

Electronic Supplementary Material

Spawning by the European eel across 2000 km of the Sargasso Sea

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1. Background, sampling methods, and additional information.

Research on the spawning area of the European eel, *Anguilla anguilla*, in the Sargasso Sea of the western North Atlantic occurred during a time period of about the last hundred years before our two sampling surveys for their larvae (leptocephali) were conducted in 2014. This began with Danish collections of larvae in the North Atlantic and the spawning area that were made by Johannes Schmidt [1-2], and those historical surveys and collection data were also overviewed and reexamined in later years [3,4]. Data from more recent surveys were also examined in combination with Schmidt's historical data, including that of the American eel, *A. rostrata*, which spawns in the southwestern Sargasso Sea in an overlapping area with the European eel [5,6]. All the survey collections and other catch data of American and European eel larvae obtained throughout the North Atlantic up to 2007 were then combined into a comprehensive database (used in main paper figure 2b,d for a specific area and time) that was analyzed in a recent monograph that also included an overview of the general history of the surveys for eel larvae [7].

As overviewed by the monograph [7] and a quantitative analysis of individual survey data [8], the next major research efforts after Schmidt's Sargasso Sea surveys in 1920–1922 [1,2] were conducted by independently organized German and American research groups in the spawning area from 1979 to 1989 [9-13]. Those collection data resulted in the formulation of the hypothesis that the northern margin of the spawning areas of both the American and European eels were defined by the northernmost of two fronts that form within the Subtropical Convergence Zone of the Sargasso Sea to the south of Bermuda [12]. These two fronts form at the same temperatures and densities each year in the spawning season during February to April [13]. The density gradients at these fronts can result in eastward frontal-jet countercurrents [14-16] that have been hypothesized to be able to transport marine eel leptocephali offshore into the Sargasso Sea from the west [13] or transport European eel leptocephali eastward [7,17]. The overall flow field within the spawning area may consist mostly of westward moving mesoscale eddies [18].

Each survey of the German and American research groups sampled for leptocephali in particular areas or in transects across one or both of the fronts [9-13], but there was never a systematic sampling grid set across the possible width of the spawning area, even if all the surveys were combined together from different years (see figure 6 of Miller et al. [7]), or combined with Schmidt's stations (see figure 2 of Westerberg et al. [8]). All of the catch data however, was consistent with spawning occurring south of the northern front in the warmer water mass. When the catch data of the smallest size classes (< 10 mm) from all the surveys was combined it showed

a pattern quite similar to Schmidt's original concept of the spawning area of the two Atlantic eel species [1,2], with the American eel spawning in the west, and the European eel spawning in the east with some overlap in where their small larvae were collected [7].

In an overlapping time period with the German and American surveys, starting in the late in the late 1970's, the panmictic populations [19-21] of the northern hemisphere anguillid eels (European, American, and Japanese eel, *A. japonica*) started showing signs of population declines. Eventually recruitment of the European eel to especially the northern part of its range reached such low levels that concern was raised among scientists and managers and efforts began to try to understand the cause of the declines and to work towards conservation measures [22,23]. There were obvious anthropogenic impacts on the eel populations from habitat loss (dams, filling in wetlands) and degradation, overfishing, or parasite introductions, but possible links to ocean-atmosphere changes in the ocean were also apparent, as was reviewed recently [24]. The European eel and the two other northern hemisphere species were all eventually listed as endangered [25], in part due to the uncertainties about what was causing the declines. In 2007 the EU introduced a recovery plan for the European eel [26] that required all member states to implement management plans, which included a primary objective of increasing the escapement of silver eels migrating to the spawning area to 40% of historical levels.

In this context, attention turned back to the Sargasso Sea spawning area to evaluate if part of the problem might be a lack of spawning eels or poor larval survival in the ocean after regime shifts had occurred. For example, fluctuations of the North Atlantic Oscillation Index (NAO) showed correlations with European eel recruitment or abundance in time series data sets, such as in the study by Friedland et al. [27]. In 2007, a Danish survey was conducted in the Sargasso Sea spawning area that examined the distribution of European eel leptocephali [17] and also studied the biological oceanography across the frontal zone [28-30] and the species composition of fish larvae [31]. In 2011, Germany began an every-3-year survey plan to monitor the larval abundance of the European eel in its spawning area during March and April, which is a season when larval abundances had been high in previous years. Larval abundances in 2011, however, were found to be significantly lower [32] compared to during the 1983 and 1985 American surveys [12]. In contrast, the typical species of marine eel leptocephali that are present in the Sargasso Sea during that season [13] did not show lower abundances [32]. A more detailed analysis of historical survey larval-catch densities in comparison to recent surveys, also concluded that larval abundances have declined and are much lower than historically [8].

In 2014, the second of the German monitoring surveys was scheduled, and a separate survey by the Danish team that conducted the 2007 survey was also independently scheduled. Once this unique opportunity of two research ships having surveys at the same time was realized, alternating transect lines were assigned to each ship to enable the entire estimated spawning area to be sampled in a short period of time, which formed the basis of the present study.

Each of the two surveys were separately planned with different objectives, sampling gear and time constraints, with both ships then moving to the Azores Islands to the northeast (main paper figure 1). The German survey using the fisheries research vessel FR/V *Walther Herwig III* was designed to replicate the sampling techniques used in the 2011 survey [32,33], which were based directly on the sampling methodology of the American 1983 and 1985 surveys for leptocephali that fished with an Isaacs-Kidd Midwater Trawl (IKMT) [12]. The German sampling in 2014 was conducted using an IKMT with a 6.2 m² mouth opening, a length of 10 m, and 500 µm mesh (Hydro-Bios Apparatebau GmbH). This type of midwater plankton trawl has a spreader-bar above the front of the net and a heavy depressor attached to the bottom of the net, which both work to keep the mouth of the net open while being connected to the ship's trawl wire. The IKMT was deployed using double oblique tows with maximum depths of 300 m during night and day, which replicates the fishing method of the earlier surveys by Kleckner and McCleave [12] as described previously [32,33]. This depth range of fishing was originally chosen because the smallest sizes of anguillid larvae can be widely distributed within the upper 300 m during day and night in the Sargasso Sea [34]. Temperature, conductivity, depth (CTD) profiles were recorded to depths of 500 m at most stations where the IKMT was deployed. All *Anguilla* and marine eel leptocephali collected during the Walther Herwig survey were measured and preliminarily identified onboard according to Smith [35]. The anguillid leptocephali were then definitively identified to a final species designation by analyzing their mitochondrial 16s rRNA gene sequences (one directional, approximately 600 base-pairs long) in comparison to aligned reference sequences of *A. rostrata* and *A. anguilla*, and 18S rDNA RFLPs were used to detect hybrids [36; with some enzyme modifications, 37]. The 2014 larval catches were used in a transport modelling study [38] and to compare larval densities among time periods in the Sargasso Sea [8].

The Danish survey was conducted on the R/V *Dana* using a 9.6 m² mouth-opening ring net with 560 µm mesh, as was used in their previous survey [17]. This type of ring net has cables attached to the metal ring that forms the mouth of the net, which are attached to the trawl wire of the ship. The ring net was fished in oblique tows to 200 or 250 m, or tows that fished the net

horizontally at one of 6 different depths for 1 hour. CTD profiles were made to 400 m at most sampling stations. The collected leptocephali were initially sorted out onboard and later identified using PCR of their mitochondrial cytochrome b sequences [39] according to Trautner [40]. Other types of sampling collections at stations were not used in the present study. These included studies of the vertical distribution of leptocephali and other fish larvae [40], analyses of spatial variability in European eel leptocephali age and growth using their otolith microstructure [41] and analyses of leptocephali gut contents for genetic comparison to environmental plankton and marine snow composition [42]. European eel (larvae) DNA was also detected in the stomach contents of common mesopelagic fishes collected during the survey [43]. The R/V *Dana* made a brief stop in the port of Bermuda to exchange scientists before returning directly to the sampling grid.

Both surveys used sea surface temperature (SST) data to estimate the latitudinal locations of the frontal positions, that were obtained from various sources by being downloaded to the ships by satellite internet/email connections during the surveys. The northern front is associated with the 22°C temperature isotherm or the 25.6 density isopycnal and the southern front is linked to 24°C and 25.0 density [13]. This was used to determine the latitudes of sampling, with some transects crossing the northern front to obtain negative data from where there were likely few or no larvae present (2 westernmost transects), but most stations were located where larvae should be present. This strategy was successful during both surveys because the same size of larvae was collected in the alternating, latitudinally overlapping transects sampled by the two ships (figure S1), and larvae were collected in all of the transects (figure S2) except for the two easternmost stations at 49°W (figure 1). The conductivity, temperature, depth (CTD) profiles made at most of the stations of both surveys were used to plot horizontal and vertical sections of temperature and salinity using the Ocean Data View (ODV) program (<https://odv.awi.de/>). Additional hydrographic data were included from ARGOS profiling floats (<http://www.argo.ucsd.edu/>) that were present in the area south of Bermuda at the time of the surveys, which allowed the geographic coverage of the hydrography to be extended beyond our station grid. The SST image used to make figure 1a was obtained from the NOAA/NESDIS Geo-Polar 5 km analysis (<https://www.ospo.noaa.gov/Products/ocean/sst/contour/>). Horizontal sections of temperature are shown at 50 m (within the pool of warmer surface water where the larvae reside at night [34]) and 150 m near possible spawning depths inferred from Japanese eel egg and preleptocephali collections in the Pacific [44,45]. European eel leptocephali were categorized into several size classes for plotting in figure 1b,c that were ≤ 12 mm (12.9 mm and smaller), ≤ 7 mm (7.9 mm and

smaller), 8 mm (up to 8.9 mm), and the smallest individual larva from both surveys (5.5 mm). The larvae were widely distributed south of the northern front (figure 1b) and were also present south of the southern front (figure S3). The length increased with eastward longitude and the linear regression had a slope of 0.17 mm/degree (Pearson's $r = 0.25$). The length increase with day of year was 0.12 mm/day (Pearson's $r = 0.30$) (figure S2). A multiple linear regression shows that time explains most of the increase in length. A t-test for the regression coefficients give $p = 0.8$ for longitude and $p = 0.0003$ for day of year.

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3) Supplementary figures.

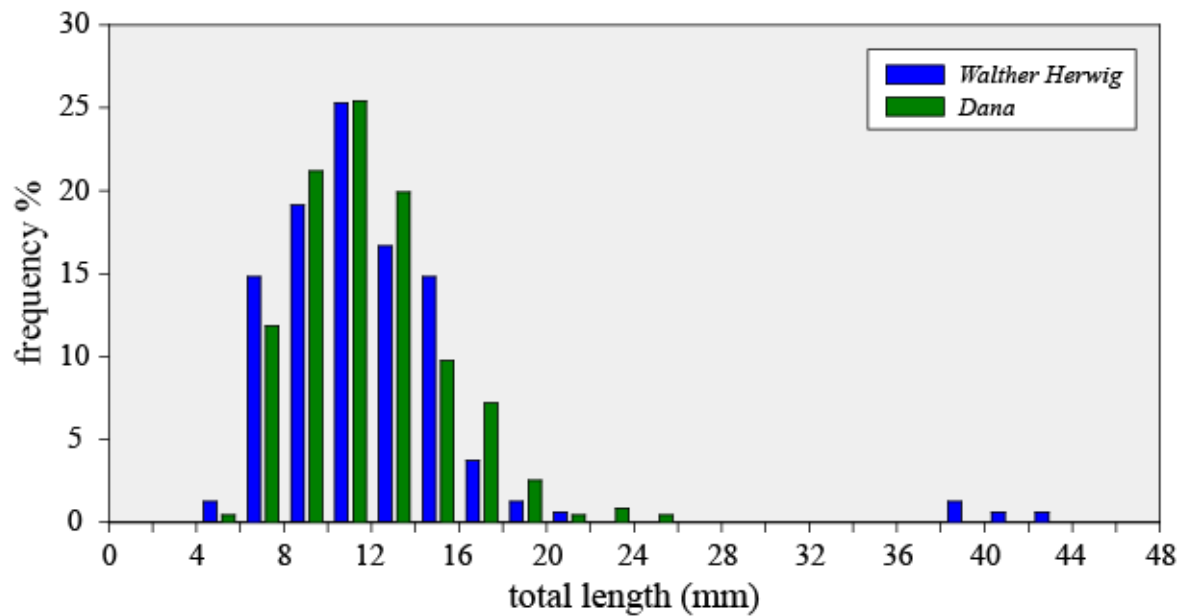


Figure S1. Length frequency distribution of the European eel, *Anguilla anguilla*, leptocephali collected by the two sampling surveys in March and April 2014 in the Sargasso Sea that were conducted by the FR/V *Walther Herwig III* (Germany) and the R/V *Dana* (Denmark). Four larger larvae not from recent spawning that were collected are included, but they are not shown in figure 2 of the main paper and were not included in the analysis of figure S2.

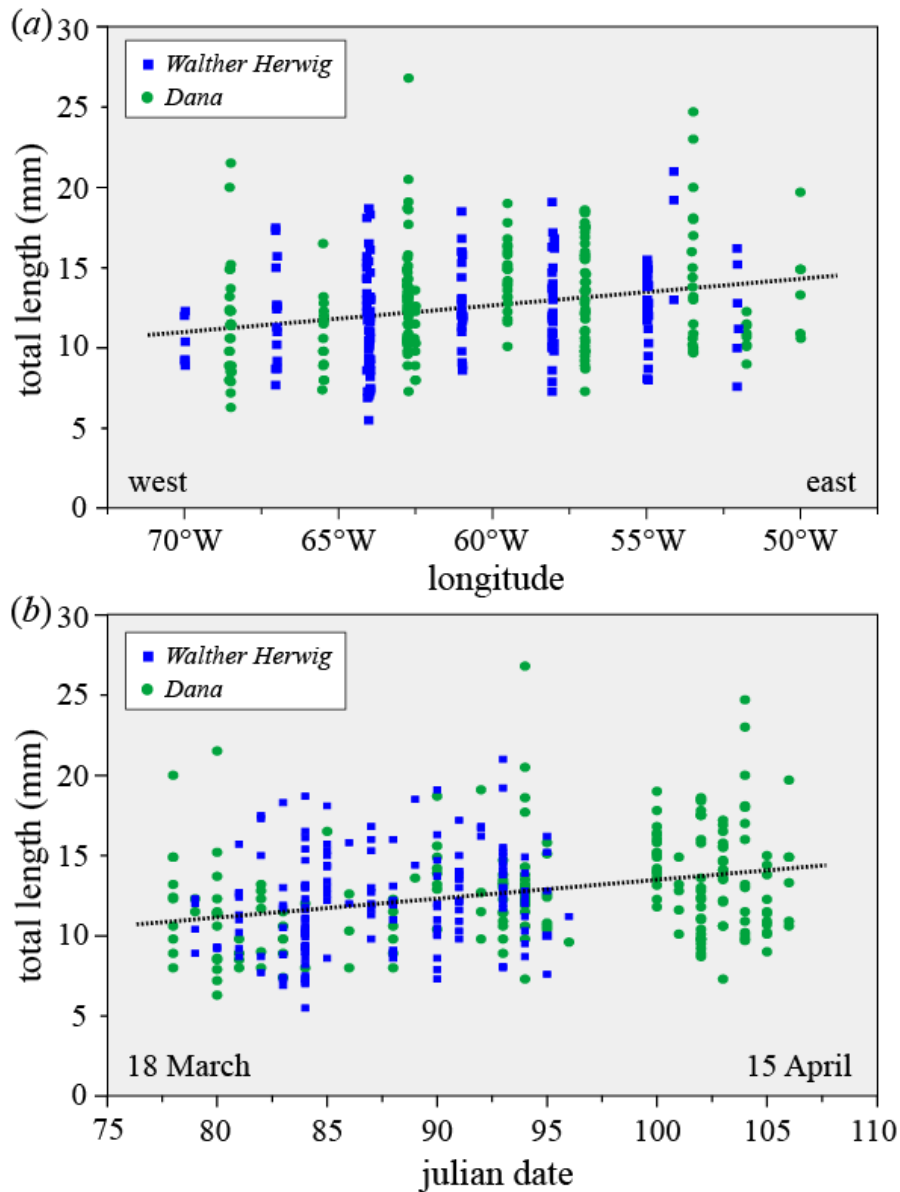


Figure S2. Lengths of the individual leptocephali of the European eel, *Anguilla anguilla*, collected by the two surveys plotted by (a) longitude, and (b) collection date, with the first and last capture dates shown. The regression lines show that the increase in size from west to east probably was due to growth of the larvae and also an apparent reduction of spawning activity in April as the two surveys moved east. The R/V *Dana* survey entered the eastern area several days after the FR/V *Walther Herwig III* survey.

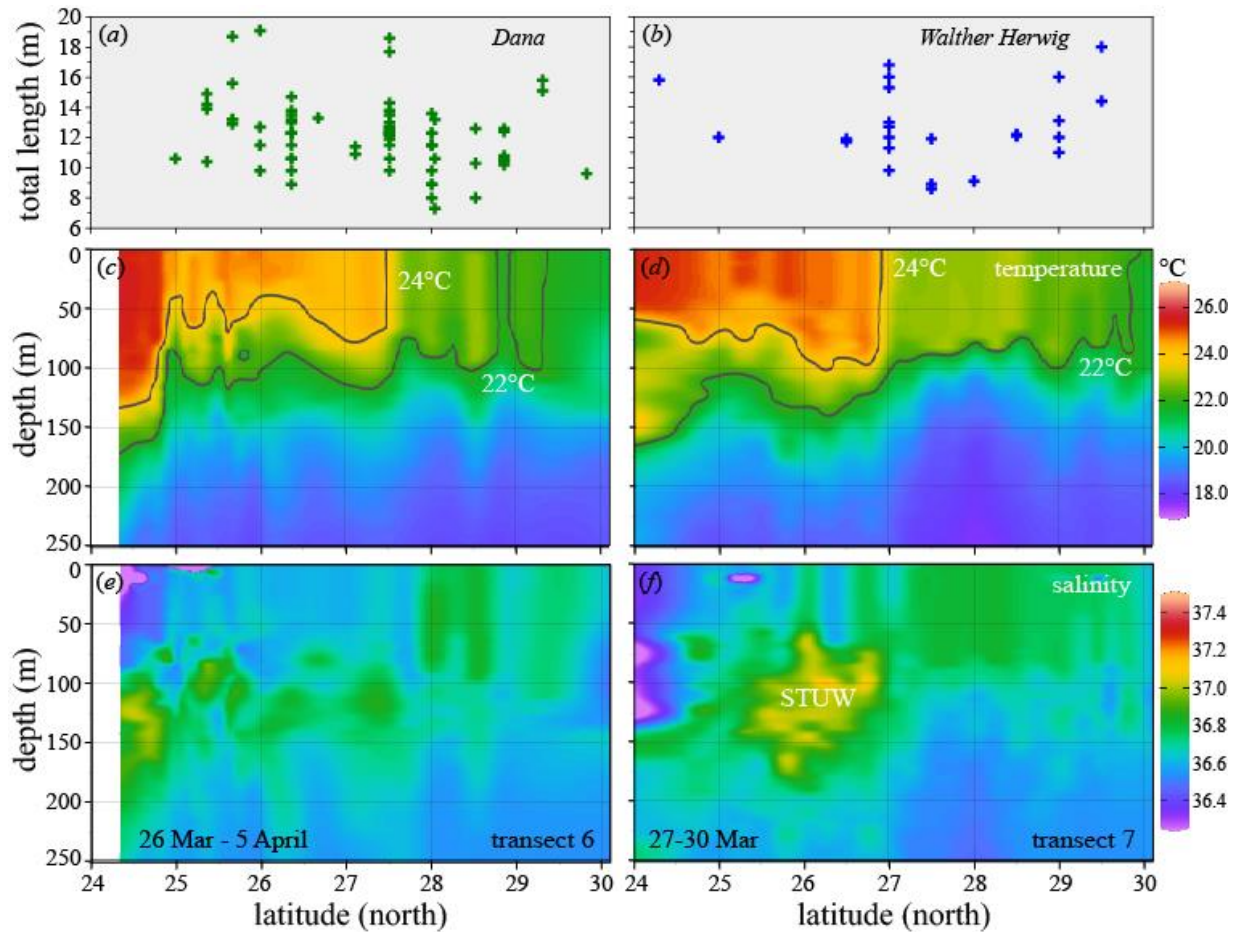


Figure S3. Distribution and size of leptocephali of the European eel, *Anguilla anguilla*, in relation to the upper 250 m hydrography in the two long transects in the central spawning area that were sampled by the R/V *Dana* along about 62.7°W (left panels) and the FR/V *Walther Herwig III* along 61°W (right panels) showing (a, b) lengths of the individual leptocephali collected in each transect plotted by latitude of sampling stations, (c, d) vertical temperature sections showing the 22 and 24°C isotherms that are associated with temperature/density fronts, and (e, f) salinity, with the subsurface core of Subtropical Underwater (STUW; see Schabetsberger et al. [46]) being more distinct in the eastern transect (f).