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SHORT NOTES

Hatching time and larval growth of Atlantic eels in the Sargasso Sea

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Abstract Several surveys and studies have examined the Atlantic anguillid eels' larval distributions, but little is known about their larval growth rates. Otoliths of 17 European eel *Anguilla anguilla* (8.8–46.0 mm) and 19 American eel *Anguilla rostrata* (9.8–59.9 mm) leptocephali collected in the Sargasso Sea (25–31°N, 58–70°W) in March and April 2011 were analyzed and their spawning times and larval growth rates were estimated. Ages calculated from the number of otolith increments of European and American eel larvae showed ranges of 10–127 days and 14–233 days, respectively. Linear relationships between age and total length indicated early larval growth rates of 0.31 mm/day for the European eel and 0.35 mm/day for the American eel. This suggested slower growth rates in low temperatures in the Sargasso Sea compared to other anguillid species in the Indo-Pacific, where water temperatures are higher. The back-calculated hatching dates of small leptocephali (8.8–26.7 mm) were in February and March 2011. More American eels hatched in February and more European eels hatched in March. The hatching times of two larger European eel leptocephali (38.7 and 46.0 mm) and a larger American eel leptocephalus (59.9 mm) were back-calculated to November and December 2010 and July 2010, respectively, suggesting hatching times outside of the primary spawning seasons. These novel observations provide important information on the timing of spawning and larval growth characteristics of Atlantic eels, which would benefit from validation by additional otolith studies of leptocephali.

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Introduction

The two Atlantic eel species of the genus *Anguilla* were both found to spawn in the southern Sargasso Sea about a century ago, through collection of their small larvae, called leptocephali (Schmidt 1922). Further collection of leptocephali confirmed spawning of eels in the Sargasso Sea about 50 years later (Schoth and Tesch 1982; Kleckner and McCleave 1988) and then again more recently (Munk et al. 2010; Hanel et al. 2014). All of these surveys collected leptocephali of both the European eel *Anguilla anguilla*, and the American eel *Anguilla rostrata* in the same overlapping areas as documented by Schmidt (1922). The earlier historical collections in the spawning area and throughout the North Atlantic or Mediterranean Sea were also re-examined (Boëtius and Harding 1985; Kleckner and McCleave 1985), and then all the historical collection data of leptocephali of the two species collected up to 2007 were analyzed (Miller et al. 2015). The collection locations of the small leptocephali indicate overlapping spawning areas (Fig. 1a) as was shown previously by McCleave et al. (1987). The leptocephali of both species then disperse outward from the spawning areas, with the larvae of European eel becoming distributed across the Atlantic basin as they are transported towards Europe and northern Africa (Schmidt 1922, 1925).

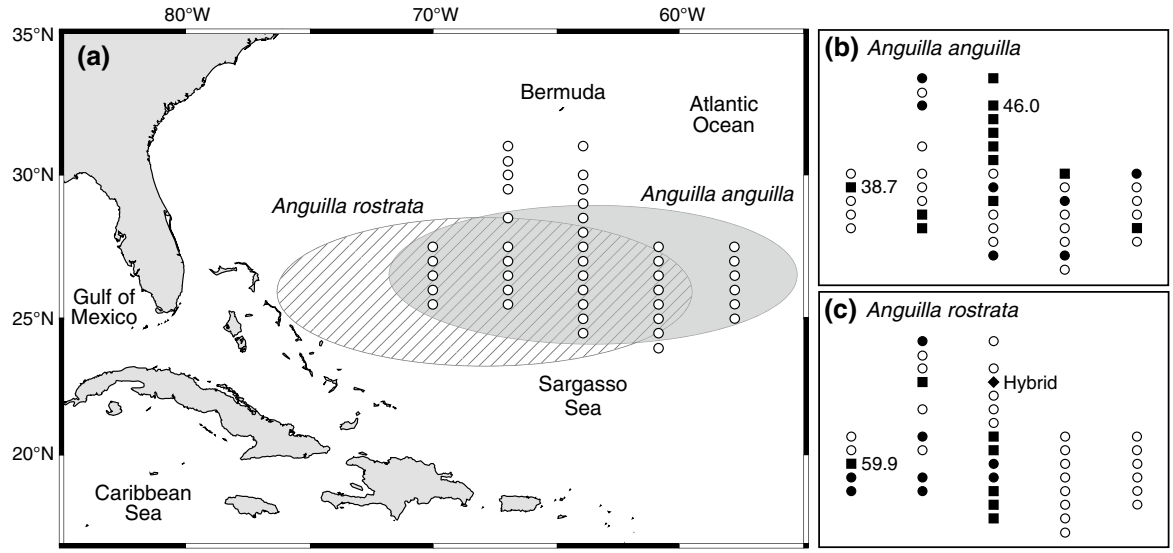

Fig. 1 Map of the sampling stations of the WH-342 survey for leptocephali in the Sargasso Sea in March and April 2011 (*open circles*), showing the estimated spawning areas of *A. anguilla* (*gray-scale*) and *A. rostrata* (*hatched*) based on the distribution of all small leptocephali collected in surveys up to 2007 (**a**) (modified from Miller et al. 2015). Stations where *A. anguilla* (**b**) and *A. rostrata* (**c**) leptocephali were collected (*closed symbols*) and where specimens used for otolith analysis were caught (*closed squares*). A hybrid leptocephalus between the two species is shown (*closed diamond*) in (**c**). Total lengths (mm) of the 3 large leptocephali are given at the stations where they were collected

Although the distributions and migratory routes of the Atlantic eel larvae were studied, few studies have focused specifically on the biology of leptocephali. The vertical distributions of leptocephali and those of marine eels were reported in the Sargasso Sea, which found that the smallest sizes are present at various depths in the upper 250 m, and as they grow larger they are present in the upper 100 m at night and then deeper during the day (Castonguay and McCleave 1987). Only one study to date (Castonguay 1987) has published the age and growth of anguillid eel leptocephali in the Sargasso Sea, based on analysis of their otolith microstructure. Castonguay (1987) analyzed the number of otolith increments of leptocephali that were almost all <60 mm in length and found an estimated somatic growth rate of 0.38 mm/day. However, not all of those leptocephali were distinguished into species. Substantially lower growth rates estimated from length frequency data, at 0.14–0.24 mm/day (Boëtius and Harding 1985; Kleckner and McCleave 1985; Righton et al. 2016), suggest that the larval growth rates of the Atlantic eels require further evaluation.

Larval growth is an important factor during the early life-history of fishes. The growth rates and larval characteristics of anguillid eel leptocephali are different depending on the species and aspects of their life histories. Temperate eel leptocephali with slower growth and larger maximum size appear to be specialized for long migrations and dispersal over a wide range of distances to higher latitudes. By contrast, tropical eels with faster growth metamorphose earlier at smaller size and tend to have shorter migration distances at low latitudes (Kuroki et al. 2006, 2008a, 2014). Studies on anguillid glass eels that recruited after metamorphosis have also indicated differences in the larval durations and sizes at recruitment within and among species (Wang and Tzeng 2000; Shiao et al. 2001; Robinet et al. 2008; Kuroki et al. 2014). However, estimates of larval growth rates from back-calculations using glass eels or elvers are not accurate due to the leptocephalus body shrinking during metamorphosis (Kuroki et al. 2010). Furthermore, the discrimination of otolith rings in glass eels that have experienced low water temperatures is difficult (Umezawa and Tsukamoto 1991; Fukuda et al. 2009).

The timing of spawning can also differ among anguillid eels. The length frequency data of small leptocephali suggests that the peak spawning time of American eels (February–March) is a little earlier than that of European eels (March–April) (Schmidt 1922, 1925; McCleave 2008). This temporal difference may contribute to reproductive isolation, although some hybridization between the two species does occur (Avise et al. 1990; Albert et al. 2006).

Our objectives here were to examine larval growth rates of European eel and American eel leptocephali collected within the Sargasso Sea in 2011, and to back-calculate hatching dates using their otolith microstructure. In doing so, we were able to directly compare our larval growth rate data to those of Castonguay's (1987) study. Otolith-inferred

data of this nature can contribute species-specific insights into the reproductive ecology and larval biology of these eels.

Materials and methods

Collection and processing of leptocephali

The leptocephali were collected between 16 March and 6 April 2011 during the WH-342 cruise of the Thünen Institute on board the fishery research vessel Walther Herwig III. An Isaacs-Kidd Midwater Trawl (IKMT) with a 6.2 m^2 mouth opening, a length of 10 m, and 0.5 mm mesh (Hydro-Bios Apparatebau GmbH) was used to collect the leptocephali. The IKMT was deployed to maximum depths of 300 m during night and day using a double oblique fishing style following Kleckner and McCleave (1988). 52 tows were made at 42 stations (Fig. 1a), distributed within five transects as described previously (Miller et al. 2013; Hanel et al. 2014). All stations also included the deployment of conductivity, temperature, depth (CTD) profiles to depths of 500 m.

The survey of the spawning area in March and April 2011 found a low abundance of anguillid larvae, with 42 *A. anguilla* (7.7–19.7, 38.7, 43.5, 46.0 mm) and 45 *A. rostrata* (7.4–26.7, 47.5, 59.9 mm) leptocephali collected at 33 stations spread across the Subtropical Convergence Zone (Fig. 1b, c) as described previously (Hanel et al. 2014). Leptocephali were sorted fresh out of the IKMT plankton samples and were measured from the tip of the teeth to the end of the tail to determine their total length (TL). All leptocephali were tentatively identified onboard based on their total myomere counts and then either preserved in ethanol or frozen. Genetic identification in the laboratory was then used to classify the leptocephali to a final species designation using their cytochrome *b* sequences (Trautner 2013) and 18S rDNA RFLPs following the protocol of Frankowski and Bastrop (2010) along with some restriction enzymes modifications as described by Prigge et al. (2013). Leptocephali were selected for otolith analyses from station locations spread across most of the areas where the larvae of both species were collected (Fig. 1b, c).

Otolith analyses

Otolith analyses were conducted using the sagittal otoliths of 17 *A. anguilla* and 19 *A. rostrata* ethanol-preserved leptocephali. The diameters of the otoliths were measured along the longest axis by optical microscopy (SMZ-1500, Nikon). The otoliths were subsequently embedded in epoxy resin, mounted on glass slides and ground to expose the core. After polishing, the otoliths were etched with 0.05 M HCl and vacuum coated with Pt–Pd in an ion-sputterer (E-1030, Hitachi) for observation with a scanning electron microscope (S-4500, Hitachi).

The successive otolith daily rings from the first feeding check to the edge were counted on the scanning electron microscope photographs to estimate the age of leptocephali. Because the daily rings between the hatching check and the first feeding check could not be clearly identified, this interval was considered to have been deposited over a period of ten days. This assumption was based on the approximate time taken for yolk absorption in artificially spawned larvae of *A. anguilla* and *A. rostrata* (Oliveira and Hable 2010; Tomkiewicz 2012; Rindom et al. 2014) and first feeding observations in Japanese eel *Anguilla japonica* (Kurokawa et al. 1995; Tanaka et al. 2001). Some scanning electron microscope photographs of small otoliths did not show clear microstructure due to over etching or uneven ground surfaces of the otolith. In these cases, Pt–Pd coating for scanning electron microscopic observation was removed by slight grinding, then age was estimated by making at least two counts of the rings of the ground otoliths under the optical microscope while moving the focus of the objective lens between 50× and 100× magnifications.

The growth rates of the leptocephali based on the examination of their otolith microstructures were determined using their age as indicated by the total number of otolith rings and TL data. The slope of the regression analysis of the TL and number of increments were used to estimate the overall early growth rates of each species excluding the three largest specimens. The difference of the slope of linear regression lines between *A. anguilla* and *A. rostrata* was tested by analysis of covariance (ANCOVA). Individual growth rates for three large leptocephali were determined as: (TL−3)/age, based on the total approximate length of 3 mm at hatching obtained for both artificial and wild eel larvae (Yamamoto and Yamauchi 1974; Oliveira and Hable 2010; Tsukamoto et al. 2011; Tomkiewicz 2012; Rindom et al. 2014), and following previous studies on the determination of age of anguillid leptocephali (Kuroki et al. 2006, 2008a).

Results

Leptocephali of *A. anguilla* ($n = 17$) ranging in size from 8.8 to 46.0 mm (Fig. 1b) and *A. rostrata* ($n = 19$) from 9.8 to 59.9 mm (Fig. 1c) collected across the Subtropical Convergence Zone in the Sargasso Sea (25–31°N, 58–70°W) were examined in the analysis. Most were smaller than 30 mm except for three leptocephali (38.7 and 46.0 mm of *A. anguilla* and 59.9 mm of *A. rostrata*). Sagittal otoliths of leptocephali observed with both optical and scanning electron microscopes had successive

rings from the core to the edge, which were regarded as daily increment rings (Fig. 2). Among them, four otoliths had atypical double cores as shown in Robinet et al. (2008). The otolith diameters showed a linear relationship with TL for both *A. anguilla* ($y = 3.79x-18.4$, $p < 0.01$, $r^2 = 0.97$) and *A. rostrata* ($y = 3.35x-8.79$, $p < 0.01$, $r^2 = 0.94$) despite a lack of intermediate sizes of larvae (Fig. 3a).

Estimated ages from otolith daily rings of leptocephali were 10–127 days in *A. anguilla* and 14–233 days in *A. rostrata*. The relationship between age and total length in the present and previous study (Castonguay 1987) is shown in Fig. 3b. The data from both studies show similar patterns of larval growth. The data from the previous study complemented these new data in that they covered a wider size range, including leptocephali collected in the summer season that had ages of around 50–100 days. Small *A. anguilla* (8.8–19.7 mm) and *A. rostrata* (10.0–26.7 mm) leptocephali from the 2011 cruise showed linear relationships between length and age ($y = 0.31x + 7.58$, $p < 0.01$, $r^2 = 0.74$ and $y = 0.35x + 5.59$, $p < 0.01$, $r^2 = 0.69$), suggesting growth rates of 0.31 and 0.35 mm/day, respectively. The growth rate of *A. anguilla* was estimated to be marginally slower than *A. rostrata* (ANCOVA, $P = 0.015$). The somatic growth rates of the three larger leptocephali were estimated separately. The individual growth rates of the two *A. anguilla* leptocephali of 38.7 and 46.0 mm were 0.28 and 0.37 mm/day and that of the 59.9 mm *A. rostrata* leptocephalus was 0.24 mm/day.

The sampling period of the present study was too short to estimate the peak spawning times of the two species. However, the hatching dates of the small larvae overlapped in February and March 2011, with more *A. rostrata* hatching in February and more *A. anguilla* hatching in March (Fig. 4). The hatching times of larger leptocephali were in November and December 2010 for *A. anguilla,* and in July 2010 for *A. rostrata* (Fig. 4).

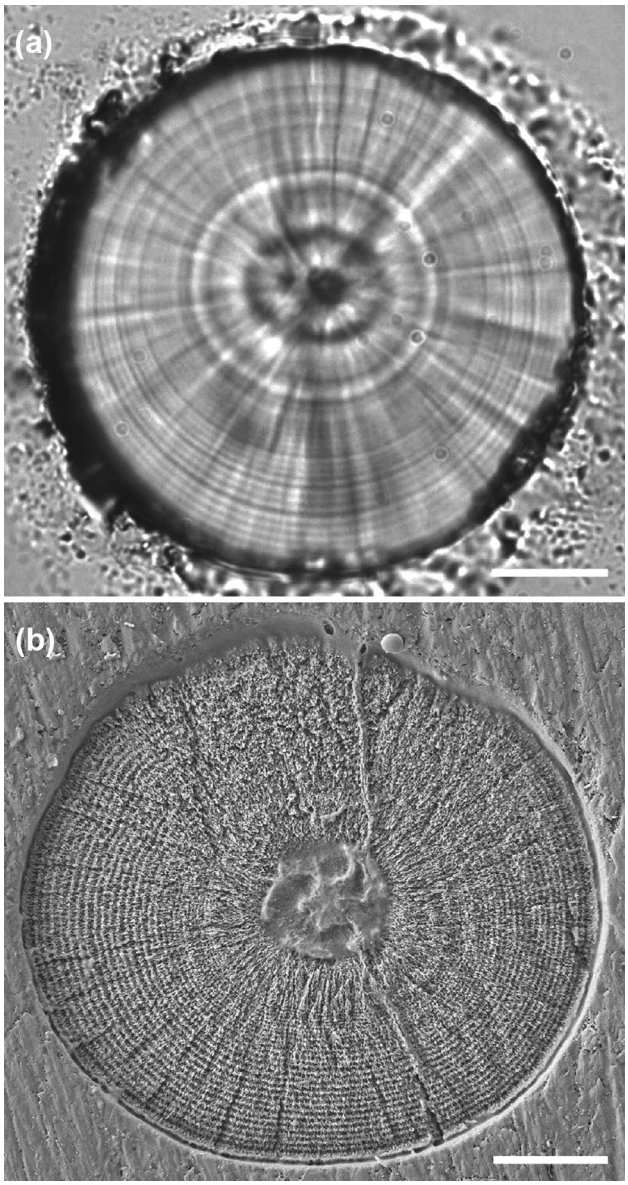

Fig. 2 Optical micrograph of a 15.0 mm *A. rostrata* leptocephalus otolith (**a**) and scanning electron micrograph of a 19.7 mm *A. anguilla* leptocephalus otolith (**b**). *Scale bars* are 10 μm

Discussion

Several studies have examined the distribution of Atlantic eel leptocephali in the Sargasso Sea (Schmidt 1922, 1925; Schoth and Tesch 1982; Kleckner and McCleave 1988; McCleave 2008; Miller et al. 2015), but only one work has previously published larval growth rates (Castonguay 1987). The otolith microstructures observed in the current study were comparable with those from leptocephali, glass eels and elvers in previous studies (e.g. Lecomte-Finiger 1992; Castonguay 1987; Kuroki et al. 2008b). The successive otolith rings of anguillid leptocephali examined here were treated as daily increment rings, based on observations of daily incremental growth in reared leptocephali (Umezawa et al. 1989; Shinoda et al. 2004). Several days under- or over-estimation may result from unreadable rings between the hatch check and first feeding check in our study, however, the growth rates we observed were similar to those described by Castonguay (1987).

Our inferred somatic growth rates of *A. anguilla* (0.31 mm/day) and *A. rostrata* (0.35 mm/day) were marginally lower than previous observations (0.38 mm/day, Castonguay 1987) based on regression data from both species combined. This difference may be due to the size

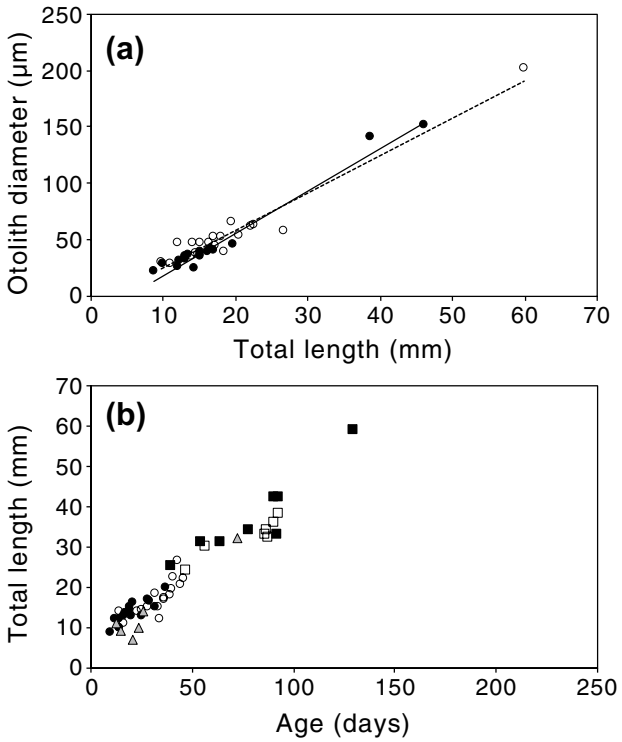

Fig. 3 Relationship between otolith diameter and total length (**a**), and age in relation to total length (**b**) of the *A. anguilla* (*closed circles*) and *A. rostrata* (*open circles*) leptocephali examined. *Solid* and *dashed lines* in (**a**) show the regression lines of *A. anguilla* and *A. rostrata*, respectively. The data points of age and total length of the leptocephali examined by Castonguay (1987) are shown with different symbols for *A. anguilla* (*closed squares*), *A. rostrata* (*open squares*), and *Anguilla* spp. (*gray triangles*) in (**b**)

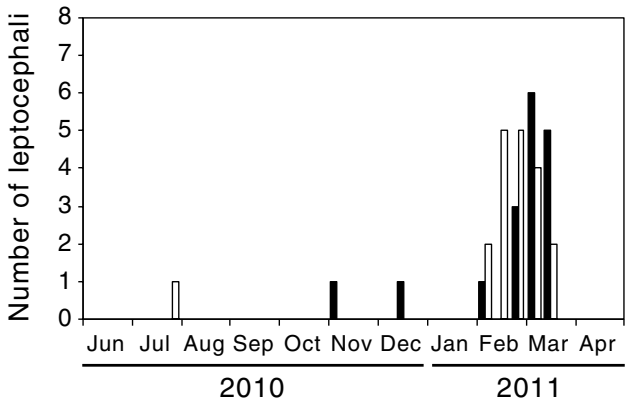

Fig. 4 Hatching dates of *A. anguilla* (*closed bars*) and *A. rostrata* (*open bars*) leptocephali represented in 10 day intervals of each month, back-calculated from collection date and age when examined

compositions of leptocephali being different between the two studies, the previous study has middle-sized larvae from 25 to 34 mm. In leptocephalus otoliths of Atlantic eels, daily rings corresponding to ages 40–60 days had the widest increments, after which they became narrow (Lecomte-Finiger 1992; Kuroki et al. 2014). Therefore, middle-sized leptocephali as examined by Castonguay (1987) may have higher growth rates than smaller individuals. Differing oceanic environmental conditions between 1984 and 1985 for Castonguay (1987) and 2010–2011 in the present study, may also have influenced larval growth. This may have manifested through reductions in productivity and feeding success, although it is noted that the abundance of anguillid larvae appears to be lower in recent years (Castonguay et al. 1994; Bonhommeau et al. 2008; Hanel et al. 2014; Miller et al. 2016).

When the anguillid leptocephali approach their maximum size, the somatic growth rate appears to slow down in this and in previous studies (Kuroki et al. 2006). The mean recruitment size of glass eels of *A. rostrata* is about 50–60 mm (Wang and Tzeng 2000; Sullivan et al. 2006) after they have experienced shrinkage during metamorphosis. Therefore, the oldest *A. rostrata* leptocephalus (59.9 mm, 233 days) in this study may have been approaching its maximum size range, and consequently, growing slower, as has been suggested for other anguillid eels (Kuroki et al. 2014). It is also worth considering that these large larvae might have had slower growth than most leptocephali due to protracted retention around the spawning area.

Both of our estimates of larval growth rates and those of Castonguay (1987) are slightly lower than has been observed in otolith microstructure studies of anguillid leptocephali on both temperate species such as *Anguilla australis* and *A. japonica* (0.4–0.5 mm/day) and tropical species such as *Anguilla borneensis*, *Anguilla celebesensis*, and *Anguilla reinhardtii* (0.5–0.6 mm/day) (Kuroki et al. 2014). Compared to the other anguillids, the slower growth rates of both *A. anguilla* and *A. rostrata* leptocephali might be related to the low temperatures (50–150 m: 20–25 °C, unpublished CTD data) experienced by the larvae in the Sargasso Sea. The oceanic water temperatures experienced by our larvae were lower than those in other areas such as the *A. japonica* spawning area at a lower latitude in the North Pacific Ocean (50–150 m: 25–28 °C, Tsukamoto et al. 2011). Leptocephali growth is influenced by water temperature in artificially spawned and reared anguillid eels (Okamura et al. 2007), although it is not known if genetic differences in larval somatic growth rates occur among anguillid species. The lower growth rate of *A. anguilla* compared to *A. rostrata* found in this study is consistent with the current hypothesis that larval growth rates are related to migration distances (Kuroki et al. 2006, 2014). Typical tropical anguillid species such as *A. borneensis* and *A. celebesensis* with adjacent growth habitats and spawning areas have the fastest

larval growth rates, whereas temperate and some tropical species with protracted larval durations have slower growth rates. Leptocephali length frequency data also suggest that shorter migrating *A. rostrata* may grow faster than *A. anguilla,* that migrate further and have longer larval durations and a larger maximum larval size (Wang and Tzeng 2000; Kuroki et al. 2008b; Miller et al. 2015).

Larval growth rates estimated from sizes of *A. anguilla* leptocephali collected in the Sargasso Sea have recently been used to estimate the peak spawning season in relation to when the maturing silver eels leave continental waters to migrate to the Sargasso Sea (Righton et al. 2016). This latter study used a growth rate of 0.14 mm/day, considerably lower than the growth rate estimated from otolith microstructure. It is possible, therefore, that the Righton et al. (2016) estimated peak spawning season may precede true peak spawning if the otolith-derived growth estimates are considered more accurate. Larval growth rate estimates from otolith analyses of leptocephali are higher and probably more accurate than those made based on the analysis of length frequency data versus the collection dates of larvae as discussed in the analysis of historical *A. japonica* data in the Pacific Ocean (Shinoda et al. 2011). The lower growth rates from length frequency data may result from size-selective advection of fast growing larvae out of the spawning area, retention of slow growing individuals within the area, and the continuous addition of small larvae during the spawning season, which would reduce the perceived overall growth rates.

Our estimated hatching season of February–March was consistent with the typical estimated spawning seasons of both species and similar-sized larvae were collected during those same months as in previous research surveys (Schmidt 1922, 1925; Schoth and Tesch 1982; Kleckner and McCleave 1988; McCleave 2008; Miller et al. 2015). However, the two larger *A. anguilla* larvae examined were back-calculated as having hatched in November and December 2010, suggesting that they originated from early season spawning. The larger *A. rostrata* larva appeared to have hatched in July 2010, which would equate to late-season spawning (McCleave and Kleckner 1987; Miller et al. 2015). Spawning outside of the primary spawning season has not previously been documented and requires confirmation through additional research efforts.

Further studies on the age, growth and hatching dates of the two species of Atlantic eel leptocephali are required to validate our observations on the early life history. The inclusion of larger larvae will provide valuable information about larval growth patterns up to the period of metamorphosis into glass eels, as the two species diverge from each other and migrate to either the eastern or western sides of the North Atlantic basin.

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Compliance with ethical standards

Conflict of interest The authors declared that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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