Supporting Information

Richardson et al. 10.1073/pnas.1525636113

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 offshelf 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 (OD_{260}/OD_{280}) 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 $MgCl_2$ (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 ddH_2O 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).

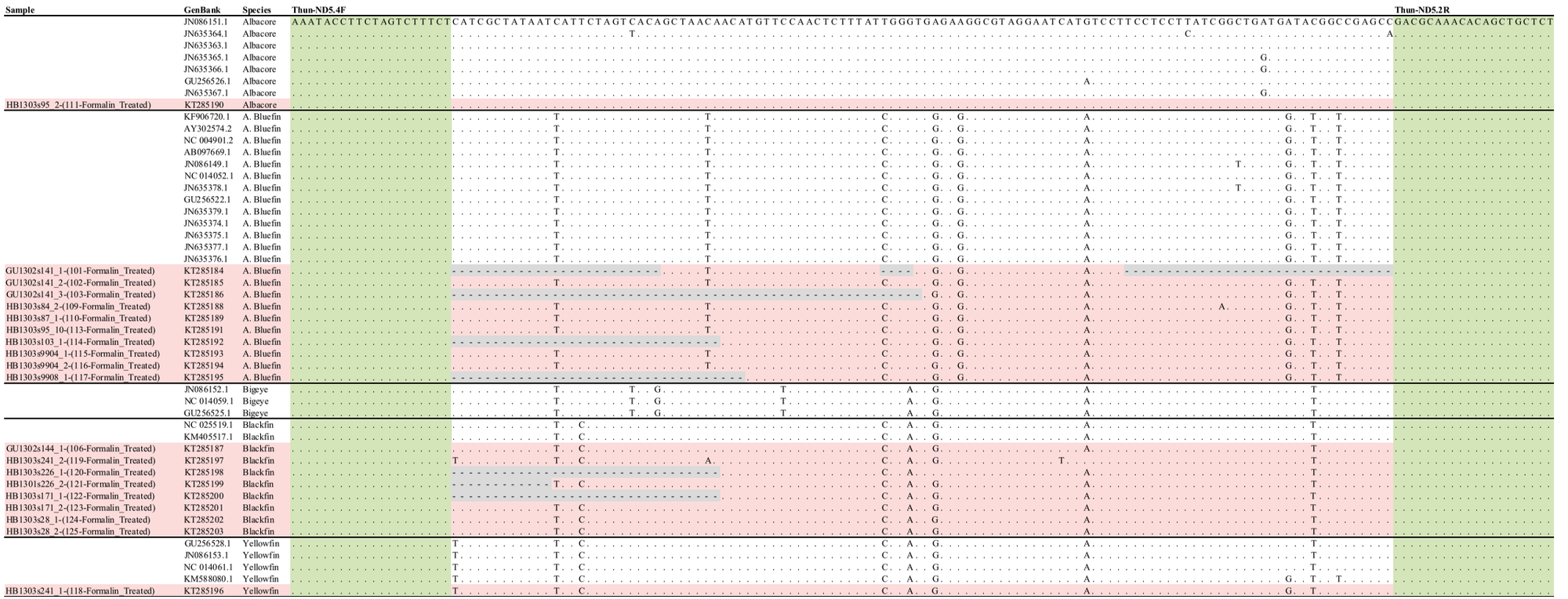

Fig. S1. First ND5 PCR product used for species identity. DNA sequence between primers Thun-ND5.4F and Thun-ND5.2R. Sample, GenBank accession number, species, PCR primers (green), matching sequence (dots), and SNP bases (letters) are noted. Species separations are designated by underlined text. Reference samples are shown with white background, and study samples are shown with a pink background. Unresolved DNA sequence is designated with a gray dash (–).

Sample	GenBank	Species	Thun-ND5.2F		Thun-ND5.3R
	JN086151.1	Albacore	GACGCAAACACAGCTGCTCT	ACAAGCGGTAGTGTACAACCGAGTGGGGGATATCGGACTCATTCTTGCCATAGCATGAATA	GCAACTAACCTAAACTCATGAGAAA
	JN635364.1	Albacore	....	T.....A.......................C..............................	....
	JN635363.1	Albacore	....	....	....
	JN635365.1	Albacore	....	........................A....................................	....
	JN635366.1	Albacore	....	....	....
	GU256526.1	Albacore	....	....	....
	JN635367.1	Albacore	....	....	....
HB1303s95_2-(111-Formalin_Treated)	KT285190	Albacore	....	............C..	....
	KF906720.1	A. Bluefin	....	............A..T........C...........G........................	....
	AY302574.2	A. Bluefin	....	............A..T........C...........G........................	....
	NC 004901.2	A. Bluefin	....	............A..T........C...........G........................	....
	AB097669.1	A. Bluefin	....	............A..T........C...........G........................	....
	JN086149.1	A. Bluefin	....	............A..T........C...........G........................	....
	NC 014052.1	A. Bluefin	....	............A..T........C...........G........................	....
	JN635378.1	A. Bluefin	....	............A..T........C...........G........................	....
	GU256522.1	A. Bluefin	....	............A..T........C...........G........................	....
	JN635379.1	A. Bluefin	....	............A..T........C...........G........................	....
	JN635374.1	A. Bluefin	....	...............T........C...........G........................	....
	JN635375.1	A. Bluefin	....	............A..T........C...........G........................	....
	JN635377.1	A. Bluefin	....	............A..T........C...........G........................	....
	JN635376.1	A. Bluefin	....	............A..T........C...........G........................	....
GU1302s141_1-(101-Formalin_Treated)	KT285184	A. Bluefin	....	............A..T.---------------....G........................	....
GU1302s141_2-(102-Formalin_Treated)	Short sequence	A. Bluefin	....	............A..T........C------------------------------------	....
GU1302s141_3-(103-Formalin_Treated)	Short sequence	A. Bluefin	....	-------------------------------.....G........................	....
HB1303s84_2-(109-Formalin_Treated)	KT285188	A. Bluefin	....	............A..T........C....................................	....
HB1303s87_1-(110-Formalin_Treated)	KT285189	A. Bluefin	....	............A..T........C...........G........................	....
HB1303s95_10-(113-Formalin_Treated)	KT285191	A. Bluefin	....	............A..T........C...........G........................	....
HB1303s103_1-(114-Formalin_Treated)	KT285192	A. Bluefin	....	............A..T........C...........G........................	....
HB1303s9904_1-(115-Formalin_Treated)	KT285193	A. Bluefin	....	............A..T........C...........G........................	....
HB1303s9904_2-(116-Formalin_Treated)	KT285194	A. Bluefin	....	............A..T........C...........G........................	....
HB1303s9908_1-(117-Formalin_Treated)	KT285195	A. Bluefin	....	............A..T........C...........G........................	....
	JN086152.1	Bigeye	....	G...........A..T..............C....................G.........	....
	NC 014059.1	Bigeye	....	G...........A..T..............C....................G.........	....
	GU256525.1	Bigeye	....	G...........A..T..............C....................G.........	....
	NC 025519.1	Blackfin	....	G........G..A..T........T..A.....T.................G.....G...	....
	KM405517.1	Blackfin	....	G........G..A..T........T..A.....T.................G.....G...	....
GU1302s144_1-(106-Formalin_Treated)	KT285187	Blackfin	....	G........G..A..T........T..A.....T.....T...........G.....G...	....
HB1303s241_2-(119-Formalin_Treated)	KT285197	Blackfin	....	G........G..A..T........T..A.......................G.....G...	....
HB1303s226_1-(120-Formalin_Treated)	KT285198	Blackfin	....	G........G..A..T........T........T.................G.....G...	....
HB1301s226_2-(121-Formalin_Treated)	KT285199	Blackfin	....	G........G..A..T........T..A.....-.................G.....G...	....
HB1303s171_1-(122-Formalin_Treated)	KT285200	Blackfin	....	G........G..A..T........T..A.....T.................G.....G...	....
HB1303s171_2-(123-Formalin_Treated)	KT285201	Blackfin	....	G........G..A..T........T..A.....T.................G.....G...	....
HB1303s28_1-(124-Formalin_Treated)	KT285202	Blackfin	....	G........G..A..T........T..A.....T.....T...........G.....G...	....
HB1303s28_2-(125-Formalin_Treated)	KT285203	Blackfin	....	G.....A..G..A..T........T........T.................G.....G...	....
	GU256528.1	Yellowfin	....	G........G..A..T........C..A.......................G.....G...	....
	JN086153.1	Yellowfin	....	G........G..A..T........C..A.......................G.....G...	....
	NC 014061.1	Yellowfin	....	G........G..A..T........C..A.......................G.....G...	....
	KM588080.1	Yellowfin	....	G........G..A..T........C..A.......................G.....G...	....
HB1303s241_1-(118-Formalin_Treated)	KT285196	Yellowfin	....	G........G..A..T........C..A.......................G.....G...	....

Fig. S2. Second consecutive ND5 PCR product used for species identity. DNA sequence between primers Thun-ND5.2F and Thun-ND5.3R. Sample, GenBank accession number, species, PCR primers (green), matching sequence (dots), and SNP bases (letters) are noted. Species separations are designated by underlined text. Reference samples are shown with white background, and study samples are shown with a pink background. Unresolved DNA sequence is designated with a gray dash (–). DNA sequence data from samples GU1302s141_2-(102-Formalin_Treated) and GU1302s141_3-(103-Formalin_Treated) were too short to be assigned an individual GenBank accession number.

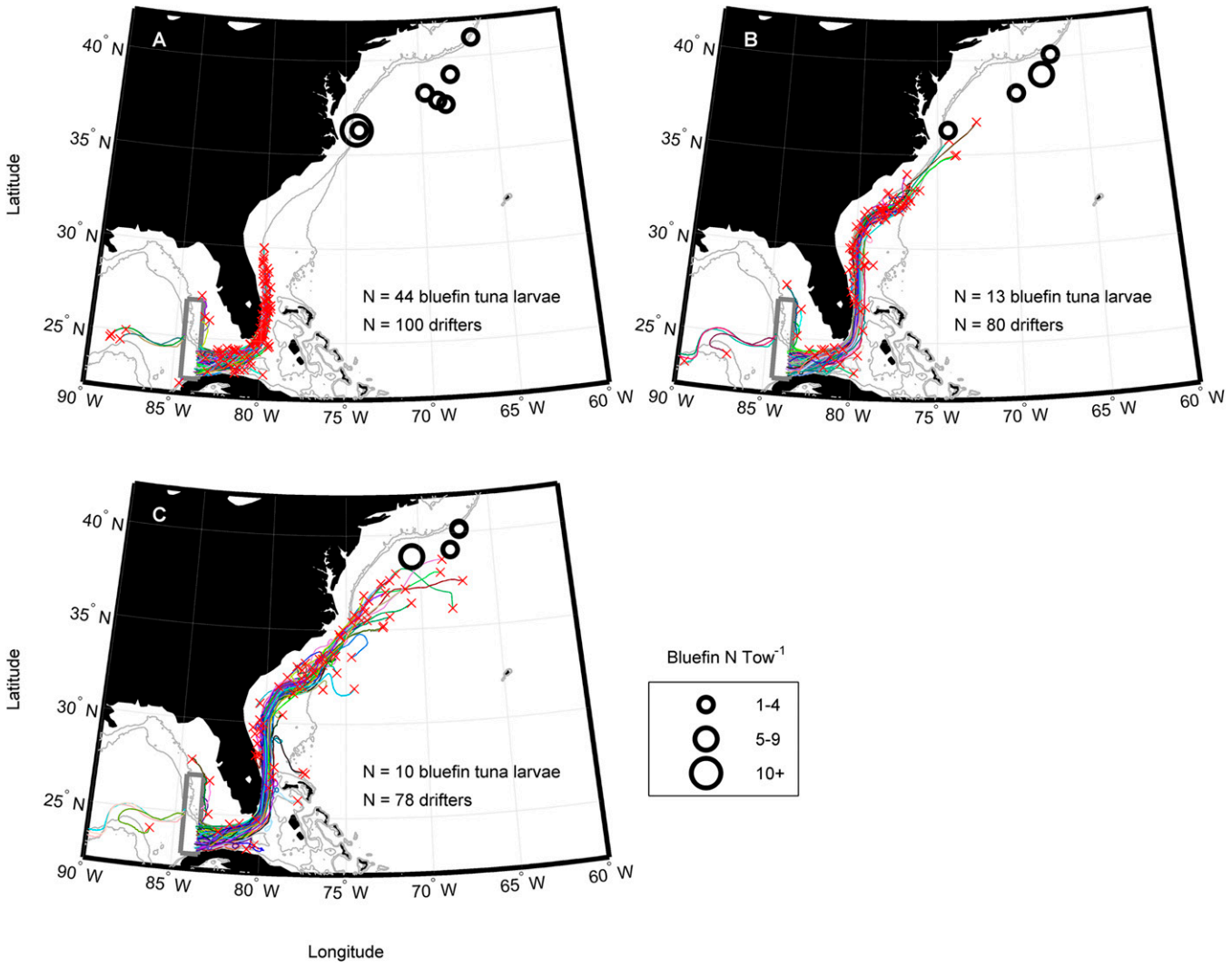

Fig. S3. Locations and tracks of satellite tracked drifting buoys that exited the Gulf of Mexico. (*A*) Drifter locations 6 d after Gulf of Mexico exit (red x) and larvae ages 1–6 d. (*B*) Drifter locations 12 d after Gulf of Mexico exit (red x) and larvae ages 7–12 d. (*C*) Drifter locations 18 d after Gulf of Mexico exit (red x) and larvae ages 13–18 d.

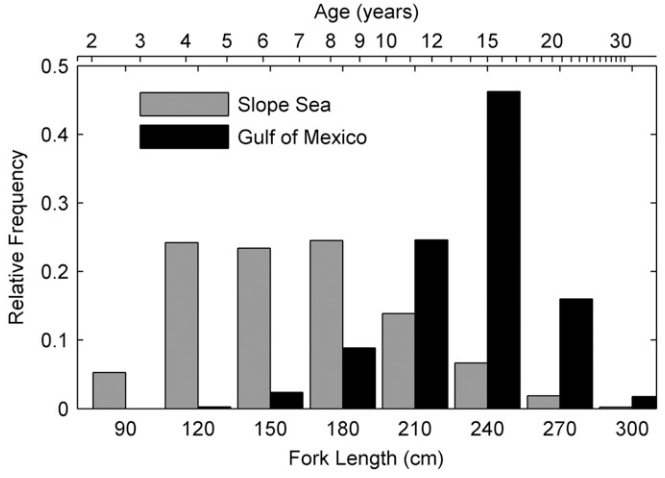

Fig. S4. Length structure of bluefin tuna on the two spawning grounds. Lengths were recorded by observers of the longline fishery, and the data are constrained to the respective spawning seasons.

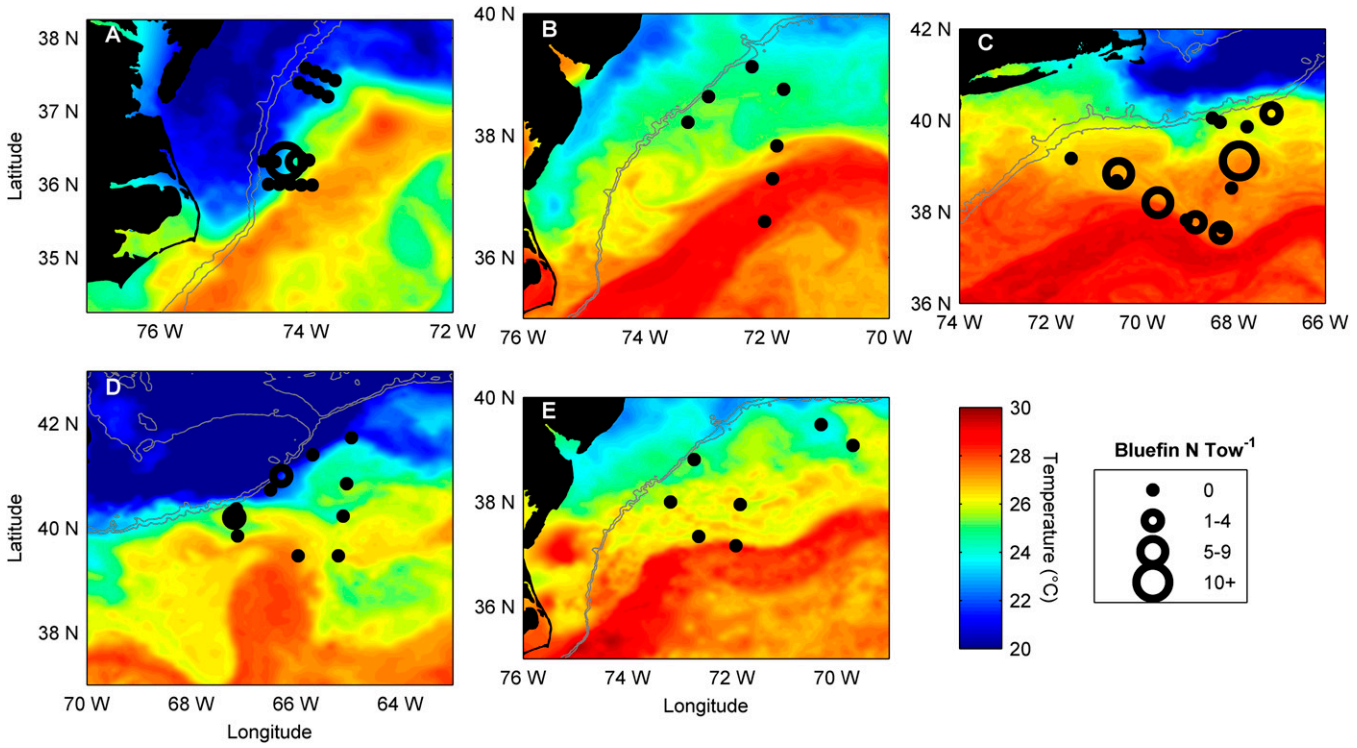

Fig. S5. Distribution of Atlantic bluefin tuna larvae relative to satellite measured and interpolated SST. Sampling is shown for (*A*) June 21–23, 2013; (*B*) July 5–8, 2013; (*C*) July 13–22, 2013; (*D*) August 1–9, 2013; and (*E*) August 12–16, 2013.

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. Larval bluefin tuna data from the 2013 Slope Sea sampling

Cruise	Station	Fixative	Fish no.	Length, mm	Genetic ID attempted	GenBank no.
GU1302	0141	Ethanol	1	2.7	Yes	Failed
GU1302	0141	Ethanol	2	2.3	Yes	KT352980
GU1302	0141	Ethanol	3	2.7	Yes	KT352982
GU1302	0141	Ethanol	4	2.7	Yes	KT352983
GU1302	0141	Ethanol	5	2.6	Yes	Failed
GU1302	0141	Ethanol	6	3.0	Yes	KT352981
GU1302	0141	Ethanol	7	2.7	Yes	KT352986
GU1302	0141	Ethanol	8	2.8	Yes	KT352984
GU1302	0141	Ethanol	9	4.0	Yes	KT352985
GU1302	0141	Ethanol	10	2.5	No	
GU1302	0141	Ethanol	11	2.3	No	
GU1302	0141	Ethanol	12	2.9	No	
GU1302	0141	Ethanol	13	2.3	No	
GU1302	0141	Ethanol	14	2.7	No	
GU1302	0141	Ethanol	15	2.4	No	
GU1302	0141	Ethanol	16	2.6	No	
GU1302	0141	Ethanol	17	2.7	No	
GU1302	0141	Ethanol	18	2.6	No	
GU1302	0141	Ethanol	19	2.4	No	
GU1302	0141	Ethanol	20	2.4	No	
GU1302	0141	Ethanol	21	2.2	No	
GU1302	0141	Formalin	1	5.0	Yes	KT285184
GU1302	0141	Formalin	2	2.4	Yes	KT285185
GU1302	0141	Formalin	3	2.3	Yes	KT285186
GU1302	0141	Formalin	4	2.4	No	
GU1302	0141	Formalin	5	2.5	Yes	Failed
GU1302	0141	Formalin	6	2.4	No	
GU1302	0141	Formalin	7	2.3	No	
GU1302	0141	Formalin	8	2.4	No	
GU1302	0141	Formalin	9	2.0	No	
GU1302	0141	Formalin	10	2.3	No	
GU1302	0141	Formalin	11	2.5	No	
GU1302	0141	Formalin	12	2.0	No	
GU1302	0141	Formalin	13	2.4	No	
GU1302	0141	Formalin	14	2.4	No	
GU1302	0141	Formalin	15	2.5	No	
GU1302	0142	Ethanol	1	2.7	Yes	KT352979
HB1303	0084	Formalin	1	3.0	Yes	Failed
HB1303	0084	Formalin	2	3.9	Yes	KT285188
HB1303	0084	Formalin	3	2.6	No	
HB1303	0084	Formalin	4	3.0	No	
HB1303	0084	Formalin	5	3.0	No	
HB1303	0087	Formalin	1	2.4	Yes	KT285189
HB1303	0088	Formalin	1	3.0	No	
HB1303	0088	Formalin	2	2.8	No	
HB1303	0095	Formalin	1	2.0	No	
HB1303	0095	Formalin	2	5.1	Yes	KT285190*
HB1303	0095	Formalin	3	4.9	No	
HB1303	0095	Formalin	4	5.0	Yes	Failed
HB1303	0095	Formalin	5	5.0	No	
HB1303	0095	Formalin	6	5.3	No	
HB1303	0095	Formalin	7	4.9	No	
HB1303	0095	Formalin	8	4.8	No	
HB1303	0095	Formalin	9	5.0	No	
HB1303	0095	Formalin	10	6.7	Yes	KT285191
HB1303	0095	Formalin	11	5.9	No	
HB1303	0095	Formalin	12	4.5	No	
HB1303	0103	Formalin	1	4.5	Yes	KT285192
HB1303	0103	Formalin	2	5.8	No	
HB1303	0200	Formalin	1	2.4	No	
HB1303	9904	Formalin	1	8.0	Yes	KT285193
HB1303	9904	Formalin	2	7.2	Yes	KT285194
HB1303	9904	Formalin	3	8.0	No	

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 (±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.