Palaeoclimate shaped Bluefish (*Pomatomus saltatrix,* L.) structure in the Northern hemisphere.

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RUNNING TITLE: Northern hemisphere population structure of Bluefish

BLURB: Three genetic units were identified across the Atlantic Ocean and the Mediterranean Sea. Paleoclimate could explain recent Bluefish population structure.

COVER TEASER: Genetic structure of Bluefish northern populations

23 ABSTRACT:

24 Bluefish (*Pomatomus saltatrix* L.), a highly migratory cosmopolitan predator, is the
25 only extant representative of the family Pomatomidae. It has been the subject of many
26 studies due to its commercial and recreational value, but much less research has been
27 conducted on its global population structure. Here we investigate the population
28 structure of this species and the effects of present and past oceanographic barriers to
29 dispersal in its North Atlantic, Mediterranean, Marmara, and Black seas. We employed
30 mitochondrial (cytochrome b and cytochrome oxidase subunit I genes) and nuclear
31 (eight microsatellite loci) DNA as molecular markers. Three main genetic units of
32 Bluefish were identified in the considered: American (West Atlantic waters), Spanish
33 (East Atlantic-Western Mediterranean regions), and Turkish (Eastern Mediterranean,
34 Marmara and Black seas). Our results suggested that Bluefish is panmictic in the
35 northwest Atlantic Ocean but not in the Mediterranean Sea. The common ancestor of
36 the studied populations was traced back to the interglacial cycle Aftonian II, while the
37 separation between clades was estimated to have occurred during glacial periods, likely
38 due to migrations to refuges and the closure of the Mediterranean Sea. In conclusion,
39 paleoclimate seems to have been fundamental for shaping the present genetic lineages
40 of *Pomatomus saltatrix.*

41

42 KEYWORDS: Bluefish, phylogeography, ocean barriers, glaciations, population
43 structure.

44

45 1. INTRODUCTION:

46 Ocean currents and the apparent lack of physical barriers in the marine realm seem to
47 facilitate extensive gene flow among marine fish populations (Palumbi 1994). Pelagic
48 and demersal fishes are expected to exhibit little intraspecific genetic structuring even
49 over large geographic distances (Ward et al. 1994) and marine environments are often
50 seen as open habitats in which isolation by distance is the main mechanism that may
51 promote speciation (Palumbi 1994). However, several studies have demonstrated the
52 existence of marine physical barriers that produce intraspecific genetic fragmentation in
53 marine systems. Population structuring of highly migratory marine fish can be promoted
54 by currents (Machado-Schiaffino et al. 2010), salinity gradients (Nielsen et al. 2004),
55 temperature boundaries (Crow et al. 2007), convergence of distinct water masses
56 (Borrero-Perez et al. 2011), behaviour (Campos-Telles 2011), or historical past events
57 (Shen et al. 2011). Some barriers to gene flow are well defined by coastal shapes and
58 features. Examples are the Gibraltar Strait for some Sparidae species (Bargelloni et al.
59 2003), the Siculo-Tunisian strait for sea bass (Bahri-Sfar et al. 2000), the Florida Keys
60 for gobies (Avise 1992), and the hydrographic isolation of the Aegean and Ionian and
61 Adriatic Seas for numerous species (Partanello et al. 2007; Perez-Losada et al. 2007).
62 On the other hand, behavioral traits such as homing can also account for population
63 differentiation in some species (e.g., Castillo et al. 2005). The genetic variability and
64 population genetic structure of a species are shaped by both present and historical
65 marine barriers and also by palaeoecological history (Partanello et al. 2007; Perez-
66 Losada et al. 2007). Hence, to better understand speciation mechanisms in the marine
67 environment, it is important to characterize not only population dynamics and structure,
68 but also life-history strategies, environmental factors from past and present, and
69 physical barriers to dispersal (Zardoya et al. 2004).

70 Bluefish (*Pomatomus saltatrix*) is a cosmopolitan, migratory, pelagic predator
71 distributed over continental shelves and in estuaries of temperate waters of the Atlantic,
72 Indian, and Pacific Oceans and adjacent seas, including the Mediterranean, Aegean, and
73 Black seas (Briggs 1960; Tortonese 1986; Pottern et al. 1989; Juanes et al. 1996). As a
74 consequence of its broad distribution and the existence of potential oceanographical
75 barriers, the species may be composed of multiple different populations but there is
76 limited information available (Goodbred and Graves 1996; Turan et al. 2006; Pardiñas
77 et al. 2010). Bluefish is one of the most important recreational and commercial species
78 along the East Coast of the United States (Robillard et al. 2008) and is the target species
79 of an important artisanal fishery in all Turkish seas: Marmara, Aegean, and Black
80 (Ceyhan et al. 2007). Failure to detect population units can lead to local overfishing and
81 ultimately to severe declines of fisheries (Hutchings 2000 QUERY: not in references;
82 Knutsen et al. 2003 QUERY: 2009?), thus an accurate definition of Bluefish population
83 structure is particularly important and necessary for fisheries management (Utter 1991;
84 Wilson 2003).

85 The contemporary distribution of Bluefish is coincident with sea surface temperatures
86 of 18-27ºC (Juanes et al. 1996) and it has been suggested that shifts in its ranges and
87 contacts between populations have resulted from historical changes in water
88 temperature (Goodbred and Graves 1996). The sensitive behavioural response of
89 Bluefish to temperature variations may provide new insights into the evolutionary
90 consequences of the glacier-interglacier cycles and migrations into refuges for marine
91 migratory species.

92 The objective of this study was to document the present population structure of Bluefish
93 in its northern distribution across the North Atlantic ocean and Mediterranean,
94 Marmara, and Black seas, identify possible ocean barriers to dispersal, and reconstruct

95 the phylogeography of Bluefish to understand the role of climate for determining
96 historical and present barriers to gene flow along the Atlantic and Mediterranean basins.
97

98 2. MATERIAL AND METHODS:

99 2.1. *Sampling*

100 A total of 120 samples of *Pomatomus saltatrix* collected from 8 different locations
101 (Figure 1) between 2004 and 2009 were analyzed. These samples represent at most two
102 consecutive generations since the maturity age is 2.4 in males and 1.9 in females (Dhieb
103 et al., 2006). Four locations were in the northwest (NW) Atlantic Ocean, on the
104 American coast: New Jersey, Maryland, North Carolina, and Florida and four locations
105 were in the East Atlantic and in the Mediterranean basin: Cadiz (Atlantic Spanish coast)
106 and Barcelona (West Mediterranean, Spanish coast), and Canakkale and Istanbul
107 (Marmara and Black seas, Turkish coast).
108

109 2.2. *DNA extraction, amplification and sequencing*

110 DNA was extracted with Chelex (Bio-Rad®) following Estoup et al. (1996). Two
111 mitochondrial genes and eight microsatellite loci were amplified. The mitochondrial
112 cytochrome b (Cyt b) gene sequences were obtained following the protocol described by
113 Kocher et al. (1989) with the primers H151 and L148 described therein. The
114 cytochrome oxidase subunit I (COI) gene was amplified with primers designed for
115 *Pomatomus saltatrix*: COI-R Pom.: 5´-AAGAATGGGGTCTCCTCCAC-3´ and COI-F
116 Pom.: 5´- TTGGTGCATGAGCTGGTATG-3´; with the software PRIMER3 (Rozen
117 and Skaletsky 2000). Several potential primers set were generated; we selected one set
118 of primers that covered the maximum number of base pairs. The polymerase chain
119 reactions (PCR) to obtain the COI sequences were performed using the GeneAmp PCR

120 system 2700 (Applied Biosystems). Total reaction volume was 40 µl and the reaction
121 mix contained approximately 50 ng of DNA, 20 pmol of each primers, 10 mM Tris-
122 HCL pH 8.8, 250 µM of each dNTP, 5U of DNA Taq polymerase (Promega, Madison,
123 Wisconsin) and 2.5 mM $MgCl_2$. The PCR conditions were: initial denaturing at 95ºC for
124 5 min, then 35 cycles of denaturing at 95ºC for 30 s, annealing at 58ºC for 30 s and an
125 extension of 72ºC for 30 s, and a final extension at 72ºC for 20 min. PCR products were
126 visualized in 2% agarose gels with 10 mg/ml of ethidium bromide. Stained bands were
127 excised from the gel and DNA fragments were purified with an Eppendorf PerfectPrep
128 Gel CleanUp® kit. Then, purified DNA was precipitated using standard 2-propanol
129 precipitation and re-suspended in formamide prior to sequencing. Sequencing was
130 performed in an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems), with
131 BigDye 3.1 Terminator system, in the Unit of Genetic Analysis of the University of
132 Oviedo (Spain).
133 Eight tetranucleotide microsatellites were assayed: elf 17, elf 19, elf 37, elf 39, elf 44,
134 elf 46, elf 49 and elf 50 (Dos Santos et al. 2008). Reaction and conditions were
135 modified from those described by the authors. PCRs consisted of: 95ºC for 5 min, 35
136 cycles of 95ºC for 30s, the annealing temperature (Table 1) for 30s, 72ºC for 30s,
137 followed by 72ºC of 20 min. Concentration of $MgCl_2$ for each loci and fluorescent label
138 were also described in Supplementary Information Table S1. PCRs were performed in
139 a total volume of 20 µl in individual PCR reactions for each locus with the GeneAmp
140 PCR system 2700 and 2720 Thermal Cycler (Applied Biosystems). Products were
141 visualized in 50 ml 2% agarose gels with 2.5 µl of ethidium bromide (10 mg/ml) for
142 verification and genotyped using an ABI PRISM 3100 Genetic Analyzer (Applied
143 Biosystems), with GS500 LIZ 3130 size standard, in the Unit of Genetic Analysis of
144 the University of Oviedo (Spain).

145

146 2.3. *Genetic diversity*

147 Sequences of each mitochondrial gene (cytb and COI) were aligned using ClustalW
148 (Thompson et al. 1994) from the BioEdit Sequence Alignment Editor (Hall 1999) and
149 were visually inspected to avoid base-calling errors. The incongruence length difference
150 (ILD) tests (Farris et al. 1995) were implemented in PAUP* 4.0 (Swofford 1999) with
151 1, 000 replicates and p-value of 0.05. The two mtDNA gene sequences were
152 concatenated and haplotypes defined with DNAsp v.4.50.3 (Rozas et al. 2003). MtDNA
153 haplotype (H) and nucleotide diversity (pi) were calculated for each location using the
154 software Arlequin version 3.0 (Excoffier et al. 2005).

155 Microsatellite allele sizes were estimated using the GeneMapper® Software Version 4.0
156 (Applied Biosystems). Loci with scoring errors, large allele dropout, and null alleles
157 were discarded employing the program MICROCHECKER (Van Oosterhout et al.
158 2004). Conformity to Hardy-Weinberg equilibrium was calculated using the exact
159 probability test with GENEPOP software (Raymond and Rousset 1995 QUERY: not in
160 references). Microsatellite variation (number of alleles per locus, allelic richness, and
161 observed and expected heterozygosity) was calculated with the programs GENETIX
162 Version 4.03 (Belkhir et al. 2004 QUERY: 2001?) and FSTAT Version 2.9.3.2 (Goudet
163 2001).

164

165 2.4. *Population differentiation and structure*

166 A median-joining (Bandelt et al. 1999) haplotype network was constructed using the
167 concatenated mtDNA genes to visualize the intra-specific relationships of the different
168 haplotypes and their relative frequencies in the sampled populations with the program
169 Network 4.5.1.6 (http://fluxus-engineering.com) with default settings. Network software

170 reconstructed all possible, shortest, least complex, phylogenetic trees (maximum
171 parsimony) from a data set under different algorithms.
172 Genetic divergence between populations was estimated using population pairwise F_{ST}
173 values calculated using nuclear and mtDNA data using the program Arlequin version
174 3.0 (Excoffier et al. 2005). The statistical significance of F_{ST} values between samples
175 was calculated with 1,000 permutations and 10,000 steps in Markov chain. This
176 software was also employed for molecular analysis of variance (locus by locus and
177 standard AMOVAs) using 1,000 permutations.
178 Fu's F_S test (Fu 1997) for selective neutrality was calculated in Arlequin v.3.0. For
179 neutral markers, this test can be employed to detect changes in population size.
180 Significant and negative F_S values can be interpreted as signatures of population
181 expansion (Dodson et al. 2007). Mismatch analysis was also used to explore the spatial
182 and demographic evolution of the studied Bluefish populations with the Raggedness
183 index (Harpending 1994) and the Sum of Squared Deviation (SSD; Schneider and
184 Excoffier 1999). Both demographic and spatial mismatch analysis were calculated with
185 Arlequin v.3.0 and were based on the null hypothesis of expansion, thus non-significant
186 values reveal population expansion.
187 Population structure across the study area was assessed using the program
188 STRUCTURE 2.3.1 (Pritchard et al. 2000) by using microsatellite loci data. This
189 software estimates the minimum number of population units with genetic identity in the
190 dataset under a Bayesian framework. This dataset was analyzed under the "Admixture
191 model" which assumes that individuals may have mixed ancestry. The parameter set
192 consisted of a burn-in period of 30,000 steps followed by 300,000 Markov Chain Monte
193 Carlo (MCMC) iterations and 7 runs for each K (number of genetic units estimated); the
194 number of K was estimated following Evanno et al. (2005).

195 Mantel tests of association between genetic differentiation values (pairwise F_{ST} values)
196 and geographical Euclidean distances (linear distances between sampling locations) was
197 calculated with Arlequin version 3.0 (Excoffier et al. 2005). Mantel tests were done to
198 determine if the considered populations follow an isolation-by-distance model. To
199 determine possible geographical barriers to dispersal, we employed the software
200 BARRIER v.2.2 (Manni et al. 2004) that can identify geographically continuous and
201 discontinuous assemblages of samples from a spatial landscape. Geographical
202 coordinates of each sampling area were mapped into a matrix connected by Delauney
203 triangulation (Brassel and Reif 1979). Barriers in the triangulation were identified using
204 mitochondrial and nuclear pairwise F_{ST} distances. The analysis employed was based on
205 Monmonier's maximum distance algorithm (Manni et al. 2004) to identify regions with
206 sharp genetic change or discontinuity.
207
208 2.5. *Phylogeny and evolutionary history of Pomatomus saltatrix*
209 Population divergence time estimations were done under a Bayesian Markov Chain
210 Monte Carlo (MCMC) framework using the two mtDNA genes with the
211 softwareBEAST version 1.6.1 (Drummond and Rambaut 2007). Following a burn-in of
212 3 million cycles, rates were sampled once every 1,000 cycles from 30 million MCMC
213 steps for an Extended Bayesian Skyline tree prior with a stepwise model for
214 mitochondrial DNA and strict clock model. Bayesian intraspecific phylogenies are
215 based on coalescent theory (Kingman 1982 QUERY: not in references) and allow the
216 inference of past population dynamics and parameters from contemporary gene
217 sequences. The best evolutionary model of both sequences (COI and Cyt b) and their
218 priors (kappa, gamma-shape, proportion of invariant sites, etc.) were defined by
219 jModeltest software version 0.11 (Posada 2009 QUERY: not in references) using the

220 Akaike information criterion (AIC; Akaike 1974). The mutation rates employed were
221 2% per million years (MY) for Cyt b (Brown et al. 1979) and 1.2% per MY for COI
222 (Bermingham et al. 1997). Tracer version 1.5 (Rambaut and Drummond 2007) was used
223 to check that chains had converged to a stationary distribution. The analysis was
224 repeated with longer runs (50 million MCMC steps) when datasets did not accomplish
225 this condition.
226
227 3. RESULTS:
228 3.1. *Genetic diversity*
229 A total of 46 haplotypes for the concatenated COI-Cyt b genes were detected among the
230 studied samples. Most (68.75%) were observed among American samples (Northwest
231 Atlantic Ocean) while the rest were from the eastern area (Table 1; GenBank ID:
232 JQ039400-JQ039435for COI and JQ039436-JQ039465 for Cyt b haplotypes). High
233 haplotype diversity and low nucleotide diversity was found in general for all the
234 regions, but differences between localities revealed a gradient from west to east across
235 the studied area. American samples exhibited higher diversities than European ones, and
236 western Mediterranean samples were more diverse than those from the eastern
237 Mediterranean (Table1). The highest number of haplotypes and haplotype and
238 nucleotide diversities corresponded to northwest Atlantic samples.
239 The eight microsatellite loci considered were amplified for the same 120 individuals
240 across the sampling area. MICROCHECKER did not detect dropouts or scoring errors,
241 but null alleles were found for one loci (Elf44) in different populations. Therefore,
242 Elf44 was excluded from the data set. High genetic variability was found at the seven
243 loci. The number of alleles per locus ranged from 18 (Elf17) to 29 (Elf37– See
244 Supplementary Information Table S1). All sampling areas were in Hardy-Weinberg

245 equilibrium and there were no significant differences between expected and observed
246 heterozygosities in any location (Table 1).
247
248 3.2. *Population differentiation and population structure*
249 Differentiation between the two sides of the Atlantic Ocean was observed in the
250 haplotype network (Figure 2), where the main separation between mitochondrial
251 lineages corresponded clearly to the geographical differentiation between the two sides
252 of the Atlantic Ocean, giving separate American and European clades, with further
253 internal division. The presence of unique haplotypes may be caused by recent
254 differentiation and factors such as geological or demographic factors and mutations
255 would explain the haplotype structure. (Verheyen et al. 2003)
256 Population structure of Bluefish based on both mitochondrial and nuclear DNA was
257 consistent with three different genetic units in the sampling area: northwest Atlantic,
258 and west and east Mediterranean. F_{ST} population pairwise comparisons revealed
259 significant differences between northwest Atlantic and other locations and also between
260 east and west Mediterranean samples (Table 2) for both types of markers. The northwest
261 Atlantic group consisted of all the North American localities: Florida, Maryland, North
262 Carolina, and New Jersey. The west Mediterranean cluster included Cadiz (from the
263 Atlantic Ocean) and Barcelona (west Mediterranean), and the east Mediterranean group
264 was composed by the two Turkish localities, Istanbul and Canakkale.
265 Bayesian analysis confirmed that Bluefish from this study belonged to three different
266 genetic units (K=3), corresponding to the same three clusters detected with the genetic
267 distances but with a moderate level of admixture between them (Figure 3). There are
268 also a secondary maximum at K=8, supported by much lower likelihood than K=3. It
269 suggested a hierarchical island model (Evanno et al. 2005) of 8 different populations

270 clustered in 3 main sets. The analysis of molecular variance confirmed these three
271 genetic units. Both AMOVAs, standard (for mtDNA) and locus by locus (for
272 microsatellite loci; Table 3), showed significant differences among the three groups and
273 within populations, but not among populations within the three previously defined
274 groups. In the standard AMOVA, the highest percentage of variation was observed
275 between groups (71%; $P<0.001$), while the highest percentage of variation in the locus
276 by locus AMOVA was within populations (93.72%; $P < 0.001$). These results were
277 consistent with STRUCTURE and mitochondrial and nuclear F_{ST} results.
278 Demographic analyses indicated that all the populations except Canakkale were in
279 expansion (Table 4). In the Mantel test, a high and significant correlation was detected
280 between genetic and Euclidean distances between samples ($r = 0.734$; $p<0.001$),
281 suggesting a pattern of isolation by distance for *Pomatomus saltatrix*. The software
282 BARRIER (Manni *et al.* 2004) identified two main boundaries coincident with the two
283 longer geographical distances between samples. The two detected barriers were
284 supported with both types of molecular markers (Figure 4). However, all the previous
285 population structure results (STRUCTURE, mitochondrial and nuclear F_{ST} and
286 AMOVAs) suggested some permeability across the three genetic units The Gibraltar
287 Strait was not a barrier to gene flow for *Pomatomus saltatrix* since the Spanish samples
288 on the two sides of the Strait (Cadiz and Barcelona) were not significantly different.
289
290 3.3. *Phylogeny and evolutionary history of Pomatomus saltatrix*
291 The time to the most recent common ancestor (TMRCA) of the sampled Bluefish was
292 estimated to be 480,000 years ago (95% Highest Posterior Density (HPD) = 0.28–0.72
293 MY), while the TMRCA for the NW Atlantic Ocean was dated as 252,000 years
294 (95%HPD=0.13–0.39 MY) and 148,300 years (95%HPD=0.05–0.27 MY) for the

295 Mediterranean samples. The population growth rate estimated with the Extended
296 Bayesian Skyline model for the North American samples reached its maximum
297 approximately 40,100 years ago, while for the European clade the maximum occurred
298 23,420 years ago (Figure 5). Both estimates suggest that the northwest Atlantic clade is
299 more ancient than the Mediterranean. The Bayesian tree obtained for the northwest
300 Atlantic Ocean suggested that there are two separated American lineages which are
301 composed of a mixture of all localities. The TMRCA of the two American lineages was
302 estimated to be approximately 175,000 and 131,000 years. The substructure detected
303 with the Bayesian methods and not with F_{ST} would be due to the most sophisticated
304 approaches of Bayesian inference. F_{ST} – based approaches are well understood, widely
305 used, and easily applied but Bayesian-based analyses allow more precise estimates (See
306 Pearse and Crandall 2005 QUERY: 2004? for a review)

307

308 4. DISCUSSION:

309 This study provides a genetic analysis of North Atlantic and Mediterranean Bluefish
310 populations. Three clear genetic units were identified, and may follow a hierarchical
311 island model. The North American samples were clustered together, without any
312 significant difference between sampling sites, suggesting that Bluefish is panmictic in
313 the northwest Atlantic Ocean. Many studies based on distribution and morphological
314 characteristics had investigated the number of stocks in the East Coast of the United
315 States. In previous studies, the number of stocks identified ranged from six to two
316 (Lund 1961; Lassiter 1962; Lund and Maltezos 1970), but other investigations
317 concluded that either two distinct spawning groups or one stock with two distinct
318 spawning periods occurred in the region (Norcross et al. 1974; Kendall and Walford
319 1979; Hare and Cowen 1993; Smith et al. 1994). Later, Graves et al. (1993) concluded

320 that there was a single population based on mitochondrial DNA, and a morphometric
321 study (Austin et al. 1999) corroborated the one-stock hypothesis despite the evidence of
322 phenotypic plasticity. Our study confirms the one-stock hypothesis based in both
323 nuclear and mitochondrial DNA, but the Bayesian mitochondrial DNA results suggested
324 that there are two different lineages within the American panmictic population that may
325 have evolved at different time.
326 Our results support two genetic discontinuities for Bluefish: one in the middle of the
327 Atlantic Ocean and other in the Mediterranean Sea. However, regional permeability and
328 migration can be inferred to occur across both of them. The Mediterranean barrier could
329 be at the level of the Siculo-Tunisian Strait, and may be due to the hydrographic
330 isolation of the Aegean and Ionian and Adriatic Seas, or either of them, but not in the
331 Gibraltar Strait or Alboran Sea. The Siculo-Tunisian strait is a barrier to gene flow for
332 other species (Stefanni and Thorley 2003; Zardoya et al. 2004), and in most cases the
333 major genetic break or limitation to larval dispersal between the Eastern and Western
334 Mediterranean occurs in the straits separating the Adriatic, Aegean, and ⁄ or Black Sea
335 (Nikula and Vainola 2003; Costagliola et al. 2004; Domingues et al. 2005; Peijnenburg
336 et al. 2006; Perez-Losada et al. 2007; Zulliger et al. 2009). The accurate location/s of
337 the barrier/s for this species cannot be deduced from the present results.
338 The most probable factor influencing Bluefish population genetic structure seems to be
339 geographical, such as the distance to continents and shallow waters. Small fish used to
340 be found in shallow coastal waters of 2 m depth (May and Maxwell 1986) and apparent
341 population genetic isolation associated with different continents has been reported
342 before (Goodbred and Graves 1996). Bluefish is a highly migratory species (Juanes et
343 al. 1996). Water temperature and photoperiod are major factors described to influence
344 movement patterns in Bluefish (Juanes et al. 1996; Olla and Studholme 1972; Wilk

345 1977) but other factors, like water salinity, could contribute to depict their population
346 structure. Nonetheless, we have compared annual sea surface temperature as well as
347 annual temperature at 200 m depth (maximum depth for *Pomatomus saltatrix*) with the
348 Bluefish genetic structure detected in this study. In this case, changes in temperature
349 might not explain the barriers detected here, nor the different Bluefish populations
350 genetic structure (See maps of temperature in Supplementary Information Image S1).
351 Additionally, salinity gradients at the surface and at 200 m depth would not explain the
352 genetic structure and barriers detected (See Supplementary Information Image S1). Both
353 factors had no differences along the genetic units detected, thus those factors are not a
354 barrier in the sampling area. In contrast, both annual salinity and water temperature
355 along North Atlantic Ocean and Mediterranean Sea could explain the permeability
356 across the two boundaries detected and could allow Bluefish to migrate. This
357 connection across barriers can prevent vicariant speciation, as *Pomatomus saltatrix* is
358 the sole member of its genus and family.
359 American Bluefish exhibited higher diversity than European populations, and west
360 Mediterranean were more diverse than east Mediterranean populations. Hewitt (1996,
361 2000) demonstrated that the last glaciations had a profound effect on genetic diversity.
362 If genetic diversity remains high or increases at the limits of the range, then these
363 populations may be composed of two or more lineages that differentiated in distinct
364 glacial refugia, which is consistent with the two American lineages detected in our
365 study. Therefore, the occurrence of high levels of genetic diversity in previously
366 glaciated areas would suggest colonization from multiple refugia (Griswold and Baker
367 2002). This could be the case of the northwest Atlantic (American Bluefish) population
368 which has the highest genetic diversity and was dated as the oldest population, having
369 necessarily passed through several glacial cycles (Riss, Würm). On the opposite side of

370 the Atlantic, the emergence of the Mediterranean clade occurred during the Riss glacial
371 cycle, a harsh period when the Gibraltar Strait was closed due to the decrease in sea
372 level (Loget and van den Driessche 2006). Later, the Mediterranean Sea was closed and
373 part of the Bluefish population was probably isolated for a long period. Further radical
374 hydrographical changes that occurred during the course of the late Pleistocene climatic
375 cycles, such as fluctuations in water level that repeatedly isolated totally or partially the
376 Black Sea, Aegean Sea, and Eastern Mediterranean (Svitoch et al. 2000) may have
377 contributed to differentiate those populations. As reported for other marine species (e.g.,
378 Borrero-Perez et al. 2011) this isolation may have been maintained until the present due
379 to the anticyclonic front located in the Peloponnese peninsula (Millot 2005). The
380 Atlantic European clade may have colonized the western Mediterranean after the
381 opening of the Strait of Gibraltar and continued its expansion until the present. The
382 palaeogenetic history of Bluefish seems therefore associated with glacial-interglacial
383 cycles, as suggested by the estimated time to the most recent common ancestors
384 (TMRCA) of the different clades. TMRCA for the Bluefish analyzed here was dated to
385 the Aftonian II, an interglacial period of favorable conditions with growth and
386 expansion of species, and the TMRCA of both south European and east Mediterranean
387 clades corresponded to glacial periods (fourth cycle of Mindel and Riss glaciation
388 respectively). Bluefish populations may have migrated to different refuges and stayed
389 isolated for long periods until the climatic conditions became favorable again.

390 In conclusion, the present study suggests that the paleoecological history (e.g.,
391 glaciations and interglacial periods in the late Pleistocene) has been crucial for shaping
392 the present genetic variability and population structure of *Pomatomus saltatrix* in the
393 North Atlantic Ocean and in the Mediterranean basin.

394

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641 FIGURE LEGENDS:

642 **Figure 1: Sampling locations:**

643 Geographical distribution of sampling points in the Atlantic Ocean and the
644 Mediterranean, Marmara, and Black Seas. NJ: New Jersey (USA); MD: Maryland
645 (USA); NC: North Carolina (USA); F: Florida (USA); C: Cadiz (Spain); B: Barcelona
646 (Spain); TC: Canakkale (Turkey); TI: Istanbul (Turkey)

647

648 **Figure 2: Haplotypes network:**

649 Median-Joining network with the relationships among the 48 haplotypes defined by
650 concatenated Cyt b and COI mitochondrial genes. Circles sizes are proportional to the
651 frequency of each haplotype. Different locations are represented in different colors.
652 Branches are proportional to mutations between haplotypes.

653

654 **Figure 3: Population structure across the sampling area:**

655 (A.) Likelihood and number of cluster detected (K) based on nuclear DNA with
656 STRUCTURE 2.3.1 (Pritchard *et al.* 2000) following Evanno *et al.* (2005).

657 (B.) Percentage of individual membership detected with STRUCTURE 2.3.1
658 (Pritchard *et al.* 2000). Each genetic unit is represented by one color (American
659 samples in blue, Spanish in green, and Turkish in red). Each vertical bar represents
660 one individual. Acronyms of sampling localities are below the plot and described in
661 Figure 1 and Table 1.

662

663 **Figure 4: Barriers to dispersal:**

664 Detected spatial genetic discontinuities along the distribution of the samples based on
665 the Monmonier's maximum difference algorithm are marked with red arrows and green
666 letters: A is the first detected barrier and B the second barrier.

667

668 **Figure 5: Maximum population growth rates for the studied Bluefish during the**
669 **last 50,000 years:**

670 Population growth rates (in percentage) estimated with the Extended Bayesian Skyline
671 of BEAST software for the European and American Bluefish clades.

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Table 1: Diversity indices for mitochondrial DNA and microsatellite loci of *Pomatomus saltatrix* samples

Localities	Acronym	N	H	(π)	Nh	NA	AR	He	Ho	F_{IS}
USA		65			33(33/21)	18.28				
New Jersey	NJ	23	0.9264 +/- 0.0388	0.0045 +/- 0.0026	14 (5/5)	13.9	33.405	0.8856	0.7018	0.1938
Maryland	MD	20	0.9692 +/- 0.0209	0.0051 +/- 0.0029	19 (11/10)	13.1	32.644	0.8714	0.7576	0.1531
North Carolina	NC	11	0.9273 +/- 0.0665	0.0051 +/- 0.0030	8 (2/2)	16.4	31.952	0.8399	0.7549	0.1269
Florida	F	11	0.9636 +/- 0.0510	0.0056 +/- 0.0033	9 (4/4)	10.1	32.921	0.8593	0.6598	0.2321
Spain		41			14 (10/8)	15.43				
Cadiz	C	35	0.8414 +/- 0.0441	0.0025 +/- 0.0016	12 (6/6)	14.6	31.130	0.8014	0.7170	0.1207
Barcelona	B	6	1.0000 +/- 0.0962	0.0031 +/- 0.0021	6 (1/1)	6.7	29.848	0.7143	0.7619	0.0243
Turkey		14			4 (2/3)	9.14				
Canakkale	TC	7	0.0000 +/- 0.0000	0.0000 +/- 0.0000	1 (-)	6.7	28.666	0.7194	0.8299	-0.0662
Istanbul	TI	7	0.5238 +/- 0.2086	0.0006 +/- 0.0006	3 (2/2)	6	28.611	0.7211	0.6922	0.1577
Total		120			55 (-/34)	23.28	33.352	0.839	0.741	

Diversity indices of the concatenated Cytb-COI genes as H, Haplotype diversity; (π), Nucleotide diversity; Nh, Number of haplotypes (private/singletons as exclusive of a locality / one single copy respectively) and for microsatellite loci asNA, average number of alleles per locus. AR, allelic richness. He and Ho, heterozygosity observed and expected respectively and F_{IS} values. N, sample size.

Table 2: Populations pairwise Fst differences:

Localities	NJ	MD	NC	F	C	B	TC	TI
NJ	-	0.01008	0.00367	0.00498	**0.02203**	**0.03900**	**0.06407**	**0.06734**
MD	0.02436	-	0.01153	0.00240	**0.01930**	**0.03542**	**0.08319**	**0.07287**
NC	0.04170	-0.02772	-	0.01777	**0.01969**	0.02785	**0.07205**	**0.06623**
F	0.05955	-0.02595	-0.05941	-	0.01357	**0.04297**	**0.06358**	**0.04482**
C	**0.75687**	**0.74427**	**0.75280**	**0.74692**	-	0.00572	**0.07385**	**0.07825**
B	**0.70537**	**0.68326**	**0.67787**	**0.65902**	-0.06545	-	**0.05565**	**0.04119**
TC	**0.74745**	**0.73286**	**0.74596**	**0.73079**	**0.19134**	**0.18157**	-	0.01869
TI	**0.75434**	**0.74041**	**0.75938**	**0.74326**	**0.23288**	**0.17656**	0.00001	-

Pairwise F_{ST} estimates between Bluefish samples based on mtDNA (below diagonal) and nuclear DNA based on microsatellite loci (above diagonal). Significant P values are in bold. Locality acronyms as described in Table 1.

Table 3: Molecular analysis of variance:

AMOVA	Variance	% Variation	ϕStatistics	p
Locus by locus (8 Microsatellite Loci)				
Among groups	0.065	2.96	$\phi_{CT} = 0.057$	**< 0.001**
Among populations within groups	0.015	0.70	$\phi_{SC} = 0.005$	0.168
Within population	2.109	96.33	$\phi_{ST} = 0.062$	**< 0.001**
Standard (881bp COI-CytB)				
Among groups	3.811	70.61	$\phi_{CT} = 0.709$	**< 0.001**
Among populations within groups	0.005	0.09	$\phi_{SC} = -0.006$	0.345
Within population	1.581	29.30	$\phi_{ST} = 0.708$	**< 0.001**

Based on microsatellite loci (locus by locus AMOVA) and mitochondrial sequence variation (standard AMOVA). Significant P values in bold.

Table 4: Population expansion test:

	Mismatch Analysis (Ho=expansion)				Neutrality test (Ho= No expansion)
	Demographic expansion		Spatial expansion		
	SSD	Raggedness	SSD	Raggedness	FS
NJ	0.002	0.011	0.003	0.011	**-5.648**
MD	**0.104**	0.016	0.005	0.016	**-10.917**
NC	0.009	0.029	0.012	0.029	-1.769
F	**0.137**	0.173	0.049	0.173	-2.774
B	0.036	0.133	0.036	0.133	**-3.576**
C	0.031	**0.120**	0.027	0.120	**-4.298**
TC	**0.000**	**0.000**	**0.000**	**0.000**	0.000
TI	0.022	0.185	0.022	0.185	-0.921

Mismatch analysis of demographic and spatial expansion and neutrality tests: SSD, Schneider and Excoffier's test of sudden expansion (Schneider and Excoffier 1999); Raggedness index (Harpending 1994); FS: Fu's test of neutrality (Fu 1997), respectively. Values indicating population expansion are marked in bold. Locality acronyms as described in Table 1.