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Comparative assessment of population genetics and demographic history of two congeneric deep sea fish species living at different depths

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ABSTRACT: The mechanisms that determine population genetic structure in the marine environment are poorly understood, as are the processes that drive population dynamics. One potential factor is depth, with especially those species living in the abyss inhabiting a distinct environment with respect to habitat complexity, pressure, the distribution of resources and environmental change over time. Here we consider a deep sea fish genus, *Coryphaenoides*, which has many named species, including 8 abyssal species. We provide data in support of the existence of 2 distinct evolutionary lineages within the genus, associated with depth, and also provide detailed population genetic data for the abyssal species (*C. brevibarbis*) for comparison with available data on a congeneric species inhabiting shallower waters (*C. rupestris*). The abyssal species showed no sign of population genetic structure across a thermal oceanographic boundary (the Sub-Polar Front), for which *C. rupestris* showed differentiation. An assessment of historical demographics suggested a decline in population size for both species, but a faster and more severe decline for the abyssal species. We consider these data in the context of environmental gradients and potential evolutionary mechanisms. Relatively low effective population size estimates for both species emphasize the importance of understanding these processes for the effective conservation and management of deep sea fish stocks.

KEY WORDS: Population genetics · Demographic decline · Marine fish · Deep sea

INTRODUCTION

The relationship between habitat, population dynamics and population structure has been investigated in various systems, and may be expected to show a dependency on aspects of habitat stability, carrying capacity and physical requirements. For example, Marten et al. (2006) found that habitat type predicts population differentiation for invertebrate species (including 150 species in a meta-analysis) in lentic (standing) versus lotic (running) freshwater habitats, though the effect was small (as expected due to the large number of possible contributing factors). The authors suggest that differences in the temporal and spatial stability of the 2 habitats explain the differentiation, with species needing to adopt strategies involving strong gene flow in the less permanent lentic habitats. There are some parallels with the deep sea, with the abyss being more uniform and perhaps less productive than shallower waters, and therefore potentially promoting greater gene flow (see Etter et al. 2005, and the 'Discussion' section). However, not all studies have found a strong relationship between habitat and population structure. Blank et al. (2007) found no effect for deciduous versus mixed-coniferous habitat on population structure comparing great tits *Parus major* and blue tits *P. caeruleus*. Further, Shikano et al. (2010) studying the European nine-spine stickleback *Pungitius pungitius* considered the relative importance of habitat type (coastal versus freshwater) compared to demographic history (in the context of postglacial colonization) and found that, although habitat type had an effect, a stronger effect was related to founder events during postglacial colonization. In the present study we consider the possible influence of

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habitat in the deep sea, and focus on 2 species inhabiting different depths with respect to their population size, structure and dynamics over time.

Deep water at the Mid-Atlantic Ridge of the North Atantic is inhabited by at least 16 macrourid species in 9 genera (Bergstad et al. 2008a). The most numerically dominant genus is *Coryphaenoides*. In particular, the species *C. rupestris*, *C. brevibarbis* and *C. armatus* rank amongst the most abundant demersal fishes on the ridge or in the deep axial valleys and fracture zones.

The round-nosed grenadier *Coryphaenoides rupestris* is a deep-sea demersal fish distributed across the North Atlantic and found primarily at a depth range of 800 to 2600 m (Coad & Reist 2004). A commercial fishery for *C. rupestris* began in the NW Atlantic in 1967 (Atkinson 1995), with declining catches since the 1970s (Koslow et al. 2000), resulting in an estimated 99.6% decline in abundance over a period of 26 yr (Devine et al. 2006). In the NE Atlantic, fisheries began in the 1970s, quickly peaking before undergoing a severe decline (Koslow et al. 2000) with an estimated loss in spawning biomass to approximately 30% of pre-fishery levels (ICES 2002).

Previous work on this species has identified geographically well-separated spawning areas in Norwegian fjords and in the Skagerrak (Bergstad 1990, Bergstad & Gordon 1994), as well as evidence for genetic structuring associated with the Sub-Polar Front (White et al. 2010a). Selection at an unknown gene linked to a neutral locus was also proposed in the context of sampling depth, predominantly in the eastern portion of its distribution in the North Atlantic (White et al. 2010a).

The shortbeard grenadier *Coryphaenoides brevibarbis* is a deep-sea fish distributed in the North Atlantic, at depths of 1500 to 4700 m (Bergstad et al. 2008b), where it feeds on copepods, amphipods and mysids (Gordon & Duncan 1987). While this species is not commercially important itself, it is a common bycatch in deep-water fisheries. Comparatively little is known about this species.

A defining characteristic of the genus *Coryphaenoides* is the use of demersal habitat at depth; however, only a proportion of the species in the genus are found in very deep (abyssal) water. Among the 65 named species in the genus, 8 are found at depths >4000 m, 16 at depths >3000 m and 33 at depths of 2000 m or greater. The other 32 species have been recorded at maximum depths of from 457 to 1870 m, though there are very few data available for some of the species in this genus (see www.fishbase.org).

Our objective in the present study is to compare 2 well-sampled species within the genus, 1 found deeper and 1 found shallower than 4000 m, and consider their population genetics and historical demographics in that context. Among the 8 species found at depths >4000 m, 6 have been sequenced for the *COI* mtDNA gene. We combined all 9 species for which *COI* sequences were available with our own data for *COI* for *Coryphaenoides rupestris* and *C. brevibarbis* to consider the phylogenetic relationship between our focal species. We tested the hypothesis that the abyssal species, with different environmental influences, will show a distinct population structure in the focal area, and different historical demographics. Each of the focal study species have been subject to recent periods of intensive fishing and likely shifts in habitat characteristics over Quaternary climatic cycles; however, differences associated with life history or depth may have impacted their consequent demographic and population genetic profiles.

MATERIALS AND METHODS

***Coryphaenoides brevibarbis* sampling and genetic analysis.** *C. brevibarbis* samples were collected on either side of the sub-polar front, spanning a distance of 780 km between the most distant points (NW2 and SE2), and 270 km at the shortest point across the front (NE2 and SE1). Samples were collected by an OTSB trawl towed on a single warp (Gordon & Bergstad 1992) from the RRS 'James Cook'. The present study shares sampling sites with our earlier study of the congeneric species *C. rupestris*, where differentiation was found across this putative boundary (White et al. 2010a) over a range of 270 km. In total, 380 individuals of *C. brevibarbis* were obtained from 7 sampling sites on the Mid-Atlantic ridge (MAR; Table 1, Fig. 1). Population genetic analysis for *C. rupestris* is reported elsewhere (White et al. 2010a). Here we report sampling and genetic analysis for *C. brevibarbis*, but the sampling was conducted during the same project as that described by White et al. (2010a), and samples were collected from 2 of the same sampling sites (NE1 & SE1) and 1 site proximate to one of the *C. rupestris* sites (SE2 near Faraday; see Fig. 1), providing the opportunity for a direct comparison.

DNA was extracted from specimens using a phenol-chloroform protocol (after Hoelzel 1998). A total of 17 microsatellite DNA loci were PCR amplified; 13 of these were previously described by White et al. (2010b): *Cbre2*, *Cbre6*, *Cbre12*, *Cbre13*, *Cbre14*, *Cbre19*, *Cbre20*, *Cbre26*, *Cbre27*, *Cbre30*, *Cbre32*, *Cbre40* and *Cbre43*. Two were described by White et al. (2008): *CorRu1* and *CorRu28*. *Crup7* was described by Knutsen et al. (2008), and *CaraC7* was described by Schneider et al. (2009). Polymerase chain reactions (PCR) were carried out using Qiagen multiplex PCR kits in a total volume of 10 µl reaction^{-1}. This volume comprised 5 µl of

Table 1. Sampling information and summary statistics for each sampling site. Values in bold indicate significant departure from Hardy-Weinberg equilibrium, assessed in Arlequin using exact tests. n: sample size; H_e and H_o: mean expected and observed heterozygosity, respectively; A: mean alleles per locus; A_{rich}: mean allelic richness; F_{IS}: mean inbreeding coefficient (p-values in parentheses); SPF: Sub-Polar Front; MAR: Mid-Atlantic Ridge

Sampling site	Latitude	Longitude	Side of SPF	Side of MAR	Broad geographic cluster	Depth (m)	n	H_e	H_o	A	A_{rich}	F_{IS}
SE1	51.55° N	30.25° W	S	E	1	1910	24	0.79	0.79	11.80	11.04	0.00 (0.120)
NE1	52.59° N	34.52° W	N	E	2	1650	23	0.74	0.69	10.73	9.97	**0.07 (0.006)**
NE2	53.02° N	33.37° W	N	E	2	3030	48	0.75	0.74	14.27	10.40	**0.02 (0.000)**
NE3	53.08° N	34.46° W	N	E	2	2350	111	0.76	0.73	19.13	10.57	**0.04 (0.000)**
NW1	53.17° N	35.32° W	N	W	3	2548	55	0.76	0.76	15.93	10.77	0.00 (0.099)
NW2	53.57° N	36.13° W	N	W	3	2627	87	0.76	0.76	17.33	10.53	0.00 (0.021)
SE2	49.06° N	27.50° W	S	E	4	2739	32	0.76	0.74	13.20	10.97	0.02 (0.029)

multiplex PCR master mix, 4 µl of primer master mix (with each primer at a concentration of 0.5 µM) and 1 µl of template DNA. PCR was performed with the profile: hot-start activation at 95°C for 15 min, 35 cycles of 30 s denaturing at 94 °C, 90 s annealing at 57°C, 60 s extension at 72°C and final extension at 60°C for 10 mins. Loci were multiplexed in 2 sets of 8 and 9 loci, respectively. Forward primers were 5′ fluorescently labelled using either FAM, HEX (Eurofin), or NED (Applied Biosystems) for screening of alleles on the ABI 3730 sequencer. Alleles were scored using PEAK SCANNER V1.0 (Applied Biosystems), with GS 500 as the internal size standard. Alleles were binned using the program FLEXIBIN V2 (Amos et al. 2006).

For each sampling site, expected heterozygosities, allelic richness and F_{IS} (Wright's inbreeding coefficient) were calculated using GENEPOP (Raymond & Rousset 2005). Significance of deviation from Hardy-Weinberg Equilibrium (HWE) was evaluated using exact tests. The program MICROCHECKER V2.2.3 (Van Oosterhout et al. 2004) was used to test for allele scoring errors and the existence of null alleles. MICROCHECKER found evidence of null alleles in >1 sample for the loci *Cbre6* and *Cbre20*. Therefore, further analyses were conducted without these loci.

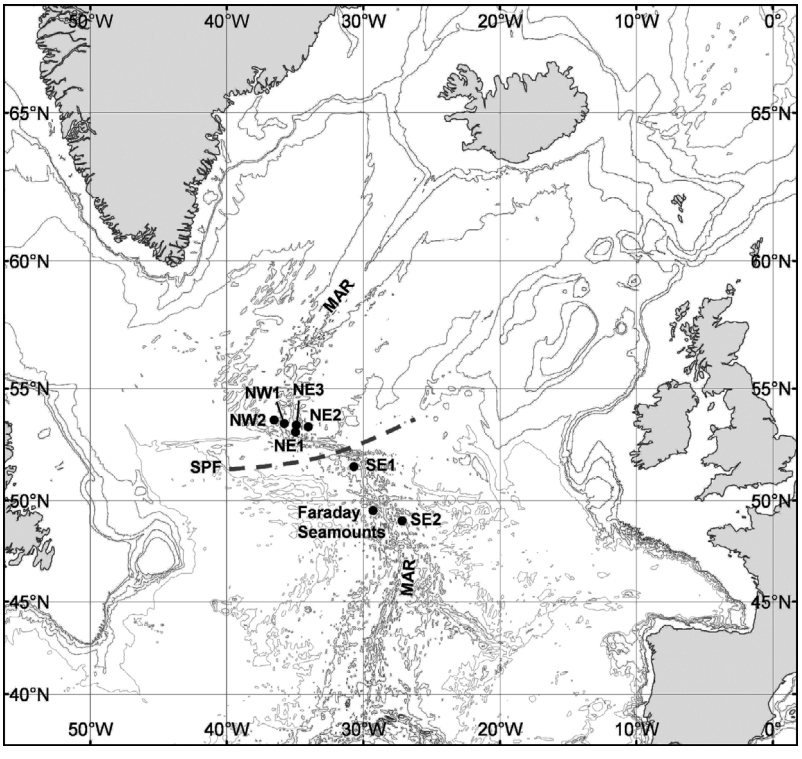

Fig. 1. Sampling sites in the North Atlantic. SPF: Sub-Polar Front; MAR: Mid-Atlantic Ridge

Including loci under selection can sometimes distort population genetic analyses (White et al. 2010a). Therefore, we used a Bayesian approach to detect loci potentially under selection, implemented in the program BAYESCAN (Foll & Gaggiotti 2008). F_{ST} (Wright's interpopulation inbreeding coefficient) values reflect contributions from locus-specific effects, such as selection, and population-specific effects, such as genetic drift and immigration rates (Balding & Nichols 1995). BAYESCAN uses a hierarchical Bayesian approach to estimate the locus and population effects on these F_{ST} values. Following the suggestion of Foll & Gaggiotti (2008), we used the 'decisive' criterion under Jeffrey's scale of evidence to identify outlier loci, in order to minimize the false-positive rate and to maximize the true-positive rate. No loci emerged as outliers under selection.

Overall and pairwise F_{ST} (Weir & Cockerham 1984) among sampling sites in the North Atlantic were calculated using ARLEQUIN 3.5 (Excoffier & Lischer 2010), and the significance of the difference between the observed F_{ST} and zero was evaluated using exact tests.

We used the program POWSIM 4.1 (Ryman & Palm 2006) to assess the statistical power of our analysis to reject the null hypothesis of genetic homogeneity for different combinations of number of samples, sample sizes, number of loci, number of alleles and allele frequencies for a hypothetical degree of true differentiation (quantified as F_{ST}).

We also conducted AMOVA in ARLEQUIN 3.5, with several different hierarchical structures relating to barriers that are thought or known to influence gene flow (White et al. 2010a). Firstly, sampling sites were grouped according to the side of the Sub-Polar Front (SPF) on which they fell (north vs. south). Secondly, they were grouped according to the side of the MAR (west vs. east). Finally, sampling sites were placed in 1 of 4 groups, based on the side of the SPF and the side of the MAR.

Phylogeny of the genus *Coryphaenoides*. Database sequences for *Coryphaenoides* species for the *COI* locus were compared with sequences derived for 3 samples each of *C. rupestris* and *C. brevibarbis*. From the database there were 2 sequences for *C. leptolepis* (EU148126-7; direct submission), 2 for *C. guentheri* (EU148124-5; direct submission), 3 for *C. carapinus* (EU148120-2; direct submission), 2 for *C. brevibarbis* (EU148118-9; direct submission), 4 for *C. armatus* (EU148115-7; FJ164497; direct submission; Steinke et al. 2009), 2 for *C. yaquinae* (GU440291-2; direct submission), 5 for *C. cinereus* (FJ164498-502; Steinke et al. 2009), 9 for *C. acrolepis* (FJ164488-96; Steinke et al. 2009) and 3 for *C. mediterraneus* (EU148128-30; direct submission). Previous phylogenies have been presented comparing some of these species (e.g. Morita 1999, Roa-Varon & Orti 2009); here we extend the comparison between abyssal and shallow-water species. Three outgroup species were included: *Macrourus holotrachys* (EU074455), *Coelorinchus marinii* (EU074385) and *Pseudonezumia flagellicauda* (EU148298). PCR primers coryco1F (ATA CCT C/T GT G/A TTT GGT GC) and coryco1R (GGT AAG G/A TTT CG G/A TCT GT) were designed from the alignment of the available sequences within the genus. PCR amplification used the Qiagen multiplex kit: 10 µl of the master mix, 2 µM of each primer and ~100 ng template DNA in a 20 µl volume. The cycle conditions were 95°C for 15 min, 94°C for 30 s, 50°C for 1 min 30 s and 72°C for 1 min, for 35 cycles, with a final elongation step at 60°C for 10 min. Sequencing was by the chain termination method on an ABI 3730, in both directions. The sequence length produced was 566 bp, and all sequences were compared at that length. The best evolution model for the trees was assessed using Modeltest 3.7 (Posada & Crandall 1998). Phylogenies were run in PAUP using the maximum parsimony (data not shown) and neighbour-joining (NJ) methods with 1000 bootstrap replicates and the Jukes-Cantor evolution model for the NJ tree (which had the highest support from Modeltest). Trees were also constructed in MR BAYES 3.1.2 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003) based on the same model to provide Bremmer support values. All estimates for the depth ranges of individual species were taken from their entries in FishBase (www.fishbase.org/).

Demographic history of *Coryphaenoides rupestris* and *C. brevibarbis*. The software DIYABC (Cornuet et al. 2008) was used to estimate the effective population sizes for *C. rupestris* and *C. brevibarbis*. For all runs of DIYABC, we defined 2 models, 1 assuming constant population size and 1 allowing a population size change at t generations in the past. For *C. rupestris*, we ran 2 trials for separate local samples. This was to guard against a Wahlund effect, even though the 2 populations chosen had shown no significant differentiation based on 11 microsatellite DNA loci (White et al. 2010a), and to provide replicate tests. The first run was based on 91 samples from SE1 (see Fig. 1) and 2 000 000 simulated data sets. The second run was based on 97 individuals from the Faraday Seamounts site and 3 000 000 simulated data sets (Fig. 1). Two runs are also presented for *C. brevibarbis*; the first includes all 380 samples and is based on 2 000 000 simulated data sets. The second was based on just 1 local sample of 111 individuals from the NE3 site (Fig. 1; the largest local sample for this species) and 2 000 000 simulated data sets. This was again to test for consistency. There was no differentiation between sites, and so no need to do all sites separately. Prior distributions were set for N_e (uniform distribution lower limit 100, upper limit 100 000), t (uniform distribution lower limit 1, upper limit 100 000), mean µ (uniform distribution lower limit 1×10^{-5}, upper limit 1×10^{-2}) and locus µ (gamma distribution lower limit 1×10^{-5}, upper limit 1×10^{-2}, shape 2.0), mean P (uniform distribution lower limit 0.1, upper limit 0.3) and locus P (gamma distribution lower limit 0.01, upper limit 0.9, shape 2.0).

RESULTS

Phylogeny of the genus *Coryphaenoides*

All trees showed the same lineage structure, and the NJ tree is shown together with support for the same nodes from the analysis in MR BAYES (Fig. 2). Two main lineages are well supported by all analyses, and these grouped according to the maximum depth at

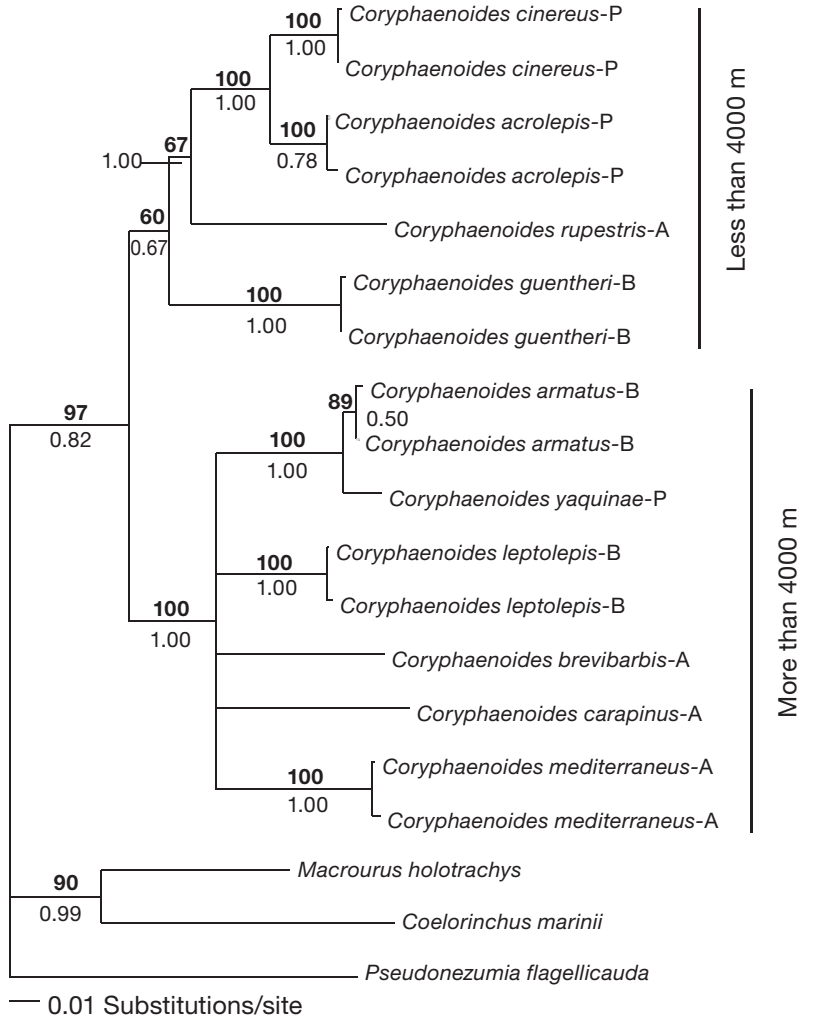

Fig. 2. Neighbour-joining tree of *COI* sequences with percent bootstrap support (in bold text above branches) and Bremmer support values from a Bayesian tree (below branches) shown; the maximum depth range is also indicated. After each *Coryphaenoides* taxon there is a code to indicate origin — A: Atlantic; P: Pacific; B: both Atlantic and Pacific

which the species within each lineage have been found (less than or more than 4000 m). Out of 45 available sequences representing 10 *Coryphaenoides* species there were 16 unique sequences. There was no apparent pattern with respect to species distribution, with species from each lineage being found in the Atlantic and in the Pacific. One of our focal study species is represented in each lineage.

Coryphaenoides brevibarbis genetic analysis

F_{ST} over all samples was 0.0002 and not significant. POWSIM suggested that with our loci and our sampling regime, we would have 99.5% power to detect significant genetic differentiation when F_{ST} = 0.002, 77.5% power to detect significant genetic differentiation when F_{ST} = 0.001 and 36% power when F_{ST} = 0.0005. F_{IS} was marginally positive and significant at several sample locations (Table 1). Significant results are from the NE region, but marginally significant results are seen at other sites and variation in F_{IS} values among sites is likely stochastic. For *Coryphaenoides brevibarbis*, ranges of expected heterozygosity and allelic richness among sampling sites were similar to the range of values seen for *C. rupestris* (H_E: 0.74 to 0.79, allelic richness: 11.34 to 12.19; White et al. 2010a).

There were no significant genetic differences between populations using F_{ST} (Table 2) or exact tests (data not shown), and none of the AMOVAs revealed significant genetic differentiation between groups (Table 3).

Demographic history of *Coryphaenoides rupestris* and *C. brevibarbis*

DIYABC suggested that both species had undergone a period of population size decline, though the support for this is strongest for *C. brevibarbis*. For the first *C. brevibarbis* run including all samples, the posterior median of current N_e was estimated to be 8280 (95% confidence limits, CL: 3100 and 21 900), for a median mutation rate of 6.02×10^{-4} (95% CL: 1.71×10^{-4} and 9.63×10^{-4}). The posterior distribution for past N_e was broad, but the 95% CL did not overlap the distribution for the current N_e estimate (past N_e: 72 000; 95% CL: 26 800 and 97 700). The distribution for *t*, the time of population change, was also broad: 4910 generations (95% CL: 807 to 39 400). The posterior distribution for mean *P* had a median of 0.30 (95% CL: 0.299 and 0.300). Support for the scenario of a change in population size was higher than for a constant population (direct estimate: 99%; logistic regression support 70% including the top 5000 simulations and 84.5% including the top 20 000 simulations). All direct estimates were based on 500 simulations.

For the single *Coryphaenoides brevibarbis* population sample at NE1, the current N_e estimate was 6750 (95% CL: 794 and 38 400), for a median mutation rate of 3.66×10^{-4} (95% CL: 5.50×10^{-5} and 5.91×10^{-3}). The posterior distributions for past N_e and *t* were

Table 2. Genetic differentiation between sampling sites. Pairwise F_{ST} values are shown below the diagonal, with p-values in parentheses. No values were significant when Bonferroni correction was applied (adjusted p = 0.0024)

Sampling site	SE1	NE1	NE2	NE3	NW1	NW2	SE2
NE1	0.003 (0.208)	–					
NE2	0.001 (0.264)	0.002 (0.237)	–				
NE3	−0.002 (0.922)	0.001 (0.469)	−0.001 (0.840)	–			
NW1	−0.001 (0.683)	0.001 (0.319)	−0.001 (0.647)	0.001 (0.276)	–		
NW2	−0.003 (0.938)	0.000 (0.566)	−0.001 (0.806)	0.000 (0.653)	0.001 (0.103)	–	
SE2	0.003 (0.094)	0.000 (0.587)	−0.002 (0.835)	0.001 (0.359)	0.005 (0.017)	0.000 (0.505)	–

broad, with past N_e having a median of 63 400 (95% CL: 14 700 and 96 700), and t having a median of 10 800 generations (95% CL: 1390 and 65 900). The posterior distribution for mean P had a median of 0.298 (95% CL: 0.245 and 0.300). The support for the changing population size scenario was 84% for the direct estimate and 78% for the logistic regression estimate for the top 5000 simulations.

For the first *Coryphaenoides rupestris* estimate based on the sample from SE1, the posterior median of current N_e was estimated to be 6280 (95% CL: 503 and 73 100), for a median mutation rate of 1.57×10^{-3} (95% CL: 8.20×10^{-5} and 9.31×10^{-3}). The posterior distributions for past N_e and t were broad, with past N_e having a median of 20 700 (95% CL: 1470 and 83 200), and t having a median of 54 700 generations (95% CL: 7300 and 95 900). The posterior distribution for mean P had a median of 0.867 (95% CL: 0.768 and 0.897). The support for the changing population size scenario was relatively weak (52% for the direct estimate and 54% for the logistic regression estimate for the top 5000 simulations). For *C. rupestris* at the second site (Faraday Seamounts), the posterior median of current N_e was estimated to be 4990 (95% CL: 406 and 69 600), for a median mutation rate of 1.88×10^{-3} (95% CL: 1.00×10^{-4} and 9.46×10^{-3}). The posterior distributions for past N_e and t were broad, with past N_e having a median of 25 200 (95% CL: 1990 and 86 400), and t having a median of 56 100 generations (95% CL: 7380 and 96 200). The posterior distribution for mean P had a median of 0.892 (95% CL: 0.847 and 0.900). The support for the changing population size scenario was stronger for this sample (55% for the direct estimate and 64% for the top 5000 simulations).

Table 3. Results of AMOVAs. Groupings are (A) north versus south of the Sub-Polar Front (SPF), (B) west and east of the Mid-Atlantic Ridge (MAR), (C) NW, NE, SW, SE and (D) broad geographic clusters given in Table 1

Source of variation	df	Sum of squares	Percentage of variation
(A) North vs. south of the SPF			
Among groups	1	4.503	−0.12
Among populations within groups	5	29.271	0.04
Among individuals within populations	753	4230.406	100.08
(B) West and east of the MAR			
Among groups	1	4.889	−0.05
Among populations within groups	5	28.886	0.03
Among individuals within populations	753	4230.406	100.02
(C) NW, NE, SW, SE			
Among groups	2	9.389	−0.11
Among populations within groups	4	24.386	0.09
Among individuals within populations	753	4230.406	100.03
(D) Broad geographic clusters			
Among groups	3	15.981	−0.06
Among populations within groups	3	17.793	0.05
Among individuals within populations	753	4230.406	100.01

DISCUSSION

Little is known about most of the species in the genus *Coryphaenoides*, but some are found primarily in water >2 km deep (including *C. brevibarbis*), which may mean that either the physical barrier of the MAR rising about 3.2 km above the surface of the ocean floor on average or its impact on deep sea currents could act as a barrier to gene flow. Our study found no genetic differentiation across the MAR for *C. brevibarbis*, and while some differentiation across the SPF (though at a low level) was seen for *C. rupestris* (White et al. 2010a), no population structure was found for *C. brevibarbis*.

White et al. (2010a) suggested that larval distribution on distinct current systems either side of the SPF may have explained the detected genetic

differentiation of *Coryphaenoides rupestris*. Surface currents in the North Atlantic create a clockwise gyre south of the SPF and a tendency for north-eastward flow north of the SPF. Deep water currents show a distinct pattern. For example, the North Atlantic Deep Water (NADW) flow runs at a depth of about 2 to 4 km north to south and into the South Atlantic. As described in the introduction, *C. brevibarbis* represents a relatively deep water species, reaching depths of up to 4700 m (range = 1500 to 4700 m), while *C. rupestris* is found mostly in shallower water (180 to 2600 m). Although the depth ranges of the 2 species overlap, if current systems dominated by flow at the surface are affecting gene flow in *C. rupestris*, it may be that *C. brevibarbis* inhabits waters too deep to be similarly affected. It is known that the eggs of *C. rupestris* are buoyant (Bergstad 1990), which could bring them into the surface current system, but little is known about the reproductive biology of *C. brevibarbis.*

As described above, a subset of species in the genus *Coryphaenoides* can live in waters deeper than 4 km (abyssal species). Morita (1999) compared 2 such species (*C. armatus* and *C. yaquinae*) with 5 shallower water species (with depth ranges that are unlikely to overlap with *C. armatus* and *C. yaquinae*) from the same genus (*C. pectoralis*, *C. acrolepis*, *C. longifilis*, *C. cinereus* and *C. nasutus*) at the 12S and *COI* mtDNA genes and showed that the 2 abyssal species grouped together. This was consistent with a study by Wilson et al. (1991) showing that *C. armatus* and another abyssal species, *C. leptolepis*, grouped together in a tree based on peptide mapping at the LDH-A_4 gene. We extend and further support this inference based on the comparison of 6 abyssal (out of 8 abyssal species in the genus) and 4 shallower water species at the *COI* locus, confirming the support for 2 lineages based on habitat depth. These analyses are preliminary (a full understanding must wait for a more inclusive set of *Coryphaenoides* species based on multiple genetic markers) and the potential relevance of overlapping depth ranges for some species is not clear, but together the current data suggest that our 2 focal species may each belong to a distinct lineage defined by the deepest range of its habitat. This is important for the present study because it suggests that aspects of habitat type with respect to depth are defining the radiations of these species, which would strengthen their utility in a comparative analysis (yet more work will be necessary to determine whether depth is the primary factor, e.g. although we found no correlation with location, relatively few species were included in the phylogeny). Relevant to this, Morita (2008) compared 2 deep water and 2 shallower water *Coryphaenoides* species and showed that myosin heavy chain proteins differ in ways that may be associated with adaptation to pressure. Some species show intraspecific neutral genetic differentiation among populations at different depths (e.g. Knutsen et al. 2009, White et al. 2010a), and for *C. rupestris* this was associated with local selection (White et al. 2010a), though the relevant functional locus could not be identified.

The precise impact of historical environmental change on ocean currents is unknown; however, it is likely that currents at depth were affected differently than those at the surface. For example, sediment core data from the Bermuda rise showed that the rapid climatic transitions at the start and end of the last interglacial period are associated with abrupt changes in deep water flow, representing changes that occurred over periods of just a few hundred years (Adkins et al. 1997). Related factors may have differentially affected species occupying habitat at different depths.

Both species showed relatively high estimated historical effective population sizes (median estimates of from 63 400 to 72 000 for *Coryphaenoides brevibarbis* and from 20 700 to 22 500 for *C. rupestris*), though the confidence limits were broad, especially for *C. rupestris*. These values are consistent with current population size estimates for some other North Atlantic species, including *Antimora rostrata* (30 000; White et al. 2010a) and *Mycteroperca microlepis* (16 500; Cushman et al. 2009). For each of the study species, however, current N_e estimates were lower (median estimates of from 6750 to 8280 for *C. brevibarbis* and from 4990 to 6280 for *C. rupestris*), more comparable to an estimated 2190 for current N_e in the orange roughy *Hoplostethys atlanticus* (White et al. 2009b). In each case, the scenario for a population decline had higher posterior support than a model for constant population size (and repeat analyses for each species gave similar results), though the support for this was stronger for *C. brevibarbis*, consistent with the indications of a more severe decline. Only *C. brevibarbis* showed non-overlapping N_e estimates for historical compared to current distributions, and this fact reinforces the likelihood that this species experienced a decline. The associated timeframes were since the last interglacial, though the median estimates were more recent for *C. brevibarbis* (4910 to 10 800 generations) than for *C. rupestris* (54 700 to 56 100 generations). Taken together the data suggest a greater decline over a shorter time span for *C. brevibarbis* (and stronger support for the signal of decline) than for *C. rupestris*, which may indicate a greater impact on the species adapted to habitat at greater depth. As a theoretical possibility, it may be consistent with a strong impact of climatic variation (e.g. in association with glacial epochs) on deep-water flow (e.g. Adkins et al. 1997), though the specific mechanism of how change in the current systems may lead to population decline is not clear. It is also not clear

what impact the potential for *C. brevibarbis* to live at depths overlapping those inhabited by *C. rupestris* may have. Both species are also taken in large numbers in deep water trawl fisheries (though not well estimated for *C. brevibarbis*, and possibly less due to its distribution in deeper water), and therefore a recent decline related to these fisheries may have also influenced our present day N_e estimates (though our estimates of the time for the start of a decline in each species are too old to reflect a fisheries impact).

The conservation and management relevance of our data depends in part on the relationship between the effective and census population size. However, this relationship is poorly understood for marine fish species. Portnoy et al. (2008) reviewed estimated N_e/N ratios for marine fish and report a range from 0.00001 to 0.5. The most extreme at the low end was based on an estimated census size of ~100 000 000 plaice *Pleuronectes platessa* compared to an N_e of from 2000 to 20 000 (Hoarau et al. 2005). At the other extreme was an estimated N_e/N ratio of about 0.5 for the sandbar shark *Carcharhinus plumbeus* (Portnoy et al. 2008). Various factors may contribute to the large variation in N_e/N ratio values, including variation in family size, unequal contributions from males and females, and non-random mating (Hedgecock 1994, Hedrick 2005). In marine fish species, it has been suggested that high variance in reproductive success among individuals ('sweepstakes breeders') in particular could greatly depress the value of N_e in relation to N (e.g. Hedrick 2005, Hoarau et al. 2005).

For our study species, each shows some indication of positive F_{IS} values, which could be consistent with a sweepstake breeding strategy, but the data on census numbers are insufficient to draw any strong conclusions about N_e/N ratios. It is known that from 7000 to 42 000 t of *Coryphaenoides rupestris* were taken each year in trawl fisheries in the North Atlantic between 1970 and 2008 (FAO 2010), and, although similar data are not available for *C. brevibarbis*, the bycatch is thought to be substantial. Therefore, given the relatively small N_e estimates, the N_e/N ratios are likely to be small. This is problematic for management since conservation should be based on N_e, while the large census numbers suggest a healthy stock.

Our data suggest that the deeper water species showed less evidence of population structure, and potentially a more extreme, faster population decline since sometime during the last glaciation. Given the investment required to acquire these data for a given species, a clearer indication of the potential role of habitat depth in determining the different patterns will likely have to wait for a meta-analysis, after data are available for a broader range of species. However, there is precedent for abyssal species showing less population genetic structure than conspecifics or related species at shallower depths. Of course, highly mobile species will in most cases require inter-specific comparisons for evidence of structure associated with genetic drift, as individuals can migrate in the water column and habitat use by depth can vary over small geographic ranges for these species (e.g. *C. rupestris*; White et al. 2010a). Comparisons among populations of the deep sea bivalve *Deminucula atacellana* showed a strong association between population differentiation and depth, with comparisons at the greatest depths (down to 3000 m) showing the lowest values of ϕ_{ST} (based on comparisons of 16S mtDNA sequences; Zardus et al. 2006). In another study, 4 bivalve species were compared, and the species found at the greatest depth (down to 4800 m) showed the shallowest radiations of 16S mtDNA sequence haplotypes and the least differentiation among populations (Etter et al. 2005). A similar effect was seen for the cosmopolitan deep sea amphipod *Eurythenes gryllus* (France & Kocher 1996). There have been various suggestions about how dispersal potential, reduced habitat complexity, or depth-related variation in mutation rates may lead abyssal species to show reduced population structure (e.g. see the discussion in Etter et al. 2005); however, little is presently known about these species or the relevant systems. The specious genus *Coryphaenoides*, which includes 8 abyssal species, should be a useful system in which to explore these questions further.

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