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

Ayala, Daniel Jiro; Munk, Peter

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

3   Daniel Jiro Ayala, Peter Munk*

4   National Institute of Aquatic Resources, Technical University of Denmark, 2800 Lyngby, Denmark

5   *Corresponding author. E-mail address: pm@aqua.dtu.dk

6

7   **Abstract**

8   The European eel (*Anguilla anguilla*) spawns in the Sargasso Sea, and the leptocephalus larvae are
9   distributed in the Subtropical Convergence Zone (STCZ), covering an area approximately 500 X
10   2000 km in extent. The STCZ is hydrographically diverse and highly dynamic, which is likely to
11   impact growth and survival opportunities of the larvae. Here we investigate the temporal and spatial
12   growth rate variability of larvae collected along seven sampling transects across the STCZ during
13   March-April 2014. Scanning electron microscopic analyses of otolith ring-formations were used to
14   estimate larval age-at-length, initial growth and growth trajectories. Analyzed larvae averaged 14
15   mm in length and 30 days of age. Age estimation indicated that the larvae were spawned during one
16   continuous period, extending from mid-February to late-March, and that spawning had peaked
17   during the new moon of March 4. Growth estimates (average: 0.38 mm d^{-1} , ~6% weight increase d^{-1})
18   ¹) showed substantial variability among individuals. There were no apparent spatial trends in this
19   variability, and no linkage to measures of temperature and salinity, while earlier-spawned
20   leptocephali showed slightly higher growth rates than later-spawned larvae. The variability in
21   growth rates at single sampling sites was as great as variability across the entire area of distribution.
22   This indicates that individual growth opportunities are strongly impacted by variable conditions in

23 the immediate environment while average conditions for larval growth and survival are basically
24 the same throughout the immense area of larval distribution.

25 Running title: Growth rate variability of eel larvae

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

28

29 **Introduction**

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

42 The spawning takes place in an extensive, hydrographically characteristic area of the southern
43 Sargasso Sea (Schmidt, 1925; Schabetsberger *et al.*, 2016) termed the Subtropical Convergence
44 Zone (STCZ). It is approximately located between 70° and 50°W, and is bounded latitudinally by

45 seasonally shifting frontal zones, generally around 24° and 28°N (Schmidt, 1922, 1925; Miller *et al.*,
46 2015). Nutrient entrainment is enhanced in this zone due to strong eddy activity (Richardson &
47 Bendtsen, 2017), providing an increase in primary production and zooplankton abundance that
48 likely enhances growth conditions for the eel larvae (Munk *et al.*, 2010; Andersen *et al.*, 2011;
49 Riemann *et al.*, 2011).

50 Insight into larval eel growth variability is a requisite for understanding the bio-physical linkages
51 influencing their life traits in this oligotrophic, open-ocean environment. Their growth rates have
52 historically been estimated from changes in average body sizes between sampling dates (Schmidt
53 1922, 1935; Boëtius & Harding, 1985), but this procedure does not provide sufficient precision and
54 resolution due to infrequent and incomplete sampling across the relevant areas of distribution
55 (Shinoda *et al.*, 2011). Alternatively, growth rates have been ascertained from ageing of larvae by
56 microstructure analysis of their otoliths, either during their earlier stages, when they are distributed
57 in the southern Sargasso Sea (Castonguay, 1987; Kuroki *et al.*, 2017), or from metamorphosed
58 (glass) eels caught in brackish or freshwater environments close to the European continent
59 (Lecomte-Finiger, 1992; 1997; Arai *et al.*, 2000; Wang & Tzeng, 2000). The glass-eel method,
60 however, suffers from uncertainties in the interpretation of peripheral ring patterns, because the
61 colder environment experienced during this stage might have depressed ring formation (Fukuda *et*
62 *al.*, 2009).

63 Based on otolith microstructure analysis and age-at-length estimations, we here describe and
64 analyze the temporal and spatial growth variability of European eel larvae during their first months
65 after hatching, when they are widely distributed across the southern Sargasso Sea. The study was
66 part of a research initiative, named the Danish Eel Expedition 2014, which focused on
67 environmental conditions and early life characteristics of the European eel larvae in the Sargasso
68 Sea. Given the large hydrodynamic variability at both local and regional scales throughout the area

69 of young leptocephalus distribution, our objective was to ascertain whether some spawning areas or
70 periods were potentially more favorable than others for larval growth. Further we wanted to
71 evaluate the length of the spawning period and relate the timing of peak spawning activity to phases
72 of the moon (ref. “New Moon Hypothesis,” Tsukamoto *et al.* (2003)).

73

74 MATERIAL AND METHODS

75 *Larval collection and identification*

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

84 On board, leptocephali were immediately sorted fresh from the plankton, and screened for presence
85 of European and American eel larvae using myomere counts of the body (*A. anguilla* ≥ 112 , *A.*
86 *rostrata* ≤ 111) in conjunction with the position of the last dorsally directed blood vessel (at
87 myomere number: *A. anguilla* 46-50, *A. rostrata* 44-48). The standard length (SL, see Sørensen *et*
88 *al.* 2016) of potential *Anguilla* larvae were measured to the nearest 0.1 mm and larvae were digitally
89 photographed, before being individually stored in 96% ethanol for later genetic species
90 identification. Remaining plankton samples from each station were also stored in 96% ethanol, and
91 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, $r^2=0.997$). Lengths (SL) of the *A. anguilla* larvae found during screening, of the preserved sample, were 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) = 1.3×10^{-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

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

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

162 ***Physical and chemical parameters***

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

169 **RESULTS:**

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

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

182 ***Otolith analysis***

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

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

202 The 223 SEM-analyzed otoliths had an average of 13.7 increments, of these 208 larvae from 7.2-
203 24.7 mm had from 2-58 growth increments, and 14 larvae in pre-leptocephalus stage and of lengths
204 from 6.3-8.9 mm, had not yet implemented deposition of primary growth. The single, larger *A.*
205 *anguilla* leptocephalus, had 96 growth increments. Overall the individual growth-rings had an
206 average width of 397 nm (range: 252-792 nm).

207 *Age and growth estimation*

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

217 Estimated spawning dates ranged from 19th January to 28th March (Fig 4a). The single larger
218 specimen had an estimated spawning date of 8th December, 2013. From daily spawning intensity
219 estimates, which considered mortality and uneven sampling coverage of later-hatched larvae (Fig.
220 4b), we assessed both mean and medium spawning date to 28th February. The new moon dates in
221 early 2014 over the Sargasso Sea were 31st January, 1st March and 31st March. The spawning
222 intensity of European eels, therefore, appeared to have peaked around the specific new moon event
223 of 1st March 2014.

224 The individual length-at-age estimates showed considerable variability among the larvae
225 investigated, with a length range of ± 2.5 mm for age intervals of 1 day. The large variability was
226 apparent for all sampled larvae (Fig. 5a), and also was observed among specimens from single
227 stations, as exemplified for station 30 (Fig. 5b). For all sampled larvae the relationship between age
228 and length appeared curvilinear, and data were represented by a non-linearly fitted Laird-Gompertz
229 curve (Fig 5a). Growth rates deduced from this curve-fit illustrate their general decline during

230 ontogeny, both for absolute growth in length and for length- and weight-specific growth rates
231 (Table 2). For the first part of the growth curve (e.g. SL < 25 mm) the relationship does not differ
232 markedly from linear, and we used a linear model (Fig. 5c, rate estimate 0.38 mm d⁻¹) for a
233 comparison to results from historical growth studies that have used linear regressions in their
234 growth rate estimates.

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

247

248 **DISCUSSION:**

249 *Otolith formation*

250 Our analysis of larval eel ages and growth required robust interpretation of otolith characteristics.
251 Particularly important were the points selected as indicative of age, the distinctions among different

ring types and the measurements of ring widths. Further, the assumption about the temporal (daily) periodicity in increments was essential.

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

275 returned to their respective continents. Incremental growth zones of glass eel otoliths are often
276 diffuse, zones apparently formed during metamorphosis or during sustained periods in lower-
277 temperature waters (Arai *et al.*, 2000; Cieri & McCleave, 2001, Fukuda, 2009). However, within
278 the eel's larval period, as examined in the present investigation, we expect warmer, weakly variable,
279 environmental conditions, and thus a generally consistent deposition patterns.

280 It is imperative for our interpretation of age, that the otolith growth increments are produced daily.
281 Daily growth ring deposition has been validated for four anguillids: two tropical species, *A.*
282 *marmorata* (Sugeha *et al.*, 2001) and *A. celebesensis* (Arai *et al.*, 2000), and two temperate species,
283 *A. japonica* (Umezawa *et al.*, 1989; Shinoda *et al.*, 2004) and *A. rostrata* (Martin, 1995). It appears
284 that daily periodicity in ring deposition is a common trait among anguillid larvae. The periodicity
285 of increments in otoliths of *A. anguilla* has not yet been validated experimentally, owing to
286 difficulties in rearing their larvae beyond first-feeding (Tomkiewicz *et al.*, 2013; Sørensen *et al.*,
287 2016). Nevertheless, we assume daily ring formation in *A. anguilla* for two reasons: (1) overall
288 otolith microstructure is closely similar among many eel species (Correia *et al.*, 2002; Lee *et al.*,
289 2008; Kuroki *et al.* 2008), including both tropical and temperate anguillids (Leander *et al.*, 2013).
290 (2) Studies by Correia *et al.* (2002) and Ma *et al.* (2005) suggest that leptocephali from regions with
291 relatively warm water, as was the case with specimens in the current study, do have daily otolith
292 increment deposition.

293 ***Age estimates***

294 The present estimates of leptocephalus ages showed high variation at given sampling sites
295 (stations), for example larvae at one station had a more than 50-day age range from youngest to
296 oldest individuals. Castonguay (1987) showed a comparably great age range for *A. anguilla*,
297 specifically 46 days at a single of his sampling stations. These variably-aged groups of leptocephali

298 are not likely to have actively traversed the distances between our sampling stations within the
299 relevant periods of time; they were apparently spawned over extended periods in basically the same
300 water masses. As we see this pattern throughout the vast geographical area of our sampling, the
301 observations imply that spawning is wide-spread in space and time. Thus spawning in the area
302 might not take place as synchronized events by large assemblies of eels, a more individual behavior
303 is indicated by the laboratory observations by Boëtius and Boëtius (1980) of courtship by male
304 *A. anguilla* towards individual females. Further, individual female eels have been shown to spawn
305 several batches of eggs over time (Tomkiewicz & Jarlbæk, 2008), and the larvae from relatively
306 narrow areas could likely stem from such series of separate egg batches spawned by the individual
307 females.

308 The spawning curve estimated from findings of the present study did not support the new moon
309 hypothesis of spawning as it has been formulated for *A. japonica*; i.e. with a restricted spawning
310 event during a few days around new moon (Ishikawa *et al.*, 2001; Tsukamoto 2006, 2009).
311 According to our estimation, the spawning took place during an extended period, however, the
312 median of the spawning curve was close to new moon, and some synchronicity, where the intensity
313 of spawning is inversely related to lunar light intensity, cannot be precluded. Our findings in respect
314 to ages of sampled larvae suggest that the earliest spawning in 2014 took place around mid-
315 February. If there had been spawning earlier in 2014, we would have expected to find some older
316 leptocephali during our investigation. There might, however, be later spawning episodes –
317 potentially related to lunar periodicity – which we did not cover during our period of sampling.
318 Prior field collections of small *A. anguilla* in the Sargasso Sea have indicated spawning throughout
319 the early half of the year (Kleckner & McCleave, 1988; McCleave & Kleckner, 1987).

320 ***Growth estimates***

321 The average estimate of absolute somatic growth rate for larvae <25 mm (0.38 mm d^{-1}) is the same
322 as estimated by Castonguay (1987) in his study, while it is somewhat above the estimate by Kuroki
323 *et al.* (2017) of 0.31 mm d^{-1} . Compared to estimates for other species of the genus *Anguilla* (range:
324 $0.35 - 0.59 \text{ mm d}^{-1}$; Tsukamoto, 1992; Arai *et al.*, 2000; Kuroki *et al.*, 2006, 2007) the estimates for
325 *A. anguilla* are all in the lower range. A number of non-anguillid eels, and elopomorph leptocephali
326 have also been shown to have quite high growth rates (range: $0.63 - 1.42 \text{ mm d}^{-1}$; Crabtree *et al.*,
327 1992; Bishop *et al.*, 2000). The higher growth rates cited were obtained in relatively warm waters,
328 and temperature is likely the main cause of differences among values.

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

337 We anticipated that the hydrographically variable environment of the STCZ would lead to a
338 significant spatial variability in the growth of eel larvae distributed across it. However, we found
339 only a weak spatial trend in one of the two measures we used for larval growth: lengths at age and
340 average width of the first otolith increments. The measure of initial growth rate tended to decline
341 towards the north in the area, a tendency which might relate to the seasonal effect on this index,
342 irrespective that we could not deduce consistent north-south trends in our estimates of average
343 spawning time (Fig. 1c). The part of variability we could ascribe to spatial, temporal or
344 oceanographic measures were in any case minor compared to the prominent, non-explained,

345 variability in larval growth rates at single sites. Large growth variability was seen on a local scale
346 (i.e. within few kilometers) while potential larger-scale changes (10-100 kilometers) did not add
347 further to this variability. It should be noted, however, that the significant mixing of larger water
348 masses due to the prominent eddies could be partly responsible for blurring potential directional
349 tendencies on the actual scale. Along the longitudinal axes the eddy patterns are quite repetitive,
350 and on the mesoscale (~ 100 km) we see basically the same hydrographic patterns from west to east
351 across the area of larval distribution.

352 ***Conclusions and Perspectives***

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

369 larvae leave the STCZ and face other environmental conditions during their drift towards the
370 European continent, and the present findings encourage continued studies incorporating these later
371 phases of the larval life.

372

373

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381

382

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Transect	Sampling period	Longitude (degrees W)	Latitude range of sampled larvae (degrees N)	Larvae analyzed (no)	Standard length (mm)		Otolith diameter (µm)	
					min	max	min	max
1	18/3 - 21/ 3	68° 30'	30° 00'- 25° 00'	28	8.0	18.4	22.6	48.2
2	22/3 - 25/3	65° 30'	28° 30'- 25° 00'	19	7.2	16.5	22.1	39.8
3	26/3 - 5/4	62° 45'	29° 50'- 24° 40'	115	7.3	34.2	23.5	132.8
4	9/4 -10 /4	59° 30'	27° 30'- 25° 40'	16	11.8	19.7	30.1	60.0
5	11/4 -12 /4	57° 00'	28° 20'- 26° 30'	15	8.9	17.5	27.4	39.6
6	13/4 -14 /4	53° 30'	28° 30'- 27° 20'	17	6.3	24.7	27.7	67.0
7	15/4 -16 /4	50° 00'	27° 20'- 26° 20'	13	9.0	19.7	25.5	41.5

554 Table 1

555

556 **Legends to figures**

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

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

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

573 Figure 4. *A. anguilla*. Contributions of leptocephali (n=208) from spawning on back-calculated
574 dates (as percent within a day of year or a calendar date). Thirteen pre-leptocephali not exhibiting
575 otolith growth increments, hence with uncertain spawning dates, and 2 leptocephali with spawning
576 dates on January 20 and December 8 (2013) are not included). Moon phases illustrated with

577 pictograms along the top axis: black circles are new moons, light circles full moons. a) direct back-
578 calculations, b) distributions re-calculated with incorporation of natural mortality and an even
579 sampling coverage

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

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

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

594

Fig. 1.

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b)

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c)

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606 Fig 4

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 611 Fig. 6
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614 [Fig. 7](#)

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