STABLE ISOTOPES ISSUE

Chris Harrod · Jonathan Grey · T. Kieran McCarthy
Michelle Morrissey

Stable isotope analyses provide new insights into ecological plasticity in a mixohaline population of European eel

Abstract Recent studies have shown that anguillid eel populations in habitats spanning the marine–freshwater ecotone can display extreme plasticity in the range of catadromy expressed by individual fishes. Carbon and nitrogen stable isotope analysis was used to differentiate between European eels (*Anguilla anguilla*) collected along a short (2 km) salinity gradient ranging from $< 1\permil$ to $\sim 30\permil$ in Lough Ahalia, a tidal Atlantic lake system. Significant differences were recorded in mean $\delta^{13}C$, $\delta^{15}N$ and C:N values from eels collected from fresh, brackish and marine-dominated basins. A discriminant analysis using these predictor variables correctly classified ca. 85% of eels to salinity zone, allowing eels to be classified as freshwater (FW), brackish (BW) or marine (MW) residents. The results of the discriminant analysis also suggested that a significant proportion of eels moved between habitats (especially between FW and BW). Comparisons of several key population parameters showed significant variation between eels resident in different salinity zones. Mean condition and estimated age was significantly lower in MW eels, whilst observed length at age (a correlate of growth) was significantly higher in MW eels, intermediate in BW and lowest in FW eels. This study has demonstrated that the ecology of eels found along a short salinity gradient can be extremely plastic and that stable isotope analysis has considerable utility in demonstrating intra-population variation in diadromous fishes.

C. Harrod · T. K. McCarthy · M. Morrissey
Department of Zoology, National University of Ireland, Galway, Ireland

J. Grey
Department of Physiological Ecology, Max Planck Institute for Limnology, Postfach 165, D-24302, Plön, Germany

Present address: C. Harrod (✉)
Department of Physiological Ecology, Max Planck Institute of Limnology, Postfach 165, D-24302, Plön, Germany
E-mail: harrod@mpil-ploen.mpg.de
Tel.: +49-4522-763283

Present address: J. Grey
School of Biological Sciences, Queen Mary, University of London, London, E1 4NS, UK

Introduction

Over the last two decades, sharp declines in indices of recruitment and escapement of several species of temperate zone *Anguilla* have led to biologists becoming increasingly concerned regarding their long-term conservation status (Dekker et al. 2003). Many reasons for their decline have been proposed, including overfishing of all life stages, habitat degradation, shifts in oceanic currents, barriers to migration (e.g. weirs, dams), accumulation of xenobiotic compounds and parasites (Feunteun 2002; Casselman 2003; Dekker 2003; Tatsukawa 2003). Interest in eel biology has not only increased recently due to conservation concern, but also because several studies have shown that the catadromous life cycle of the eel is even more complicated than was previously considered. Eels were originally thought to make an obligate migration into freshwater to fulfil their catadromous life cycle. However, the development of high-resolution otolith Sr:Ca techniques (Thresher 1999), has allowed workers to track the migration histories of individual eels from otolith microchemistry and has shown that an eel stock may include several divergent migratory components, where some individuals may complete their life cycle without entering freshwater, and may reside in marine or brackish waters till maturation (Tsukamoto et al. 1998; Tzeng et al. 2000; Tsukamoto and Arai 2001; Jessop et al. 2002; Tsukamoto et al. 2002; Arai et al. 2003; Shiao et al. 2003; Tzeng et al. 2003; Jessop et al. 2004). It has become apparent that the so-called freshwater eels can actually make significant use of marine and mixohaline habitats

(Tsukamoto and Arai 2001; Tsukamoto et al. 2002), and Tsukamoto et al. (1998) even suggested that marine eels might form the bulk of the spawning stock. That many anguillid eels demonstrate facultative catadromy (movements into freshwater were previously thought to be obligate) challenges the widely accepted version of eel life cycles and has significant implications for those working on their biology and management. For instance, as managers and scientists work to manage and conserve anguillid eels throughout their distribution (Moriarty and Dekker 1997; Feunteun 2002; Casselman and Cairns 2003), they should be aware that eel stocks could include several components. The potential existence of different migratory components is likely to be particularly problematic for those attempting the stock assessment of eels in open marine and coastal ecosystems.

Much of our knowledge regarding the ecology of the most-studied anguillid species, e.g. European (*A. anguilla*), American (*A. rostrata*) and Japanese (*A. japonica*) eels, relates to freshwater populations (Aoyama and Miller 2003; Tesch 2003). Published records of the ecology of anguillid eels in mixohaline or marine waters are characterised by plasticity, with many workers demonstrating significant contrasts in the structure (e.g. sex ratio, age and size structure), behaviour (activity, home range size) and performance (e.g. growth rates, condition, mortality rates, production) of eels along salinity gradients (Hansen and Eversole 1984; Helfman et al. 1984a, b; Bozeman et al. 1985; Helfman et al. 1987; Svedäng et al. 1996; Jessop et al. 2002; Morrison and Secor 2003; Jessop et al. 2004). In order to identify divergent components or any ecologically distinct groups of eels along a salinity gradient, there is a requirement for a reliable marker. Unfortunately, no differences in external morphology have yet been recorded between eels found along salinity gradients, and biologists must rely on internal 'tags'. The most high-resolution technique for identifying membership of different salinity groups is examination of otolith Sr:Ca (Secor et al. 1995; Tzeng et al. 1997; Campana et al. 2000; Campana and Thorrold 2001; Tsukamoto and Arai 2001). However, the capital costs involved in purchasing equipment are considerable, and its use is limited to highly trained operators, which has limited its accessibility to many workers. Many studies that have used this technique have had limited sample sizes, which obviously has implications regarding the robustness of conclusions. It seems apparent therefore that there is a need for alternative techniques to reliably and more readily identify the existence of divergent components in eel populations. A range of potential methods exist e.g. mark recapture and biotelemetry (Lucas and Baras 2000), but these are either labour-intensive or time-consuming. We were interested in the use of stable isotopes of nitrogen and of carbon as an indicator of the existence of different components within the eel stock that differ with regard to their long-term habitat use (Fry and Sherr 1984; Peterson and Fry 1987). This method has been used in good effect to detect differential usage of waters varying in salinity, both in fishes (e.g. Dufour et al. 1998; Kline et al. 1998; Doucett et al. 1999a; b; McCarthy and Waldron 2000; Chanton and Lewis 2002; Litvin and Weinstein 2004) and other aquatic taxa (Smith et al. 1996; Chanton and Lewis 2002; Fry et al. 2003).

The use of stable isotope analysis to identify differential use of salinity habitats relies on the existence of predictable differences in stable isotope signatures along salinity gradients. For instance, a predictable gradient of ^{13}C exists across the marine–freshwater ecotone due to relative depletion of ^{13}C in fresh waters (Fry and Sherr 1984; Fry 2002). Fish feeding in different locations along a salinity gradient should therefore have contrasting carbon isotope signatures. Nitrogen stable isotope ratios ($\delta^{15}N$) provide some information on the source of food, as unpolluted fresh waters are depleted relative to marine waters, but with less resolution than that offered by $\delta^{13}C$. The principal benefit in using $\delta^{15}N$ is to identify consumer trophic level, as enrichment of ca. 3.5‰ occurs with each trophic level (Post 2002). C and N isotopes in consumer tissues reflect a temporal record of that which is assimilated from diet, and are accumulated in an organism's tissues over long periods of time—hence in poikilotherms such as fish, an individual's stable isotope signature represents a long-term, time-integrated measure of diet or habitat use (Hesslein et al. 1993). The ratio of carbon to nitrogen (C:N) in a consumer's tissues provides an indication of body composition: e.g. C:N can be considered a proxy of lipid content (McConnaughey and McRoy 1979).

The aim of the current study was to investigate the utility of stable isotope analysis to identify the existence of different ecotypes similar to those described by other workers using otolith Sr:Ca ratios (e.g. freshwater, brackish/estuarine and marine). If eels could be separated according to their use of different salinities, we were interested in how their ecology differed along a marine–freshwater gradient. This study forms part of a larger study examining the population biology and behaviour of European eels in marine and mixohaline waters.

Methods

Site

We sampled eels along a short (~2 km) marine–freshwater salinity gradient in three basins of Lough (lake) Ahalia in County Galway, western Ireland (53° 23′ N 9° 34′ W: Fig. 1). Lough Ahalia is an Atlantic coastal lake that consists of a series of basins forming the lower section of the River Screebe catchment. The system flows into the Atlantic Ocean via a single exit in Camus bay. The lowest basin (surface area ~25 ha, mean depth = 1.6 m) receives twice daily saline intrusions on
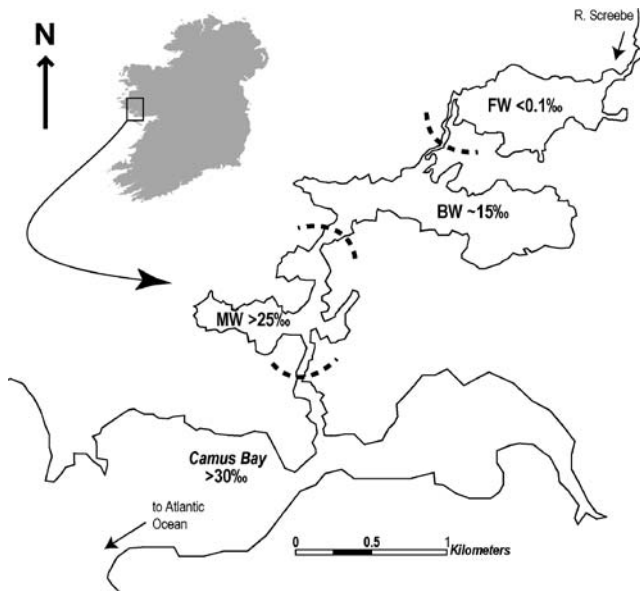
Fig. 1 Lough Ahalia, showing location in Ireland and the three distinct salinity zones referred to in this study: freshwater (*FW*), brackish water (*BW*) and marine dominated (*MW*)

flood tides. The saline influence continues, but intensity weakens in the central basin (surface area ~60 ha, mean depth = 2.5 m), which receives saline input on spring tides (i.e. fortnightly). The upper basin (surface area ~40 ha, mean depth = 1.7 m) is entirely freshwater, with a single significant inflow from the Screebe River, which drains the upper part of the catchment. The scale and intensity of the saline influence on the lower and central sections varies with tidal cycle (e.g. spring to neap) and also the strength and direction of the wind. The Screebe system supports a recreational fishery for migratory salmon (*Salmo salar*) and brown trout (*S. trutta*). This system was chosen for the study because it displays a very marked salinity gradient (0.5 − ~30‰) over a relatively short linear distance (~2 km) and importantly, the eel stock is unexploited.

Salinity

To further characterise typical salinity values within the three basins of Lough Ahalia, we collected bimonthly (September 2003–June 2004) salinity data from a series of locations over a 200-m grid ($n = 30$). Salinity values were recorded every 50 cm throughout the water column using a Hydrolab 4a Datasonde (Hydrolab INC., USA). Significant variation between salinity values were recorded within each basin so using 90th percentile benthic salinity, we classified them into three distinct salinity zones: freshwater (FW: 0.1‰); brackish water (BW: 15‰); and a marine-dominated zone (MW: 29.9‰). As eels are typically benthivorous, we consider benthic salinities to be most representative of their habitats.

Sample collection

European eels were sampled in September and October 2003 using unbaited summer fyke nets (Poole 1990) at sites along the salinity gradient (see Fig. 1). Fyke nets are selective for eels of TL > 300 mm (McCarthy et al. 1999). Nets were initially set for 24 h, but some nets were reset for an additional 24-h period until ca. 30 fish were captured from each of the three salinity zones (Actual *n*: FW = 37, BW = 27, MW = 34). All eels captured were yellow-phase (sub-adult) individuals, and showed no external signs of silvering [i.e. metamorphosis into the silver adult phase prior to the spawning migration (Aoyama and Miller 2003)]. On capture fish were placed in labelled bags and transported to the laboratory on ice, where they were individually frozen. Samples of the invasive gastropod *Potamopyrgus antipodarum* ($n > 50$ individuals per site) were collected from areas adjacent to those sampled for eels in order to provide an isotopic baseline against which eel trophic level could be calculated for each specific salinity zone (Cabana and Rasmussen 1996; Post 2002). *P. antipodarum* was selected as our isotopic baseline as it is both abundant and ubiquitous across the different salinity zones.

Laboratory analyses

Fish were thawed, and their total length (TL in millimetres), blotted wet weight (± 0.1 g) and sex recorded. Eel sex was assessed visually following Beullens et al. (1997). Sagittal otoliths were excised, cleaned and dried with tissue and placed in microtitre plates for storage. Subsequently, age of each fish was estimated from one otolith by counting of annuli under a binocular microscope after burning and cracking in a gas flame. Estimated ages are given as years following the otolith elver-transition check (Poole and Reynolds 1996).

Stable isotope analysis and C:N

Muscle tissue was excised from the left flank of each eel, macerated in pre-combusted glass vials and oven dried at 65°C for 24 h. Tissues were then pulverised using an agate pestle and mortar, and each sample was divided into two: one was treated to remove lipids according to the protocol of Bligh and Dyer (1959); the remainder was left untreated. Lipid stores tend to be ^{13}C-depleted because lipid synthesis favours the lighter carbon isotope (DeNiro and Epstein 1977), but the protocol used to remove lipids may adversely affect nitrogen isotope integrity (Grey et al. 2002), so analysis of both treated and untreated samples provides the best measures of carbon and nitrogen stable isotope ratios.

Potamopyrgus antipodarum were maintained alive in filtered water for over 24 h to allow for gut evacuation (cf. Feuchtmayr and Grey 2003), then oven dried, pul-

verised and divided as above. However, treatment for one set of samples involved acidification for 6 h using 0.1 M HCl to remove inorganic carbon from shell material. Samples were rinsed copiously with deionised water and oven dried for a further 24 h prior to storage in a desiccator.

For stable isotope analyses, samples were weighed ($\sim$0.7 mg) into tin cups prior to combustion in a Eurovector elemental analyser (Eurovector, Milan, Italy) coupled to a Micromass Isoprime continuous flow isotope ratio mass spectrometer (Micromass, Manchester, UK). Isotope ratios are given using the δ notation expressed in units of per mil (‰). The reference materials used were secondary standards of known relation to the international standards of Vienna PDB (for carbon) and atmospheric N_2 (for nitrogen). Repeat analyses of internal standards inserted after every six samples resulted in typical precision of < 0.1‰ for carbon and < 0.3‰ for nitrogen. The C:N values were calculated from the percentage carbon and nitrogen in each sample determined from an exact weight of material analysed in the elemental analyser.

Statistical analyses

Stable isotope data ($\delta^{13}C$ untreated, $\delta^{13}C$ lipid-treated and baseline-corrected $\delta^{15}N$) and C:N ratios were compared using one-way ANOVA, with salinity zone as a factor. Prior to ANOVA, data were tested for normality (Lillefor's test) and homogeneity of variance (Levene's test). All stable isotope-derived data failed these assumptions and were subsequently Box–Cox transformed in order to stabilise variance and normalise data (Sokal and Rohlf 1995). Paired t tests were used to test for effects of lipid treatment on $\delta^{13}C$ and C:N ratio values. Mean $\delta^{13}C$ values were compared between the different salinity zones to test whether or not eels were utilising the same putative food source. Mean baseline-corrected $\delta^{15}N$ values were tested to examine differences in eel trophic level. Where ANOVA showed significant differences, we used Fisher LSD tests to make post-hoc comparisons of differences between different salinity zones.

We employed multiple discriminant analysis (DA) in order to examine the reliability of our classification of eels into FW, BW or MW groups (SPSS 1998; Tabachnick and Fidell 2001). Three variables derived from the stable isotope analyses (SIA) were used as predictors of membership into three distinct salinity groups. Predictors were $\delta^{13}C$, C:N (both variables from lipid-treated samples) and baseline-corrected $\delta^{15}N$ (all predictors were Box–Cox transformed), and groups were FW, BW and MW. The performance of the DA was examined using jack-knifed classification procedures, with sample proportions used as prior probabilities. Finally, the DA was validated by the use of cross-validation, where two-thirds of the data were used as a learning sample, and the remainder were run as a test sample. The DA indi-

cated that several eels may have been wrongly classified (i.e. any observations whose classifications were closer to a group other than that which they had been originally grouped). We examined predicted group membership as an indicator of possible movement by eels between salinity zones.

The mean estimated age, length and weight of eels collected in each of the salinity zones was compared using one-way ANOVA. Where ANOVA showed significant differences, we used Fisher LSD tests to make post-hoc comparisons of differences between different salinity zones. Prior to ANOVA, data were tested for normality (Lillefor's test) and homogeneity of variance (Levene's test). We also examined differences in the relative weight of fish from each of the salinity zones (i.e. condition) using ANCOVA to test for differences in the parameters of $\log_{10}$-transformed length–weight regressions. When tests indicated significant differences (e.g. in intercepts), Fisher's LSD tests were used to make post-hoc comparisons. A similar approach was used to compare relationships between age and length at capture in order to test for differences in relative growth performance between eels from the different salinities. Fish from the MW zone had an increased proportion of younger fish (< 5 years) which could have skewed the results, so we only included fish > 5 years in our comparisons. Finally, the ratio of eels with female, male and indeterminate gonads (F:M:I) captured in each salinity zone was compared using a G test (Sokal and Rohlf 1995).

Statistical analyses were conducted in SYSTAT v8.0 (SPSS 1998). $P = 0.05$ was used as a critical level of significance in all tests.

Results

Isotope results

$\delta^{13}C$

Non lipid-treated mean $\delta^{13}C$ values were significantly different in eels from all three salinity zones ($F_{2,95} = 209.6$, $P < 0.001$. Table 1). However, there was a marked effect of lipid treatment on eel muscle $\delta^{13}C$ values in all three salinity zones, (Paired t test: FW $t = -7.48$, df $= 36$, $P < 0.001$; BW $t = -6.91$, df $= 26$, $P < 0.001$; MW $t = -6.51$, df $= 33$, $P < 0.001$). Paired differences between untreated and lipid-treated $\delta^{13}C$ values were largest in FW eels, and smallest in MW eels (compare mean values in Table 1), indicating that FW eel muscle tissue contained a relatively larger proportion of lipid. However, there was considerable variance in each salinity zone and an ANOVA comparing mean differences between salinity zones was insignificant ($F_{2,95} = 1.51$, $P = 0.23$). Due to the potentially confounding effect of differences in lipid concentration between the salinity zones, all subsequent analyses of $\delta^{13}C$ data refer to lipid-treated samples. Mean lipid-treated

Table 1 Summary statistics of yellow-phase European eel δ^{13}C, δ^{15}N and C:N ratios collected in fresh (*FW*), brackish (*BW*) and marine (*MW*) salinity zones

	n	δ^{13}C (‰)			δ^{15}N (‰)			C:N		
		Uncorrected			Uncorrected			Uncorrected		
		Mean	95% CI	SD	Mean	95% CI	SD	Mean	95% CI	SD
FW	37	−25.2[a]	0.63	1.9	12.1[g]	0.21	0.62	4.44[k]	0.578	1.73
BW	27	−23.9[b]	0.7	1.8	11.1[h]	0.54	1.37	3.76[k,m]	0.27	0.68
MW	34	−17.3[c]	0.49	1.4	10.9[h]	0.34	0.98	3.83[m]	0.476	1.36

	n	δ^{13}C (‰)			δ^{15}N (‰)			C:N		
		Lipid-treated			Baseline-corrected			Lipid-treated		
		Mean	95% CI	SD	Mean	95% CI	SD	Mean	95% CI	SD
FW	37	−23.6[d]	0.34	1.0	5.75[i]	0.21	0.62	3.23[n]	0.032	0.09
BW	27	−22.6[e]	0.52	1.3	4.76[j]	0.54	1.37	3.18[o]	0.038	0.10
MW	34	−16.3[f]	0.32	0.9	5.78[i]	0.34	0.98	3.27[n]	0.023	0.06

NB Differences in the superscript characters following mean values indicate significant differences ($P < 0.05$) between different salinity zones following Fisher's LSD post-hoc tests of Box-Cox transformed data (untransformed data shown)

δ^{13}C values differed significantly between salinity zones ($F_{2,95} = 457.1$, $P < 0.0001$. See Table 1 and Fig. 2). Mean δ^{13}C values were most depleted in FW eels (−23.6‰), intermediate (−22.6‰) in BW and least depleted in MW eels (−16.3‰).

$\delta^{15}N$

Prior to all analyses, eel δ^{15}N data were baseline-corrected against *P. antipodarum* δ^{15}N. Snail δ^{15}N values could be considered isotopically identical in FW and

Fig. 2 Isotope bi-plot comparing yellow eel δ^{13}C and δ^{15}N collected from three salinity zones in Lough Ahalia. Gaussian bivariate 95% confidence ellipses are centred on mean δ^{13}C and δ^{15}N values for each category

BW (6.3‰) but were lower in MW (5.1‰). Mean eel muscle δ^{15}N differed between some of the salinity zones (ANOVA of Box–Cox transformed data: $F_{2,95} = 8.30$, $P < 0.001$). Post-hoc comparisons showed that the mean baseline-corrected δ^{15}N values of FW and MW eels were similar (Table 1, Fig. 2), indicating that eels in these areas were feeding at similar trophic levels (mean δ^{15}N ∼5.8‰). However, BW eels had a reduced mean (±95% CI) δ^{15}N signature of 4.76‰ (±0.54), suggesting that eels in the central basin fed at a slightly lower trophic level. However, BW eels showed more variation in their nitrogen isotope composition than in either the FW or MW salinity zone (see 95% CI in Table 1 and range in δ^{15}N values in Fig. 2). A plot of δ^{13}C and δ^{15}N values recorded from individual eels collected in each salinity zone (Fig. 2) shows that eels can be most easily separated using δ^{13}C, and less easily using δ^{15}N. However, a simple bivariate approach seems limited due to overlap in δ^{13}C and δ^{15}N values between FW and BW eels.

C:N ratios

Untreated mean C:N values differed between the salinity zones ($F_{2,95} = 5.25$, $P = 0.007$, Table 1). Post-hoc comparisons revealed that mean C:N differed significantly between eels from FW [Mean (±SE) = 4.44 (±0.29)] and MW [3.83 (±0.23)]. There was a significant effect of lipid treatment on C:N values in all three salinity zones, (Paired t test: FW $t = 4.39$, df = 36, $P < 0.001$; BW $t = 4.75$, df = 26, $P < 0.001$; MW $t = 2.48$, df = 33, $P = 0.018$). Differences between untreated and lipid-treated C:N values recorded from individual eels were significantly lower in MW eels ($F_{2,95} = 8.07$, $P < 0.001$), but similar in FW and BW fishes. Significant differences still existed between mean C:N ratios following lipid treatment ($F_{2,95} = 7.66$, $P = 0.001$). Post-hoc comparisons showed that lipid-treated C:N ratios were signifi-
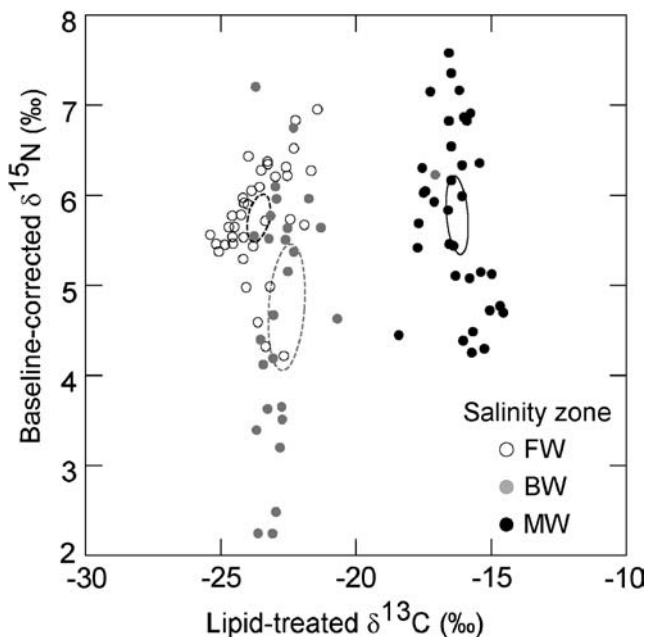
cantly lower in BW eels [mean ($\pm$ SE) = 3.18 (± 0.02)] than FW [3.23 (± 0.02)]) or MW eels [3.27 (± 0.01)]. These differences were due to slightly reduced percentage of carbon content in BW eels (Kruskal-Wallis test: $H = 1.9$, $P < 0.05$).

Correlation analyses were used to examine relationships between measures of fish size (eel TL, weight and age) and the results of SIA analyses (baseline-corrected $\delta^{15}N$ and lipid-treated $\delta^{13}C$ and C:N) for eels captured in each salinity zone. Significant correlations were consistently recorded between fish mass and $\delta^{15}N$ across the salinity gradient (FW $r = 0.50$, $P = 0.002$; BW $r = 0.70$, $P < 0.001$; and MW $r = 0.57$, $P < 0.001$, All habitats combined: $r = 0.40$, $P < 0.001$). Correlations between $\delta^{13}C$, C:N, fish age and size were extremely variable between salinity zones and typically non-significant.

Multivariate analysis of SIA data

Discriminant analysis, based on baseline-corrected $\delta^{15}N$ and lipid-treated $\delta^{13}C$ and C:N values (Tables 2–4), allowed clear separation of fish from each of the three salinity zones (Wilk's lambda $\lambda = 0.078$, $\chi^2 = 239.7$, df = 6, $P < 0.001$: see Fig. 3). Although the first discriminant function ($\delta^{13}C$) explained the bulk of the variation between the salinity groups (Table 2: 98%), the second discriminant function (Table 2: $\delta^{15}N$ and C:N) was still highly significant ($\chi^2 = 15.1$, df = 2, $P < 0.0001$). Table 3 shows the classification functions for each group, permitting future classification of Lough Ahalia eels into different salinity groups.

The classification analyses indicated that our use of SIA data to classify eels was robust (Table 4). On average, 85% of eels were correctly classified, but the ability of the model to classify eels varied with habitat. For instance, all eels caught in MW were correctly classified, whilst 25% of FW and BW eels were misclassified. By examining the cases that the DA model suggests were misclassified, it seems likely that from their stable isotope signatures there were several eels caught outside of their typical habitat. Of the 38 eels

classified by the DA as FW, 30 (79%) were captured in freshwater, and 8 (21%) were collected from the BW salinity zone. Of the 25 eels classified by the model as BW, 18 (72%) were taken in BW, 7 (28%) in FW. A single DA-classified MW eel was captured in the BW salinity zone (3%); all others (97%) were collected in the MW zone.

Population structure

Yellow eels ranged in TL between 123 mm and 681 mm, but ANOVA comparisons showed that eels in each of the three salinity zones had similar mean lengths (Table 5: $F_{2,95} = 1.32$, $P = 0.273$). The overall mean TL ($\pm 95\%$ C.I.) of eels collected was 377 (± 15.3) mm. The mean weight of eels collected in the three salinity zones did not differ significantly at the 5% level (Table 2: $F_{2,95} = 0.38$, $P = 0.687$).

Estimated ages of eels ranged from 1+ to 48+, with an overall mean (± 95 C.I.) estimated age of 16.5 (± 0.57) years since the glass eel metamorphosis. Mean age differed amongst the salinity zones (Table 5: $F_{2,95} = 15.22$, $P < 0.001$), and post-hoc comparisons of mean ages ($\pm 95\%$ CI) showed that FW (20+ ± 2.7) and BW (19+ ± 4.0) eels were on average older than MW (11+ ± 1.8) eels. There was no evidence that the contribution of females or males increased along the salinity gradient as F:M:I sex ratios were similar (Table 5 G test: $G_{adj} = 5.65$, df = 4, $P = 0.227$).

Comparisons of $\log_{10}$-transformed length–weight relationships (Table 6) using ANCOVA demonstrated that the intercept parameter for MW eels was significantly lower than that calculated for FW and BW eels (ANCOVA$_{intercepts}$: $F_{2,90} = 5.811$, $P = 0.004$). This shows that eels collected from the most saline part of the

Table 3 Classification functions for the three salinity zones: FW, BW and MW. NB: Data were Box–Cox transformed prior to analysis

Predictor	FW	BW	MW
Lipid-treated $\delta^{13}C$	−20.76	−20.34	−17.67
Baseline-corrected $\delta^{15}N$	4.71	4.59	4.45
Lipid-treated C:N	12706.96	12690.80	12206.28
Constant	−3872.94	−3841.13	−3482.13

Table 2 Standardised canonical coefficients for each of the two discriminant functions used to classify eels into FW, BW and MW salinity groups

Predictor variable	Discriminant function I	Discriminant function II
Lipid-treated $\delta^{13}C$	1.034	0.013
Baseline-corrected $\delta^{15}N$	−0.010	−0.926
Lipid-treated C:N	−0.152	0.410
Eigenvalue	9.91	0.17
Canonical correlation	0.95	0.39
% Variation	98%	2%
$\chi^2 =$	239.7	15.1
$df =$	6	2
$P =$	< 0.0001	< 0.0001

NB Data were Box–Cox transformed prior to analysis

Table 4 Classification matrix functions for the three salinity zones: FW, BW and MW

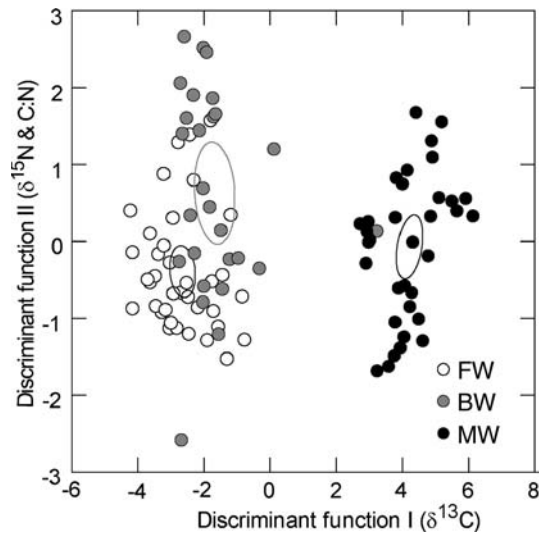|		% Correctly classified				
	Observed	FW	BW	MW	Mean %	n
% Correct classification	FW	81	70	100	85	37
% Correct (jack-knifed)	BW	78	70	100	84	24
% Correct (training set)	MW	78	85	100	88	34

Fig. 3 Canonical plot scores from discriminant analysis of SIA-derived data of yellow-phase European eels collected from three salinity zones in Lough Ahalia. Gaussian bivariate 95% confidence ellipses are centred on group centroids. The discriminant analysis was based on three Box–Cox- transformed variables: $\delta^{13}C$, C:N (both lipid-treated) and baseline-corrected $\delta^{15}N$

Table 6 Parameters of Log10 transformed linear regression of length–weight (NB: FW and BW data pooled as ANCOVA showed no significant differences)

Salinity zone	A ($\pm$SE)	B ($\pm$SE)	df	R^2	P
FW BW	-5.60 (± 0.17)	2.94 (± 0.07)	60	0.97	< 0.001
MW	-5.30 (± 0.19)	2.81 (± 0.07)	30	0.98	< 0.001

gradient (Hesslein et al. 1991; Doucett et al. 1999a; McCarthy and Waldron 2000; Fry 2002). We feel that this provides good evidence for the existence of several discrete components within the L. Ahalia eel population (sensu Secor 1999). The analysis of carbon and nitrogen stable isotopes may not provide a life-time record (cf. otolith Sr:Ca), but does provide a long-term, integrated picture of residence through consistent patterns of consumption (Hesslein et al. 1993). Estimating the actual residence times of eels in the different salinity zones is difficult as little is known about protein turnover rates in wild fishes (Houlihan et al. 1995). Beyond studies of cultured eels (e.g. Owen 2001), data regarding protein turnover times in eels are rare and it is difficult to estimate the half-life of white muscle, which is required to put a definite figure on residence times. Owen (2001) predicted that in a cultured eel of 100 g, whole body fractional rates of protein synthesis in eels fed at maximum ration at 25°C are ca. 2% d^{-1}. However, rates of protein synthesis in poikilotherms will vary with temperature, and individual age and growth status (Houlihan et al. 1995), and the relatively slow growth of Lough Ahalia eels will reduce protein turnover times in comparison to those recorded from cultured eels. Hesslein et al. (1993) suggest that for muscle tissue collected from adult fishes, protein turnover rates in white muscle are such that the stable isotope ratio values we present may relate to periods of ca. 1 year.

As might be expected, the most distinct differences in $\delta^{13}C$ were recorded between eels collected from extreme ends of the salinity gradient (FW and MW: Table 1, Fig. 2). Although differences in mean $\delta^{13}C$ values were less extreme between FW and BW eels, they were still highly significant ($P < 0.001$). The results of the discriminant analysis indicate that a small, but significant number of eels were captured in a salinity zone at odds with their isotopic composition. If we assume that eels are in, or near to, close to isotopic equilibrium with their diets, this indicates that some individuals had moved

Lough Ahalia system had lower weight for a given length (i.e. condition was lower in marine eels).

Length at age was extremely variable, both within and between sites, suggesting significant variation in individual growth rates. As the assumption of parallel slopes was met, differences in the intercepts of the log$_{10}$-transformed length at age relationships for eels collected in each salinity zone suggested that eels inhabiting different salinities grew at different rates (ANCOVA intercepts: $F_{2,90} = 13.07$, $P < 0.001$). Post-hoc comparisons showed that age-adjusted mean lengths were greatest in MW eels, intermediate in BW and lowest in FW eels, implying that growth rates were positively associated with increased salinities.

Discussion

We used SIA to identify consistent differences between eels collected along a short (< 2 km) salinity gradient. Mean $\delta^{13}C$ values recorded from eels in each salinity zone were indicative of consumers consistently feeding at different locations along a freshwater–marine salinity

Table 5 Summary statistics of various population parameters of yellow European eel collected in fresh (*FW*), brackish (*BW*) and marine (*MW*) salinity zones

Salinity zone	n eels	Mean TL ($\pm$95% CI)	Mean weight ($\pm$95 CI)	Mean age ($\pm$95 CI)	Sex ratio (F:M:I)
FW	37	366 (± 24.9)	101 (± 30.8)	20[a] (± 2.7)	0.54:0.41:0.05
BW	27	397 (± 27.1)	112 (± 26.6)	19[a] (± 4.0)	0.74:0.19:0.07
MW	34	372 (± 29.0)	96 (± 17.6)	11[b] (± 1.8)	0.53:0.32:0.15

TL Total length (mm), weight (g), estimated age (years post elver check). *NB* Superscript letters following mean age values indicate significant differences following Fisher's LSD post-hoc tests

between salinity zones, with movements most apparent between BW and FW eels. Other studies of mixohaline eels that have shown that movements between salinity habitats can take place over the short- (< 24 h) and long term (> 1 year) (Dutil et al. 1988; Jessop et al. 2002). We have used biotelemetry and mark-recapture of eels to examine the nature of these movements and have evidence for inter-basin movements by some Lough Ahalia eels (C Harrod, unpublished data).

An alternative explanation to the patterns shown by our data is that those individuals with isotopic signatures suggestive of movements between salinity zones are outliers, and simply reflect natural variation within these zones rather than evidence of movements. Although we cannot discount the alternative hypothesis outright, in our study such fishes were identified using a combination of three separate measures of salinity ($\delta^{13}C$, $\delta^{15}N$ and C:N) in the DA, and we therefore feel that our results provide evidence for individual movements between salinity zones rather than being an effect of individual variation within the different basins.

During the current study, we employed a range of techniques to examine ecological variation in eels collected along a FW–MW salinity gradient. Several of these have shown that the ecology of eels can differ significantly along such a gradient (e.g. mean age, relative condition and growth), and reinforce the patterns shown in the $\delta^{13}C$ data. There is a strong argument that the L. Ahalia eel population can be separated into different salinity components (freshwater, brackish and marine-type eels) similar to those identified in *Anguilla* using otolith Sr:Ca ratios (e.g. Tsukamoto et al. 1998; Tzeng et al. 2000; Tsukamoto and Arai 2001; Jessop et al. 2002; e.g. Tsukamoto et al. 2002; Morrison et al. 2003; Tzeng et al. 2003). Although we reiterate that carbon and nitrogen SIA does not provide a life-time record of individual habitat use, the increasing availability of SIA service providers means that the method offers an alternative to otolith Sr:Ca as a means of identifying long-term residency by eels (and other diadromous fishes) in contrasting salinity zones. SIA sample preparation is far less onerous than that of Sr:Ca, and analysis requires less equipment or operator time, allowing larger and probably more representative sample sizes to be analysed.

Although anguillid eels are widely distributed and can play important functional roles in the systems where they are found (Aida et al. 2003; Tesch 2003), there have been relatively few uses of SIA to study their trophic ecology. Rounick and Hicks (1985) examined $\delta^{13}C$ of various tissues of two stream-dwelling species of Pacific eel (*A. dieffenbachii* and *A. australis*) in relation to their invertebrate prey. Jones and Waldron (2003) reported $\delta^{13}C$ and $\delta^{15}N$ values from a small sample ($n = 3$) of European eels collected from a productive shallow lake in Britain. Bardonnet and Riera (2005) used variation in $\delta^{13}C$ to infer that some glass eels (*A. anguilla*) actively fed during their estuarine migration. The current study represents the first detailed use of stable isotope analysis

to examine the trophic ecology of yellow-phase European eels. It has shown that eel trophic level can vary relative to a common baseline across different salinity zones. Brackish water eels fed at a slightly lower mean trophic level (mean baseline-corrected $\delta^{15}N = 4.76$) that their counterparts in FW and MW, which displayed similar mean values (mean baseline-corrected $\delta15N$: FW = 5.75 and MW = 5.78). It is difficult to speculate on the actual differences between the diets of BW and FW/MW eels, as we do not have detailed $\delta^{15}N$ data for potential prey. Observations of stomach contents from eels captured in all three salinity zones suggest that the diet of eel is catholic and includes benthic invertebrates (amphipods, isopods, molluscs) and fishes (C Harrod, Unpublished data). The reduced mean $\delta^{15}N$ in BW eels may indicate an increased dependence on benthic macroinvertebrates rather than fish. Interestingly, not only did BW eels feed at a lower mean trophic level, but differences between individual BW eels were considerable, with baseline-corrected $\delta^{15}N$ values ranging between 2.2‰ and 7.2‰ (see Fig.2). That eels in the most heterogeneous salinity zone should display greater trophic plasticity is interesting, and may indicate that prey diversity is greater in the BW zone, or that BW eels exhibit different foraging patterns to FW or BW eels. A more detailed analysis of trophic plasticity in L. Ahalia eels will require serial measurement of SIA signatures of the same individual (e.g. fin clips or blood samples) or by comparing the $\delta^{15}N$ values of a range of tissues with different turnover times (Fry et al. 2003; Bearhop et al. 2004).

Our results showed that the somatic composition of eels differed along the salinity gradient. The variation in untreated C:N suggests that lipid content was significantly lower in the muscle of MW fish (McConnaughey 1978). However, when we analysed the lipid-treated C:N data, we again showed differences in body composition, with BW eels having a lower mean lipid-treated C:N than eels from FW or MW. These differences were due to a small, but significant reduced mean carbon content in BW fish. Usually, intraspecific differences in somatic carbon content are related to variation in lipid concentration due to season, age, or maturation status rather than habitat (Gorokhova and Hansson 2000; Tanner et al. 2000). However, in the current study, significant differences exist after lipids were removed, and it is unclear why eels from BW should have reduced muscle carbon content relative to FW or MW fish. We can probably discount the role of differences in age structure, growth rate, somatic condition or sex ratio, as BW fish show values that typically overlap with either MW or FW fish. In the current study, the greatest contrast between BW eels and eels from other salinity zones was dietary, with BW eels feeding at a lower trophic level. We have suggested that their diet may include an increased proportion of macroinvertebrates relative to eels from the other salinity zones, and the most abundant littoral macroinvertebrate taxa in the BW zone are isopods (e.g. *Sphaeroma* Spp) and amphipods (*Gammarus*

Spp.). These taxa have low mean carbon content relative to values typically recorded from other benthic invertebrates (Salonen et al. 1976; Penczak 1985; Frost et al. 2003) or fishes, both from Lough Ahalia (C Harrod, Unpublished data), or other systems (Penczak 1985; Tanner et al. 2000). The reduced muscle carbon content in BW eels relative to FW or MW fish may therefore be related to differences in diet.

We report a consistent positive correlation between the weight of individual eels and their trophic level. A similar pattern has been recorded from several other fishes (Kline et al. 1998; Doucett et al. 1999a; Fry et al. 1999; Overman and Parrish 2001; Grey et al. 2002) and is generally considered as evidence that larger consumers feed at a higher trophic level (but see Overman and Parrish 2001 who demonstrate a more significant effect of age). Interestingly, there was no such relationship with length or age in eels from Lough Ahalia, suggesting that in eels, weight may be a better indicator of predatory scope than length. Relationships between eel δ^{13}C and size within each salinity zone were all nonsignificant, showing that although larger eels may have been feeding at different trophic levels, it is likely that they were feeding in similar areas to smaller conspecifics.

It is increasingly important that those attempting to manage these vulnerable fishes are aware that the typical view of the life cycle of many Anguilla Spp. (i.e. obligate catadromy) represents a gross oversimplification. As managers and scientists struggle to manage and conserve anguillid eels worldwide (Moriarty and Dekker 1997; Feunteun 2002; Casselman and Cairns 2003), they will have to consider the possibility that eel stocks may include several components which could differ in their fundamental biology (Jessop et al. 2002; Tsukamoto et al. 2002; Tzeng et al. 2003: this study). The identification of different population components is going to be particularly important to groups involved in the assessment of eel populations in coastal and estuarine ecosystems. Although the samples examined in the current study are relatively small, we have provided more evidence of considerable ecological plasticity in the European eel. Eels in L. Ahalia fed in distinct areas (δ^{13}C data), and to a degree at different trophic levels (δ^{15}N data). They also differed in somatic composition (C:N data). The eels were structured according to age and differed in both growth rates and relative condition along the salinity gradient. Much of the intra-population variation in other eel populations has been associated with asymmetry between male and female life-history strategies. Helfman et al. (1987), have proposed that American male eels have a time-limiting strategy (i.e. to mature quickly) whilst female conspecifics follow a size-maximisation strategy (in order to maximise individual fecundity). However, the potential confounding effects of sex (Oliveira et al. 2001; Oliveira and McCleave 2002) were limited in the current study, because sex ratios were similar between each of the salinity zones. Growth in all three salinity zones was relatively slow (evidenced by the elevated mean estimated age for a given length), and may be representative of an unexploited eel stock in oligotrophic Irish waters (Poole and Reynolds 1996). However, there is some evidence that in Lough Ahalia, faster growth (in terms of length, but not mass) was associated with increased salinity. Similar patterns of enhanced growth in brackish waters has been observed in A. anguilla (Fernandez-Delgado et al. 1989) and other species of Anguilla, e.g. A. rostrata (Morrison and Secor 2003; Jessop et al. 2004) and A. japonica (Tzeng et al. 2003). Tzeng et al. (2003) have suggested that the energetic costs of osmoregulation are minimised in eels in salinities closest to that of their body fluids (10.5–14‰), and propose that this may contribute to the better growth seen in marine and brackish eels. However, marine and estuarine productivity tends to be higher than in adjacent freshwaters at higher latitudes (Gross 1987; Gross et al. 1988) and mixohaline eels may simply have access to greater food resources.

We recommend the use of multivariate classification techniques such as discriminant analysis to ecologists using analysis of multiple stable isotopes to examine differences between consumers that can be classified into two or more groups e.g. anadromous and non-anadromous fishes (Hesslein et al. 1991; Doucett et al. 1999a, b; Charles et al. 2004). The advantages of DA include robust tests for differences between different groups (Table 2), and also the identification of those variables that are most efficient in separating different groups (Table 3). Further useful outputs are classification functions (Table 4), which can be used to classify new observations into groups. For instance, it is currently very difficult to estimate relative production or escapement of eels from different parts of a catchment. However, if the δ^{13}C, δ^{15}N and C:N values of silver eels migrating out of a catchment are recorded, a DA model such as that described here could easily provide an estimate of relative contribution to escapement by silver eels originating from different salinity components (sensu Charles et al. 2004).

References

Aida K, Tsukamoto K, Yamauchi K (eds) (2003) Eel Biology. Springer-Verlag, Tokyo

Aoyama J, Miller MJ (2003) The silver eel. In: Aida K, Tsukamoto K, Yamauchi K (eds) Eel Biology. Springer-Verlag, Tokyo, pp 107–117

Arai T, Kotake A, Ohji M, Miyazki N, Tsukamoto K (2003) Migratory history and habitat use of Japanese eel Anguilla japonica in the Sanriku Coast of Japan. Fish Sci 69:813–818

Bardonnet A, Riera P (2005) Feeding of glass eels (*Anguilla anguilla*) in the course of their estuarine migration: new insights from stable isotope analysis. Estuar Coast Shelf Sci 63:201–209

Bearhop S, Adams CE, Waldron S, Fuller RA, Macleod H (2004) Determining trophic niche width: a novel approach using stable isotope analysis. J Appl Ecol 73:1007–1012

Beullens K, Eding EH, Gilson P, Ollevier F, Komen J, Richter CJJ (1997) Gonadal differentiation, intersexuality and sex ratios of European eel (*Anguilla anguilla* L.) maintained in captivity. Aquaculture 153:135–150

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911–917

Bozeman EL, Helfman GS, Richardson T (1985) Population size and home range of American eels in a Georgia tidal creek. Trans Am Fish Soc 114:821–825

Cabana G, Rasmussen JB (1996) Comparison of aquatic food chains using nitrogen isotopes. Proc Natl Acad Sci USA 93:10844–10847

Campana SE, Thorrold SR (2001) Otoliths, increments, and elements: keys to a comprehensive understanding of fish populations? Can J Fish Aquat Sci 58:30–38

Campana SE, Chouinard GA, Hanson JM, Frechet A, Brattey J (2000) Otolith elemental fingerprints as biological tracers of fish stocks. Fish Res 46:343–357

Casselman JM (2003) Dynamics of resources of the American eel, *Anguilla rostrata*: declining abundance in the 1990s. In: Aida K, Tsukamoto K, Yamauchi K (eds) Eel biology. Springer-Verlag, Tokyo, pp 255–274

Dekker W, Casselman JM, Cairns DK, Tsukamoto K, Jellyman D, Lickers H (2003) Worldwide decline of eel resources necessitates immediate action: Québec Declaration of Concern. Fisheries 28:28–30

Chanton J, Lewis FG (2002) Examination of coupling between primary and secondary production in a river-dominated estuary: Apalachicola Bay, Florida, USA. Limnol Oceanogr 47:683–697

Charles K, Roussel J-M, Cunjak RA (2004) Estimating the contribution of sympatric anadromous and freshwater resident brown trout to juvenile production. Mar Freshwat Res 55:185–191

Dekker W (2003) Status of the European eel stock and fisheries. In: Aida K, Tsukamoto K, Yamauchi K (eds) Eel biology. Springer-Verlag, Tokyo, pp 237–254

DeNiro M, Epstein S (1977) Mechanism of carbon isotope fractionation associated with lipid synthesis. Science 197:261–263

Doucett RR, Hooper W, Power G (1999a) Identification of anadromous and nonanadromous adult brook trout and their progeny in the Tabusintac River, New Brunswick, by means of multiple-stable-isotope analysis. Trans Am Fish Soc 128:278–288

Doucett RR, Power M, Power G, Caron F, Reist JD (1999b) Evidence for anadromy in a southern relict population of Arctic charr from North America. J Fish Biol 55:84–93

Dufour V, Pierre C, Rancher J (1998) Stable isotopes in fish otoliths discriminate between lagoonal and oceanic residents of Taiaro Atoll (Tuamotu Archipelago, French Polynesia). Coral Reefs 17:23–28

Dutil J-D, Giroux F, Kemp A, Lavoie G, Dallaire J-P (1988) Tidal influence on movements and on daily cycle of activity of American eels. Trans Am Fish Soc 117:488–494

Fernandez-Delgado C, Hernando JA, Herrera M, Bellido M (1989) Age and growth of yellow eels, *Anguilla anguilla*, in the estuary of the Guadalquiver river (south-west Spain). J Fish Biol 34:561–570

Feuchtmayr H, Grey J (2003) Effect of preparation and preservation procedures on carbon and nitrogen stable isotope determinations from zooplankton. Rapid Commun Mass Spectrom 17:2605–2610

Feunteun E (2002) Management and restoration of European eel population (*Anguilla anguilla*): an impossible bargain. Ecol Eng 18:575–591

Frost PC, Tank SE, Turner MA, Elser JJ (2003) Elemental composition of littoral invertebrates from oligotrophic and eutrophic Canadian lakes. J N Am Benthol Soc 22:51–62

Fry B (2002) Conservative mixing of stable isotopes across estuarine salinity gradients: a conceptual framework for monitoring watershed influences on downstream fisheries production. Estuaries 25:264–271

Fry B, Sherr EB (1984) δ^{13}C measurements as indicators of carbon flow in marine and freshwater ecosystems. Contrib Mar Sci 27:13–47

Fry B, Mumford PL, Tam F, Fox DD, Warren GL, Havens KE, Steinman AD (1999) Trophic position and individual feeding histories of fish from Lake Okeechobee, Florida. Can J Fish Aquat Sci 56:590–600

Fry B, Baltz DN, Benfield MC, Fleeger JW, Gace A, Haas HL, Quiñones-Rivera ZJ (2003) Stable isotope indicators of movement and residency for brown shrimp (*Farfantepenaeus aztecus*) in coastal Louisiana marshscapes. Estuaries 26:82–97

Gorokhova E, Hansson S (2000) Elemental composition of *Mysis mixta* (Crustacea, Mysidacea) and energy costs of reproduction and embryogenesis under laboratory conditions. J Exp Mar Biol Ecol 246:103–123

Grey J, Thackeray SJ, Jones RI, Shine A (2002) Ferox trout (*Salmo trutta*) as 'Russian dolls': complementary gut content and stable isotope analyses of the Loch Ness foodweb. Freshwat Biol 47:1235–1243

Gross MR (1987) Evolution of diadromy in fishes. Am Fish Soc Symp 1:14–25

Gross MR, Coleman RM, McDowall RM (1988) Aquatic productivity and the evolution of diadromous fish migration. Science 239:1291–1293

Hansen RA, Eversole AG (1984) Age, growth and sex ratio of American eels in brackish-water portions of a South Carolina river. Trans Am Fish Soc 113:744–749

Helfman GS, Bozeman EL, Brothers EB (1984a) Comparison of American eel growth rates from tag returns and length-age analyses. Fish Bull 82:519–522

Helfman GS, Bozeman EL, Brothers EB (1984b) Size, age and sex of American eels in a Georgia river. Trans Am Fish Soc 113:132–141

Helfman GS, Facey DE, Hales LS, Bozeman EL (1987) Reproductive ecology of the American eel. Am Fish Soc Symp 1:42–56

Hesslein RH, Capel MJ, Fox DE, Hallard KA (1991) Stable isotopes of sulfur, carbon, and nitrogen as indicators of trophic level and fish migration in the lower Mackenzie River basin, Canada. Can J Fish Aquat Sci 48:2258–2265

Hesslein RH, Hallard KA, Ramlal P (1993) Replacement of sulfur, carbon, and nitrogen in tissues of growing broad whitefish (*Coregonus onus*) in response to a change in diet traced by δ^{34}S, δ^{13}C, and δ^{15}N. Can J Fish Aquat Sci 50:2071–2076

Houlihan DF, Carter CG, McCarthy ID (1995) Protein synthesis in fish. In: Hochachka P, Mommsen T (eds) Biochemistry and molecular biology of fishes, vol 4. Elsevier, Amsterdam, pp 191–220

Jessop BM, Shiao J-C, Iizuka Y, Tzeng WN (2002) Migratory behaviour and habitat use by American eels *Anguilla rostrata* as revealed by otolith microchemistry. Mar Ecol Prog Ser 233:217–229

Jessop BM, Shiao JC, Iizuka Y, Tzeng WN (2004) Variation in the annual growth, by sex and migration history, of silver American eels *Anguilla rostrata*. Mar Ecol Prog Ser 272:231–244

Jones IJ, Waldron S (2003) Combined stable isotope and gut contents analysis of food webs in plant-dominated, shallow lakes. Freshwat Biol 48:1396–1407

Kline TC, Wilson WJ, Goering JJ (1998) Natural isotope indicators of fish migration at Prudhoe Bay, Alaska. Can J Fish Aquat Sci 55:1494–1502

Litvin SY, Weinstein MP (2004) Multivariate analysis of stable-isotope ratios to infer movements and utilization of estuarine organic matter by juvenile weakfish (*Cynoscion regalis*). Can J Fish Aquat Sci 61:1851–1861

Lucas MC, Baras E (2000) Methods for studying spatial behaviour of freshwater fishes in the natural environment. Fish Fish 1:283–316

McCarthy ID, Waldron S (2000) Identifying migratory *Salmo trutta* using carbon and nitrogen stable isotope ratios. Rapid Commun Mass Spectrom 14:1325–1331

McCarthy TK, Cullen P, O'Conner W (1999) The biology and management of River Shannon eel populations. Fish Bull (Dub) 17:9–20

McConnaughey T (1978) Ecosytems naturally labelled with carbon-13: applications to the study of food webs. M.Sc Thesis, University of Alaska

McConnaughey T, McRoy CP (1979) Food-web structure and the fractionation of carbon isotopes in the Bering Sea. Mar Biol 53:257–262

Moriarty C, Dekker W (1997) Management of the European eel. Fish Bull (Dub) 15:1–110

Morrison WE, Secor DH (2003) Demographic attributes of yellow-phase American eels (*Anguilla rostrata*) in the Hudson River estuary. Can J Fish Aquat Sci 60:1487–1501

Morrison WE, Secor DH, Piccoli PM (2003) Estuarine habitat use by Hudson River American eels as determined by otolith strontium:calcium ratios. In: Dixon DA (ed) Biology, management, and protection of catadromous eels. American Fisheries Society, Bethesda, pp 87–100

Oliveira K, McCleave JD (2002) Sexually different growth histories of the American eel in four rivers in Maine. Trans Am Fish Soc 131:203–211

Oliveira K, McCleave JD, Wippelhauser GS (2001) Regional variation and effect of lake:river area on sex distribution of American eels. J Fish Biol 58:943–952

Overman NC, Parrish DL (2001) Stable isotope composition of walleye: ^{15}N accumulation and area-specific differences in δ^{13}C. Can J Fish Aquat Sci 58:1253–1260

Owen SF (2001) Meeting energy budgets by modulation of behaviour and physiology in the eel (*Anguilla anguilla* L.). Comp Biochem Physiol A 128:631–644

Penczak T (1985) Phosphorus, nitrogen and carbon cycling by fish populations in two small lowland rivers in Poland. Hydrobiologia 120:159–165

Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies. Annu Rev Ecol Syst 18:293–320

Poole WR (1990) Summer fyke nets as a method of eel capture in a salmonid fishery. Aquacult Fish Manage 21:259–262

Poole WR, Reynolds JD (1996) Age and growth of yellow eel, *Anguilla anguilla* (L.), determined by two different methods. Ecol Freshwat Fish 5:86–95

Post DM (2002) Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83:703–718

Rounick JS, Hicks BJ (1985) The stable carbon isotope ratios of fish and their invertebrate prey in four New Zealand rivers. Freshwat Biol 15:207–214

Salonen K, Sarvala J, Hakala I, Viljanen M-L (1976) The relation of energy and organic carbon in aquatic invertebrates. Limnol Oceanogr 21:724–730

Secor DH (1999) Specifying divergent migrations in the concept of stock: the contingent hypothesis. Fish Res 43:13–34

Secor DH, Henderson-Arzapalo A, Piccoli PM (1995) Can otolith microchemistry chart patterns of migration and habitat utilization in anadromous fishes? J Exp Mar Biol Ecol 192:15–33

Shiao J-C, Iizuka Y, Chang CW, Tzeng WN (2003) Disparities in habitat use and migratory behavior between tropical eel *Anguilla marmorata* and temperate eel *A. japonica* in four Taiwanese rivers. Mar Ecol Prog Ser 261:233–242

Smith RJ, Hobson KA, Koopman HN, Lavigne DM (1996) Distinguishing between populations of fresh- and salt-water harbour seals (*Phoca vitulina*) using stable-isotope ratios and fatty acid profiles. Can J Fish Aquat Sci 53:272–279

Sokal RR, Rohlf FJ (1995) Biometry, 3rd edn. W.H. Freeman & Co., New York

SPSS (1998) Systat 8.0 Statistics. Prentice Hall, New Jersey

Svedäng H, Neumann E, Wickström H (1996) Maturation patterns in female European eel: age and size at the silver eel stage. J Fish Biol 48:342–351

Tabachnick BG, Fidell LS (2001) Using multivariate statistics, 4th edn. Allyn and Bacon, Boston & others

Tanner DK, Brazner JC, Brady VJ (2000) Factors influencing carbon, nitrogen, and phosphorus content of fish from a Lake Superior coastal wetland. Can J Fish Aquat Sci 57:1243–1251

Tatsukawa K (2003) Eel resources in East Asia. In: Aida K, Tsukamoto K, Yamauchi K (eds) Eel biology. Springer-Verlag, Tokyo, pp 293–298

Tesch F-W (2003) The eel, 5th edn. Blackwell, Oxford

Thresher RE (1999) Elemental composition of otoliths as a stock delineator in fishes. Fish Res 43:165–204

Tsukamoto K, Arai T (2001) Facultative catadromy of the eel *Anguilla japonica* between freshwater and seawater habitats. Mar Ecol Prog Ser 220:265–276

Tsukamoto K, Nakai I, Tesch W-V (1998) Do all freshwater eels migrate?. Nature 396:635–636

Tsukamoto K, Aoyama J, Miller MJ (2002) Migration, speciation, and the evolution of diadromy in anguillid eels. Can J Fish Aquat Sci 59:1989–1998

Tzeng WN, Severin KP, Wickström H (1997) Use of otolith microchemistry to investigate the environmental history of European eel *Anguilla anguilla*. Mar Ecol Prog Ser 149:73–81

Tzeng WN, Wang CH, Wickström H, Reizenstein M (2000) Occurrence of the semi-catadromous European eel *Anguilla anguilla* in the Baltic Sea. Mar Biol 137:93–98

Tzeng WN, Iizuka Y, Shiao J-C, Yamada Y, Oka HP (2003) Identification and growth rates comparison of divergent migratory contingents of Japanese eel, (*Anguilla japonica*). Aquaculture 216:77–86