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Historical separation and present-day structure of common dolphinfish (*Coryphaena hippurus*) populations in the Atlantic Ocean and Mediterranean Sea

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The common dolphinfish (*Coryphaena hippurus*) is an epipelagic, mid-trophic level, highly migratory species distributed throughout the world's tropical and subtropical oceans in waters greater than 20°C. Life-history variables, migratory behaviour, and genetic markers have been used to define major stocks in the central Atlantic Ocean and Mediterranean Sea. Here, we used the mitochondrial DNA gene NADH subunit 1 (688 bp) to test for differences between population groups. A total of 103 haplotypes were detected among 203 fish. Gene diversities in samples were large and similar among populations (mean $h = 0.932$; range 0.894–0.987), but nucleotide diversities varied widely among samples (range $\pi = 0.004$–0.034) and appear to reflect population histories. Principal component analysis revealed two large populations groups, and the analysis of molecular variation and pairwise values of Φ_{ST} resolved population structure within these groups. Populations in the eastern Atlantic and Mediterranean showed the largest amounts of divergence from one another ($\Phi_{CT} = 0.331$). Adult movement and biophysical barriers to larval dispersal may explain contemporary differences between stocks, but the divergent populations in the

Mediterranean Sea are likely due to isolations by cold temperature barriers during Pleistocene glaciations. The geographically large stock groupings require international cooperation in the harvest management and conservation of local dolphinfish populations.

Keywords: common dolphinfish, migratory species, mtDNA, population connectivity.

Introduction

Geographical surveys of molecular markers in population samples can be used to estimate genetic relationships among populations, and these results can be used to indirectly estimate patterns of dispersal (Broquet and Petit, 2009). Marine organisms that move long distances as adults or that spawn pelagic larvae capable of drifting in ocean currents are expected to show little genetic population structure because of the high potential for gene flow (Cowen and Sponaugle, 2009; Nanninga and Manica, 2018). However, behaviour and biophysical processes can limit dispersal and produce genetic differences between populations (Selkoe *et al.*, 2016). On long time scales, climate and oceanic variability can lead to separations between populations, or to local population extinctions and colonizations. A wealth of oceanographic and paleo-oceanographic and paleo-climatic studies can be used to understand the origins of present-day genetic population structure.

Dolphinfish (*Coryphaena hippurus*, L.) are widely distributed in the world's oceans in tropical and subtropical waters warmer than 20°C (Palko *et al.*, 1982; Oxenford, 1999; Sinopoli and Andaloro, 2017) and support important recreational, artisanal, and commercial fisheries wherever they occur. In particular in the western and central Mediterranean Sea, young of the year support small-scale commercial fisheries based on fish aggregating devices (FADs) along the Maltese, Tunisian, Sicilian, and Balearic coasts (Morales-Nin *et al.*, 2000; Massutí and Reñones, 2005; Andaloro *et al.*, 2007) constituting a valuable seasonal resource for Mediterranean traditional small fisheries. In the western Atlantic, dolphinfish have been recorded as far north as Georges Bank and as far sound as Rio de Janeiro, Brazil, but are common only from North Carolina, throughout the Gulf of Mexico and the Caribbean to the northeast coast of Brazil (Oxenford, 1999). In the eastern Atlantic, dolphinfish occur from the Bay of Biscay to Angola, and throughout the Mediterranean Sea (http://www.fao.org/fishery/species/3130/).

Importantly for interpreting genetic patterns, archival tags indicate that these fish are highly migratory making large circuits in the open ocean before returning to near shore areas where they are harvested (Merten *et al.*, 2016). Dolphinfish prefer epipelagic surface waters (0–10 m in depth) with temperatures as warm as 28°C, but can briefly tolerate temperatures as low as 16°C at depth during feeding excursions (Farrell *et al.*, 2014). Dolphinfish are a mid-trophic level fish with a maximal longevity of about 4 years (Beardsley, 1967). However, catches usually consist of fish 2 years of age or less (Gatt *et al.*, 2015), and some commercial fisheries target fish 2–8 months of age (Benseddik *et al.*, 2011). Dolphinfish have a type III life history with the production of large numbers of eggs and with high early and juvenile mortality as high as 99% (Oxenford, 1999). Female dolphinfish are highly fecund, spawning batches of 20 000 to 620 000 eggs throughout most of the year (McBride *et al.*, 2012). Annual fecundities of females in the western Atlantic are estimated to be 15–174 million eggs, depending on the size of a fish (McBride *et al.*, 2012). Larvae drift in surface waters, and juveniles grow about 4 mm per day for the first 6 months. Dolphinfish become sexually mature in their first year; 50% of females mature at 46 cm in fork length and 50% of males at 47–48 cm (Schwenke and Buckel, 2007).

Tagging studies show that dolphinfish are highly migratory, moving offshore in winter in response to seasonal changes in temperature and food availability and onshore in spring (Massutí and Morales-Nin, 1997a; Hammond, 2008). Individual dolphinfish have been documented to move in a circular pattern over 1000 km along the Atlantic coasts of North, Central, and South America (Hammond, 2008; Merten *et al.*, 2014a, b). Using information from surface drifters, Merten *et al.* (2014b) confirmed that movements in the western Atlantic generally followed the Gulf Stream. Geographic patterns of temporal and spatial CPUE in the NW Atlantic, summarized from pelagic longline logbooks 1999–2007, indicated that sea surface temperature and chlorophyll-*a* concentrations were the most important variables explaining the distribution of dolphinfish (Farrell *et al.*, 2014). CPUE was largest at 22–25°C in longline fishery and at chlorophyll-*a* concentration greater than 0.2 mg m^{-3}.

Despite the potential for long-distance migration, discrete populations have been postulated on the basis of regional differences in growth inferred from otoliths rings (Oxenford and Hunte, 1983; Duarte-Neto *et al.*, 2008; Chang *et al.*, 2013), seasonal migration patterns (Arocha *et al.*, 1999), regional catch data (Oxenford and Hunte, 1986; Duarte-Neto *et al.*, 2008), and biophysical ocean conditions (Farrell *et al.*, 2014; Merten *et al.*, 2016). Together these data indicate the presence of at least three large stocks in the western Atlantic: (i) eastern coast of Brazil (Duarte-Neto *et al.*, 2008), (ii) northeastern coast of Brazil, and Venezuela as far as Puerto Rico and including the Caribbean Sea (Oxenford and Hunte, 1986; Arocha *et al.*, 1999; Duarte-Neto *et al.*, 2008), and (iii) northward from Puerto Rico including the Bahamas, Florida and the Carolinas, and likely including the Gulf of Mexico (Oxenford and Hunte, 1986; Oxenford, 1999).

Fewer studies of stock structure based on life-history traits and tagging studies have been made in the eastern Atlantic. Only a single fish that, tagged in the western Atlantic has been recovered in the eastern Atlantic (Merten *et al.*, 2015; Maroso *et al.*, 2016; https://beyondourshores.org/regional-dolphinfish-reports/).
Estimates of growth, based on limited population sampling, were similar between northern western Atlantic populations and Mediterranean populations, but differed between southern western Atlantic populations (Chang *et al.*, 2013). Several studies show a skewed sex ratio, in which females predominate among younger fish, but males among older fish. This pattern is consistent among harvested dolphinfish in the eastern Atlantic and Mediterranean (Massutí and Morales-Nin, 1997b; Castro *et al.*, 1999; Potoschi *et al.*, 1999; Gatt *et al.*, 2015; Maroso *et al.*, 2016). Even though the distributions of juveniles have been thoroughly documented within the Mediterranean (Sinopoli *et al.*, 2012; Cillari *et al.*, 2018), little is known about the spatial and temporal distributions of adults. A distinct sub-population may overwinter in the central Mediterranean in lieu of migrating to open-ocean waters (Gatt *et al.*, 2015).

Several studies have attempted to resolve genetic stock structure with geographical surveys of molecular markers. An early study of

Table 1. Sampling locations, location codes, and sample sizes (*N*) of *Coryphaena hippurus*.

Group	Location	Sample code	*N*
MED	Antalya Bay (Levantine Sea)	LevS	19
	Malta (Strait of Sicily)	SS	20
	Gulf of Castellammare (Tyrrhenian Sea)	Tyr	33
	From Alboran Sea to Levante Sea (Western Mediterranean)	AlbS	22
ATL	Azores Island (Eastern Atlantic)	AzoI	22
	Cabo Verde (Eastern Atlantic)	Cver	23
	North Carolina (Western Atlantic)	NCar	21
	Barbados Island (Caribbean Sea)	Barb	20
	Louisiana (Gulf of Mexico)	Lous	23

MED, Mediterranean; ATL, Atlantic.

allozyme markers concluded that populations in the eastern Atlantic and Mediterranean were genetically indistinguishable (Pla and Pujolar, 1999). Subsequent studies of DNA markers in populations around the globe have provided greater resolution. These studies show that western Atlantic populations are similar to Indo-Pacific populations, but that Mediterranean populations differ markedly from other global populations (Díaz-Jaimes *et al.*, 2010). Some genetic differences have been detected among populations in the Mediterranean Sea (Maroso *et al.*, 2016; Sacco *et al.*, 2017).

The goal of this study was to examine genetic variability among populations using mitochondrial (mt) DNA NADH I sequences to understand broad-scale genetic population structure in the Atlantic Ocean and Mediterranean Sea. Samples were collected in all the major putative stocks in the western (except eastern Brazil) and eastern Atlantic Ocean and in the Mediterranean Sea to test whether stocks defined by phenotypic variables also show genetic differences with neutral molecular markers. We also focus on biophysical isolation mechanisms and historical events that may be responsible for genetic divergence of Mediterranean dolphinfish from Atlantic populations.

Methods

Collection of samples

Specimens of *C. hippurus* were collected from nine regions throughout the North Atlantic Ocean and Mediterranean Sea (Table 1; Figure 1). Adults in the Mediterranean were collected as by-catch in the swordfish longline fishery; juveniles were caught by purse seine in commercial and recreational fisheries. Atlantic specimens were captured by recreational fishers by trolling or spear fishing. A piece of muscle or a fin clip from each fish was preserved in either ethanol (90–99%) or frozen at −20°C.

Tissue samples were collected from fish intended for personal consumption or public sale. All fish were dead prior to the collection of tissues so that live animals were not subjected to stress during sampling; all methods were in accordance with relevant guidelines and regulations.

DNA extraction, amplification, and sequencing

Samples preserved in ethanol were thoroughly rinsed in sterile distilled water to remove traces of ethanol before processing. Total DNA was extracted following Tagliavia *et al.* (2016) and stored at −20°C for subsequent analysis. The polymerase chain reaction (PCR) was used to amplify a fragment of the mtDNA nicotinamide adenine dinucleotide (NADH) dehydrogenase subunit 1 (ND1) gene using primers NADH163 (5-TAA TCC TGC CGC AAT TAT CC-3) and NADH128 (5-AGG CCT TCC AGG TTA GGT GT-3) (Díaz-Jaimes *et al.*, 2006). The thermal cycling profile and the PCR condition followed Díaz-Jaimes *et al.* (2006). Amplified products were resolved on a 1% agarose gel purified using a QIAquick PCR purification Kit (QIAGEN) and sequenced on an ABI PRISM 310 automated sequencer (Applied Biosystems).

Data analyses

DNA sequences were aligned using CLUSTAL X (Thompson *et al.*, 1994) with default settings. Sequence alignments were manually checked. Fish from a sampling location were considered to belong to the same population. Intra-population diversities were estimated with the total number of haplotypes per sample, shared and singleton haplotypes, and the number of conserved, variable, and informative sites, and with haplotype (h) and nucleotide (π) diversities with DNAsp (Librado and Rozas, 2009). Estimates of haplotype diversity are based on haplotype frequencies and are analogous to heterozygosities for diploid loci. Nucleotide diversities are also estimated from haplotype frequencies, but also incorporate sequence divergence between haplotypes. A minimum spanning network (MSN) was constructed with Arlequin 3.0 (Excoffier *et al.*, 2005).

Population differentiation was assessed using a hierarchical analysis of molecular variance (AMOVA) with Arlequin 3.0 and with various geographical models. Populations in the Atlantic and Mediterranean were examined for genetic differentiation by sample location (Table 1). AMOVAs yielded estimates of the proportion of variation among groups (Φ_{CT}), the proportion of variation among populations within groups (Φ_{SC}), and the proportion of variation among fish within groups (Φ_{ST}). Atlantic samples were further sub-divided into localized sampling areas in the western Atlantic (WATL), including North Carolina (NCar), Barbados Islands (Barb), and Louisiana (Lous), and compared with samples from the eastern Atlantic (EATL), including Azores Islands (AzoI) and Cabo Verde (Cver). Samples from the Atlantic Ocean were compared with samples from the Mediterranean Sea (MED) (Table 1). The following comparisons were made using a proximity criterion: MED vs. EATL and EATL vs. WATL. AMOVAs were also performed by adding GenBank sequences from fish of known geographic origins in the Atlantic–Mediterranean region and by using the same partitioning schemes as above (Supplementary Table S1).

Pairwise Φ_{ST} between samples was estimated and tested for significance with probability values adjusted for multiple pair-wise tests with the sequential Bonferroni correction (Rice, 1989). A pairwise genetic distance matrix between individuals was generated using GenAlEx 6.1 (Peakall and Smouse, 2006) and used for

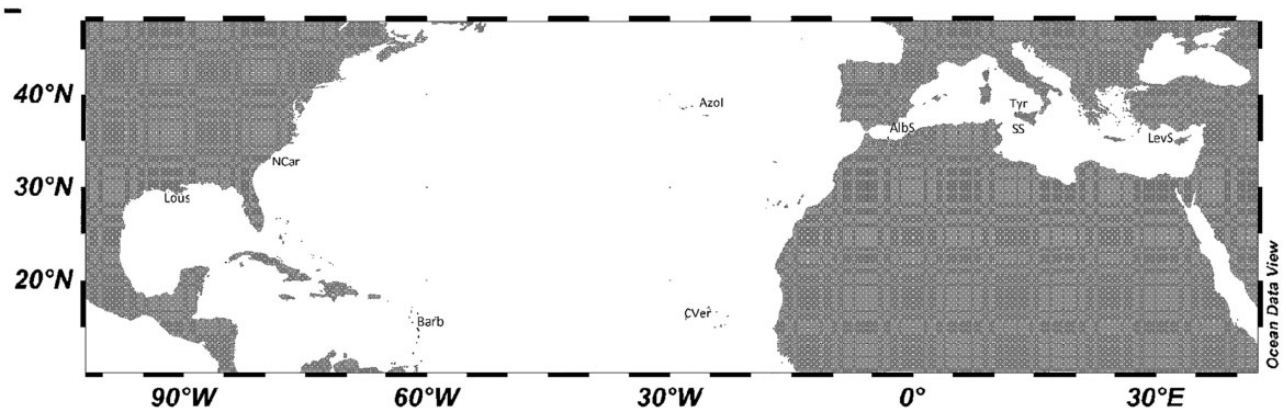

Figure 1. Location of sampling sites for *Coryphaena hippurus* from the Atlantic Ocean and Mediterranean Sea. Modified from Schlitzer, R., Ocean Data View, http://odv.awi.de, 2017 version number ODV4, Version: ODV 4.7.10.

principal coordinates analysis (PCoA). PCoA is a metric multidimensional scaling technique that permits the positioning of objects in a low-dimensional, Euclidean space, whilst attempting to represent inter-object similarity (Gower, 1966; Legendre and Legendre, 2012). Isolation by distance (IBD) was tested with a Mantel permutation test for a correlation between genetic distance and geographic distance and with a reduced major axis regression to estimate the intercept and slope of genetic distance vs. geographic distance (IBDWS web service, Jensen *et al.*, 2005). IBD was tested for Mediterranean and Atlantic populations together and for only Atlantic populations. We made additional tests with PCoA and IBDWS by including relevant GenBank sequences.

Indirect estimates of gene flow (N_em) were computed using DNAsp and the following measures: G_{ST} (Nei, 1973; equation 9), Φ_{ST} (Hudson *et al.*, 1992; equation 3); N_{ST} (Lynch and Crease, 1990; equation 36) with the Jukes and Cantor correction (Jukes and Cantor, 1969), delta (δ_{ST}; equation 4), and gamma (γ_{ST}; equation 5) (Nei, 1982). N_em estimates were based on the island model of population structure $F_{ST} = 1/(1 + 2N_em)$ (Wright, 1951).

Further, migration rates (M) between MED, EATL, and WATL (as defined for AMOVA) were estimated with Bayesian inference in MIGRATE 3.0 (Beerli, 2002). This approach uses Markov chain Monte Carlo (MCMC) simulations to estimate population sizes ($\theta = N_e\mu$) and migration rates between regions ($M = m/\mu$). We used BARRIER 2.2 (Manni *et al.*, 2004) to detect genetic discontinuities between populations. This method uses computational geometry and a Monmomier's maximum difference algorithm to identify barriers to gene flow. The user specifies the number of barriers and a multiple matrices approach provides a test the significance of the barrier.

Results

A 688-bp mtDNA ND1 fragment was successfully amplified in all 203 specimens. Polymorphisms at 318 nucleotide sites defined 103 haplotypes, of which 51 were shared among fish. The remaining 52 haplotypes occurred in single fish. Nucleotide frequencies were 0.242 (A), 0.307 (T), 0.268 (C), and 0.183 (G), showing a thymine bias. The overall transition/transversion ratio was $R = 1.24$, showing a bias toward transitions, as is common in protein-coding genes.

Table 2. Genetic diversity of *Coryphaena hippurus* at seven locations across the Atlantic and Mediterranean, based on mtDNA ND1 gene sequences (688 bp).

Location	*N*	N_{hap}	*H*	π
LevS	19	9	0.912	0.017
SS	20	11	0.921	0.012
Tyr	33	27	0.987	0.017
AlbS	22	12	0.953	0.015
Azol	23	14	0.965	0.025
Cver	22	17	0.978	0.034
NCar	21	12	0.957	0.023
Barb	20	8	0.894	0.004
Lous	23	11	0.905	0.007
Tot	203	103	Avg 0.932	Avg 0.020

Location codes as in Table 1 and locations in Figure 1. *N* = sample size. N_{hap} = the number of haplotypes. *H* = haplotype diversity. π = nucleotide diversity.

Haplotype diversities (h) were large, ranging from 0.894 (Barb) to 0.987 (Tyr) (Table 2), with a mean diversity of 0.969. Nucleotide diversities (π) were more variable among samples, ranging from 0.004 (Barb) to 0.034 (CVer); mean nucleotide diversity was 0.018, indicating a mean sequence divergence of 1.8% between haplotypes (Table 2).

A MSN among haplotypes indicated that only two haplotypes were shared among western and eastern Atlantic, and Mediterranean samples (Figure 2). These two haplotypes had overall frequencies of 0.194 and 0.058.

All of the fixation indices (F-statistics) in the AMOVA tests differed significantly from zero ($p < 0.001$) for Φ_{ST}, $F_{CT,}$ and F_{SC} (Table 3). Most of the variation occurred among individuals within populations on average (88.21%). Populations of dolphinfish in the Western (WATL) and eastern Atlantic (EATL) differed significantly from one another ($\Phi_{CT} = 0.072$, $p < 0.001$), as did populations within these two groups ($\Phi_{SC} = 0.011$, $p < 0.001$). A three-way comparison of all the Atlantic with samples from the Mediterranean (WATL × EATL × MED) highly significant ($\Phi_{CT} = 0.231$, $p < 0.001$), but the comparison between the eastern Atlantic (EATL) and the MED samples showed the largest amount of divergence among populations ($\Phi_{CT} = 0.331$, $p < 0.001$). The results of AMOVAs that included GenBank

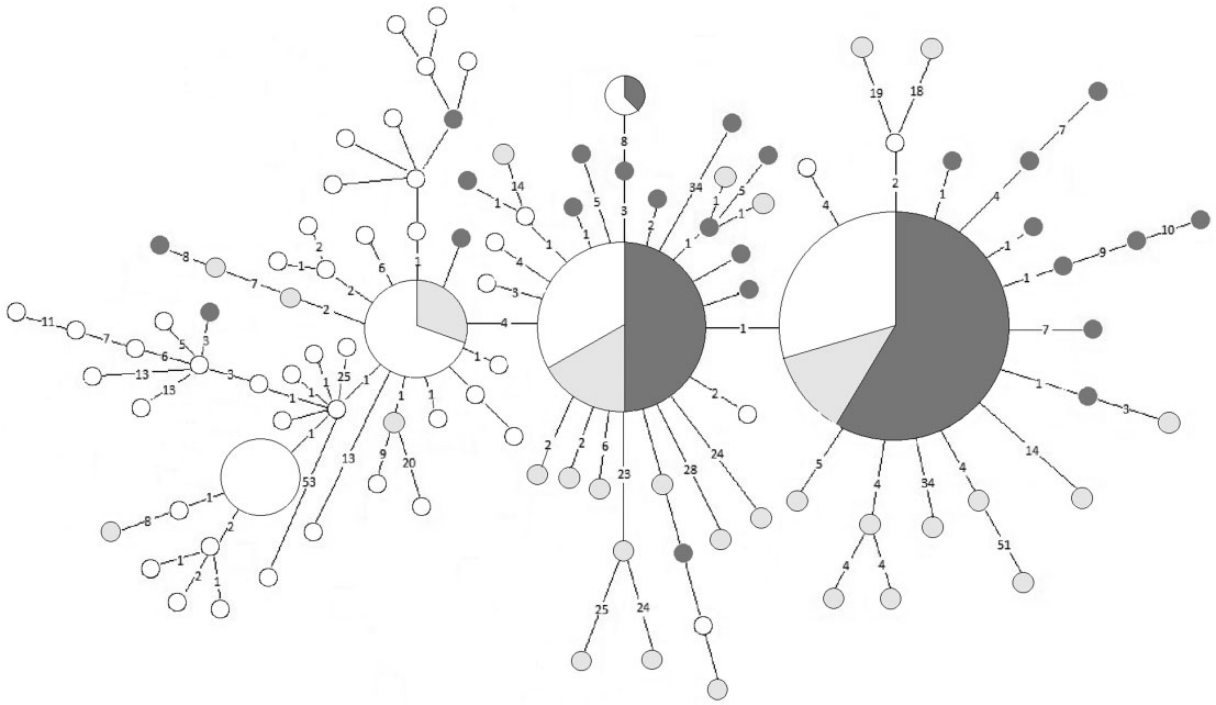

Figure 2. MSN among all 103 mitochondrial haplotypes of the ND1 gene of mtDNA of *Coryphaena hippurus*. Circles represent haplotypes and diameters are proportional to the frequency. Coloured wedges indicate haplotype frequencies by sample location. Haplotypes (circles) are connected by the least number of pairwise substitutions between all possible sequence comparisons. Numbers on connecting lines indicate the number substitutions separating two adjacent haplotypes. An unnumbered line represents one nucleotide substitution. White=MED. Grey=EATL. Dark Grey=WATL

Table 3. Results of analyses of molecular variance (AMOVA) of mtDNA ND1 haplotype frequencies among samples of *Coryphaena hippurus* from the Atlantic and Mediterranean.

Comparison		Percentage of variation	*F*-statistic	*p*
Pooled	Among populations	11.79	Φ_{ST} 0.118	<0.001
	Within populations	88.21		
ATL vs. MED	Among groups	9.64	Φ_{CT} 0.096	<0.001
	Among pops within groups	5.87	Φ_{SC} 0.065	<0.001
	Within population	84.49	Φ_{ST} 0.155	<0.001
WATL vs. EATL vs. MED	Among groups	23.10	Φ_{CT} 0.231	<0.001
	Among pops within groups	1.51	Φ_{SC} 0.019	<0.001
	Within populations	75.39	Φ_{ST} 0.246	<0.001
EATL vs. MED	Among groups	33.06	Φ_{CT} 0.331	<0.001
	Among pops within groups	3.41	Φ_{SC} 0.050	<0.001
	Within populations	65.53	Φ_{ST} 0.365	<0.001
EATL vs. WATL	Among groups	7.22	Φ_{CT} 0.072	<0.001
	Among pops within groups	1.06	Φ_{SC} 0.011	<0.001
	Within populations	91.78	Φ_{ST} 0.082	<0.001

sequences from the Mediterranean (Sacco *et al.*, 2017) and Atlantic Ocean (Merten *et al.*, 2015) showed similar patterns of significant divergence (Supplementary Table S2).

Pairwise values of Φ_{ST} between samples from the Atlantic Ocean ranged from 0.001 to 0.095 and within the Mediterranean from 0.025 to 0.099, but between Atlantic and Mediterranean samples, distances ranged from 0061 to 0.297 (Table 4). Distances between the Atlantic and Mediterranean samples were significant, except for comparisons between LevS and Tyr in the Mediterranean, between AzoI and Cver in the eastern Atlantic, and between Lous and Barb in the western Atlantic. The PCoA of sequence divergences between individuals showed two well-differentiated groups: one consisting of fish from the Mediterranean Sea and one including fish from the Atlantic Ocean (Figure 3). Some fish from the western Mediterranean Sea (AlbS, Cver) and eastern Atlantic Ocean were positioned between the two groups in the PCoA.

Table 4. Pairwise-sample Φ_{ST} values, based on mtDNA ND1 gene sequences of *Coryphaena hippurus* from the Atlantic and Mediterranean.

	LevS	SS	Tyr	AlbS	AzoI	Cver	NCar	Barb
SS	0.045*							
Tyr	0.025	0.071**						
AlbS	0.081**	0.045*	0.099**					
AzoI	0.129**	0.061**	0.142**	0.044*				
Cver	0.164**	0.094**	0.199**	0.066**	0.046			
Ncar	0.166**	0.119**	0.184**	0.106**	0.077*	0.075**		
Barb	0.288**	0.187**	0.297**	0.131*	0.095**	0.041**	0.087**	
Lous	0.231**	0.139**	0.231**	0.067**	0.072*	0.050**	0.085**	0.001

*$p < 0.05$; **$p < 0.001$.

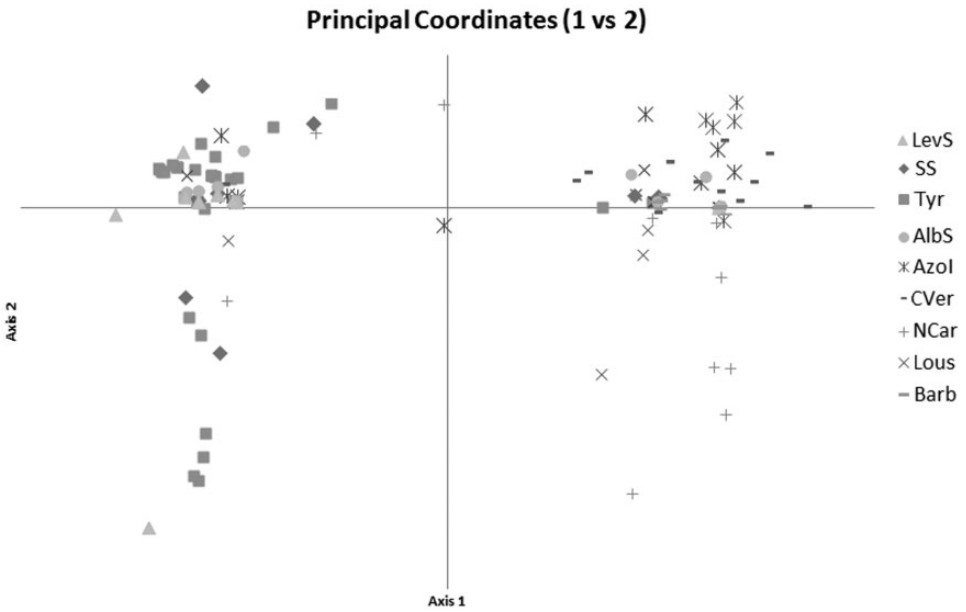

Figure 3. PCoA via distance matrix with data standardization. Different symbols correspond to sampling locations.

The Mantel test and reduced major axis regression revealed a significant correlation between genetic and geographic distances indicating an IBD pattern between the Atlantic and Mediterranean populations ($r = 0.681$; $p < 0.001$), and among just the Atlantic populations ($r = 0.591$; $p < 0.001$) (Figure 4).

Indirect estimates of gene flow between populations indicate that gene flow between Atlantic and Mediterranean populations ($N_em = 1.7$–8.4, by various methods) was generally much smaller than it was between populations within the Mediterranean ($N_em = 6.5$–15.9) (Table 5). The average θ estimated with MIGRATE was 0.065 in MED, 0.093 in EATL, and 0.050 in WATL (Table 6). Bayesian asymmetrical migration rates of gene flow (M) were greatest from MED to EATL and WATL.

Results from the BARRIER analyses identified three genetic discontinuities. The first and second areas did not separate MED from ATL, but divided Cver from AzoI and Cver from Barb. The third area was between AlbS and AzoI. Additional genetic discontinuities were detected, but were only supported by values less than 50%.

Discussion

The results of this study are broadly consistent with estimates of stock boundaries in the Atlantic Ocean and Mediterranean Sea, based on tagging, abundance, growth, and migratory behaviour. The results also confirm previous genetic studies, which showed little genetic differentiation between populations within regions, but large amounts of divergence between Atlantic and Mediterranean populations. Our results also provide additional insights into the genetic consequences of contemporary and historical barriers to dispersal.

Before discussing these results in detail, we provide the following cautions in the interpretations of the data. First, our samples were small and were widely spaced so that only a rough resolution of genetic population structure is possible. Nevertheless, the samples covered most of the range of dolphinfish in the central Atlantic and Mediterranean and provide an opportunity to test models of stock structure on a broad geographical scale. In any case, only geographically large stocks are expected because of the high potential for gene flow at adult and larval life-history stages. Second, our conclusions are based on a single, maternally inherited genetic marker and hence can provide only one view of the genetic population structure of dolphinfish (Karl *et al.* 2012). The analysis of large numbers of single-nucleotide polymorphisms (SNPs) or high-mutation markers, such as microsatellite DNA, may show additional resolution of small-scale structure that reflects population responses to decadal climatic shifts and

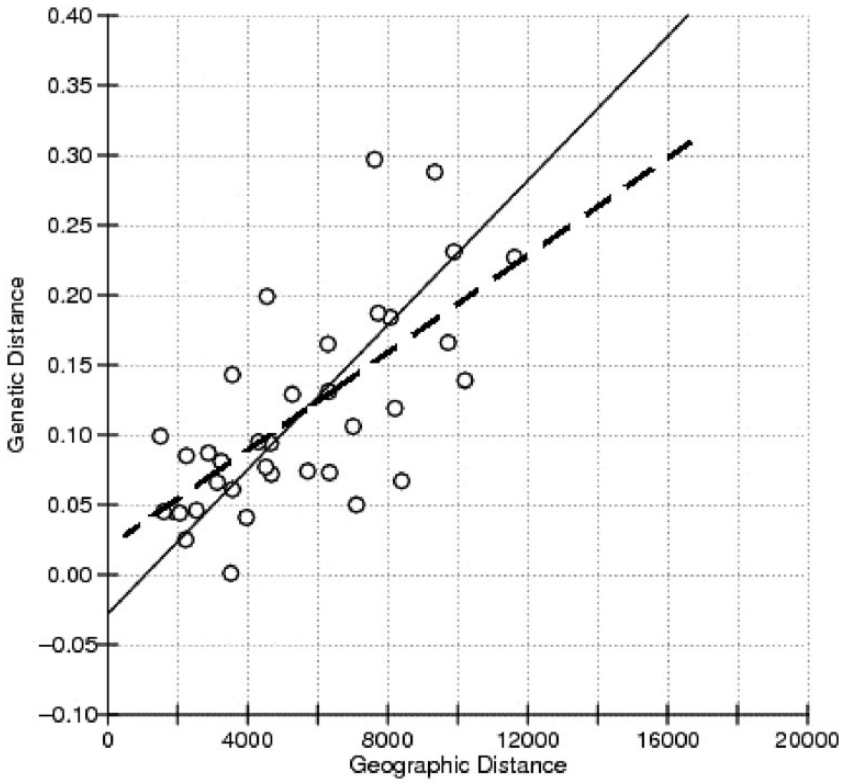

Figure 4. Relationship between genetic and geographic distances showing an isolation. By Distance (IBD) pattern. Continuous line represents the relationships between Mediterranean and Atlantic populations; dashed line the relationship within the Atlantic populations.

Table 5. Gene flow estimates (as number of specimens) for *Coryphaena hippurus* across all samples from the Atlantic and Mediterranean and among samples within the Mediterranean.

	Nei (1973)	Nei (1982)	Lynch and Crease (1990)	Hudson *et al.* (1992)
MED vs. ATL	8.4	1.7	2.0	2.0
MED vs. EATL	25.2	2.6	1.5	1.5
MED vs. WATL	11.6	1.6	0.9	0.9
EATL vs. WATL	30.0	10.2	11.8	10.7
MED	15.85	4.1	6.4	6.5

EATL includes AzoI and CVer; WATL includes NCar, Barb and Lous.

Table 6. Gene flow estimates conducted with MIGRATE software.

		M		
Population	θ	**MED**	**EATL**	**WATL**
MED	0.065		393.37	448.55
EATL	0.093	768.42		284.15
WATL	0.050	738.00	462.45	

M is migration rate; columns are donor populations whereas rows are receiving populations.

changes in oceanic conditions. Third, the inferences made from the statistical analyses in this study are based on the assumption that mtDNA variants are neutral to natural selection and are influenced only by random drift and gene flow. Random drift is an inverse function of the effective size of a population (the number of reproductively successful individuals) and gene flow is accomplished by the successful reproduction of immigrants in a population.

Finally, we were unable to use nucleotide mismatch and coalescence analyses to reconstruct historical demographies because of small local sample sizes. These methods assume that a sample comes from a single panmictic populations with a unique demographic history. Pooling samples to achieve large sample sizes violates this assumption and biases historical reconstructions by over-estimating the timing and magnitudes of demographic events. Additionally, some methods of historical reconstruction use the bifurcating Fisher–Wright model to sample the distribution of genealogies from an observed set of DNA sequences. However, dolphinfish have a type-III life-history pattern and are thus prone to reproductive skew among families, a condition that leads to multiple coalescences in a genealogy (Eldon *et al.*, 2015; Niwa *et al.*, 2016). Nevertheless, the genetic patterns reported here, together with paleo-climate and paleo-ocean histories, can be used to test models of population history.

The results of this study are largely consistent with previously hypothesized stocks of dolphinfish. In the western central

Atlantic, our sample from North Carolina represents the northern stock and was genetically divergent from a sample from Barbados, which is located in the southern stock. This southern stock presumably extends to the northwestern shores of Brazil. The boundary between these two, large migratory northern and southern circuits is assumed to be Puerto Rico (Oxenford and Hunte, 1986; Oxenford, 1999). However, a previous study failed to find a generic difference between dolphinfish in waters north and south of Puerto Rico (Merten *et al.*, 2015). The genetic similarity between the samples from the Gulf of Mexico and Barbados in the present study (Table 4) indicates that the relationship of fish in the Caribbean Sea and Gulf of Mexico to larger stock groups is uncertain. Further, genetic studies are required to better resolve the population structure in these areas.

Our study included two samples from the eastern central Atlantic Ocean (Azores off the Iberian Peninsula and Cabo Verde off West Africa) that were genetically similar. The results of tagging studies or stock characteristics are unavailable to be able to define migratory patterns, but these two areas appear to be part of the same widely distributed stock.

In the present study, the largest genetic differences between populations were between populations in the eastern Atlantic and those in the Mediterranean Sea. Significant levels of genetic divergence were detected between these two areas in the AMOVA (Table 3) and pairwise comparisons (Table 4). This genetic discontinuity may result from processes on different time scales. On contemporary time scales, Gibraltar Strait and especially the Oran-Almeria ocean front between Atlantic and Mediterranean water masses may limit the dispersal of passively drifting larvae and the migration of adults (Tintoré *et al.*,1988). This ocean front marks a major genetic boundary between populations of numerous marine organisms (Patarnello *et al.*, 2007). However, the results of the present and previous genetic studies show that at least some gene flow occurs between eastern Atlantic and Mediterranean populations. The four equilibrium-based methods we used to estimate migration between eastern Atlantic populations and Mediterranean populations ranged from 1.5 to 25.2 genetically effective individuals per generation (Table 5). The MIGRATE analysis indicates that gene flow into the Mediterranean from the Atlantic is twice as large as it is in the reverse direction (Table 6).

Sea surface temperatures throughout much the Mediterranean Sea drop below 20°C during the winter. As a result, it is often presumed that adult dolphinfish move to either warmer areas in the Mediterranean, such as Gulf of Gabes, Sardinian waters, or the Balearic Sea, or migrate to the eastern Atlantic Ocean along the west African coastline where Sea Surface Temperature (SST) is warm year-round (Kocheď *et al.*, 2015). Thus, movements of adults through the Strait of Gibraltar may be restricted to late spring through late summer, a time coinciding with the seasonal reproduction in the western Mediterranean (Massutí, 1997; Sinopoli and Andaloro, 2017). Migration-rate estimates in the present study support the hypothesis that adults migrate from the Mediterranean Sea to the warmer waters of the eastern Atlantic Ocean. However, conventional and satellite tagging studies are needed identify migratory pathways between overwintering and spawning grounds in the Mediterranean.

Evidence for the genetic influence of Atlantic dolphinfish populations on western Mediterranean populations is apparent in the pattern of genetic divergences between populations. The sample from the Alboran Sea showed a smaller amount of divergence from the two eastern Atlantic samples (mean $\Phi_{ST} = 0.055$) than did the three samples from the central and eastern Mediterranean Sea (mean $\Phi_{ST} = 0.128$). The mean genetic distance between the Alboran Sea population and the three Mediterranean samples was nearly twice as large ($\Phi_{ST} = 0.075$) as the mean genetic distance between the three remaining Mediterranean samples (mean $\Phi_{ST} = 0.047$). The genetic influence of eastern Atlantic dolphinfish may extend as far as the Balearic Islands in the western Mediterranean. In a study of single nucleotide polymorphisms in samples of dolphinfish from eight localities in the Mediterranean, Maroso *et al.* (2016) found that the western most sample from the Balearic Islands off the east coast of Spain differed substantially from other samples collected in the central and eastern Mediterranean Sea. Although no Atlantic-Ocean samples were included in that study, the genetically divergent western Mediterranean sample likely reflects the influence of gene flow from Atlantic populations.

The large amount of genetic divergence between Mediterranean and Atlantic dolphinfish populations indicates periods of isolation of the Mediterranean population. Two mechanisms may have periodically isolated dolphinfish in the Mediterranean over the glacial cycles of the Pleistocene. First, lower sea levels may have restricted dispersal across the Strait of Gibraltar. During glacial maxima, global sea levels dropped about 120 m below present-day seal levels (Rohling *et al.*, 2014). Restriction to dispersal by lower sea level was postulated by Díaz-Jaimes *et al.* (2010) as barrier to dispersal that led to the reproductive isolation of the Mediterranean population of dolphinfish and hence to genetic divergence of this population from populations in the Atlantic Ocean. However, the sill across the strait is about 300 m below present-day sea levels, so that passage across the Strait for an epipelagic fish would not have been completely blocked.

A second, more likely isolating mechanism was the drop in sea surface temperature in the eastern Atlantic Ocean and in the western Mediterranean Sea during Pleistocene glaciations (Jouzel *et al.*, 2007). During the last glacial maxima in the Pleistocene, ice-rafted debris as far south as Portugal (40°N) indicated that the polar front reached nearly to the Strait of Gibraltar (Rogerson *et al.*, 2012). The appearance of a cold-water foraminifera (*Neogloboquadrina pachyderma*) in the sediments of the Alboran Sea in the far western Mediterranean corresponds to cold events in the North Atlantic over the past 50 000 years (Cacho *et al.*, 1999). Reconstructions of sea surface temperatures at the Last Glacial Maximum (LGM) from the analysis of sediment cores shows a strong east-west temperature gradient across the Mediterranean. Winter LGM SST ranged from 8°C to 10°C in the Alboran Sea to 14°C in the southeastern Mediterranean and summer temperatures ranged from 14°C to 16°C in the west to over 24°C in the far eastern Mediterranean (Hayes *et al.*, 2005). Similar drops in SST have occurred during other glacial maxima and stadials (Rohling *et al.*, 1998).

Since present-day dolphinfish are found only in waters with temperatures well above 20°C, populations could have survived only in the eastern Mediterranean. These populations were isolated for several hundred to thousands of years during periods of northern hemisphere glaciation. In the eastern Atlantic, dolphinfish populations were displaced several degrees of latitude to the south along West Africa where warmer conditions prevailed. The most recent period of isolation in the Mediterranean was likely during the brief climatic reversal in post-LGM warming that

occurred 11.5 thousand years ago (Younger Dryas) (Xiaodong *et al.*, 2014).

Geographical patterns in genetic diversity among populations can be used to infer population histories (Wilkins and Wakeley, 2002). Large values of nucleotide diversity are expected in populations with long continuous histories that promote the accumulation of numerous mutations. In our samples, the largest nucleotide diversities occurred in eastern Atlantic samples (mean $\pi = 0.030$) and in the sample from North Carolina ($\pi = 0.023$), and the smallest values in the Gulf of Mexico and the Barbados (mean $\pi = 0.006$). Nucleotide diversities in the Mediterranean were intermediate (mean $\pi = 0.015$). This pattern may indicate that eastern Atlantic populations are at the centre of an ideal habitat, but that populations in the southwestern Atlantic stock are at the edge of optimal habitat conditions. The intermediate diversities in the Mediterranean likely reflect population instability during Pleistocene climate variability that led to repeated isolations and smaller effective population sizes. The enclosed Mediterranean populations could not follow optimal habitats as could dolphinfish populations in western and eastern Atlantic.

In summary, the results of this study, and previous genetic studies, provide evidence for the existence of genetically discrete stocks of dolphinfish in the western Atlantic and of a large eastern Atlantic stock. The results also confirm that the Mediterranean population is genetically differentiated from Atlantic populations and has likely been isolated from the eastern Atlantic numerous time by Pleistocene drops in temperature that prevented dolphinfish from moving out of the Mediterranean. Our results point to the need not only to manage western and eastern Atlantic populations separately, but also the need to manage populations in the central and eastern Mediterranean as a single shared stock among Mediterranean nations (CopeMed II, 2016). Given the highly migratory nature of dolphinfish, informed management decisions must involve multijurisdictional supranational agencies, as well as the FAO-General Fisheries Commission for the Mediterranean.

Supplementary data

Supplementary material is available at the *ICESJMS* online version of the manuscript.

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Data availability

Datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

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