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Japanese eel *Anguilla japonica* do not assimilate nutrition during the oceanic spawning migration: evidence from stable isotope analysis

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ABSTRACT: During 2008 and 2009, a total of 12 adult Japanese eels *Anguilla japonica* were captured in the southern part of the West Mariana Ridge, the presumed spawning area. We compared the stable carbon and nitrogen isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) between the 'Mariana silvers' (terminal phase) and those of yellow and silver eels caught in rivers, lakes and coastal areas of Japan (initial phase). Profiles of stable isotope signatures between the initial and terminal phases were similar; both characteristically had a wide range for $\delta^{13}C$ (–24.9 to –12.0‰ and –20.5 to –11.3‰ for the initial and terminal phases, respectively) and $\delta^{15}N$ (6.5 to 18.4‰ and 9.0 to 18.1‰, respectively). Mesopelagic fishes, including several other anguillid species caught near the West Mariana Ridge, characteristically had a very narrow range of $\delta^{13}C$ (–16.9 to –15.3‰) and a wide but lower range of $\delta^{15}N$ (5.3 to 11.1‰) than the Japanese eels. The very similar profiles in stable isotopic signatures between the initial and terminal phase eels, distinct from those of Mariana mesopelagic fishes, indicate that Japanese eels do not assimilate nutrition from the marine environment during long (ca. 6 mo) spawning migration and retain the initial isotopic values of where they ceased feeding.

KEY WORDS: $\delta^{13}C$ · $\delta^{15}N$ · Starvation · Spawning migration · *Anguilla japonica*

INTRODUCTION

It has been presumed that silver stage freshwater eels of the genus *Anguilla* do not feed during the oceanic migration and are totally dependent on their fat stores to fuel the migration and gonad development (Tsukamoto 2009), since the alimentary tracts of silver eels caught in rivers and coastal areas are morphologically and histologically degenerating (Pankhurst & Sorensen 1984, Durif et al. 2005). Hence, freshwater eels have to accomplish very long oceanic migration without feeding and being reliant on stored reserves: 2000–3000 km for the Japanese and American eels (*Anguilla japonica* and *Anguilla rostrata*, respectively) and 5000–6000 km for the European eel *Anguilla anguilla* (Tsukamoto et al. 2002, Aoyama 2009). However, Svedäng & Wickström (1997) suggested that silver stage eels may arrest maturation and resume feeding during migration, since they observed many silvering female European eels with fat contents that

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were insufficient to reach the Sargasso Sea. Some tagged and released silver stage European eels were recaptured >4 yr later (Westin 1990); regeneration of alimentary tracts and feeding has also been observed in male silver stage eels (Dollerup & Graver 1985). On the other hand, Van Ginneken & Van den Thillart (2000) showed that the energy cost for the European eels to reach the Sargasso Sea may theoretically be lower than expected. In fact, laboratory experiments successfully demonstrated that European eels are capable of swimming over a distance of 5500 km without resting and feeding (Van Ginneken et al. 2005, 2007). Yet, the swim trial alone does not assure absence of feeding during the spawning migration, and it would be inevitable that freshwater eels caught in the spawning area would be analyzed too.

Research cruises were performed in June and August 2008 and June 2009, and a total of 12 adult Japanese eels (6 males and 6 females) were captured by a large mid-water trawl net in the southern part of the West Mariana Ridge (Chow et al. 2009, H. Kurogi unpubl. data), the supposed spawning area (Tsukamoto et al. 2003, Tsukamoto 2006). We compared the stable carbon and nitrogen isotope ratios ($\delta^{13}C$ and $\delta^{15}N$, indicators of food source) between these 'Mariana silvers' (terminal phase) and the Japanese eels at yellow and silver stages collected in rivers, lakes and coastal areas of Japan (initial phase) to ascertain the presence/absence of feeding during the reproductive migration.

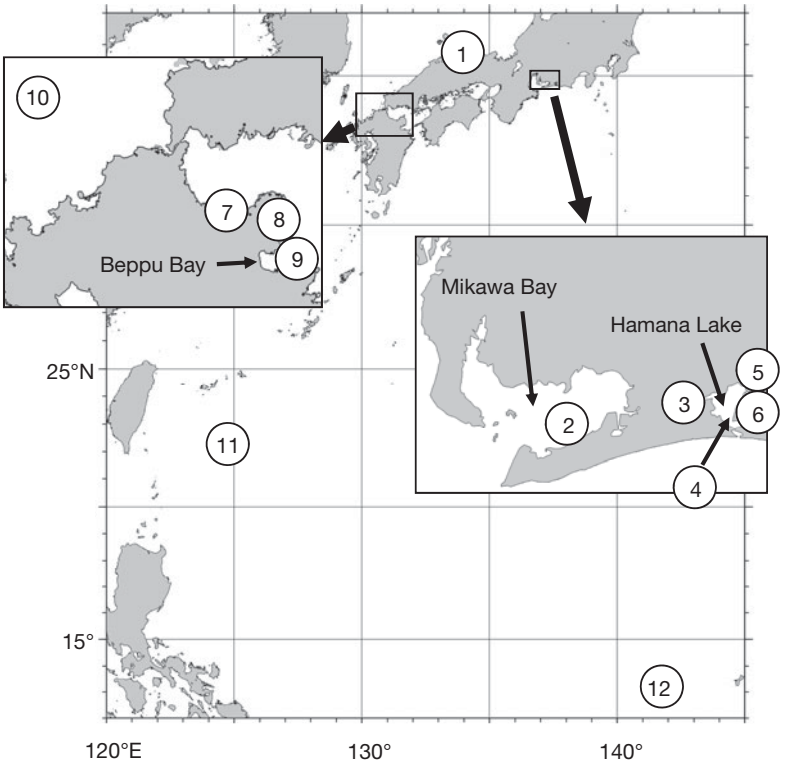

Fig 1. Locations of fish samples collected. (1) Koyama River, (2) Mikawa Bay, (3) Irideohta River, (4) Hamana Lake, (5) Miyakoda River, (6) Sanaru Lake, (7) Yakkan River, (8) Aki River, (9) Beppu Bay, (10) Okinoshima Is., (11) northwest Pacific (26° 00′ N and 126° 30′–127° 00′ E), (12) southern part of the West Mariana Ridge (see Chow et al. 2009)

MATERIALS AND METHODS

Fish samples. The catch localities and biological data of the fish samples used in the present study are presented in Fig. 1 and Table 1. A total of 72 adult Japanese eels *Anguilla japonica* at the initial phase were collected from 10 locales in Japan (Fig. 1, positions 1–10), which were classified into 37 yellow and 35 silver stage eels (Okamura et al. 2007). All initial phase eels (IPE) were adult (60.5 ± 13.0cm in body length) and were either caught downstream and in river estuaries, or in coastal lakes and other coastal areas. Leptocephali of Japanese eel (AJL) and a conger eel (*Conger* sp.: CON) were collected in the south of the Ryukyu Archipelago (Fig. 1, position 11). Terminal phase silver eels (TPE) were caught in the southern part of the West Mariana Ridge (Fig. 1, position 12). Body length of these 12 TPE ranged from 44.7 to 76.7 cm (mean of 67.4 ± 9.3 cm for female, n = 6; 52.2 ± 7.2cm for male, n = 6). Juveniles and adults of 8 mesopelagic fishes, *Derichthys* sp. (longneck eel: KUB), *Dolichosudis fuliginosa* (barracudina: HSN), *Emmelichthys struhsakeri* (rover: RSC), *Lepidocybium flavobrunneum* (escolar: ABS), *Nemichthys* sp. (snipe eel: SIG), *Ruvettus pretiosus* (oilfish: BRM), *Scombrolabrax heterolepis* (longfin escolar: MKK) and *Serrivomer* sp. (sawtooth eel: SERA) and leptocephali of a sawtooth eel (*Serrivomer* sp.: SERL), were collected where the TPE were caught (Fig. 1, position 12).

Stable isotope analysis. Dorsal muscle tissues dissected from the central part of the body were lyophilized and homogenized to a fine powder, and then defatted using 2:1 or 1:1 chloroform-methanol solution (v/v) and centrifugation (Folch et al. 1957). Four IPE samples (IPE3–6) of Hamana Lake system were defatted using 2:1 chloroform-methanol solution, and the other samples using 1:1 solution (v/v). The defatted samples were oven-dried and sample aliquots (ca. 0.8 mg) were placed in tin containers. $\delta^{13}C$ and $\delta^{15}N$ were analyzed using an EA-1108 elemental analyzer (Carlo Erba) coupled with an isotope ratio mass spectrometer (Finnigan Mat ConFlo II, Mat 252). The

Table 1. Collection data of freshwater eel *Anguilla japonica* and other marine fish species used in this study. Catch locality: see Fig. 1 for more specific location. Parentheses: no. of ind. at silver (s) or yellow (y) stages; (–) no information available

Species	ID	Catch locality	Fig. 1	Stage	Date	n	Body length (cm ± SD)	Sex
Anguilliformes								
Anguilla japonica	IPE1	Koyama River	1	Adult (1s, 3y)	Jun 2007, 2008 Nov 2008	4	66.1 ± 15.4	–
	IPE2	Mikawa Bay	2	Adult (1s)	Dec 2008	1	69.0	1♀
	IPE3	Irideohta River	3	Adult (7y)	Sep, Nov 2007	7	61.0 ± 5.7	5♀, 2♂
	IPE4	Hamana Lake	4	Adult (2s, 4y)	Nov 2007	6	57.5 ± 6.7	5♀, 1♂
	IPE5	Miyakoda River	5	Adult (11s, 9y)	Sep, Nov 2007	20	70.5 ± 7.9	18♀, 2♂
	IPE6	Sanaru Lake	6	Adult (4y)	Sep, Nov 2007	4	66.8 ± 2.7	4♀
	IPE7	Yakkan River	7	Adult (11s, 10y)	Oct–Dec 2008	21	49.0 ± 13.8	–
	IPE8	Aki River	8	Adult (3s)	Oct, Dec 2008	3	52.0 ± 7.6	–
	IPE9	Beppu Bay	9	Adult (3s)	Dec 2008	3	63.4 ± 2.2	3♀
	IPE10	Okinoshima Isl.	10	Adult (3s)	Dec 2008	3	69.9 ± 8.0	3♀
	AJL	NW Pacific[a]	11	Larva	Oct 2005	5	5.0 ± 0.4	–
	TPE	WMR[b]	12	Adult (12s)	Jun, Aug 2008 Jun 2009	12	52.9 ± 14.4	6♀, 6♂
Conger sp.	CON	NW Pacific[a]	11	Larva	Oct 2005	4	3.4 ± 1.4	–
Derichthys sp.	KUB	WMR[b]	12	Adult	Aug 2008	3	29.6 ± 7.3	–
Nemichthys sp.	SIG	WMR[b]	12	Adult	Aug 2008	3	65.4 ± 6.4	–
Serrivomer sp.	SERA	WMR[b]	12	Adult	Aug 2008	3	39.9 ± 16.1	–
Serrivomer sp.	SERL	WMR[b]	12	Larva	Aug 2008	2	4.6 ± 2.2	–
Aulopiformes								
Dolichosudis fuliginosa	HSN	WMR[b]	12	Adult	Jun 2008	5	30.7 ± 2.8	–
Perciformes								
Emmelichthys struhsakeri	RSC	WMR[b]	12	Juvenile	Jun 2008	4	9.7 ± 1.8	–
Lepidocybium flavobrunneum	ABS	WMR[b]	12	Juvenile	Jun 2008	8	12.2 ± 6.4	–
Ruvettus pretiosus	BRM	WMR[b]	12	Juvenile	Jun 2008	2	13.8 ± 4.0	–
Scombrolabrax heterolepis	MKK	WMR[b]	12	Juvenile	Jun 2008	7	11.8 ± 3.6	–

[a]26° 00′ N and 126° 30′–127° 00′ E
[b]13° N, 142° E (see Chow et al. 2009)

isotope ratios were expressed as per mille (‰) deviation from international standard (i.e. the Vienna Pee Dee Belemnite for carbon and air N_2 for nitrogen), in which $\delta^{13}C$ or $\delta^{15}N = (R_{sample}/R_{standard} - 1) \times 1000$, where R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$. Instrumental precision was 0.2‰. The Mann-Whitney *U*-test was used to compare the $\delta^{13}C$ and $\delta^{15}N$ values between 2 groups such as yellow and silver stage eels. Kruskal-Wallis nonparametric procedure was used to investigate the overall heterogeneity in the $\delta^{13}C$ and $\delta^{15}N$ values among samples of the Japanese eel and among species. *t*-statistics for significance were used to test Pearson's correlation coefficient (r) between body length of fish samples and $\delta^{15}N$ values; p-values <0.05 were considered statistically significant.

RESULTS

Stable isotope signatures of the Japanese eel

Dual plots of $\delta^{13}C$ and $\delta^{15}N$ values of IPE and TPE individuals are shown in Fig. 2, and the summary of mean values are presented in Table 2. Japanese eels are characterized by large variation in both isotope ratios within and among samples. $\delta^{13}C$ values of IPE (n = 72) ranged from –24.9 to –12.0‰ (mean of –17.0 ± 3.4‰ SD), and $\delta^{15}N$ ranged from 6.5 to 18.4‰ (mean of 13.3 ± 2.1‰). $\delta^{15}N$ values were independent of the body length in IPE (r = 0.078, p > 0.1). A weak but significant

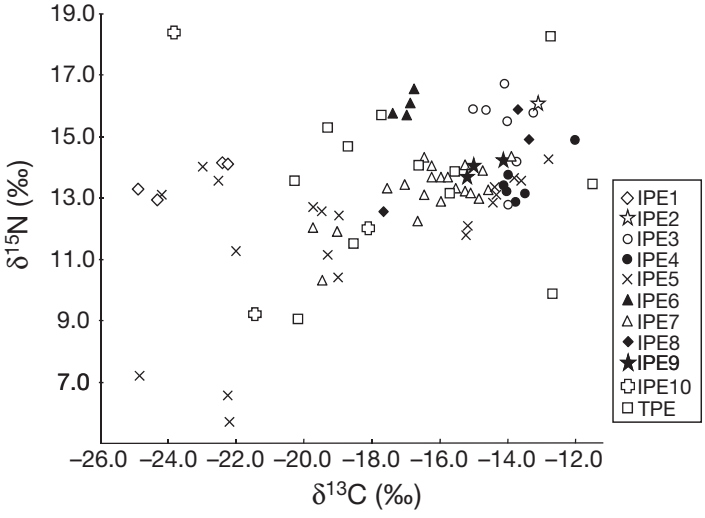

Fig. 2. *Anguilla japonica*. Dual plots of $\delta^{13}C$ and $\delta^{15}N$ values of the initial phase eels (IPE) from 10 local samples and terminal phase eels (TPE). See Table 1 for the abbreviated locality ID

Table 2. *Anguilla japonica*. Summary of mean values (±SD) of $\delta^{13}C$ and $\delta^{15}N$ values in adult Japanese eel samples. See Table 1 for ID information

ID	n	$\delta^{13}C$	$\delta^{15}N$
IPE[a]	72	−17.0 ± 3.4	13.3 ± 2.1
yellow	37	−16.2 ± 3.3	13.6 ± 1.9
silver	35	−17.7 ± 3.5	13.0 ± 2.3
♀	41	−17.2 ± 3.7	13.4 ± 2.6
♂	5	−14.3 ± 0.6	13.2 ± 1.4
IPE1	4	−23.4 ± 1.3	13.6 ± 0.6
IPE2	1	−13.1	16.1
IPE3	7	−14.1 ± 0.6	15.2 ± 1.3
IPE4	6	−13.6 ± 0.8	13.5 ± 0.7
IPE5	20	−18.6 ± 4.0	11.8 ± 2.5
IPE6	4	−17.0 ± 0.3	16.0 ± 0.4
IPE7	21	−16.3 ± 1.6	13.2 ± 0.9
IPE8	3	−14.9 ± 2.4	14.5 ± 1.7
IPE9	3	−14.8 ± 0.6	14.0 ± 0.3
IPE10	3	−21.1 ± 2.8	13.3 ± 4.7
TPE	12	−16.6 ± 3.1	13.4 ± 2.4
♀	6	−16.4 ± 3.7	13.9 ± 3.0
♂	6	−16.9 ± 2.6	12.9 ± 1.8

[a]All IPE samples were pooled

difference was observed in $\delta^{13}C$ between yellow (−16.2 ± 3.3, n = 37) and silver (−17.7 ± 3.5, n = 35) stages ($p = 0.044$), while differences in both $\delta^{13}C$ and $\delta^{15}N$ values between 41 females and 5 males were not significant ($p > 0.12$). Significant heterogeneity was detected in both $\delta^{13}C$ and $\delta^{15}N$ values among 9 local samples (IPE1, IPE3–10: $p < 0.001$) and even among closely located 4 samples from Hamana Lake system (IPE3–6: $p < 0.002$). Heterogeneous $\delta^{13}C$ values and the large variation within some samples indicate that the carbon pool system is heterogeneous among localities; some samples consisted of individuals from a number of different carbon pool systems. TPE (n = 12) also showed large variations in $\delta^{13}C$ and $\delta^{15}N$ values, ranging from −20.5 to −11.3‰ (mean of −16.6 ± 3.1‰) and 9 to 18.1‰ (mean of 13.4 ± 2.4‰), respectively. No significant difference was observed in both $\delta^{13}C$ and $\delta^{15}N$ values between sexes of TPE ($p > 0.4$). Comparison among yellow and silver stages of IPE and TPE detected no significant difference in both $\delta^{13}C$ and $\delta^{15}N$ values ($p > 0.12$).

Stable isotope ratios of mesopelagic fishes

Dual plots of $\delta^{13}C$ and $\delta^{15}N$ values of 10 marine fishes are shown in Fig. 3, and the summary of mean values are presented in Table 3. A very narrow range of $\delta^{13}C$ values (−17.6 to −15.3‰) was observed in 8 species of juvenile and adult mesopelagic fish (including 3 anguillid species), indicating that they belong to the same carbon pool system. The variance of $\delta^{13}C$ values in these mesopelagic fishes was considerably smaller than that in TPE (*F*-test, $p < 0.001$). On the other hand, $\delta^{15}N$ values varied largely (5.2–12.6‰) among the mesopelagic species, and positive enrichment with body length was observed within species such as longneck eel (KUB: $r = 0.976$, $p > 0.1$),

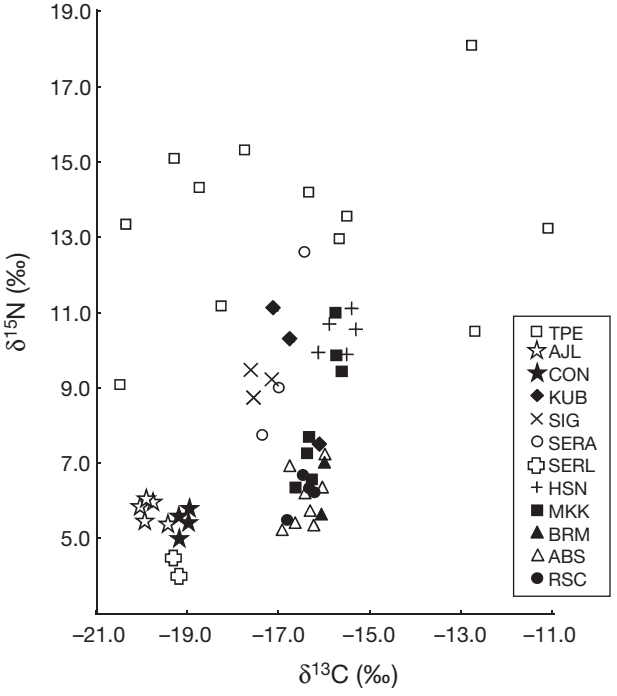

Fig. 3. Dual plots of $\delta^{13}C$ and $\delta^{15}N$ values of 10 marine fishes. See Table 1 for the abbreviated species ID

Table 3. Summary of mean values (± SD) of $\delta^{13}C$ and $\delta^{15}N$ data in 10 marine fish species used in this study. See Table 1 for the location information

Fish species	ID	Stage	n	$\delta^{13}C$	$\delta^{15}N$
Anguilliformes					
Anguilla japonica	AJL	Larva	5	−19.8 ± 0.2	5.7 ± 0.3
Conger sp.	CON	Larva	4	−19.1 ± 0.1	5.4 ± 0.3
Derichthys sp.	KUB	Adult	3	−16.6 ± 0.5	9.7 ± 1.9
Nemichthys sp.	SIG	Adult	3	−17.4 ± 0.2	9.2 ± 0.4
Serrivomer sp.	SERA	Adult	3	−16.9 ± 0.5	9.8 ± 2.5
Serrivomer sp.	SERL	Larva	3	−19.2 ± 0.1	4.2 ± 0.3
Aulopiformes					
Dolichosudis fuliginosa	HSN	Adult	5	−15.7 ± 0.4	10.4 ± 0.5
Perciformes					
Emmelichthys struhsakeri	RSC	Juvenile	4	−16.5 ± 0.2	6.1 ± 0.5
Lepidocybium flavobrunneum	ABS	Juvenile	8	−16.4 ± 0.3	6.0 ± 0.7
Ruvettus pretiosus	BRM	Juvenile	2	−16.0 ± 0.0	6.3 ± 1.0
Scombrolabrax heterolepis	MKK	Juvenile	7	−16.1 ± 0.4	8.3 ± 1.8

sawtooth eel (SERA: r = 0.941, p > 0.1) and longfin escolar (MKK: r = 0.971, p < 0.001), indicating different trophic levels by species and developmental stages. Anguillid leptocephali (AJL, CON and SERL) represented a further depleted and narrow range of $\delta^{13}C$ (–20.0 to –19.0‰) and $\delta^{15}N$ (4.0–6.0‰) values. Therefore, mesopelagic species at juvenile and larval stages and those showing positive enrichment of $\delta^{15}N$ with body length were not included in subsequent statistic analysis, and significant heterogeneity among HSN, SIG and TPE was observed (p = 0.007).

DISCUSSION

Harrod et al. (2005) found much more depleted $\delta^{13}C$ values (–23.6‰ average) in European eels (> 30cm in body length) collected in the freshwater habitat than those in the marine habitat (–16.3‰ average). Likewise, Bardonnet & Riera (2005) observed a significant shift of isotopic signatures from marine glass eels to river pigmented eels, where the pigmented eels, after starting to assimilate terrigenous organic matter sources, were observed to have lower $\delta^{13}C$ (–26.7 to –22.7‰) than the marine glass eels (–21.4 ± 0.3‰). Thus, the large $\delta^{13}C$ variation of IPE observed in the present study may correspond to the relative extent of assimilation of marine and terrigenous organic matter. The silver stage IPE with depleted $\delta^{13}C$ may have ceased feeding in the freshwater habitat; those with elevated $\delta^{13}C$ did so in habitats with greater marine influence (i.e. in estuarine or coastal areas).

Based on the stomach content analysis in shortfin eel *Anguilla australis* of Lake Ellesmere, Ryan (1986) observed that smaller eels (≤40 cm) fed primarily on invertebrates and became progressively more piscivorous as they grew; large eels (> 50.1 cm) were almost entirely piscivorous. Kelly & Jellyman (2007) observed a similar diet shift in shortfin eel, with progressive enrichment of the $\delta^{15}N$ value. Since no such correlation between body length and $\delta^{15}N$ value was observed in the present study, the individual eels used may already have been too large to influence the $\delta^{15}N$ value any further. The large $\delta^{15}N$ variation in IPE indicates that the trophic level of eels may vary among localities and that individual eels may consume diverse food sources within a locality. Although $\delta^{15}N$ of TPE was higher than that of adult mesopelagic species and comparable with values reported for sharks, swordfish and tunas (Estrada et al. 2005, MacNeil et al. 2005, Ménard et al. 2007), it is unlikely that TPE share a similar trophic level with these top marine predators.

The large variation observed in both isotope ratios of IPE appears to be inherited by TPE. If the silver eels have assimilated marine nutrition, isotopic signatures of TPE would have markedly shifted downward in $\delta^{15}N$ and been skewed in $\delta^{13}C$. Yet, isotopic fractionation and turnover rates may vary considerably among species (Suring & Wing 2009), and turnover rates of migrating eels may be low as suggested by the low metabolic rate experimentally indicated by Van Ginneken et al. (2005). However, Suring & Wing (2009) observed isotope turnover in blue cod *Parapercis colias* to be comparable with other fish species, although the growth rate of the blue cod was much slower than the fish used in previous studies. Furthermore, the long oceanic spawning migration required about half a year for the Japanese eel (Tsukamoto et al. 2003) which would be enough to yield a significant shift in the isotopic signature between IPE and TPE. Thus, the almost identical profiles in the stable isotopic values between IPE and TPE indicate that the Japanese eels during the spawning migration do not assimilate marine food sources, keeping the initial isotopic signatures obtained at the habitat where they ceased feeding.

Investigating otolith strontium and calcium (Sr:Ca) ratios, Tsukamoto et al. (1998) suggested that most silver stage Japanese eels caught in the coastal areas of Japan were 'sea eels' (spending most of their life in the sea without entering freshwater areas). Subsequently using a larger sample size, Tsukamoto & Arai (2001) and Chino & Arai (2009) observed 3 types of silver eels; 'sea eels', 'estuarine eels' (inhabiting estuaries or switching between different habitats), and 'river eels' (remaining in freshwater river habitats after arrival in the estuary), in which they found 'estuarine eels' to be the most predominated type (> 50%) followed by 'sea eels' and 'river eels'. These results derived from Sr:Ca data may be corroborated by our $\delta^{13}C$ data showing that many of our silver eels had intermediate $\delta^{13}C$ value (Fig. 4). Furthermore, the similarly scattered $\delta^{13}C$ data profiles of IPE and TPE indicate that eels from different habitats are successfully contributing to the reproduction.

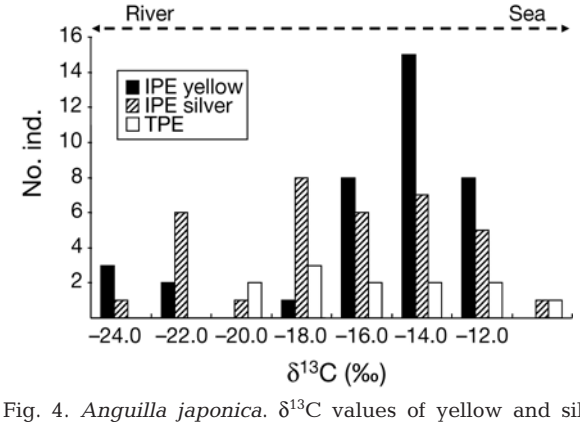

Fig. 4. *Anguilla japonica*. $\delta^{13}C$ values of yellow and silver stages of initial (IPE) and terminal phase eels (TPE)

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