Supporting Information for

Ancient DNA suggests a historical demographic decline and genetic erosion in the Atlantic bluefin tuna

Adam Jon Andrews[*1,2], Emma Falkeid Eriksen[3], Bastiaan Star[3], Kim Præbel[4], Antonio Di Natale[5], Estrella Malca[6,7], Vedat Onar[8], Veronica Aniceti[9], Gabriele Carenti[10], Gäel Piques[11], Svein Vatsvåg Nielsen[12], Per Persson[13], Federica Piattoni[1], Francesco Fontani[14], Lane Atmore[3,15], Oliver Kersten[3], Fausto Tinti[*2], Elisabetta Cilli[†14], Alessia Cariani[†2]

1Norwegian Institute of Water Research, Oslo, Norway
2Department of Biological, Geological and Environmental Sciences, University of Bologna, Ravenna, Italy
3Centre for Ecology and Evolutionary Synthesis, University of Oslo, Oslo, Norway
4NFH, UiT Arctic University of Norway, Tromsø
5Aquastudio Research Institute, Messina, Italy
6Cooperative Institute for Marine and Atmospheric Studies, University of Miami, Miami, Florida, United States of America
7NOAA Fisheries, Populations and Ecosystems Monitoring Division, Miami, Florida, United States of America
8Faculty of Veterinary Medicine, Muğla Sıtkı Kocman University, Milas, Turkey
9Consejo Superior de Investigaciones Científicas, Institució Milà i Fontanals (CSIC-IMF), Barcelona, Spain
10CEPAM, CNRS, Université Côte d'Azur, Nice, France
11ASM, CNRS, Université Paul Valéry, Montpellier, France
12Stavanger Maritime Museum, Stavanger, Norway
13Museum of Cultural History, University of Oslo, Norway
14Department of Cultural Heritage, University of Bologna, Ravenna, Italy
15Departmet of Anthropology, University of British Colombia, Vancouver, Canada

Corresponding authors: Adam Jon Andrews & Fausto Tinti
Email: adam@palaeome.org, fausto.tinti@unibo.it

This PDF files includes:

An extension of the Methods
Figures S1 to S17
Tables S1 to S2
Supplementary References

Sample Details

Table S1

Table S1. Sampling and sequencing details of genomic samples resequenced for WG analyses												
Sample ID	**Species**	**Sample Group**	**Sample type**	**Location**	**Longitude**	**Latitude**	**Life stage**	**Year**	**Reads Obtained**	**Endogenous (%)**	**Read Length (bp)**	**Nuclear Coverage (fold)**
TUN006	Thunnus thynnus	3000BCE	Archaeological	Jortveit, Norway	58.27	8.51	Adult	3000BCE	376804642	60.30	76	30.98
TUN055	Thunnus thynnus	3000BCE	Archaeological	Jortveit, Norway	58.27	8.51	Adult	3000BCE	239118563	63.90	69	19.16
IST_913C_16	Thunnus thynnus	800-1300	Archaeological	Yenikapi	28.95	41.01	Adult	800-1300	129536799	50.00	73	6.80
IST_913C_23	Thunnus thynnus	800-1300	Archaeological	Yenikapi	28.95	41.01	Adult	800-1300	231830205	46.90	66	9.66
IST_913C_13	Thunnus thynnus	800-1300	Archaeological	Yenikapi	28.95	41.01	Adult	800-1300	259277713	61.00	73	5.95
IST_913C_12	Thunnus thynnus	800-1300	Archaeological	Yenikapi	28.95	41.01	Adult	800-1300	386660574	53.00	78	22.39
IST_913C_10	Thunnus thynnus	800-1300	Archaeological	Yenikapi	28.95	41.01	Adult	800-1300	160138093	59.00	68	9.05
IST_913C_05	Thunnus thynnus	800-1300	Archaeological	Yenikapi	28.95	41.01	Adult	800-1300	141218038	57.00	72	7.89
IST_913C_04	Thunnus thynnus	800-1300	Archaeological	Yenikapi	28.95	41.01	Adult	800-1300	237180201	45.00	85	11.56
IST_913C_02	Thunnus thynnus	800-1300	Archaeological	Yenikapi	28.95	41.01	Adult	800-1300	85247221			3.56
IST_913C_01	Thunnus thynnus	800-1300	Archaeological	Yenikapi	28.95	41.01	Adult	800-1300	109403461			5.54
IST_913C_24	Thunnus thynnus	800-1300	Archaeological	Yenikapi	28.95	41.01	Adult	800-1300	132066947	54.70	84	8.51
SAN_10C_10	Thunnus thynnus	900-1300	Archaeological	Sant Antonio	13.36	38.11	Adult	900-1300	163296705	59.00	66	9.89
CDM_10C_07	Thunnus thynnus	900-1300	Archaeological	Corso dei Mille	13.37	38.11	Adult	900-1300	437023789	17.00	75	6.93
CDM_10C_06	Thunnus thynnus	900-1300	Archaeological	Corso dei Mille	13.37	38.11	Adult	900-1300	404338085	19.00	60	6.07
CDM_10C_04	Thunnus thynnus	900-1300	Archaeological	Corso dei Mille	13.37	38.11	Adult	900-1300	524748537	17.00	72	8.68
CDM_10C_11	Thunnus thynnus	900-1300	Archaeological	Corso dei Mille	13.37	38.11	Adult	900-1300	333454528	20.70	80	7.49
MZ_13C_12	Thunnus thynnus	900-1300	Archaeological	Mazara del Vallo	12.59	37.65	Adult	900-1300	162081768	52.41	79	9.81

MZ_13C_13	Thunnus thynnus	900-1300	Archaeological	Mazara del Vallo	12.59	37.65	Adult	900-1300	346826115	21.62	86	9.13
MZ_13C_15	Thunnus thynnus	900-1300	Archaeological	Mazara del Vallo	12.59	37.65	Adult	900-1300	224210622	58.47	67	12.07
PF_1618_02	Thunnus thynnus	1600-1800	Archaeological	Pedras de Fogu	8.62	40.86	Adult	1600-1800	275133758	57.30	71	16.65
PF_1618_05	Thunnus thynnus	1600-1800	Archaeological	Pedras de Fogu	8.62	40.86	Adult	1600-1800	203126677	39.40	77	9.10
PF_1618_23	Thunnus thynnus	1600-1800	Archaeological	Pedras de Fogu	8.62	40.86	Adult	1600-1800	84770223			4.60
PF_1618_21	Thunnus thynnus	1600-1800	Archaeological	Pedras de Fogu	8.62	40.86	Adult	1600-1800	180357636	56.73	73	10.32
PF_1618_19	Thunnus thynnus	1600-1800	Archaeological	Pedras de Fogu	8.62	40.86	Adult	1600-1800	234745788	49.20	73	11.37
PF_1618_18	Thunnus thynnus	1600-1800	Archaeological	Pedras de Fogu	8.62	40.86	Adult	1600-1800	170354508	57.90	85	9.52
PF_1618_24	Thunnus thynnus	1600-1800	Archaeological	Pedras de Fogu	8.62	40.86	Adult	1600-1800	189521908	35.63	81	7.41
PF_1618_07	Thunnus thynnus	1600-1800	Archaeological	Pedras de Fogu	8.62	40.86	Adult	1600-1800	208238351	39.90	76	8.72
PF_1618_10	Thunnus thynnus	1600-1800	Archaeological	Pedras de Fogu	8.62	40.86	Adult	1600-1800	204549484	39.40	74	8.20
ML_18C_02B	Thunnus thynnus	1750-1800	Archaeological	Marseille Leca	5.37	43.30	Adult	1750-1800	185930362	35.20	80	7.40
ML_18C_04B	Thunnus thynnus	1750-1800	Archaeological	Marseille Leca	5.37	43.30	Adult	1750-1800	328889414	21.70	78	7.71
ML_18C_09	Thunnus thynnus	1750-1800	Archaeological	Marseille Leca	5.37	43.30	Adult	1750-1800	441434027	25.00	66	8.91
ML_18C_07B	Thunnus thynnus	1750-1800	Archaeological	Marseille Leca	5.37	43.30	Adult	1750-1800	356090645	27.50	66	8.57
ML_18C_01	Thunnus thynnus	1750-1800	Archaeological	Marseille Leca	5.37	43.30	Adult	1750-1800	278236664	34.00	62	8.04
ML_18C_10B	Thunnus thynnus	1750-1800	Archaeological	Marseille Leca	5.37	43.30	Adult	1750-1800	374549711	17.90	71	9.86
ML_18C_05B	Thunnus thynnus	1750-1800	Archaeological	Marseille Leca	5.37	43.30	Adult	1750-1800	346448656	23.70	67	7.65
ION_1925_40	Thunnus thynnus	1925	Archival	Zliten	14.66	33.25	Adult	1925	157680443	49.00	69	7.75
ION_1925_27	Thunnus thynnus	1925	Archival	Zliten	14.66	33.25	Adult	1925	197619377	45.00	57	7.49
ION_1925_22	Thunnus thynnus	1925	Archival	Zliten	14.66	33.25	Adult	1925	217493567	37.00	58	6.52
ION_1925_11	Thunnus thynnus	1925	Archival	Zliten	14.66	33.25	Adult	1925	397637695	46.00	59	13.96
ION_1925_09	Thunnus thynnus	1925	Archival	Zliten	14.66	33.25	Adult	1925	370419792	40.00	72	13.37

ION_1925_08	Thunnus thynnus	1925	Archival	Zliten	14.66	33.25	Adult	1925	302195775	33.00	56	8.02
ION_1925_04	Thunnus thynnus	1925	Archival	Zliten	14.66	33.25	Adult	1925	264014574	36.00	62	8.09
ION_1925_01	Thunnus thynnus	1925	Archival	Zliten	14.66	33.25	Adult	1925	579104421	38.00	58	17.42
IST_1941_02	Thunnus thynnus	1941	Archival	Istanbul	28.95	41.00	Adult	1941	277052784	27.00	63	6.32
IST_1941_01	Thunnus thynnus	1941	Archival	Istanbul	28.95	41.00	Adult	1941	1321362576	11.00	69	13.03
N18021808	Thunnus thynnus	Gulf of Mexico 2014-2018	Modern	Gulf of Mexico	-87.25	28.33	Larvae	2018	139541381	51.35	88	10.52
N18021809	Thunnus thynnus	Gulf of Mexico 2014-2018	Modern	Gulf of Mexico	-87.25	28.33	Larvae	2018	172754062	58.41	87	12.80
N18021810	Thunnus thynnus	Gulf of Mexico 2014-2018	Modern	Gulf of Mexico	-87.25	28.33	Larvae	2018	116693740	58.08	115	10.29
N17040501	Thunnus thynnus	Gulf of Mexico 2014-2018	Modern	Gulf of Mexico	-88.13	25.84	Larvae	2017	187146187	42.51	82	10.53
N17040108	Thunnus thynnus	Gulf of Mexico 2014-2018	Modern	Gulf of Mexico	-87.78	26.12	Larvae	2017	188911025	69.58	63	10.37
W14050096	Thunnus thynnus	Gulf of Mexico 2014-2018	Modern	Gulf of Mexico	-87.76	28.00	Larvae	2014	152783501	61.98	62	13.11
W14050097	Thunnus thynnus	Gulf of Mexico 2014-2018	Modern	Gulf of Mexico	-87.76	28.00	Larvae	2014	105816601	63.54	90	8.98
W14050029	Thunnus thynnus	Gulf of Mexico 2014-2018	Modern	Gulf of Mexico	-93.00	27.04	Larvae	2014	175222894	68.44	60	5.83
W14050007	Thunnus thynnus	Gulf of Mexico 2014-2018	Modern	Gulf of Mexico	-93.58	26.53	Larvae	2014	108745683	70.76	73	7.61
W14050014	Thunnus thynnus	Gulf of Mexico 2014-2018	Modern	Gulf of Mexico	-93.58	26.53	Larvae	2014	182858567	72.00	70	10.05
IEO-BA-104	Thunnus thynnus	Western Medierranean 2013	Modern	Western Mediterranean	2.07	39.27	YoY	2013	143129257	72.70	76	11.12
IEO-BA-107	Thunnus thynnus	Western Medierranean 2013	Modern	Western Mediterranean	2.07	39.27	YoY	2013	144642227	73.53	106	15.62
IEO-BA-109	Thunnus thynnus	Western Medierranean 2013	Modern	Western Mediterranean	2.07	39.27	YoY	2013	96241790	65.12	91	8.64
IEO-BA-110	Thunnus thynnus	Western Medierranean 2013	Modern	Western Mediterranean	2.07	39.27	YoY	2013	97782879	73.43	76	7.40
IEO-BA-113	Thunnus thynnus	Western Medierranean 2013	Modern	Western Mediterranean	2.07	39.27	YoY	2013	155133760	70.13	114	17.70

IEO-BA-121	Thunnus thynnus	Western Medierranean 2013	Modern	Western Mediterranean	2.07	39.27	YoY	2013	160129608	68.84	68	9.82
IEO-BA-63	Thunnus thynnus	Western Medierranean 2013	Modern	Western Mediterranean	2.07	39.27	YoY	2013	207719516	63.24	57	9.78
IEO-BA-67	Thunnus thynnus	Western Medierranean 2013	Modern	Western Mediterranean	2.07	39.27	YoY	2013	166718453	61.82	54	6.92
IEO-BA-68	Thunnus thynnus	Western Medierranean 2013	Modern	Western Mediterranean	2.07	39.27	YoY	2013	171334592	71.36	87	14.90
IEO-BA-77	Thunnus thynnus	Western Medierranean 2013	Modern	Western Mediterranean	2.07	39.27	YoY	2013	217771014	69.63	62	11.86
UNIB-SI-65	Thunnus thynnus	Central Mediterranea n 2013	Modern	Central Mediterranean	13.18	36.93	YoY	2013	224072082	36.86	91	15.90
UNIB-SI-67	Thunnus thynnus	Central Mediterranea n 2013	Modern	Central Mediterranean	13.18	36.93	YoY	2013	187286567	39.77	90	13.12
UNIB-SI-70	Thunnus thynnus	Central Mediterranea n 2013	Modern	Central Mediterranean	13.18	36.93	YoY	2013	173517705	67.00	59	9.02
UNIB-SI-74	Thunnus thynnus	Central Mediterranea n 2013	Modern	Central Mediterranean	13.18	36.93	YoY	2013	195783542	46.52	100	14.07
UNIB-SI-77	Thunnus thynnus	Central Mediterranea n 2013	Modern	Central Mediterranean	13.18	36.93	YoY	2013	135696808	65.49	61	7.44
UNIB-SI-79	Thunnus thynnus	Central Mediterranea n 2013	Modern	Central Mediterranean	13.18	36.93	YoY	2013	145693992	75.30	100	14.89
UNIB-SI-81	Thunnus thynnus	Central Mediterranea n 2013	Modern	Central Mediterranean	13.18	36.93	YoY	2013	179052561	59.80	87	13.92
UNIB-SI-84	Thunnus thynnus	Central Mediterranea n 2013	Modern	Central Mediterranean	13.18	36.93	YoY	2013	191490646	45.23	101	16.61
UNIB-SI-89	Thunnus thynnus	Central Mediterranea n 2013	Modern	Central Mediterranean	13.18	36.93	YoY	2013	194564289	63.46	66	10.85
UNIB-SI-92	Thunnus thynnus	Central Mediterranea n 2013	Modern	Central Mediterranean	13.18	36.93	YoY	2013	156174048	62.00	51	6.27
CYPR-LS-315	Thunnus thynnus	Eastern Mediterranea n 2013	Modern	Eastern Mediterranean	33.42	35.51	YoY	2013	97896709	70.20	133	11.55

CYPR-LS-330	Thunnus thynnus	Eastern Mediterranean 2013	Modern	Eastern Mediterranean	33.42	35.51	YoY	2013	107324817	69.75	143	13.60
CYPR-LS-331	Thunnus thynnus	Eastern Mediterranean 2013	Modern	Eastern Mediterranean	33.42	35.51	YoY	2013	104811752	70.80	137	12.94
CYPR-LS-343	Thunnus thynnus	Eastern Mediterranean 2013	Modern	Eastern Mediterranean	33.42	35.51	YoY	2013	103487327	64.40	134	11.36
CYPR-LS-347	Thunnus thynnus	Eastern Mediterranean 2013	Modern	Eastern Mediterranean	33.42	35.51	YoY	2013	173458587	55.22	95	14.18
CYPR-LS-352	Thunnus thynnus	Eastern Mediterranean 2013	Modern	Eastern Mediterranean	33.42	35.51	YoY	2013	149595935	75.87	97	15.22
CYPR-LS-41	Thunnus thynnus	Eastern Mediterranean 2013	Modern	Eastern Mediterranean	33.42	35.51	YoY	2013	147452659	75.60	97	15.44
CYPR-LS-45	Thunnus thynnus	Eastern Mediterranean 2013	Modern	Eastern Mediterranean	33.42	35.51	YoY	2013	115427698	70.70	129	13.26
CYPR-LS-49	Thunnus thynnus	Eastern Mediterranean 2013	Modern	Eastern Mediterranean	33.42	35.51	YoY	2013	153052848	77.21	99	16.62
CYPR-LS-34	Thunnus thynnus	Eastern Mediterranean 2013	Modern	Eastern Mediterranean	33.42	35.51	YoY	2013	47828983	78.40	119	7.53
M-TUN005	Thunnus thynnus	Norway 2018	Modern	Møre, Norway	63.65	7.95	Adult	2018	106652464	69.20	105	11.97
M-TUN006	Thunnus thynnus	Norway 2018	Modern	Møre, Norway	63.65	7.95	Adult	2018	92809018	72.60	99	10.16
M-TUN007	Thunnus thynnus	Norway 2018	Modern	Møre, Norway	63.65	7.95	Adult	2018	88264024	71.70	99	9.58
M-TUN008	Thunnus thynnus	Norway 2018	Modern	Møre, Norway	63.65	7.95	Adult	2018	120629503	76.00	101	14.09
M-TUN009	Thunnus thynnus	Norway 2018	Modern	Møre, Norway	63.65	7.95	Adult	2018	106263898	76.00	101	12.37
M-TUN010	Thunnus thynnus	Norway 2018	Modern	Møre, Norway	63.65	7.95	Adult	2018	123372801	74.80	100	14.13
M-TUN011	Thunnus thynnus	Norway 2018	Modern	Møre, Norway	63.65	7.95	Adult	2018	113959487	76.60	100	13.25
M-TUN012	Thunnus thynnus	Norway 2018	Modern	Møre, Norway	63.65	7.95	Adult	2018	115930638	75.50	101	13.40
M-TUN013	Thunnus thynnus	Norway 2018	Modern	Møre, Norway	63.65	7.95	Adult	2018	140002724	76.10	101	16.23
M-TUN014	Thunnus thynnus	Norway 2018	Modern	Møre, Norway	63.65	7.95	Adult	2018	145862994	75.10	100	16.60

Modern samples

Young-of-the-year Mediterranean samples were randomly selected from those caught in spawning areas as part of a separate study(26).

Gulf of Mexico larval samples were collected as part of the NOAA Restore project (https://restoreactscienceprogram.noaa.gov/projects/bluefin-tuna-larvae). Larvae were randomly selected for sequencing from three sampling surveys conducted in 2014, 2017 and 2018 from the north and western shelf of the Gulf of Mexico. Multiple years and locations were sampled to obtain maximum genomic variability among few final samples analysed (n=10). Full details of sampling are found in Table S2.

Table S2. Sampling details of the Gulf of Mexico (GoM) 2014-2018 samples sequenced herein, collected across multiple years and locations as part of the NOAA Restore project.

ID	*Station*	*Ship*	*Gear*	*Sample_No*	*SL_mm etoh*	*developmental stage*	*depth collected, m*	*Longitude*	*Latitude*	*Date*
N17040108	*4*	*NOAA SHIP NANCY FOSTER*	*90 cm quadrangular bongo, 505 mesh*	*D03760*	*7.19*	*postflexion*	*0-25*	*-87.7773*	*26.1198*	*10-May-17*
N17040501	*21*	*NOAA SHIP NANCY FOSTER*	*90 cm quadrangular bongo, 505 mesh*	*D03791*	*5.5*	*flexion*	*0-25*	*-88.1328*	*25.8438*	*12-May-17*
N18021808	*91*	*NOAA SHIP NANCY FOSTER*	*90 cm quadrangular bongo, 505 mesh*	*D04664*	*not measured*	*larvae*	*0-25*	*-87.2497*	*28.3327*	*15-May-18*
N18021809	*91*	*NOAA SHIP NANCY FOSTER*	*90 cm quadrangular bongo, 505 mesh*	*D04664*	*not measured*	*larvae*	*0-25*	*-87.2497*	*28.3327*	*15-May-18*
N18021810	*91*	*NOAA SHIP NANCY FOSTER*	*90 cm quadrangular bongo, 505 mesh*	*D04664*	*not measured*	*larvae*	*0-25*	*-87.2497*	*28.3327*	*15-May-18*
W14050007	*23*	*F.G. Walton Smith (UNOLS Ship)*	*1x2m rectangular net, 505 mesh*	*47654*	*4.28*	*larvae*	*0-10*	*-93.582*	*26.5346*	*7-May-14*
W14050014	*23*	*F.G. Walton Smith (UNOLS Ship)*	*1x2m rectangular net, 505 mesh*	*47654*	*4.32*	*larvae*	*0-10*	*-93.582*	*26.5346*	*7-May-14*
W14050029	*27*	*F.G. Walton Smith (UNOLS Ship)*	*1x2m rectangular net, 505 mesh*	*47659*	*5.05*	*larvae*	*0-10*	*-93.0033*	*27.0388*	*8-May-14*
W14050096	*69*	*F.G. Walton Smith (UNOLS Ship)*	*1x2m rectangular net, 505 mesh*	*47701*	*6.68*	*larvae*	*0-10*	*-87.761*	*27.9963*	*20-May-14*
W14050097	*69*	*F.G. Walton Smith (UNOLS Ship)*	*1x2m rectangular net, 505 mesh*	*47701*	*5.94*	*larvae*	*0-10*	*-87.761*	*27.9963*	*20-May-14*

A total of 10 bluefin tuna were analysed from catches off Ålesund in 2018. These were adult individuals ca. 2 m in fork length (FL) and were analysed as part of a separate study, for full details see (92)

Ancient samples

Archival and archaeological sample details are listed below along with body size estimates calculated using the online tool https://tunaarchaeology.org/lengthestimations/ as detailed in the publication (39)

1925 & 1941 tuna trap archived samples

We analysed specimens collected from two locations in the early 20th century by the ecologist Massimo Sella (50). All specimens consist of vertebrae that were air-dried by the collator after capture and processing at tuna traps (Tonnare). A total of 8 vertebrae specimens were obtained from BFT captured in the tonnara at Zilten, Libya (Ionian Sea) in 1925, estimated to represent BFT between 158-204 cm FL, average 182 cm FL. Two large (275 and 278 cm FL) specimens were also sampled from tuna traps in the Bosporus, Istanbul, Turkey in 1941.

1750-1800 Marseille Harbour, Marseille, France

A total of seven bluefin tuna bones (6 opercula and 1 vertebra) were obtained from the excavation Marseille-22 rue Jean François Leca, of the ancient port of Marsille, France, which was dated to between the late 18th and early 19th century (128). An approximate date of 1750-1850 CE is shown for this sample group in analyses. FL estimates were not made for these individuals as the vertebra selected was fragmented and could not be assigned to rank or accurately measured. Specimens represented large ca. 2 m sized adult bluefin tuna.

1600-1800 Pedras de Fogu, Sassari, Italy

Ten vertebrae samples were obtained from the archaeological site of 'Pedras de Fogu' (Sassari, Sardinia, Italy). A tuna trap (tonnara) operated at this location from the end of the 16th to the end of the 18th century where BFT vertebrae have been recovered in a midden at the back of the beach after they were revealed by coastal erosion (129). These specimens were estimated to range from 115-231 FL, average 178 cm FL.

900-1200 Sicily, Italy

A total of 3 specimens (2 vertebrae and 1 cranial element) were obtained from the archaeological site of 'Mazara del Vallo' situated in the town (southwestern Sicily, Italy). Samples were recovered from urban 10-13th century layers, each dated by context as detailed in (130), and identified as different individuals according to their range of sizes. FL estimates were not made for these individuals as the vertebrae selected were fragmented and could not be assigned to rank or accurately measured. Broadly, specimens represented small-large sized adult bluefin tuna. A total of 3 samples (2 vertebrae and 1 cranial element) were selected for analyses from urban 9-10th century layers in two different excavations in settlements in the city of Palermo, Sicily; Sant'Antonino and Corso dei Mille. The layers were dated by context as detailed in (130). Samples were estimated to represent individuals ranging from 101-185 cm FL, average 130 cm FL, believed to have been caught locally.

800-1200 Yenikapi, Istanbul, Turkey

Ten vertebrae specimens were selected for analyses from an excavation at a Byzantine era site in the Yenikapi neighbourhood of Istanbul, Turkey. The Port of Theodosius operated at this site from 4-11th century before being filled in the 15th century (131). The 800-1200 CE origin of the samples is proposed from carbon dating achieved in a separate study(15). It is unknown whether specimens were fished locally or transported to the city of Constantinople, which was a major trading hub throughout the Byzantine period.

3000 BCE Jortveit, Norway

A total of two samples were used from Jortveit, southern Norway. Accurate FL estimates could not be made for these individuals as the vertebrae were fragmented and could not be assigned to rank or measured accordingly. Nonetheless, the bones were of considerable size, likely representing specimens ca. 2 m sized adult BFT. These individuals were carbon dated to ca. 3000 BCE. For full details see (92)

Supplementary Figures

Mapping and sample quality

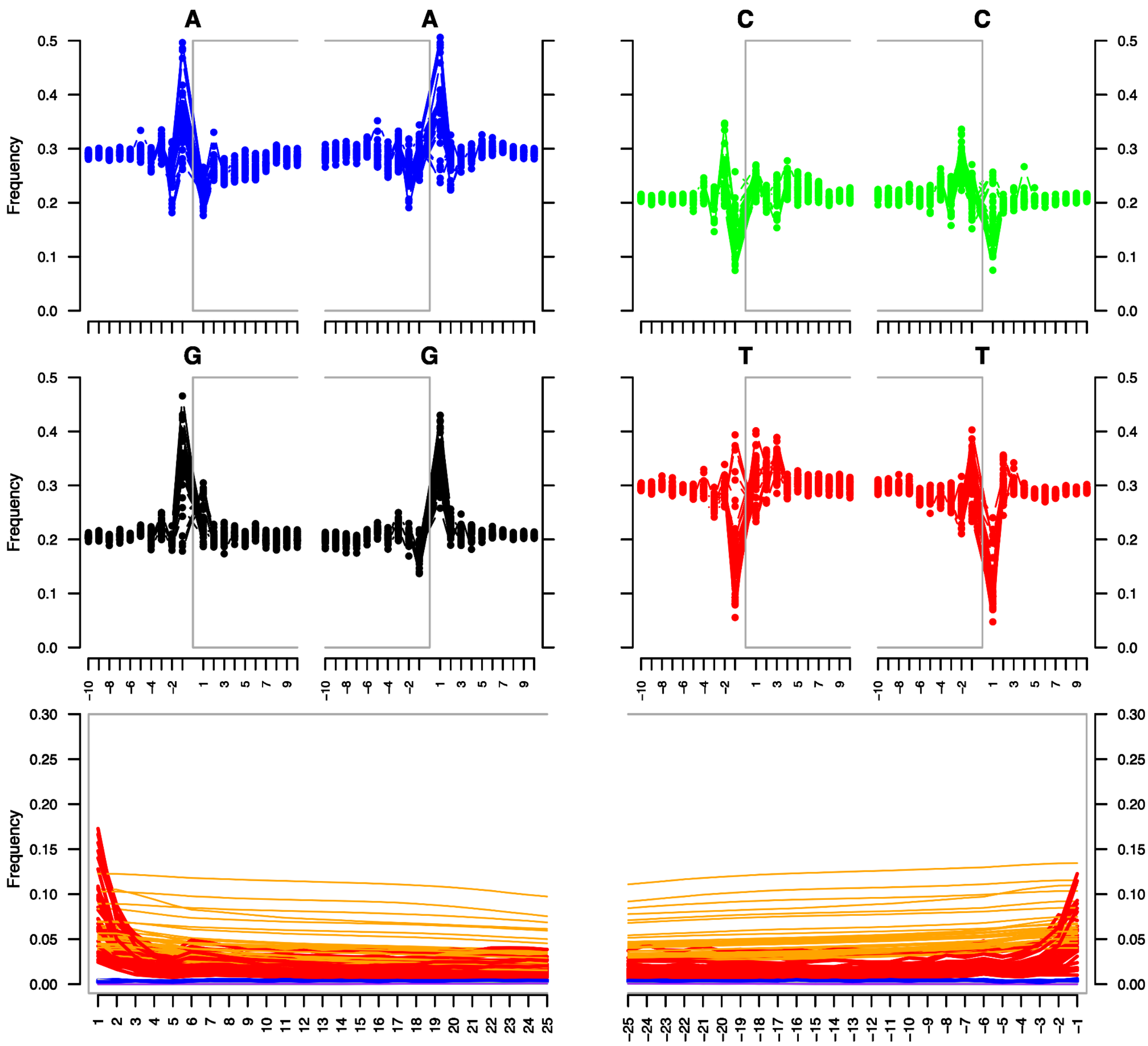

Figure S1. MapDamage fragment length plots for ancient Atlantic bluefin tuna (*Thunnus thynnus*) samples (n=45). X-axes represent base pair distance from terminal ends of sequence reads.

Demographic analyses

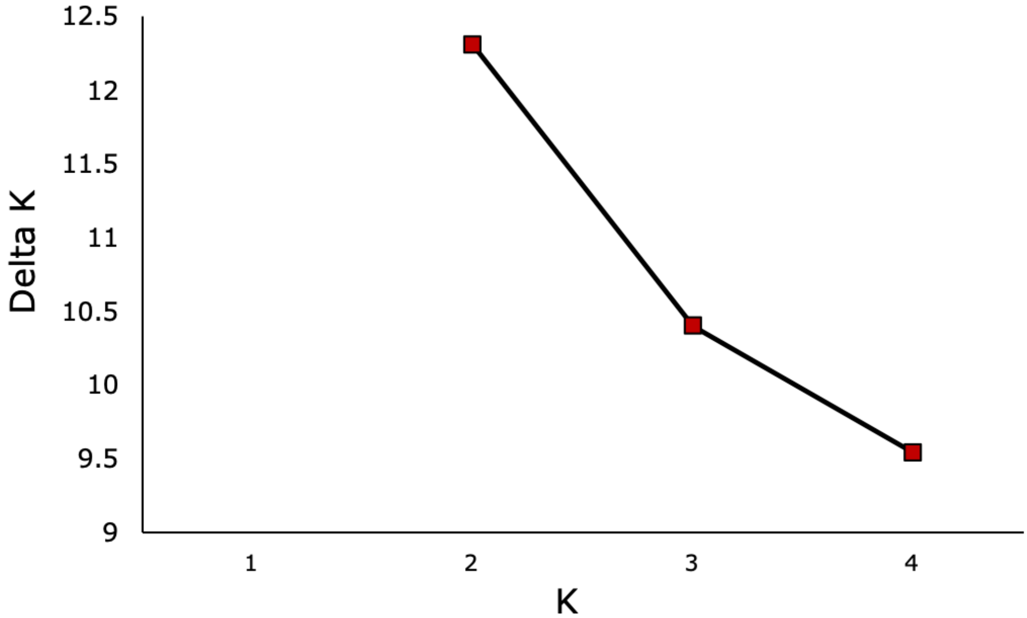

Figure S2. Delta K of 2 was selected for the optimal number of Atlantic bluefin tuna (*Thunnus thynnus*) populations using the modern spawning site dataset (n=39).

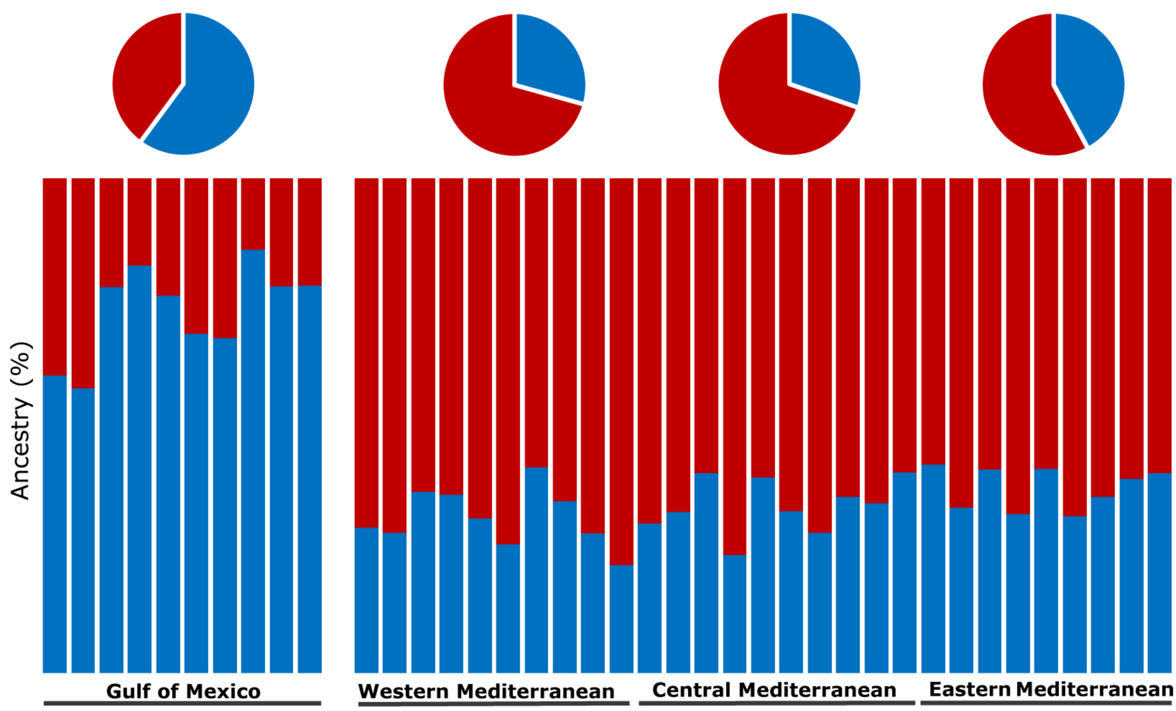

Figure S3. STRUCTURE bar plots and pie charts for the modern Atlantic bluefin tuna (*Thunnus thynnus*) (n=39) spawning site pruned dataset at 386,963 SNPs. Illustrating that at a greater number of loci not using F_{ST} site-filtering methods, the Eastern Mediterranean has a slightly greater quantity of Gulf of Mexico ancestry than Western and Central Mediterranean bluefin tuna, as discovered using the modern and ancient combined dataset in the main paper (Figure 1).

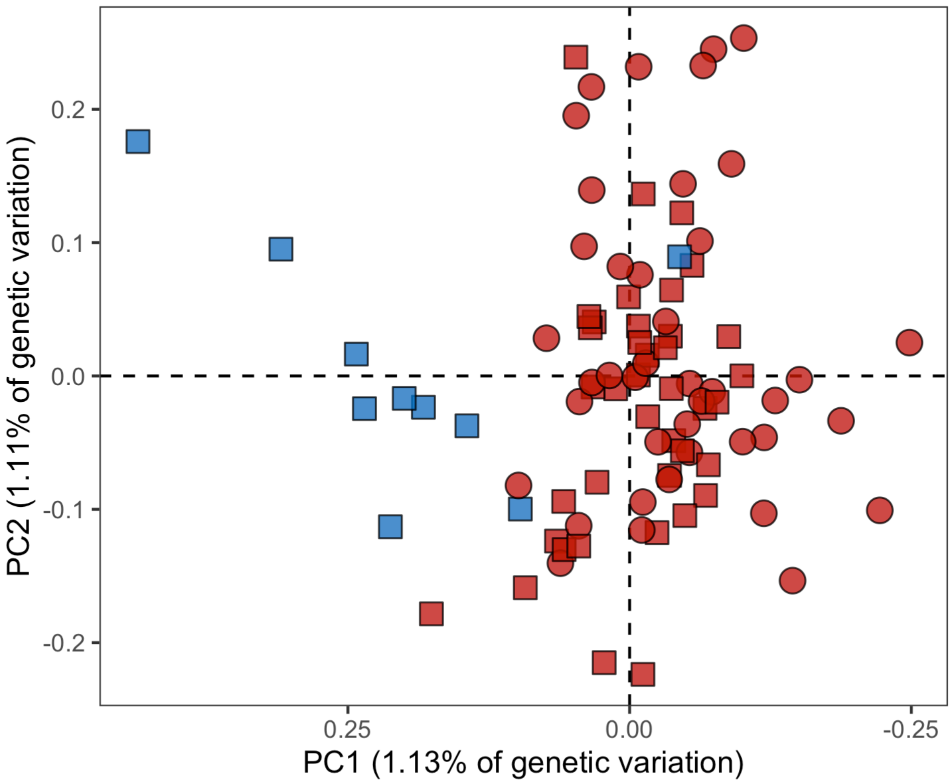

Figure S4. Principal components analysis (PCA) of the modern and ancient dataset at 27,882 SNPs which have not been filtered using the F_{ST} site-filtering approach. Illustrating that without our approach, PCAs cannot clearly separate the two populations of Atlantic bluefin tuna (*Thunnus thynnus*).

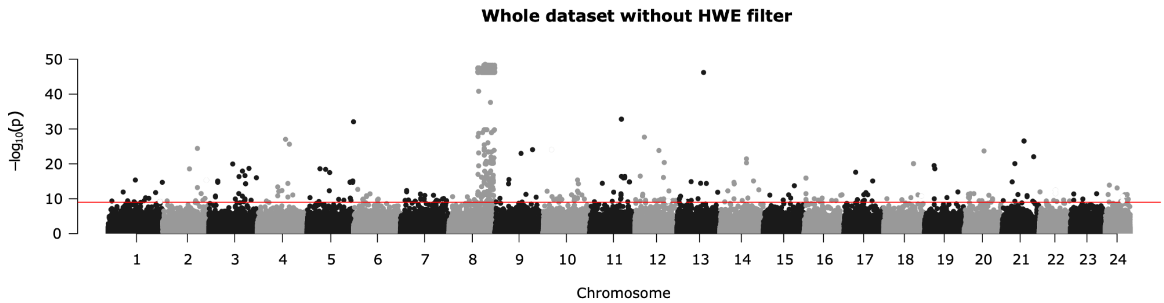

Figure S5. Manhattan plot of pcadapt outlier detection results for Atlantic bluefin tuna (*Thunnus thynnus*) (n=39) using the modern spawning site dataset to show introgression signals on Chromosome 8 when including loci that deviate from Hardy Weinberg Equilibrium.

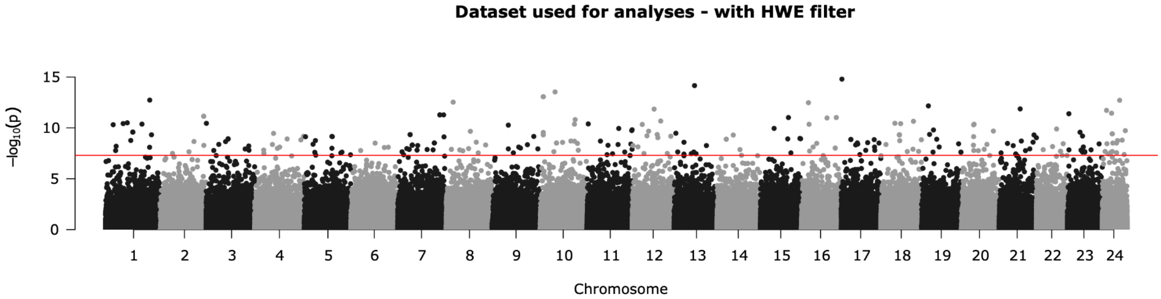

Figure S6. Manhattan plot of pcadapt outlier detection results for Atlantic bluefin tuna (*Thunnus thynnus*) (n=39) using the modern spawning site dataset to show the removal of introgression signals on Chromosome 8 when removing loci that deviate from Hardy Weinberg Equilibrium. See Methods. This approach was used for all demographic analyses.

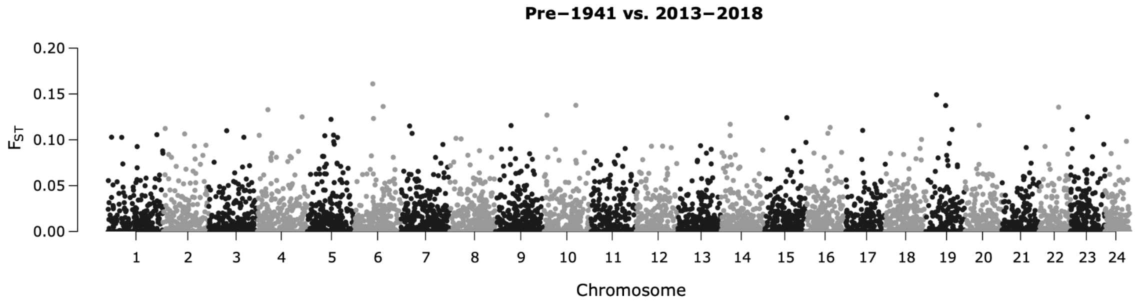

Figure S7. Manhattan plot of Pcadapt outlier detection results for Atlantic bluefin tuna (*Thunnus thynnus*) (n=90) using the combined modern and ancient dataset to show the distribution of F_{ST} temporal differences across the genome being relatively uniform and low F_{ST}.

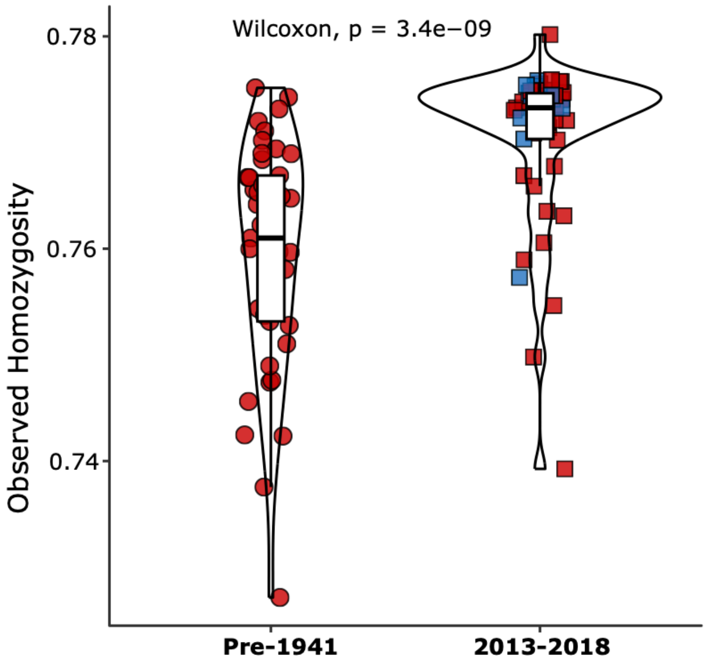

Figure S8. Violin plots show observed homozygosity (fraction of sites) differences when F_{ST} site-filtering is not applied to the dataset and 10% missing data is allowed at 2,890,241 SNPs between pooled ancient (Pre-1941, n=41) and modern (2013-2018, n=49), coloured by population (blue: western stock, red: eastern stock) and with shapes depicting time period (circle: ancient, square: modern).

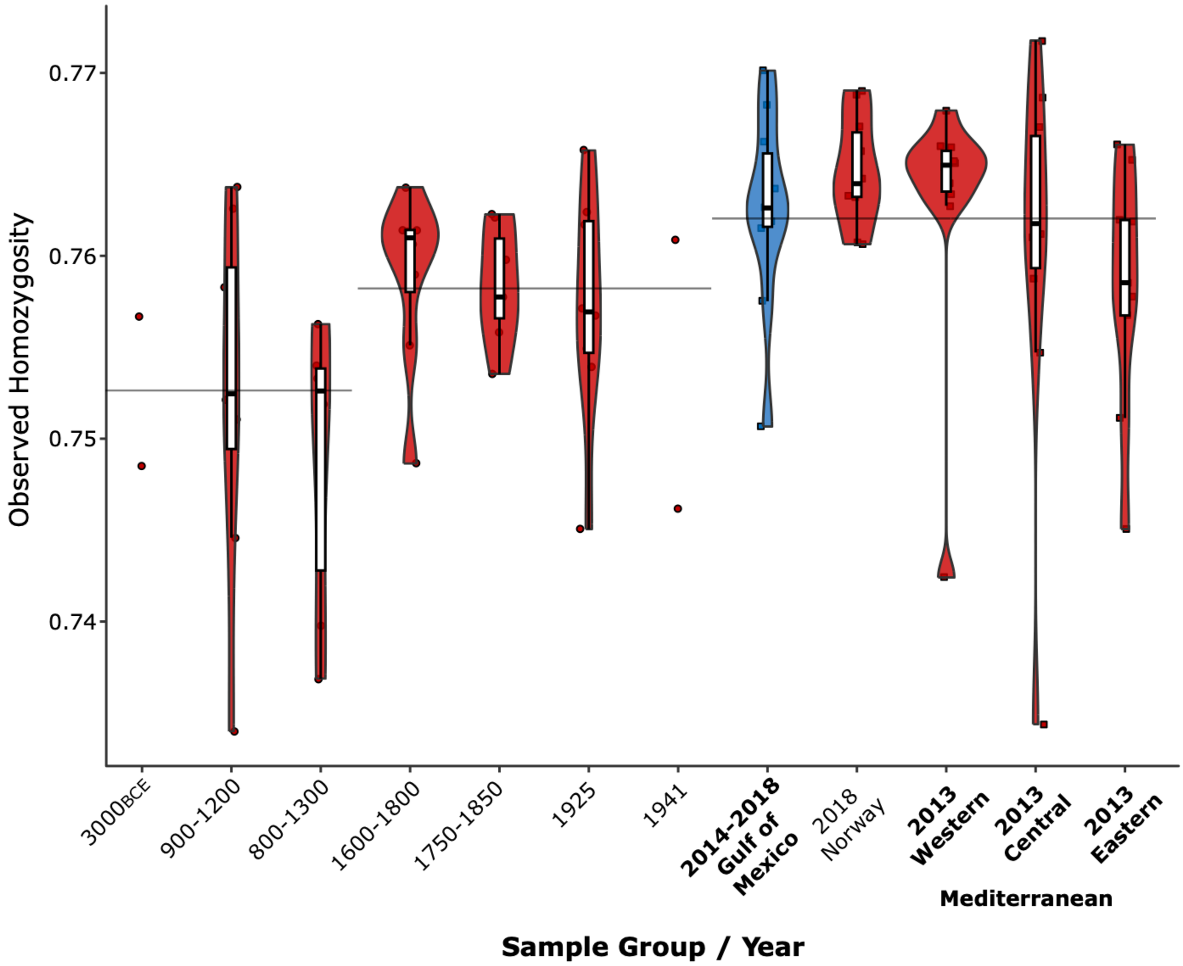

Figure S9. Violin plots show step-wise loss of observed homozygosity (fraction of sites) over time at 12,838 SNPs between grouped ancient and modern Atlantic bluefin tuna (*Thunnus thynnus*) samples (n=90), coloured by stock (blue: western stock, red: eastern stock) and with shapes depicting time period (circle: ancient, square: modern). Boxplots are illustrated for groups >2 sample size, with group medians, 25th and 75th percentile as outer edges and 95th percentiles (black whiskers). Horizontal black lines between groups of time point samples (3000BCE to 900-1300; 1600-1800 to 1941; modern 2013-2018 samples) show averages.

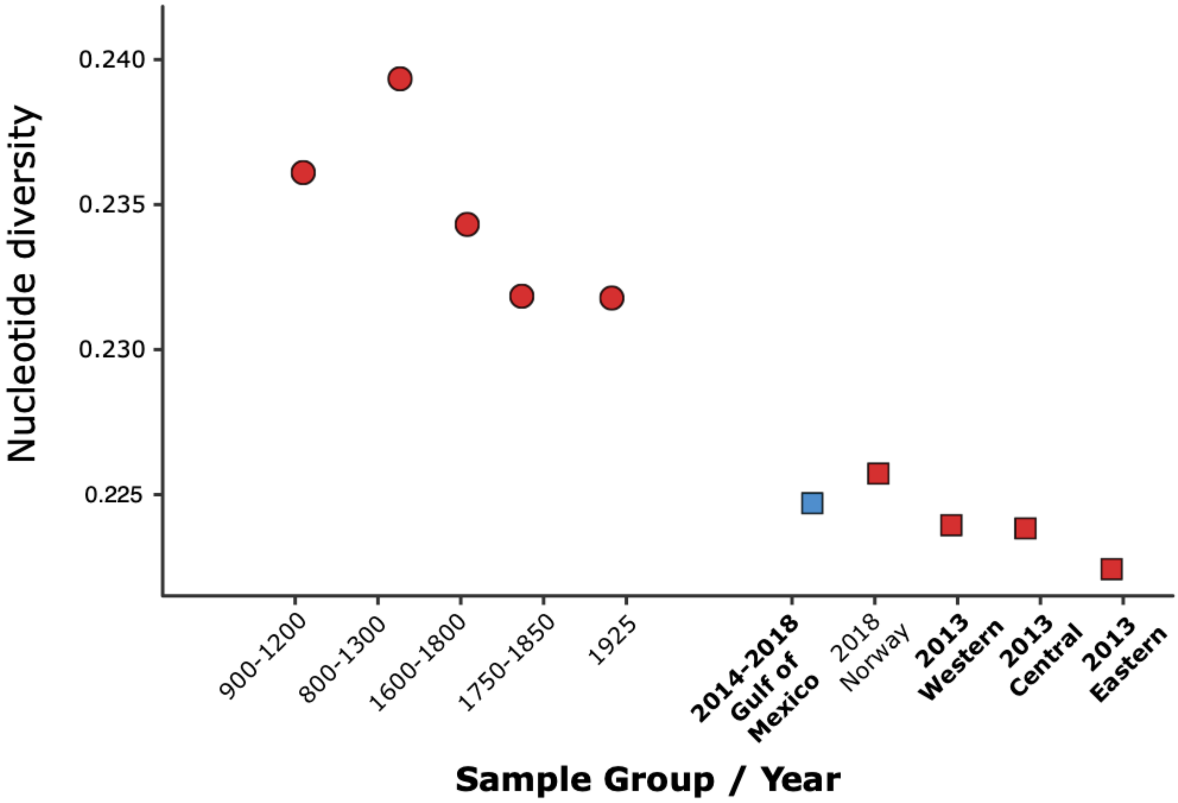

Figure S10. Nucleotide diversity estimates for each sample group show the trajectory of pre-industrial decline between ancient and modern Atlantic bluefin tuna (*Thunnus thynnus*), calculated including missing data at 2,890,241 loci, estimating nucleotide diversity average of 50Kb windows and not showing groups with sample size <2. Groups coloured by stock (blue: western stock, red: eastern stock).

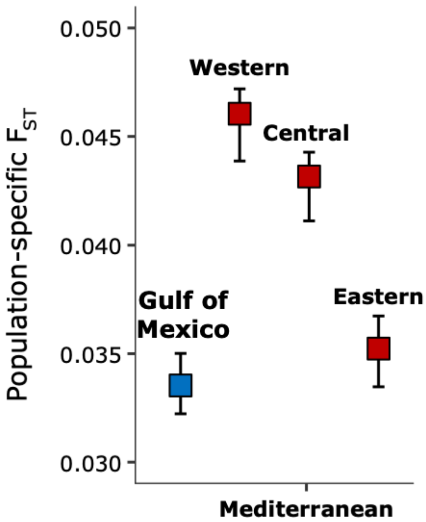

Figure S11. Error bars show population-specific F_{ST} estimates for each modern spawning site sample at 386,963 SNPs, with 95% confidence intervals (CIs, black whiskers).

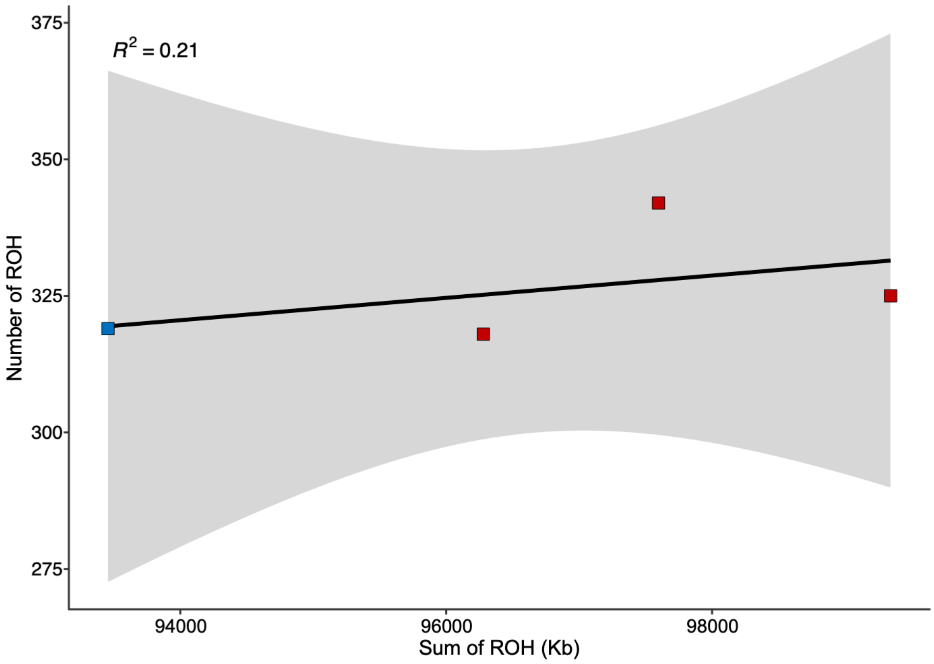

Figure S12. Scatter plot of the number of runs of homozygosity (NROH) vs the sum of runs of homozygosity (SROH) with geom_smooth fit line (black, grey shading as 95% confidence interval) for modern Atlantic bluefin tuna (*Thunnus thynnus*) samples analysed (n=39). Correlation between NROH and SROH) was weak as shown by R2=0.21 as a result of no difference between modern samples.

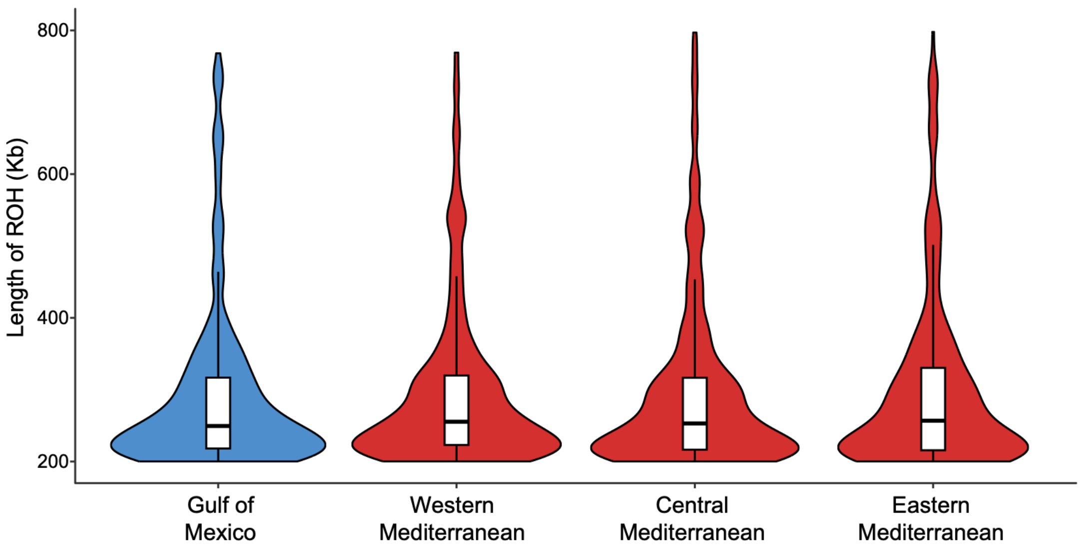

Figure S13. Violin plots no difference between Atlantic bluefin tuna (*Thunnus thynnus*) modern spawning site samples (n=39) in lengths of runs of homozygosity (ROH), coloured by stock (blue: western stock, red: eastern stock). Boxplots are illustrated with group medians, 25th and 75th percentile as outer edges and 95th percentiles (black whiskers).

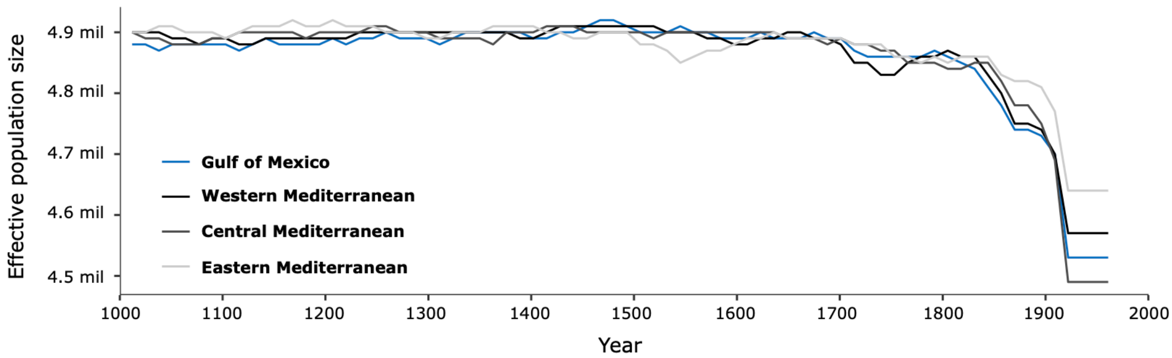

Figure S14. GONE estimates of historical effective population size (N_e) for grouped Atlantic bluefin tuna (*Thunnus thynnus*) samples (n=39) confirm similar trajectories between Mediterranean sites as when pooled (main paper, Figure 2). Mediterranean spawning sites (grey scale) and Gulf of Mexico (blue), representing the previous 100 generations from 1000 CE to 1964, using the most likely generation length of 13 years.

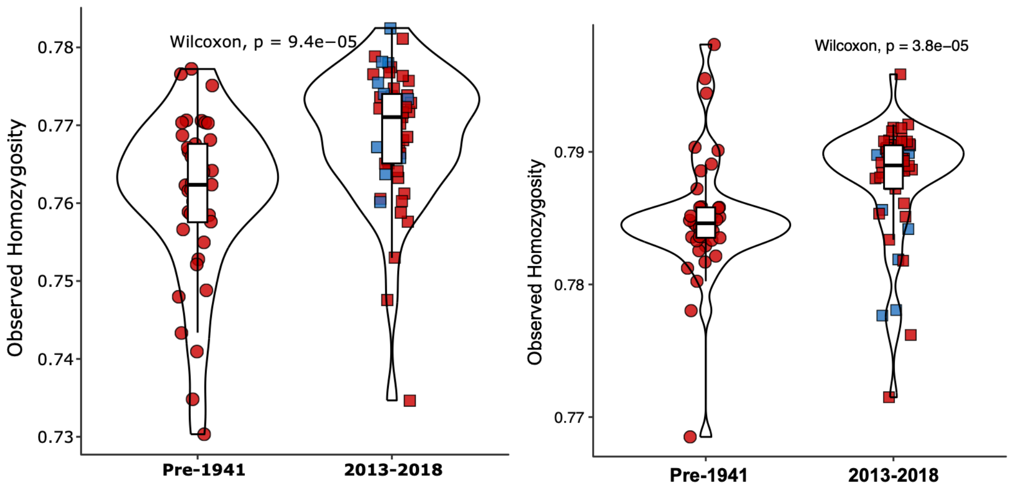

Figure S15. Violin plots show observed homozygosity (fraction of sites) differences when (left) transition sites potentially subject to ancient DNA damage are excluded from the dataset at 5822 SNPs, and (right) when the main dataset is filtered to contain only SNPs with greater coverage than 10 and GQ >25 at 334,783 SNPs, between pooled ancient (Pre-1941, n=41) and modern (2013-2018, n=49), coloured by population (blue: western stock, red: eastern stock) and with shapes depicting time period (circle: ancient, square: modern).

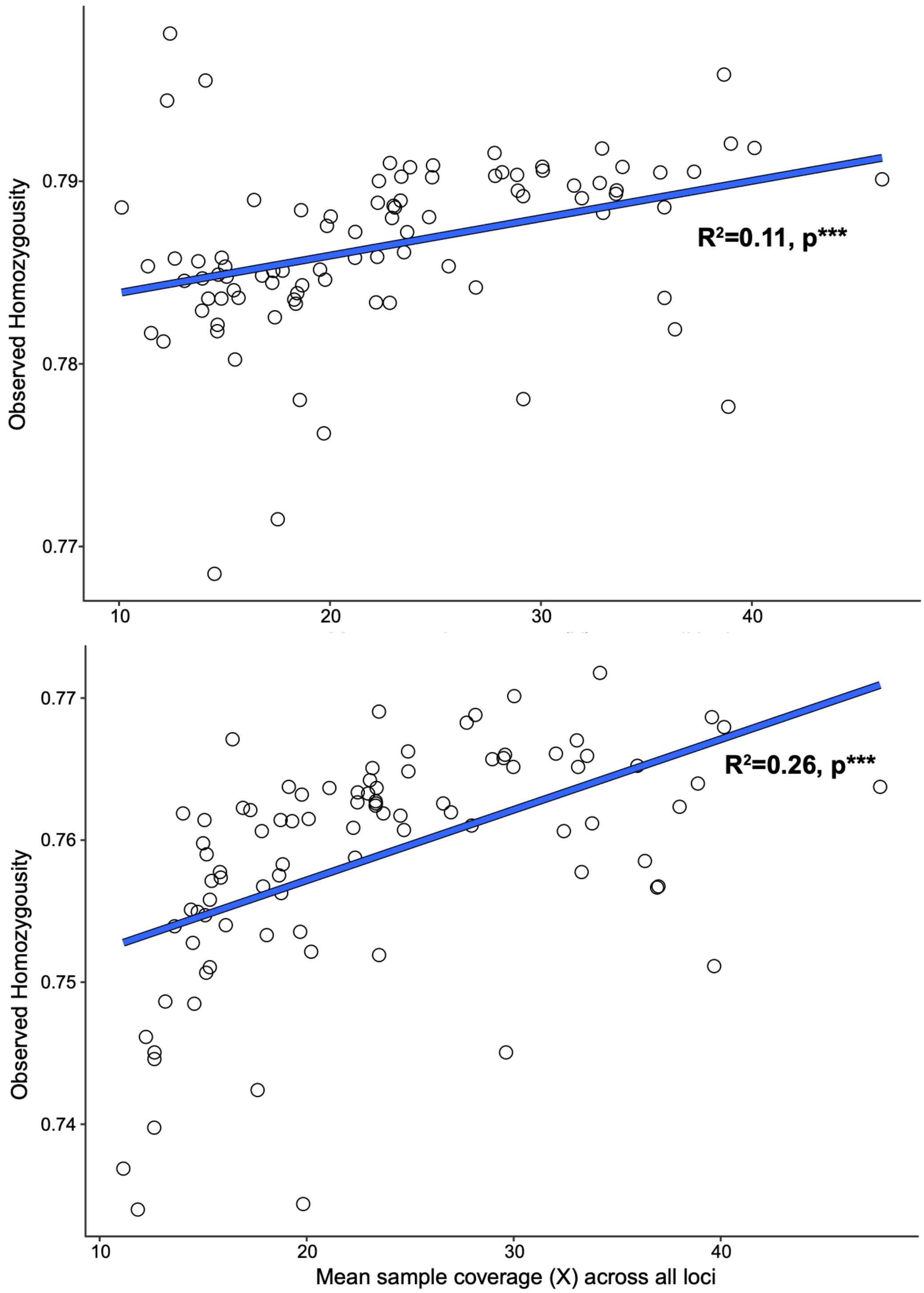

Figure S16. Scatterplots show relationships between Observed Homozygosity (fraction of sites) and mean sample coverage (fold) calculated per individual. Patterns in (Pearsons) correlation and significance are consistent whether using the main FST-filtered dataset shown in Figure 2 (bottom), or the dataset filtered to increase quality further (top) by removing loci <10x coverage and with genotype quality scores below 25, at 334,783 loci. Significance of Pearson's correlation was illustrated using asterisk (***: $p<0.001$)

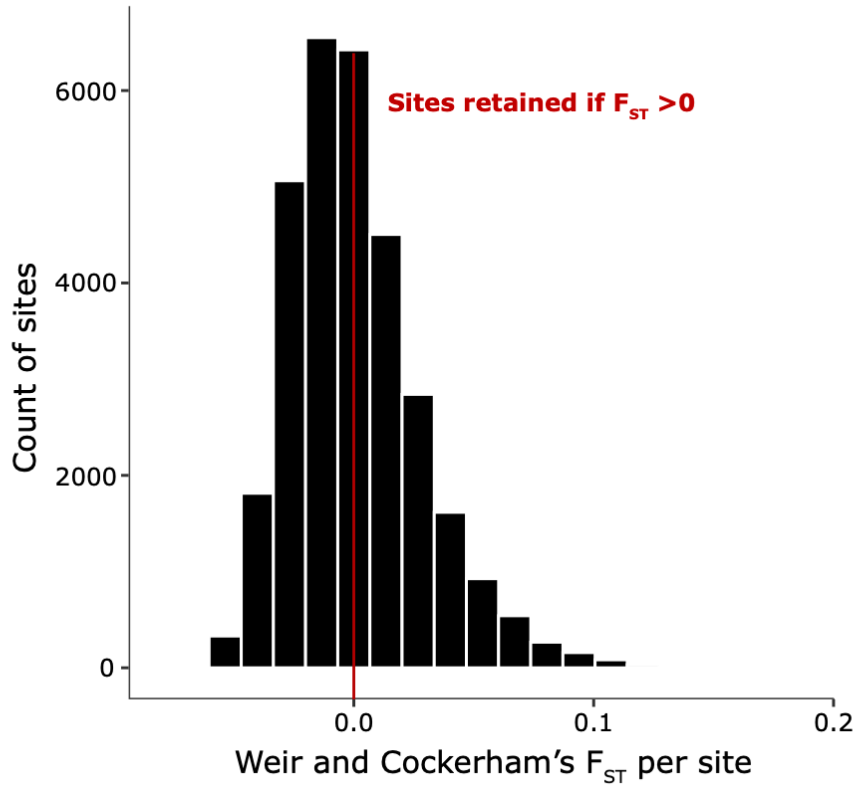

Figure S17. Histogram of per-site F_{ST} values calculated using Weir and Cockerhams F_{ST} in VCFTools. Illustrated is the F_{ST} distribution of the modern and ancient dataset at 30,264 SNPs showing cut off used for filtering (0 F_{ST}).

Supplementary References

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