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Size distribution and genetic diversity of the offshore rockfish (*Pontinus kuhlii*) from three Atlantic archipelagos and seamounts

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ABSTRACT

The offshore rockfish (*Pontinus kuhlii*) is a widespread demersal fish in the temperate eastern Atlantic. This species inhabits hard bottoms at depths between 100 and 600 m and it is an important resource for the Azorean commercial fishing fleet. During several research fishing surveys in the Azores, Madeira and Cape Verde archipelagos diverse biological data on this species were collected. The degree of geographical population differentiation across the three Atlantic archipelagos was examined using two mitochondrial markers, control region (CR) and cytochrome b (cyt b). A total of 44 specimens were sequenced for each marker revealing high haplotypic diversity (CR: $H_d=0.9736$; cyt b: $H_d=0.8520$) and low nucleotide diversity (CR: $\pi=0.0171$; cyt b: $\pi=0.0059$). The sample size from the different subareas was limited but sufficient to reveal that no genetic structure was evident ($\Phi_{ST}=-0.0465$ to -0.0224), suggesting the existence of one panmictic population. Despite very different exploitation rates between archipelagos, the size structure of *P. kuhlii* was very similar and inter-annual variation was also low. Exploitation rates are probably too low to significantly affect the size structure, even in the Azores where the species is a secondary target of the commercial fishery. In the Azores region this species is more abundant on seamounts, however bigger fishes tend to occur on island slope than on seamounts.

1. Introduction

Marine populations are large, often highly fecund, and pelagic eggs and/or larvae can disperse over long distances. This is also the case for most marine fishes, and it is often expected that gene flow is high and geographical differentiation low (Hoarau and Borsa, 2000; Friess and Sedberry, 2011; Sedberry et al., 1996; Theisen et al., 2008; White et al., 2009, 2011). Several studies have however demonstrated the existence of different types of barriers or other mechanisms limiting gene flow and promoting genetic differentiation in marine species with wide ranges, e.g. retention rather than dispersal by ocean currents, bathymetry features limiting dispersal and migration, preference for fragmented habitats, isolation by distance, and dispersal mechanisms, that can condition gene flow between populations and promote genetic differentiation (Aboim et al., 2005; Carlsson et al., 2004; Domingues, et al., 2007; Riginos and Victor, 2001; Rocha-Olivares and Vetter, 1999; Stefanni and Thorley, 2003; Stockley et al., 2005). To study such features further, the Atlantic seamounts and the Macaronesian Islands (Azores, Madeira, Canaries and Cape Verde archipelagos) constitute very interesting study areas since they have many species in common and are discontinuous habitats. The archipelagos are separated by very deep ocean areas which could limit the rates of exchange of demersal fish species with restricted vertical distributions and may also be distant enough to limit mixing of pelagic life stages.

The offshore rockfish (*Pontinus kuhlii*, Scorpaenidae) is one of the widespread and commercially exploited demersal fish in the temperate eastern Atlantic archipelagos, but the scientific information needed to facilitate management advice is limited, e.g. knowledge of population structure and population effects of varying exploitation rates. The geographic range of the species extends from Portugal to São Tomé Island in the gulf of Guinea (Afonso et al., 1999), including Morocco, Mauritania and the Macaronesian archipelagos (Menezes et al., 2004). The species has also been sporadically reported in the Mediterranean Sea (Manent and Abella, 2005; Massutí and Massó, 1975; Mercader and Garcia-Rubies, 2010; Merella et al., 1998; Pais et al., 2005). *P. kuhlii* inhabits hard bottoms at depths between 100 and 600 m and it appears to have a relatively sedentary behaviour.

Most of the published studies on the species focus on age, growth and reproduction (Abellan et al., 2001; Ferreira et al., 2008; Isidro, 1990, 1996; Monteiro et al., 1991; Pereira et al., 2012). It is an oviparous species (Koya and Muñoz, 2007) with a moderate life span, reaching an age of 18 years in the case of

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males and 14 years in case of females (Abellan et al., 2001). No data regarding spawning and larvae dispersal are found in the literature. It is unknown if different archipelagos have distinct populations of offshore rockfish, and our study is the first looking to the genetic population structure of the species.

Population size structure is potentially affected by exploitation, and the rockfish is exploited at different rates in the three archipelagos. Although the main targets of the Azorean demersal fleet are towards the more valuable and abundant blackspot seabream (*Pagellus bogaraveo*) and bluemouth rockfish (*Helicolenus dactylopterus*), the offshore rockfish is abundant in Azorean waters and it is an important species landed in the harbours of the archipelago (Menezes et al., 2006).

In the Azores most of the commercial fisheries are conducted with handlines and longlines targeting demersal species mainly to 600 m depth (Menezes et al., 2006). In Madeira there is a high diversity but low abundance of demersal species, which means that the handline and longline fisheries for such species are less developed than in the Azores. The deepwater fishery targeting the black scabbardfish (*Aphanopus carbo*) with suspended longlines is the most important fishery in the region (Menezes, 2003; Stefanni and Knutsen, 2007). In the Cape Verde archipelago demersal fisheries are also less developed, and most of the fishing is restricted to the island shelf, targeting species that live shallower than 200 m (Medina et al., 2007; Menezes, 2003; World Bank, 2008). The differences in the fisheries development in each archipelago suggest that species may be subject to different fishing pressure, which makes it interesting to compare more heavily exploited Azorean population with the relative pristinely Cape Verde population. Menezes (2003) pointed out that fishes tend to be bigger in Cape Verde than in the Azores, which could reflect differences in fishing pressure (Horwood, 2010; Maunder et al., 2006). A decrease in the occurrence of large fishes was observed for the offshore rockfish at Condor seamount, probably due to the intense fishery in the area (Menezes et al., 2013).

We investigated the population structure of *P. kuhlii*, i.e. population differentiation and size structure. The sedentary behaviour of the species and its restricted vertical distribution result in a patchy distribution across islands and seamounts, and such a pattern of distribution suggest that depth can be acting as a barrier to population connectivity. To test this hypothesis, genetic analyses were performed using two types of molecular markers displaying different levels of polymorphism. In order to further evaluate spatial patterns amongst the three Atlantic archipelagos in terms of relative abundance, population structure and habitat choices, we also compared length distributions, biological data, and catch levels.

2. Material and methods

2.1. Sampling

Specimens of *P. kuhlii* were collected during several scientific fishing campaigns in the Azores, Madeira and Cape Verde archipelagos (Fig. 1), by the Azorean R/V "*Arquipélago*". All these samples were collected using a "stone-buoy" type of bottom long-line gear (Menezes, 2003; Menezes et al., 2006, 2009). A total of 4483, 347 and 346 individuals were caught during the cruises in the Azores (from 1995 to 2011), Madeira (1995–97, 2003) and Cape Verde (2000), respectively. All the fishes were measured onboard. A subsample of the specimens caught in each longline set was used for more detailed biological sampling (e.g. sex, maturation, otoliths, etc; Table 1). Sex was determined by macroscopic observation of the gonads. For molecular analyses small portions of white muscle were sampled and preserved in 95% ethanol until used for genomic DNA extractions.

2.2. DNA extraction and PCR amplification

The number of samples available for molecular screening was 44 in total: 10 from the Voador seamount (Azores), 10 from the Condor seamount (Azores), 4 from Madeira, 10 from Seine seamount (Madeira) and 10 from Cape Verde (Table 2).

Total genomic DNA was extracted from small (1–3 mg) portions of tissue by SDS proteinase K procedure following a phenol–chloroform protocol based on Sambrook et al. (1989), with slight modifications (Stefanni, 2000).

The mitochondrial Control Region (CR) was partially amplified by polymerase chain reaction (PCR) using the primers L-Pro1 and H-DL1 (Ostellari et al., 1996). The cyt b region was partially amplified by the primers CYTB-GLU-L-CP (Palumbi et al., 1991) and H15149 (Kocher et al., 1989). The thermal cycle parameters for both markers started with an initial denaturation of 2 min at 94 °C, followed by 30 cycles of denaturation of 30 s at 94 °C, annealing for 1 min 10 s at 51 °C, extension for 1 min 35 s at 72 °C, with a final extension of 7 min at 72 °C. Controls consisting of template-free mix were run in each instance to detect possible contamination. Electrophoresis of PCR products on a 1% agarose gel was performed to evaluate the integrity of the products. Finally, all amplified products were purified using ExoSAP-IT (USB Corporation) and sent for sequencing to the BMR Genomics (Padua, Italy) facility.

2.3. Sequence alignment and molecular analyses

Sequences from each PCR products were used to search in the National Centre for Biotechnology Information (NCBI) database for similarity using the BLAST tool (Altschul et al., 1990; Zhang et al., 2000). The most similar sequences for mtDNA CR was the partial sequence of *Sebastolobus macrochir* (AF161805), while for mtDNA cyt b the results obtained were 3 sequences of *P. kuhlii* from Canary Islands (AF100921–AF100923) and 1 from *P. furcirhinus* (AF030712). All these sequences were used during the alignment and *S. macrochir* as well as *P. furcirhinus* were included as outgroups for phylogenetic analyses. All sequences were aligned using SEAVIEW (Galtier et al., 1996) and CLUSTAL_X (version 1.8.3.; Thompson et al., 1997).

For phylogenetic investigation, the most appropriate nucleotide substitution model was selected from the hierarchical series of likelihood ratio tests, implemented in MEGA (version 5.0; Tamura et al., 2011), to identify the best fit of nucleotide substitution models. The Tamura 3-parameter model of nucleotide substitution with non-uniformity of evolutionary rates among sites ($G=0.65$ and $I=0.73$) had the lowest Bayesian Information Criterion (BIC, Schwarz, 1978) value for the mtDNA CR, while the Kimura 2-parameter modelled by a discrete Gamma distribution ($G=0.08$) was the one identified for the mtDNA cyt b region. Neighbour-Joining Trees were inferred using MEGA and implementing the automatic option for the parameters as suggested by Tamura et al. (2011).

Comparisons between sampling localities and relationships among haplotypes of both mitochondrial genes were analysed using the Median Joining Network (MJN) method (Bandelt et al., 1999) estimated with Network (version 4.6.1.0.; fluxus-engineering.com) based on default parameters. Three additional sequences from the Canary Islands were available for cyt b at the NCBI Database as mentioned above. These sequences were added to the cyt b network analyses for geographical comparison, but not included in any further analyses.