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Do differences in mating behaviour lead to differences in connectivity patterns of reef fishes? Insights from two sympatric surgeonfish species in the Indian Ocean

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ABSTRACT

Disentangling the contribution of biotic and abiotic factors in the structuring of the genetic diversity of reef species is critical to illuminate the diversification of evolutionary lineages in marine environment. Howevr, previous studies have mainly focused on determining the influence of pelagic larval duration on the connectivity and demographic history of reef fishes, whereas few studies have examined the effects of other biotic factors, such as mating behaviour and habitat preference. Here, we use mitochondrial DNA (ATPase 6/8) and ten microsatellite loci to compare the population genetic structure and demographic history of the spawning aggregating *Acanthurus triostegus* with the monogamous spawning *Acanthurus leucosternon*. Pairwise comparisons and discriminant analysis of principal components showed that the genetic structuring patterns of the two species are not consistent with the influence of mating behaviour, suggesting the possible role of other biotic and abiotic factors. However, demographic history estimates revealed that these species may have responded differently to sea level fluctuations during the glacial maxima.

1. Introduction

Understanding dispersal in the marine environment is essential because it has a profound influence on species evolution and persistence (Mora and Sale, 2002). For most shallow water marine species with a bipartite life cycle, dispersal through the pelagic larval stage represents the only mechanism of linking populations between distant sites. However, tracking dispersal in the marine environment remains a major challenge, because marine larvae are minute and suffer high rates of mortality (Sale et al., 2005). Consequently, the application of genetic markers to infer dispersal in marine organisms is increasingly a common practice (Hellberg et al., 2002; Jones et al., 2009). Because larvae of most marine species spend times ranging from days to months in the pelagic marine environment (Sale et al., 2005; Almany et al., 2007), it has long been thought that species with a long pelagic larval duration (PLD) will have a high dispersal and weak genetic structure. Indeed, previous studies have shown a correlation between PLD and gene flow (Dawson et al., 2002; Faurby and Barber, 2012; Riginos et al., 2014). However, there is a growing number of studies which demonstrate that the influence of PLD on dispersal distance is often overestimated (Barber et al., 2002; Weersing and Toonen, 2009; Selkoe and Toonen, 2011; Riginos et al., 2013). Furthermore, other features such as past biogeographic events (Barber et al., 2002; Otwoma and Kochzius, 2016), ocean currents, larval swimming ability (DiBattista et al., 2017), differences in habitat (Rocha et al., 2002), distance (Otwoma et al., 2018a), mating behaviour (Jackson et al., 2014), and local adaptation (Imron et al., 2007) have been found to profoundly affect the genetic population structure of marine species.

Comparative phylogeography offers invaluable insights into the factors that drive spatial genetic structuring in sympatric taxa (Papadopoulou and Knowles, 2016). This approach uses the concordance-discordance criterion to determine whether the genetic structure of sympatric species is impacted by abiotic or biotic factors (Papadopoulou and Knowles, 2016). The assumption of most comparative phylogeographic studies is that taxa evolving in a certain environment should respond the same way to extrinsic and intrinsic factors that cause genetic divergence. Nevertheless, co-occurring taxa often show discordant phylogeographic structure, suggesting that every species respond uniquely to environmental changes (Crandall et al., 2008; DiBattista et al., 2012; Weber et al., 2015; Puritz et al., 2017).

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According to Papadopoulou and Knowles (2016), taxon-specific traits need to be incorporated into comparative phylogeography studies, so as to provide a better understanding of the mode and rate of phylogeographic diversification. For example, Puritz et al. (2017) compared the population genetics of the planktonic-developing seastar *Meridiastra calcar* and benthic-developing dwarf cushion seastar *Parvulastra exigua* in the temperate waters of Australia and linked their divergent responses to Pleistocene glacial cycles to species-specific traits. Similarly, Weber et al. (2015) found that the brooding lineages of the brittle star *Ophioderma longicauda* display a greater genetic structure than the broadcast spawner lineage. These studies confirm that integrating species-specific traits into comparative phylogeographic tests can help in understanding the influence of biotic and abiotic factors on the genetic structuring of marine species (Papadopoulou and Knowles, 2016).

Among biotic factors that are predicted to affect the genetic structuring of marine species, significant progress has been made in our ability to understand the relationship between PLD and realized dispersal distance of marine taxa (Weersing and Toonen, 2009). However, relatively few empirical studies have investigated the relationship between gene structuring and other biotic factors, such as mating behaviour (monogamous pairing vs. spawning aggregation) and habitat preferences (generalists vs. specialists). Although there is still no clear consensus on whether mating behaviour and habitat preferences can affect the population genetics and demographic history of marine species (Craig et al., 2010; Reece et al., 2011), it is likely that their influence is governed by interactions with environmental factors such as, ocean currents, large-scale climatic variations, and geological features. In other cases, the influence of these environmental factors may even override the influence of the two biotic factors on the genetic structuring of marine species (Ayre et al., 2009).

To further test whether mating behaviour and habitat preference can predict genetic structuring and demographic history of reef species, respectively, we focus on two phylogenetically related surgeonfishes, the powder blue-tang *Acanthurus leucosternon* and convict surgeonfish *Acanthurus triostegus* (Sorenson et al., 2013). These two species, like other *Acanthurus* species, are primarily herbivores, feeding on benthic algae that inhibit coral recruitment (Randall, 1956). They are sympatric in large parts of the Indian Ocean; but have clear differences in their range-sizes (Randall, 1956). While *A. triostegus* occurs throughout the Indo-Pacific, *A. leucosternon* is mainly restricted in the Indian Ocean (Randall, 1956).

They also differ considerably in their ecological and reproductive behaviour. *Acanthurus leucosternon* is a habitat specialist that is often restricted in coral reefs (Randall, 1956). It is extremely territorial and forms monogamous pairs, which are dispersed over broad areas of the reef (Robertson et al., 1979; Kuiter and Debelius, 2001). In contrast, *A. triostegus* forms resident spawning aggregation and is a habitat generalist that can also be found inhabiting turbid waters in lagoons, bays, and harbours (Randall, 1956; Hartup et al., 2013). Generally, resident spawning aggregation sites occur on top of deep coral reef ridges that are found near the shelf edge and have specific oceanic currents or strata that enhance larval retention and survival (Colin, 1992; Heyman et al., 2005; Starr et al., 2007; Claydon et al., 2014). Previous studies have also found that connectivity between multiple spawning aggregation sites may be restricted due to philopatry and larval homing behaviour (Lobel and Robinson, 1988; Cherubin et al., 2011; Jackson et al., 2014), suggesting that substantial genetic differentiation between various spawning aggregations may describe a general pattern of spawning aggregating reef fishes (Jackson et al., 2014) but see (Zatcoff et al., 2004; Portnoy et al., 2013; Bernard et al., 2016). On the other hand, spawning sites of monogamous spawning species are usually dispersed throughout the reef such that the fertilized pelagic eggs (Robertson et al., 1979; Kuiter and Debelius, 2001) and larvae may be exposed to average ocean currents conditions that could facilitate long-distance dispersal (Portnoy et al., 2013). Thus, assuming that the site fidelity and larval retention associated with spawning aggregation does limit dispersal; species forming spawning aggregations would be expected to have lower connectivity patterns than monogamous pairing spawners (Portnoy et al., 2013; Jackson et al., 2014).

Sea level fluctuations during the Pleistocene are suspected to have primarily influenced the demographic histories of marine taxa, including crabs (He et al., 2010), gastropods (Crandall et al., 2008), corals (Woodroffe et al., 2010), and fish (Craig et al., 2010; Ludt et al., 2012). However, species with narrow niches (habitat specialists) such as *A. leucosternon* may have been more sensitive and likely to experience population declines than their congeners with high ecological plasticity (habitat generalists) such as *A. triostegus*, when sea level dropped during the glacial maxima (Crandall et al., 2008; Craig et al., 2010; Ludt et al., 2012). This is because habitat generalists with broad niches have usually better chances to survive in adverse conditions than habitat specialists.

In this study, we compared the population genetic structure of *A. leucosternon* and *A. triostegus*, to determine whether reproductive mating behaviour has an effect on the genetic structuring of these reef fishes. Given that PLD estimates among *Acanthurus* species are not remarkably different (maximum = 70 days: Thresher, 1984; McCormick, 1999; Rocha et al., 2002), we expected greater genetic structuring among populations of *A. triostegus* than *A. leucosternon* in the Indian Ocean, if spawning aggregation in the former does enhance larval retention. However, if the long PLD suffice to ensure large-scale dispersal, then we predict similar geographic genetic homogeneity in the two species whose adults differ in reproductive mating behaviour. In addition, we reconstructed the demographic history of *A. triostegus* and *A. leucosternon* to determine whether habitat preferences played a role in shaping their present phylogeographic structure. If habitat preference did influence the species response to sea level fluctuations during the Pleistocene, then these two species should exhibit different demographic history.

2. Materials and methods

2.1. Sampling and DNA extraction

Samples of adult *A. triostegus* and *A. leucosternon* were collected at 15 locations in the Indian Ocean, between 2011 and 2015 (Fig. 1). Fin clips from individual fishes were obtained from local fishermen and stored in 96% ethanol or saturated salt-DMSO solution. DNA extraction was done following the standard salting-out protocol (Sunnucks and Hales, 1996).

2.2. Amplification and sequencing of mitochondrial DNA fragment

A partial fragment spanning the mitochondrial ATPase8 and ATPase6 gene regions was amplified from 179 *A. leucosternon* and 94 *A. triostegus* through the polymerase chain reaction (PCR) using ATP8.2 (5AAAGCRTYRGCCTTTTAAGC 3′) and CO3.2 (5′ GTTAGTGGTCAKGGGCTTGGRTC 3′) primers (Lessios and Robertson, 2006). The PCR reactions were conducted according to the original protocol (Lessios and Robertson, 2006). Purification of the PCR products was done by incubating with exonuclease and alkaline phosphatase (both from Thermo Scientific), following the manufacturer's protocol. Thereafter, sequencing was performed using DyeDeoxy terminators (Applied Biosystems) and an automatic sequencer (ABI PRISM 310 and 3100, Applied Biosystems). The new ATPase dataset of 94 *A. triostegus* was supplemented with 75 published Indian Ocean sequences from Liggins et al. (2016) [49 sequences: GenBank accession numbers KJ779682-KJ779696, KJ779801-KJ779818, and KJ77840-KJ779855] and Otwoma et al. (2018a) [26 sequences: GenBank accession numbers MF139586-MF139611] (Table 1).

Because of the close proximity of sampling stations along the Kenyan and Tanzanian coastlines (Fig. 1), only sub-samples comprising of the key sampling stations was used in the ATPase analysis.

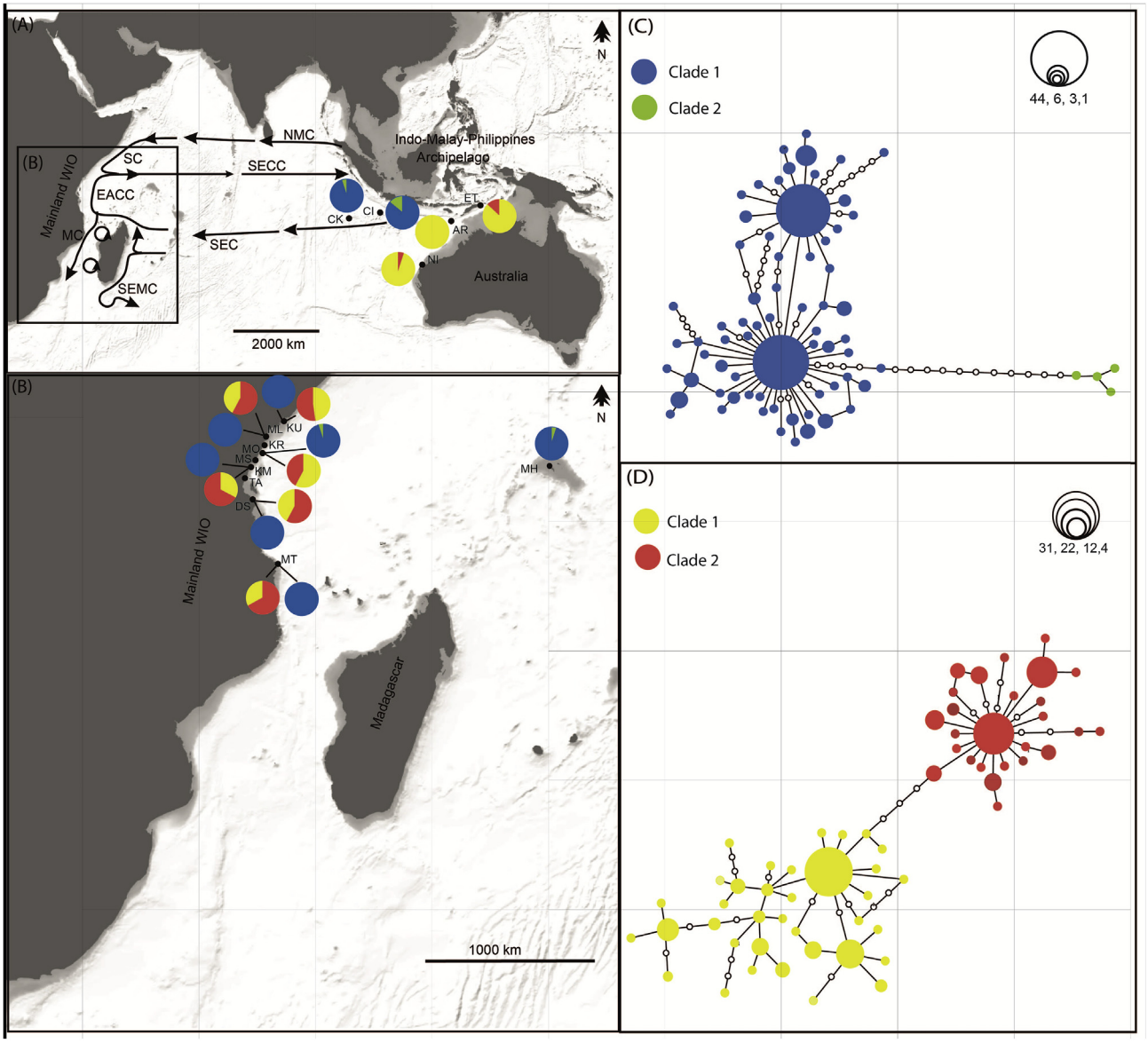

Fig. 1. Maps showing *A. leucosternon* and *A. triostegus* sample sites in (A) the Eastern Indian Ocean (EIO), (B) Western Indian Ocean (WIO), and dominant surface ocean currents (For sample sites abbreviations see Tables 1 and 2). Panel C: The four specimen forming clade 2 (green dots) are cryptic hybrids (*A. leucosternon* x *A. nigricans*) as shown in supplemental Figure A3. NMC; Northeast Monsoon Current, SECC; South Equatorial Counter Current, SEC; South Equatorial Current, SEMC; Southeast Madagascar Current, MC; Mozambique Current, EACC; East African Coastal Current, and SC; Somali Current (Schott and McCreary, 2001). Haplotype networks for (C) *A. leucosternon* and (D) *A. triostegus* constructed from 785bp fragment spanning the ATPase6 and ATPase8 gene regions. Large circles and lines represent haplotypes and one mutational step, respectively, while small circles represent intermediate missing haplotypes. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2.3. Amplification and genotyping of nuclear microsatellites

Genomic DNA of 320 *A. triostegus* was amplified through PCR, using 10 published microsatellite loci: Ahy49, Ahy54, Ahy65, Ahy75, Ahy112, Ahy119, Ahy170, Ahy178, Ahy182, and Ahy203 (DiBattista et al., 2011). PCR reactions and conditions followed the protocol described by Otwoma et al. (2018b). Labelled PCR products were pooled for genotyping and resolved on ABI 3730 genetic analyser alongside a labelled internal size standard (AlexaFluor 660 (IBA GmbH) following, DeWoody et al. (2004). Microsatellite allele sizes were manually scored using Geneious version 8.1.6 (Kearse et al., 2012). From all scored genotypes, those from six loci (Ahy54, Ahy65, Ahy75, Ahy112, Ahy182, and Ahy203) were discarded due to low amplification success in > 95% of the samples. Genotyping of the remaining four loci was repeated for 80 randomly chosen individuals to check for possible misamplification and scoring errors. All microsatellite dataset of *A. leucosternon* specimens was obtained from a previously published study (Otwoma et al., 2018b) (Table 2).

It should be noted that only samples from nine WIO samples sites were genotyped (Table 2), because the remainder of the samples either became available at a later stage of the study (Seychelles, Christmas Island, and Cocos-Keeling) or were unavailable to us (Ningaloo, Ashmore Reef, and East Timor).

2.4. Data analysis

2.4.1. ATPase

ATPase sequences were assembled and trimmed using Geneious. Thereafter, newly-generated sequences were deposited in GenBank. Arlequin version 3.5 (Excoffier and Lischer, 2010) was used to calculate

haplotype and nucleotide diversities at each sampling location for each species. Genetic differentiation among and between sample sites was tested using single-level analysis of molecular variance (AMOVA), hierarchical AMOVA, and pairwise comparison in Arlequin. All analyses were permuted 10,000 times at a significance level of 0.05. We used the online IBDWS services to test the relationship between geographic distance and all Indian Ocean pairwise Φ_{ST} estimates in both species. The sequential Bonferroni correction was used to adjust the confidence interval of all analysis involving multiple tests (Rice, 1989).

Corrected Akaike Information Criterion (AICc) implemented in jModelTest version 2.1.9 (Darriba et al., 2012) indicated the HKY + G to be the best substitution model for the data set for both species. The neutral evolution of the ATPase marker was tested by Fu, 1997 F_S test for each species. Significant negative Fu's F_S values indicate either selective sweeps, purifying selection or population expansion (Fu, 1997). The signature of population expansion after a bottleneck was confirmed by comparing simulated and observed mismatch distribution in Arlequin (Fu, 1997; Schneider and Excoffier, 1999). A unimodal mismatch distribution indicates a population that has undergone a recent and fast demographic expansion, while a multimodal mismatch distribution suggests a population under demographic equilibrium. The Bayesian Skyline Plot (BSP) in BEAST version 1.8.4 (Drummond and Rambaut, 2007) was used to examine changes in female effective population size (Nef) through time. The BSP analyses were run under HKY + G substitution models for both species, employing a strict clock. We used the ATPase 8 and 6 average within species substitution rate of 1.3×10^{-8} per site per year (Lessios and Robertson, 2006) under a uniform prior distribution. The program Tracer version 1.5 was employed to visualize the BSP (Drummond et al., 2005).

Newly-generated and all publicly available ATPase6/8 sequences from the genus *Acanthurus* (*A. line*atus: EU273284.2, *A. leucosternon*: EU136032, *A. nigricans* (32): DQ111095.1-DQ111126.1, *A. triostegus* from Pacific Ocean (179): KJ779697.1-K779800.1, KJ779819.1-KJ779840.1, KJ779871.1-KJ779856.1, DQ111127.1-DQ111163.1) were aligned using Mafft (Katoh et al., 2002) with the default options (*-linsi*) and using *Paracanthurus hepatus* (GenBank: KT826539.1) as an outgroup. The resulting alignment of 561 sequences was trimmed to the same length of 785 bp in BioEdit (Hall, 1999). The software ALTER (Glez-Pena et al., 2010) was used to collapse identical haplotypes resulting in the final alignment of 217 unique sequences. Subsequently, a phylogenetic tree was constructed using MrBayes version 3.2.6 × 64 (Huelsenbeck and Ronquist, 2001) with priors being set according to the suggested HKY model with a gamma distribution. Four Markov chains (three heated and one cold), searching from a random starting tree, were run in parallel. All eight chains were run simultaneously for 10 million generations, sampling every 1000 generations. The first 25% of the trees were discarded as burn-in after confirming convergence of likelihood values of each chain using the command *sump*. The majority-rule consensus tree with posterior probabilities was determined from the remaining 60,002 trees using the command *sumt conformat = simple* and visualized in Mega 6.0 (Tamura et al., 2013).

For each species, a haplotype network of Indian Ocean sequences was constructed using the minimum spanning algorithm in the software PopART version 1.7 (Bandelt et al., 1999).

2.4.2. Microsatellites

Possible deviations from the expectations of Hardy-Weinberg Equilibrium (HWE) and linkage disequilibrium (LD) were examined for each locus and sample site using GENEPOP version 4.2 (Raymond and Rousset, 1995; Rousset, 2008). Micro-checker version 2.2.3 (van Oosterhout et al., 2004) was used to screen for the presence of null alleles and large allele dropout. For each sample site, the mean number of alleles (Na), expected heterozygosity (He), observed heterozygosity (Ho), and private alleles were estimated in GenAlex version 6.5 (Peakall and Smouse, 2012). The average allelic richness (Ar) and inbreeding coefficient (F_{IS}) were calculated for each sample site using FSTAT version 2.9.3.2 (Goudet, 1995).

The hypothesis of homogeneous allele frequency and genotype distributions among sample sites was tested using FreeNA (Chapuis and Estoup, 2007). FreeNA was chosen because it uses the ENA (Excluding Null Alleles) method to provide for an accurate estimation of F_{ST} in the presence of null alleles (Chapuis and Estoup, 2007). Additionally, the relationship between genotypes and geographical locations was evaluated using the discriminant analysis of principal components (DAPC) in Adegenet version 2.0.2 (Jombart et al., 2010). Unlike Bayesian clustering methods, DAPC can be performed in situations where the assumptions of HWE and LD have not been met. The sequential Bonferroni correction was used to adjust the confidence interval of all analysis involving multiple tests (Rice, 1989). The relationship between linear geographic and genetic distance was evaluated using a Mantel (1967) test in GenAlex for both species. The distance between sampling locations was measured to the nearest 5 km in Google Earth.

3. Results

3.1. Genetic diversity

A total of 179 *A. leucosternon* and 169 *A. triostegus* individuals were analysed. The sequence alignments were trimmed to 785 bp for both species, revealing 72 and 62 unique haplotypes in *A. leucosternon* and *A. triostegus*, respectively. Haplotype diversity was almost similar between the two species, ranging from 0.8 to 0.98 (across all sample sites = 0.89) in *A. leucosternon* and 0.71 to 1 (across all sample sites = 0.94) in *A. triostegus* sampling sites. Nevertheless, the overall nucleotide diversity was twofold higher in *A. triostegus* than *A. leucosternon* (0.0074 vs 0.0034). A two-sample *t*-test confirmed the significant difference between the nucleotide diversities of the two species ($t = 2.11$, $df = 16$, $P = 0.0006$) (Table 1).

The haplotype network revealed two clades for both species (Fig. 1; Fig. S3; and Fig. S4) but the phylogenetic analysis showed almost all individuals of *A. leucosternon* as members of clade 1, while clade 2 comprised of *A. leucosternon* individuals with introgressed *A. nigricans* genes. In *A. triostegus*, clade 1 is found in both EIO (Eastern Indian Ocean) and WIO, while clade 2 is mainly dominant in the WIO and only appears at a lower frequency in the EIO.

All the ten loci amplified successfully in 305 *A. leucosternon*, while only four (Ahy 49, Ahy 119, Ahy 170, and Ahy 178) amplified consistently in 320 *A. triostegus*. After Bonferroni correction, one out of 36 loci in *A. triostegus* and 19 out of 90 loci in *A. leucosternon* deviated from the expectations of HWE. Analysis in Micro-checker suggested that deviations at five markers (one in *A. triostegus* [Ahy170 (Tanga)] and four in *A. leucosternon* [Ahy 54 (all populations), Ahy 75 (Malindi, Kuruwitu, Kisite-Mpunguti, and Kiunga) Ahy 182 (Mombasa, Tanga, and Kiunga), and Ahy 203 (Kisite-Mpunguti, Tanga, and Kiunga]) could be due to the presence of null alleles. Nevertheless, there was no evidence of linkage disequilibrium between the loci in both *A.triostegus* and *A. leucosternon* datasets. The mean allelic richness varied between 9.03 (Kuruwitu) and 10.9 (Dar es Salaam) in *A. leucosternon*, and between 5.75 (Tanga) and 6.53 (Mtwara) in *A. triostegus*. Observed and expected heterozygosity in *A. leucosternon* (Ho = 0.81–0.88 and He = 0.84–0.89) were slightly higher than those of *A. triostegus* (Ho = 0.63–0.85 and He = 0.66–0.73) (Table 2).

3.2. Genetic differentiation

Analysis of molecular variance (AMOVA) based on the ATPase marker indicated genetic homogeneity among the samples of *A. leucosternon* ($\Phi_{ST} = -0.0047$, $P = 0.72$) and *A. triostegus* ($\Phi_{ST} = 0.0035$, $P = 0.35$) in the WIO. Correspondingly, pairwise comparisons between and among WIO locations were all non significant for both species (Tables 3 and 4). However, AMOVA involving all the Indian Ocean locations, WIO and EIO, revealed significant Φ_{ST} value ($\Phi_{ST} = 0.15$,

Table 1
Genetic diversity of *A. leucosternon* and *A. triostegus* deduced from a fragment spanning a 785 bp gene region of ATPase8 and ATPase6. (n) the number of sequences, (Nhp) number of haplotypes, (h) haplotype diversity, (π) nucleotide diversity, Fu's F_S, (SSD) sum of square deviations, (HRI) Harpendig's raggedness index, [#] = data taken from Liggins et al. (2016), and [$] = data taken from Otwoma et al. (2018a).

Location	code	Biogeographical region	n	Nhp	h	π	Fu's F_S	SSD	HRI
Acanthurus leucosternon									
Kiunga	KU	WIO	25	14	0.86	0.0035	−7.27*	0.133^{*}	0.025^{ns}
Malindi	ML	WIO	21	15	0.94	0.0035	−10.54*	0.002^{ns}	0.033^{ns}
Mombasa	MO	WIO	20	9	0.80	0.0034	-2.16^{ns}	0.006^{ns}	0.047^{ns}
Kisite-Mpunguti	KM	WIO	19	10	0.84	0.0022	−5.71*	0.004^{ns}	0.071^{ns}
Dar es Salaam	DS	WIO	15	13	0.98	0.0037	−9.88*	0.023^{ns}	0.101^{ns}
Mtwara	MT	WIO	25	15	0.89	0.0026	−11.31*	0.004^{ns}	0.064^{ns}
Mahe	MH	WIO	25	13	0.88	0.0039	−4.99*	0.008^{ns}	0.049^{ns}
Cocos-Keeling Island	CK	EIO	22	15	0.92	0.0049	−7.16*	0.039^{*}	0.152^{ns}
Christmas Island	CI	EIO	7	6	0.95	0.0032	−2.71*	0.044^{ns}	0.224^{ns}
All sample sites			**179**	**72**	**0.89**	**0.0034**	**−26.49***	**0.002^{ns}**	**0.041^{ns}**
Acanthurus triostegus									
Kiunga	KU[$]	WIO	20	11	0.91	0.0065	-1.45^{ns}	0.039^{ns}	0.051^{ns}
Malindi	ML	WIO	19	14	0.94	0.0062	−5.72*	0.039^{ns}	0.051^{ns}
Mombasa	MO[$]	WIO	12	12	1.00	0.0084	−6.74*	0.015^{ns}	0.026^{ns}
Kisite-Mpunguti	KM	WIO	21	15	0.94	0.0076	−4.94*	0.008^{ns}	0.011^{ns}
Dar es Salaam	DS	WIO	24	20	0.99	0.0085	−10.61*	0.011^{ns}	0.013^{ns}
Mtwara	MT	WIO	24	14	0.93	0.0066	-3.49^{ns}	0.018^{ns}	0.021^{ns}
East Timor	ET[#]	EIO	16	7	0.74	0.0059	0.69^{ns}	0.052^{ns}	0.131^{ns}
Ashmore Reef	AR[#]	EIO	15	6	0.71	0.0023	-1.06^{ns}	0.059^{ns}	0.171^{ns}
Ningaloo	NI[#]	EIO	18	9	0.84	0.0048	-1.31^{ns}	0.019^{ns}	0.041^{ns}
All sample sites			**169**	**62**	**0.94**	**0.0074**	**−25.01***	**0.005^{ns}**	**0.007^{ns}**

Ns: not significant; *P ≤ 0.005 (after Bonferroni correction).

Table 2
Microsatellite genetic diversity characteristics of *A. leucosternon* (ten microsatellites loci) and *A. triostegus* (four microsatellites loci). (n) number of individuals, (Na) number of alleles, (Ne) number of effective alleles, (Ar) allelic richness, (Ho) observed heterozygosity, (He) expected heterozygosity, (PVA) private alleles, (F_{IS}) inbreeding index, and [$] = data taken from Otwoma et al. (2018b).

Location	Code	n	Na	Ne	Ar	Ho	He	PVA	F_{IS}
Acanthurus leucosternon									
Kiunga	KU[$]	25	10.3	6.62	9.08	0.82	0.86	1	0.05
Malindi	ML[$]	40	13.5	7.17	10.1	0.86	0.87	1	0.01
Kuruwitu	KR[$]	35	11.7	6.18	9.03	0.86	0.84	3	−0.02
Mombasa	MO[$]	33	13.7	1.73	10.8	0.87	0.89	0	0.02
Msambweni	MS[$]	35	13.3	7.04	10.2	0.84	0.86	3	0.03
Kisite-Mpunguti	KI[$]	51	15.0	7.21	10.3	0.82	0.86	7	0.05
Tanga	TA[$]	29	11.5	6.65	9.8	0.81	0.85	3	0.05
Dar es Salaam	DS[$]	16	11.2	6.32	10.9	0.86	0.86	2	−0.01
Mtwara	MT[$]	41	14.7	7.06	10.3	0.88	0.86	5	0.01
All sample sites		**305**	**12.7**	**6.93**	**10.1**	**0.84**	**0.86**	**25**	**0.01**
Acanthurus triostegus									
Kiunga	KU	32	10.8	5.56	6.33	0.82	0.72	1	−0.15
Malindi	ML	47	12.5	5.76	6.05	0.68	0.66	1	−0.02
Kuruwitu	KR	46	11.3	6.16	6.19	0.79	0.68	2	−0.11
Mombasa	MO	23	8.8	5.55	6.48	0.72	0.73	1	0.02
Msambweni	MS	43	11.0	6.22	6.21	0.79	0.71	4	−0.11
Kisite-Mpunguti	KI	34	12.0	5.92	6.24	0.77	0.69	3	−0.17
Tanga	TA	26	6.8	3.95	5.75	0.63	0.67	0	0.06
Dar es Salaam	DS	33	10.0	5.66	5.96	0.85	0.71	1	−0.19
Mtwara	MT	36	12.5	6.22	6.53	0.69	0.69	7	0.01
All sample sites		**320**	**10.6**	**5.67**	**6.31**	**0.75**	**0.69**	**20**	**−0.07**

Ns: not significant; *P ≤ 0.005 (after Bonferroni correction).

P < 0.0001) among samples of *A. triostegus*, but this remained non significant in *A. leucosternon* (Φ_{ST} = −0.00067, P = 0.49). Hierarchical AMOVA and pairwise comparisons (Table 3) suggested that the heterogeneity in *A. triostegus* Indian Ocean sampling sites was due to the differentiation between EIO and WIO (Φ_{CT} = 0.27, P = 0.01). The relationship between genetic and geographic distances indicated a significant pattern of isolation-by-distance in *A. triostegus* (r^2 = 0.75 P < 0.0001), but not in *A. leucosternon* (r^2 = 0.0082, P = 0.59).Fig. S2).

For microsatellites, ENA corrected estimates from the AMOVA revealed low but significant F_{ST} values among WIO sampling locations of *A. leucosternon* (F_{ST} = 0.0025 P < 0.001) and *A. triostegus* (F_{ST} = 0.011 P < 0.001). Nevertheless, the majority of the variation was explained by differences within locations (*A. leucosternon* 99% and *A. triostegus* 95%). For *A. leucosternon*, the ENA corrected pairwise F_{ST} estimates ranged from 0 to 0.0081 and were all non significant after Bonferroni adjustment (significance level = 0.001) (Table S1).

For *A. triostegus*, the ENA corrected pairwise Fst estimates ranged between 0 and 0.0127, with only one pairwise comparison (between Malindi and Kuruwitu) remaining significant after Bonferroni adjustment (Table S2). The DAPC assignment also supported the lack of significant spatial structure among the WIO sample sites in both species (K = 1, Fig. 2). Similarly, the isolation-by-distance test using all the nine WIO samples sites analysed with microsatellites was not significant in both species (*A. triostegus* r^2 = 0.03 P = 0.28 and *A. leucosternon* r^2 = 0.07 P = 0.15) (data not shown), rejecting the hypothesis of distance restricted dispersal in the WIO region.

3.3. Demographic analysis

For *A. leucosternon*, the neutral evolution of the ATPase marker was rejected for all sample sites, with the exception of Mombasa in the WIO. On the contrary, negative and significant Fu's F_S values were only revealed in five out of nine *A. triostegus* sampling sites. However, the mismatch distribution analysis, using both the SSD and HRI goodness-of-fit, indicated that the model of sudden population expansion could not be rejected for all the Indian Ocean populations of both species (Table 1). Similarly, BSP did not support a constant Ne (female effective population size), indicating a population expansion that began ~ 200,000 years ago in *A. leucosternon* (Late Pleistocene) and < 300,000 years ago in *A. triostegus* (Mid-Pleistocene) (Fig. 3).

4. Discussion

The present study investigated the genetic population structure of *A. triostegus* and *A. leucosternon*, to determine whether differences in their

Table 3
Pairwise comparison among Indian Ocean populations of *A. triostegus* based on ATPase derived Φ_{ST} estimates. For sample site, abbreviations see Tables 1 and 2

	KU	ML	MO	KM	DS	MT	ET	AR
ML	0.007^{ns}							
MO	0.048^{ns}	0.049^{ns}						
KM	0.009^{ns}	-0.028^{ns}	0.046^{ns}					
DS	-0.001^{ns}	-0.015^{ns}	-0.026^{ns}	-0.009^{ns}				
MT	0.007^{ns}	-0.022^{ns}	0.076^{ns}	-0.025^{ns}	0.006^{ns}			
ET	$\mathbf{0.191}^{*}$	$\mathbf{0.286}^{*}$	0.095^{ns}	$\mathbf{0.237}^{*}$	$\mathbf{0.146}^{*}$	$\mathbf{0.275}^{*}$		
AR	$\mathbf{0.315}^{*}$	$\mathbf{0.454}^{*}$	$\mathbf{0.272}^{*}$	$\mathbf{0.396}^{*}$	$\mathbf{0.288}^{*}$	$\mathbf{0.428}^{*}$	0.092^{ns}	
NI	$\mathbf{0.241}^{*}$	$\mathbf{0.359}^{*}$	$\mathbf{0.173}^{*}$	$\mathbf{0.312}^{*}$	$\mathbf{0.214}^{*}$	$\mathbf{0.346}^{*}$	-0.023^{ns}	-0.002^{ns}

Ns: not significant; *P ≤ 0.0014 (after Bonferroni correction).

mating behaviour could lead to differing connectivity patterns in the Indian Ocean. Based on our results, the genetic structuring patterns showed in *A. triostegus* and *A. leucosternon* at both local and broad geographical scale were not consistent with the influence of mating behaviour, suggesting the possible role of other biotic and abiotic factors. Nevertheless, attempts to determine whether habitat preferences played a role in shaping their present demographic histories revealed that *A. triostegus* and *A. leucosternon* may have responded differently to sea level fluctuations during the glacial maxima.

4.1. WIO connectivity

Contrary to expectations, pairwise comparisons and DAPC showed that both species exist as single panmictic populations in the WIO, rejecting the hypothesis that populations of *A. triostegus* are more structured than *A. leucosternon*. Similar patterns of connectivity in these two *Acanthurus* species can be explained by two common factors. First, the long PLD and year-round spawning of acanthurids (Randall, 1956; Thresher, 1984; Craig, 1998; McCormick, 1999; Rocha et al., 2002) could expose the larvae of these two species to the full spectrum of the prevailing ocean currents in the WIO, promoting long-distance dispersal. Interestingly, almost all the WIO sample sites are located in the vicinity of the permanent north-flowing East African Coastal Current (EACC), which flows faster (mean velocity of EACC = 100 cm/s; Swallow et al., 1991) than the average swimming speed of *A. triostegus* (55.7 cm/s) or other *Acanthurus* species larvae (24.7 cm/s) (Leis and Carson-Ewart, 1997). This suggests that the effect of ocean currents (e.g., EACC) could override the influence of other factors in determining the dispersal distances of larvae for both species. Second, the linear arrangements of coral reef habitats along the Eastern African coastline may act as stepping stones for active larval dispersal (through directed larval swimming) between the different sampling locations or multiple spawning aggregations, leading to genetic connectivity within the two acanthurid populations. However, such a dispersal mechanism often results in isolation-by-distance, which was not detected in our microsatellite datasets for the two species. Nonetheless, the magnitude of microsatellite F_{ST} and mtDNA Φ_{ST} values revealed by the overall AMOVA in the WIO were far higher for *A. triostegus* (F_{ST} = 0.01 and Φ_{ST} = 0.0035, P = 0.35) than for *A. leucosternon* (F_{ST} = 0.0025 and Φ_{ST} = −0.0047, P = 0.72), indicating there are additional factors that might affect dispersal that differs between these two species. Previous studies on other shallow water marine species have also shown a lack of genetic differentiation between multiple spawning aggregations (Bernard et al., 2016; Carson et al., 2011; Portnoy et al., 2013; Shaw et al., 2010; Zatcoff et al., 2004 but see Jackson et al., 2014).

4.2. Indian Ocean connectivity

The survey of the two surgeonfishes across the Indian Ocean (EIO and WIO) reveal a divergent population structure. Populations of *A. triostegus* display significant genetic differentiation in the Indian Ocean, while *A. leucosternon* exhibits no genetic structure. Although these results are generally consistent with our predictions that *A. triostegus* will have a higher genetic differentiation than *A. leucosternon*, it seems unlikely that these differences stem from behaviour related to their mating strategies. Spawning aggregation events in *A. triostegus* draw individuals to a spawning site located approximately 2 km away from the adult home range (Robertson et al., 1979; Claydon et al., 2014), suggesting that each sampling location analysed for this species (in the present study) may represent a spawning aggregation site. Therefore, if the signature of genetic differentiation in *A. triostegus* is driven by fidelity to spawning aggregation sites, we would expect spatial genetic differences between nearby, as well as distant sampling locations. These expectations are contradicted by *A. triostegus* pairwise comparison (Table 3) estimates, which show that most of the significant pairwise Φ_{ST} values were between distant sites (between EIO and WIO sampling localities), rather than within biogeographical regions.

A more feasible explanation for the disparity in the phylogeographic structure could be that the 2 species differ in their larval swimming capabilities. Leis and Carson-Ewart (1997) determined the average swimming speed of *A. triostegus* larvae (55.7 cm/s) to be twofold higher than that of other *Acanthurus* species (24.7 cm/s). Given that East Timor, Ashmore Reef, Christmas Island, and Cocos-Keeling are located in the slow flowing South Equatorial Current (6.5° S - 12° S, mean velocity = 20–24 cm/s) (Schott and McCreary, 2001; Lumpkin and Johnson, 2013), it is possible that the larvae of *A. triostegus* interacting with this current have the potential to limit their dispersal distances, while *A. leucosternon* larvae may be transported to the WIO. The finding

Table 4
Pairwise comparison among Indian Ocean populations of *A. leucosternon* based ATPase derived Φ_{ST} estimates. For sample site, abbreviations see Tables 1 and 2.

	KU	ML	MO	KM	DS	MT	MH	CI
ML	-0.012^{ns}							
MO	0.009^{ns}	-0.005^{ns}						
KM	0.015^{ns}	-0.009^{ns}	-0.016^{ns}					
DS	0.028^{ns}	0.006^{ns}	-0.003^{ns}	-0.014^{ns}				
MT	0.002^{ns}	-0.008^{ns}	-0.006^{ns}	-0.018^{ns}	0.001^{ns}			
MH	0.002^{ns}	-0.017^{ns}	-0.026^{ns}	-0.014^{ns}	-0.005^{ns}	-0.009^{ns}		
CI	-0.006^{ns}	-0.019^{ns}	-0.026^{ns}	0.002^{ns}	-0.013^{ns}	-0.011^{ns}	-0.019^{ns}	
CK	$\mathbf{0.046}^{*}$	0.023^{ns}	-0.024^{ns}	0.011^{ns}	0.014^{ns}	0.026^{ns}	-0.014^{ns}	-0.023^{ns}

Ns: not significant; *P ≤ 0.0014 (after Bonferroni correction).

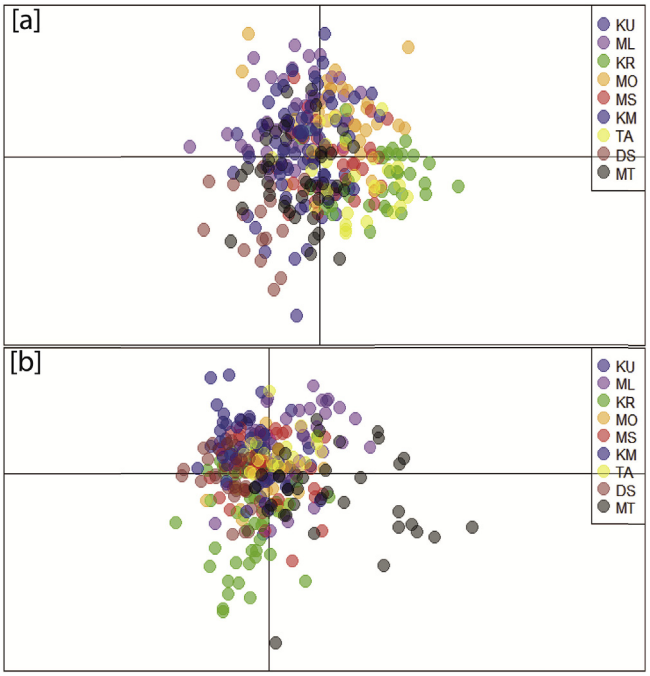

Fig. 2. Scatter plots of the Discriminant Analysis of Principal Components (DAPC) of K = 1 for (a) *A. leucosternon* and (b) *A. triostegus*, indicating a homogeneous panmictic population for each species in the Western Indian Ocean. The corresponding locations are indicated in the legend and given in Table 1.

of an isolation-by-distance signature in *A. triostegus* seems to support this prediction, indicating that its strong swimming larvae may favour dispersal between geographically near populations, while long-distance dispersal may be more sporadic (Puebla et al., 2009). This prediction of high self-recruitment in *A. triostegus* is also consistent with the genetic divergence reported between two geographically close sites (Moorea and Bora-Bora separated by approximately 259 km) in the Pacific Ocean (Planes and Fauvelot, 2002). *Acanthurus leucosternon*, on the other hand, does not exhibit a pattern of significant isolation-by-distance, possibly due to substantial long-distance dispersal. In fact, declining populations of *A. leucosternon* at Cocos Keeling and Christmas Island (Marie et al., 2007) may indicate that long-distance dispersal (passive dispersal) exceeds self-recruitment (active dispersal) at these sites, because the latter is usually required to sustain stable populations at a given location (Cowen et al., 2006). In general, findings on the Indian Ocean scale are consistent with emerging empirical and biophysical models, which suggest that active larval dispersal favour philopatry, larval retention, and self-recruitment (Jones et al., 1999; Cowen et al., 2000; Gerlach et al., 2007; Burgess et al., 2016). Nevertheless, without direct estimates of larval dispersal in *A. triostegus* and *A. leucosternon*, this hypothesis remains largely speculative.

The phylogenetic analysis revealed two clades for each species. In *A. triostegus*, clade 1 is distributed throughout the Indian Ocean, while clade 2 is dominant in the WIO and occurs at a lower frequency in the EIO. The dominance of clade 2 in the WIO could suggest that it developed there, after a long-term absence of gene flow between the EIO and WIO. However, its occurrence in the EIO (at lower frequencies) and the wide-distribution of clade 1 in the Indian Ocean, suggest that separation between EIO and WIO populations of *A. triostegus* was not absolute. In *A. leucosternon*, the two clades are not geographically-restricted. Clade 1 is dominant in all sampling locations, while Clade 2 is rare and appears to be individuals with introgressed *A. nigricans* genes as shown in Fig. 1, Fig. S3, and Fig. S4. The occurrence of clade 2 at Mombasa and Mahe in the WIO is consistent with available evidence, suggesting that the introgression of *A. leucosternon* with *A. nigricans* genes is more widespread (DiBattista et al., 2016; Otwoma et al., 2018b) than previously thought and may result in the merging of the two species into one (Marie et al., 2007).

4.3. Demographic history

Both species experienced demographic expansion that dates back to the mid-Pleistocene period when sea-level fluctuations profoundly affected habitat availability (Lambeck and Chappell, 2001; Lambeck et al., 2002). In the Indian Ocean, reef habitats may have been reduced by approximately 90%, when the sea level dropped up to 130 m below present levels (Ludt and Rocha, 2014). This loss of habitat could have restricted the population growth of *A. triostegus* and *A. leucosternon*, which may have started to expand after the habitats were restored as the sea-level rose. However, the demographic expansion seems to have been more dramatic and recent in *A. leucosternon* (expansion time ~ 200,000 years ago: mid-Pleistocene) than in *A. triostegus* (expansion time < 300,000 years ago: mid-Pleistocene), possibly due to the differences in species-specific habitat requirements. Unlike *A.*

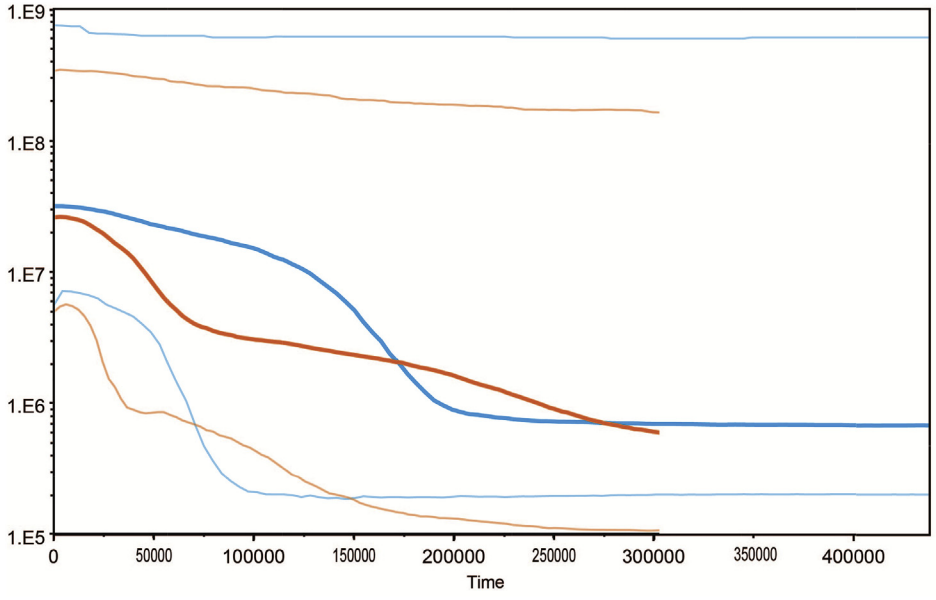

Fig. 3. Bayesian skyline plot based on ATPase sequences showing the female effective population size (Nef) fluctuation throughout time. Solid lines: median estimations; transparent lines: 95% confidence interval; Blue = *A. leucosternon*, Orange = *A. triostegus*. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

leucosternon, which is often restricted to coral reef habitats, *A. triostegus* can be found inhabiting turbid waters in bays, harbours, and tide pools (Randall, 1956; Robertson et al., 1979). According to Kotiaho et al. (2005), species with narrow niche breadth are usually sensitive to habitat disturbance and face a higher risk of extinction. It is, thus, possible that the strict dependence of *A. leucosternon* on coral reefs may have caused its population expansion to lag until suitable habitats were available. In contrast, the older expansion time in *A. triostegus* suggests that it may have been able to colonize the unstable and low-quality habitats that became available immediately when the sea-level started to rise. This inference is supported by the findings of higher nucleotide diversity in *A. triostegus* than in *A. leucosternon* (Table 1), which suggests that the former might have had multiple isolated populations in different refugia that came into contact as sea-level rose inflating its genetic diversity (Ludt et al., 2012).

In principle, the differences in the levels of nucleotide diversity values may also indicate divergent evolutionary histories in the two *Acanthurus* species (Delrieu-Trottin et al., 2017). *Acanthurus leucosternon* is a young species that diverged from its ancestral clade in the mid-Pleistocene (~600,000 years ago) (Sorenson et al., 2013; DiBattista et al., 2016) and low nucleotide diversity could suggest recent extinction or recolonization events in the Indian Ocean (Pellissier et al., 2014). In contrast, *A. triostegus* diverged from the *Acanthurus* and *Ctenochatus* clade in the Miocene (> 20 Million years ago) (Sorenson et al., 2013) and the high nucleotide diversity may suggest that it has had a stable and long demographic history in the Indian Ocean (Pellissier et al., 2014).

Estimates of trends in female effective population size show that the two acanthurid species have almost similar contemporary population sizes (Fig. 3) contrary to the IUCN assessment records, which indicate that *A. triostegus* might be more abundant than *A. leucosternon* (Abesamis et al., 2012; Mcllwain et al., 2012). This suggest that the BSP estimates might not give a clear answer to the question of what is the contemporary population sizes of *A. triostegus* and *A. leucosternon* and, thus should be interpreted with caution.

In conclusion, the mating behaviour seems to be of minor importance to the evolutionary history of the two acanthurids as spawning aggregations and pair spawnings are not fixed to *A. triostegus* and *A. leucosternon,* respectively. Both modes of mating behaviour (pair and aggregation spawning) are repeatedly found in species of the family Acanthuridae, sometimes triggered by population density as in *Zebrasoma scopas*, a species where both reproductive behaviours are known (Thresher, 1984). Such different mating strategies result in significant differences in testes sizes between males of each category (Robertson et al., 1979), because pair-spawning males do not compete for fertilization (but for females), whereas aggregate-spawning males invest in their gonads. Therefore, these mating strategies appear to be an adaptation to overcome reproductive constraints, but with minor or no influence on the genetic structuring of Acanthuridae species.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2019.104760.

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