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Growth rate variability of larval European eels (*Anguilla anguilla*) across the extensive eel spawning area in the southern Sargasso Sea

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Abstract

The European eel (*Anguilla anguilla*) spawns in the Sargasso Sea, and the leptocephalus larvae are distributed in the Subtropical Convergence Zone (STCZ), covering an area approximately 500 X 2000 km in extent. The STCZ is hydrographically diverse and highly dynamic, which is likely to impact growth and survival opportunities of the larvae. Here we investigate the temporal and spatial growth rate variability of larvae collected along seven sampling transects across the STCZ during March-April 2014. Scanning electron microscopic analyses of otolith ring-formations were used to estimate larval age-at-length, initial growth and growth trajectories. Analyzed larvae averaged 14 mm in length and 30 days of age. Age estimation indicated that the larvae were spawned during one continuous period, extending from mid-February to late-March, and that spawning had peaked during the new moon of March 4. Growth estimates (average: 0.38 mm d\(^{-1}\), ~6% weight increase d\(^{-1}\)) showed substantial variability among individuals. There were no apparent spatial trends in this variability, and no linkage to measures of temperature and salinity, while earlier-spawned leptocephali showed slightly higher growth rates than later-spawned larvae. The variability in growth rates at single sampling sites was as great as variability across the entire area of distribution. This indicates that individual growth opportunities are strongly impacted by variable conditions in...
the immediate environment while average conditions for larval growth and survival are basically the same throughout the immense area of larval distribution.

Running title: Growth rate variability of eel larvae

Keywords: Anguillid eels, Subtropical Convergence Zone, temporal and spatial variability, specific growth rate, otolith microstructure, spawning period, new moon.

Introduction

The European eel, *Anguilla anguilla*, is an important fisheries species and has significant public and scientific interest. There are still several unknown aspects of its life cycle, especially during the early life history. The hatched larvae transform to a transparent, elongated “willow-leaf” form (termed a leptocephalus) shortly after their yolk sac is exhausted, and they stay in that stage for an extended period (Miller, 2009). The European eel spawns in the Sargasso Sea and its leptocephali drift across the North Atlantic toward adult habitats on the European and North African continents (Tesch, 2003). During the larval period, they obtain lengths of approximately 7-8 cm, but then shrink to 6-7 cm when metamorphosing to a juvenile, glass-eel form, before entering estuarine and freshwater habitats where they transform into yellow eels. Years later another transformation takes place; the eels attain larger eyes and a silvering of the skin (Pankhurst, 1982; Tesch, 2003), whereupon they start a spawning migration back to the Sargasso Sea (Aarestrup *et al*., 2009; Amilhat *et al*., 2016).

The spawning takes place in an extensive, hydrographically characteristic area of the southern Sargasso Sea (Schmidt, 1925; Schabetsberger *et al*., 2016) termed the Subtropical Convergence Zone (STCZ). It is approximately located between 70° and 50°W, and is bounded latitudinally by
seasonally shifting frontal zones, generally around 24° and 28°N (Schmidt, 1922, 1925; Miller et al., 2015). Nutrient entrainment is enhanced in this zone due to strong eddy activity (Richardson & Bendtsen, 2017), providing an increase in primary production and zooplankton abundance that likely enhances growth conditions for the eel larvae (Munk et al., 2010; Andersen et al., 2011; Riemann et al., 2011).

Insight into larval eel growth variability is a requisite for understanding the bio-physical linkages influencing their life traits in this oligotrophic, open-ocean environment. Their growth rates have historically been estimated from changes in average body sizes between sampling dates (Schmidt 1922, 1935; Boëtius & Harding, 1985), but this procedure does not provide sufficient precision and resolution due to infrequent and incomplete sampling across the relevant areas of distribution (Shinoda et al., 2011). Alternatively, growth rates have been ascertained from ageing of larvae by microstructure analysis of their otoliths, either during their earlier stages, when they are distributed in the southern Sargasso Sea (Castonguay, 1987; Kuroki et al., 2017), or from metamorphosed (glass) eels caught in brackish or freshwater environments close to the European continent (Lecomte-Finiger, 1992; 1997; Arai et al., 2000; Wang & Tzeng, 2000). The glass-eel method, however, suffers from uncertainties in the interpretation of peripheral ring patterns, because the colder environment experienced during this stage might have depressed ring formation (Fukuda et al., 2009).

Based on otolith microstructure analysis and age-at-length estimations, we here describe and analyze the temporal and spatial growth variability of European eel larvae during their first months after hatching, when they are widely distributed across the southern Sargasso Sea. The study was part of a research initiative, named the Danish Eel Expedition 2014, which focused on environmental conditions and early life characteristics of the European eel larvae in the Sargasso Sea. Given the large hydrodynamic variability at both local and regional scales throughout the area
of young leptocephalus distribution, our objective was to ascertain whether some spawning areas or periods were potentially more favorable than others for larval growth. Further we wanted to evaluate the length of the spawning period and relate the timing of peak spawning activity to phases of the moon (ref. “New Moon Hypothesis,” Tsukamoto et al. (2003)).

MATERIAL AND METHODS

Larval collection and identification

Leptocephali larvae were sampled at 72 Stations from 16 March to 20 April 2014, from the research vessel DANA (Technical University of Denmark) (Fig. 1, Table 1). Nine latitudinal transects were sequentially sampled from west to east, and collections were used to examine leptocephalus abundance and provide specimens for further analysis. The larvae were sampled with a 3.5 m diameter, ring net, equipped with a 25 m long, 560 µm mesh net, with 300 µm mesh in the hindmost 1 m of the net and in the cod-end container. Larval sampling was conducted during both daytime and nighttime. At a ship speed of 2.5 knots, the net was hauled obliquely to a maximum depth of 200 m. Flowmeters in the opening measured water flow into the net.

On board, leptocephali were immediately sorted fresh from the plankton, and screened for presence of European and American eel larvae using myomere counts of the body (A. anguilla ≥112, A. rostrata ≤111) in conjunction with the position of the last dorsally directed blood vessel (at myomere number: A. anguilla 46-50, A. rostrata 44-48). The standard length (SL, see Sørensen et al. 2016) of potential Anguilla larvae were measured to the nearest 0.1 mm and larvae were digitally photographed, before being individually stored in 96% ethanol for later genetic species identification. Remaining plankton samples from each station were also stored in 96% ethanol, and re-examined post-cruise for Anguilla larvae that were missed during the initial screening. Thirty
larvae selected from the first-screening were measured before being preserved in ethanol for later re-measurement and calculation of a shrinkage factor (relationship: fresh length (mm) = 1.163* preserved length (mm) - 4.356, r²=0.997). Lengths (SL) of the *A. anguilla* larvae found during screening, of the preserved sample, where subsequently converted to the lengths of newly caught larvae using the shrinkage regression. For calculation of weight specific growth rates, we used a regression based on leptocephali of several species measured and weighed by Deibel *et al.* (2012) (relationship: Wet weight (g) = 1.3 x 10^{-5} SL^{2.34} (mm)).

Genetic species-identity confirmation of all anguillid larvae, differentiating among European eels, American eels and their hybrids, was carried out based on analysis of the mitochondrial cytochrome b gene and microsatellite genotyping (see Jacobsen *et al.*, 2016).

**Scanning electron microscopic analysis (SEM)**

A subset of the genetically-confirmed European eel leptocephali was selected for otolith microstructure analysis. Leptocephali were subsampled ensuring a representative coverage from each station where European eel leptocephali were caught. The sagittal otoliths were extracted using acupuncture needles under a dissecting microscope, and embedded in epoxy resin (Epofix®; Struers, Copenhagen, Denmark) on glass slides. Otoliths were hand polished to the core using a succession of fine-grained silicon carbide/aluminum oxide polishing papers (3M®; St.Paul, Minnesota, USA - final grain size 0.3 microns).

Other studies on eel larvae have shown the increment widths of the individual daily growth rings to be below the resolving capability of light microscopy, around 200 nm (e.g. Castonguay, 1987; Arai *et al.*, 1999; Kuroki *et al.*, 2017). Therefore, we examined all otoliths with scanning electron microscopy (SEM) that resolves spatially to ~ 1 nm (Goodhew *et al.*, 2000). To prepare the mounted and polished otoliths for SEM, they were etched with 0.05M HCl for approximately 20
seconds and coated with gold in an ion-sputterer. Subsequently, high-resolution digital pictures of each otolith were made using an FEI Quanta FEG 650 scanning electron microscope and stored for later analysis. Analysis of the left otolith was always attempted first; if no good-quality pictures could be made of it, the right otolith was used. Some otoliths required subsequent re-polishing and re-imaging when the initial SEM analysis showed they were not polished to the core. Final SEM photos, in which the magnification range generally was from 5000 to 20000, were visually inspected and growth increments, defined as alternating darker and lighter ring patterns, were counted. A range of measurements was made on the otoliths (see below).

An inner heavy, dark ring circumscribing the nucleus was regarded as the hatch check (HC; Umezawa et al., 1989) and was used to delineate the nucleus (Fig. 2). A crystalline crown (CC; Shinoda et al., 2004) region could be seen surrounding the hatch check. This ended in another heavy, dark zone, which often was composed of two distinct and more heavily imprinted rings immediately adjacent to each other. We refer to this second characteristic pattern as the first-feeding check (FFC, Fig. 2; Shinoda et al., 2004), and we assume the FFC demarcates the start of exogenous feeding (Lecomte-Finiger, 1992; Shinoda et al., 2004). The region within the FFC will be referred to as the core region. In some instances isolated and unclear ring patterns could be seen within the radius of the crystalline crown; these were not included in the estimate of total number of rings. Only the concentric growth increments from the FCC (inclusive) to the otolith edge were counted and interpreted as individual growth increments. (Fig. 2). Measurements were carried out of maximal otolith diameter, nucleus diameter, core region diameter, as well as the accumulated widths of the first 10 and the total number of growth increments. All measurements were made along each otolith’s longest radius (core to edge).

Age and growth estimations
The number of growth rings outside the FCC was assumed to represent the age of larvae in days from first feeding (see discussion for arguments for this assumption). In order to estimate age from spawning, we added 14 days to account for a 2-day embryonic period (Sørensen et al., 2016, Politis et al. 2017) and a 12-day post-hatch period (at assumed optimal temperature of 18 C) before a larva would be capable of exogenous feeding (Politis et al. 2017). Spawning dates (date of fertilization) were back-calculated by subtracting the age estimate from the date of capture of each individual. A “spawning curve” was obtained showing frequency of larvae along a “day of hatch” axis, and it was subsequently adjusted to account for differences in accumulated mortality among differently aged larvae and to account for the under-representation of the late-spawned larvae that were not available to the gear during our first period of sampling. In the first case we incorporate an arbitrarily set mortality rate of 10 % d⁻¹, in the latter case the frequency of larvae from each sampling date was weighted by the inverse number of stations where larvae from that date could be covered by our station sampling.

Growth rates were expressed in three ways. First, estimates of absolute and specific growth rates for the entire population were made using all length-at-age information and regressing those data by non-linear fitting to the Laird-Gompertz growth function (Laird, 1969). Second, as the relationship between larval length and age appeared approximately linear for larvae below 25 mm, we assumed linear growth in that range and estimated individual growth rates from IGR= (SL – LH)*ADPH⁻¹, where LH is the mean length at hatching (set to 3.6 mm, Sørensen et al., 2016), and ADPH is age at hatching (set to increment number plus 12). Lastly, to assess temporal differences in growth (earlier-spawned versus later-spawned leptoccephali), we used the apparent relationship between otolith growth (ring widths) and increase in larval length to define an index of initial growth rate (IGI) as the width of the initial 10 otolith increments (when at least 10 increments after FCC were apparent).
**Physical and chemical parameters**

CTD casts by a Seabird SBE11 (9+) were carried out at all stations of sampling, from surface to 400 m. Measures of temperature and salinity at depths 50 m and 150 m, which are the depth strata where larvae are found aggregating at night and day, respectively (Castonguay and McCleave 1987; Munk et al. in prep), were used for analyses of linkages between growth estimates and oceanography. For illustration of basic oceanographic characteristics in the sampling area measures were spatially interpolated by the nearest-neighbor method in the program Surfer ©.

**RESULTS:**

European eel larvae were collected at 52 stations along the first seven transects (Table 1); no larvae were found at stations along transects 8 or 9 (along 44°00′W and 37°40′W, respectively, Fig. 1a). Larval lengths (SL) ranged from 6-20 mm, except for seven larvae of 21-26 mm and a single larva of 34.2 mm. Larval mean lengths at different stations showed large spatial variation, but there were no general spatial trends (Fig. 1b). However, patterns along specific transects differed to some extent: along transects 1, 2, 4 and 5 mean lengths showed a slight increase toward the more northerly stations, whereas the opposite tendency was seen along transects 3, 6 and 7.

The temperature measured at 50m and 150 m depth are contoured in Fig. 1b, c. The gradients in temperature change illustrate the significant hydrographic variability in the area; temperature decrease from south to north on each transect and the steep change in temperature (frontal zone) is positioned differently, dependent on depth of measurement. Larvae are in many cases sampled across the frontal zone(s).

**Otolith analysis**
Preparation for SEM-imaging was carried out on sagittal otoliths of 312 of the larvae; however, 89 otoliths were rendered unexaminable by combinations of overpolishing, cracking/splintering and over-etching. Thus, 223 had sufficient quality for SEM with a clearly discernible ring increment pattern across a full section from the core to the periphery of the otolith.

Otoliths were generally circular in shape, and in larvae old enough to have begun increment deposition we could see distinctive and clear concentric growth rings circumscribing the bipartite core region (Fig. 2). The core region had an average diameter of 23.5 ± 2.7 µm, and was always divided into two subregions: an inner nucleus and a crystalline growth-crown. Most nuclei were roughly circular in shape, but infrequently (~15% of otoliths) 2-3 conglomerated-nuclei resulted in oblong {nucleus + crystalline crown} assemblages. The crystalline growth-crown is, according to Shinoda et al. (2004), produced in the yolk-sac stage during the first two weeks post-hatch. The individual growth increments outside the defined FFC are composed of a calcium carbonate-rich ring abutting a protein-rich ring. Together these pairs produce a distinctive and characteristic, translucent-opaque concentric ring pattern in SEM after the polishing and etching (Lecomte-Finiger, 1992, Leander et al., 2013). Only infrequently (~5% of otoliths) could ring patterns be distinguished within the FFC. These increments were much fainter relative to the distinct growth increments outside the FCC, and often they did not form a complete ring. Otolith diameters ranged from 22.1 to 132.8 µm (Table 1), and these, like the widths of the incremental sections, showed a curvilinear relationship to larval length (Fig. 3).

The 223 SEM-analyzed otoliths had an average of 13.7 increments, of these 208 larvae from 7.2-24.7 mm had from 2-58 growth increments, and 14 larvae in pre-leptocephalus stage and of lengths from 6.3-8.9 mm, had not yet implemented deposition of primary growth. The single, larger A. anguilla leptocephalus, had 96 growth increments. Overall the individual growth-rings had an average width of 397 nm (range: 252-792 nm).
Age and growth estimation

Fourteen specimens that did not have primary growth rings and did not show evidence of an FCC were taken to be $\leq 15$ days from spawning, based on our assumption that initial increment deposition begins at the onset of first-feeding, 15 days post-fertilization. The largest 34.2 mm larva was estimated to 110 days old. Not including it or the 14 no-growth-increment specimens, an average age of 30 days from spawning (range: 17-73 days) was estimated. The largest difference in age between youngest and oldest eel larvae for any single station was 52 days. Average age at given stations (corrected for differences in sampling time) showed some spatial tendencies. Ages increased toward the north along four of the transects, and the larvae in the eastern part of the sampling area were generally younger (spawned later) than those from western parts (Fig. 1c).

Estimated spawning dates ranged from 19th January to 28th March (Fig 4a). The single larger specimen had an estimated spawning date of 8th December, 2013. From daily spawning intensity estimates, which considered mortality and uneven sampling coverage of later-hatched larvae (Fig. 4b), we assessed both mean and medium spawning date to 28th February. The new moon dates in early 2014 over the Sargasso Sea were 31st January, 1st March and 31st March. The spawning intensity of European eels, therefore, appeared to have peaked around the specific new moon event of 1st March 2014.

The individual length-at-age estimates showed considerable variability among the larvae investigated, with a length range of $\pm 2.5$mm for age intervals of 1 day. The large variability was apparent for all sampled larvae (Fig. 5a), and also was observed among specimens from single stations, as exemplified for station 30 (Fig. 5b). For all sampled larvae the relationship between age and length appeared curvilinear, and data were represented by a non-linearly fitted Laird-Gompertz curve (Fig 5a). Growth rates deduced from this curve-fit illustrate their general decline during
ontogeny, both for absolute growth in length and for length- and weight-specific growth rates (Table 2). For the first part of the growth curve (e.g. SL < 25 mm) the relationship does not differ markedly from linear, and we used a linear model (Fig. 5c, rate estimate 0.38 mm d$^{-1}$) for a comparison to results from historical growth studies that have used linear regressions in their growth rate estimates.

The estimates of individual growth rates (IGR), showed high variability, with neither latitudinal nor longitudinal spatial trends (both tests: n=223, p>0.20; Fig. 6 a,b). The growth indices of early larval growth, based on the total widths of the first 10 increments (IGI) showed a variability of the same magnitude and spatial trends in these are either not significant (test of longitude: n=223, p>0.20; Fig. 6 c) or showed a weak tendency of decline in northward direction (test of latitude: n=223, P<0.05; Fig. 6 d). The statistical tests of potential relationships between growth rate estimates and hydrographic measures, here chosen as temperature and salinity at 50 and 150m, did neither show significant trends (all IGR tests: n=221, p>0.5; all IGI tests: n=144, p>0.07). A temporal trend appeared in IGI; when compared to estimated individual hatching dates, these indices of initial growth rates showed a significant decline during the spawning period (n=147, p< 0.05; Fig. 7). This decline in initial growth rates was also significant for a subset of data which were from a one station only (station 30, 62°45’, 25° 30’) (n= 53, p< 0.05); subset indicated in Fig. 7).

DISCUSSION:

Otolith formation

Our analysis of larval eel ages and growth required robust interpretation of otolith characteristics. Particularly important were the points selected as indicative of age, the distinctions among different
In the majority of our examined otoliths we could ascertain both a hatch check (HC) and a first-feeding check (FFC) (Fig. 2). The mean diameters of HC and FFC from the present study (10.4 µm and 23.4 µm, respectively) are in close correspondence to diameters reported by Arai et al. (2000) and Wang & Tzeng (2000), and our definition of check-marks also appears in good agreement with those described for glass eel otoliths from A. anguilla (Lecomte-Finiger, 1992) and for leptocephali of other species (Shinoda et al., 2004).

The duration from hatching to FFC could not be estimated based on microstructure patterns. Thus, in the back-calculation to an estimated date of spawning, we used a mean duration from hatching to FFC of 12 days from laboratory studies. Laboratory growth trials by Politis et al. 2017 showed time to first feeding of 12 days post-hatching at 18°C, and biomechanical modeling studies (Bouilliart et al., 2015) likewise indicate that A. anguilla leptocephali are capable of transitioning from endogenous nutrition to exogenous feeding at 12-13 days post-hatching. The estimate we used from the study by Politis et al. (2017) was obtained at the temperature which these find optimal for larval development. At suboptimal, but viable, temperatures, 16 and 20°C the durations are 14 or 10 days respectively (Politis et al. 2017), thus our mean spawning date could vary ±2 days, dependent on assumed temperature.

Ring widths in otoliths from larvae of anguillid eel species are generally very narrow, below the resolution of light microscopy (Kuroki et al., 2014), so we used SEM for adequate resolution of ring patterns. In otoliths that have been properly ground and etched, the rings were distinct as a sequence of alternating darker and brighter bands. Most studies of increments in the otolith anguillid larvae have been carried out on the larger otoliths from glass eels caught when they have
returned to their respective continents. Incremental growth zones of glass eel otoliths are often diffuse, zones apparently formed during metamorphosis or during sustained periods in lower-temperature waters (Arai et al., 2000; Cieri & McCleave, 2001, Fukuda, 2009). However, within the eel’s larval period, as examined in the present investigation, we expect warmer, weakly variable, environmental conditions, and thus a generally consistent deposition patterns.

It is imperative for our interpretation of age, that the otolith growth increments are produced daily. Daily growth ring deposition has been validated for four anguillids: two tropical species, A. mormorata (Sugeha et al., 2001) and A. celebesensis (Arai et al., 2000), and two temperate species, A japonica (Umezawa et al., 1989; Shinoda et al., 2004) and A. rostrata (Martin, 1995). It appears that daily periodicity in ring deposition is a common trait among anguillid larvae. The periodicity of increments in otoliths of A. anguilla has not yet been validated experimentally, owing to difficulties in rearing their larvae beyond first-feeding (Tomkiewicz et al., 2013; Sørensen et al., 2016). Nevertheless, we assume daily ring formation in A. anguilla for two reasons: (1) overall otolith microstructure is closely similar among many eel species (Correia et al., 2002; Lee et al., 2008; Kuroki et al. 2008), including both tropical and temperate anguillids (Leander et al., 2013). (2) Studies by Correia et al. (2002) and Ma et al. (2005) suggest that leptocephali from regions with relatively warm water, as was the case with specimens in the current study, do have daily otolith increment deposition.

**Age estimates**

The present estimates of leptocephalus ages showed high variation at given sampling sites (stations), for example larvae at one station had a more than 50-day age range from youngest to oldest individuals. Castonguay (1987) showed a comparably great age range for A. anguilla, specifically 46 days at a single of his sampling stations. These variably-aged groups of leptocephali
are not likely to have actively traversed the distances between our sampling stations within the relevant periods of time; they were apparently spawned over extended periods in basically the same water masses. As we see this pattern throughout the vast geographical area of our sampling, the observations imply that spawning is wide-spread in space and time. Thus spawning in the area might not take place as synchronized events by large assemblies of eels, a more individual behavior is indicated by the laboratory observations by Boëtius and Boëtius (1980) of courtship by male A.anguilla towards individual females. Further, individual female eels have been shown to spawn several batches of eggs over time (Tomkiewicz & Jarlbæk, 2008), and the larvae from relatively narrow areas could likely stem from such series of separate egg batches spawned by the individual females.

The spawning curve estimated from findings of the present study did not support the new moon hypothesis of spawning as it has been formulated for A. japonica; i.e. with a restricted spawning event during a few days around new moon (Ishikawa et al., 2001; Tsukamoto 2006, 2009). According to our estimation, the spawning took place during an extended period, however, the median of the spawning curve was close to new moon, and some synchronicity, where the intensity of spawning is inversely related to lunar light intensity, cannot be precluded. Our findings in respect to ages of sampled larvae suggest that the earliest spawning in 2014 took place around mid-February. If there had been spawning earlier in 2014, we would have expected to find some older leptocephali during our investigation. There might, however, be later spawning episodes – potentially related to lunar periodicity – which we did not cover during our period of sampling.

Prior field collections of small A. anguilla in the Sargasso Sea have indicated spawning throughout the early half of the year (Kleckner & McCleave, 1988; McCleave & Kleckner, 1987).

_Growth estimates_
The average estimate of absolute somatic growth rate for larvae <25 mm (0.38 mm d\(^{-1}\)) is the same as estimated by Castonguay (1987) in his study, while it is somewhat above the estimate by Kuroki et al. (2017) of 0.31 mm d\(^{-1}\). Compared to estimates for other species of the genus *Anguilla* (range: 0.35 – 0.59 mm d\(^{-1}\); Tsukamoto, 1992; Arai et al., 2000; Kuroki et al., 2006, 2007) the estimates for *A. anguilla* are all in the lower range. A number of non-anguillid eels, and elopomorph leptocephali have also been shown to have quite high growth rates (range: 0.63 – 1.42 mm d\(^{-1}\); Crabtree et al., 1992; Bishop et al., 2000). The higher growth rates cited were obtained in relatively warm waters, and temperature is likely the main cause of differences among values.

We assessed the potential seasonal variation in growth from the rates during the first 10 days of life based on the otolith widths for that period. We found a negative correlation of early growth rates with estimated spawning dates (Fig. 7); thus, specimens spawned earlier in the year showed higher average initial growth rates. The magnitude of this seasonal variation was, however, small relative to the substantially large overall variation in growth for larvae with the same spawning dates. There is no straightforward explanation for this weak temporal trend; it appears not simply to linked to primary productivity changes, while the productivity in the Sargasso area generally is increasing during the period January to April (Mentzel & Ryther 1961).

We anticipated that the hydrographically variable environment of the STCZ would lead to a significant spatial variability in the growth of eel larvae distributed across it. However, we found only a weak spatial trend in one of the two measures we used for larval growth: lengths at age and average width of the first otolith increments. The measure of initial growth rate tended to decline towards the north in the area, a tendency which might relate to the seasonal effect on this index, irrespective that we could not deduce consistent north-south trends in our estimates of average spawning time (Fig. 1c). The part of variability we could ascribe to spatial, temporal or oceanographic measures were in any case minor compared to the prominent, non-explained,
variability in larval growth rates at single sites. Large growth variability was seen on a local scale (i.e. within few kilometers) while potential larger-scale changes (10-100 kilometers) did not add further to this variability. It should be noted, however, that the significant mixing of larger water masses due to the prominent eddies could be partly responsible for blurring potential directional tendencies on the actual scale. Along the longitudinal axes the eddy patterns are quite repetitive, and on the mesoscale (~ 100 km) we see basically the same hydrographic patterns from west to east across the area of larval distribution.

Conclusions and Perspectives

Our findings of wide individual variability in both age and growth rates of European eel larvae at given restricted locations, combined with a lack of clear spatial trends in average growth rates across the vast area of larval distribution, point to several remarkable characteristics of the spawning and early life of European eel. Spawning is protracted in time and space, and the large variability in ages at given locations indicates that several spawning events had taken place within restricted areas, possibly by multiple spawnings of individual females. Due to the differences in time of spawning, and apparently also due to local variability in the hydrographic and biological environments, marked differences in life trajectories of individual larvae were apparent within relatively restricted areas. The large variability in growth rates shows that most larvae are far from optimal growth, indicating that conditions for early life are harsh at any given location in the STCZ. On the other hand, the average opportunities for larval life across the 2000 km wide area used for spawning by European eels did not differ significantly. Thus, the species has a huge area available, the STCZ, with conditions which can support the early life, and the spawners apparently need not direct their migration and spawning to specific longitudes along the STCZ in order to further promote growth opportunities of their larvae. Our study showed a decline in growth rate for the older larvae that are still distributed in the STCZ. Further changes in growth is to be expected when
larvae leave the STCZ and face other environmental conditions during their drift towards the European continent, and the present findings encourage continued studies incorporating these later phases of the larval life.

Acknowledgements

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Atlantic eels (*Anguilla anguilla* and *A. rostrata*). Heridity **118**: 266–275 (doi.org/10.1038/hdy.2016.96)


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<td>13/4 -14/4</td>
<td>53° 30’</td>
<td>28° 30’- 27° 20’</td>
<td>17</td>
<td>6.3</td>
<td>24.7</td>
</tr>
<tr>
<td>7</td>
<td>15/4 -16/4</td>
<td>50° 00’</td>
<td>27° 20’- 26° 20’</td>
<td>13</td>
<td>9.0</td>
<td>19.7</td>
</tr>
</tbody>
</table>

Table 1
Legends to figures

Figure 1. a) Locations of stations in the Sargasso Sea sampled during March-April 2014 by the Danish Eel Expedition. Transects are numbered 1-9. Rectangle encloses area shown in b) and c). b) Mean lengths of *A. anguilla* larvae illustrated for all stations were these larvae were sampled; symbols indicate length intervals (mm) as in legend to the right. Symbols are imposed on an isopleth map of measured temperature at 50 m depth, isotherms show temperature in 0.25°C intervals, c) Mean ages of *A. anguilla* larvae estimated for the 1st April; symbols illustrate age intervals (d) as in legend to the right. Symbols are imposed on an isopleth map of measured temperature at 150 m depth, isotherms show temperature in 0.25°C intervals.

Figure 2: *A. anguilla*. Scanning electron microscopy images of sagittal otoliths from two specimens. a) otolith from a 23.0 mm leptocephalus, b) otolith from a 14.9 mm leptocephalus, c) illustration of the hatch check (HC), first-feeding check (FFC) and the crystalline core (CC) from the 23.0 mm leptocephalus.

Figure 3. *A. anguilla*. Relationships between larval length (mm) and otolith sagitta measures (µm). Upper curve (filled circles) illustrates relationship to maximal diameter of otolith (OD), lower curve (open triangles) illustrates distances along radius measured from start of ring formation at FFC to the edge of otolith (ORF). Curves are nonlinear fits as indicated by the equations.

Figure 4. *A. anguilla*. Contributions of leptocephali (n=208) from spawning on back-calculated dates (as percent within a day of year or a calendar date). Thirteen pre-leptocephali not exhibiting otolith growth increments, hence with uncertain spawning dates, and 2 leptocephali with spawning dates on January 20 and December 8 (2013) are not included). Moon phases illustrated with
pictograms along the top axis: black circles are new moons, light circles full moons. a) direct back-calculations, b) distributions re-calculated with incorporation of natural mortality and an even sampling coverage

Figure 5. *A. anguilla*. Relationships between otolith increments and length measures. a) Larval length versus increments, larvae from all stations. b) As for a), but only for larvae from station 30, c) As for a), only for larvae ≤25mm. d) Maximal otolith diameter (µm) versus increments. Curves are fitted Laird-Gompertz curves (a and b), or linear regressions (b and d), regression equations and $r^2$ values are inserted in the graphs. Prediction and confidence bands illustrated by line types listed upper left in a).

Figure 6. *A. anguilla*. Spatial variation in growth rate estimates of leptocephali. a-b) Growth rate in length (mm d$^{-1}$) during period from hatching to catch, related to c) sampling longitude or d) sampling latitude. c-d) Growth in otolith radius (µm) during the first 10 days after first feeding, related to a) sampling longitude and b) sampling latitude.

Figure 7. *A. anguilla*. Growth in larval otolith radius (µm) during the first 10 days after first feeding, plotted along an axis representing estimated date of spawning. Closed symbols denote all available data (n=147). Open circles indicate larvae from just station 30 (N=53). Linear regressions for all data, and for station 30 data only, are shown by hatched and full lines, respectively.

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Fig. 1.
Fig. 2

c)
Fig. 3

OD = 15.1*exp(0.06*L), R^2 = 0.80

ORF = 0.59*exp(0.16*L), R^2 = 0.81
L = 8.2 \cdot \exp(1.23(-\exp(-0.04\text{Incr})))
\hat{r}^2 = 0.89

L = 8.0 \cdot \exp(0.96(-\exp(-0.06\text{Incr})))
\hat{r}^2 = 0.66

L = 8.3 + 0.38\text{Incr}
\hat{r}^2 = 0.85

L = 23.1 + 0.87\text{Incr}
\hat{r}^2 = 0.87

Fig. 5
Fig. 6