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## **Migratory life history of European eel *Anguilla anguilla* from freshwater regions of the River Asi, southern Turkey and their high otolith Sr:Ca ratios**

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Otolith Sr:Ca ratios from 32 of 34 European eel *Anguilla anguilla* collected from three freshwater sites in the River Asi, southern Turkey, indicated that they were resident in fresh water without apparent exposure to salt water since the elver stage. The Sr:Ca ratio criterion indicative of residence in fresh water was more than twice that of values from other European countries. Otolith Sr:Ca ratios of *A. anguilla* from fresh waters can vary among regions, possibly reflecting regional-specific water chemistry. Hence, the use of Sr:Ca ratios determined in one region to interpret results from a different region might lead to misclassification of migratory life-history types. © 2011 The Authors

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Key words: freshwater residence; life-history types; water chemistry.

### **INTRODUCTION**

The European eel *Anguilla anguilla* (L.) is a catadromous species widely distributed in Europe (Dekker, 2003; Tesch, 2003). It is also found in Turkish rivers and streams draining into the Mediterranean Sea, Aegean Sea and parts of the Black Sea (Küçük *et al.*, 2005; Yalçın-Özdilek *et al.*, 2006; Yalçın-Özdilek & Solak, 2007). The otolith Sr:Ca ratio is a powerful tool to distinguish the migratory life histories of fishes moving between freshwater and saltwater habitats (Campana, 1999). It has been used to reveal the complicated migratory life histories of *A. anguilla* in the yellow eel stage in many European countries (Tzeng *et al.*, 1997, 2000; Arai *et al.*, 2006; Daverat & Tomás, 2006; Shiao *et al.*, 2006; Lin *et al.*, 2009).

It is unclear, however, whether the migratory life histories of *A. anguilla* in Turkish waters, the eastern limit of the species distribution, are similar to those from other countries. To classify the migratory life histories of *A. anguilla*, an otolith Sr:Ca ratio indicative of residence in fresh water, brackish water or sea water is needed.

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Extrapolating the criterion ratio from one region to another may, however, not be appropriate because of differences between regions in water chemistry, as found for the American eel *Anguilla rostrata* (Lesueur) (Jessop *et al.*, 2008). Therefore, the objectives of this study were: (1) to identify the otolith Sr:Ca ratio indicative of residence in fresh water using *A. anguilla* collected from three freshwater sites in the River Asi in southern Turkey, (2) to examine their migratory life histories and (3) to compare the otolith Sr:Ca ratios used to classify migratory history types of *A. anguilla* among European regions.

## MATERIALS AND METHODS

The River Asi (named Orontes in Syria) is *c.* 380 km in length (94 km in Turkey) and originates in central Lebanon and drains into the Mediterranean Sea at Samandağ (Hatay) in Turkey (36° 02' N; 35° 57' E). Annual precipitation is 400–800 mm, but rainfall is restricted to the rainy season from October to May, when the water temperature can drop to *c.* 16° C. In the dry season (July to August), total precipitation is <50 mm with water temperatures varying around 27° C (Yılmaz & Doğan, 2008; Hatay Directorate of Agriculture, 2003; unpubl. data). The width and depth of the river change considerably with the season. In the rainy season, the depth of the river may increase to over 2.5 m at a width of 35–45 m while in the hot and arid summer the depth is <1 m, with the riverbed often drying up completely. Some small freshwater streams feed into the lower Asi from the Ansariye Mountain, such that the mixing region of fresh water and sea water is restricted to <1 km upstream of the river mouth (S. Yalçın-Özdilek, pers. obs.). Within the delta of the River Asi, *A. anguilla* is one of the most important commercial species for local consumption or export (Yalçın, 1997).

A total of 34 yellow eels were collected from Demirköprü on 10 April and from Güzelburç and Samandağ on 10 July in 2007 (Fig. 1). Samples were collected by local fishermen using drift nets in Demirköprü and fyke nets in Güzelburç and Samandağ. The mean  $\pm$  s.d. electrical conductivity was  $81.8 \pm 7.0$ ,  $77.1 \pm 6.6$  and  $68.6 \pm 4.8$  mS m<sup>-1</sup> in Demirköprü, Güzelburç and Samandağ, respectively (Taşdemir & Göksu, 2001), corresponding to a salinity of <0.5 (Anderson & Cummings, 1999). Therefore, these sampling sites were all in fresh water.

The total length ( $L_T$ ) of *A. anguilla* was measured to the nearest cm and mass ( $M_T$ ) to the nearest g. Sexes were determined by macroscopic examination (Tesch, 2003). The largest otolith (sagitta) was removed, air dried, embedded in Epofix resin (www.emsdiasum.com), then ground and polished until the core was exposed. The Sr:Ca ratios of the polished otoliths were measured by an electron probe microanalyser (EPMA, JEOL JXA-8900R; www.jeol.com) with a spot size of 10  $\mu$ m and interval of 10  $\mu$ m, as detailed in Lin *et al.* (2009). The time represented by one spot might range from several days to perhaps months, depending on the distance from the core.

Fish otoliths are deposited daily (Campana & Thorrold, 2001), and thus the otolith edge is deposited on the day when the fish are caught. In other words, the nearer the measuring points are to the otolith edge, the better these points represent the water chemistry of the collection site. As the *A. anguilla* were all collected in freshwater habitats and the otolith Sr:Ca ratios were measured from the primordium to the edge, the last three points were used to calculate the value indicative of residence in fresh water.

Otolith Sr:Ca ratios were measured consecutively, meaning that the last three points were not independent and should be compared by repeated measure ANOVA (Elsdon *et al.*, 2008). The ratios were not significantly affected by site, sex or site–sex interaction (all  $P > 0.5$ ), and so the results were pooled. The mean  $\pm 2$  s.d. of the otolith Sr:Ca ratio from the last three points was used as the criterion for residence in fresh water following Shiao *et al.* (2006) and Tabouret *et al.* (2010). Sr:Ca ratios with two consecutive points above this value were considered as indicative of saltwater experience (Jessop *et al.*, 2008). All computations were completed in R (version 2.8.1) (www.R-project.org). The significance level ( $\alpha$ ) was set at 0.05.

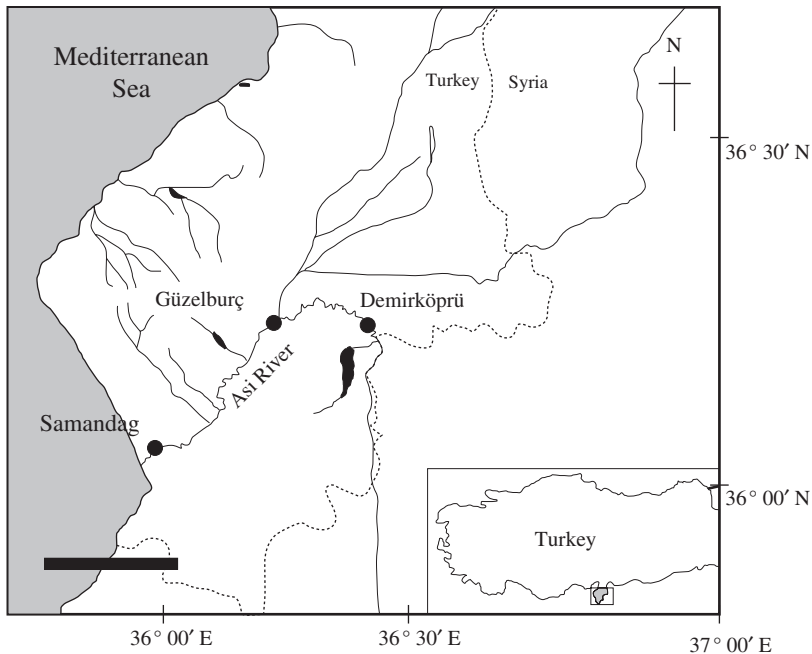


FIG. 1. Sampling sites (Samandağ, Güzelburç and Demirköprü) for *Anguilla anguilla* in the River Asi, southern Turkey. Scale bar = 10 km.

## RESULTS

A total of 34 yellow *A. anguilla*, including 14 females, 15 males and five individuals with undeveloped gonads, were collected from three sites in the River Asi (Table I). The otolith Sr:Ca ratios from the primordium to the edge were similar among individuals from the three sampling sites (Fig. 2). The ratios ranged from  $9$  to  $11 \times 10^{-3}$  in the primordium, increased considerably and reached a peak of  $17$  to  $19 \times 10^{-3}$  at a distance from the primordium of *c.*  $100 \mu\text{m}$ , corresponding to the onset of metamorphosis from leptocephalus to glass eel. After this peak, the ratios decreased drastically, varying between  $2$  and  $3 \times 10^{-3}$  near  $200 \mu\text{m}$ , corresponding

TABLE I. Sample sizes (*n*), means  $\pm$  s.d. and ranges (in parentheses) of total length ( $L_T$ ) and mass ( $M_T$ ) of *Anguilla anguilla* collected from Demirköprü, Güzelburç and Samandağ in the River Asi, Turkey by sex: females (F), males (M) and fish with undeveloped gonads (U)

Location	Demirköprü		Güzelburç		Samandağ	
	F	F	M	F	M	U
<i>n</i>	10	3	3	1	12	5
$L_T$ (mm)	$45.7 \pm 7.3$ (39.2–60.0)	$56.5 \pm 7.4$ (52.2–65.0)	$38.8 \pm 3.2$ (36.7–42.4)	$45.5$ (34.8–40.5)	$37.4 \pm 2.1$ (33.0–40.2)	$35.8 \pm 3.2$ (33.0–40.2)
$M_T$ (g)	$113.7 \pm 57.5$ (66.2–228.8)	$439.7 \pm 198.2$ (308.0–667.6)	$134.5 \pm 27.7$ (116.1–166.4)	$171.6$ (81.2–139.5)	$108.3 \pm 18.5$ (81.2–139.5)	$96.2 \pm 42.4$ (58.3–160.9)

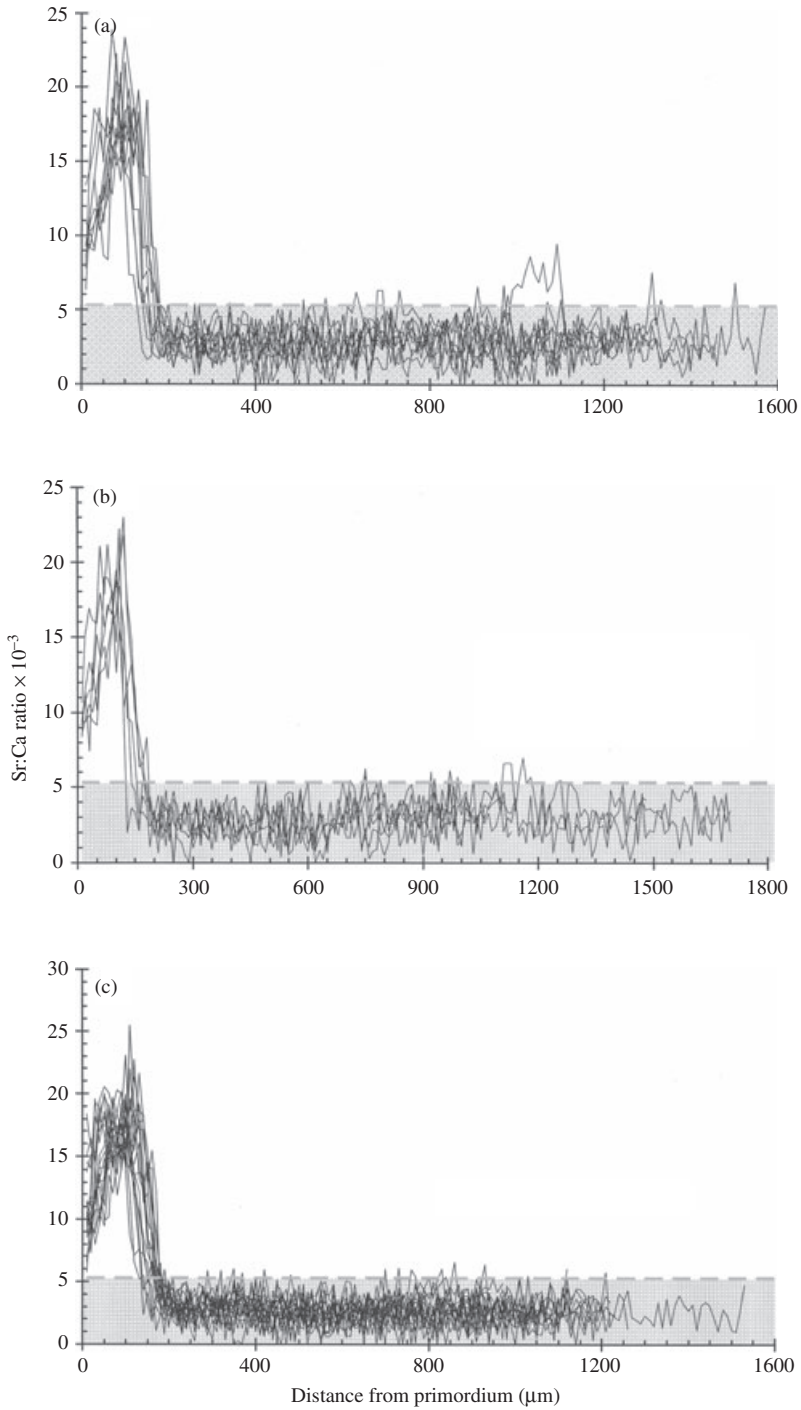


FIG. 2. Otolith Sr:Ca ratios ( $\times 10^{-3}$ ) of *Anguilla anguilla* collected from (a) Demirköprü, (b) Güzelburç and (c) Samandığ. ----, the mean + 2 s.d. of the otolith Sr:Ca ratios of the last three points ( $5.29 \times 10^{-3}$ ), indicating residence in fresh water (grey region).

to the end of metamorphosis. After metamorphosis, the otolith Sr:Ca ratios fluctuated around  $3 \times 10^{-3}$ , and ranged from 1 to  $5 \times 10^{-3}$ . The mean  $\pm$  s.d. of the last three points is  $2.85 \pm 1.22 \times 10^{-3}$  and the corresponding mean  $+2$  s.d. value is  $5.29 \times 10^{-3}$ . Therefore, two consecutive points with ratios  $>5.29 \times 10^{-3}$  were considered as saltwater exposure.

After becoming glass eels, the otolith Sr:Ca ratios of collected individuals were generally  $<5.29 \times 10^{-3}$  except that one individual from Demirköprü had ratios  $>5.29 \times 10^{-3}$  at an otolith radius *c.* 1000–1100  $\mu\text{m}$  [Fig. 2(a)], and one in Güzelburç had two points  $>5.29 \times 10^{-3}$  at an otolith radius *c.* 1100  $\mu\text{m}$  [Fig. 2(b)]. Therefore, all but two individuals from three sampling sites in the River Asi spent their entire yellow eel stage in fresh water and did not return to salt water after they recruited to the River Asi. For the two individuals having a short period of saltwater experience, they had lived in fresh water for most of their yellow eel stage.

The mean otolith, s.d. and coefficient of variation of Sr:Ca ratios after the elver stage of *A. anguilla* collected from fresh waters in different published studies and the ratios indicative of residence in freshwater, brackish-water and seawater habitats in different regions are listed in Table II. The mean Sr:Ca ratio of *A. anguilla* from the River Asi in southern Turkey is  $2.79 \times 10^{-3}$ , close to  $2.70 \times 10^{-3}$  for *A. anguilla* from the freshwater Fumemorte Canal in southern France. It is, however, approximately two times higher than that from the Garonne and Dordogne Rivers ( $1.4 \times 10^{-3}$ ) and Adour River ( $1.59 \times 10^{-3}$ ), two to three times higher than from five Irish rivers ( $0.98$ – $1.78 \times 10^{-3}$ ), eight times higher than from Lake Mälaren and Ommen in southern Sweden ( $0.37 \times 10^{-3}$ ), four times higher than from Lake Fardume in Gotland in Sweden ( $0.71 \times 10^{-3}$ ) and also four times higher than from Lake Baluošai in Lithuania ( $0.72 \times 10^{-3}$ ). Consequently, it is reasonable to conclude that the otolith Sr:Ca ratio indicative of residence in fresh water was highest in the River Asi ( $5.29 \times 10^{-3}$ ), followed by that in Lake Baluošai, Lithuania ( $2.24 \times 10^{-3}$ ) and in the Gironde watershed ( $2.0 \times 10^{-3}$ ).

## DISCUSSION

Prolonged residence in fresh water without saltwater experience in anguillids appears to be particularly affected by distance of riverine or lacustrine habitats from the tidal limit, by migration barriers, or related to stocking or escapes of cultured anguillids from farms into upstream reaches (Tesch, 2003; Arai *et al.*, 2006; Shiao *et al.*, 2006; Lin *et al.*, 2010). In this study, the distances from the sampling sites to the river mouth are relatively short (2, 20 and 30 km for Samandağ, Güzelburç and Demirköprü, respectively). There has not been any *A. anguilla* stocking or farming in the Asi catchment and there are no migration barriers, such as dams or hydro-electric power plants, downstream of the riverine sampling sites (S. Yalçın-Özdilek, pers. obs.). It is possible that the sampled *A. anguilla* remained in freshwater habitats with a limited home range (Laffaille *et al.*, 2005; Tabouret *et al.*, 2010). The high salinity of the Mediterranean Sea (Pinet, 2009) or high interspecific competition in the river mouth might inhibit the use of brackish habitats.

Habitat use by *A. anguilla* after the elver stage is facultative and highly variable among different European regions. Nearly all the *A. anguilla* in the present study lived in fresh water, except two that evidently resided in salt water for a short time.

TABLE II. Mean, s.d. and coefficient of variation (c.v.) of otolith Sr:Ca ratios ( $\times 10^{-3}$ ) of *Anguilla anguilla* after elver stage from different European fresh waters. Sr:Ca<sub>Crit</sub> is the criterion Sr:Ca ratio for residence in sea water (SW), brackish water (BW) and fresh water (FW). The methods used to construct criterion Sr:Ca ratios from individuals from freshwater habitats: A, mean  $\pm 2$  s.d. of otolith Sr:Ca ratios of last three measuring points of individuals from freshwater habitats; B, mean  $\pm 2$  s.d. of otolith Sr:Ca ratios of freshwater-resident individuals after the elver stage; C, linear discriminant analysis using the first point after metamorphosis for brackish water and the last measuring point for fresh water; D, from rearing experiment in the laboratory

Location	Mean	s.d.	c.v.	Sr:Ca <sub>Crit</sub>			Method	Reference
				SW	BW	FW		
River Asi, southern Turkey	2.79	1.21	43	>5.29	>5.29	<5.29	A	This study
Adour River, south-western France	1.59	0.32	20	>4.93	2.23–4.93	<2.23	A	Tabouret <i>et al.</i> (2010)*
Lake Baluošai, Lithuania	0.72	0.76	106	>3.23	2.24–3.23	<2.24	B	Shiao <i>et al.</i> (2006)
Fumemorte Canal, Camargue, southern France	2.70	0.48	18				C	J. Panfili, unpubl. data
Garonne and Dordogne Rivers, western France	1.40			>6	2–5	<2	D	Daverat & Tomás (2006)
Five Irish freshwater rivers	0.98–1.78							Arai <i>et al.</i> (2006)
Lake Mälaren and Ommen, southern Sweden	0.37	0.02	5					Tzeng <i>et al.</i> (1997)
Lake Fardume, Sweden	0.71	0.89	126					Tzeng <i>et al.</i> (2000)
Lake Kīšezers, Latvia	0.84	0.77	92					Lin <i>et al.</i> (2009)

\*Otolith Sr:Ca and Ba:Ca ratios are simultaneously used to classify different types of migratory life histories.

More than 85% of *A. anguilla* from the Baltic Sea had at least one brackish-water experience (Tzeng *et al.*, 2000; Shiao *et al.*, 2006), while nearly all individuals from five Irish rivers were resident in fresh water (Arai *et al.*, 2006). Daverat *et al.* (2006) found a gradient of migratory histories in the Gironde River basin of western France where residence in fresh water of *A. anguilla* increased towards the upper reaches of the Gironde River, but the proportion of freshwater residents also increased towards the sea. A gradient of migratory life histories was, however, not found in individuals from three sites in the River Asi, not even for Samandağ. Similarly, no gradient was found in the River Shannon between the upper (Lough Derrevarga), middle (Castleconnell) and lower parts (Killaloe) (Arai *et al.*, 2006), although the upstream and downstream movement of *A. anguilla* in the River Shannon might be affected by power plants (*e.g.* Ardnacrusha power station). The situation in the River Asi can be considered as an exception in relation to gradients in migratory life-history strategies.

It is apparent that the otolith Sr:Ca ratios of *A. anguilla* living in fresh waters can vary substantially between regions. This may be due to region-specific spatial and temporal variation in Sr:Ca concentrations in water (Kraus & Secor, 2004; Munro *et al.*, 2005), different equipment used for measurement of otolith Sr:Ca ratios, water temperatures (Campana, 1999; Elsdon & Gillanders, 2002; Martin *et al.*, 2004) or fish growth rates (Lin *et al.*, 2007).

In a preliminary examination, the Sr:Ca ratios from one of the paired otoliths (sagitta) of *A. anguilla* from the Fumemorte Canal were measured by electron probe microanalyser (EPMA) and the other otolith by laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS). Sr:Ca ratios were not significantly different ( $2.67 \pm 0.59 \times 10^{-3}$  and  $2.72 \pm 0.32 \times 10^{-3}$  for EPMA and LA-ICPMS, respectively,  $P > 0.5$ ) between the two machines (J. Panfili, pers. comm.). Therefore, the observed variation in otolith Sr:Ca ratio among fresh waters was probably not due to different measuring machines, at least not for EPMA and LA-ICPMS.

Water temperature can affect otolith Sr:Ca ratios in fishes (Campana, 1999; Elsdon & Gillanders, 2002; Martin *et al.*, 2004). The water temperature in the River Asi ( $16\text{--}27^\circ\text{C}$ ) and the growth rate of *A. anguilla* ( $90\text{ mm year}^{-1}$ ) are high compared to other European regions (Yalçın-Özdilek *et al.*, 2006; Yılmaz & Doğan, 2008). High water temperature might lead to elevated otolith Sr:Ca ratios (Martin *et al.*, 2004), but the temperature effects on Sr:Ca ratios are not consistent among studies (Campana, 1999) and otolith Sr:Ca ratios were negatively (Tzeng, 1994) or not affected by temperature in Japanese eel *Anguilla japonica* Temminck & Schlegel (Tzeng, 1996). Meanwhile, otolith Sr:Ca ratios decreased with increasing somatic growth rate (Lin *et al.*, 2007), unlike the case in the River Asi. Therefore, high water temperature and growth rate may not be the main reasons for the high otolith Sr:Ca ratio.

Otolith Sr:Ca ratios and criterion ratios for freshwater *v.* seawater exposure may be affected by local water chemistry. The water in the River Asi is hard and alkaline (pH range 7.1–8.3), with relatively high concentrations of alkaline earth elements and other heavy metals (*e.g.* Ag, Cd and Cr). During the rainy season, the water alkalinity further increases due to the rain dissolving basic calcareous layers (Odemis & Evrendilek, 2007; Yılmaz & Doğan, 2008). High water hardness and geological leaching of rockbeds might enhance Sr concentrations in water, possibly leading to the observed high otolith Sr:Ca ratios.

A sample-specific Sr:Ca ratio indicative of residence in freshwater or saltwater habitats should be calculated from the individuals collected from freshwater sites or controlled experiments. Applying a criterion from another region *a priori* might lead to misclassification of the migratory life histories (Elsdon *et al.*, 2008). For example, if the criterion ratio from Lithuanian waters ( $2.24 \times 10^{-3}$ ) was applied, all *A. anguilla* from the River Asi might be mistakenly classified as having frequently moved to or been caught in saltwater habitats, whereas they were collected from fresh waters.

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