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Genetic structure and history of populations of the deep-sea fish *Helicolenus dactylopterus* (Delaroche, 1809) inferred from mtDNA sequence analysis

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Abstract

***Helicolenus dactylopterus* is an Atlantic benthopelagic fish species inhabiting high-energy habitats on continental slopes, seamounts and islands. Partial sequences of the mitochondrial control region (D-loop) and cytochrome *b* (cyt *b*) were used to test the hypothesis that *H. dactylopterus* disperses between continental margin, island and seamount habitats on intraregional, regional and oceanic scales in the North Atlantic. Individuals were collected from five different geographical areas: Azores, Madeira, Portugal (Peniche), Cape Verde and the northwest Atlantic. D-loop (415 bp) and cyt *b* (423 bp) regions were partially sequenced for 208 and 212 individuals, respectively. Analysis of variation among mitochondrial DNA sequences based on pairwise *F*-statistics and AMOVA demonstrated marked genetic differentiation between populations in different geographical regions specifically the Mid-Atlantic Ridge (Azores)/northeast Atlantic (Portugal, Madeira) compared to populations around the Cape Verde Islands and in the northwest Atlantic. Some evidence of intraregional genetic differentiation between populations was found. Minimum-spanning network analysis revealed star-shaped patterns suggesting that populations had undergone expansion following bottlenecks and/or they have been colonized by jump dispersal events across large geographical distances along pathways of major ocean currents. Mismatch distribution analysis indicated that Azores and northwest Atlantic populations fitted a model of historical population expansion following a bottleneck/founder event estimated to be between 0.64 and 1.2 million years ago (Ma).**

Keywords: colonization, expansion, *Helicolenus dactylopterus*, mtDNA, North Atlantic, population structure

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Introduction

Many bathyal fish species (living at 200–2000 m depths) have large geographical ranges that span one or more oceans. Populations of benthopelagic species inhabit continental slopes, the slopes of oceanic islands and seamounts that may be separated from each other by thousands of kilometres of deep ocean. The question arises as to whether such species have life histories that are characterized by extremely high dispersal or if their present-day distributions have resulted from past dispersal events when oceanic conditions and the configuration of geographical features were different. Such historic structuring of populations is exhibited by shallow-water fish such as anchovies (reviewed in Grant & Bowen 1998).

Fish species, typical of high-energy deep-benthic habitats, located on seamounts and continental slopes, often have 'K-type' life strategies. They are generally long-lived, with low natural rates of mortality and sporadic recruitment to populations (Rogers 1994; Koslow *et al*. 2000). The life history of many deep-sea fish species, however, includes an extensive larval phase. This suggests that they may exhibit high levels of dispersal and, as a result, should exhibit a lack of stock structure on oceanic, regional and subregional scales. However, seamounts and oceanic islands maybe

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isolated from each other and from continental margins by large geographical distances. Current-topography interactions may also generate trapped parcels of water around these features (e.g. Taylor columns on seamounts) acting as larval retention mechanisms (Rogers 1994).

Many studies of seamount and oceanic island-associated species to date suggest that populations do exhibit panmixia across large geographical distances on regional or even oceanic scales, as expected from aspects of life history, including slender armourhead (*Pseudopentaceros wheeleri* Hardy, 1983), wreckfish (*Polyprion americanus* Bloch & Schneider, 1801) and alfonsino (*Beryx splendens* Lowe, 1834) (Martin *et al*. 1992; Sedberry *et al*. 1996; Hoarau & Borsa 2000). For some deep-water species of fish there is, however, evidence for genetic differentiation among populations at the trans-oceanic, oceanic and regional scales including roundnose grenadier (*Coryphaenoides rupestris* Gunnerus, 1765), Greenland halibut (*Reinhardtius hippoglossoides* Jordan & Snyder, 1901), rockfish (*Sebastolobus alascanus* Bean, 1890 and *Sebastolobus alascanus altivelis* Gilbert, 1896), ling (*Genypterus blacodes* Forster, 1801), hoki (*Macruronus novaezealandiae* Hector, 1871), oreos (*Allocyttus niger* James, Inada & Nakamura, 1988, *Allocyttus verrucosus* Gilchrist, 1906) and others (reviewed in Creasey & Rogers 1999; Rogers 2003).

The bluemouth, *Helicolenus dactylopterus* (Delaroche, 1809), is a benthopelagic fish of the order Scorpaeniformes and belongs to the family Sebastidae. Many fish in this family occur on continental and oceanic island slopes and seamounts. They are heavy-bodied muscular species, typical of the guild of benthic or benthopelagic deep-sea fish identified from these habitats (Koslow *et al*. 2000). The bluemouth is commonly found between 200 and 1000 m depths (Kelly *et al*. 1999), along the edge of the continental shelf and upper continental slope of the eastern Atlantic (from Norway to the Gulf of Guinea), the western Atlantic (from Canada to Brazil), and off the west coast of South Africa. It is also distributed on the slopes of the Macaronesian islands (Azores, Madeira, Canaries and Cape Verde), on seamounts of the Mid-Atlantic Ridge (Hureau & Litvinenko 1986), and non-axial seamounts such as the Josephine Bank (Maul 1976).

There have been few studies focusing on the morphological, ecological, and geographical separation of populations of bluemouth, though there have been studies on other members of the family Sebastidae. The only genetic studies on bluemouth have been restricted to studies of phylogeny (Johansen *et al*. 1993; Kai *et al*. 2003). However, Eschemeyer (1969) divided *Helicolenus dactylopterus* in to two subspecies on the basis of morphological characteristics: *Helicolenus dactylopterus lahillei* and *Helicolenus dactylopterus dactylopterus*. The latter is thought to occur in four different populations located off South Africa, the Gulf of Guinea, the northeast (NE) Atlantic (from Norway to North Africa and Mediterranean) and northwest (NW) Atlantic (Nova Scotia to Venezuela). Barsukov (1980) proposed the further subdivision of the species into six subspecies also based on morphological measurements and geographical distribution: *H. d. dactylopterus*, *Helicolenus dactylopterus maderensis*, *Helicolenus dactylopterus maculatus*, *Helicolenus dactylopterus gouphensis*, *Helicolenus dactylopterus angolensis* and *H.d.lahillei*.

The existence of morphologically different geographical populations of bluemouth is inconsistent with features of its life history. This species is known to have internal fertilization with brooding of eggs until the early stage of development (early embryo) consistent with zygoparity (Sequeira *et al*. 2003). Spawning occurs multiple times in a single season and fecundity is relatively high for a scorpaenid (11 000–87 000 eggs compared to, for example a maximum of 20 000 for *Scorpaena porcus* and 33 000 for *Scorpaena notata*) (Muñoz & Casadevall 2002). Eggs and larvae of the bluemouth are pelagic (Muñoz & Casadevall 2002) and larvae occur in the zooplankton between the months of March and September in the NE Atlantic (Russell 1976). Tagging experiments around the Azores archipelago strongly suggest that and bluemouth leads a very sedentary lifestyle, as many tagged specimens have been recaptured, after more than 1 year, exactly in the same places as they were originally caught and tagged (G.M. Menezes, unpublished). This is consistent with seabed observations of individuals of this species remaining in place at the bottom even in the proximity of a submersible. This type of adult lifestyle is associated with an ambush mode of predation where the predator waits on the seabed, attacking prey as they pass (Uiblein *et al*. 2003). Thus, although adults are sedentary, larval-mediated dispersal is likely to occur in this species allowing genetically effective migration over considerable distances.

The aim of this study is to investigate the genetic population structure of the bluemouth in the North Atlantic Ocean to determine whether this species can disperse over large (interregional) distances or whether larval transport is limited. Extensive larval dispersal will result in panmixia among populations at the regional and oceanic scale whereas limited dispersal will lead to marked genetic structure among populations. Historical factors, such as the effects of the Last Glacial Maximum (LGM), may have also played a role in determination of the current distribution of this species and the genetic structure of populations (e.g. Muus *et al*. 2001; Marko 2004). To test these hypotheses, *H. dactylopterus* was collected using a stratified sampling scheme from the Macaronesian archipelagos (Azores, Madeira, Cape Verde), continental Portugal (Peniche), and the NW Atlantic (off the coast of South Carolina, USA). Single samples were collected from localities on an oceanic scale; multiple samples were collected on a regional scale in the NE Atlantic and on a subregional scale in the Azores Archipelago including the three island groups and the Azores Bank (38°10′N, 29°00′W). Genetic population structure was analysed among populations by comparisons of haplotype frequencies in

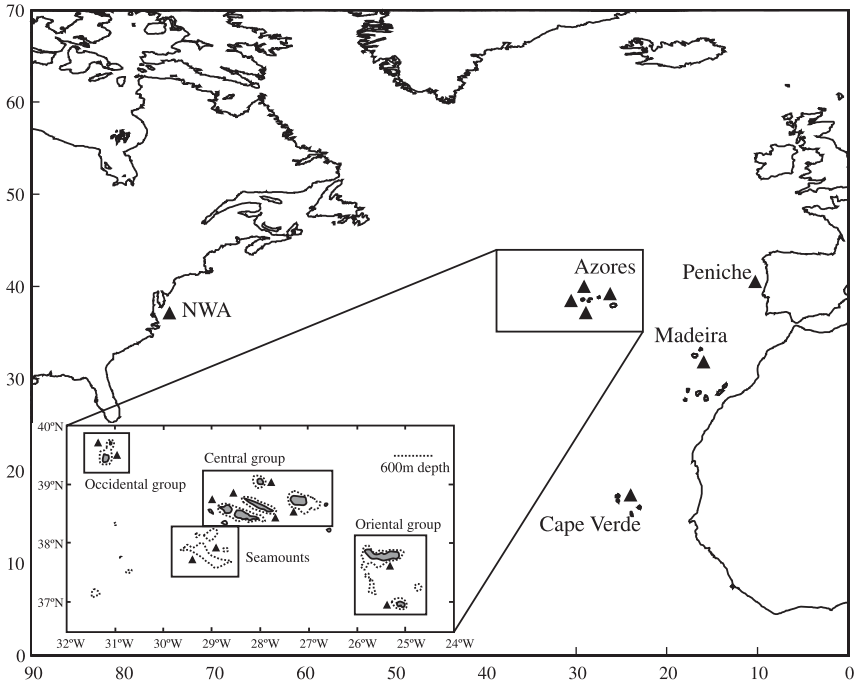

Fig. 1 North Atlantic map with Azores archipelago detail. (▲), sampling sites.

partial sequences of the mitochondrial cytochrome *b* gene (cytb) and mitochondrial control region (D-loop).

Materials and methods

Sampling and DNA extractions

The majority of *Helicolenus dactylopterus* individuals were collected in the Madeira, Azores and Cape Verde archipelagos during the ARQDMAD-P97, ARQDAÇO-P00 and ARQDCAB-P00 cruises on-board the RV 'Arquipélago' of the Department of Oceanography and Fisheries, University of Azores (Menezes *et al.* 1998; map Fig. 1). These samples were collected using a 'stone-buoy' type of bottom long-line gear (Menezes *et al*. 1998). Other individuals were collected at the fisheries market of Peniche (Portugal) and samples from the NW Atlantic were sent by Dr George Sedberry from the South Carolina Department of Natural Resources.

Liver and muscle samples were removed from fresh fish and stored in 95% ethanol immediately after collection. In the case of the NW Atlantic samples, fins were collected and stored in a sarcosyl/urea preservative solution. Total genomic DNA was extracted from small (1–3 mg) sections of tissue following a phenol–chloroform protocol based on Sambrook *et al*. (1989). The extracted DNA was resuspended in elution buffer and stored at –20 °C until further utilization.

PCR amplification and sequencing

The mitochondrial control region (415 bp) was partially amplified for 208 individuals by polymerase chain reaction (PCR) using two universal primers: L-Pro-1 (5′-ACTCTCACCCCTAGCTCCCAAAG-3′) and H-DL-C-1 (5′-CCTGAAGTAGGAACCAGATGCCAG-3′) described by Ostellari *et al*. (1996). Reactions of 10-μL total volume containing 1 μL of 10 × buffer (QIAGEN; Tris-HCl, KCl, $MgCl_2$, pH 7.8); 1.25 μL of $MgCl_2$ (Qiagen); 0.6 μL of DNTP mix (Applied Biosystems); 0.1 μL of *Taq* polymerase (QIAGEN); 1.5 μL of template (10–20 ng) DNA; 5.05 μL of H_2O and 0.5 μL of each primer (10 pmol/μL), were conducted on a Perkin-Elmer DNA Thermal Cycler 480 under conditions as follows: 4 min at 94 °C, followed by 30 cycles of denaturing at 92 °C for 60 s, annealing at 50 °C for 60 s and extension at 72 °C for 60 s; finishing with an extension step at 72 °C for 5 min.

The cyt *b* region (423 bp) was partially amplified for 212 individuals using the same PCR reagents and quantities as for D-loop but using the universal primers CYB-GLU-L-CP (5′-TGACTTGAAGAACCACCGTTG– 3′) and CB2-H (5′-CCCTCAGAATGATATTTGTCCTCA-3′) described by Palumbi *et al*. (1991). PCR cycles were performed on an MWG-Biotech Primus 96 Plus thermocycler under the following conditions: 94 °C for 4 min, followed by 30 cycles of 94 °C for 50 s, 56 °C for 30 s and 72 °C for 50 s; finishing with an extension step at 72 °C for 5 min.

All amplified products were purified using QIAquick PCR Purification kits (QIAGEN) following the supplier's instructions. Each purified PCR product was used in a cycle sequencing reaction using Applied Biosystems BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) under the following conditions: 10-μL reactions (4 μL Dye, 0.16 μL primer, 1 μL sample and 4.84 μL H_2O) at

96 °C for 10 s, 50 °C for 5 s, 60 °C for 4 min for 25 cycles and a holding step at 4 °C. The resulting cycle sequencing fragments were purified using DyeEx Spin kits (QIAGEN) following the supplier's instructions. Finally, products were visualized using an Applied Biosystems Prism 377 automated sequencer.

Sequence alignment

Both mitochondrial partial sequences were sequenced in both directions to check the validity of the sequence data. One example of cyt *b* and D-loop sequences were used to search GenBank for similar sequences using the BASIC LOCAL ALIGNMENT SEARCH TOOL (BLAST) available at the NCBI website (http://www.ncbi.nih.gov/BLAST/). The most similar sequences obtained from BLAST searches were added as outgroups for phylogenetic analysis to the data sets obtained for *Helicolenus dactylopterus* in the present study. These included cyt *b* partial sequence data for *Sebastes emphaeus* (AF030725), *Sebastes hopkinsi* (AF030751), *Hozukius emblemarius* (AB096132) and *Helicolenus hilgendorfii* (NC003195, AB096133, AB096134). D-loop sequences were obtained from *Sebastes inermis* (AB071270.1) and *Helicolenus hilgendorfii* (AP002948). All sequences were aligned using CLUSTAL_X (Thompson *et al*. 1997). Alignments were checked by eye and repeated using different values for parameters. For cyt *b* all parameters were as default with pairwise parameters set at gap opening penalty 10, gap extension penalty 0.1 and multiple alignment parameters set at gap opening penalty of 10, gap extension 0.2. D-loop alignment parameters were default except for pairwise and multiple alignment parameters that were both set at gap opening penalty 10 and gap extension penalty 5.

Population genetic analysis

Eight different geographical populations were defined a priori: NW Atlantic, Cape Verde, Peniche (continental Portugal), Madeira, Azores central group, Azores oriental group, Azores occidental group and Azores seamounts (Azores Bank).

Intrapopulation diversity was analysed by estimating gene diversity (h), the probability that two randomly chosen haplotypes are different (Nei 1987), and nucleotide diversity (π), the probability that two randomly chosen homologous nucleotides are different (Tajima 1983; Nei 1987).

The overall genetic differentiation between each sample population was tested using pairwise *F*-statistics (Wright 1951). This approach does not allow the specific testing of hypotheses that relate geographical proximity of populations to genetic population structure. To achieve this, genetic differentiation was tested in the framework of a predefined geographical structure of the samples on the oceanic, interregional and intraregional levels using analysis of molecular variance (AMOVA) based on pairwise squared-Euclidean distances between haplotypes (Excoffier *et al*. 1992). All population analyses were performed using ARLEQUIN version 2.0 (Schneider *et al*. 2000).

Phylogeographical analysis

Phylogenetic analysis was carried out on both cyt *b* and D-loop sequences along with outgroup taxa in order to ascertain how populations were related to each and whether there was any evidence of historical dispersal and colonization between regions (i.e. whether one population was derived from another). Because mitochondrial DNA sequences often have unequal nucleotide base frequencies and transitions/transversion (ti/tv) ratios markedly different from 2, these parameters were estimated using the program TREE-PUZZLE version 5.0 (Strimmer & Von Haesler 1996). This program also identified sequences that were identical and these were reduced to a single sequence for subsequent analyses to save on computing time and to minimize the size of output trees.

Sequence data were subsequently analysed using distance (neighbour joining) and maximum-parsimony methods. All analyses used default parameters except for expected ti/tv ratios and nucleotide frequencies that were estimated from the data. The HKY85 model of sequence evolution (Hasegawa *et al*. 1985) was used in all analyses where appropriate as this most closely simulates the evolution of mitochondrial DNA sequences. It was assumed that mutation rates were similar across the analysed partial sequences and gamma distribution was set at default. The sampling error of neighbour-joining (NJ) and maximum-parsimony (MP) trees was analysed using bootstraps of 10 000 replicates where possible followed by the construction of majority-rule trees. For maximum-parsimony analysis, for D-loop sequences, only 100 bootstraps were possible because of limitations in computational power. In order to attempt to reduce homoplasy, cyt *b* sequences were also analysed using transversions only, again using 100 bootstraps, because of computational limitations. All phylogenetic analyses were carried out using PHYLIP version 3.6a3 (Felsenstein 2002).

Because phylogenetic analysis makes assumptions that are invalid at the population level (ancestral haplotypes are extinct) data were also analysed using phylogeographical techniques based on haplotype networks as implemented by the software package TCS version 1.13 (Clement *et al*. 2000).

Neutrality and demographic history

Demographic history was investigated by analysing mismatch distributions of pairwise differences between all

individuals of each population using ARLEQUIN version 2.000 software package (Schneider *et al*. 2000). This kind of analysis can discriminate whether a population has undergone a rapid population expansion (possibly after a bottleneck) or has remained stable over time. The mismatch distribution will appear unimodal (like a Poisson curve) if accumulation of new mutations is greater than the loss of variation through genetic drift, and multimodal if the generation of new mutations is offset by random genetic drift (Rogers & Harpending 1992).

ARLEQUIN was also used to test for departures from mutation-drift equilibrium with Tajima's *D*-test (Tajima 1989). The time of possible population expansions (t) was calculated through the relationship $\tau = 2ut$ (Rogers & Harpending 1992), where τ is the mode of the mismatch distribution, u is the mutation rate of the sequence considering that $u = 2\mu k$ (μ is the mutation rate per nucleotide and k is the number of nucleotides). A mutation rate of 2% per nucleotide per million years (Myr) was used for cyt *b* as the mean rate for vertebrate mtDNA (Brown *et al*. 1979). The D-loop region evolves faster than this rate in fish and a mean value of 3.6% per Myr was selected as the mean mutation rate estimated from species pairs located at either side of the Isthmus of Panama (Donaldson & Wilson 1999). The generation time for *H. dactylopterus* was taken as approximately 14 years, as ageing studies on otoliths have indicated that most fish live up to 13–14 years. Note that this study indicated that ages of more than 30 years were not uncommon for the bluemouth and individuals reaching a maximum age of 43 years for males and 37 years for females in the NE Atlantic (Kelly *et al*. 1999). Fourteen years is probably therefore a conservative figure for generation time in this species.

Results

Control region

Control region sequence variation. A total of 208 individuals were sequenced for the mtDNA control region (415 bp) and the overall diversity was high with 179 different haplotypes. This agrees with previous works on teleostean fish (Lee *et al*. 1995). Only 14 haplotypes (9%) were shared among different individuals, the other 160 were singletons. Nine of the shared haplotypes were represented at more than one site, while the other five were only shared between individuals restricted to the same geographical site. The most common haplotype sequence was registered in GenBank (Accession no. AY563096). Sequence comparisons revealed 138 divergent sites resulting mainly from transitions, followed by transversions and some insertions/deletions of single base pairs. The expected ti/tv ratio was 5.38 and nucleotide frequencies were T = 0.314, C = 0.166, A = 0.396 and G = 0.124.

Population variability. The haplotype diversity (h) of the analysed populations was very high, with observed values between 0.9108 in the NW Atlantic and 1.000 in several other populations. In contrast, nucleotide diversity (π) within each population was moderate to low, ranging from 0.0059 in the NW Atlantic to 0.033 in Cape Verde population (Table 1).

Phylogeographical relationships of populations. The analysis of molecular variance (Table 2) on five specified groups (Madeira, Peniche, Azores, Cape Verde and NW Atlantic) indicated that a high proportion of the total variance was attributed to differences between the defined groups of populations with a significant value ($P < 0.0127$), indicating geographical structure in haplotype frequencies for D-loop between regions. Only 0.01% of the variation was attributed to differences between populations within groups but this was also significant suggesting that, while the populations within the NE Atlantic did not contain a large component of the variance at the subregional scale, there were significant differences within the region.

Estimates of genetic differentiation between all eight predefined populations, using *F*-statistics, are given in Table 3. The populations from the NW Atlantic and Cape Verde Islands showed high levels of genetic differentiation from all the other populations. F_{ST} values were high for all pairwise comparisons with the Cape Verde Islands and the

Table 1 Intrapopulation nucleotide (π) and haplotype (h) diversities for *Helicolenus dactylopterus* control region and cyt *b*

	Control region		Cyt *b*	
Populations	Nucleotide diversity (π)	Haplotype diversity (h)	Nucleotide diversity (π)	Haplotype diversity (h)
Cape Verde	0.033	0.976	0.006	0.788
NWA	0.006	0.911	0.002	0.426
Madeira	0.031	1.000	0.007	0.936
Peniche	0.029	1.000	0.007	0.860
Azores	0.029	0.996	0.005	0.865

Table 2 Hierarchical analysis of molecular variance (AMOVA) results for *Helicolenus dactylopterus*

Molecular Marker	Source of variation	Total variance (%)	Fixation indices	*P* value
D-loop	Among groups	39.86	$F_{CT} = 0.3986$	< 0.05
	Among populations within groups	0.01	$F_{SC} = 0.0001$	< 0.001
	Within populations	60.13	$F_{ST} = 0.3987$	< 0.001
Cyt *b*	Among groups	42.52	$F_{CT} = 0.4252$	< 0.001
	Among populations within groups	−0.07	$F_{SC} = -0.0013$	< 0.001
	Within populations	57.56	$F_{ST} = -0.4244$	< 0.001

Table 3 *Helicolenus dactylopterus*. Population pairwise F_{ST} values for control region (above diagonal) and cyt *b* sequences (below the diagonal) and *P* values: *** = significant at $P < 0.001$

F_{ST} values	Azores (oriental)	Azores (ocidental)	Azores (central)	Azores Seamounts	Peniche	Madeira archipelago	NW Atlantic	Cape Verde archipelago
Oriental	—	−0.0115	−0.0117	−0.0116	0.0048	−0.0075	0.7583***	0.3036***
Ocidental	0.0002	—	0.0167	−0.0085	0.0034	−0.0101	0.7326***	0.2711***
Central	−0.0123	0.0052	—	0.0083	0.7173	0.0191	0.7665***	0.3317***
Seamounts	0.0082	−0.0043	−0.0004	—	−0.0077	−0.0189	0.6904***	0.2346***
Peniche	0.0270	0.0279	0.0188	0.0096	—	−0.0162	0.7173***	0.2434***
Madeira	−0.0027	−0.0068	0.0053	0.0013	0.0069	—	0.7272***	0.2349***
NWA	0.7910***	0.7408***	0.7714***	0.7315***	0.7159***	0.7531***	—	0.6587***
Cape Verde	0.4154***	0.3668***	0.3778***	0.3119***	0.2509***	0.3440***	0.6845***	—

NW Atlantic and the *P* values associated with these comparisons were significant ($P < 0.0005$). Significant genetic differentiation was not detected between populations within the NE Atlantic region (Azores, Peniche, Madeira).

Phylogenetic analysis. Haplotype networks were inconclusive for D-loop and were excluded from this section as a result of high variability and high level of homoplasy among sequences. As a result of poor resolution and low bootstrap support NJ trees are not shown. However, it is noted that Cape Verde individuals were mostly contained within 3–4 clades depending on the type of analysis and were most closely related to NW Atlantic individuals that were always contained within a single clade. A few Cape Verde individuals were detected among NE Atlantic haplotypes and a few NE Atlantic individuals were detected among Cape Verde clades or unresolved haplotypes. *Sebastes inermis* appeared in different parts of the tree with different analyses suggesting that this sequence was so distantly related to *H. dactylopterus* that it was effectively acting as random data (e.g. Wheeler 1990).

Demographic history and neutrality. Because no evidence of genetic differentiation was observed within the Azores, all populations within this archipelago were pooled as a single group to conduct tests of selective neutrality and demographic history as for intrapopulation diversity. Pairwise mismatch distributions and results of Tajima's *D*-test performed on each population are given in Fig. 2. The parameters of the model of sudden expansion (Rogers & Harpending 1992) and the goodness-of-fit test to the model are given in Table 4. All histograms presented multimodal curves characteristic of populations with constant size over time. Most of the populations presented moderate to highly negative Tajima's *D*-test values although only one, the NW Atlantic, was significant. This population presents a unimodal curve with a significantly negative Tajima *D*-test value, indicating a sudden expansion in population size. A time of this expansion was estimated at approximately 642 000 BP.

Cyt b *region*

Cyt b *sequence variation.* The 5'end of the cyt *b* mtDNA region (423 bp) was amplified from a total of 212 individuals altogether. The alignment of the sequences revealed 69 different genotypes defined by 70 divergent nucleotide sites. Most nucleotide variation resulted from transitions followed by transversions with an expected ti/tv ratio of 5.63. A single insertion/deletion was detected as a result of an extra nucleotide found in one individual. This may represent the rare occurrence of this type of mutation in the cyt *b* region but may also have resulted from experimental error. The nucleotide frequencies were T = 0.302, C = 0.287, A = 0.249 and G = 0.162. The most common haplotype (GenBank Accession no. AY563095) was found in 50

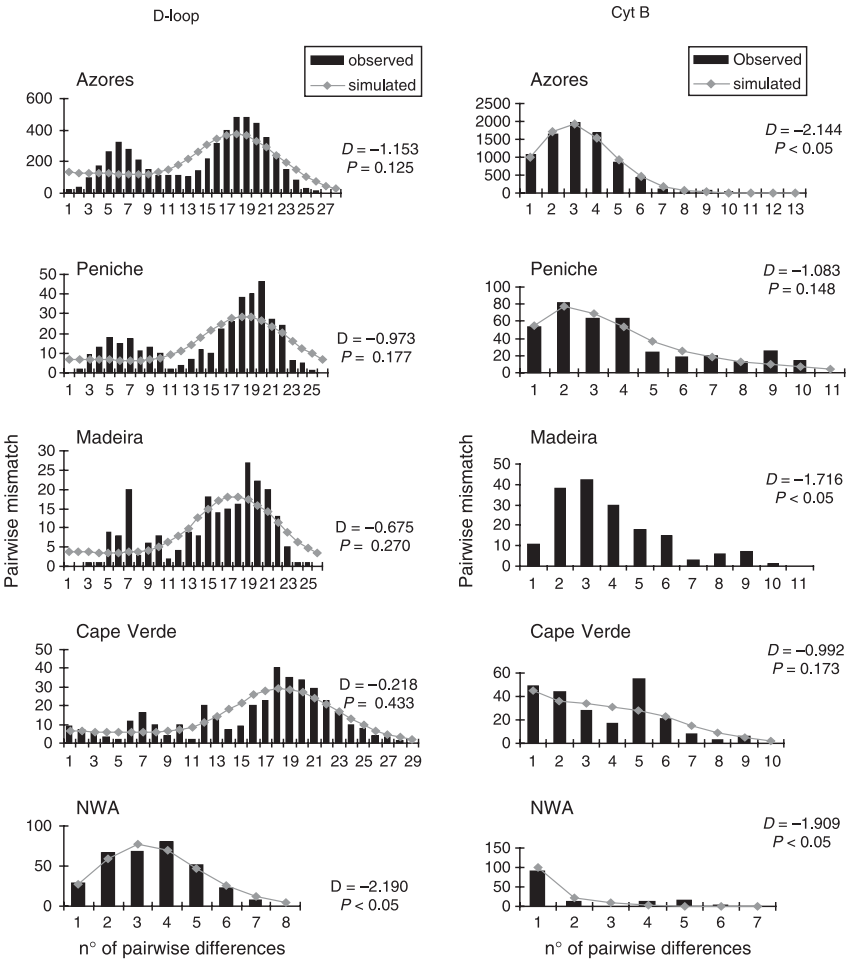

Fig. 2 Pairwise mismatch distributions (Rogers & Harpending 1992), simulated model of sudden expansion (Rogers 1994) and results of Tajima's *D*-test with associated probability (Tajima 1989) for each population with both molecular markers.

	Populations	Azores	Madeira	Peniche	Cape Verde	NWA
D-loop	Parameters					
	S	105	57	67	60	24
	θ_0	3	0	2	5	2
	θ_1	38.037	60.469	54.258	59.102	35.039
	τ	18.172	17.875	18.478	18.630	2.728
	Goodness-of-fit test					
	SSD	0.008	0.013	0.012	0.006	0.003
	P	0.238	0.223	0.173	0.513	0.595
cyt *b*	*S*	42	19	17	13	7
	θ_0	3		2.472	5	404
	θ_1	15.079		620.625	4.073	0.405
	τ	2.473		701	4.544	3.000
	Goodness-of-fit test					
	SSD	0.001	No fit	0.005	0.022	0.034
	P	0.852		0.727	0.561	0.135

Table 4 *Helicolenus dactylopterus*. Parameters of the sudden expansion model and goodness-of-fit test to the model with respective significance for each population. *S*, number of polymorphic sites; θ_0, pre-expansion population size; θ_1, postexpansion population size; τ, time in number of generations; SSD, sum of squared deviations

individuals from the NE Atlantic populations (Azores, Madeira, Peniche), but this was not detected in Cape Verde and NW Atlantic individuals. Fourteen other haplotypes were also found shared among individuals from different areas.

Population variability. Haplotype diversity (*h*), within the geographical populations was high ranging from 0.426 in the NW Atlantic population to 0.936 in Madeira. Nucleotide diversity (π) was generally low ranging from 0.002 in the NW Atlantic population to 0.007 in continental Portugal (Peniche) (Table 1).

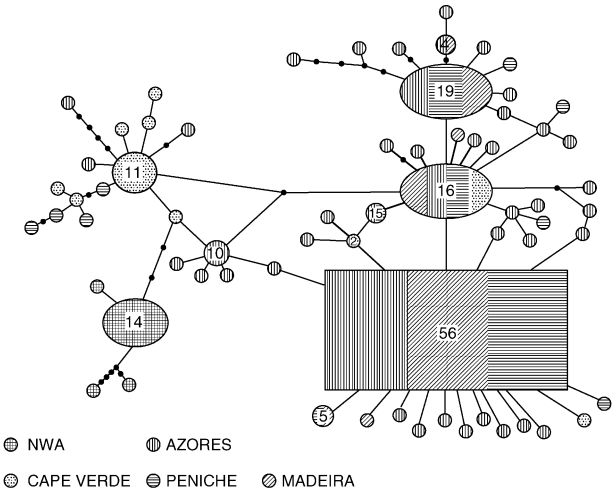

Fig. 3 *Helicolenus dactylopterus*. Cyt *b*. Minimum spanning network analysis of haplotypes for all populations. Black dots represent putative mutational steps between haplotypes.

Phylogeographical relationships of populations. The hierarchical partition of variance among populations tested using AMOVA (Excoffier *et al*. 1992) was performed as previously presented for the control region (Table 2). The proportion of 'among regions' variance component is large (42.52%) and significant. Again, only a small variance component was attributable to populations within regions but this was also significant.

The estimates of pairwise F_{ST} values between the different geographical areas are given in Table 3 together with respective *P* values. High F_{ST} values, were found in pairwise comparisons between all NE Atlantic populations and the Cape Verde and NW Atlantic. *P* values were all significant ($P < 0.0005$), as for the results obtained with the control region.

Phylogenetic analysis. The haplotype network derived from cyt *b* partial sequences is presented in Fig. 3. The most common haplotype, represented by a square-shaped box (size of squares and circles is proportional to the number of haplotypes) represents individuals from the NE Atlantic (Azores, Madeira, Peniche). The fourth and fifth most common haplotypes were restricted to the NW Atlantic and Cape Verde, respectively. The star-shaped phylogenies for three primarily NE Atlantic clades, and the Cape Verde and NW Atlantic clades are consistent with recent population expansion. That specific clades contain haplotypes from single geographical regions is consistent with the significant 'among regions' variance component from (F_{CT}) AMOVA and significant pairwise *F*-statistics. This suggests strong genetic differentiation between the regions of the NE Atlantic, Cape Verde and NW Atlantic (but see succeeding discussion).

Neighbour-joining and maximum-parsimony analysis of the cyt *b* haplotype data also showed poor resolution and poor bootstrap support for the trees as a whole and so these trees are not presented. However, there were similarities in the topology of all trees for cyt *b* analyses, including tranversion-only analysis, in that NW Atlantic haplotypes formed a single clade and this was most closely related to Cape Verde haplotypes that were found mostly in three clades. A few Cape Verde individuals exhibited NE Atlantic haplotypes and a few Cape Verde haplotypes were found in the NE Atlantic. A single small clade of haplotypes from the Azores only was also consistently resolved but with poor support. This may contribute to the small but significant variance component detected by AMOVA at the subregional scale. *Sebastes* spp. did not consistently occur in one place on the tree for NJ analysis if *H. hilgendorfii* was included in the outgroup. This is consistent with a distant relationship between *Sebastes* spp. and *Helicolenus*.

Demographic history and neutrality. Pairwise mismatch distributions and results of Tajima's *D*-test performed on each population — Azores, Madeira, Peniche, Cape Verde and NWA — are given in Fig. 2. The parameters of the model of sudden expansion (Rogers & Harpending 1992) and the goodness-of-fit test to the model are given in Table 4. All populations exhibited moderate to highly negative *d* values but only the Azores and NW Atlantic populations were significant. The Madeira population could not be fitted to an expansion model. The estimated time of expansion for populations from the Azores and the NW Atlantic were 1.02 and 1.24 Myr, respectively.

Discussion

Genetic variation

Nucleotide and haplotype diversities can provide some information on the history of bluemouth populations in the North Atlantic. High genetic variation (h) and low to moderate nucleotide diversity (π) were found in all populations analysed for both mtDNA markers. This pattern of genetic diversity can be attributed to a recent population expansion after a low effective population size caused by bottlenecks or founder events (Grant & Bowen 1998). Such an explanation is also consistent with the star-shaped haplotype networks detected for cyt *b* in populations of *Helicolenus dactylopterus* (Fig. 3). Mismatch distribution analysis further supports a population expansion, for populations in the Azores and the NW Atlantic (Fig. 2). In such cases, the rapid growth of a population leads to the retention of new mutations especially in mtDNA sequences, known to evolve several times faster than nuclear DNA (Brown *et al*. 1979). Such patterns of diversity, haplotype networks and mismatch distribution strongly suggest a

historical influence on the genetic structure of *H. dactylopterus* populations, as estimated by analysis of haplotype frequencies.

Population structure

F-statistics, AMOVA, haplotype network and phylogenetic analyses all indicated marked genetic structure in *H. dactylopterus* populations at the interregional scale. There appears to be no effective gene flow between the NE and NW Atlantic populations of this species and little or no gene flow between the Cape Verde Islands and populations on the Mid-Atlantic Ridge (Azores), Madeira and the European continental slope (Peniche). However, a few 'Cape Verde' haplotypes were sampled from NE Atlantic populations and vice versa. This may represent occasional migrants between these localities, though the overall level of genetic exchange must be below that required to homogenize populations, or there is a barrier to gene flow between these populations (i.e. they are separate species). Occasional long-distance migration has been detected in other deep-water species that can even span the entire length of oceans (e.g. Møller *et al*. 2003). These haplotypes may also represent historical migration events or result from homoplasy. In contrast to studies on some seamount-associated species (wreck fish, alfonsino, slender armourhead), the larval-dispersive phase in *H. dactylopterus* is not sufficient to allow gene flow between populations at the interregional scale.

Within the geographical region of the NE Atlantic there is some evidence for genetic differentiation between the island groups and seamounts of the Azores, Madeira and the European continental slope. AMOVA showed that only a small component of variance was attributable to populations within regions, however, this was significant. It must also be noted that phylogenetic analysis for both D-loop and cyt *b* data both revealed a small clade of haplotypes that only occurred in the Azores. This is suggestive of some degree of reproductive isolation of Azores populations but the limited sample sizes in this investigation, and the poor resolution of phylogenetic analyses, limit the interpretation of these data. Migration between these populations may occur by larval transport in the Azores and Canaries currents. Adult migration is not consistent with behavioural observations for this species but these data are limited at present so this cannot be ruled out. It is possible that the large geographical distance between the Mid-Atlantic Ridge and the European continental slope and Madeira is a barrier to gene flow within this region. More detailed investigations of the populations within this region, using high-resolution genetic markers to detect fine-scale genetic structure, are being conducted. Migration between the NE Atlantic and Cape Verde Islands is limited in the present day and, if it exists at all, it is sporadic. Dispersal routes may include larval dispersal via the Canaries current and adult dispersal along the continental slope.

The polarity of the relationships between the NW Atlantic and Cape Verde and other NE Atlantic populations inferred by using *Helicolenus hilgendorfii* as the outgroup taxon suggest that the eastern Atlantic populations are likely to have been the source of migrants for the NW Atlantic population of *H. dactylopterus*. This is consistent with data that suggest that current western Atlantic populations of some marine invertebrates such as *Asterias rubens*, *Littorina obtusata* and *Nucella lapillus* were founded from the eastern Atlantic (e.g. Wares & Cunningham 2001). For these species, trans-Atlantic migration is thought to have occurred after the LGM, which was much more severe in the NW Atlantic than the NE, resulting in elimination of populations of marine organisms on the eastern coast of North America (Wares & Cunningham 2001; Hewitt 2003). Intuitively, it may seem unlikely that deep-sea species were strongly impacted by previous glaciation events, but the larvae of *H. dactylopterus* are planktonic and would therefore be vulnerable to changes in sea-surface temperatures and other physical factors. In addition, food chains in the deep sea, with the exception of chemosynthetic communities, are dependent on surface productivity. Any changes that impact surface productivity patterns are likely to have knock-on effects on food webs as indicated by recent changes in community structure of deep-sea animals (e.g. Billett *et al*. 2001; Wigham *et al*. 2003). An east to west migration is counter to evidence that some tropical Atlantic taxa migrated from the western Atlantic to the eastern Atlantic via the Atlantic Equatorial Undercurrent (Muus *et al*. 2001). Historical dispersal for *H. dactylopterus*, across the Atlantic would have to occur at the larval stage via the Northern Equatorial Current.

A strong historical influence on the genetic population structure of marine organisms in the North Atlantic has been suggested for other demersal fish species (e.g. Carr *et al*. 1995; Pogson *et al*. 1995; Muus *et al*. 2001; Bargelloni *et al*. 2003; Stockley *et al*. in press). In particular, the LGM is thought to have been responsible for the extermination or reduction of populations of marine organisms in both the North Atlantic and the North Pacific. Mismatch distribution tests and significant Tajima's *D* values are indicative of population bottlenecks followed by expansion in at least two of the sample locations, the Azores and the United States. The haplotype network and phylogenetic analyses of the NE Atlantic, Cape Verde and NW Atlantic populations suggest historical dispersal events between these populations followed by population expansions. The phylogenetic trees and minimum-spanning networks are similar to those presented for historical extinction-recolonization models of other fish taxa (e.g. sardine evolution; Grant & Bowen 1998). The low genetic diversity associated with NW Atlantic populations is

also consistent with historical long-distance or jump-dispersal events between the eastern and western Atlantic rather than stepping-stone or Gaussian modes of dispersal (reviewed by Hewitt 2003). This emphasizes the importance of occasional long-distance migration events in shaping the distribution and genetic structure of marine fish populations.

The estimated times for expansion in *H. dactylopterus* are not consistent with the end of the LGM, but are much older ranging from 0.64 to 1.2 Myr for the NW Atlantic population to 1.02 Myr for the Azores population. These times are more in agreement with data available for populations of decapod crustaceans from the North Atlantic/Mediterranean region (200 000–400 000 years; Stammatis *et al*. 2004) but even exceed these estimates markedly. The period encompassed by the expansion times for *H. dactylopterus* populations in the NW Atlantic and Azores coincide with a period of major global climatic change, known as the mid-Pleistocene revolution. This period began with a major advance in ice sheets, detected through marine oxygen isotope ($\delta^{18}O$) records around 900 000 BP. This climatic change also coincided with major shifts in ocean biogeochemistry (Becquey & Gersonde 2002). This was followed by a shift to 100 000-year glacial cycles at approximately 650 000 BP (Mudelsee & Schulz 1997). A further significant climatic change event, known as the mid-Brunhes event occurred 300 000–400 000 BP and was associated with an increase in glaciation of the Northern Hemisphere (Jansen *et al*. 1986; Becquey & Gersonde 2002). While the estimates of times of population expansion from this study may be subject to considerable margins of error, it is clear that the time of expansion for *H. dactylopterus* populations, in the North Atlantic, is much older than the LGM. The extensive time period since expansion may explain the morphological differences between populations of this species and together with phylogeographical data may even cast doubt on the conspecificity of at least some *H. dactylopterus* subspecies (see Emerson *et al*. 1999 for a comparable terrestrial example). Significantly it suggests that glaciations prior to the LGM may strongly influence the genetic structure of populations of marine species in the North Atlantic/Mediterranean. Some of these events were more severe than the LGM and may have had geographically wider and more profound impacts on marine organisms.

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