R3, 118, route de Narbonne,
31062 Toulouse Cedex 9, France*

The potential trophic impact of introduced brook trout *Salvelinus fontinalis* on native brown trout *Salmo trutta* in a mountain stream (south-west France) was investigated using stable isotope analysis (SIA). The isotopic signatures ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of *S. fontinalis* were similar regardless of the absence or presence of *S. trutta*, and SIA mixing models revealed that *S. fontinalis* diet consisted mainly of terrestrial invertebrates. Conversely, a significant shift in *S. trutta* isotopic signatures (depletion of 1.6‰ $\delta^{13}\text{C}$ and enrichment of 0.6‰ $\delta^{15}\text{N}$) was observed in sympatry with *S. fontinalis*; this may be due to a dietary shift towards terrestrial invertebrates. Contrary to an expected dietary divergence in sympatry, an elevated level of dietary overlap was observed between the non-native and native salmonids when in co-occurrence. This dietary convergence is more likely to be due to behavioural interactions than to variations in food availability or fish displacements.

© 2007 The Authors

Journal compilation © 2007 The Fisheries Society of the British Isles

Key words: alien species impacts; diet shift; mixing model; salmonids; terrestrial and aquatic invertebrates.

INTRODUCTION

Biological invasions are a pervasive and costly environmental problem (Vitousek *et al.*, 1996, 1997; Lake & Leishman, 2004), especially those involving fresh waters (Moyle & Light, 1996). The most frequently introduced freshwater organisms in Europe are fishes (Copp *et al.*, 2005a; García-Berthou *et al.*, 2005), but detailed studies on the impacts exerted by non-native fishes on native species are lacking (García-Berthou, 2007) except for a few species (Townsend, 2003; Koehn, 2004). Because of their recreational value for angling, salmonids have been widely introduced throughout the world (Fausch *et al.*, 2001), but the effect of these introductions on native species

*Author to whom correspondence should be addressed. Tel.: +33 561 556 730; fax: +33 561 556 096; email: julien.cucherousset@cict.fr

†Present address: INRA, UMR Ecobiop, Quartier Ibarron, 64310 St Pée sur Nivelle, France.

and ecosystem function is less well documented for Europe than elsewhere (Cunjak & Power, 1986; Fausch, 1988; Nakano *et al.*, 1998; Baxter *et al.*, 2004; Peterson *et al.*, 2004; Hasegawa & Maekawa, 2006; Spens *et al.*, 2007). Besides the introduction of novel diseases (Gozlan *et al.*, 2006), the most obvious impact of introduced fishes on native species is through predation and competition for food and habitat, and this can lead to a decline in the native species (Moyle & Light, 1996).

Conventional methods for assessing predation and food competition are based on the analysis of gut contents, which generally provide a mere snapshot of competitive interactions, with short-comings such as a high rate of empty stomachs and variability in the data collected due to diel and seasonal fluctuations in resource use (Clarke *et al.*, 2005; Copp *et al.*, 2005b; McHugh & Budy, 2006). Whereas, stable isotope analysis (SIA) is based on the predictable relationship between the isotopic composition of consumers and their diet and can be implemented as a sensitive, cost-effective and temporally integrative tool to analyse long-term dietary records in tissues with a minimal handling of studied organisms (Peterson & Fry, 1987; Post, 2002; Fry, 2006). SIA provides a means of assessing the ecological impacts of non-native species upon aquatic food webs (Vander Zanden *et al.*, 1999; Gorokhova *et al.*, 2005; Maguire & Grey, 2006). Recently, SIA has been used as a complementary tool of stomach contents and population monitoring (*e.g.* growth, survival and displacement) to study the interactions between non-native and native salmonids (Clarke *et al.*, 2005; McHugh & Budy, 2006; McHugh *et al.*, 2007). Because the impact of non-native species on biological interactions and ecosystem functioning may be negligible or not easily perceived (Townsend, 2003), the ability of SIA to detect subtle ecological interactions between non-native and native salmonids should be further evaluated.

The aim of the present study was to assess, using SIA, the potential impact of the introduced North American salmonid, brook trout *Salvelinus fontinalis* (Mitchill, 1815), on the trophic status of native brown trout *Salmo trutta* L., 1758, in a mountain stream. The brook trout was introduced in 1936 to streams in the Pyrénées Mountains, where it now co-occurs with native brown trout. In North America, the adverse impacts of non-native *S. trutta* on native *S. fontinalis* have been demonstrated (Nyman, 1970; Fausch & White, 1981), but the reciprocal effects on native *S. trutta* of *S. fontinalis* introductions in Europe have received little detailed study (Delacoste *et al.*, 1997; Blanchet *et al.*, 2007; Spens *et al.*, 2007). Therefore, the specific objectives of the present study were to: (1) test for differences in the stable isotope signatures of the two species under conditions of allopatry and sympatry and (2) determine whether observed differences in isotope signature may be interpreted as shifts in dietary resource use.

MATERIALS AND METHODS

The study was undertaken in the River Oriège, a torrential tributary stream in the Pyrénées Mountains (south-west France, 1°57' E; 42°39' N) that flows into the River Ariège at 815 m a.s.l. (Fig. 1). Discharge varies from 1 m³ s⁻¹ (winter and summer) to 15 m³ s⁻¹ (spring thaw), and water temperatures range from 0 to 13.5° C. The upstream

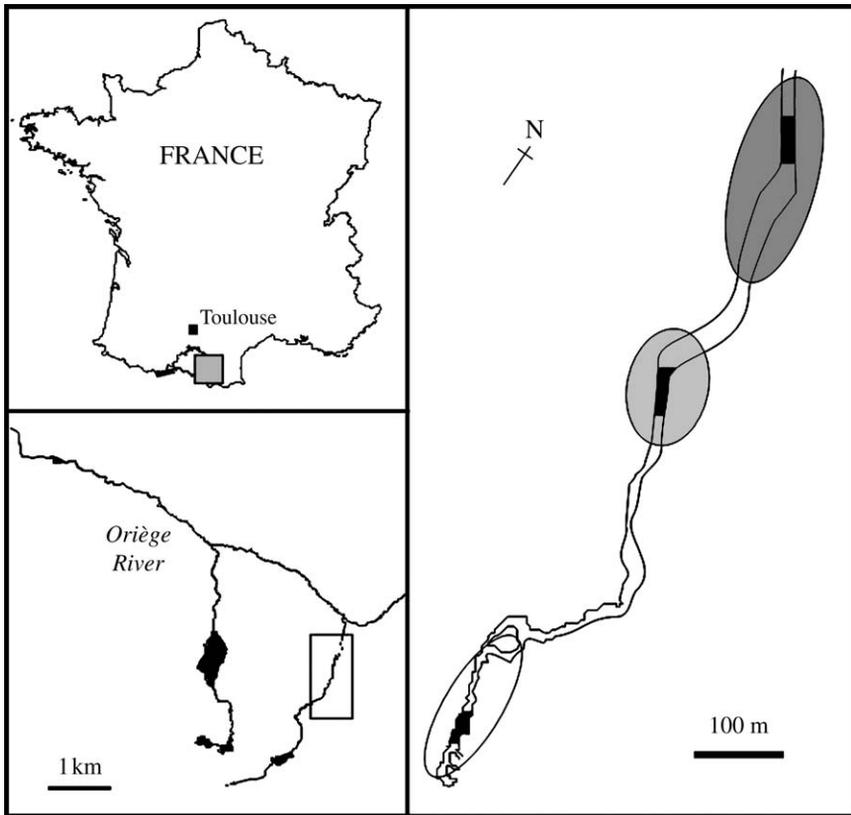


FIG. 1. Map of the River Oriège (France) with location of the study area and the stretches sampled for stable isotope analyses in the three treatments: allopatry *Salmo trutta* (●), sympatry (○) and allopatry *Salvelinus fontinalis* (○).

section of the River Oriège (1480 m a.s.l) was surrounded by a 35 ha homogeneous grassland composed of *Poaceae* and *Equisetum* sp. This area belongs to a 4000 ha national reserve, where angling and fish stocking have been prohibited for at least 20 years. Only two fish species were present, *S. trutta* and *S. fontinalis* of which the latter was introduced in 1950s by a local angling society.

Based on preliminary estimates of fish density and size-class structure (Table I), carried out in early July 2006 by electrofishing (two-pass removal sampling, using

TABLE I. Mean densities of *Salmo trutta* and *Salvelinus fontinalis*, based on a two-pass removal sampling by electrofishing, length of the study stretches, mean \pm s.d. channel width and mean \pm s.d. water depth of the River Oriège (France) on 7 July 2006

Treatment	<i>S. trutta</i> density (individuals m^{-2})	<i>S. fontinalis</i> density (individuals m^{-2})	Length (m)	Width (m)	Depth (cm)
Allopatry <i>S. fontinalis</i>	0.006	0.925	28	6.0 \pm 3.2	29.8 \pm 8.8
Sympatry	0.081	0.066	50	5.2 \pm 1.3	31.8 \pm 13.1
Allopatry <i>S. trutta</i>	0.118	0.010	51	5.8 \pm 0.8	20.9 \pm 9.1

a Type EFKO FEG 1500 backpack electroshocker), the study area could be divided into three sites ('treatment'): an upstream site where brook trout is predominant ('allopatry *S. fontinalis*'), a downstream site where brown trout is predominant ('allopatry *S. trutta*') and an intermediate site where the two species co-occur with similar densities ('sympatry'). The three stretches were comprised within a 700 m stretch of river (Fig. 1) of uniform width and depth (Table I) and homogeneous terrestrial landscape. Furthermore, there were no potential sources of variation in isotopic signature of food sources such as tributary confluences, forest inputs or human disturbance between the study stretches.

STABLE ISOTOPE ANALYSIS

On 25 July and 4 August 2006, *S. fontinalis* and *S. trutta* were collected by electrofishing at the three sites of the River Oriège, with samples of available prey collected on 25 July. Following French regulations for ethical treatment of wild animals, the fishes were anaesthetized in a solution of 2-phenoxy-ethanol (0.04 ml l^{-1}), and then measured for total length ($L_T \pm 1 \text{ mm}$). Based on the L_T and age relationship established in this population (Aymes, 2005), age 2 and age 3 year fishes, *i.e.* from 152 to 244 mm (Table II), were fin clipped for SIA. Fin tissues were selected because stable isotopes of this tissue correlate closely with those of muscle tissue in salmonids and allow non-lethal sampling for quantifying their diet with SIA (Jardine *et al.*, 2005). Twenty individuals of each species in each treatment (allopatry and sympatry) were fin clipped (*i.e.* $n = 80$ samples, Table II), and samples were frozen for SIA. The main food items consumed by fishes in the study area (*i.e.* aquatic and terrestrial invertebrates) were known from previous dietary studies (Lagarrigue *et al.*, 2002; Aymes, 2005). They were collected at randomly selected locations within the three study stretches using hand-nets. The specimens were identified and combined according to taxonomic group (family for aquatic invertebrates; order for terrestrial invertebrates; $n = 3\text{--}30$) regardless of the sampling sites, kept alive for 24 h to ensure clearance of the gut, killed by freezing and then subjected to SIA.

All samples (invertebrates and salmonids fin clips) were oven dried (60°C for 48 h) and ground into a homogeneous powder using a mixer mill (Retsch MM 200). Approximately 0.2 mg of sample was weighed (Sartorius M2P) into a tin cup and combusted in a Carlo Erba NC2500 elemental analyser at high temperature for C and N isotope analysis. Resultant gases were delivered *via* continuous flow to a Finnigan Mat Delta XP isotope ratio mass spectrometer (Thermo Finnigan, Bremen, Germany). Isotope ratios are reported in delta notation as per international standards: peedee belemnite carbonate (C) and atmospheric nitrogen (N). Data were corrected using working standards (bass muscle, bovine liver, nicotinamide) that were previously calibrated against International Atomic Energy Agency (IAEA) standards. Isotopic analyses were performed at the Stable Isotopes in Nature Laboratory, University of New Brunswick, Canada.

DATA ANALYSES

After tests of normality and homoscedasticity, the data were subjected to ANCOVA, as per Vila-Gispert & Moreno-Amich (2001) and Jardine & Curry (2006), to assess changes in isotopic signature between species (*S. trutta* *v.* *S. fontinalis*) and treatments (allopatry *v.* sympatry). ANCOVAs were performed separately for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ as the response variables, with L_T as the covariate, and the treatment or species (separately) as the factor [*i.e.* species (or treatment) $\times L_T$, and species (or treatment) $\times L_T$ interaction term]. The models were run initially with the interaction term and again without interaction if it was not significant ($\alpha = 0.05$).

To approximate the contributions of invertebrates to fish diet, the IsoSource mixing model (version 1.3.1) developed by Phillips & Gregg (2003) was applied, using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of invertebrates. The stable isotope data for taxonomic groups were pooled within four potential sources following Phillips *et al.* (2005) so as to reduce the number

TABLE II. Mean \pm s.e. and range total length (L_T) of *Salmo trutta* and *Salvelinus fontinalis* from the preliminary fish survey (7 July 2006) of the River Oriège (France) and for the 20 specimens per treatment used in stable isotope analysis (29 July and 5 August 2006), with the mean \pm s.e. and range $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. Test results for differences in L_T among treatments are indicated: NS, no significant difference (Kolmogorov–Smirnov test) between treatments (*S. trutta* and *S. fontinalis*, both $P > 0.05$) and for mean L_T (ANOVA: both $P > 0.05$); *, significant differences (ANOVA) between treatments, $P < 0.05$; †, significant differences (Kolmogorov–Smirnov test) between treatments ($P < 0.05$)

Treatment	Preliminary survey			Stable isotope analyses					
	<i>n</i>	L_T (mm)	<i>n</i>	L_T (mm)	Range (mm)	$\delta^{13}\text{C}$ (‰)	Range (‰)	$\delta^{15}\text{N}$ (‰)	Range (‰)
<i>S. trutta</i> allopatry	34	167.4 \pm 55.7 ^{NS}	20	187.3 \pm 5.4*	152–229†	-21.21 \pm 0.16	-23.15 to -20.37	5.40 \pm 0.13	4.29–6.73
<i>S. trutta</i> sympatry	20	149.9 \pm 49.0 ^{NS}	20	208.4 \pm 6.5*	158–244†	-22.81 \pm 0.18	-24.05 to -21.24	5.99 \pm 0.12	5.03–6.97
<i>S. fontinalis</i> sympatry	138	140.2 \pm 28.4 ^{NS}	20	193.7 \pm 4.0*	168–227†	-23.64 \pm 0.15	-24.84 to -22.01	5.91 \pm 0.10	4.90–6.70
<i>S. fontinalis</i> allopatry	15	143.9 \pm 50.9 ^{NS}	20	178.9 \pm 4.6*	155–225†	-24.01 \pm 0.18	-25.56 to -22.93	5.49 \pm 0.16	4.72–7.44

of sources for modelling (provided that the pooled sources are isotopically and functionally similar). Consequently, the four potential food sources were aggregated based on their known diets (C  r  ghino, 1997) and origins. Aquatic invertebrates were separated into herbivores and carnivores. Herbivores consisted of two grazers (Ephemeroptera genus *Ecdyonurus* and Coleoptera genus *Halesus*), one filterer (Simuliidae), one shredder (Leuctra). Carnivores were comprised of three predators Diptera family Limoniidae, Plecoptera genus *Perla* and Trichoptera family Rhyacophilidae). Terrestrial invertebrates were comprised of the taxonomic orders commonly known to fall into temperate streams and to be consumed by salmonids (Baxter *et al.*, 2005): herbivorous (Thysanoptera, Hymenoptera, Coleoptera, Orthoptera and Homoptera) and carnivorous (Diptera and Formicidae) insects. The estimates were calculated after the adjustment of fish isotopic values for trophic fractionation between food and consumers tissues using two different values. Indeed, mixing models are known to be somewhat sensitive to these enrichment values, and the choice of enrichment values is a source of uncertainty in the mixing models (Phillips & Gregg, 2003). Consequently, enrichment factors of *S. fontinalis* from McCutchan *et al.* (2003) were used (*i.e.* +3.3‰ for $\Delta\delta^{13}\text{C}$ and +3.8‰ for $\Delta\delta^{15}\text{N}$) and of *S. trutta* calculated from Grey (2001) (*i.e.* +3.75‰ for $\Delta\delta^{13}\text{C}$ and +1.65‰ for $\Delta\delta^{15}\text{N}$). Finally, mixing models were considered for each of the eight possible combinations (*i.e.* two enrichment factors \times two species \times two treatments).

RESULTS

Although fish size structure and mean L_T did not differ between treatments in the preliminary fish survey for either species, significant differences in fish size structure and mean L_T were observed between treatments for specimens of both species subjected to SIA (Table II). The effect of treatment varied between the two species. *Salmo trutta* had significantly more enriched $\delta^{13}\text{C}$ signatures than *S. fontinalis* in both the allopatry and sympatry treatments (Tables II and III). This difference, however, tended to be less when the two species co-occurred (Fig. 2). The $\delta^{13}\text{C}$ signature of *S. fontinalis* did not change significantly between the two treatments, whereas *S. trutta* was significantly $\delta^{13}\text{C}$ depleted (*c.* 1.6‰) in sympatry compared to allopatry. No significant effect of L_T on $\delta^{13}\text{C}$ was detected (Table III). Similarly, the $\delta^{15}\text{N}$ signature of *S. fontinalis* did not change significantly between the two treatments, whereas *S. trutta* was significantly $\delta^{15}\text{N}$ enriched (*c.* 0.6‰) in sympatry compared to allopatry (Tables II and III). No significant difference in $\delta^{15}\text{N}$ values was observed between the two species when they co-occurred (Table III). An interactive effect of L_T and species on the trophic position of individual fish was observed when the two species were in allopatry. $\delta^{15}\text{N}$ increased with increasing L_T for each species in each treatment but more steadily in allopatry than in sympatry (Table III and Fig. 3). Although no differences between species were observed in sympatry, *S. fontinalis* were more enriched than *S. trutta* in $\delta^{15}\text{N}$ at large L_T in allopatry (Fig. 3).

The stable isotope signatures of taxonomic groups of invertebrates ranged from -29.33 to -23.04 ‰ for $\delta^{13}\text{C}$ and -0.74 to 7.51 ‰ for $\delta^{15}\text{N}$ (Fig. 2). Terrestrial invertebrates were significantly $\delta^{13}\text{C}$ depleted and $\delta^{15}\text{N}$ enriched compared to aquatic invertebrates (Kruskall–Wallis test, $n = 14$, $P < 0.01$). The enrichment factors between herbivorous and carnivorous terrestrial invertebrates were 0.89 ‰ $\Delta\delta^{13}\text{C}$ and 3.63 ‰ $\Delta\delta^{15}\text{N}$ and 0.20 ‰ $\Delta\delta^{13}\text{C}$ and 1.45 ‰ $\Delta\delta^{15}\text{N}$ for aquatic invertebrates. Stable isotope signatures were functionally consistent

TABLE III. ANCOVA results of stable isotope analyses ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of *Salmo trutta* and *Salvelinus fontinalis* (species) in allopatry and sympatry (treatment) in the River Oriège (France) collected in July 2006. Probability (*P*) values in bold are significant

Situation	Source of variation	d.f.	MS	<i>F</i>	<i>P</i>
$\delta^{13}\text{C}$ (‰)					
Allopatry	Intercept	1	290.34	489.37	< 0.001
	Species	1	75.42	127.13	< 0.001
	L_T	1	0.01	0.02	0.880
	Error	37	0.59		
Sympatry	Intercept	1	311.35	547.40	< 0.001
	Species	1	5.55	9.76	0.003
	L_T	1	0.22	0.38	0.539
	Error	37	0.57		
<i>S. trutta</i>	Intercept	1	335.54	552.56	< 0.001
	Treatment	1	22.53	37.09	< 0.001
	L_T	1	0.01	0.02	0.886
	Error	37	0.61		
<i>S. fontinalis</i>	Intercept	1	247.72	448.71	< 0.001
	Treatment	1	0.80	1.45	0.236
	L_T	1	0.32	0.58	0.450
	Error	37	0.55		
$\delta^{15}\text{N}$ (‰)					
Allopatry	Intercept	1	2.16	9.35	0.004
	Species	1	1.02	4.40	0.043
	L_T	1	6.79	29.37	< 0.001
	Species \times L_T	1	1.22	5.29	0.027
	Error	36	0.23		
Sympatry	Intercept	1	10.17	45.46	< 0.001
	Species	1	0.02	0.70	0.794
	L_T	1	1.49	6.67	0.014
	Error	37	0.22		
<i>S. trutta</i>	Intercept	1	12.18	42.41	< 0.001
	Treatment	1	1.59	5.54	0.024
	L_T	1	1.52	5.28	0.027
	Error	37	0.29		
<i>S. fontinalis</i>	Intercept	1	0.93	5.67	0.023
	Treatment	1	0.06	0.38	0.540
	L_T	1	7.19	43.97	< 0.001
	Error	37	0.16		

L_T , total length.

and isotopically similar within the four potential food sources (Phillips *et al.*, 2005). Regardless of the enrichment factors used, mixing models indicated consistent ranges of feasible solutions (Table IV). The absence of significant $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ differences among treatments for *S. fontinalis* was confirmed by similar ranges of feasible solutions for the four potential sources, and this species tended to incorporate mainly herbivorous terrestrial invertebrates in its diet (Table IV). Conversely, *S. trutta* almost exclusively incorporated carnivorous

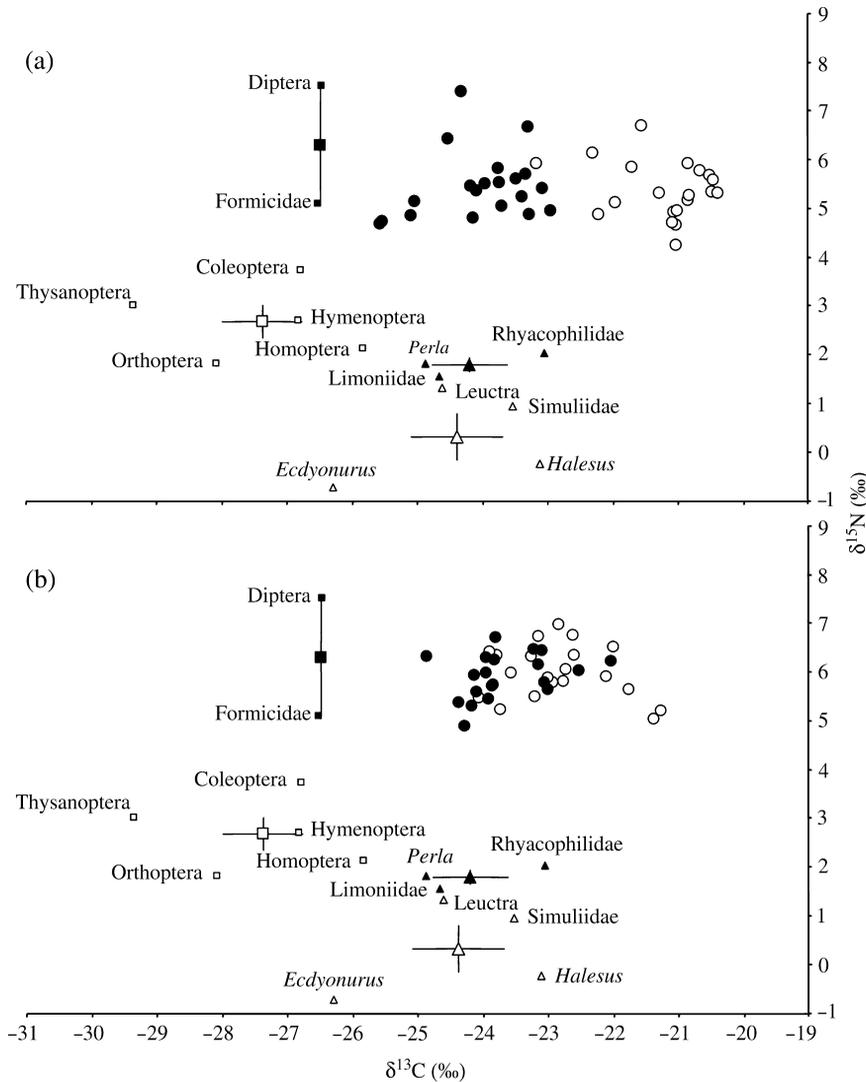


FIG. 2. Combined $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of *Salvelinus fontinalis* (●) and *Salmo trutta* (○) in the (a) allopatry and (b) sympatry treatments in the River Oriège (France) in July 2006. The potential prey items are aquatic herbivorous (△), aquatic carnivorous (▲), terrestrial herbivorous (□) and terrestrial carnivorous (■) invertebrates. The large symbols with error bars (S.E.) represent the mean for each group.

aquatic invertebrates in its diet in the allopatry situation. The significant changes in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of *S. trutta* among treatments were the consequences of a significant diet shift towards terrestrial invertebrates (Table IV).

DISCUSSION

Overlaps in trophic resource use, suggesting dietary competition, have been demonstrated previously in co-occurring native and non-native salmonid

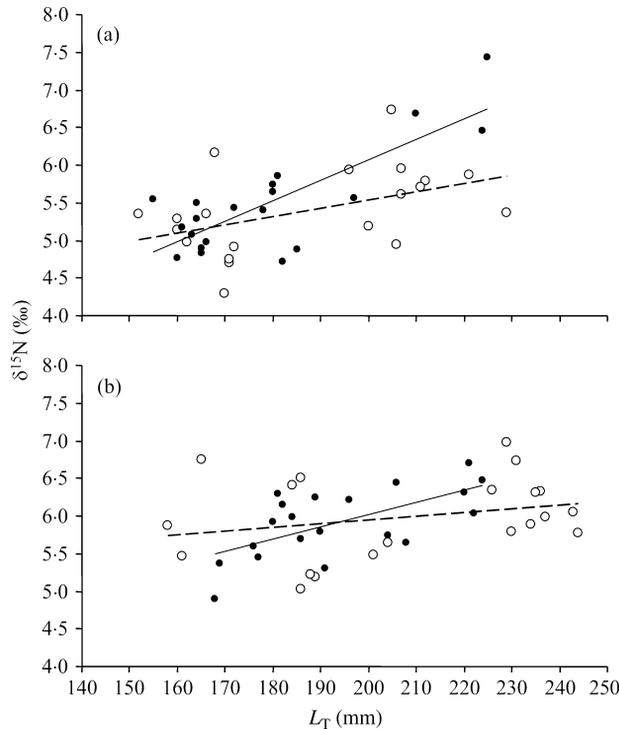


FIG. 3. Plot of $\delta^{15}\text{N}$ as a function of total length (L_T) for *Salvelinus fontinalis* (●) and *Salmo trutta* (○) in the (a) allopatry and (b) sympatry treatments in the River Oriège (France) in July 2006 (see Table III for detailed statistics).

species (Dunham *et al.*, 2000; Hilderbrand & Kershner, 2004; Clarke *et al.*, 2005). In the present study, dietary overlap was also observed, but a shift in the diet of native *S. trutta* due to the presence of introduced *S. fontinalis* was suggested by changes in the stable isotope signature of *S. trutta* between allopatry and sympatry with *S. fontinalis* and the absence of changes in *S. fontinalis*. The observed changes in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in native *S. trutta* under sympatry indicate a higher proportion of terrestrial prey in its diet. A different effect of fish size on $\delta^{15}\text{N}$ was not observed between species under sympatry, but it was under allopatry, though $\delta^{15}\text{N}$ increased more steadily with fish size in *S. fontinalis* than in *S. trutta*. These results demonstrate the existence of trophic interactions when the species co-occurred, subsequently suggesting a potential interspecific competition in sympatry.

The observed trophic shift in *S. trutta* was somewhat unexpected since interspecific competition between closely related species is typically thought to induce a partitioning of food resources rather than convergence in species diets (Griffith, 1974; Nakano *et al.*, 1999; Miyasaka *et al.*, 2003). Theoretically, the similarity in *S. trutta* and *S. fontinalis* diet should have been greater under allopatry than sympatry, with trophic competition more likely to occur when food resources are limited (Hilderbrand & Kershner, 2004). The presence of a non-native salmonid may decrease the abundance of terrestrial prey along a river

TABLE IV. Mixing model estimates (mean \pm s.d. and ranges of feasible solutions) of the contribution of aquatic (herbivorous and carnivorous) and terrestrial (herbivorous and carnivorous) invertebrates to the diet of *Salmo trutta* and *Salvelinus fontinalis* in allopatry and sympatry in the River Oriège (France) in July 2006

Species	Treatment	Aquatic invertebrates						Terrestrial invertebrates					
		Herbivorous			Carnivorous			Herbivorous			Carnivorous		
		Mean \pm s.d. (%)	Range	Range	Mean \pm s.d. (%)	Range	Range	Mean \pm s.d. (%)	Range	Range	Mean \pm s.d. (%)	Range	Range
<i>S. trutta</i> *	Allopatry	31.4 \pm 8.8	18–41	49–73	58.9 \pm 9.3	49–73	4.6 \pm 3.0	1–9	5.1 \pm 3.4	0–9			
	Sympatry	23.7 \pm 9.9	9–34	5–31	15.6 \pm 10.4	5–31	54.9 \pm 3.4	51–60	5.8 \pm 3.9	0–10			
<i>S. fontinalis</i> *	Sympatry	17.0 \pm 1.8	13–20	0–7	2.0 \pm 1.9	0–7	80.5 \pm 1.5	78–84	0.5 \pm 0.7	0–2			
	Allopatry	17.9 \pm 1.8	14–21	0–7	2.0 \pm 1.9	0–7	79.6 \pm 1.5	77–83	0.5 \pm 0.7	0–2			
<i>S. trutta</i> †	Allopatry	2.4 \pm 2.2	0–8	50–60	56.3 \pm 2.6	50–60	0.4 \pm 0.6	0–2	40.9 \pm 0.9	39–42			
	Sympatry	9.5 \pm 2.1	8–11	0–3	1.5 \pm 2.1	0–3	36.5 \pm 0.7	36–37	52.5 \pm 0.7	52–53			
<i>S. fontinalis</i> †	Sympatry	1.2 \pm 1.3	0–5	0–5	1.3 \pm 1.3	0–5	59.8 \pm 4.2	48–69	37.7 \pm 4.8	31–52			
	Allopatry	0.2 \pm 0.4	0–1	0–1	0.2 \pm 0.4	0–1	81.2 \pm 1.7	78–84	18.4 \pm 1.8	16–22			

*Enrichment factors of *S. fontinalis* from McCutchan *et al.* (2003).

†Enrichment factors of *S. trutta* from Grey (2001); see text for details.

stretch (Baxter *et al.*, 2004), and this could partially explain why *S. trutta* fed almost exclusively on aquatic invertebrates under allopatry. No evidence of food limitation, however, was observed in the study system, where both terrestrial and aquatic invertebrates were very abundant (Aymes, 2005). Moreover, fish densities were two to four times lower than in the study of Baxter *et al.* (2004).

Competitive interferences for habitat use might also provide explanations for the observed results. In North America, considerable habitat shifts by native *S. fontinalis* have been observed in the presence of introduced *S. trutta*, and this was attributed to high aggressive behaviour of *S. trutta* (Nyman, 1970; Fausch & White, 1981). Whereas in Europe, *S. trutta* habitat use was little affected by *S. fontinalis* (Blanchet *et al.*, 2007; Spens *et al.*, 2007). *Salmo trutta* aggressively dominates *S. fontinalis*, and this territorial defence may induce changes in the activity rhythm and foraging behaviour of *S. trutta*, which correspond to variations in terrestrial invertebrate availability (Baxter *et al.*, 2005). Foraging mode shifts from benthos to drift foragers have already been proposed as a mechanism to explain the co-occurrence of salmonids (Nakano *et al.*, 1999).

Despite the strengths of the present study, it should be noted that a possible bias may have occurred from fish displacement between treatments, leading to the observed changes in isotopic signatures. Indeed, *S. trutta* and *S. fontinalis* are known to display long-range movements within fresh waters (Gowan *et al.*, 1994; Gowan & Fausch, 1996; Cucherousset *et al.*, 2005). Permanent fish exchanges between treatments, however, would have led to a general dilution of the isotopic signatures (Cunjak *et al.*, 2005), and the clear segregation of isotopic signatures between treatments suggests that this was not the case, especially for *S. trutta*. Hence, the stable isotope signatures appear to reflect the prey consumed by fishes at their main feeding locations (Cunjak *et al.*, 2005). Also of note is that mixing models are highly sensitive to the enrichment values (Phillips & Gregg, 2003), which may vary when calculated under different nutritional conditions, *i.e.* wild invertebrates in Grey (2001) and trout chow in McCutchan *et al.* (2003) and could lead to modified enrichment values (McCutchan *et al.*, 2003; Vanderklift & Ponsard, 2003). The mixing models returned similar dietary mixtures regardless of the species-specific fractionation estimates used (Table IV).

Although SIA can detect changes in fish diet when salmonid species co-occur, the behavioural interactions that induce these changes remain poorly understood. To address this, the combined use of SIA and individual tagging protocols (*e.g.* passive integrated transponder technology) could providing new insights into fish foraging and movements (Cunjak *et al.*, 2005) as well as the ecological mechanisms and processes (*e.g.* displacements, diel and ontogenetic shifts in diet and habitat) associated with the co-occurrence of native and non-native fishes.

We thank the 'Office National de la Chasse et de la Faune Sauvage', the 'Agence de l'Eau' and the 'Direction Régionale de l'Environnement' for supporting this study (contract n°2004/071617/N°1413467.00, ONCFS-FEDER). We are grateful to G. H. Copp and two anonymous reviewers for the valuable comments on earlier versions of the manuscript.

References

- Aymes, J.-C. (2005). Interactions trophiques entre salmonidés: étude de la stratégie alimentaire et du comportement trophique d'une espèce indigène, *Salmo trutta* fario, et d'une espèce exotique, *Salvelinus fontinalis*, en situation allopatrique et sympatrique. Master's Thesis, University of Toulouse III, France.
- Baxter, C. V., Fausch, K. D., Murakami, M. & Chapman, P. L. (2004). Fish invasion restructures stream and forest food webs by interrupting reciprocal prey subsidies. *Ecology* **85**, 2656–2663.
- Baxter, C. V., Fausch, K. D. & Saunders, W. C. (2005). Tangled web: reciprocal flows of invertebrates prey link streams and riparian zones. *Freshwater Biology* **50**, 201–220.
- Blanchet, S., Loot, G., Grenouillet, G. & Brosse, S. (2007). Competitive interactions between native and exotic salmonids: a combined field and laboratory demonstration. *Ecology of Freshwater Fish* **16**, 133–143.
- Céréghino, R. (1997). Influence des éclusées hydroélectriques sur la structure et la dynamique des populations d'invertébrés d'une rivière pyrénéenne de moyenne montagne. PhD Thesis, University of Toulouse III, France.
- Clarke, L. R., Vidergar, D. T. & Bennett, D. H. (2005). Stable isotopes and gut content show diet overlap among native and introduced piscivores in a large oligotrophic lake. *Ecology of Freshwater Fish* **14**, 267–277.
- Copp, G. H., Bianco, P. G., Bogutskaya, N. G., Erős, T., Falka, I., Ferreira, M. T., Fox, M. G., Freyhof, J., Gozlan, R. E., Grabowska, J., Kováč, V., Moreno-Amich, R., Naseka, A. M., Peňáz, M., Povž, M., Przybylski, M., Robillard, M., Russell, I. C., Stakénas, S., Šumer, S., Vila-Gispert, A. & Wiesner, C. (2005a). To be, or not to be, a non-native freshwater fish? *Journal of Applied Ichthyology* **21**, 242–262.
- Copp, G. H., Spathari, S. & Turmel, M. (2005b). Consistency of diel behaviour and interactions of stream fishes and invertebrates during summer. *River Research and Applications* **21**, 75–90. doi: 10.1002/rra.833
- Cucherousset, J., Ombredane, D., Charles, K., Marchand, F. & Baglinière, J.-L. (2005). A continuum of life history tactics in a brown trout (*Salmo trutta*) population. *Canadian Journal of Fisheries and Aquatic Sciences* **62**, 1600–1610.
- Cunjak, R. A. & Power, G. (1986). Winter habitat utilization by stream resident brook trout (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*). *Canadian Journal of Fisheries and Aquatic Sciences* **43**, 1970–1981.
- Cunjak, R. A., Roussel, J.-M., Gray, M. A., Dietrich, J. P., Cartwright, D. F., Munkittrick, K. R. & Jardine, T. D. (2005). Using stable isotope analysis with telemetry or mark-recapture data to identify fish movement and foraging. *Oecologia* **144**, 636–646.
- Delacoste, M., Baran, P., Lascaux, J. M., Abad, N. & Besson, J. P. (1997). Bilan des introductions de salmonidés dans les lacs et ruisseaux d'altitude des Hautes-Pyrénées. *Bulletin Français de la Pêche et de la Pisciculture* **344/345**, 205–219.
- Dunham, J. B., Rahn, M. E., Schroeter, R. E. & Breck, S. W. (2000). Diets of sympatric Lahontan cutthroat trout and nonnative brook trout: implications for species interactions. *Western North American Naturalist* **60**, 304–310.
- Fausch, K. D. (1988). Tests of competition between native and introduced salmonids in stream: what have we learned? *Canadian Journal of Fisheries and Aquatic Sciences* **45**, 2238–2246.
- Fausch, K. D. & White, R. J. (1981). Competition between brook trout (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*) for position in a Michigan stream. *Canadian Journal of Fisheries and Aquatic Sciences* **38**, 1220–1227.
- Fausch, K. D., Taniguchi, Y., Nakano, S., Grossman, G. D. & Townsend, C. R. (2001). Flood disturbance regimes influence rainbow trout invasion success among five holartic regions. *Ecological Applications* **11**, 1438–1455.
- Fry, B. (2006). *Stable Isotope Ecology*. New York: Springer Science.

- García-Berthou, E. (2007). The characteristics of invasive fishes: what has been learned so far? *Journal of Fish Biology* **71** (Suppl. D), 33–53.
- García-Berthou, E., Alcaraz, C., Pou-Rovira, Q., Zamora, L., Coenders, G. & Feo, C. (2005). Introduction pathways and establishments rates of invasive aquatic species in Europe. *Canadian Journal of Fisheries and Aquatic Sciences* **62**, 453–463.
- Gorokhova, E., Hansson, S., Hoglander, H. & Andersen, C. M. (2005). Stable isotopes show food web changes after invasion by the predatory cladoceran *Cercopagis pengoi* in a Baltic Sea bay. *Oecologia* **143**, 251–259.
- Gowan, C. & Fausch, K. D. (1996). Mobile brook trout in two high-elevation Colorado streams: re-evaluating the concept of restricted movement. *Canadian Journal of Fisheries and Aquatic Sciences* **53**, 1370–1381.
- Gowan, C., Young, M. K., Fausch, K. D. & Riley, S. C. (1994). Restricted movement in resident stream salmonids: a paradigm lost? *Canadian Journal of Fisheries and Aquatic Sciences* **51**, 2626–2637.
- Gozlan, R. E., Peeler, E. J., Longshaw, M., St-Hilaire, S. & Feist, S. W. (2006). Effect of microbial pathogens on the diversity of aquatic populations, notably in Europe. *Microbes and Infection* **8**, 1358–1364.
- Grey, J. (2001). Ontogeny and dietary specialization in the brown trout (*Salmo trutta* L.) from Loch Ness, Scotland, examined using stable isotopes of carbon and nitrogen. *Ecology of Freshwater Fish* **10**, 168–176.
- Griffith, J. S. (1974). Utilization of invertebrates drift by brook trout (*Salvelinus fontinalis*) and cutthroat trout (*Salmo clarki*) in small streams in Idaho. *Transactions of the American Fisheries Society* **103**, 440–447.
- Hasegawa, K. & Maekawa, K. (2006). The effects of introduced salmonids on two native stream-dwelling salmonids through interspecific competition. *Journal of Fish Biology* **68**, 1123–1132.
- Hilderbrand, R. H. & Kershner, J. L. (2004). Influence of habitat type on food supply, selectivity, and diet overlap of Bonneville cutthroat trout and nonnative brook trout in Beaver Creek, Idaho. *North American Journal of Fisheries Management* **23**, 33–40.
- Jardine, T. D. & Curry, R. A. (2006). Unique perspectives on the influence of size and age on consumer $\delta^{15}\text{N}$ from a rainbow smelt complex. *Journal of Fish Biology* **69**, 215–223.
- Jardine, T. D., Gray, M., McWilliam, S. M. & Cunjak, R. A. (2005). Stable isotope variability in tissues of temperate stream fishes. *Transactions of the American Fisheries Society* **134**, 1103–1110.
- Koehn, J. D. (2004). Carp (*Cyprinus carpio*) as a powerful invader in Australian waterways. *Freshwater Biology* **49**, 882–894.
- Lagarigue, T., Céréghino, R., Lim, P., Reyes-Marchant, P., Chappaz, R., Lavandier, P. & Belaud, A. (2002). Diel and seasonal variations in brown trout (*Salmo trutta*) feeding patterns and relationship with invertebrate drift under natural and hydropeaking conditions in a mountain stream. *Aquatic Living Resources* **15**, 129–137.
- Lake, J. C. & Leishman, M. R. (2004). Invasion success of exotic in natural ecosystems: the role of disturbance, plant attributes and freedom from herbivores. *Biological Conservation* **117**, 215–226.
- Maguire, C. M. & Grey, J. (2006). Determination of zooplankton dietary shift following a zebra mussel invasion, as indicated by stable isotope analysis. *Freshwater Biology* **51**, 1310–1319.
- McCutchan, J. H., Lewis, W. M., Kendall, C. & McGrath, C. C. (2003). Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos* **102**, 378–390.
- McHugh, P. & Budy, P. (2006). Experimental effects of nonnative brown trout on the individual- and population-level performance of native Bonneville cutthroat trout. *Transactions of the American Fisheries Society* **135**, 1441–1455.
- McHugh, P., Budy, P., Thiede, G. & VanDyke, E. (2007). Trophic relationships of nonnative brown trout, *Salmo trutta*, and native Bonneville cutthroat trout,

- Oncorhynchus clarkii* utah, in a northern Utah, USA river. *Environmental Biology of Fishes* (in press). doi: 10.1007/s10641-006-9171-8
- Miyasaka, H., Nakano, S. & Furukawa-Tanaka, T. (2003). Food habit divergence between white-spotted charr and masu salmon in Japanese mountain streams: circumstantial evidence for competition. *Limnology* **4**, 1–10.
- Moyle, P. B. & Light, T. (1996). Biological invasions of fresh water: empirical rules and assembly theory. *Biological Conservation* **78**, 149–161.
- Nakano, S., Kitano, S., Nakai, K. & Fausch, K. D. (1998). Competitive interactions for foraging microhabitat among introduced brook char, *Salvelinus fontinalis*, and native bull charr, *S. confluentus*, and westslope cutthroat trout, *Oncorhynchus clarkii*, in a Montana stream. *Environmental Biology of Fishes* **52**, 345–355.
- Nakano, S., Fausch, K. D. & Kitano, S. (1999). Flexible niche partitioning via foraging mode shift: a proposed mechanism for coexistence in stream-dwelling charrs. *Journal of Animal Ecology* **68**, 1079–1092.
- Nyman, O. L. (1970). Ecological interaction of brown trout, *Salmo trutta* L., and brook trout, *Salvelinus fontinalis* (Mitchill), in a stream. *Canadian Field-Naturalist* **84**, 343–350.
- Peterson, B. J. & Fry, B. (1987). Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics* **18**, 293–320.
- Peterson, P. P., Fausch, K. D. & White, G. C. (2004). Population ecology of an invasion: effects of brook trout on native cutthroat trout. *Ecological Applications* **14**, 754–772.
- Phillips, D. L. & Gregg, J. W. (2003). Source partitioning using stable isotopes: coping with too many sources. *Oecologia* **136**, 261–269.
- Phillips, D. L., Newsome, S. D. & Gregg, J. W. (2005). Combining sources in stable isotope mixing models: alternative methods. *Oecologia* **144**, 520–527.
- Post, D. M. (2002). Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology* **83**, 703–718.
- Spens, J., Alanärä, A. & Eriksson, L.-O. (2007). Nonnative brook trout (*Salvelinus fontinalis*) and the demise of native brown trout (*Salmo trutta*) in northern boreal lakes: stealthy, long-term patterns? *Canadian Journal of Fisheries and Aquatic Sciences* **64**, 654–664.
- Townsend, C. (2003). Individual, population, community, and ecosystem consequences of fish invaders in New Zealand streams. *Conservation Biology* **17**, 38–47.
- Vander Zanden, M. J., Casselman, J. M. & Rasmussen, J. B. (1999). Stable isotope evidence for the food web consequences of species invasions in lakes. *Nature* **401**, 464–467.
- Vanderklift, M. A. & Ponsard, S. (2003). Sources of variation in consumer-diet $\delta^{15}\text{N}$ enrichment: a meta-analysis. *Oecologia* **136**, 169–182.
- Vila-Gispert, A. & Moreno-Amich, R. (2001). Mass-length relationship of Mediterranean barbel as an indicator of environmental status in South-west European stream ecosystems. *Journal of Fish Biology* **59**, 824–832.
- Vitousek, P. M., D'Antonio, C. M., Loope, L. L. & Westbrooks, R. (1996). Biological invasions as global environmental change. *American Scientist* **84**, 468–478.
- Vitousek, P. M., Mooney, H. A., Lubchenco, J. & Melillo, J. M. (1997). Human domination of Earth's ecosystems. *Science* **277**, 494–499.