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REPORT

Life history, larval dispersal, and connectivity in coral reef fish among the Scattered Islands of the Mozambique Channel

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Abstract The Western Indian Ocean harbors one of the world's most diverse marine biota yet is threatened by exploitation with few conservation measures in place. Primary candidates for conservation in the region are the Scattered Islands (Îles Éparses), a group of relatively pristine and uninhabited islands in the Mozambique Channel. However, while optimal conservation strategies depend on the degree of population connectivity among spatially isolated habitats, very few studies have been conducted in the area. Here, we use highly variable microsatellite markers from two damselfishes (*Amphiprion akallopisos* and *Dascyllus trimaculatus*) with differing life history traits [pelagic larval duration (PLD), adult habitat] to compare genetic structure and connectivity among these islands using classic population structure indices as well as Bayesian clustering methods. All classical fixation indexes F_{ST}, R_{ST}, G'_{ST}, and Jost's D show stronger genetic differentiation among islands for *A. akallopisos* compared to *D. trimaculatus*, consistent with the former species' shorter PLD and stronger adult site attachment, which may restrict larval dispersal potential. In agreement with these results, the Bayesian analysis revealed clear genetic differentiation among the islands in *A. akallopisos*, separating the southern group (Bassas da India and Europa) from the center (Juan de Nova) and northern (Îles Glorieuses) islands, but not for *D. trimaculatus*. Local oceanographic patterns such as eddies that occur along the Mozambique Channel appear to parallel the results reported for *A. akallopisos*, but such features seem to have little effect on the genetic differentiation of *D. trimaculatus*. The contrasting patterns of genetic differentiation between species within the same family highlight the importance of accounting for diverse life history traits when assessing community-wide connectivity, an increasingly common consideration in conservation planning.

Keywords Population genetics · Population structure · *Amphiprion akallopisos* · *Dascyllus trimaculatus* · Pomacentridae · Îles Éparses

James L. O'Donnell and Ricardo Beldade have contributed equally to this work.

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Introduction

The Western Indian Ocean is one of the most biodiverse yet understudied regions of the sea (McClanahan et al. 2000; Roberts et al. 2002). In addition to high levels of local diversity and endemism, there is substantial turnover in species composition between this region and the epicenter of marine biodiversity, the Coral Triangle (Allen 2008; Briggs and Bowen 2012; Obura 2012, 2015). These attributes make the Western Indian Ocean an important

priority for conservation, yet presently its coral reefs are afforded little protection from exploitation (Mora et al. 2006). Human populations in this region are growing rapidly, increasingly relying on marine organisms as a food source and as an economic resource. The combined threat of global climate change (McClanahan et al. 2014; Mills et al. 2015) and anthropogenic disturbances has generated growing interest in creating reserves that will help sustain populations of marine organisms both for their economic and intrinsic values. Optimization of the size, spatial arrangement, and management of ecological reserves so that they effectively serve their intended purpose requires an understanding of the movement of individuals among populations in the area of interest (Halpern and Warner 2003).

The majority of marine organisms exhibit a bipartite life history, where adults are sedentary and larvae are pelagic, living in the water column for days to months before they settle (Leis 1991). This pelagic stage facilitates the movement of individuals among spatially isolated populations (population connectivity), though multiple factors may contribute to the realized dispersal of larvae from their population of origin. Traditionally, it has been assumed that the most important of these is the pelagic larval duration (PLD) (Riginos and Victor 2001; Selkoe and Toonen 2011; Luiz et al. 2013), where a short PLD results in larval settlement close to the natal habitat with increased population differentiation over fine scales (Planes et al. 2001), and a long PLD allows for long-distance larval dispersal with reduced population differentiation. It is increasingly recognized that while PLD may influence larval dispersal, realized dispersal should be mediated by both complex and dynamic oceanographic features. Simulations have shown that even modest abilities for larvae to modulate their position in the water column can obscure the relationship between PLD, oceanographic features, and realized larval dispersal (Cowen et al. 2006; Leis 2007). Indeed, larval dispersal of marine species is most likely modulated by some combination of PLD, oceanographic features, and larval behavior, as well as environmental factors such as temperature and food availability.

In this study, we brought together the necessity for understanding the level of connectivity in the Mozambique Channel (Western Indian Ocean), and the use of genetic techniques on two coral reef damselfish (family Pomacentridae), the three-spot dascyllus, *Dascyllus trimaculatus,* and the skunk clownfish, *Amphiprion akallopisos*. The two species exhibit differences in both PLD and habitat use. Reported PLD ranges from 7 to 22 d for *Amphiprion* species and from 20 to 30 d for *D. trimaculatus* (Thresher et al. 1989; Wellington and Victor 1989; Robitzch et al. 2015). Both species are symbiotic mutualists of sessile sea anemones as juveniles. As subadults, however, while clownfish remain with their host, *D. trimaculatus* leave the anemones altogether to feed on plankton in the water column (Fautin and Allen 1997), which may increase the potential number of lifetime mates and thus the probability of mating events with individuals from distant populations that increase genetic homogeneity. Clownfish larval dispersal distances have been shown to vary from <1 km (Beldade et al. 2012; Saenz-Agudelo et al. 2012) to >100 km (Simpson et al. 2014). However, despite only small differences in PLDs compared to clownfishes, *D. trimaculatus* have been shown to have large-scale genetic homogeneity, spanning >1000 km (Bernardi et al. 2001, 2002, 2003; Leray et al. 2010).

The Mozambique Channel is an arm of the Western Indian Ocean spanning approximately 500 km between Mozambique and Madagascar and 1200 km between its limits to the north (the Comoros Islands) and south (the southern end of Madagascar). A string of four oceanic island groups runs along its center from north to south: Îles Glorieuses (an archipelago of two nearby islands, Grande Glorieuse and Île du Lys), Juan de Nova, Bassas da India, and Europa (Fig. 1). These islands, together with the island of Tromelin, which lies outside of the Mozambique Channel to the east of Madagascar, make up the Îles Éparses (English translation: Scattered Islands), an overseas administrative division of France. They harbor moderate levels of coral reef biodiversity (Fricke et al. 2013) and are relatively pristine because there are no permanent

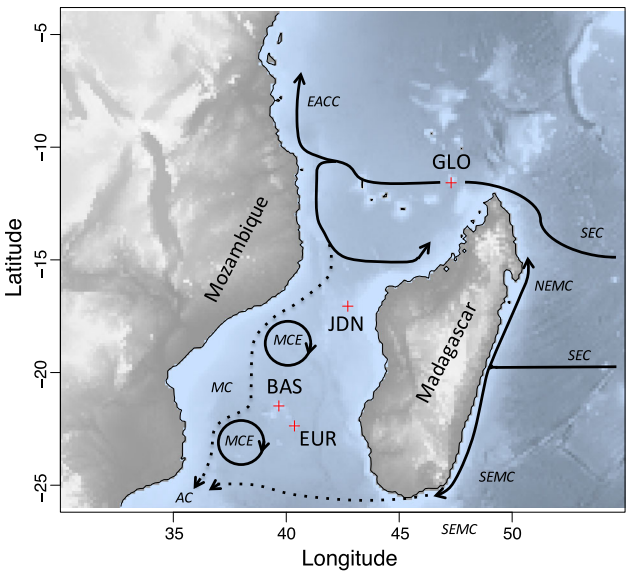

Fig. 1 Location of collection sites in the Mozambique Channel (GLO: Îles Glorieuses, JDN: Juan de Nova, BAS: Bassas da India, EUR: Europa). Main currents and flow features are shown: South Equatorial Current (SEC), the Northeast and Southeast Madagascar Currents (NEMC and SEMC), the East African Coastal Current (EACC), the Agulhas Current (AC), the Mozambique Current (MC), and the Mozambique Channel Eddies (MCE). Poorly documented currents are presented with *dashed lines* (Redrawn from Schouten et al. 2003)

human settlements, making them primary candidates for marine conservation. Following the most recent biogeographic division of this area's eco-regions (Spalding et al. 2007; Obura 2012), the southern Europa, Bassas da India, and Juan de Nova islands are separate from the northern Îles Glorieuses. These islands provide habitat for coastal marine organisms close enough for potential migration via larval dispersal, but distant enough to prevent adult dispersal. The optimal strategy for marine conservation of the Scattered Islands depends on the degree of population connectivity among them, making it important to know whether or not population structure follows the same pattern as community structure.

In this region, the oceanographic circulation patterns have been relatively better studied than genetic connectivity of coral reef fishes. The Mozambique Channel lies in the shadow of the South Equatorial Current (Fig. 1). Strong eddies form approximately four times per year following cyclonic and anticyclonic directions (Schouten et al. 2003; Lutjeharms and Bornman 2010; Swart et al. 2010; Lutjeharms et al. 2012). Though the sites included in our study are small and isolated, these eddies may serve to enhance larval retention, restricting connectivity and therefore potentially increasing genetic structure among sites. In this region, changes in local ocean circulation may take place over timescales from seasonal to decadal (Schouten et al. 2003; Lutjeharms et al. 2012) adding another element of stochasticity in the prediction of population structure between the islands in the channel.

Very few studies have examined population connectivity of coral reef fishes in the Mozambique Channel. For the bluestripe snapper, *Lutjanus kasmira*, the blotcheye soldierfish, *Myripristis berndti,* and the honeycomb grouper, *Epinephelus merra,* while population structure was found at the level of the Western Indian Ocean, no population structure was found within the Mozambique Channel (Muths et al. 2011, 2012, 2015). In this study, we used microsatellite markers to assess the level of connectivity exhibited by *A. akallopisos* and *D. trimaculatus* that were collected at all four of the Scattered Islands groups of the Mozambique Channel. We tested models of genetic structure and migration among the four Scattered Islands in light of the previously identified divide in eco-regions and the presence and location of known eddies as potential barriers to gene flow.

Methods

Sample collection

Adult and subadult fish were collected at all four island groups in the Mozambique Channel: Îles Glorieuses (GLO), Juan de Nova (JDN), Bassas da India (BAS), and Europa (EUR; Fig. 1). Individuals were captured using hand nets, and a small piece of fin tissue was collected before returning fish to their host anemone. Tissue samples were placed in 95% ethanol and stored at −20 °C as soon as possible.

Laboratory methods

Genomic DNA was extracted using a chloroform-based protocol (Sambrook et al. 1989). Microsatellite loci were originally isolated and developed for *Amphiprion chrysopterus* and *Amphiprion polymnus* (Quenouille et al. 2004; Beldade et al. 2009) and for *D. trimaculatus* (Leray et al. 2008). From the existing libraries, we chose 20 microsatellites per species to test for variability on 32 individuals. Nine polymorphic loci for *A. akallopisos* and 13 for *D. trimaculatus* were selected based on their variability and amplification success rate (Table 1). Each reaction contained 5 µl of Qiagen Multiplex PCR mastermix, 0.2 ρmol of each primer, and 0.8 µl of DNA diluted with RNAase-free water to a total reaction volume of 10 µl. The following temperature profile was used: 15 min at 95 °C, followed by 40 cycles of 30 s at 94 °C, 1 min and 30 s at 57 °C, and 1 min at 72 °C, with a final extension of 7 min at 72 °C. PCR product was diluted with 50 µl of water, and 0.5 µl of this diluted PCR product was added to 9.74 µl of HiDi formamide and 0.26 µl ROX 500 size standard (Qiagen, Valencia, California). Fragment size was analyzed on an ABI 96 capillary 3730XL DNA Analyzer (Applied Biosystems, Darmstadt, Germany).

Data analysis

We genotyped individuals using GeneMapper version 3.7 (Applied Biosystems), and Convert v1.31 (Glaubitz 2004) was used to generate input files for various analyses programs. We checked for null alleles and scoring errors using Micro-checker v.2.2.3 (Van Oosterhout et al. 2004). Arlequin v3.11 (Excoffier et al. 2005) was used to calculate expected and observed heterozygosities, test for deviation from Hardy–Weinberg Equilibrium (HWE) using a Markov chain of length 100,000, and test for linkage disequilibrium. Using Weir and Cockerham (1984) formulae, we calculated F_{IS} using the function f_{st} in the R package pegas version 0.9 (Paradis 2010; R core Team 2015) and calculated global significance values using Goudet's *G* statistic Monte Carlo test on 1000 iterations with the function gstat.randtest in the R package hierfstat version 0.04–22 (Goudet and Jombart 2015; R Core Team 2015). Several measures of genetic structure were used, including F_{ST} and R_{ST} implemented in Arlequin v3.11, and G'_{ST} and Jost's *D* implemented in the R package diveRsity (Keenan et al.

Table 1 Summaries of the microsatellite loci used in this study. Number of alleles (Na) and fragment lengths (RR, given in base pairs) for microsatellite loci used in this study for *Amphiprion akallopisos* (top panel) and *Dascyllus trimaculatus* (bottom panel), as well as number of alleles (Na), observed (Ho) and expected (He) heterozygosities for each locus at each sampled site

A. akallopisos

Locus	Na	RR (bp)	Îles Glorieuses ($n = 31$)			Juan de Nova ($n = 37$)			Europa–Bassas ($n = 41$)			Total	
			Na	Ho	He	Na	Ho	He	Na	Ho	He	% NA	FIS
B6	8	141–159	5	0.781	0.691	8	0.757	0.746	6	0.829	0.686	0	−0.120*
D114	6	202–222	4	0.613	0.632	5	0.621	0.639	5	0.732	0.675	0.9	−0.006*
44	11	241–256	8	0.548	0.728	6	0.5	0.673	5	0.548	0.606	10.9	0.249*
34	34	296-359	21	0.844	0.885	19	0.703	0.871	19	0.829	0.881	0	0.098*
120	2	454–456	2	0.375	0.347	2	0.216	0.195	2	0.146	0.217	0	0.039
D103	15	250–306	13	0.969	0.887	11	0.811	0.882	11	0.829	0.888	0	0.026*
CF11	19	184–214	10	0.774	0.714	13	0.838	0.792	11	0.78	0.702	0.9	−0.081
10TCTA	35	500–558	26	1	0.937	20	0.914	0.891	19	0.846	0.909	3.6	−0.010*
A130	15	171–194	9	0.08	0.811	9	0.194	0.49	10	0.132	0.691	10	0.787*

D. trimaculatus

Locus	Na	RR (bp)	Îles Glorieuses ($n = 45$)			Juan de Nova ($n = 32$)			Europa–Bassas ($n = 42$)			Total	
			Na	Ho	He	Na	Ho	He	Na	Ho	He	% NA	FIS
A7	8	236–250	7	0.689	0.77	8	0.719	0.788	7	0.61	0.658	0.8	0.097
A101	32	108–188	28	0.8	0.965	24	0.733	0.965	24	0.707	0.947	2.5	0.222
A103	27	181–259	21	0.689	0.945	20	0.688	0.931	21	0.561	0.937	0.8	0.315
A105	23	133–181	20	0.889	0.926	17	0.966	0.917	19	0.853	0.918	0.8	0.251
A111	37	243–389	31	0.578	0.967	24	0.581	0.962	30	0.548	0.963	0	0.033
A114	16	222–290	14	0.644	0.916	14	0.75	0.9	15	0.675	0.885	1.7	0.348
A115	21	165–251	19	0.262	0.916	13	0.16	0.891	14	0.313	0.909	3.4	0.028
A120	24	164–222	19	0.778	0.93	19	0.71	0.911	18	0.595	0.918	6.7	0.151
B103	15	280–308	11	0.889	0.879	13	0.844	0.889	14	0.833	0.888	0.8	0.413
B105	29	173–251	25	0.711	0.956	17	0.531	0.884	18	0.55	0.918	1.7	0.247
B109	30	184–256	27	0.778	0.941	21	0.929	0.927	20	0.737	0.944	5	0.36
B113	28	226–316	24	0.738	0.948	16	0.5	0.906	20	0.512	0.914	0	0.12
C12	9	251–315	8	0.289	0.32	5	0.281	0.306	5	0.214	0.26	16.8	0.729

Sample sizes are given next to each site name. For each locus across sites, percent missing data (% NA) and F_{IS} values are given, with significance of a global Goudet's *G* statistic Monte Carlo test indicated with an asterisk (*)

2013). Arlequin was also used to calculate the significance of F_{ST} values based on 1000 permutations. Since individuals were collected in anemones, where sibling individuals have been shown to occasionally co-recruit (Bernardi et al. 2012), we also tested for kinship of individuals (Loiselle et al. 1995) implemented in GENODIVE (Meirmans and Van Tienderen 2004), with a conservative cutoff value for full siblings at $k > 0.4$.

As an alternative approach to describing the genetic structure of the two pomacentrids, Bayesian clustering analyses were conducted using the software STRUCTURE (version 2.3) (Pritchard et al. 2000) and GENELAND v.4.0.3 (Guillot et al. 2005a, b). STRUCTURE implements a Markov chain Monte Carlo (MCMC) simulation approach to estimate the posterior probability that a sampled individual belongs to each of *K* clusters based on its multi-locus genotype. We conducted analyses for values of *K* from 1 to 10, each consisting of 20 independent runs of MCMC length 1,000,000. The first 10,000 iterations of each chain were discarded as burn-in. We calculated an optimal value of *K* using the method described by Evanno et al. (2005) implemented by STRUCTURE HARVESTER (Earl and vonHoldt 2012); however, because a strict abiding of such results has recently been questioned (Meirmans 2015), we also present the results under alternate scenarios of *K*. GENELAND uses a model similar to STRUCTURE, but allows inclusion of the samples' geographic positions as a prior in the analysis in order to assess the posterior probability of cluster membership not only for individuals, but for each cell of a spatial grid, thus identifying geographic boundaries

between the identified clusters. Ten MCMC runs were conducted, each including 1,000,000 iterations with a thinning of 1000, based on the uncorrelated frequency model with no uncertainty of the sample coordinates, and where the number of clusters (K) was allowed to vary between 1 and 4, based on the STRUCTURE output. Excluding the first 200 values out of the 1000 saved iterations as a burn-in, MCMC convergence was assessed by comparing the number of clusters across replicate runs, with a single run chosen for presentation on the basis of the mean posterior probability used as a criterion to choose the best run.

Finally, we used a discriminant analysis of principal components (DAPC) procedure described by Jombart et al. (2010) as an independent method for identifying the number of clusters (K) and the probability of individual membership to each cluster. Briefly, we transformed the allele size data using principal component analysis, performed successive K means clustering (for $K = 1$–10) on the transformed data retaining all principal components, and calculated the Bayesian information criterion (BIC) of each K. We chose an optimal value of K using Ward's hierarchical agglomerative clustering method on the differences in BIC between successive steps. Values were assigned to one of two groups representing large or small differences, and the value of K just before the first group switch was chosen as the optimum. Because the method for choice of an optimal K is debated, especially in the present context where groups are in reality not completely distinct, we also evaluated K based on the value at which a LOWESS curve began increasing, as well as the raw minimum BIC. If the optimum K was greater than 1, we evaluated the probability of membership to each cluster using discriminant function analysis. These operations were performed using the functions find.clusters and dapc in the R package adegenet version 2.0.1 (Jombart 2008).

Results

Microsatellite analysis

We successfully amplified nine microsatellite loci from 110 *A. akallopisos* and 13 microsatellites for 119 *D. trimaculatus* from four sites in the Mozambique Channel (Table 1; Fig. 1). Loci used in this study showed sufficient variability for a population study (2–37 alleles, mean = 20.2, SD = 10.4). Within sites, for *D. trimaculatus*, there was evidence of significant deviation from HWE in seven cases, but no site or microsatellite showed consistent deviation from HWE across populations, indicating that all sites and microsatellite loci were usable for this analysis. Kinship analysis identified two *A. akallopisos* individuals from Îles Glorieuses as being full siblings ($R = 0.617$). Therefore, one individual from the pair was removed from the subsequent analyses. No other full-sib relationships were detected (not shown).

Population structure of *D. trimaculatus* and *A. akallopisos*

Values of population differentiation were always greater in *A. akallopisos* than in *D. trimaculatus* (Table 2). Indeed, average population pairwise comparisons were higher in *A. akallopisos* than in *D. trimaculatus* for all metrics used in this study: F_{ST} (0.09 vs. 0.003), R_{ST} (0.08 vs. 0.00), G'_{ST} (0.03 vs. 0.00), and Jost's D (0.07 vs. 0.02). For *A. akallopisos*, while pairwise F_{ST} values were relatively low (<0.05), all three comparisons were found to be statistically significant (Table 2). In addition, for *A. akallopisos*, the Juan de Nova population was always most distinct from the two other populations (Table 2).

Both of the Bayesian clustering approaches were consistent with the classical population structure analyses presented above. Indeed, both indicated clear population structure in *A. akallopisos*, and less so for *D. trimaculatus*. The Evanno method identified two and four STRUCTURE clusters for *D. trimaculatus* and *A. akallopisos*,

Table 2 Classical fixation indices among study sites. Pairwise F_{ST}, R_{ST}, G'_{ST}, and Jost's D values for sites sampled in this study

	Îles Glorieuses	Juan de Nova	Europa–Bassas
F_{ST}			
Îles Glorieuses	–	0.047*	0.033*
Juan de Nova	0.001	–	0.014*
Europa–Bassas	0.001	0.006	–
R_{ST}			
Îles Glorieuses	–	0.032*	− 0.004
Juan de Nova	0.013	–	0.031*
Europa–Bassas	0.026	0.074*	–
G'_{ST}			
Îles Glorieuses	–	0.031*	0.016*
Juan de Nova	0.01	–	0.023*
Europa–Bassas	0.008	0.013	–
Jost's D			
Îles Glorieuses	–	0.112*	0.056
Juan de Nova	0.121	–	0.079*
Europa–Bassas	0.105	0.141	–

Values for *Amphiprion akallopisos* italicized above the diagonal, values for *Dascyllus trimaculatus* are below the diagonal. For F_{ST} and R_{ST} values, asterisks indicate significance ($p < 0.05$) based on 100 permutations computed in ARLEQUIN v3.1 (Excoffier et al. 2005). Values for G'_{ST} and Jost's D were calculated using the R package diveRsity (Keenan et al. 2013), and asterisks indicate estimates for which the 95% confidence interval does not contain zero

respectively (Electronic Supplementary Materials, ESM, Fig. 1). Results with no structure at all ($K = 1$) for *D. trimaculatus* were not significantly different than the chosen ones ($K = 2$) thus being consistent with the classical population genetics approach. Finally, for easier comparative visualization, Evanno's method results ($K = 2$ and $K = 4$) and keeping clusters at three ($K = 3$) for both species are presented in Fig. 2. This visualization illustrates the clear difference between the two species; *A. akallopisos* show stronger population structure than *D. trimaculatus*. In addition, as for the classical population genetic approach, the Juan de Nova *A. akallopisos* population was most different from the two other populations (Fig. 2).

GENELAND's spatially explicit algorithm also clearly showed a difference between the two species. While it only identified a single panmictic population for *D. trimaculatus*, three clusters were identified for *A. akallopisos*. GENELAND specifically assigned those clusters to the three regions sampled here (north, central, and south Mozambique Channel), with the geographically close Bassas da India and Europa grouped together (Fig. 3). The accuracy of classification of spatial grid cells to genetic clusters is highest when the spatial origins of individual samples are dense and uniformly distributed over the area of interest. Our samples were collected from only four sites over a very large area, making the boundaries of genetic clusters unlikely to be truly representative of the distribution of our study species' larvae in the ocean. Nevertheless, the analysis of GENELAND provides a useful visualization of the genetic clusters in space and provides a baseline against which to test genetic breaks in future studies. We present the results after removal of individuals for which no data were available for at least one locus. A preliminary analysis using individuals with missing data (not shown) yielded qualitatively similar results, but did not appropriately sample the cluster parameter or establish convergence after multiple runs.

The results of DAPC (ESM, Figs. 2, 3) are largely consistent with those of the Bayesian clustering algorithms implemented by STRUCTURE and GENELAND. For *D. trimaculatus*, BIC increased monotonically from $K = 1$ to $K = 10$; that is, the analysis was unable to identify any distinct groups from the data. For *A. akallopisos*, the value of BIC declined sharply from $K = 1$ to $K = 2$, but remained very similar for $K = 2$–4. While a clustering-based approach identified $K = 2$ to be optimal, the LOWESS and minimum BIC approaches suggest an

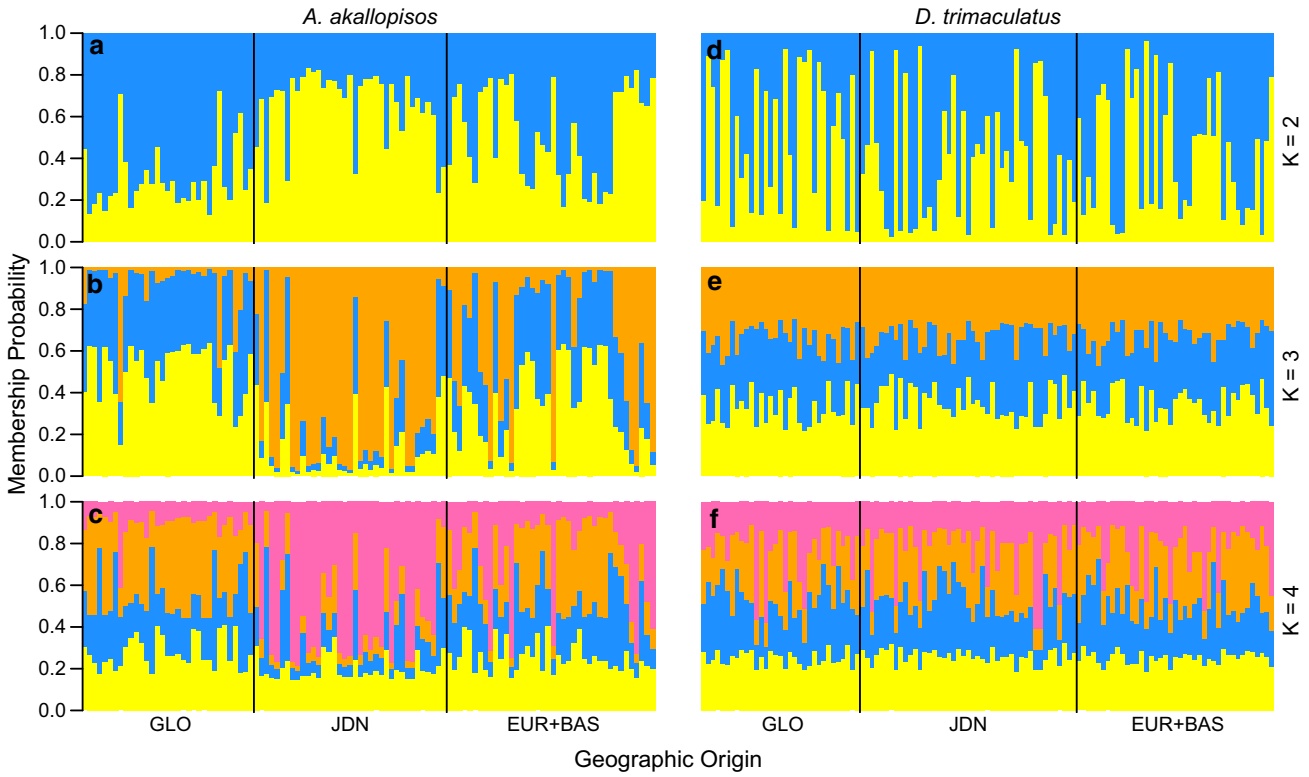

Fig. 2 Plots representing output of Bayesian clustering algorithm implemented by STRUCTURE for *Amphiprion akallopisos* (**a**, **b**, **c**) and *Dascyllus trimaculatus* (**d**, **e**, **f**). Each *vertical bar* represents an individual, and the *colors* represent the posterior probability of its membership to each cluster. The number of clusters selected ($K = 2$, 3, and 4) is indicated to the right. Populations are separated by *black vertical bars* as Îles Glorieuses (GLO), Juan de Nova (JDN), and Europa + Bassas da India (EUR + BAS)

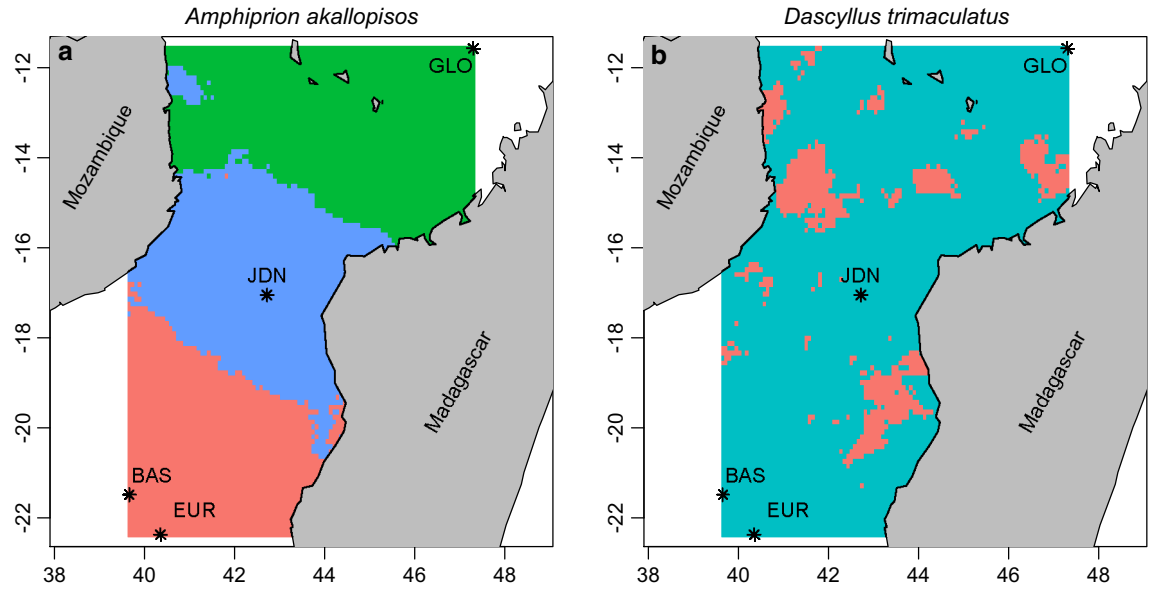

Fig. 3 Maps representing the output of GENELAND for *A. akallopisos* (**a**) and *D. trimaculatus* (**b**). The 100 by 100 grid cells are *colored* by modal estimated cluster membership based on posterior probabilities of 1 million iterations. This represents the dataset after removal of individuals for which no data were available for at least one locus. A preliminary analysis using individuals with missing data (not shown) yielded qualitatively similar results, but did not appropriately sample the cluster parameter or establish convergence after multiple runs

optimum *K* of 3 and 4, respectively. DAPC assigned nearly all individuals to one of the genetic clusters with almost complete certainty, while the Bayesian approaches generally split the uncertainty when the number of clusters was greater than the most likely, as expected.

Discussion

We found discordant patterns of population connectivity between two damselfish species that, while closely related, differ in strategies at both the larval and adult stage. Notably, the pattern of genetic structure over our study's scale differs from a prediction of simple isolation by distance and from previously defined eco-regions yet resemble the patterns from other fish species in the area.

Population structure patterns

While we found no evidence of genetic structure among any of the Îles Éparses for *D. trimaculatus,* the species with the longest PLD, low levels of genetic structure were revealed in *A. akallopisos* based on any and all metrics used in this study. These results were consistent using metrics of population differentiation (F_{ST}, R_{ST}, G'_{ST}, and Jost *D*), as well as Bayesian clustering analyses.

The shorter PLD (7–22 d) and more restricted adult movement of *A. akallopisos* adults might have played a determinant role in the genetic differentiation found across islands. Our results for *D. trimaculatus* with a longer PLD are also consistent with the lack of structure found in species from previous studies. Indeed, *D. trimaculatus* exhibits a PLD (20–30 d) that is similar or shorter than the three other species studied in the region, *L. kasmira* (30 d), *E. merra* (30 d), and *M. berndti* (55 d) (Muths et al. 2011, 2012, 2015). These three species are broadcast spawners, with both eggs and larvae being pelagic, while both species included in our study are benthic spawners (laying eggs directly on the substrate, like all pomacentrids) with pelagic larvae. While egg type and nesting strategy have been evoked to predict population structure in marine fishes, our results do not indicate a significant difference in structure between the broadcast spawners *L. kasmira*, *E. merra*, and *M. berndti* and the benthic spawning *D. trimaculatus* with a similar PLD.

Similar to our results for *A. akallopisos*, higher F_{ST} values between Îles Glorieuses and Juan de Nova compared to those between Îles Glorieuses and Europa were reported for the soldierfish *M. berndti* (Muths et al. 2011). This pattern contrasts with the eco-regions proposed by Spalding et al. (2007) and reviewed by Obura (2012). These regions are delimited on the basis of differences in community composition driven by taxonomic turnover at or above the species level, implicating a barrier that creates or maintains the range limit of many taxa. Other species' ranges span such boundaries, but may or may not exhibit genetic differentiation. A lack of population genetic differentiation across eco-regions indicates that the forces driving population connectivity differ from those responsible for community-level patterns. Studies of such cases

can help identify the processes that are important in maintaining diversity across and within spatially isolated communities. But perhaps even more interesting than the lack of concordance with eco-regions is that individuals from a site situated between the others (Juan de Nova) are assigned to a different genetic cluster than those on either side.

Oceanography and connectivity

One factor that may explain this unusual pattern of disjunct genetic structure is the complex oceanographic features of the region (see Fig. 1 in Schouten et al. 2003), which will influence the fate of the migrating larvae between the islands. The Mozambique Channel lies in the shadow of the south equatorial current, and while previously thought to be dominated by the southward flowing Mozambique Current (Sætre 1985), it has now been shown that strong eddies form within the channel approximately four times per year (Schouten et al. 2003). Though the sites included in our study are small and isolated, these eddies may serve to enhance self-recruitment, especially for species with short PLDs, and therefore increase genetic structure among sites. Oceanographic features such as seasonal eddies have been shown to drive genetic connectivity of reef fishes elsewhere (e.g., Beldade et al. 2014). The eddies in the narrowest part of the Mozambique Channel near Juan de Nova may increase retention rates of larvae with short durations, such as *A. akallopisos*. Both species in this study are known to spawn approximately twice per lunar cycle, increasing the likelihood that larvae are distributed during periods of both eddy presence and absence.

The Scattered Islands are conservation priorities because of their species richness, isolation, and very low anthropogenic pressure. The present study and recent work in this region encompassing a total of five coral reef fish species with different life history traits (free or attached eggs, duration of the PLD, mobility as adults, life span, fecundity, etc.) will inform conservation and management plans for this region. The contrasting patterns of genetic structure and connectivity found for *M. berndti* and *A. akallopisos* showing a shallow level of genetic structure and weaker connectivity between Juan de Nova and the other Scattered islands, and *D. trimaculatus*, *L. kasmira*, and *E. merra* showing virtually no structure at this spatial scale, highlight the need for different strategies depending on the target species considered. Given the putative dispersal routes among the Îles Ésparses, it would be very interesting to determine the origin of larvae in the descending current (along Mozambique) and the northward current (along Madagascar). The proximity and likely sharing of genetic material between species in the Scattered Islands, Mozambique, and Madagascar encourages the inclusion of samples from sites on either side of the channel, as well as the comparison of these results to other species.

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