

Discovery of a spawning ground reveals diverse migration strategies in Atlantic bluefin tuna (*Thunnus thynnus*)

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Atlantic bluefin tuna are a symbol of both the conflict between preservationist and utilitarian views of top ocean predators, and the struggle to reach international consensus on the management of migratory species. Currently, Atlantic bluefin tuna are managed as an early-maturing eastern stock, which spawns in the Mediterranean Sea, and a late-maturing western stock, which spawns in the Gulf of Mexico. However, electronic tagging studies show that many bluefin tuna, assumed to be of a mature size, do not visit either spawning ground during the spawning season. Whether these fish are spawning in an alternate location, skip-spawning, or not spawning until an older age affects how vulnerable this species is to anthropogenic stressors including exploitation. We use larval collections to demonstrate a bluefin tuna spawning ground in the Slope Sea, between the Gulf Stream and northeast United States continental shelf. We contend that western Atlantic bluefin tuna have a differential spawning migration, with larger individuals spawning in the Gulf of Mexico, and smaller individuals spawning in the Slope Sea. The current life history model, which assumes only Gulf of Mexico spawning, overestimates age at maturity for the western stock. Furthermore, individual tuna occupy both the Slope Sea and Mediterranean Sea in separate years, contrary to the prevailing view that individuals exhibit complete spawning-site fidelity. Overall, this complexity of spawning migrations questions whether there is complete independence in the dynamics of eastern and western Atlantic bluefin tuna and leads to lower estimates of the vulnerability of this species to exploitation and other anthropogenic stressors.

ichthyoplankton | Scombridae | large pelagic fish | pop-up satellite archival tag | population structure

Long-distance migrations pose a unique challenge to fisheries management, as conservation actions taken on a regional scale can be undermined if less stringent measures are implemented across other parts of the migratory pathway. Few species exemplify this problem better than Atlantic bluefin tuna (*Thunnus thynnus*). This species is harvested by the fisheries of over 20 nations, from the tropics to subarctic and coastal to international waters. Contentious international disputes have persisted for decades over how many bluefin tuna to harvest and how to allocate catch among nations. By the start of the 21st century, intense fishing pressure had driven this species to historically low population levels, a decline that has since reversed as fishing mortality has decreased under stricter management (1). However, despite this recent positive trend, many challenges remain in developing an ecologically sustainable fishery for bluefin tuna that also provides economic and social benefits to the fishing communities throughout its range. Among the most prominent of these challenges is the need for stock assessment models and management regulations that better account for the complex movements of this species.

The movements of Atlantic bluefin tuna are among the best documented of any highly migratory species, but how to interpret these migrations within the broader context of life history and population structure remains controversial. Currently, Atlantic bluefin tuna are assessed by the International Commission for the Conservation of Atlantic Tunas as an eastern stock, which spawns in the Mediterranean Sea, and a western stock, which spawns in the Gulf of Mexico. Based on sampling on these two spawning grounds, the eastern bluefin tuna stock assessment uses an age at 50% maturity of 4 y and the western bluefin tuna stock assessment uses a “knife-edge” age at maturity (i.e., all fish reach maturity at the same age) of 9 y. Electronic tagging shows that many bluefin tuna much older than these estimated ages at maturity do not occupy either known spawning ground during the spawning season (2–5). This contradiction has been attributed to fish not maturing until an older age than assumed in the assessment (age at 50% maturity: eastern fish, 6–10 y; western fish, 14–16 y) or not spawning every year (6–8). Alternatively, energetic and life history modeling (9), reproductive studies (10–12), and analyses of tag data (3, 4) provide evidence for undocumented spawning grounds, and an age at 50% maturity of 4–5 y throughout the Atlantic. These two depictions of bluefin tuna life history have vastly different implications for management. The documentation

Significance

We present unequivocal evidence that Atlantic bluefin tuna spawn in the Slope Sea, counter to the current assumption that the Gulf of Mexico and Mediterranean Sea are the exclusive spawning grounds. We also demonstrate that age at maturity of western bluefin tuna is currently overestimated, that this stock exhibits size-structured spawning migrations, and that migratory connections exist between western and eastern Atlantic spawning grounds. Atlantic bluefin tuna support a highly contentious international fishery, and our results present an alternate life history model to inform the management of this species. The implications of our work are most pronounced for western Atlantic bluefin tuna, which have a life history less vulnerable to overexploitation and extinction than is currently estimated.

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of an additional spawning ground would require that bluefin tuna population structure be reevaluated, possibly leading to different conclusions concerning the suitability of proposed and implemented spatial management strategies. Additionally, populations that mature late and spawn in restricted areas are considered more vulnerable to overexploitation and environmental change than earlier maturing populations with broader spawning distributions (13, 14).

Over 40 y ago, an area named the Slope Sea, north of the Gulf Stream and south of the northeast US continental shelf (15), was suggested to be an Atlantic bluefin tuna spawning ground (10, 16). The primary support for this hypothesis came from an exploratory longline cruise in this area from June to July 1957 that found bluefin tuna in spawning condition (10, 17). Recent reproductive studies on adjacent foraging grounds in the Gulf of Maine (11, 12), electronic tagging data analyses (2, 4), and energetic modeling studies (9) provided further circumstantial evidence for spawning in this area. However, targeted surveys for bluefin tuna larvae in the Slope Sea were never performed, and most research over the past few decades has dismissed the idea that substantial levels of spawning occur in the western Atlantic outside of the Gulf of Mexico. Here, we use opportunistic ichthyoplankton sampling to present unequivocal evidence that the Slope Sea is an important bluefin tuna spawning ground. We then use this information, coupled with electronic tagging, to reinterpret the life history, migration pathways, and population structure of Atlantic bluefin tuna.

Results and Discussion

We found larval bluefin tuna in the Slope Sea demonstrating an additional western Atlantic spawning ground (Fig. 1A). A total of 67 bluefin tuna larvae was collected during sampling from June 23 to August 9, 2013, across a broad area of the western Slope Sea (Fig. 1B and Tables S1 and S2). Diagnostic morphological characters were used to identify each of these larvae to species, with the identity of 18 larvae, including 10 fixed in formalin, verified using genetic sequencing (Fig. 1C and D, and Figs. S1 and S2; details are given in SI Text). Sequence from one additional larva, identified morphologically as a bluefin tuna, was consistent with albacore (*Thunnus alalunga*). This larva was not necessarily misidentified, as ~3% of bluefin tuna from the Mediterranean Sea contain introgressed albacore mitochondrial DNA (7).

Nearly all larvae collected in the Slope Sea were unequivocally spawned in the Slope Sea, rather than being transported into the area from the Gulf of Mexico. Larval sizes and published growth rates (18) indicate that about 40% of the larvae were spawned in July when adult bluefin tuna are not present in the Gulf of Mexico (6). Additionally, >60% of the larvae were ≤ 3.0 -mm standard length (SL), and were thus spawned within 6 d of collection (18). Based on an analysis of satellite-tracked drifters (details are given in SI Text), the minimum transport time from the easternmost point in the Gulf of Mexico to the southernmost latitude of the Slope Sea is 10.5 d, with less than 25% of drifters covering this distance in fewer than 20 d (Fig. S3).

Our results indicate that the length and age at maturity for western Atlantic bluefin tuna has long been overestimated due to an incomplete understanding of the full distribution of spawning. Currently, a knife-edge maturity of 190-cm fork length (FL) (age, 9) is used in the assessment based on the smallest mature individual found in the Gulf of Mexico. Electronic tagging data shows that larger fish undertake extensive annual migrations between the Gulf of Mexico in the winter and spring and Atlantic Canada in the summer and fall, whereas smaller fish undertake shorter migrations between the North Sargasso Sea and the northeast United States continental shelf (Fig. 2A–C). Only the largest individuals migrate into the Gulf of Mexico, with just 50% doing so by 240-cm FL (age, 15) (Fig. 2D). Potential Slope Sea spawners were classified as those fish that spent ≥ 20 d in the Slope Sea from June 1 to August 15; 20 d was chosen based on estimates of bluefin tuna spawning duration (8, 19). Over 75% of individuals 133- to 212-cm FL (age, 5–11) were classified as potential Slope Sea spawners (Fig. 2D). The difference in tuna size structure on the two spawning grounds during the spawning season is also evident in longline catch data (Fig. S4).

Our assertion of a younger age at maturity for western Atlantic bluefin tuna is supported by three additional lines of evidence. First, endocrine measurements indicated that all >131-cm FL (age, 5) fish caught in the Gulf of Maine, an area adjacent to the Slope Sea, were mature (11). Second, microscopic examination of gonads sampled in the Gulf of Maine found that females 185- to 235-cm FL (age, 9–14) had atretic follicles in June and July, indicative of recent and proximate spawning, whereas fish >235-cm FL (age, 15+) had primary-stage oocytes indicative of earlier and

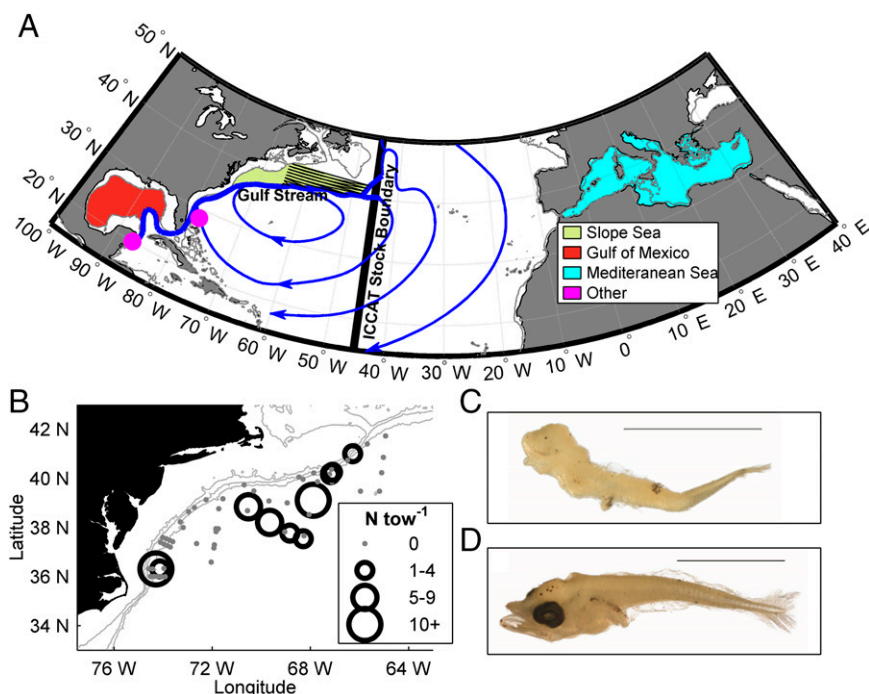


Fig. 1. Distribution of Atlantic bluefin tuna spawning and larvae. (A) Known spawning regions in the Gulf of Mexico, Mediterranean Sea, and Slope Sea. Hatched area of the Slope Sea was not sampled. A few larvae have also been collected in the Yucatan Channel and Blake Plateau (22, 23). Blue arrows indicate general circulation patterns. (B) Collection locations of larvae in 2013. (C and D) Genetically identified formalin-fixed bluefin tuna larvae collected in the Slope Sea. (C) GU1302-Station 141-Fish 3; 2.3-mm SL; GenBank accession no. KT285186. (D) HB1303-Station 084-Fish 2; 3.9-mm SL; GenBank accession no. KT285188. (Scale bars: C and D, 1 mm.) Background debris has been digitally removed from images.

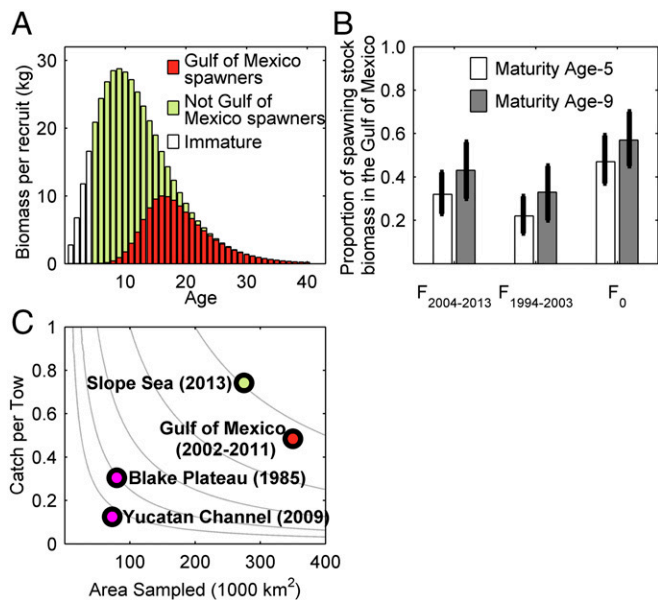


Fig. 3. Estimated proportion of western Atlantic spawning that occurs in the Gulf of Mexico. (A) Relative biomass of Gulf of Mexico spawners using the 2004–2013 average estimated fishing mortality rate and the age pattern of Gulf of Mexico migration (Fig. 2D). (B) Sensitivity of the proportion of Gulf of Mexico spawning to different ages at maturity and levels of fishing mortality (medium: 2004–2013; high: 1994–2003; F_0 : no fishing). CIs (95%) are based on the uncertainty in the proportion at age migrating to the Gulf of Mexico. (C) Relative total larval abundance (a product of $N \text{ tow}^{-1}$ and area sampled), in the Gulf of Mexico (2002–2011) (21), the western Slope Sea (2013), the Yucatan Channel (2009) (23), and the Blake Plateau (1985) (22). Each contour line represents a doubling of total larval abundance. All sampling used the same protocol.

24), and these features are hypothesized to enhance larval survival (25). The optimal 23–28 °C temperature for bluefin tuna spawning (24) occurs about 2 mo later in the Slope Sea versus the Gulf of Mexico, and the differences in timing of spawning are consistent with the difference in timing of optimal temperature (Fig. 4).

Size-structured migrations, a type of differential migration, are common in the animal kingdom (13), but the partitioning of both spawning and feeding areas is comparatively rare and less well explored. The ability of larger fish to swim faster and at less relative energetic cost than smaller fish (9, 13), provides larger fish more flexibility in spawning location choice, but on its own does not explain if and how these fish benefit from spawning in the Gulf of Mexico rather than the Slope Sea. One possibility is that large fish are able to arrive at northern feeding grounds earlier in the summer (10) by taking advantage of their fast swimming speed and the 2-mo difference in the timing of optimal spawning temperatures between regions (Fig. 4). Alternatively, the Gulf of Mexico may provide better feeding or reduced predation for larvae, or the earlier spawning time may allow juveniles to achieve a larger size at the end of the first year, factors that may increase survival through the early life stages.

The discovery of the Slope Sea spawning ground requires a reevaluation of the nature and levels of mixing between the eastern and western Atlantic stocks. Otolith stable isotopes have indicated that bluefin tuna exhibit high levels of natal homing to eastern or western Atlantic spawning grounds (26), a conclusion generally supported by genetic analyses of fish from the Mediterranean Sea and Gulf of Mexico (7). The absence of tagged fish moving between the Gulf of Mexico and Mediterranean Sea has also previously been used to support the hypothesis of complete reproductive isolation between the two stocks (5, 7). However, some ~200-cm FL fish have migrated to the Mediterranean Sea after an extended period of western Atlantic residency. These fish exhibited the same seasonal migration as similar-size fish in our study (Fig. 2B), including the occupation of the Slope Sea

during the spawning season (2, 5, 27). These migratory tracks suggest that reproductive mixing between the eastern and western stocks may occur in the Slope Sea and that the population structure of bluefin tuna may be more complex than is currently depicted (4, 28). To fully evaluate bluefin tuna population structure, biological samples from spawning fish and larvae collected in the Slope Sea need to be included in future analyses.

Our results have four important implications for the assessment and management of Atlantic bluefin tuna. First, the western Atlantic bluefin tuna stock assessment should use a younger age at maturity (11). Lowering the age at maturity will increase estimates of spawning stock biomass and will likely lead to higher estimates of sustainable fishing mortality rates (14, 29, 30). Second, analyses of the vulnerability of Atlantic bluefin tuna to climate change (31), the *Deepwater Horizon* oil spill (32), as well as the location of fishery closures to protect spawning fish, assume that the Gulf of Mexico and Mediterranean Sea are the only spawning grounds. These quantitative analyses and decisions need to be revisited. On a conceptual level, a diversity of migration strategies exposes a population to a variety of environmental conditions, and should confer added long-term stability in the face of climate and ecosystem variability (13). Third, the level and size selectivity of fishing mortality drives the ratio of spawning in the Gulf of Mexico versus the Slope Sea. Determining the relative quality of these two regions as nursery habitat is important for understanding long-term recruitment variability. Fourth, estimates of the nature and extent of mixing from tagging data need to be reevaluated to account for Slope Sea spawning. Spatially explicit population models show that changes in the distribution of catch can help achieve management goals, assuming levels of mixing in different areas of the ocean are known (33).

Overall, the discovery of a bluefin tuna spawning ground highlights the need to further integrate traditional shipboard sampling with electronic tagging studies in testing many of the long-held assumptions that underlie the management of this iconic species. Two priorities for field studies on the Slope Sea spawning ground are to evaluate how consistent the 2013 distribution and abundance of larvae is in additional years, and to refine information on the reproductive status of different size classes of fish. More broadly, this work reveals how limited plankton sampling has been in the open ocean, and of this sampling, how little has been analyzed with the taxonomic expertise necessary to resolve spawning by economically valuable fishes. The possibility that there are additional undocumented bluefin tuna spawning grounds should continue to be evaluated.

Methods

Ichthyoplankton Sampling. Two National Oceanic and Atmospheric Administration (NOAA) Northeast Fisheries Science Center (NEFSC) cruises sampled ichthyoplankton in the Slope Sea in 2013. From June 9 to June 24, 2013, an

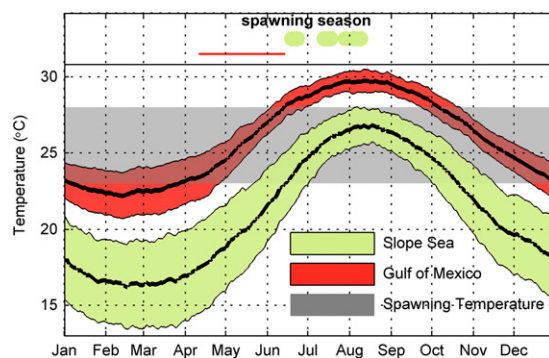


Fig. 4. Mean (\pm SD) SST cycles across the bluefin tuna spawning grounds in the northern Gulf of Mexico and southwestern Slope Sea. Spawning temperatures (23–28 °C) for bluefin tuna are denoted in gray. The reported timing of spawning is based on available larval collections in the Gulf of Mexico (24) and Slope Sea.

Ecosystem Monitoring (ECOMON) cruise on the NOAA Ship *Gordon Gunter* (GU1302) sampled the northeast US continental shelf using a random stratified design (34). Four offshore transects into the Slope Sea from June 21 to June 23, 2013, were added to this cruise. The second cruise occurred from July 1 to August 18, 2013, on the NOAA Ship *Henry B. Bigelow* (HB1303). This cruise was part of the Atlantic Marine Assessment Program for Protected Species (AMAPPS), which evaluates the abundance and distribution of marine mammals, seabirds, and sea turtles. Plankton sampling on HB1303 was scheduled around the visual surveys for protected species. For our study, we only considered stations offshore of the 1,000-m isobaths on both cruises; these stations occur outside of the area on the continental shelf and shelf break that is typically sampled by the NEFSC.

Plankton was sampled at most stations with a double-oblique tow of a 61-cm-diameter bongo net equipped with 333- μ m mesh nets on each side of the frame (34). The net was deployed to 200-m depth at stations off the continental shelf. The ship's speed through the water during the plankton tows was \sim 1.5 kn (2.8 km/h), and 300–400 m³ of water was filtered for tows to 200 m. A 1-m² multiple opening/closing net and environmental sampling system (MOCNESS) was deployed at additional stations during the HB1303 cruise. Details of ichthyoplankton sample processing, morphological and molecular approaches to larval bluefin tuna identification, and full station data and larval bluefin tuna counts and measurements (Tables S1 and S2) are available in *SI Text*.

Oceanographic Data. In situ oceanographic data were collected with a Seabird Electronics SBE Model 19+ V2 profiling CTD (conductivity, temperature, depth) attached above the bongo net, or directly by the MOCNESS sensors (Table S1). All data collected by the CTD have been uploaded to the National Oceanographic Data Center (<https://www.nodc.noaa.gov/>) and can also be accessed at the NEFSC ftp site (<ftp://ftp.nefsc.noaa.gov/pub/hydro/>). Seasonal cycles of sea surface temperature (SST) for the Slope Sea and Gulf of Mexico spawning grounds were developed using the NOAA 1/4° daily optimal interpolation SST (OISST) data (<https://www.ncdc.noaa.gov/oisst>) (35) from 1982 to 2014 in the areas of high larval abundance in the southwestern Slope Sea (south of 38.5°N and west of 65°W; north of the mean Gulf Stream position) and the northern Gulf of Mexico (26–28°N, 95–85°W).

Electronic Tag Deployment and Data Processing. Electronic tagging of Atlantic bluefin tuna was conducted from 2002 to 2014, with >90% of tags deployed during the months of July to November. Full details of different tagging campaigns, tagging protocols, and tag functionality are described elsewhere (3, 4, 36, 37). The majority of the deployed tags were pop-up satellite archival tags (PSATs), which are designed to release from fish after a pre-determined length of time and transmit data via satellite [Microwave Telemetry, Inc., models PTT-100 ($n = 348$) and X-Tag ($n = 219$); and Wildlife Computers models Mk10 ($n = 10$) and MiniPAT ($n = 19$)]. Most PSATs were programmed for 1-y deployments. Additionally, 132 implanted archival tags were deployed [Wildlife Computers MK-9 ($n = 20$); Lotek LTD 2310 ($n = 82$) and LTD 2350 ($n = 30$)]. This tagging approach requires the recapture of the fish and the return of the tag. Four archival tags were recovered.

Position estimates from electronic tags use light-based geolocation that require measurements of day length and time of sunrise and sunset. Position estimates were refined using a state-space Kalman filter that also incorporates SST and depth (4, 38–40). Geolocation analysis was carried out using the R statistical software, except for tagging years 2002–2006, which were completed by Collecte Localisation Satellites (CLS) using proprietary software.

Electronic Tagging Analysis. We characterized the annual migrations patterns of two size classes (>220- and \leq 220-cm FL) of bluefin tuna using electronic tagging data. Tag locations were assigned to one of six regions in the western Atlantic or a seventh region encompassing the eastern Atlantic (Fig. 2). Boundaries among regions followed meridians of longitude, parallels of latitude, or bathymetric contours, with the exception of the Slope Sea, which was defined as a polygon with (i) the southern boundary formed by the mean location of the Gulf Stream to the bifurcation point at 47°W (41); (ii) the northwestern boundary formed by the 500-m isobath from Cape Hatteras, NC, to 62°W; and (iii) the northeastern boundary separating Slope Sea water from Labrador Sea water formed by a line between 43°N 62°W and 42°N 46°W (15). The first 30 d of locations were excluded from the analyses to limit the influence of tag deployment location. The proportion of locations within each region was calculated for each day of year. A total of 212 fish <220-cm FL and 104 fish >220-cm FL were included in the analysis, although the number of fish with active tags varied by day of year. The most tag locations were available for December and the least for September.

Electronic tagging data were used to characterize the size structure of bluefin tuna that were potential Slope Sea and Gulf of Mexico spawners. Our focus was on western Atlantic spawning, and thus we did not consider tagged

bluefin tuna that were resident in the eastern Atlantic (east of 45°W) for the entirety of both the Gulf of Mexico and Slope Sea spawning seasons (April to August). Fish with tags attached through at least April 30 were classified as potential Gulf of Mexico spawners if they visited waters west of 81°W during any time from March to June. For Slope Sea spawners, we only included fish in the analysis if the tag remained attached through at least July 15. Most (>95%) of the tagged fish occupied the Slope Sea at some point during the spawning season, including many that rapidly passed through the area during their migration north from the Gulf of Mexico to the United States or Canadian continental shelf. We considered a bluefin tuna a potential Slope Sea spawner if it occupied the Slope Sea for \geq 20 d from June 1 to August 15. The 20-d duration was based on published reports that bluefin tuna have a spawning period of 18 d (7 d SD) in the Gulf of Mexico (8) and 23.9 d (range, 19–31 d) in the Mediterranean Sea (19).

We fit polynomial logistic functions to characterize the proportion of fish at length classified as potential Slope Sea spawners and potential Gulf of Mexico spawners. The Akaike information criterion was used to select between a first-order ($P = 1/[1 + \exp[-(b_0 + b_1L)]]$) and second-order ($P = 1/[1 + \exp[-(b_0 + b_1L + b_2L^2)]]$) polynomial logistic function for each spawning ground. Lengths used in this model were projected forward from the length at tagging to May 1 for the Gulf of Mexico analysis and July 1 for the Slope Sea analysis using the established growth equation (42).

Proportion of Spawning in the Gulf of Mexico. We used the following equation to estimate the proportion of western Atlantic bluefin tuna spawning that occurs in the Gulf of Mexico (P_{GOMEX}) under different scenarios of fishing mortality and age at maturity:

$$P_{GOMEX} = \frac{\sum_{t=1}^{40} N_t W_t m_t P_{t,GOMEX}}{\sum_{t=1}^{40} N_t W_t m_t} \quad [1]$$

where N_t is the relative number of fish at age t , W_t is the weight at age t , m_t is the maturity at age t for the population as a whole, and $P_{t,GOMEX}$ is the proportion of fish at age t that migrate to the Gulf of Mexico. Weight at age (W_t) was calculated using a two-step process. First, the Von Bertalanffy growth function was used to calculate length at age (42):

$$L_t = L_\infty * [1 - \exp[-k(t - t_0)]] \quad [2]$$

with $L_\infty = 314.9$, $k = 0.089$, and $t_0 = -1.13$. Second, weights were calculated from lengths:

$$W_t = aL_t^b \quad [3]$$

with $a = 1.59 * 10^{-5}$ and $b = 3.02$ (1). For simplicity, maturity at age (m_t) was assumed to be knife edge at age 5 or age 9, the latter consistent with the current stock assessment. The logistic function characterizing the length structure of fish that migrate to the Gulf of Mexico, provided an estimate of $P_{t,GOMEX}$, with lengths converted to ages. Cls for the proportion of spawning in the Gulf of Mexico were developed by bootstrapping the fish used in developing the logistic function.

The relative age structure of a population averaged across years can be calculated using an age-specific total mortality rate (Z_t) with the number at age 1 (recruitment) set to 1:

$$N_{t+1} = N_t * e^{-Z_t} \quad [4]$$

Total mortality (Z_t) is the sum of natural mortality (M_t) and fishing mortality (F_t). We used $M = 0.14$ for all ages to remain consistent with the stock assessment, and evaluated three scenarios for age-specific fishing mortality. The first scenario was no fishing mortality on any age class (i.e., $F = 0$). The second scenario was the average estimated fishing mortality rate (0.04–0.06 for ages 3–14, and 0.076 for ages \geq 15) from the stock assessment for the most recent decade (2004–2013) when fishing mortality rates are thought to have reached 40-y lows. The third fishing mortality scenario corresponded to 1994–2003 when fishing mortality was higher (0.05 at age 3–0.16 at ages \geq 15). Estimates of both natural and fishing mortality rates in bluefin tuna are uncertain (1, 28, 43). The proportion of spawning in the Gulf of Mexico will be underestimated if total mortality is overestimated. Notably, <5% of bluefin tuna caught in the fishery from 1996 to 2007 were >20 y in age (44), suggesting that a substantial overestimate of total mortality is unlikely, unless the fishery is selective against older fish.

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Supporting Information

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SI Text

Plankton Sample Processing and Larval Identification. All ichthyoplankton samples were fixed in 5% (vol/vol) formalin buffered with seawater upon collection. The exceptions to this were the samples collected with the second bongo net during the 18 off-shelf stations of the GU1302 cruise, which were fixed in 95% (vol/vol) ethanol. Initial processing of the formalin-preserved samples occurred at the Morski Instytut Rybacki (MIR) in Szczecin, Poland. All fish larvae were removed from the samples, identified, and measured. Samples were returned to the NEFSC Narragansett Laboratory for further verification of larval fish identifications. For the HB1303 cruise, one of the two nets at each station was processed for ichthyoplankton; the other net was used to quantify zooplankton abundance. For the GU1302 cruise, the ichthyoplankton from the formalin-preserved samples was processed at MIR, and the matching ethanol-preserved samples were processed at the NEFSC Narragansett Laboratory. Station data and larval bluefin tuna data are available in Tables S1 and S2, respectively.

Morphological identifications of Atlantic bluefin tuna (*Thunnus thynnus*) larvae were verified or performed by K.E.M. and D.E.R., both of whom have extensive larval identification experience and have worked specifically with larval scombrids. Established morphological criteria were used to identify bluefin tuna larvae (45). However, some identification guides also state that the geographic distribution and time of spawning must be used to assign species level identities within the genus *Thunnus* (46). These two criteria preclude identifying western Atlantic larval *Thunnus* as bluefin tuna unless they were collected during the springtime in the Gulf of Mexico. Additionally, a recent review has noted errors and limitations in published descriptions of Atlantic bluefin tuna larvae, and has questioned many reported occurrences of larval bluefin tuna. This review urged researchers to integrate morphological and molecular approaches in identifying larval bluefin tuna (47).

We used molecular identification to confirm the accuracy of our morphological identifications. We chose a representative subset of 25 larvae for molecular identification, to maintain a sufficient intact sample archive for future work. We pursued two separate molecular identification approaches, one for the ethanol-preserved samples implemented at the Canadian Centre for DNA Barcoding (www.ccdb.ca) and one for formalin-preserved samples implemented at the Alaska Fisheries Science Center's Auke Bay Laboratory. Samples submitted to both laboratories included *Thunnus* species other than bluefin tuna to test the genetic identification approach. Due to concern about possible cross-contamination and false-positive readings, no well-preserved (e.g., ethanol-fixed) samples of bluefin tuna tissue were ever handled in the Auke Bay Laboratory that ran the formalin-fixed larvae.

Ten ethanol-preserved larvae were subjected to a standard DNA barcoding protocol using a 650-bp portion of the cytochrome *c* oxidase 1 (COI) gene. Standard protocols were used for DNA extraction (48), the PCR, and bidirectional sequencing (49). An additional 184-bp fragment of the COI gene was also sequenced. Eight out of 10 submitted specimens of morphologically identified bluefin tuna sequenced successfully (GenBank accession nos. KT352979–KT352986). We evaluated these sequences using the Barcode of Life Datasystem (BOLD) (www.boldsystems.org) database and the BOLD Identification System, and through the manual implementation of a character-based identification approach with 10 reference sequences of each

Thunnus species (50). Both approaches to sequence analysis yielded the same results, with all of the COI sequences of the morphologically identified bluefin tuna larvae consistent with bluefin tuna.

For the identification of formalin-fixed larvae, reference tuna mitochondrial DNA sequences were downloaded from GenBank on December 8, 2014. Through comparative analysis, the NADH dehydrogenase 5 (ND5) gene was determined to be among the most diagnostic among Atlantic *Thunnus* species (Figs. S1 and S2). Because of known difficulty of PCR amplifying large fragments from formalin-treated samples (51), the analysis focused on two small adjacent sequences that showed high divergence between species. Although the numbers of reference sequences were limited for each species [the smallest number was 2 for blackfin tuna (*T. atlanticus*), and the largest number was 13 for Atlantic bluefin tuna], a number of DNA single-nucleotide polymorphisms (SNPs) were identified to corroborate the morphological identification performed previously.

Of the 24 formalin-preserved tuna larvae processed for DNA sequencing, 15 were identified morphologically as Atlantic bluefin tuna and 9 were identified as species other than bluefin tuna and were considered controls. Genetic analyses were performed blind to the morphological species identifications. Tissue was prepared as described previously, and DNA was isolated using a QIAamp DNA FFPE Tissue Kit (QIAGEN). Extraction protocols were as described by the manufacturer except that proteinase K digestion was extended to 1.5 h, after which the sample was incubated at 90 °C for 2 h to encourage reversal of the formaldehyde linkages within the nucleic acids. DNA extractions were processed in three groups of eight samples, and two elutions of each sample were made. Elution 1 DNA concentrations ranged from 1 to 89 ng/μL (mean of 26 ng/μL), and elution 2 ranged from 1 to 53 ng/μL (mean of 13 ng/μL) as assayed using a Nanodrop Lite Spectrophotometer (Thermo Fisher). The optical density ratios (OD₂₆₀/OD₂₈₀) for the elutions ranged from 1.28 to 2.04, with a mean of 1.74. Agarose gel electrophoresis suggested an average DNA size of ~500 bp.

Based on the position of species-specific SNPs identified within the ND5 gene, DNA primers for PCR were developed to span two small heterogenetic consecutive regions. PCR samples were prepared as follows: 1 μL of DNA template, 4 μL of Colorless GoTaq Reaction buffer (Promega), 1.24–3.1 mM MgCl₂ (final), 0.25 mM/nucleotide dNTP mixture (final), 0.5 μL of 20 μM forward primer (0.5 μM final), 0.5 μL of 20 μM reverse primer (0.5 μM final), 0.5 μL of Taq DNA polymerase (5 U/μL), and ddH₂O to 20 μL. PCR conditions included an initial denaturation step (94 °C for 2 min), 40 cycles (94 °C for 45 s, 54 °C for 45 s, 72 °C for 1 min), and a final polymerization step (72 °C for 5 min).

Following PCR amplification, an aliquot from each sample was analyzed on a 2.2% agarose gel to check product formation. Unique to the formalin-treated samples, a small by-product, the size of a primer–dimer, was often also visible, although this by-product did not interfere with DNA sequencing. PCR products were Sanger sequenced, and the products were aligned to reference tuna DNA sequences using CodonCode Aligner and MEGA6 (52) software. Species confirmation was determined by homology (Figs. S1 and S2). Of the 15 samples identified morphologically as bluefin tuna, 4 did not sequence, 10 had sequences consistent with bluefin tuna, and 1 had a sequence consistent with albacore (*T. alalunga*). The albacore sequence may indicate either a morphological misidentification or a bluefin

tuna with introgressed albacore mtDNA (7). All nine samples identified morphologically as species of *Thunnus* other than bluefin tuna sequenced. The sequences from eight were consistent with blackfin tuna and one with yellowfin tuna (*T. albacares*) (Figs. S1 and S2).

Drifter Analysis. We used the Global Drifter Program database (June 2014 update downloaded at <ftp://ftp.aoml.noaa.gov/phod/pub/buoydata/>) of NOAA's Atlantic Oceanographic and Meteorological Laboratory to evaluate the larval transport times from the Gulf of Mexico to the Slope Sea (53). These satellite-tracked drifting buoys are drogued at 15-m depth and thus provide a good match to the expected trajectories of early-stage *Thunnus* spp. larvae, which occupy the upper 25 m of the water column (54). Transport times from the Gulf of Mexico were calculated from the last recorded location in a box defined by 22.8–27°N and 84.5–83.5°W, an area at the entrance to the Straits of Florida where the eastward Florida Current predominates. We calculated the minimum transport time for one of these drifters to reach 36°N and also present the trajectories and final locations of drifters still active at 6, 12, and 18 d after leaving the Gulf of Mexico (Fig. S3). For comparison, we used an established age-length key (18) to estimate the age of each bluefin tuna larvae collected in the Slope Sea.

Notably, our approach was designed to underestimate expected larval transport times, providing a conservative evaluation of whether larvae could have been transported from known spawning grounds. Larval bluefin tuna are generally not collected in the fast-moving Loop Current in the Gulf of Mexico (24) and were not collected in the fast-moving Gulf Stream south of the Slope Sea. The estimated transport times encompass transport in the fast-moving western boundary currents, but do not account for the additional time required for a larva to become entrained in the Loop Current or to exit the Gulf Stream to Slope Sea waters.

Observer Data. We used 1992–2014 data from the Pelagic Longline Observer Program (55) as one means of evaluating the length structure of bluefin tuna in the Gulf of Mexico and the Slope Sea during the spawning seasons of April to June and June to August, respectively (Fig. S4). Regulations dictate that many

bluefin tuna are not retained, and thus lengths are often estimated to the nearest 30-cm interval, rather than directly measured. These estimated lengths reduce the precision of the reported length frequency distributions. However, discarded fish are typically smaller than kept fish, and using only directly measured fish would have biased length frequency distributions.

Satellite Data. Remote sensing SST data were used to visualize the broader oceanographic context for each of our sampling stations (Fig. S5). We used the Multiscale ultrahigh-resolution SST product (mur.jpl.nasa.gov/), which is gridded at a 1-km resolution, and integrates data from MODIS, AMSR-E, and AVHRR.

Allometric Egg Production and the Proportion of Spawning in the Gulf of Mexico. One assumption in most analyses of fisheries data is that stock-wide egg production is proportional to the biomass of mature fish, regardless of the underlying size structure of the population. In some species, larger fish produce proportionately more eggs for their weight than smaller fish, which can be characterized by the following function:

$$F = aL^b,$$

where F is fecundity, L is length, and a value of b greater than ~ 3 indicates allometric egg production. We tested the sensitivity of our estimates of the relative proportion of spawning in the Gulf of Mexico to allometric egg production. An estimate of the parameter a in the above equation, which scales fecundity to an absolute measure, is not needed to calculate the proportion of spawning in the Gulf of Mexico. For bluefin tuna in the Mediterranean Sea, batch fecundity and spawning frequency were found to be isometric (spawning duration was not estimated) (56). In contrast, in a limited sample size of Pacific bluefin tuna (*T. orientalis*), batch fecundity was estimated to be 9.5 million eggs at 190-cm FL and 25.7 million eggs at 240-cm FL (57), corresponding to an exponent of about 4.2, although an exponential regression was not used. We evaluated an allometric scalar of 4.2 for fecundity as an additional factor influencing the proportion of spawning in the Gulf of Mexico (Table S3).

Table S1. Station data for the 2013 Slope Sea sampling

Cruise	Station	Date	Latitude	Longitude	SST, °C	SSS	Gear	Preservative	No. of bluefin tuna larvae
GU1302	0114	21-Jun-13	37.59	-74.01	20.40	34.22	Bongo	EtOH and formalin	0
GU1302	0115	21-Jun-13	37.54	-73.88	20.03	33.73	Bongo	EtOH and formalin	0
GU1302	0116	21-Jun-13	37.48	-73.74	19.93	33.44	Bongo	EtOH and formalin	0
GU1302	0117	21-Jun-13	37.42	-73.61	19.94	33.47	Bongo	EtOH and formalin	0
GU1302	0118	21-Jun-13	37.20	-73.71	22.13	34.08	Bongo	EtOH and formalin	0
GU1302	0119	21-Jun-13	37.26	-73.84	20.85	33.37	Bongo	EtOH and formalin	0
GU1302	0120	21-Jun-13	37.32	-73.97	19.75	33.38	Bongo	EtOH and formalin	0
GU1302	0121	21-Jun-13	37.39	-74.10	20.90	34.73	Bongo	EtOH and formalin	0
GU1302	0139	22-Jun-13	36.32	-74.59	22.12	32.98	Bongo	EtOH and formalin	0
GU1302	0140	23-Jun-13	36.31	-74.43	21.90	33.02	Bongo	EtOH and formalin	0
GU1302	0141	23-Jun-13	36.31	-74.29	24.28	34.03	Bongo	EtOH and formalin	21 EtOH, 15 formalin
GU1302	0142	23-Jun-13	36.31	-74.13	24.70	35.38	Bongo	EtOH and formalin	1 EtOH
GU1302	0143	23-Jun-13	36.33	-73.97	25.97	36.03	Bongo	EtOH and formalin	0
GU1302	0144	23-Jun-13	35.99	-73.92	27.75	35.89	Bongo	EtOH and formalin	0
GU1302	0145	23-Jun-13	35.99	-74.07	27.12	35.59	Bongo	EtOH and formalin	0
GU1302	0146	23-Jun-13	36.00	-74.22	22.82	32.96	Bongo	EtOH and formalin	0
GU1302	0147	23-Jun-13	36.00	-74.37	22.97	32.46	Bongo	EtOH and formalin	0
GU1302	0148	23-Jun-13	36.00	-74.52	22.18	33.00	Bongo	EtOH and formalin	0
HB1303	0024	05-Jul-13	38.22	-73.30	24.12	32.00	Bongo	Formalin	0
HB1303	0025	05-Jul-13	38.64	-72.96	24.62	31.23	Bongo	Formalin	0
HB1303	0028	06-Jul-13	36.59	-72.04	28.39	36.02	Bongo	Formalin	0
HB1303	0029	06-Jul-13	37.29	-71.91	28.34	36.02	Bongo	Formalin	0
HB1303	0034	07-Jul-13	37.83	-71.84	25.57	33.10	Bongo	Formalin	0
HB1303	0035	07-Jul-13	38.76	-71.73	24.63	32.05	Bongo	Formalin	0
HB1303	9901	07-Jul-13	37.95	-71.75	24.85	N/A	MOCNESS	Formalin	0
HB1303	0041	08-Jul-13	39.13	-72.25	24.78	32.39	Bongo	Formalin	0
HB1303	0063	11-Jul-13	39.73	-70.70	23.56	33.18	Bongo	Formalin	0
HB1303	0079	13-Jul-13	39.17	-71.56	24.95	32.36	Bongo	Formalin	0
HB1303	9904	13-Jul-13	38.84	-70.52	24.81	34.10	MOCNESS	Formalin	6
HB1303	0083	14-Jul-13	38.68	-70.56	25.54	33.61	Bongo	Formalin	0
HB1303	0084	14-Jul-13	38.20	-69.66	26.61	34.80	Bongo	Formalin	5
HB1303	0087	15-Jul-13	37.77	-68.84	26.31	35.04	Bongo	Formalin	1
HB1303	0088	15-Jul-13	37.54	-68.29	26.19	34.30	Bongo	Formalin	2
HB1303	9905	15-Jul-13	37.82	-69.04	26.82	34.05	MOCNESS	Formalin	0
HB1303	0091	16-Jul-13	37.66	-68.26	25.97	34.38	Bongo	Formalin	0
HB1303	0092	16-Jul-13	38.52	-68.05	26.32	34.63	Bongo	Formalin	0
HB1303	0095	17-Jul-13	39.11	-67.88	26.42	34.67	Bongo	Formalin	12
HB1303	0096	17-Jul-13	39.86	-67.71	25.64	34.59	Bongo	Formalin	0
HB1303	0103	18-Jul-13	40.15	-67.18	26.31	36.19	Bongo	Formalin	2
HB1303	0111	19-Jul-13	39.96	-68.30	25.85	34.61	Bongo	Formalin	0
HB1303	0137	22-Jul-13	40.05	-68.47	25.39	34.09	Bongo	Formalin	0
HB1303	0163	03-Aug-13	40.37	-67.14	25.29	34.69	Bongo	Formalin	0
HB1303	0167	04-Aug-13	40.17	-67.22	25.49	34.94	Bongo	Formalin	0
HB1303	0168	04-Aug-13	40.73	-66.49	21.14	32.24	Bongo	Formalin	0
HB1303	9908	04-Aug-13	40.20	-67.18	25.47	34.90	MOCNESS	Formalin	1
HB1303	0170	05-Aug-13	39.85	-67.12	25.15	34.47	Bongo	Formalin	0
HB1303	0171	05-Aug-13	39.47	-65.96	27.11	35.67	Bongo	Formalin	0
HB1303	0179	06-Aug-13	39.47	-65.19	25.77	35.39	Bongo	Formalin	0
HB1303	0180	06-Aug-13	40.23	-65.10	23.95	33.56	Bongo	Formalin	0
HB1303	0186	07-Aug-13	40.85	-65.03	24.35	34.77	Bongo	Formalin	0
HB1303	0187	07-Aug-13	41.73	-64.94	19.63	31.74	Bongo	Formalin	0
HB1303	0198	08-Aug-13	41.40	-65.68	19.28	32.33	Bongo	Formalin	0
HB1303	0200	09-Aug-13	41.00	-66.28	21.92	32.44	Bongo	Formalin	1
HB1303	0218	12-Aug-13	38.81	-72.73	25.15	33.22	Bongo	Formalin	0
HB1303	0221	13-Aug-13	38.00	-73.18	25.77	32.20	Bongo	Formalin	0
HB1303	0222	13-Aug-13	37.34	-72.64	26.04	32.69	Bongo	Formalin	0
HB1303	0226	14-Aug-13	37.16	-71.93	28.23	35.3	Bongo	Formalin	0
HB1303	0227	14-Aug-13	37.95	-71.85	25.95	34.04	Bongo	Formalin	0
HB1303	0238	16-Aug-13	39.48	-70.30	23.55	33.58	Bongo	Formalin	0
HB1303	0239	16-Aug-13	39.08	-69.69	26.04	34.73	Bongo	Formalin	0
HB1303	0241	16-Aug-13	39.00	-68.95	26.22	34.00	Bongo	Formalin	0
HB1303	0244	17-Aug-13	40.22	-67.86	24.16	34.39	Bongo	Formalin	0

For the GU1302 cruise, both the net preserved in formalin and the net preserved in 95% ethanol (EtOH) were processed.

Table S2. Cont.

Cruise	Station	Fixative	Fish no.	Length, mm	Genetic ID attempted	GenBank no.
HB1303	9904	Formalin	4	7.1	No	
HB1303	9904	Formalin	5	6.6	No	
HB1303	9904	Formalin	6	7.0	No	
HB1303	9908	Formalin	1	8.4	Yes	KT285195

*Sequence KT285190 consistent with albacore (*Thunnus alalunga*).

Table S3. Estimated proportion of spawning ($\pm 95\%$ CI) that occurs in the Gulf of Mexico

Fishing mortality	Age at maturity, y	Fecundity exponent	Proportion of spawning in the Gulf of Mexico
F _{2004–2013}	5	Proportional	0.32 (0.22–0.41)
F _{2004–2013}	5	4.2	0.40 (0.29–0.50)
F _{2004–2013}	9	Proportional	0.43 (0.30–0.56)
F _{2004–2013}	9	4.2	0.49 (0.34–0.62)
F _{1994–2003}	5	Proportional	0.22 (0.13–0.30)
F _{1994–2003}	5	4.2	0.27 (0.17–0.37)
F _{1994–2003}	9	Proportional	0.33 (0.21–0.46)
F _{1994–2003}	9	4.2	0.37 (0.24–0.50)
F ₀	5	Proportional	0.47 (0.35–0.57)
F ₀	5	4.2	0.56 (0.43–0.66)
F ₀	9	Proportional	0.57 (0.44–0.69)
F ₀	9	4.2	0.63 (0.47–0.74)

Different scenarios of fishing mortality, age at maturity, and fecundity at length relationships were evaluated.

Implications of alternative spawning for bluefin tuna remain unclear

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The discovery of 67 bluefin tuna larvae in waters off the northeast US continental shelf is certainly of considerable scientific interest, but the paper in PNAS by Richardson et al. (1) makes several broad assertions that go beyond what the data support. The authors extrapolate differences in larval catch rates to conclude that the majority of spawning occurs outside of the Gulf of Mexico. This contention equates 1 y of opportunistic samples with 32 y of a design-based survey, fails to apply standard larval data corrections (2, 3), and ignores the high variance that makes comparison of these catch rates unreliable. The situation resembles the 1985 discovery of larvae offshore of the Carolinas (4), a similar northward range extension presented with the caveat that “low catches limit the precision of results” and a need to “rein in unwarranted speculation.” Similar imprecision in Slope Sea catch rates warrants similar caution until the current and historical importance of spawning in this area to the population can be confirmed with additional research.

More concerning is the claim that western Atlantic bluefin tuna mature earlier, and are therefore less vulnerable to exploitation. Stock assessments routinely consider younger (4 y of age) maturity as a sensitivity evaluation (5). Under that assumption, spawning stock biomass estimates are higher, but the number of recruits produced per spawner is lower. Stock recruitment relationships derived externally from these estimates indicate proportionally higher spawning biomass and lower fishing mortality rates are needed to achieve the maximum

sustainable yield, resulting in less optimistic appraisals of stock status (Fig. 1). In other words, maintaining the same catch, size composition, and index trends with a greater spawning biomass implies that the stock is less productive, and therefore more vulnerable to exploitation, exactly the opposite conclusion of Richardson et al. (1).

Although early-maturing species often exhibit greater resilience to fishing, vulnerability assessments of bluefin tuna do not apply this generalization in isolation of other information. The Convention on International Trade in Endangered Species (CITES) (6) and US Endangered Species Act (7) listings recently considered for Atlantic bluefin tuna placed primary consideration of vulnerability upon products of population dynamic models: specifically and respectively, the rate of historical decline (6) and probability of extinction (7).

In summary, the paper by Richardson et al. (1) could have important implications for resilience of the species to fishing and climate change, but it makes several premature claims that carry considerable risk of being misused. Any conclusions regarding vulnerability should only be made in a population dynamics context with full consideration of the wealth of information now available through enhanced research programs. Furthermore, comprehensive larval and reproductive sampling, tagging, oceanographic modeling, and genetic analyses should be initiated not only for the Slope Sea but also for other areas of known or potential spawning.

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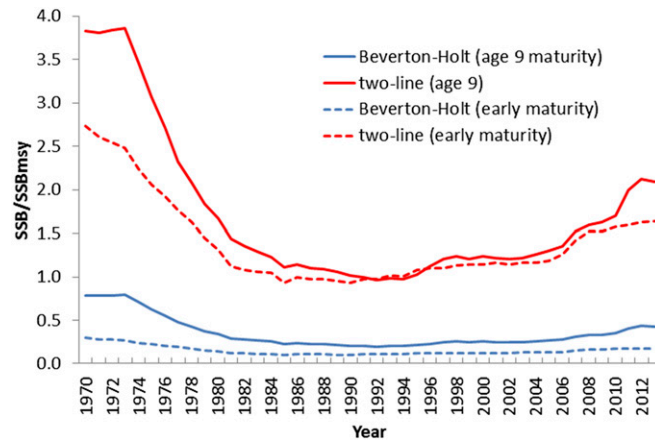


Fig. 1. Estimates of Western Atlantic bluefin tuna spawning stock biomass relative to spawning stock biomass at maximum sustainable yield (SSB/SSBmsy) under two age at maturity assumptions and two alternative stock-recruitment hypotheses. SSB/SSBmsy values less than 1 indicate that the stock is overfished.

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- 6 CITES (1994) *Convention on International Trade in Endangered Species of Wild Fauna and Flora*. 85 Resolution Conf. 9.24 (Rev. CoP16) (Fort Lauderdale, FL).
- 7 Federal Register 76(105) (2011) pp 31556–31570.

Data do not support new claims about bluefin tuna spawning or abundance

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Richardson et al. (1) assert, but do not adequately demonstrate, that western-stock Atlantic bluefin tuna spawn off the northeastern United States, the northeastern area is their main spawning area, and they spawn younger and are less vulnerable to fishing than believed. Further, their assertions lead logically to a conclusion about vulnerability that is opposite the authors' conclusion.

First, Richardson et al. (1) found larval bluefin tuna in an area not previously sampled. Previous researchers found larvae north to the Carolinas (2). The Gulf Stream could have transported some of the new-found larvae from previously known areas off the Carolinas. The origin remains equivocal.

Second, Richardson et al. (1) say two-thirds of western-stock spawning occurs outside the Gulf of Mexico, "assuming recent fishing mortality and maturity at age 5." Absent genetics, it is possible that Gulf Stream larvae are spawned by eastern-stock fish, which are more abundant.

Larvae spawned months later than in the Gulf of Mexico and facing cooling water months earlier may all die. McGowan and Richards (3) believed Atlantic conditions "not... favorable for... survival of bluefin tuna larvae."

Third, Richardson et al. (1) say their "assertion of a younger age at maturity for western Atlantic bluefin tuna is supported by three lines of evidence." Gulf of Maine fish older than 5 y had "endocrine measurements" indicating maturation. However, they might have been eastern-stock fish. Additionally, large Gulf of Maine fish "had atretic follicles... indicative of recent and proximate spawning." Atretic follicles can also indicate egg resorption from malnutrition, causing

a skipped spawning year (ref. 4, pp. 211–212). Finally, in 1957, Slope Sea fish aged 4–12 y had developing to ripe gonads (5). It is unsurprising that fish aged 4–12 y include individuals with gonads "developing" or mature. In addition, Mather et al. (5) warned, "Estimates of spawning areas and seasons from gonad condition are subject to... serious errors."

Richardson et al. (1) believe smaller, younger fish must be breeding somewhere. So they "classify" fish that spent more than 20 d in the Slope Sea as "breeders." Thus, "Over 75% of individuals 133- to 212-cm (age 5–11) were classified as potential Slope Sea spawners." Brief residence does not imply breeding. Small fish in an area with large fish and larvae is not evidence of small fish spawning. They conclude, "evidence indicates that the western stock matures around age 4–5" (1). Their data do not support it, and several studies refute it.

Richardson et al.'s suspected "reproductive mixing" (1) is inconsistent with genetic studies (6), stable isotopes (7), and behavior (8).

Fourth, Richardson et al. (1) acknowledge that "By the start of the 21st century, intense fishing pressure had driven this species to historically low population levels." If we simply accept their claims *i–iii* above, then depletion occurred despite a vastly larger and wider distributed breeding population than known, making vulnerability higher than thought; thus, rebuilding targets must be revised far upward of current targets. A depleted species cannot withstand more stress than it has withstood simply because larvae have been found adjacent to a region where larvae were previously found.

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REPLY TO SAFINA AND WALTER ET AL.:

Multiple lines of evidence for size-structured spawning migrations in western Atlantic bluefin tuna

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Walter et al. (1) and Safina (2) raise numerous concerns regarding our study (3). Specifically, they question our conclusions that (i) a majority of spawning occurs outside the Gulf of Mexico, (ii) western North Atlantic bluefin tuna mature earlier than currently estimated, and (iii) additional spawning locations and younger age at maturity mean that the western Atlantic bluefin tuna are less vulnerable to anthropogenic impacts, including exploitation.

There are two alternative hypotheses regarding the life history of western Atlantic bluefin tuna: (i) a late-maturing stock (age of 9+ y) that spawns only in the Gulf of Mexico and (ii) an early-maturing stock (age of 4–5 y) that spawns in multiple locations during age-structured migrations (3). The hypothesis that western Atlantic bluefin tuna spawn only in the Gulf of Mexico is refuted by our finding of very young larvae in the Slope Sea; the assertion that these larvae were spawned off the Bahamas or in the Gulf of Mexico is not supported by oceanographic studies. The hypothesis that western Atlantic bluefin tuna mature at the age of 9 y or older is refuted by direct reproductive studies. We encourage those individuals interested in determining the value of this evidence to read the maturity studies cited in our work (4, 5), as well as the study by Mather et al. (6), rather than relying on the edited quote in the letter of Safina (2). Previously, the absence of younger fish (<9 y of age) in the Gulf of Mexico (i.e., the only known spawning ground) was used as indirect evidence for an older age at maturity and to question the direct reproductive studies (7, 8). With the discovery of a Slope Sea spawning ground, this indirect argument is no longer valid.

Our conclusion that a majority of spawning occurs outside the Gulf of Mexico is based on the conclusion of a lower age at maturity; our analysis of tagging data; and estimates of total mortality, including values from the stock assessment model used by Walter et al. (1). A central component of this analysis, the proportion of fish migrating to the Gulf of Mexico by size (or age), is remarkably consistent across four separate datasets: two independent electronic tagging datasets (3, 8) and analyses of two fisheries-dependent longline catch datasets (7, 9). Abundance of larvae in the Slope Sea compared with the Gulf of Mexico provides secondary support for the conclusion; the referenced collection of larvae in other areas outside the Gulf of Mexico (10, 11) provides additional support. We understand that this conclusion is challenging, and we thus provided numerous sensitivity analyses for readers to evaluate this claim, including one consistent with age at maturity and mortality from the current stock assessment.

Based on our findings supporting a lower age at maturity and spawning in areas outside the Gulf of Mexico, we conclude that western Atlantic bluefin tuna are less vulnerable to anthropogenic impacts than previously thought. Formal assessments of species vulnerability involve evaluating intrinsic species attributes, population trends, and extrinsic factors (e.g., climate change, oil spills), along with the uncertainty in each. The Convention on International Trade in Endangered Species (CITES) standard lists a set of intrinsic vulnerability factors, the first two of which are as follows: (i) “life history (e.g., low fecundity, slow growth rate of the individual, high age at first maturity, long generation time)” and (ii) “low absolute numbers or biomass or restricted

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area of distribution" (at any life stage) (12). According to these criteria, an additional spawning ground, younger age at maturity, and larger mature population all reduce vulnerability. Further, the analyses presented by Walter et al. (1) and the International Commission for the Conservation of Atlantic Tunas (ICCAT) (13) indicate that lowering the age at maturity within the stock assessment model reduces the maximum decline in spawning stock biomass over the observed 1970–2013 period from an >80% decline (age of 12–16 y at maturity), to an ~74% decline (age of 9 y at maturity), to an ~66% decline (age of 4–5 y at maturity). A lower rate of decline in the face of fishing is one definition of lower vulnerability.

Safina (2) raises the possibility that the Slope Sea is an eastern Atlantic stock spawning ground. This statement further questions the central Atlantic stock separation line that forms the basis of Atlantic bluefin tuna assessment and management. Stock boundaries should encompass the spawning grounds of a population. We do not believe that this explanation is the most likely one for currently available data; however, as we state in our paper, "to fully evaluate bluefin tuna population structure, biological samples from spawning fish and larvae collected in the Slope Sea need to be included in future analyses."

The available data support the hypothesis that bluefin tuna in the western Atlantic have size-structured spawning migrations

across a wide latitudinal range. As Walter et al. (1) state in the conclusion of their letter, further testing of this model will require additional research using multiple techniques. Longline sampling of adult fish in the Slope Sea for studies of reproduction and population structure is a top priority. We also agree with Walter et al. (1) that larval surveys and analyses should be designed that allow for the implementation of the larval production method (14) in both the Slope Sea and the Gulf of Mexico. This approach can provide an independent rigorous comparison of the relative magnitude of spawning in the two regions. Finally, as with the testing of the Gulf of Mexico-only spawning hypothesis, exploratory sampling is critical to answering whether there are other undocumented spawning grounds that may further change our perception of the life history of this species.

The process by which we engage in bluefin tuna science is important. We emphasize the benefits of advancing collaborative scientific approaches that value the insights of fishermen, many of whom had deduced Slope Sea spawning from their own observations. Furthermore, the highly migratory nature and oceanic habitat of bluefin tuna require that multiple types of sampling and data across a wide geographic range be used to address even the most basic life history questions. Improving and implementing open access standards for all types of data will accelerate progress in understanding bluefin tuna life history.

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