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Genetic structure of *Sufflogobius bibarbatus* in the Benguela upwelling ecosystem using microsatellite markers

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Abstract

The bearded goby *Sufflogobius bibarbatus* is an abundant endemic small fish species on the continental shelf of the northern Benguela. The goby habitat is characterised by generally low bottom oxygen concentrations that vary spatially and seasonally. In the present study of population structure, 13 samples of *S. bibarbatus* from inner and outer shelf areas between 19°S and 32°S were screened using ten microsatellite loci. The genetic data were analysed in relation to isolation by distance and depth. Furthermore, for the first time, this study examined genetic data in relation to bottom oxygen concentration at the sampling locations. The data show low but significant genetic heterogeneity (*G*-test; F_{ST} = 0.007, $p < .05$). There was weak but significant genetic differentiation along a latitudinal gradient across all sampling sites from 19.50°S to 32.37°S (Mantel test; $r = .464$, $p = .001$), but this disappeared when the southernmost sample was removed. On the other hand, a positive correlation of bottom oxygen concentration with pairwise F_{ST} ($r = .336$; $p = .017$) was observed among the sampling sites from the Northern Benguela shelf area. Overall, the data are complex but suggest that isolation by distance and bottom oxygen concentration may play a role in the genetic structuring of *S. bibarbatus*. The findings are discussed in relation to the species' life history features and oceanographic characteristics of the Benguela upwelling ecosystem.

KEYWORDS
Gobiidae, hypervariable markers, hypoxia tolerant, upwelling area

1 | INTRODUCTION

Despite ample opportunities for marine connectivity owing to the absence of physical barriers (Weersing & Toonen, 2009), many marine species show low but significant genetic population structure (Palumbi, 1994). Possible reasons for population differentiation in the marine environment include maintenance of discrete spawning sites and larval retention, historical vicariance events, present day biogeographic discontinuities that prevent indiscriminate adult or larval dispersal, and isolation by distance among populations (Palumbi, 1994; Planes & Fauvelot, 2002; Riginos & Nachman, 2001; Wright, Bishop, Matthee, & Heyden, 2015). Furthermore, weak currents on spawning sites may restrict the advective transport of eggs and larvae away from the spawning site (Hellberg, 2009; Palumbi, 1994) leading to geographic isolation, while oceanic currents may also create isolated circulation cells (White et al., 2009) and cline formation in species (Knutsen, Jorde, Albert, Hoelzel, & Stenseth, 2007). Other drivers of genetic structuring in marine populations include local adaptation or selection in response to environmental gradients (Ingram, 2011; Palumbi, 1994; Wang et al., 2013; Waples, 1998), habitat related

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ecological factors such as depth, bottom topography (Ingram, 2011; Prada & Hellberg, 2013; Saha et al., 2016) and bottom oxygen concentration (van der Meer et al., 2005), and also physical discontinuities in basin circulations that limit connectivity (Cadrin et al., 2010; Knutsen et al., 2007; Pampoulie et al., 2004; Stefánsson et al., 2009). On the other hand, if the currents distribute early life stages over large areas, this can lead to panmixia over broad geographic ranges preventing any genetic structuring (Naro-Maciel et al., 2011).

The Benguela current in the eastern boundary of the South Atlantic Ocean creates one of the most productive upwelling ecosystems of the world (Agenbag & Shannon, 1988; Boyer, Cole, & Bartholomae, 2000). Over the shelf areas of the Benguela, the oceanographic conditions are characterised by perennial upwelling that varies spatially and seasonally in intensity (Boyer et al., 2000; Hutchings et al., 2009). Many studies have suggested that the strong upwelling centred on the shelf off Lüderitz (27–28°S) with powerful offshore advection could represent a semi-permanent hydrographic barrier, mostly against longshore transport of pelagic life stages of some species inhabiting the region (Boyer et al., 2000; Duncombe Rae, 2005; Hutchings et al., 2002, 2009). Upwelling also creates a contemporary biogeographic and genetic discontinuity in some ecologically important fishes including cape hakes (*Merluccius paradoxus* and *M. capensis*) (Henriques et al., 2016) and *Atractoscion aequidens* (Henriques, Potts, Santos, Sauer, & Shaw, 2014) in the region. However, hydrographical studies predict that long distance transport of eggs and larvae across the upwelling system in deeper parts is still possible (Boyer et al., 2000; Lett, Veitch, Lingen, & Hutchings, 2007), and therefore population connectivity can disrupt spatial genetic structuring of species across the Benguela region (Mas-Riera, Lombarte, Gordoa, & MacPherson, 1990). Genetic discontinuity and its determinants can be followed in species having a wide distribution across the area affected by the Benguela current and the associated perennial upwelling.

The bearded goby, *Sufflogobius bibarbatus* von Bonde, 1923, is an abundant small fish species endemic to the Benguela upwelling ecosystem (Crawford, Shannon, & Pollock, 1987). Adults have a wide distribution across the Benguela region, with the highest densities mainly between 22°S and 27°S (Crawford et al., 1987; Salvanes et al., 2018; Seivåg, Salvanes, Utne-Palm, & Kjesbu, 2016). *S. bibarbatus* is well known for being uniquely adapted to severe hypoxia (Currie, Utne-Palm, & Salvanes, 2018; Salvanes, Utne-Palm, Currie, & Braithwaite, 2011; Utne-Palm et al., 2010); it occurs naturally on shelf areas with severely hypoxic conditions (Salvanes et al., 2015), and also inshore where hydrogen sulphide rich sediments and sulphide eruptions occur (Brüchert, Currie, & Peard, 2009; Brüchert et al., 2006; Currie et al., 2018). It has a benthic egg stage and pelagic larvae (Skrypzeck, Salvanes, Currie, & Kotze, 2014). The larvae are distributed over the shelf area of Namibia between 17.5–33.5°S above the 50–300 m isobaths (Olivar & Fortuño, 1991; Olivar & Shelton, 1993; O'Toole, 1978; Shelton, 1986). Highest concentrations of the young larvae are recorded between the 100–200 m isobaths, indicating that spawning areas are located on the outer shelf (Olivar, Rubiés, & Salat, 1992).

After the collapse of the sardine population *Sardinops sagax*, *S. bibarbatus* has partially replaced the sardine as the main prey item in the diet of predators, where it can represent over 50% of the prey consumed by seabirds, seals and hake *Merluccius* spp. (Crawford, Cruickshank, Shelton, & Kruger, 1985; Crawford et al., 1987; van der Lingen et al., 2006; Mecenero, Roux, Underhill, & Kirkman, 2006; Salvanes & Gibbons, 2018). As a result, it has become vital to understand their population genetic structure across the region. While the reported wide distribution of *S. bibarbatus* could allow for a panmictic population across the region, the complex circulation in the Benguela may, by contrast, generate hydrographic barriers for the exchange of early life stages, leading to genetic discontinuities as observed in the cape hakes and *A. aequidens* (Henriques et al., 2016, 2014). Moreover, this region-wide distribution provides an ideal opportunity to test whether habitat-related ecological factors such as hypoxia (Salvanes et al., 2015, 2018), depth, and isolation by distance may play a role in population structuring.

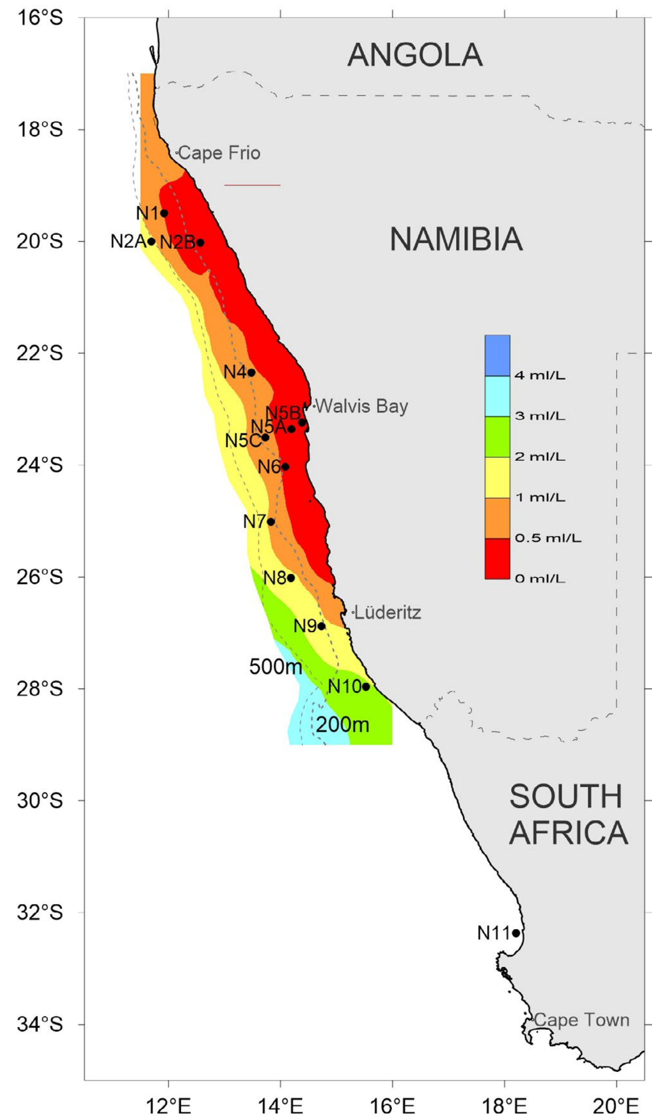

FIGURE 1 Bottom dissolved Oxygen concentration (ml DO L^{-1}) along the coast of Namibia averaged from 1990 to 2012. Extreme hypoxic areas are identified by red color code (0–0.5 ml DO L^{-1}). The sampling sites of *Sufflogobius bibarbatus* are shown with relevant location labels (N1–N11)

Little is yet known on the genetic structure of *S. bibarbatus*. Two earlier studies suggested genetic heterogeneity in *S. bibarbatus* among several sites off Namibia, using allozyme markers (Ndudane, 2004) and PCR-RFLP analysis of mitochondrial DNA (mtDNA) (Gunawickrama, Silva, Johansen, Salvanes, & Nævdal, 2010); these indicated a complex pattern of structuring that could not be explained by geographic distance or hydrodynamics alone. The present study aims to further investigate the population genetic structure of *S. bibarbatus* and to examine if there is a genetic correlation with geographic distance, depth, and ambient oxygen in the Benguela ecosystem. We obtained samples from a wider range than the aforementioned studies and have chosen ten highly variable microsatellite markers developed for *S. bibarbatus* by Gunawickrama, Westgaard, Salvanes, and Johansen (2012). Unlike allozymes and mtDNA markers, microsatellites are highly polymorphic nuclear markers; thus, they are a useful tool for the detection of any fine-scale population structure that may be present.

2 | MATERIALS AND METHODS

2.1 | Samples

Adult *Sufflogobius bibarbatus* were collected from 18 sampling sites during bottom trawl surveys onboard the RV Dr. Fridtjof Nansen during January - February in years 2003–2011 and onboard RV G.O. Sars in April 2008. At least one sampling site from each latitude was selected on the continental shelf of the northern Benguela along the Namibian coast between 19°S and 32°S. A total of 13 sample groups were considered (Figure 1, Table 1) with a depth range spanning 56–552 m. Gill tissues of individual fish (*n* = 1,115) were collected and fixed in 96% ethanol for genetic analysis.

2.2 | Bottom oxygen concentration

Environmental data used were collected from the Benguela region using Seabird CTD-O during the Namibian hake biomass surveys in January-February and cover 23 years of survey from 1990 to 2012. These were pre-screened to obtain data on dissolved oxygen concentration (ml DO L^{-1}) representing the ambient conditions of the area 5–20 m above the bottom. These data were used to obtain an overall overview of the distribution of anoxic and hypoxic water masses on the continental shelf area off Namibia in January-February. Bottom oxygen concentration was also measured at the time of sampling at each site, and these measures are used in the correlation analysis.

2.3 | Microsatellite analysis

DNA was extracted using OMEGA E-Z 96™ Tissue DNA kit (Promega). Ten selected tetranucleotide microsatellites (*A4*, *A105*, *B103*, *B104*, *C102*, *C110*, *D1*, *D102*, *D106*, and *D108*) were organized into two multiplexes as described in Gunawickrama et al. (2012) along with the PCR conditions. The PCR products were analyzed on an ABI 3130XL Genetic Analyzer, and alleles at each locus were scored by GENEMAPPER 4.0 (Applied Biosystems).

2.4 | Genetic diversity and structuring

The individuals with missing genotype data for three or more loci were removed from the data set prior to analysis. All population-wise microsatellite data were initially examined for possible genotyping errors (Kelly et al., 2011) by testing for the presence of null alleles and allele drop-outs by the software Micro-Checker 2.2.3 (van Oosterhout, Hutchinson, Wills, & Shipley, 2004). If null alleles were detected, this was adjusted for using the genotypes option of the software, and null alleles were excluded from the main analysis. Loci were also tested for the occurrence of positive and balancing selection using LOSITAN-selection workbench (Antao,Lopes, Lopes, Beja-Pereira, & Luikart, 2008; Beaumont & Nichols, 1996) using the default parameters and 50,000 simulations. Each sample group and locus were tested for deviation from Hardy-Weinberg Equilibrium (Weir & Cockerham, 1984) by exact G-test using the Markov chain method (dememorisation 10,000; 20 batches; 5,000 iterations per batch) in GENEPOP 4.2 (Rousset, 2008). The False Discovery Rate (FDR) correction of Benjamini and Hochberg (1995) was applied to p-values to control for Type I errors. Allelic variation, observed and expected average heterozygosity, and fixation (F_{IS}) indices were obtained using GENEPOP. Locus-wise F_{ST} and pairwise population differentiation (F_{ST}) were tested using exact G-test and Analysis of Molecular Variance (AMOVA) with 999 permutations in GenAlEx 6.5 (Peakall & Smouse, 2012). P-values from the G-test for pairwise F_{ST} and AMOVA were subjected to an adjusted FDR correction for multiple comparisons (Benjamini & Yekutieli, 2001). Spatial genetic structuring was investigated using two methods. First, a Discriminant Analysis of Principal Components (DAPC, Jombart, Devillard, & Balloux, 2010) was used as implemented in Adegenet (v. 2.1.1, Jombart, 2008) in R (v 3.6.1), with the optimal number of clusters evaluated using the Bayesian Information Criterion (BIC). Second, a Bayesian clustering method using the program STRUCTURE (v. 2.3.4, Pritchard, Stephens, & Donnelly, 2000) was performed using an admixture model with correlated allele frequencies, with a burn-in length of 300,000 and 500,000 MCMC runs for five iterations at each prior sub-population number *K* (ranging from *K* = 1 to K = 10). The most likely K-value was estimated using the Evanno, Regnaut, and Goudet (2005) method, as implemented in STRUCTURE Harvester (Earl & vonHoldt, 2012), and the results were summarized using CLUMPAK (Kopelman, Mayzel, Jakobsson, Rosenberg, & Mayrose, 2015). The mean number of migrants was estimated using private alleles (GENEPOP 4.2), and locus wise estimates of migrants were calculated by using the island model equation implemented in GenAlEx 6.5 (cited in Barton & Slatkin, 1986). The hypothesis of isolation by distance (Slatkin, 1993) was tested

TABLE 1 Collection localities of *Sufflogobius bibarbatus* samples along the West African coast and relevant information on sampling year, depth, and average bottom oxygen concentration (n = sample size genotyped for ten microsatellite loci; total 1,082)

Location code	Latitude/Longitude	*n*	Average depth (m)[a]	Year	Sample group	Average bottom oxygen (ml/L)
N1	19.50°S, 11.92°E	84	197	2010–2011	N1*	0.447
N2-A	20.00°S, 11.69°E	62	552	2011	N2A	1.086
N2-B	20.02°S, 12.57°E	59	143	2003	N2B	0.290
N4	22.34°S, 13.48°E	86	118	2003–2010	N4*	0.550
N5-A	23.36°S, 14.20°E	95	120	2008	N5A	0.370
N5-B	23.24°S, 14.38°E	89	56	2008	N5B	0.264
N5-C	23.51°S, 13.73°E	92	176	2008	N5C	0.628
N6	24.03°S, 14.09°E	94	170	2011	N6	0.441
N7	25.01°S, 13.83°E	65	238	2011	N7	0.890
N8	26.02°S, 14.22°E	81	265	2010–2011	N8*	1.230
N9	26.88°S, 14.73°E	89	193	2003–2011	N9*	1.170
N10	27.96°S, 15.53°E	91	82	2011	N10	2.065
N11	32.37°S, 18.20°E	95	70	2005	N11	0.748

Note: N1* includes 2 samples from nearby locations 19.20°S, 12.17°E (n = 21) and 19.79°S, 12.22°E (n = 28); N4* includes a sample from 22.34°S, 14.16°E (n = 48); N8* includes a sample from 26.02°S, 14.19°E (n = 37); N9* includes a sample from 26.68°S, 14.85°E (n = 48)

[a]Average depth of sampling sites are given for sample groups denoted by an asterisk.

using Mantel test (Manly, 1994) and matrices of pairwise geographic distance (km, linear distance between location coordinates of sampling sites) and pairwise F_{ST} in GenAlEx 6.5. Mantel tests were further used to compare pairwise F_{ST} with differences in oxygen, as well as with differences in bottom depth. All Mantel tests were repeated with the exclusion of the southernmost sampling site N11, and with the exclusion of the deepest sampling site N2A, to observe their influence on the results.

3 | RESULTS

3.1 | Bottom oxygen concentration in the Benguela system

During January-February, the average conditions for the period 1990–2012 show anoxic/hypoxic bottom waters with dissolved oxygen < 1.0 ml DO L^{-1} distributed over most of the continental shelf area off northern Namibia (19°S to 25°S; Figure 1). Hypoxic bottom oxygen prevailed for 9 out of 13 sample groups of *S. bibarbatus* including N11 at 32°S (Table 1).

3.2 | Genetic differentiation

A final genetic data set (n = 1,082) from 13 sample groups was produced from the genetic analysis (Table 2). Among ten microsatellite loci analysed, data did not show evidence for large allele dropouts, stuttering, or scoring errors. Selection at any of the loci was not evident based on LOSITAN (Figure S1). In addition, only at three instances a deviation from Hardy-Weinberg Equilibrium after False Discovery Rate (FDR) correction was found, each involving a different sample group and a different locus (Table 2). As there was no consistent pattern of disequilibrium among loci, all 10 loci were used in the analyses. Allele diversity (*Na*) across loci varied greatly, where the highest number of alleles (ranged 32–41) was observed at locus D106. There were only two loci that had lower than 10 alleles per locus; locus A105 (*Na* range: 5–9) and C110 (*Na* range: 4–10; Table 2). Average *Na* for sample groups ranged from 20.6 to 24.9 (Table 2), and was quite similar across groups, though N2B (20.6) and N2A (22.0) appeared to have the lowest *Na*. Expected heterozygosity (H_e) values were high with across-loci mean for the sample groups ranging from 0.825 to 0.844 (Table 2). However, for the Locus C110, there was a remarkably low H_e across all groups (range 0.263–0.390), coupled with its low allelic diversity.

The number of private alleles varied between 1 and 6 across sample groups (Table 2), and were mostly found at four loci (B103, B104, D1, and D106). The mean frequency of private alleles for a mean sample size of 79.1 was 0.007, and the respective number of migrants after correction for size was 23.2 by private allele method. The number of migrants (N_m) that was estimated locus-wise across all sample groups ranged 18.6–48.8 with a mean of 37.2 without correcting for sample size (Table 3). Since the N11 group (32°S) was from southern Benguela, a separate analysis was done after excluding N11, and locus-wise N_m then ranged between 25.2 and 48.3 with a mean of 37.6 (±2.4, *SE*).

Overall, there was significant population structuring across the study area, indicated by the G-test (p < .05), with a low F_{ST} value of 0.007 (±0.001, *SE*). Pairwise F_{ST} ranged from 0 to 0.009. Pairwise group analyses with the G-test revealed significant differences in seven pairs of sample groups (Table 4). The AMOVA revealed more pairwise differences with 51 (Table 5), representing 9% and 65% of all

TABLE 2 Genetic variability at ten microsatellite loci in 13 putative populations of *Sufflogobius bibarbatus* in the Benguela system with results of the test for deviation from Hardy-Weinberg Equilibrium

sampling sites		A4	A105	B103	B104	C102	C110	D1	D102	D106	D108	Total across loci*
N1	N	72	66	84	84	82	84	84	81	79	65	
	N_a	16	9	31	18	21	8	31	35	37	25	23.1
	N_p	0	0	3	0	0	0	0	0	1	1	5
	H_o	0.903	0.606	0.929	0.798	0.915	0.381	0.929	0.951	0.937	0.892	0.824
	H_e	0.870	0.629	0.935	0.854	0.922	0.372	0.945	0.956	0.960	0.943	0.839
	F	−0.038	0.037	0.007	0.066	0.008	−0.025	0.017	0.006	0.024	0.054	0.016
N2A	N	62	51	62	62	60	62	62	50	61	52	
	N_a	17	6	29	17	24	7	30	26	38	26	22.0
	N_p	0	0	0	1	1	0	1	0	1	0	4
	H_o	0.823	0.569	0.887	0.823	0.883	0.339	0.984	0.900	0.967	0.865	0.804
	H_e	0.862	0.652	0.929	0.829	0.926	0.378	0.947	0.947	0.956	0.932	0.836
	F	0.045	0.128	0.045	0.007	0.046	0.104	−0.038	0.050	−0.012	0.071	0.045
N2B	N	59	49	55	58	59	59	59	57	59	59	
	N_a	16	6	23	14	25	4	24	31	34	29	20.6
	N_p	0	0	0	1	0	0	1	0	0	0	2
	H_o	0.864	0.592	0.855	0.862	0.966	0.237	0.983	0.930	0.966	0.915	0.817
	H_e	0.869	0.635	0.919	0.870	0.939	0.263	0.927	0.954	0.962	0.952	0.829
	F	0.005	0.067	0.070	0.009	−0.029	0.099	−0.060	0.025	−0.004	0.038	0.022
N4	N	85	67	80	86	85	86	86	77	75	76	
	N_a	17	8	31	21	27	9	29	31	32	24	22.9
	N_p	0	0	3	0	0	1	0	0	0	1	5
	H_o	0.859	0.582	0.913	0.872	0.894	0.314	0.895	0.961	0.933	0.895	0.812
	H_e	0.865	0.683	0.936	0.861	0.917	0.293	0.944	0.953	0.954	0.940	0.835
	F	0.007	0.147	0.026	−0.013	0.025	−0.073	0.052	−0.008	0.021	0.048	0.023
N5A	N	95	70	93	93	95	95	95	84	95	82	
	N_a	18	8	29	19	25	7	30	38	39	28	24.1
	N_p	0	0	0	0	0	0	1	2	1	0	4
	H_o	0.863	0.543	0.892	0.806	0.916	0.358	0.937	0.917	0.947	0.915	0.809
	H_e	0.882	0.614	0.927	0.859	0.930	0.390	0.948	0.959	0.960	0.949	0.842
	F	0.022	0.116	0.037	0.061	0.015	0.083	0.012	0.044	0.013	0.036	0.044

(Continues)

TABLE 2 (Continued)

sampling sites		A4	A105	B103	B104	C102	C110	D1	D102	D106	D108	Total across loci*
N5B	N	86	66	85	87	87	89	89	71	78	85	
	N_a	16	7	29	21	27	7	34	29	36	30	23.6
	N_p	1	0	0	3	0	0	0	0	0	0	4
	H_o	0.849	0.545	0.906	0.816	0.920	0.360	0.921	0.915	0.962	0.906	0.810
	H_e	0.893	0.645	0.933	0.867	0.936	0.313	0.950	0.947	0.956	0.948	0.839
	F	0.049	0.155	0.029	**0.059***	0.018	−0.148	0.030	0.033	−0.006	0.044	0.026
N5C	N	92	74	89	88	74	92	92	92	90	77	
	N_a	17	8	28	16	27	7	31	34	38	29	23.5
	N_p	0	0	0	0	0	0	2	0	0	0	2
	H_o	0.837	0.554	0.899	0.795	0.878	0.359	0.924	0.913	0.944	0.909	0.801
	H_e	0.890	0.649	0.938	0.857	0.927	0.355	0.947	0.950	0.959	0.952	0.843
	F	**0.060***	0.146	0.042	0.072	0.053	−0.011	0.025	0.039	0.015	0.045	0.049
N6	N	94	70	92	94	93	94	93	93	92	86	
	N_a	19	8	32	16	26	10	34	34	38	29	24.6
	N_p	0	1	0	1	0	1	1	0	1	1	6
	H_o	0.830	0.571	0.935	0.840	0.925	0.426	0.935	0.935	0.957	0.907	0.826
	H_e	0.886	0.658	0.932	0.838	0.933	0.377	0.946	0.952	0.965	0.951	0.844
	F	0.063	0.131	−0.003	−0.003	0.008	−0.129	0.011	0.017	0.009	0.046	0.015
N7	N	61	65	64	63	61	65	65	65	62	52	
	N_a	17	5	26	17	25	8	27	33	36	27	22.1
	N_p	0	0	0	0	0	1	0	0	1	0	2
	H_o	0.787	0.508	0.891	0.778	0.902	0.431	0.954	0.954	0.935	0.885	0.802
	H_e	0.856	0.602	0.935	0.852	0.944	0.385	0.944	0.955	0.955	0.945	0.837
	F	0.081	0.157	0.048	0.087	0.045	−0.118	−0.010	0.001	0.020	0.064	0.037
N8	N	81	77	81	77	81	81	81	78	77	72	
	N_a	18	6	32	15	26	7	32	35	38	30	23.9
	N_p	0	0	0	0	0	0	2	2	0	0	4
	H_o	0.877	0.481	0.914	0.779	0.901	0.309	0.926	0.910	0.948	0.931	0.797
	H_e	0.888	0.560	0.929	0.830	0.934	0.302	0.952	0.947	0.954	0.952	0.825
	F	0.013	0.143	0.017	0.061	0.035	−0.021	0.027	0.039	0.007	0.022	0.034

(Continues)

TABLE 2 (Continued)

sampling sites		A4	A105	B103	B104	C102	C110	D1	D102	D106	D108	Total across loci*
N9	N	89	73	85	84	86	89	89	86	88	82	
	N_a	18	5	28	17	27	6	31	31	37	29	22.9
	N_p	1	0	0	0	1	0	0	0	1	0	3
	H_o	0.899	0.534	0.918	0.762	0.895	0.382	0.944	0.919	0.932	0.902	0.809
	H_e	0.878	0.629	0.930	0.834	0.925	0.354	0.951	0.953	0.958	0.947	0.836
	F	−0.024	0.150	0.013	0.087	0.032	−0.080	0.008	0.036	**0.027***	0.047	0.030
N10	N	91	91	91	91	86	91	91	91	90	82	
	N_a	17	8	29	20	27	9	31	37	41	30	24.9
	N_p	0	0	2	1	1	0	0	1	1	0	6
	H_o	0.879	0.549	0.890	0.857	0.907	0.352	0.934	0.945	0.922	0.902	0.814
	H_e	0.883	0.635	0.924	0.870	0.932	0.377	0.943	0.956	0.961	0.946	0.843
	F	0.005	0.134	0.037	0.015	0.026	0.067	0.010	0.012	0.041	0.046	0.039
N11	N	95	91	89	87	93	95	93	88	89	92	
	N_a	18	7	28	19	25	8	31	33	41	28	23.8
	N_p	0	0	0	0	1	0	0	0	0	0	1
	H_o	0.842	0.484	0.899	0.793	0.882	0.337	0.925	0.932	0.955	0.913	0.796
	H_e	0.880	0.534	0.933	0.838	0.922	0.382	0.949	0.958	0.955	0.948	0.830
	F	0.043	0.094	0.037	0.054	0.044	0.118	0.025	0.027	0.000	0.037	0.048

Note: Number of alleles (N_a), Number of private alleles (N_p), Observed heterozygosity (H_o), Expected heterozygosity (H_e) and fixation index (F) are calculated in GenAlEx.
F values in bold with an asterisk (*)are out of H-W equilibrium after false discovery rate correction.

TABLE 3 Locus-wise summary of the genetic indices of differentiation across 13 sample groups of *Sufflogobius bibarbatus* (significant values of F_{ST} are indicated by asterisk, $p < .05$, P = P (rand>= data) based on 9,999 permutations)

Locus	N_a (mean)[a]	F_{IS}[b]	F_{IT}[c]	F_{ST}[d]	N_m[e]
A4	17.2	0.026	0.031	0.006	44.5
A105	7.0	0.124	0.136	0.013*	18.6
B103	28.8	0.031	0.038	0.007	36.0
B104	17.7	0.043	0.048	0.005	46.3
C102	25.5	0.025	0.032	0.007*	34.5
C110	7.5	−0.009	−0.004	0.005	48.8
D1	30.4	0.008	0.014	0.006	42.0
D102	32.8	0.025	0.031	0.007	37.9
D106	37.3	0.012	0.019	0.007*	34.8
D108	28.0	0.046	0.054	0.009*	28.7
All loci mean	23.2	0.033	0.040	0.007*	37.2
±*SE*	±0.9	±0.011	±0.012	±0.001	±2.8

[a]No of alleles.
[b]Fis, Allele diversity = (Mean H_e − Mean H_o)/Mean H_e.
[c]Fit = (H_t − Mean H_o)/H_t.
[d]Fst = (H_t − Mean H_e)/H_t.
[e]N_m number of migrants = $[(1/F_{st}) - 1]/4$.

TABLE 4 Pairwise genetic differentiation between sites for *Sufflogobius bibarbatus* from the Benguela, shown by *p*-values from a *G*-test

	N1	N2-A	N2-B	N4	N5-A	N5-B	N5-C	N6	N7	N8	N9	N10	N11
N1	–												
N2-A	*.0126*	–											
N2-B	.1104	*.0019*	–										
N4	***.0001***	***.0002***	*.0023*	–									
N5-A	*.0052*	.1824	*.0100*	*.0022*	–								
N5-B	.3071	.0875	.2532	.4393	.1036	–							
N5-C	.3474	.4116	*.0095*	*.0428*	.1588	*.0154*	–						
N6	.1219	.5306	.0555	*.0168*	*.0168*	.7625	.1347	–					
N7	.4790	.6582	.0678	*.0019*	.4012	.4422	.9046	.9095	–				
N8	.3511	.1537	*.0175*	*.0036*	.6908	.4377	.0881	.7240	.9849	–			
N9	.0793	*.0481*	*.0012*	***.0001***	*.0411*	.2061	*.0275*	*.0390*	.2425	*.0494*	–		
N10	.2784	.0889	.0452	.2820	*.0358*	.9241	.4970	.7954	.7671	.5405	.2089	–	
N11	*.0131*	***.0001***	***.0002***	***.0004***	***.0009***	.0699	*.0492*	*.0120*	.0656	.2528	.0586	.6125	–

Note: Significant *p*-values are shown in italics, and those remaining significant after Benjamini-Yekutieli false discovery rate (FDR) correction are shown in bold.

pairwise comparisons in both tests respectively, after false discovery rate (FDR) correction. Four of the significant pairwise comparisons in the G-test involved N11. In the AMOVA, significant differences were found in comparisons between groups north and south of 25°S, with N7, N8, N10, and N11 showing significant differentiation from the groups further north (Table 5). Significant differences were also present between some neighboring groups, such as N2A and N2B (Table 5). There was no change in overall G-test results nor in locus-wise F_{ST} ($F_{ST} = 0.007$, $p < .001$) when the extremely remote group N11 or the deepest group N2A were removed from the analysis. The output of probabilities from STRUCTURE Harvester indicated a value of $K = 1$ or $K = 2$ as the most likely number of clusters from the data (Figure S2). The DAPC also showed some possible minor level of clustering (Figure 2) depicting differentiation between groups N2A and N2B, and a slight separation of N11. The lack of strong structuring overall suggests that the population structure is too discrete and weak to be detectable by STRUCTURE, as is indicated by the very low yet significant F_{ST} values obtained in the G-test and AMOVA.

There was a positive correlation between the genetic distance (pairwise F_{ST}) and the geographic distance when all sample groups were included (Mantel test, $r = .464$, $p = .001$, Figure 3a), suggesting that genetic differences increase with distance between groups. This disappeared when the analysis was run excluding group N11 from 32°S (Mantel test, $r = .190$, $p = .090$). For all sampling sites

TABLE 5 Pairwise genetic differentiation between sites for *Sufflogobius bibarbatus* from the Benguela, shown by F_{ST} (below diagonal) and *p*-values (above diagonal) from an analysis of molecular variance (AMOVA)

	N1	N2-A	N2-B	N4	N5-A	N5-B	N5-C	N6	N7	N8	N9	N10	N11
N1	–	*.007*	***.001***	***.006***	***.005***	***.001***	***.005***	*.010*	***.001***	***.003***	*.008*	***.001***	***.001***
N2-A	.003	–	***.004***	***.005***	.154	*.016*	***.005***	*.013*	***.001***	***.001***	*.014*	***.001***	***.001***
N2-B	.005	.004	–	*.014*	*.019*	*.010*	***.003***	.139	***.004***	***.001***	.102	***.002***	***.001***
N4	.003	.003	.003	–	***.004***	.230	***.001***	*.041*	***.001***	***.001***	***.003***	***.001***	***.001***
N5-A	.003	.001	.003	.003	–	.241	***.001***	.190	***.001***	***.001***	.324	***.001***	***.001***
N5-B	.004	.003	.003	.001	.001	–	***.001***	*.032*	***.001***	***.001***	***.001***	***.001***	***.001***
N5-C	.003	.003	.004	.005	.003	.006	–	*.009*	***.005***	***.001***	*.016*	***.001***	***.001***
N6	.002	.002	.001	.002	.001	.002	.002	–	***.001***	***.001***	.482	***.001***	***.001***
N7	.004	.005	.005	.008	.006	.009	.003	.006	–	.334	***.004***	.280	***.001***
N8	.004	.006	.004	.008	.004	.006	.006	.005	.000	–	***.005***	.082	.141
N9	.002	.003	.001	.003	.000	.003	.002	.000	.003	.003	–	*.012*	***.003***
N10	.006	.006	.004	.007	.006	.008	.004	.005	.000	.001	.002	–	*.013*
N11	.008	.008	.006	.009	.006	.007	.008	.008	.004	.001	.003	.002	–

Note: Significant *p*-values are shown in italics, and those remaining significant after Benjamini-Yekutieli false discovery rate (FDR) correction are shown in bold.

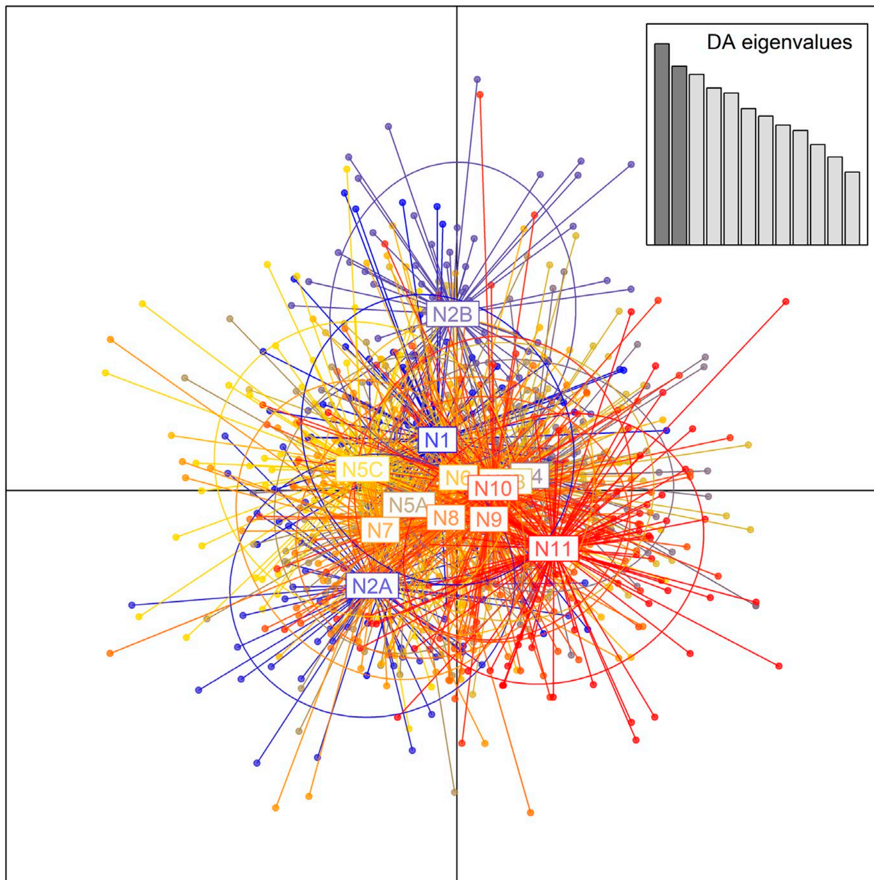

FIGURE 2 Population differentiation among 13 sampling groups of bearded goby *Sufflogobius bibarbatus* in the Benguela region using 10 microsatellite markers, depicted using Discriminant analysis of principal components (DAPC). Individuals from different sample sites are represented by coloured dots, and inertia ellipses are centred on the mean of each population

included, there was no significant correlation with bottom oxygen concentration (r = .150, p = .160, Figure 3b) or bottom depth (r = .084, p = .211, Figure 3c). However, the correlation with bottom oxygen concentration turned significant when the analysis was run excluding N11 (r = .336, p = .017). The exclusion of N2A had no influence on the results.

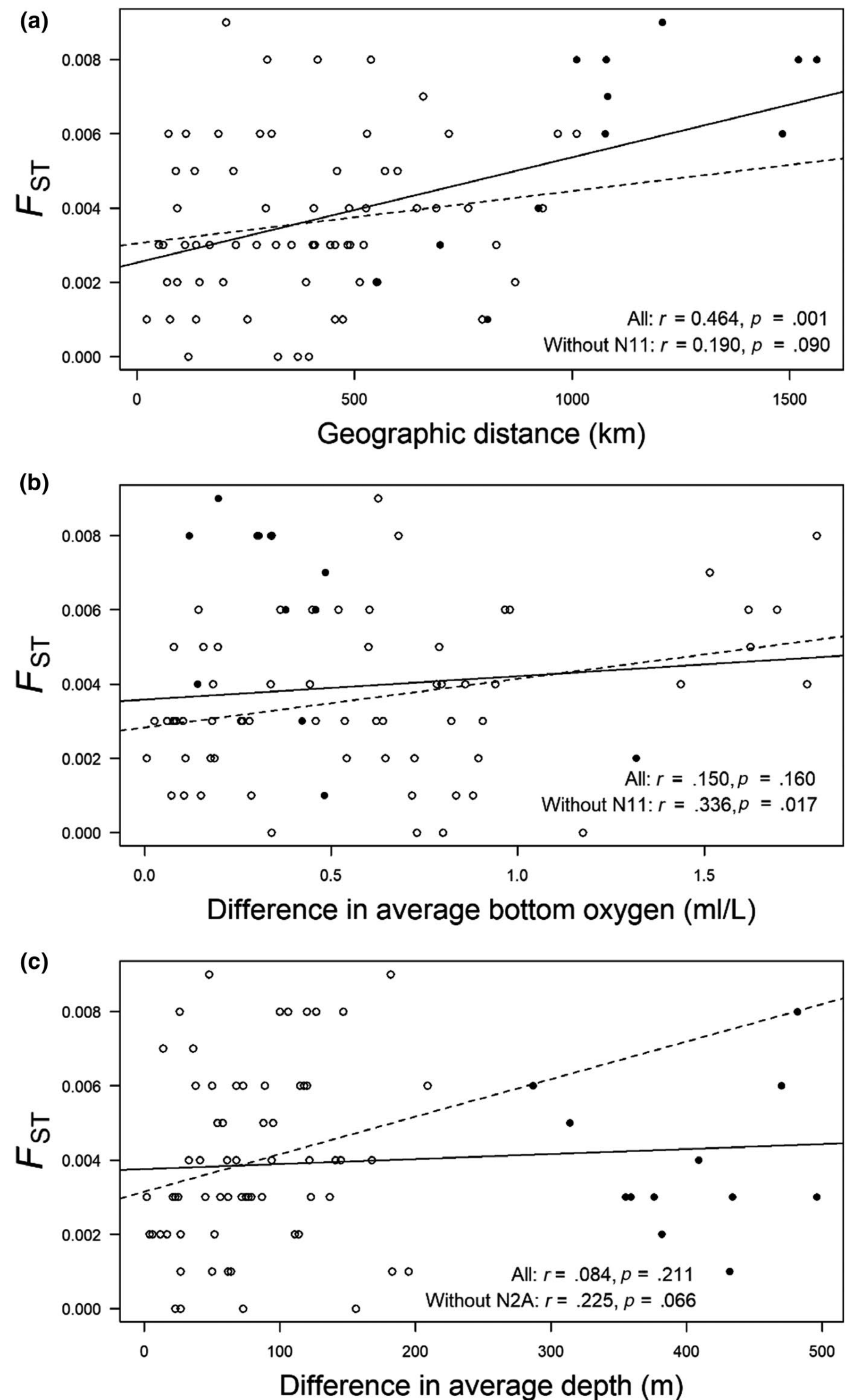

FIGURE 3 Relationship of pairwise genetic distance (F_{ST}) with linear geographic distance (a), with pairwise differences in bottom oxygen concentration (ml/L) (b), and with pairwise depth differences (c) for *Sufflogobius bibarbatus* among 13 sampling sites in Benguela. Correlation factors (*r*) and *p*-values from Mantel tests involving all pairwise comparisons are shown, and regressions are shown by the solid line. Filled circles show comparisons with site N11 (figures a and b) and site N2A (figure c), and dashed lines show regressions with these sites excluded

4 | DISCUSSION

The present study demonstrates that populations of the endemic bearded goby *Sufflogobius bibarbatus* are not panmictic in the Benguela upwelling ecosystem. The results corroborate earlier findings based on allozymes (Ndudane, 2004) and mtDNA markers (Gunawickrama et al., 2010) in suggesting that the population structure of *S. bibarbatus* in this region is weak, complex and without a clear geographic pattern. The results indicate however, an influence of isolation by distance and hydrographic conditions in restricting gene flow, and a possible influence of ambient oxygen levels that warrants further investigation.

Isolation by distance explained the genetic structure only when the most southern sampling site from south of the strong Lüderitz upwelling (N11: 32°S) was included in the analysis. This suggests that there are some mechanisms that limit the mixing of *S. bibarbatus*

from the southern Benguela with those from the north. One explanation could be that the strong upwelling off Lüderitz, characterized by strong Ekman-transport offshore (Agenbag & Shannon, 1988; Hutchings et al., 2009; Nelson & Hutchings, 1983) limits the mixing of early life stages of *S. bibarbatus* from the south with early life stages north of the strong and permanent upwelling cell (Olivar et al., 1992). Previous genetic studies on other fish species including hake (Henriques et al., 2016; Henriques, Potts, Sauer, & Shaw, 2012), and other fauna (Zardi, McQuaid, Teske, & Barker, 2007) report evidence in support of an oceanographic barrier around the Lüderitz upwelling cell generating a genetic discontinuity in populations across this upwelling cell. Our findings support this, however, our analysis does not reveal whether it is the upwelling cell itself that represents a barrier or the distance that prevents mixing of mature individuals and/or early planktonic larval stages. As the larval stages of *S. bibarbatus* are planktonic (Olivar et al., 1992; O'Toole, 1978), the differentiation within the northern area may be limited due to possible mixing of early life stages via advection in the northward running Benguela Current (Mann & Lazier, 1991). Our data show that there was no change in the effective number of migrants (cf. N_m 37.6) or genetic differentiation (cf. F_{ST} 0.007) when the southernmost remote sampling site (N11) was excluded. This indicates that regardless of the underlying mechanisms, the genetic structuring of the goby can be ascertained to exist within the Benguela ecosystem.

The data show a positive correlation between genetic distance and differences in bottom oxygen concentration among the sampling sites in the northern Benguela (Figure 3b), and therefore the influence of ambient oxygen levels on genetic structuring of *S. bibarbatus* could be important. Only four sampling sites (N2A, N8, N9 and N10) were at bottom oxygen concentration above 1.0 ml DO L^{-1}, of which N10 was from well-oxygenated waters, and these were generally located on the outer shelf area. However, our results do not show genetic differentiation between that group of sampling sites and the hypoxic sites. Low bottom oxygen concentration is typical for the Benguela upwelling ecosystem, but the extent and distribution of hypoxic water masses vary seasonally and over shelf areas (Boyd, Salat, & Masó, 1987; Rouault, 2012). The average bottom oxygen concentration over 23 years has confirmed that the northern Benguela shelf waters experience low bottom oxygen well below 1.0 ml DO L^{-1} and therefore, long term hypoxia is essentially a habitat characteristic for most of the coastal sites where the goby lives. Previous studies also report a stable perennial occurrence of low bottom oxygen concentration along most of the Namibian shelf area and that typically higher bottom oxygen occurs in the south; however, additional low oxygen sites and seeps also occur in the south (Bartholomae & van der Plas, 2007; Emeis et al., 2004).

Loss of oxygen in marine habitats may compress habitable areas for marine organisms (Gallo & Levin, 2016). This can lead to divergent selection of tolerant genotypes that have better fitness to the prevailing extremes in the habitat (Sultan & Spencer, 2002; Tobler et al., 2008), and in favor of physiological tolerance to hypoxia (Chapman, Galis, & Shinn, 2000; Martinez, Chapman, & Rees, 2009), and local adaptation. The remarkable hypoxia and anoxia tolerance of *S. bibarbatus* (Salvanes et al., 2015; Utne-Palm et al., 2010), and also ability to tolerate sulphide shocks (Currie et al., 2018; Salvanes et al., 2011), indicate that low bottom oxygen concentration does not limit the distribution and reproduction of *S. bibarbatus* (Salvanes et al., 2018; Seivåg et al., 2016). Some genetic studies of other fish species have shown that long term adaptive response to hypoxia is reflected in gene expression (van der Meer et al., 2005), which in turn can be expressed as physiological and biochemical adaptation (Chapman et al., 2000; Martinez et al., 2009). The present findings are unable to confirm the genetic basis for adaptation to hypoxia in *S. bibarbatus*, but the study system provides a unique opportunity to investigate the matter using targeted genetic approaches.

The present study based on hypervariable microsatellites verifies the findings of weak population structure of *S. bibarbatus* found in the two previous studies (Gunawickrama et al., 2010; Ndudane, 2004). Although clustering-based analyses do not show any clear structure, the *G*-test and F_{ST}-based AMOVA analysis show there is some discrete level of structuring going on, but this is very weak. The DAPC shows some possible minor level of structuring too. Whereas neither mtDNA (Gunawickrama et al., 2010) nor allozymes (Ndudane, 2004) reported evidence of isolation by distance, present results, which include samples from a wider north-south range including one group (N11) much further south of the Lüderitz upwelling zone do indicate isolation by distance as a driver of population heterogeneity in *S. bibarbatus*. Furthermore, we add preliminary evidence of a possible contribution of hypoxia in structuring these populations. We did not observe any isolation by depth, but this finding may be expected for *S. bibarbatus* given the life history features of the species. On the other hand, neutral loci that we used may not have adequately captured genetic divergence due to local adaptation. Other studies have reported that depth and bottom topography can play important roles in seascape genetics in respect to adaptive divergence using microsatellites (Saha et al., 2016).

The neutral microsatellites are known to have a high-resolution power to detect fine-scale genetic structuring in marine species, but the polymorphic nature of the marker often results in very low F_{ST} values (Waples, 1998). This Index of genetic differentiation in *S. bibarbatus* was apparently lower using microsatellite markers (F_{ST} = 0.007) than when using PCR-RFLP of mtDNA (Gunawickrama et al., 2010: F_{ST} = 0.137). Although the reasons behind such discordant values are not straightforward, MtDNA often tends to show higher values of genetic differentiation compared to microsatellites in marine species (e.g. Gariboldi et al., 2016; Sailant, Renshaw, Cummings, & Gold, 2012; Seyoum et al., 2018). The estimates of genetic differentiation reported on *S. bibarbatus* by Gunawickrama et al. (2010) may have been inflated by the presence of private mtDNA haplotypes in high frequencies in some sample groups and relatively small sample sizes (ranged 20–49 individuals). But the greater differentiation in the mtDNA could also reflect possible higher site fidelity for females than males as shown for saithe by both nuclear markers (Saha et al., 2015) and mtDNA (Eríksson & Árnason, 2014). We did not record sex in our samples and could therefore not test this hypothesis.

Our data show that fine-scale weak genetic structure is present in *S. bibarbatus* in the Benguela despite many opportunities for gene flow. The distance, hydrography, ambient oxygen levels, and adaptive life history features may be among the drivers of genetic differentiation. Determining the exact drivers of seascape genetics is always challenging due to the simultaneous influence of many factors (Selkoe et al., 2016). The reproductive behavior of *S. bibarbatus* could add to the complexity of its genetic structuring. For example, if the sex of mature fish were known, we could have tested if there was site fidelity in females compared to males, which has been observed for Saithe in the North Atlantic (Saha et al., 2015). Furthermore, the presence of alternative reproductive tactics in *S. bibarbatus* (Salvanes et al., 2018; Seivåg et al., 2016) and eggs attached to a substrate and cared for by territorial males (Skrypzeck et al., 2014), could be taken as evidence for low connectivity between some of the sampling groups. On the contrary, selection of spawning grounds on outer shelf areas in an upwelling zone would benefit larval transport to suitable nursery areas inshore, representing a mechanism increasing connectivity. Given the oceanographic and environmental characteristics of this upwelling ecosystem on one hand, and the unique behavioural and ecological adaptations of *S. bibarbatus* on the other, better knowledge of the mechanisms behind the genetic structuring could be achieved if future genetic studies include selective markers, and samples from females and males on the spawning sites and from early life stages from the entire shelf area.

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REFERENCES

Agenbag, J. J., & Shannon, L. V. (1988). A suggested physical explanation for the existence of a biological boundary at 24° 30' S in the Benguela system. *South African Journal of Marine Science*, *6*, 119–132. https://doi.org/10.2989/025776188784480726

Antao, T., Lopes, A., Lopes, R. J., Beja-Pereira, A., & Luikart, G. (2008). LOSITAN: A workbench to detect molecular adaptation based on a *FST*-outlier method. *BMC Bioinformatics*, *9*, 323.

Bartholomae, C., & van der Plas, A. K. (2007). Towards the development of environmental indices for the Namibian shelf, with particular reference to fisheries management. *African Journal of Marine Science*, *29*, 25–35. https://doi.org/10.2989/AJMS.2007.29.1.2.67

Barton, N. H., & Slatkin, M. (1986). A Quasi-equilibrium theory of the distribution of rare alleles in a subdivided population. *Heredity*, *56*, 409–415. https://doi.org/10.1038/hdy.1986.63

Beaumont, M. A., & Nichols, R. A.. (1996). Evaluating loci for use in the genetic analysis of population structure. *Proceedings of the Royal Society B*, *263*, 1619–1626.

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society B*, *57*, 289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x

Benjamini, Y., & Yekutieli, D. (2001). The control of the false discovery rate in multiple testing under dependency. *Annals of Statistics*, *29*, 1165–1188.

Boyd, A. J., Salat, J., & Masó, M. (1987). The seasonal intrusion of relatively saline water on the shelf off northern and central Namibia. *South African Journal of Marine Science*, *5*, 107–120. https://doi.org/10.2989/025776187784522577

Boyer, D., Cole, J., & Bartholomae, C. (2000). Southwestern Africa: Northern Benguela Current Region. *Marine Pollution Bulletin*, *41*(1–6), 123–140. https://doi.org/10.1016/S0025-326X(00)00106-5

Brüchert, V., Currie, B., & Peard, K. R. (2009). Hydrogen sulphide and methane emissions on the central Namibian shelf. *Progress in Oceanography*, *83*, 169–179. https://doi.org/10.1016/j.pocean.2009.07.017

Brüchert, V., Currie, B., Peard, K. R., Lass, U., Endler, R., Dübecke, A., … Zitzmann, S. (2006). An integrated assessment of shelf anoxia and water column hydrogen sulphide in the Benguela coastal upwelling system off Namibia. In L. N. Neretin (Ed.), *Past and Present Marine Water Column Anoxia*, *NATO Science Series IV- Earth and Environmental Sciences* (pp. 161–194). Berlin Heidelberg: Springer.

Cadrin, S. X., Bernreuther, M., Daníelsdóttir, A. K., Hjörleifsson, E., Johansen, T., Kerr, L., … Stransky, C. (2010). Population Structure of beaked redfish, *Sebastes mentella*: Evidence of divergence associated with different habitats. *ICES Journal of Marine Science*, *67*(8), 1617–1630. https://doi.org/10.1093/icesjms/fsq046

Chapman, L. J., Galis, F., & Shinn, J. (2000). Phenotypic plasticity and the possible role of genetic assimilation: Hypoxia-induced trade-offs in the morphological traits of an African cichlid. *Ecology Letters*, *3*, 387–393. https://doi.org/10.1046/j.1461-0248.2000.00160.x

Crawford, R. J. M., Cruickshank, R. A., Shelton, P. A., & Kruger, I. (1985). Partitioning of a goby resource amongst four avian predators and evidence for altered trophic flow in the pelagic community of an intense, perennial upwelling system. *South African Journal of Marine Science*, *3*(b), 215–228. https://doi.org/10.2989/025776185784461252

Crawford, R. J. M., Shannon, L. V., & Pollock, D. E. (1987). The Benguela Ecosystem 4. The major fish and invertebrate resources. *Oceanography and Marine Biology: An Annual Review*, *25*, 353–505.

Currie, B., Utne-Palm, A. C., & Salvanes, A. G. V. (2018). Winning ways with hydrogen sulphide on the Namibian shelf. *Frontiers in Marine Science*, *5*, https://doi.org/10.3389/fmars.2018.00341

Duncombe Rae, C. M. (2005). A demonstration of the hydrographic partition of the Benguela upwelling ecosystem at 26° 40' S. *South African Journal of Marine Science*, *27*, 617–628.

Earl, D. A., & vonHoldt, B. M. (2012). STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, *4*(2), 359–361. https://doi.org/10.1007/s12686-011-9548-7

Emeis, K. C., Brüchert, V., Currie, B., Endler, R., Ferdelman, T., Kiessling, A., … Vogt, T. (2004). Shallow gas in shelf sediments of the Namibian coastal upwelling ecosystem. *Continental Shelf Research*, *24*, 627–642. https://doi.org/10.1016/j.csr.2004.01.007

Eríksson, G. M., & Árnason, E. (2014). Gene flow across the N-Atlantic and sex-biased dispersal inferred from mtDNA sequence variation in saithe, Pollachius virens. *Environmental Biology of Fishes*, *98*, 67–79.

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software structure: A simulation study. *Molecular Ecology*, *14*, 2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

Gallo, N. D., & Levin, L. A. (2016). Fish ecology and evolution in the world's oxygen minimum zones and implications of ocean deoxygenation. *Advances in Marine Biology*, *74*, 117–198.

Gariboldi, M. C., Túnez, J. I., Failla, M., Hevia, M., Panebianco, M. V., Paso Viola, M. N., & Cappozzo, H. L. (2016). Patterns of population structure at microsatellite and mitochondrial DNA markers in the franciscana dolphin (*Pontoporia blainvillei*). *Ecology and Evolution*, *6*(24), 8764–8776. https://doi.org/10.1002/ece3.2596

Gunawickrama, K. B. S., De Silva, P. M. C. S., Johansen, T., Salvanes, A. V. G., & Nævdal, G. (2010). Preliminary evidence for genetic heterogeneity of the goby (*Sufflogobius bibarbatus*) in the Benguela ecosystem. *Journal of Applied Ichthyology*, *26*, 110–112.

Gunawickrama, K. B. S., Westgaard, J. I., Salvanes, A. G. V., & Johansen, T. (2012). Characterization of polymorphic microsatellite markers for the bearded goby *Sufflogobius bibarbatus*. *Conservation Genetics Resources*, *4*, 187–189. https://doi.org/10.1007/s12686-011-9505-5

Hellberg, M. (2009). Gene flow and isolation among populations of marine animals. *Annual Review of Ecology and Systematics*, *40*, 291–310. https://doi.org/10.1146/annurev.ecolsys.110308.120223

Henriques, R., Potts, W. M., Santos, C. V., Sauer, W. H. H., & Shaw, P. W. (2014). Population Connectivity and Phylogeography of a Coastal Fish, *Atractoscion aequidens* (Sciaenidae), across the Benguela Current Region: Evidence of an Ancient Vicariant Event. *PLoS ONE*, *9*(2), e87907. https://doi.org/10.1371/journal.pone.0087907

Henriques, R., Potts, W. M., Sauer, W. H. H., & Shaw, P. W. (2012). Evidence of deep genetic divergence between populations of an important recreational fishery species, *Lichia amia* L. 1758, around southern Africa. *African Journal of Marine Science*, *34*(4), 585–591.

Henriques, R., von der Heyden, S., Lipinski, M. R., du Toit, N., Kainge, P., Bloomer, P., & Matthee, C. A. (2016). Spatio-temporal genetic structure and the effects of long-term fishing in two partially sympatric offshore demersal fishes. *Molecular Ecology*, *25*, 5843–5861. https://doi.org/10.1111/mec.13890

Hutchings, L., Beckley, L. E., Griffiths, M. H., Roberts, M. J., Sundby, S., & van der Lingen, C. (2002). Spawning on the edge: Spawning grounds and nursery areas around the Southern African coastline. *Marine and Freshwater Research*, *53*, 307–318.

Hutchings, L., van der Lingen, C. D., Shannon, L. J., Crawford, R. J. M., Verheye, H. M. S., Bartholomae, C. H., … Monteiro, P. M. S. (2009). The Benguela Current: An ecosystem of four components. *Progress in Oceanography*, *83*, 15–32. https://doi.org/10.1016/j.pocean.2009.07.046

Ingram, T. (2011). Speciation along a depth gradient in a marine adaptive radiation. *Proceedings of the Royal Society B: Biological Sciences*, *278*(1705), 613–618.

Jombart, T. (2008). adegenet: A R package for the multivariate analysis of genetic markers. *Bioinformatics*, *24*, 1403–1405. https://doi.org/10.1093/bioinformatics/btn129

Jombart, T., Devillard, S., & Balloux, F. (2010). Discriminant analysis of principal components: A new method for the analysis of genetically structured populations. *BMC Genetics*, *11*, 94. https://doi.org/10.1186/1471-2156-11-94

Kelly, A. C., Mateus-Pinilla, N. E., Douglas, M., Douglas, M., Shelton, P., & Novakofski, J. (2011). Microsatellites behaving badly: Empirical evaluation of genotyping errors and subsequent impacts on population studies. *Genetics and Molecular Research*, *10*(4), 2534–2553. https://doi.org/10.4238/2011.October.19.1

Knutsen, H., Jorde, P. E., Albert, O. T., Hoelzel, A. R., & Stenseth, N. C. (2007). Population genetic structure in the North Atlantic Greenland halibut (*Reinhardtius hippoglossoides*): Influenced by oceanic current systems? *Canadian Journal of Fisheries and Aquatic Sciences*, *64*(6), 857–866.

Kopelman, N. M., Mayzel, J., Jakobsson, M., Rosenberg, N. A., & Mayrose, I. (2015). CLUMPAK: A program for identifying clustering modes and packaging population structure inferences across K. *Molecular Ecology Resources*, *15*(5), 1179–1191. https://doi.org/10.1111/1755-0998.12387

Lett, C., Veitch, J., van der Lingen, C., & Hutchings, L. (2007). Assessment of an environmental barrier to transport of ichthyoplankton from the southern to the northern Benguela ecosystem. *Marine Ecology Progress Series*, *347*, 247–259.

Manly, B. F. J. (1994). *Multivariate statistical methods: A primer*. New York: Chapman and Hall.

Mann, K. H., & Lazier, J. R. N. (1991). *Dynamics of marine ecosystems – biological-physical interactions in the oceans*. London: Blackwell Scientific Publications.

Martinez, M. L., Chapman, L. J., & Rees, B. B. (2009). Population variation in hypoxic responses of the cichlid *Pseudocrenilabrus multicolor victoriae*. *Canadian Journal of Zoology*, *87*, 188–194.

Mas-Riera, J., Lombarte, A., Gordoa, A., & MacPherson, A. (1990). Influence of the Benguela upwelling on the structure of demersal fish populations off Namibia. *Marine Biology*, *104*, 175–182.

Mecenero, S., Roux, J. P., Underhill, L. G., & Kirkman, S. P. (2006). Diet of Cape Fur Seals *Arctocephalus Pusillus* Pusillus at Three Mainland Breeding Colonies in Namibia. 2. Temporal variation. *African Journal of Marine Science*, *28*, 73–88.

Naro-Maciel, E., Reid, B., Holmes, K. E., Brumbaugh, D. R., Martin, M., & DeSalle, R. (2011). Mitochondrial DNA sequence variation in spiny lobsters: Population expansion, panmixia, and divergence. *Marine Biology*, *158*(9), 2027–2204. https://doi.org/10.1007/s00227-011-1710-y

Ndudane, S. (2004). *Population genetic studies of* Sufflogobius bibarbatus *in the Benguela ecosystem*. MS thesis, Department of Fisheries and Marine Biology, University of Bergen.

Nelson, G., & Hutchings, L. (1983). The Benguela upwelling area. *Progress in Oceanography*, *12*, 333–356. https://doi.org/10.1016/0079-6611(83)90013-7

Olivar, M. P., & Fortuño, J. M. (1991). Guide to Ichthyoplankton of the Southeast Atlantic (Benguela Current Region). *Scientia Marina*, *55*(1), 1–383.

Olivar, M. P., Rubiés, P., & Salat, J. (1992). Horizontal and vertical distribution patterns of ichthyoplankton under intense upwelling regimes off Namibia. *South African Journal of Marine Science*, *12*(1), 71–82. https://doi.org/10.2989/02577619209504692

Olivar, M. P., & Shelton, P. A. (1993). Larval fish assemblages of the Benguela current. *Bulletin of Marine Sciences*, *53*, 450–474.

O'Toole, M. J. (1978). *Development, distribution and relative abundance of the larvae and early juveniles of the pelagic goby* Sufflogobius bibarbatus *(von Bonde) off South West Africa, 1972–1974* (pp. 116–128). South Africa: Report, Sea Fisheries Branch.

Palumbi, S. R. (1994). Genetic divergence, reproductive isolation, and marine speciation. *Annual Review of Ecology and Systematics*, *25*, 547–572. https://doi.org/10.1146/annurev.es.25.110194.002555

Pampoulie, C., Gysels, E. S., Maes, G. E., Hellemans, B., Leentjes, V., Jones, A. G., & Volckaert, F. A. M. (2004). Evidence for fine-scale genetic structure and estuarine colonisation in a potential high gene flow marine goby (*Pomatoschistus minutus*). *Heredity*, *92*, 434–445. https://doi.org/10.1038/sj.hdy.6800438

Peakall, R., & Smouse, P. E. (2012). GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics*, *28*(19), 2537–2539.

Planes, S., & Fauvelot, C. (2002). Isolation by distance and vicariance drive genetic structure of a coral reef fish in the Pacific Ocean. *Evolution*, *56*(2), 378–399. https://doi.org/10.1111/j.0014-3820.2002.tb01348.x

Prada, C., & Hellberg, M. E. (2013). Long pre-reproductive selection and divergence by depth in a Caribbean candelabrum coral. *PNAS*, *110*(10), 3961–3966. https://doi.org/10.1073/pnas.1208931110

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, *155*, 945–959.

Riginos, C., & Nachman, M. W. (2001). Population subdivision in marine environments: The contributions of biogeography, geographical distance and discontinuous habitat to genetic differentiation in a blennioid fish, *Axoclinus nigricaudus*. *Molecular Ecology*, *10*, 1439–1453. https://doi.org/10.1046/j.1365-294X.2001.01294.x

Rouault, M. (2012). Bi-annual intrusion of tropical water in the northern Benguela upwelling. *Geophysical Research Letters*, *39*(12), https://doi.org/10.1029/2012GL052099

Rousset, F. (2008). Genepop'007: A complete re-implementation of the Genepop software for Windows and Linux. *Molecular Ecology Resources*, *8*, 103–106. https://doi.org/10.1111/j.1471-8286.2007.01931.x

Saha, A., Hauser, L., Kent, M., Planque, B., Neat, F., Kirubakaran, T. G., … Johansen, T. (2015). Seascape genetics of saithe (*Pollachius virens*) across the North Atlantic using single nucleotide polymorphisms. *ICES Journal of Marine Science*, *72*(9), 2732–2741. https://doi.org/10.1093/icesjms/fsv139

Saha, A., Johansen, T., Hedeholm, R., Nielsen, E. E., Westgaard, J. I., Hauser, L., … Boje, J. (2016). Geographic extent of introgression in *Sebastes mentella* and its effect on genetic population structure. *Evolutionary Applications*, *10*, 77–90. https://doi.org/10.1111/eva.12429

Sailant, E. A., Renshaw, M. A., Cummings, M. J., & Gold, J. R. (2012). Conservation genetics and management of yellowtail snapper, *Ocyurus chrysurus*, in the US Caribbean and South Florida. *Fisheries Management and Ecology*, *19*, 301–312. https://doi.org/10.1111/j.1365-2400.2011.00840.x

Salvanes, A. G. V., Bartholomae, C., Yemane, D., Gibbons, M. J., Kainge, P., Krakstad, J. O., … Sundby, S. (2015). Spatial dynamics of the bearded goby and its key fish predators off Namibia vary with climate and oxygen availability. *Fisheries Oceanography*, *24*(Suppl 1), 88–101. https://doi.org/10.1111/fog.12068

Salvanes, A. G. V., Christiansen, H., Taha, Y., Henseler, C., Seivåg, M. L., Kjesbu, O. S., … Gibbons, M. J. (2018). Variation in growth, morphology and reproduction of the bearded goby (*Sufflogobius bibarbatus*) in varying oxygen environments of northern Benguela. *Journal of Marine Systems*, *188*, 81–97. https://doi.org/10.1016/j.jmarsys.2018.04.003

Salvanes, A. G. V., & Gibbons, M. J. (2018). Adaptation to hypoxic environments; bearded gobies *Sufflogobius bibarbatus* in the Benguela upwelling ecosystem. *Journal of Fish Biology*, *92*, 752–772.

Salvanes, A. G. V., Utne-Palm, A. C., Currie, B., & Braithwaite, V. A. (2011). Behavioural and physiological adaptations of the bearded goby: A key fish species of the extreme environment of Benguela. *Marine Ecology Progress Series*, *425*, 193–202.

Seivåg, M. L., Salvanes, A. G. V., Utne-Palm, A. C., & Kjesbu, S. O. (2016). Reproductive tactics of male bearded goby (*Sufflogobius bibarbatus*) in anoxic and hypoxic waters. *Journal of Sea Research*, *109*, 29–41. https://doi.org/10.1016/j.seares.2016.01.002

Selkoe, K. A., D'Aloia, C. C., Crandall, E. D., Iacchei, M., Liggins, L., Puritz, J. B., … Toonen, R. J. (2016). A decade of seascape genetics: Contributions to basic and applied marine connectivity. *Marine Ecology Progress Series*, *554*, 1–19. https://doi.org/10.3354/meps11792

Seyoum, S., McBride, R. S., Tringali, M. D., Villanova, V. L., Puchutulegui, C., Gray, S., & Van Bibber, N. (2018). Genetic population structure of the spotted seatrout (*Cynoscion nebulosus*): Simultaneous examination of the mtDNA control region and microsatellite marker results. *Bulletin of Marine Science*, *94*(1), 47–71.

Shelton, P. A. (1986). *Fish spawning strategies in the variable southern Benguela Current region*. Doctoral dissertation. University of Cape Town.

Skrypzeck, H., Salvanes, A. G. V., Currie, B., & Kotze, A. (2014). First records of reproductive behaviour and early development for the bearded goby *Sufflogobius bibarbatus*. *Journal of Fish Biology*, *84*, 1256–1261.

Slatkin, M. (1993). Isolation by distance in equilibrium and non-equilibrium populations. *Evolution*, *47*, 264–279. https://doi.org/10.1111/j.1558-5646.1993.tb01215.x

Stefánsson, M. Ö., Reinert, J., Sigurðsson, Þ., Kristinsson, K., Nedreaas, K., & Pampoulie, C. (2009). Depth as a potential driver of genetic structure of *Sebastes mentella* across the North Atlantic Ocean. *ICES Journal of Marine Science*, *66*, 680–690.

Sultan, S. E., & Spencer, H. G. (2002). Metapopulation structure favors plasticity over local adaptation. *The American Naturalist*, *160*(2), 271–283. https://doi.org/10.1086/341015

Tobler, M. N., DeWitt, T. J., Schlupp, I., de Leo, F. J. G., Herrmann, R., Feulner, P. G. D., … Plath, M. (2008). Toxic hydrogen sulfide and dark Caves: Phenotypic and genetic divergence across two abiotic environmental gradients in *Poecilia mexicana*. *Evolution*, *62*(10), 2643–2659.

Utne-Palm, A. C., Salvanes, A. G., Currie, B., Kaartvedt, S., Nilsson, G. E., Braithwaite, V. A., … Gibbons, M. J. (2010). Trophic structure and community stability in an overfished ecosystem. *Science*, *329*(5989), 333–336.

van der Lingen, C., Shannon, L. J., Cury, P., Kreiner, A., Moloney, C. L., Roux, J. P., & Vas-Velho, F. (2006). Resource and ecosystem variability, including regime shift, in the Benguela current system. In V. Shannon, G. Hempel, P. Mallanotte-Rizzoli, C. Moloney, & J. Woods (Eds.), *Large marine Ecosystems*, Vol. *14* (pp. 147–184). Amsterdam: Elsevier.

van der Meer, D. L., den Thillart, G. E., Witte, F., de Bakker, M. A., Besser, J., Richardson, M. K., … Bagowski, C. P. (2005). Gene expression profiling of the long-term adaptive response to hypoxia in the gills of adult zebrafish. *American Journal of Physiology. Regulatory, Integrative, and Comparative. Physiology*, *289*(5), R1512–R1519. https://doi.org/10.1152/ajpregu.00089.2005

van Oosterhout, C., Hutchinson, W. F., Wills, D. P. M., & Shipley, P. (2004). Micro-Checker: Software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, *4*, 535–538. https://doi.org/10.1111/j.1471-8286.2004.00684.x

Wang, L., Liu, S., Zhuang, Z., Guo, L., Meng, Z., & Lin, H. (2013). Population genetic studies revealed local adaptation in a high gene-flow marine fish, the small yellow croaker (*Larimichthys polyactis*). *PLoS ONE*, *8*(12), e83493. https://doi.org/10.1371/journal.pone.0083493

Waples, R. S. (1998). Separating the wheat from the chaff: Patterns of genetic differentiation in high gene flow species. *Journal of Heredity*, *89*, 439–450. https://doi.org/10.1093/jhered/89.5.438

Weersing, K., & Toonen, R. J. (2009). Population genetics, larval dispersal, and connectivity in marine systems. *Marine Ecology Progress Series*, *393*, 1–12. https://doi.org/10.3354/meps08287

Weir, B. S., & Cockerham, C. C. (1984). Estimating F-statistics for the analysis of population structure. *Evolution*, *38*, 1358–1370.

White, C., Selkoe, K. A., Watson, J., Siegel, D. A., Zacherl, D. C., & Toonen, R. J. (2009). Ocean currents help explain population genetic structure. *Proceedings of the Royal Society B*, *277*, 1685–1694. https://doi.org/10.1098/rspb.2009.2214

Wright, D., Bishop, J. M., Matthee, C. A., & Heyden, S. (2015). Genetic isolation by distance reveals restricted dispersal across a range of life histories: Implications for biodiversity conservation planning across highly variable marine environments. *Diversity and Distributions*, *21*, 698–710. https://doi.org/10.1111/ddi.12302

Zardi, G. I., McQuaid, C. D., Teske, P. R., & Barker, N. P. (2007). Unexpected genetic structure of mussel populations in South Africa: Indigenous *Perna perna* and invasive *Mytilus galloprovincialis*. *Marine Ecology Progress Series*, *337*, 135–144.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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