Population structure of the Dory snapper, *Lutjanus fulviflamma*, in the western Indian Ocean revealed by means of AFLP fingerprinting

M. Dorenbosch[1], B.J.A. Pollux[2,4], A.Z. Pustjens[1], S. Rajagopal[1], I. Nagelkerken[1,*], G. van der Velde[1] & S.Y. Moon-van der Staay[3]

[1]*Department of Animal Ecology and Ecophysiology, Institute for Water and Wetland Research, Faculty of Science, Radboud University Nijmegen, Toernooiveld 1, NL-6525 ED, Nijmegen, The Netherlands*
[2]*Department of Aquatic Ecology and Environmental Biology, Institute for Water and Wetland Research, Faculty of Science, Radboud University Nijmegen, Toernooiveld 1, NL-6525 ED, Nijmegen, The Netherlands*
[3]*Department of Evolutionary Microbiology, Institute for Water and Wetland Research, Faculty of Science, Radboud University Nijmegen, Toernooiveld 1, NL-6525 ED, Nijmegen, The Netherlands*
[4]*Department of Plant–Animal Interaction, Centre for Limnology, Netherlands Institute of Ecology (NIOO-KNAW), Maarssen, The Netherlands*
(*Author for correspondence: E-mail: I.Nagelkerken@science.ru.nl)

Abstract

The genetic structure of spatially separated populations of the Dory snapper, *Lutjanus fulviflamma*, was investigated in seven areas along the East African coast and one area in the Comoros archipelago in the western Indian Ocean, using amplified fragment length polymorphism (AFLP). Phylogenetic and multi-dimensional scaling analyses did not show any clear clustering of individuals into the spatially separated populations. The analysis of molecular variance clearly showed that the variation was partitioned within populations and not between populations, leading to low genetic differentiation among populations. No clear relationship between genetic distance and geographic distance between populations was observed. These observations suggest that populations of *Lutjanus fulviflamma* have an open structure and are possibly genetically connected on a large geographic scale in the western Indian Ocean.

Introduction

Many coral reef fish species have a pelagic larval stage, and it is thought that dispersal between populations of these fishes predominantly occurs during this life stage. When larvae passively drift with the flow of ocean currents, dispersal can take place over both small and large geographic distances (Roberts, 1997). Since the degree of larval dispersal is likely to determine gene flow between populations of marine organisms (Warner, 1997), this may result in low levels of genetic differentiation between populations of reef fishes that are connected by these passive larvae (i.e., open populations). However, it has also been described that larvae of reef fishes have significant swimming capabilities and can actively migrate in reaction to physical or environmental variables (Kingsford et al., 2002; Leis, 2002; Simpson et al., 2004). Instead of randomly drifting with ocean currents they may actively select their settlement habitats relatively close to their natal areas. In this case, dispersal of these 'active' larvae is most likely to be more restricted than that of 'passive' larvae. This may give rise to a reduced gene flow and higher levels of genetic differentiation of populations of reef fishes that are characterized by these types of larvae (i.e., closed populations).

Various molecular studies testing genetic differentiation of populations of reef fishes that have pelagic larvae, showed low population differentiation, suggesting open population structures (Shulman & Bermingham, 1995; van Herwerden et al., 2003; Bay et al., 2004; Geertjes et al., 2004; Rivera et al., 2004). However, other molecular studies did show significant differentiation between populations of reef fishes with pelagic larvae, suggesting that gene flow between populations of these species can also be limited (Planes, 1993; Planes et al., 1998; McCartney et al., 2003; Ramon et al., 2003; Rhodes et al., 2003; Taylor & Hellberg, 2003). Studies based on fish otoliths indicate that dispersal of larvae of some reef fishes is limited and that these larvae remain close to their natal reefs (Jones et al., 1999; Swearer et al., 1999). Planes et al. (1996) and Planes & Fauvelot (2002) showed low genetic differentiation of populations on small geographic scales but significant differentiation on large geographic scales suggesting only reduced gene flow over large distances. Since the results of genetic studies dealing with population differentiation of reef fishes are not similar, generalizations on the population structure of coral reef fishes that have pelagic larvae are still unequivocal and at present the extent to which reef fish populations are open or closed must be regarded as unknown (Leis, 2002; Mora & Sale, 2002).

In the present study, we used amplified fragment length polymorphism (AFLP) to study genetic differentiation between various populations of the snapper *Lutjanus fulviflamma* along the East African coast in the western Indian Ocean. This molecular technique provides a high resolution in resolving differences in genetic structures between populations (de Bruin et al., 2003; Garcia et al., 2004). The technique has been used successfully to analyze genetic diversity in freshwater fish (Mickett et al., 2003) but has, to our knowledge, not yet been used in coral reef fishes. *Lutjanus fulviflamma* is a common snapper species that inhabits coral reefs in the entire Indian Ocean and is assumed to have a pelagic larval stage (Leis & Rennis, 2000). We studied the genetic structure of eight populations in the western Indian Ocean along the East African coast and in the Comoros archipelago that were arranged in a 1000 km north-south gradient and that were connected by the East African Coastal Current (EACC, Richmond, 2002). Spawning of *Lutjanus fulviflamma* is most pronounced in August–September (Nzioka, 1979) and is in accordance with the period when the EACC is strongest. Three hypotheses can be formulated that may explain the genetic structure between populations of *Lutjanus fulviflamma* in the above gradient: (1) Larval exchange between populations is low and most larvae remain near their natal reefs. Gene flow between populations is therefore limited and results in genetic differentiation of populations (closed populations). (2) Larval exchange occurs on small geographic scales between neighboring populations in conjunction with the flow of the EACC. Gene flow between populations is therefore structured by the EACC and the genetic structure between populations is likely to be correlated with geographic distance resulting in isolation by distance: populations close to each other have a stronger genetic connectivity than populations situated far from each other. (3) The EACC in combination with other ocean currents in the region (e.g., South Equatorial Current and Equatorial Counter Current) result in larval exchange on large geographic scales (i.e., on the level of the western Indian Ocean). Gene flow between populations is therefore high on both a small and large scale, and ocean currents result in gene flow on the level of a large geographic region resulting in one gene pool where populations cannot be genetically distinguished. In the present study we tried to resolve at which geographic scale eight spatially separated populations of *Lutjanus fulviflamma* in the western Indian Ocean are genetically connected.

Materials and methods

Sampling and DNA isolation

A total of 77 individuals of *Lutjanus fulviflamma* were collected at seven locations along the East African coast (Kenya and Tanzania) and at one location in the Comoros archipelago (Grande Comoros, Fig. 1, Table 1). Fishes were collected using either seine nets or hook and line. Since this method is predominantly suited to catch smaller snappers in relatively shallow water, all fishes that
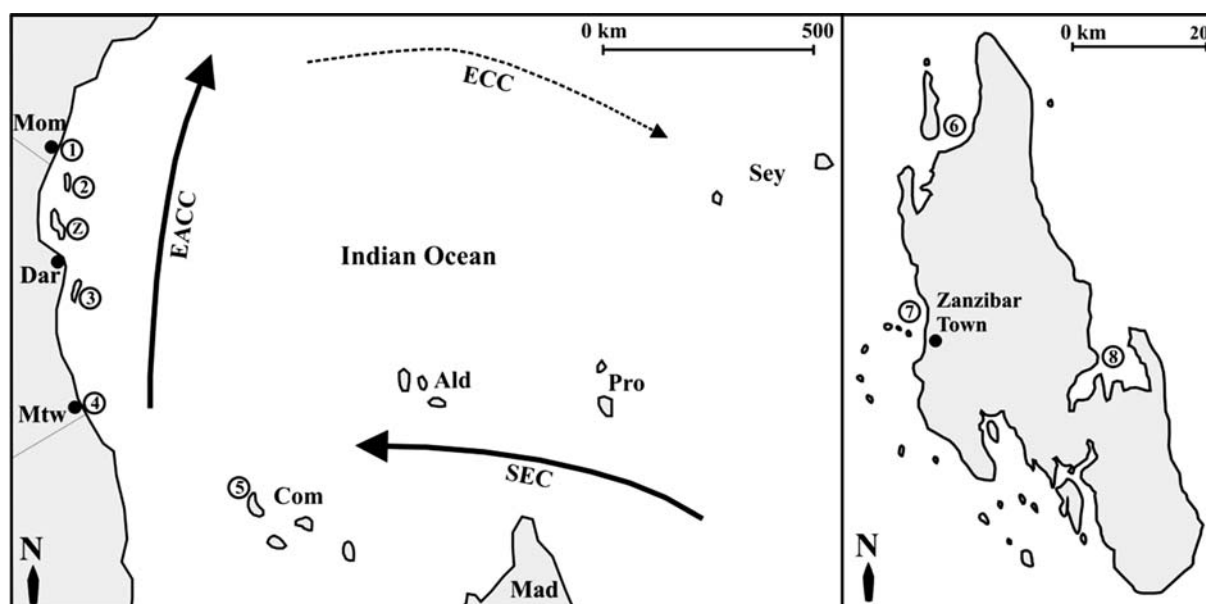
Figure 1. Sampling locations of eight populations of *Lutjanus fulviflamma* and major ocean surface current patterns, as derived from Richmond (2002) along the East African coast (Kenya and Tanzania) and the Comoros archipelago. Abbreviations: Mom: Mombasa (Kenya); Dar: Dar es Salam (Tanzania); Mtw: Mtwara (Tanzania); Com: Comoros (Grande Comoros); Ald: Aldabra; Mad: Madagascar; Pro: Providence; Sey: Seychelles. Ocean currents: EACC: East African Coastal Current; SEC: South Equatorial Current; ECC: Equatorial Counter Current (only November–March). Numbers show locations of sampled populations: (1) Mombasa; (2) Pemba; (3) Mafia; (4) Mtwara and (5) Comoros. The locations of the sampled populations on Zanzibar (Z) are shown on the detailed map: (6) Zanzibar North, (7) Zanzibar West and (8) Zanzibar East.

were collected were juveniles (fork length ranged between 5 and 20 cm). Dorsal muscle tissue of freshly killed fishes was immediately put into a saturated CTAB-20% DMSO solution and was stored at 4 °C. Total genomic DNA was isolated using the DNeasy Tissue Kit (QIAGEN Genomics Inc.). Extracted DNA was dissolved in 200 μl AE buffer (supplied) and checked for integrity by agarose gel-electrophoresis.

AFLP protocol

AFLP analysis was performed according to Vos et al. (1995) with modifications as described in de Roos (2003). Restriction–Ligation reactions were performed in a single reaction at 37 °C for 2 h, in a total volume of 10 μl containing 100 ng DNA, $1 \times$ T4 Ligase Buffer (Life Technology, Invitrogen), 0.05 M NaCl, 5U *Eco*RI – Enzyme (New England BioLabs), 5U *Mse*I – Enzyme (New England BioLabs), 0.045 M bovine serum albumine (BSA, New England BioLabs), 0.2 μM *Eco*RI – adapter (5′-CTCGTAGACTGCGTACC, CATCTGACG CATGGTTAA-′5), 2.0 μM *Mse*I – adapter (5′-GACGATGAGTCCTGAG, TACTCAGGACT-CAT-′5) and 1U T_4 – Ligase (Life Technology, Invitrogen). PCR amplifications were performed on a T-gradient thermocycler (Biometra) in two separate amplification steps. Pre-amplification PCR

Table 1. Characteristics of the sampled *Lutjanus fulviflamma* populations along the East African coast and the Comoros archipelago (N is the number of sampled individuals, N_{pl} is the number of polymorphic loci ($N_{total} = 191$), P_{pl} (%) is the percentage of polymorphic loci per population and I is Shannon's diversity index)

Population	Code	N	N_{pl}	P_{pl} (%)	I ($\pm$SE)
Comoros	COM	10	162	84.8	0.33 (0.21)
Mtwara	MTW	10	122	63.9	0.26 (0.24)
Mafia	MAF	9	146	76.4	0.33 (0.24)
Zanzibar West	ZAN-W	10	130	68.1	0.27 (0.24)
Zanzibar East	ZAN-E	10	135	70.7	0.30 (0.24)
Zanzibar North	ZAN-N	9	138	72.3	0.30 (0.24)
Pemba	PEM	9	150	78.5	0.34 (0.24)
Mombasa	MOM	10	155	81.2	0.36 (0.24)

reactions were conducted in a 20 μl volume (containing 4 μl 20 × diluted Restriction–Ligation mix, 0.5 μM EcoRI/ + A primer (Applied Biosystems), 0.5 μM MseI/ + C primer (Applied Biosystems) and 15 μl AFLP Amplification Core Mix (Applied Biosystems), with the following temperature profile: an initial denaturation step of 2 min 94 °C; 20 cycles with 20 s 94 °C, 30 s 56 °C, 2 min 72 °C; followed by 2 min 72 °C and 30 min 60 °C. Selective amplifications were performed in a 10 μl volume (containing 1.5 μl 10 × diluted Pre-amplification product, 0.05 μM Dye labeled EcoRI/ + 3 primer (Applied Biosystems), 0.25 μM MseI/ + 3 primer (Applied Biosystems) and 7.5 μl AFLP Amplification Core Mix (Applied Biosystems), with the following temperature profile: an initial denaturation step of 2 min 94 °C; 10 cycles with 20 s 94 °C, 30 s 66 °C decreasing with 1 °C per cycle, 2 min 72 °C; 25 cycles with 20 s 94 °C, 30 s 56 °C, 2 min 72 °C; followed by 30 min 60 °C. Selective amplifications were performed using two primer pairs EcoRI/ + ACC-MseI/ + CTA and EcoRI/ + AAC − MseI/ + CTA. Amplified fragments were analyzed on a CEQ™ 8000 Genetic Analysis System (Beckman Coulter Inc. 2002). Fragments between 100 and 350 bp were scored as present (1) or absent (0) using the Fragment Analysis Software Module of the CEQ™ 8000 (AFLP settings: bin width = 1.0 nt; $Y_{threshold}$ = 400 RFU). The data was assembled in a binary data matrix. Loci with a band frequency below 5% were considered as potential artifacts and omitted from further analysis.

Data analysis

Genetic variation within populations was assessed by calculating Shannon's index of diversity (I), the number of polymorphic loci (N_{pl}) and the proportion of polymorphic loci (P_{pl}; 99% criterion), using the software program POPGENE version 1.31 (Yeh & Boyle, 1999). Phylogenetic analyses of the AFLP data were performed by three different approaches: neighbour-joining distance (NJ, Saitou & Nei, 1987), maximum parsimony, and Bayesian likelihood (Huelsenbeck & Ronquist, 2001) methods. The NJ and maximum parsimony analyses were carried out in PAUP* (Swofford, 2002). For the NJ analysis, the genetic distances were estimated using the algorithm of Nei & Li (1979). Unweighted parsimony heuristic search

was conducted using a TBR branch swapping and the simple taxon addition. The monophyly of the clusters was assessed by using bootstrap replicates. The likelihood model chosen is the one implemented in the program, with the rates from gain to loss of AFLP bands equal to the rate from loss to gain. The Bayesian inference that evaluates posterior probabilities of clades was performed using the program MRBAYES version 3.0 (Huelsenbeck & Ronquist, 2001). Markov chain Monte Carlo from a random starting tree was initiated and run for 200,000 generations. Trees were sampled every 100th generation. The first 25% of the samples were discarded as 'burn-in', and the rest of the samples were used for inferring a Bayesian tree. Examination of the log-likelihood suggests that the run reached a stationarity and that these burn-in periods were sufficiently long. Finally, to visualize the clustering of individuals based on the Nei–Li distances between individuals a multidimensional scaling (MDS) was performed using SPSS (Version 11.5, SPSS Inc.). MDS analysis was performed with 1–5 dimensions to assess the improvement in stress score with additional dimensions.

Analysis of molecular variance (AMOVA) was performed to assess the degree of molecular variation within and among populations, using the program WINAMOVA (Analysis of Molecular Variance version 1.55; Excoffier et al., 1992) which performs a nested ANOVA using the matrix of Euclidean genetic distances as input (Excoffier et al., 1992). The program AMOVA-prep version 1.01 (Miller, 1998) was used to transform the dominant binary data set into input files for the program WINAMOVA. The level of genetic population subdivision was estimated by calculating pairwise genetic distances between populations using Φ statistics (in WINAMOVA) that are directly analogous to Wright's F statistics (Excoffier et al., 1992). Exact tests of population differentiation (Raymond & Rousset, 1995) were calculated with TFPGA (Tools for Populations Genetic Analysis version 1.3; Miller, 1999). Analyses were performed with pairwise combinations of populations (using 20 batches and 2000 permutations), based on observed marker frequencies and assuming linkage equilibrium among loci (Miller, 1999). The relationship between genetic distance (Φ_{ST}) and geographic distance was analyzed with a Mantel

test implemented in FSTAT version 2.9.3 (Goudet, 1995).

Assignment tests are a useful tool for estimating dispersal among populations at current (ecological) timescales (Berry et al., 2004; Manel et al., 2005). Assignment of individual genotypes to the most likely population was carried out using the frequency based assignment test of Paetkau et al. (1995) implemented in the Doh-Assignment test calculator (Brzustowski, 2002). Individuals were assigned to populations displaying the highest log-likelihood. Individuals assigned to the source population were defined as correctly assigned (CA), whereas individuals assigned to a population other than the source population were defined as miss-assigned (MA). However, assignment of genotypes was only made if the log-likelihood was at least 10-fold higher from any of the other candidate populations; otherwise the individual was defined to be ambiguously assigned (AA), i.e., not reliably assignable to any population.

Results

Descriptive analysis

The two primer combinations used in the present study revealed a total of 191 loci (i.e., clear and reproducible AFLP fragments). Population-specific loci were not observed in any of the populations. The overall proportion of polymorphic loci was 70.7% and did not differ greatly among populations (varying between 63.9 and 84.8%, Table 1).

Phylogeography & MDS analysis

In general, the data did not show a clear phylogenetic pattern. There was no clear clustering of individuals in either the phylogenetic analysis (Fig. 2) or the MDS analysis (Fig. 3). The phylogenetic trees inferred from the NJ, parsimony, and Bayesian analyses were found to have very similar topologies (only the NJ tree is shown). However, the bootstrap percentages from both the NJ and the parsimony analysis and the posterior probabilities from the Bayesian analysis showed that the monophyly of the produced clusters were very low (Fig. 2), consistent with the MDS analysis.

The two dimensional MDS shown in Figure 3, had an S-stress (often also referred to as 'strain') of 0.145. An increase to three dimensions resulted in an improvement of S-stress with 0.080, while S-stress improvement of additional dimensions was <0.010. Most individuals of populations sampled along the East African coast were concentrated in one large cluster and individuals showed similar genetic distances. Only five individuals from Zanzibar North, three individuals from Mombasa, one individual from Mafia and one individual from Pemba showed larger genetic distances from this main cluster (Fig. 3). Although six individuals of the Comoros population showed larger genetic distances from the main cluster of East African individuals, this did not result in a separate cluster containing the individuals from the Comoros, and overlap with the other individuals was high.

To asses the relationship between populations along the East African coast without the model-fitting effects of the Comoros population, MDS analysis was re-run without the Comoros individuals (results not shown). In the absence of these individuals, still most individuals formed one large cluster with comparable genetic distances. The five individuals form Zanzibar North and the three individuals from Mombasa again showed larger genetic distances from the main cluster of East African individuals.

Population subdivision

Population structure analysis closely matched the population phylogeographic analysis presented above. The 2-level hierarchical AMOVA analysis indicated that >93% of genetic variation was contained within populations and very little partitioned among the populations (Table 2). Pairwise Φ_{ST} values (Table 3; AMOVA) were very low and exact tests (implemented in TFPGA) showed no significant differentiation among any pair of populations ($p > 0.05$), indicating little differentiation between populations. Although the relationship between pairwise genetic distance and geographical distances was significant, the relationship was very weak and the model could not explain much of the observed variance (Mantel test: $r = 0.397$, $R^2 = 0.157$, $p = 0.04$; Fig. 4). The absence of population subdivision was confirmed by the results
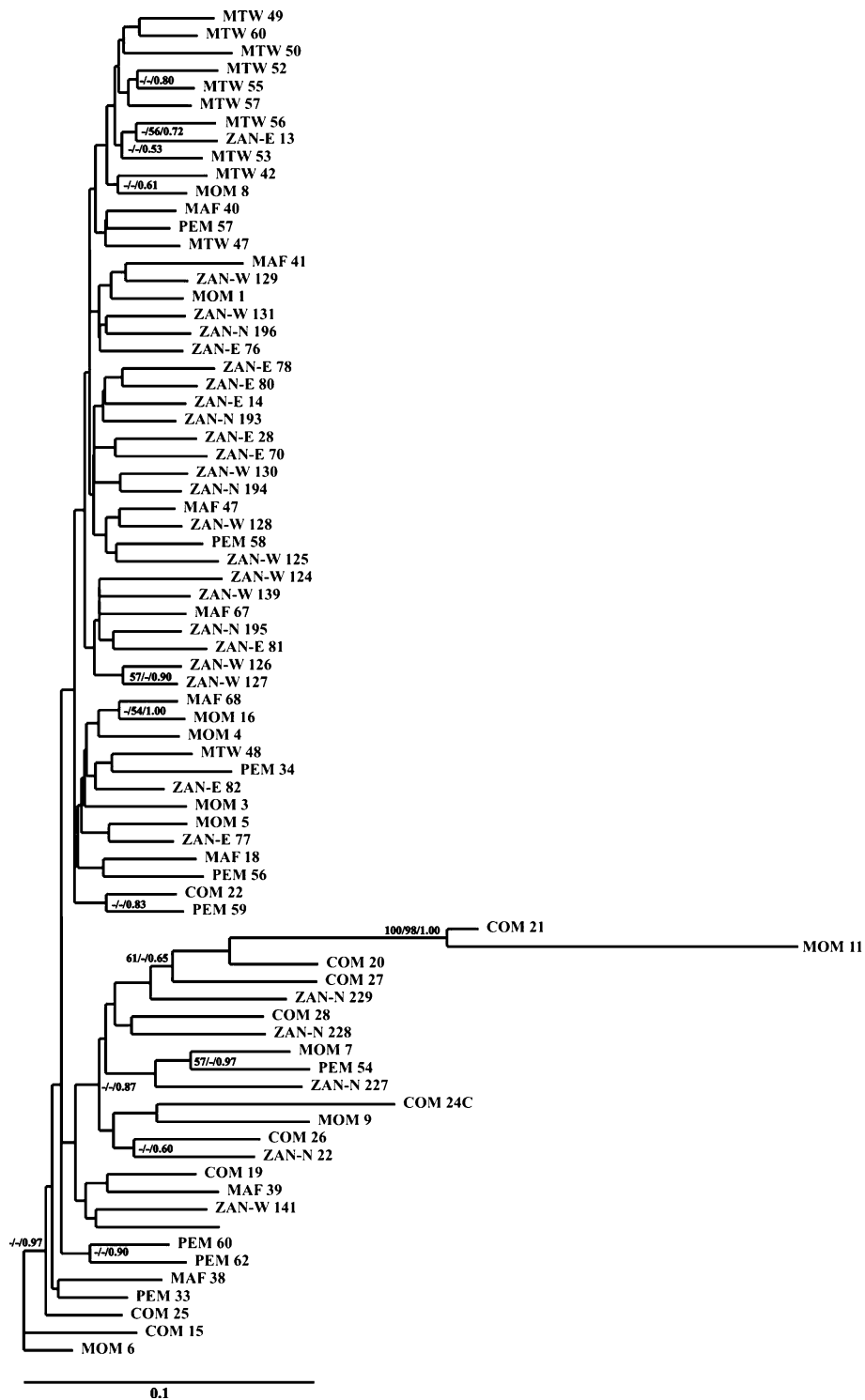
48

Figure 2. Neighbour-joining (NJ) tree of the *Lutjanus fulviflamma* populations based on 191 AFLP loci. The numbers at nodes represent the bootstrap percentages (values < 50 not shown) from 100 replicates obtained from NJ, maximum parsimony analyses, and the posterior probabilities (values < 0.5 not shown.) from Bayesian inference, respectively. The scale bar indicates the estimated evolutionary distance. Populations are encoded following Table 1, numbers refer to original sample numbers.
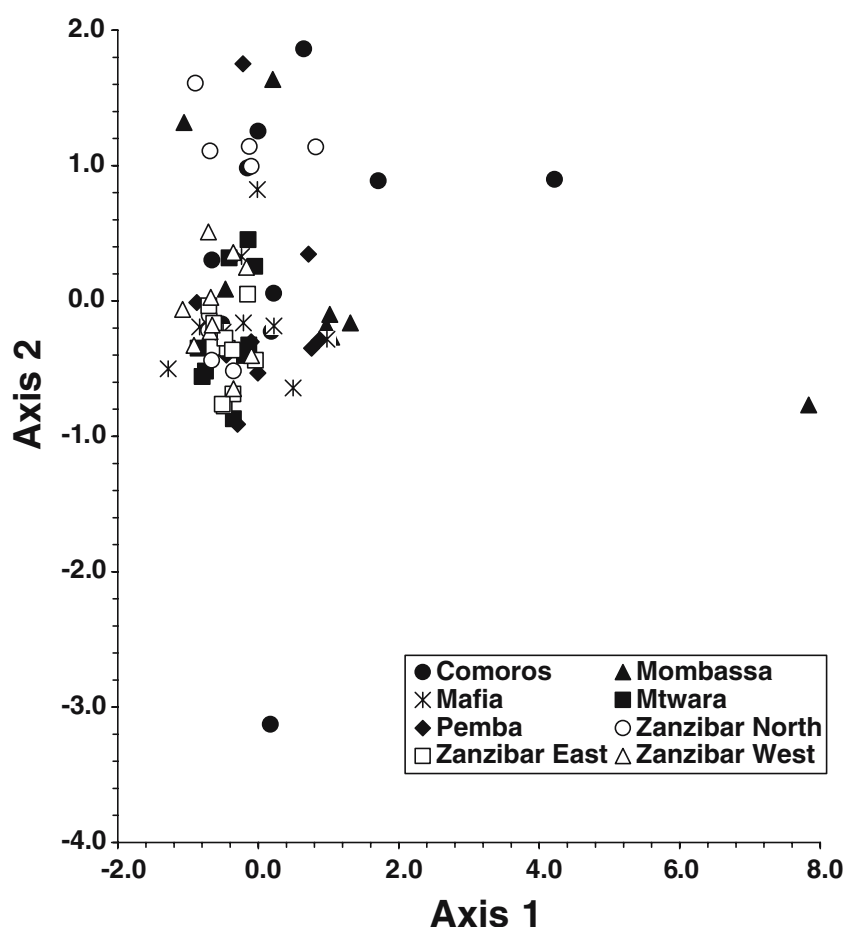
Figure 3. Multidimensional scaling (MDS) plot of all sampled *Lutjanus fulviflamma.*

from the population assignment procedure (Table 4), which showed low assignment percentages of individuals into their source population (CA_{mean} = 25%, Table 4), high assignment percentages of individuals into populations other than the source populations (MA_{mean} = 55%) and relatively high percentages of ambiguously assigned individuals (AA_{mean} = 20%).

Discussion

The results suggest little genetic differentiation among populations of *Lutjanus fulviflamma* in the western Indian Ocean. Neither the phylogenetic nor the MDS analyses showed any correlation between the clustering of individuals and their geographic origins. The analysis of molecular

variance showed that the variation was partitioned within populations and not between populations, resulting in a very low and non-significant differentiation among populations. The assignment procedure showed a relatively high percentage of ambiguously assigned individuals, indicating low differentiation among populations. The assignment procedure furthermore showed that most individuals were assigned to other populations than their source population, suggesting dispersal between populations. The results of the present

Table 2. Analysis of molecular variance (AMOVA) of *Lutjanus fulviflamma* populations

Source of variation	df	SS	MS	V	%
Among populations	7	287.29	41.04	1.63	6.07
Within populations	69	1746.61	25.31	25.31	93.93

Table 3. Pairwise genetic (Φ_{ST}, in bold below diagonal) and geographic distances (km, above diagonal) between *Lutjanus fulviflamma* populations along the East African coast and Comoros archipelago (NS indicates that the populations are genetically not significantly different). For site abbreviations see Table 1

| Population	COM	MTW	MAF	ZAN-W	ZAN-E	ZAN-N	PEM	MOM
COM		325	575	780	770	829	800	950
MTW	**0.1617**[NS]		264	500	495	544	585	731
MAF	**0.0994**[NS]	**0.0759**[NS]		275	268	318	314	468
ZAN-W	**0.1035**[NS]	**0.1157**[NS]	**0.0312**[NS]		129	40	110	249
ZAN-E	**0.1349**[NS]	**0.0650**[NS]	**0.0219**[NS]	**0.0225**[NS]		81	130	255
ZAN-N	**0.0302**[NS]	**0.1346**[NS]	**0.0665**[NS]	**0.0219**[NS]	**0.0468**[NS]		91	211
PEM	**0.0587**[NS]	**0.0807**[NS]	**0.0028**[NS]	**0.0237**[NS]	**0.0390**[NS]	**0.0307**[NS]		154
MOM	**0.0145**[NS]	**0.1045**[NS]	**0.0183**[NS]	**0.0538**[NS]	**0.0503**[NS]	**0.0271**[NS]	**0.0070**[NS]	
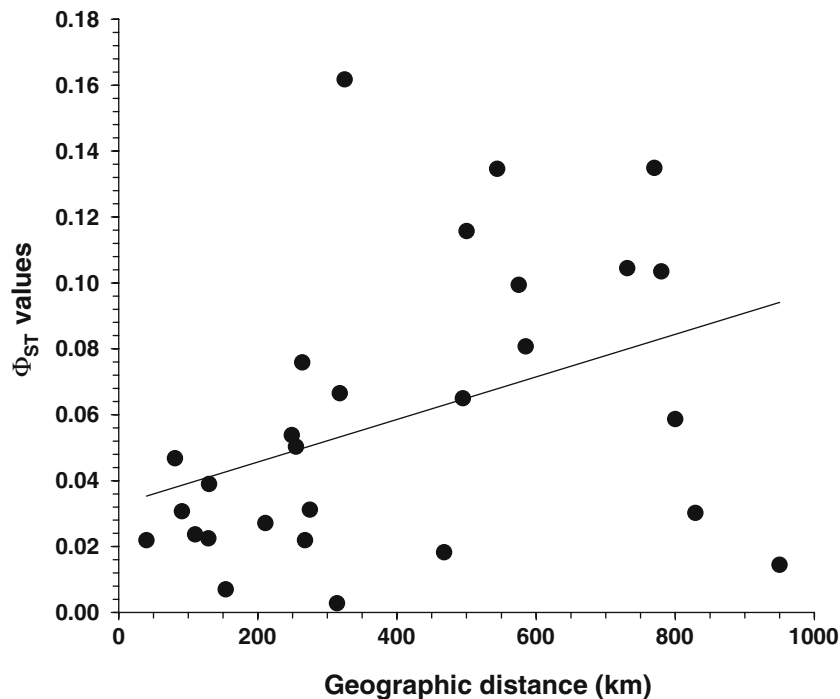
Figure 4. Relationship between pairwise genetic distance and geographical distance (Mantel test, $p = 0.035$, $R^2 = 0.16$).

study are therefore in favor of the hypothesis of open populations, with a high level of gene flow between all populations.

Considering the fact that none of the pairwise genetic distances (Φ_{ST} values) were significant and the relationship between genetic and geographic distance was weak, no clear pattern of isolation by distance among the populations in the study area could be established. Moreover, the results of the phylogenetic and MDS analysis revealed a lack of any clear genetic pattern along the investigated geographic gradient that follows the EACC, arguing against gene flow in the flow direction of the EACC. This could be the result of the geographic scale of the study area: gene flow among populations may likely encompass the whole study area (i.e., nearly a 1000 km gradient). During the southeast monsoon, the EACC is strongest, viz. approximately four knots (Richmond, 2002). Since Lutjanidae are known to spawn in this period

Table 4. Assignment of # of individuals into populations using a frequency-based assignment test (Paetkau et al., 1995). For site abbreviations see Table 1

Source population	Assigned population								CA (%)	MA (%)	AA (%)
	COM	MTW	MAF	ZAN-W	ZAN-E	ZAN-N	PEM	MOM			
COM	**3**			2		2		2	30	60	10
MTW		**8**	1						80	10	10
MAF	1	1		2	1				0	56	44
ZAN-W				**1**	3	1	1		10	50	40
ZAN-E		1	1	2	**4**				40	40	20
ZAN-N	2			2	2	**2**		1	22	67	11
PEM	1	1	2	3				1	0	89	11
MOM	3		2	1	1			**2**	20	70	10

Given is the percentage correctly assigned individuals (CA), the percentage 'mismatched' individuals, i.e., individuals assigned into another than the source population (MA), and the percentage of ambiguously assigned individuals (AA).

(Nzioka, 1979), larvae may be transported passively over distances of hundreds of kilometers in only a few days period.

The observed genetic patterns in the present study suggest a random genetic differentiation model according to Wright's island model (Wright, 1931): no relationship between the geographical distance and genetic differentiation is present and larvae from one population are likely to move to any other population down current of the flow direction of the EACC. This was also suggested by Fauvelot & Planes (2002) to explain a similar genetic population structure in seven coral reef fish species in French Polynesia. The results of the present study are therefore most in line with the hypothesis that suggests larval exchange on large geographic scales (i.e., on the level of the western Indian Ocean).

This is in accordance with other studies that applied other molecular techniques to address the question of 'openness' of marine systems and which showed open population structures and high levels of gene flow in coral reef fishes with pelagic life stages in the Caribbean (Gold & Richardson, 1994; Shulman & Bermingham, 1995; Geertjes et al., 2004; Zatcoff et al., 2004), the eastern Pacific (Stepien et al., 1994; Fauvelot & Planes, 2002; Bay et al., 2004; Rivera et al., 2004) and the Great Barrier reef (Doherty et al., 1995; van Herwerden et al., 2003). This suggests that in various regions of the world, populations of coral reef fish that have pelagic larval life stages show dispersal over long distances. In such fish species,

genetic differentiation is most likely to occur on larger geographic scales (i.e., between oceans), such as has been shown for *Dascyllus trimaculatus* (Bernardi et al., 2001), *Chlorurus sordidus* (Bay et al., 2004) and *Megalops atlanticus* (McMillen-Jackson et al., 2005). Nevertheless, there are studies that reported species with a pelagic life stage displaying retention of larvae near the origin of spawning, leading to genetic differentiation among populations, hence suggesting a closed rather than an open population structure (Planes et al., 1998; McCartney et al., 2003; Ramon et al., 2003; Taylor & Hellberg, 2003). This would mean that besides having a pelagic larval life-stage, also other variables such as larval behavior (Leis & Carson-Ewart, 2000), the duration of the pelagic stages (Doherty et al., 1995) or settlement habitat choice (Schmitt & Holbrook, 2002) can play an important role in dispersal and gene flow of reef fishes.

Acknowledgements

52

References

Bay, L. K., J. H. Choat, L. van Herwerden & D. R. Robertson, 2004. High genetic diversities and complex genetic structure in an Indo-Pacific tropical reef fish (*Chlorurus sordidus*): evidence of an unstable evolutionary past? Marine Biology 144: 757–767.

Beckman Coulter, 2002. CEQ™ 8000 Genetic Analysis System User's Guide. Beckman Coulter Inc., Fullerton, California, USA.

Bernardi, G., S. J. Holbrook & R. J. Schmitt, 2001. Gene flow at three spatial scales in a coral reef fish, the three-spot dascyllus, *Dascyllus trimaculatus*. Marine Biology 138: 457–465.

Berry, O., M. D. Tocher & S. D. Sarre, 2004. Can assignment tests measure dispersal? Molecular Ecology 13: 551–561.

Brzustowski, J. 2002. Doh assignment test calculator. Available at http://www2.biology.ualberta.ca/jbrzusto/Doh.php.

De Bruin, A., B. W. Ibelings & E. van Donk, 2003. Molecular techniques in phytoplankton research: from allozyme electrophoresis to genomics. Hydrobiologia 491: 47–63.

De Roos, K., 2003. CEQ™ 8000 AFLP Protocol. Beckman Coulter Netherlands BV, Mijdrecht, The Netherlands.

Doherty, P. J., S. Planes & P. Mather, 1995. Gene flow and larval duration in seven species of fish from the Great Barrier reef. Ecology 76: 2373–2391.

Excoffier, L., P. E. Smouse & J. M. Quattro, 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131: 479–491.

Fauvelot, C. & S. Planes, 2002. Understanding origins of present-day genetic structures in marine fish: biologically or historically driven patterns? Marine Biology 141: 773–788.

Garcia, A. A. F., L. L. Benchimol, A. M. M. Barbosa, I. O. Geraldi, C. L. Souza & A. P. de Souza, 2004. Comparison of RAPD, RFLP, AFLP and SSR markers for diversity studies in tropical maize inbred lines. Genetics and Molecular Biology 27: 579–588.

Geertjes, G. J., J. Postema, A. Kamping, W. van Delden, J. J. Videler & L. van de Zande, 2004. Allozymes and RAPDs detect little genetic population substructuring in the Caribbean stoplight parrotfish *Sparisoma viride*. Marine Ecology Progress Series 279: 225–235.

Gold, J. R. & L. R. Richardson, 1994. Mitochondrial DNA variation among 'red' fishes from the Gulf of Mexico. Fishery Research 20: 137–150.

Goudet, J., 1995. FSTAT version 1.2: a computer program to calculate *F*-statistics. Journal of Heredity 86: 485–486.

Huelsenbeck, J. P. & F. Ronquist, 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17: 754–755.

Jones, G. P., M.J. Milicich, M. J. Emslie & C. Lunow, 1999. Self-recruitment in a coral reef fish population. Nature 402: 802–804.

Kingsford, M. J., J. M. Leis, A. Shanks, K. C. Lindeman, S. G. Morgan & J. Pineda, 2002. Sensory environments, larval abilities and local self-recruitment. Bulletin of Marine Science 70: 309–340.

Leis, J. M., 2002. Pacific coral-reef fishes: the implications of behaviour and ecology of larvae for biodiversity and conservation, and a reassessment of the open population paradigm. Environmental Biology of Fishes 65: 199–208.

Leis, J. M. & B. M. Carson-Ewart, 2000. The Larvae of Indo-Pacific Coastal Fishes. An Identification Guide to Marine Fish Larvae. Fauna Malesiana Handbook 2, Brill, Leiden.

Leis, J. M. & D. S. Rennis, 2000. Lutjanidae. In Leis, J. M. & B. M. Carson-Ewart (eds), The Larvae of Indo-Pacific Coastal Fishes. An Identification Guide to Marine Fish Larvae. Fauna Malesiana Handbook 2, Brill, Leiden: 329–337.

Manel, S., O. E. Gaggiotti & R. S. Waples, 2005. Assignment methods: matching biological questions with appropriate techniques. Trends in Ecology & Evolution 20: 136–142.

McCartney, M. A., J. Acevedo, C. Heredia, C. Rico, B. Quenoville, E. Bermingham & W. O. McMillan, 2003. Genetic mosaic in a marine species flock. Molecular Ecology 12: 2963–2973.

McMillen-Jackson, A. L., T. M. Bert, H. Cruz-Lopez, S. Seyoum, T. Orsoy & R. E. Crabtree, 2005. Molecular genetic variation in tarpon (*Megalops atlanticus* Valenciennes) in the northern Atlantic Ocean. Marine Biology 146: 253–261.

Mickett, K., C. Morton, J. Feng, P. Li, M. Simmons, D. Cao, R. A. Dunham & Z. Liu, 2003. Assessing genetic diversity of domestic populations of channel catfish (*Ictalurus punctatus*) in Alabama using AFLP markers. Aquaculture 228: 91–105.

Miller, M. P., 1998. AMOVA-PREP: a program for the preparation of analysis of molecular variance input files from dominant-markers raw data, version 1.01. Available at http://www.marksgeneticssoftware.net/amovaprep.htm.

Miller, M. P., 1999. Tools for population genetic analyses (TFPGA): a windows ™ program for the analysis of allozyme and molecular population genetic data. Available at http://www.marksgeneticssoftware.net/_vti_bin/shtml.exe/tfpga.htm.

Mora, C. & P. F. Sale, 2002. Are populations of coral reef fish open or closed? Trends in Ecology & Evolution 17: 422–428.

Nei, M. & W. H. Li, 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Science USA 76: 5269–5273.

Nzioka, R. M., 1979. Observations on the spawning seasons of East African reef fishes. Journal of Fish Biology 14: 329–342.

Paetkau, D., W. Calvert, I. Sterling & C. Strobeck, 1995. Microsatellite analysis of population structure in Canadian polar bears. Molecular Ecology 4: 347–354.

Planes, S., R. Galzin & F. Bonhomme, 1996. A genetic metapopulation model for reef fishes in oceanic islands: the case of the surgeonfish, *Acanthurus triostegus*. Journal of Evolutionay Biology 9: 103–117.

Planes, S., 1993. Genetic differentiation in relation to restricted larval dispersal of the convict surgeonfish *Acanthurus triostegus* in French Polynesia. Marine Ecology Progress Series 98: 237–246.

Planes, S., P. Romans & R. Lecomte-Finiger, 1998. Genetic evidence of closed life cycles for some coral reef fishes within Taiaro Lagoon (Tuamotu Archipelago, French Polynesia). Coral Reefs 17: 9–14.

Planes, S. & C. Fauvelot, 2002. Isolation by distance and vicariance drive genetic structure of a coral reef fish in the Pacific Ocean. Evolution 56: 378–399.

Ramon, M. L., P. S. Lobel & M. D. Sorenson, 2003. Lack of mitochondrial genetic structure in hamlets (*Hypoplectrus* spp.): recent speciation or ongoing hybridization? Molecular Ecology 12: 2975–2980.

Raymond, M. & F. Rousset, 1995. GENEPOP version 1.2: population genetics software for exact tests and ecumenicism. Journal of Heredity 86: 248–249.

Richmond, M. D., 2002. A Field Guide to the Seashores of Eastern Africa and the Western Indian Ocean Islands. Sida/SAREC-UDSM, Dar es Salaam, Tanzania.

Rivera, M. A. J., C. D. Kelley & G. K. Roderick, 2004. Subtle population genetic structure in the Hawaiian grouper, *Epinephelus quernus* (Serranidae) as revealed by mitochondrial DNA analysis. Biological Journal of the Linnean Society 81: 449–468.

Rhodes, K. L., R. I. Lewis, R. W. Chapman & Y. Sadovy, 2003. Genetic structure of the camouflage grouper, *Ephinephelus polyphekadion* (Pisces: Serranidae), in the western central Pacific. Marine Biology 142: 771–776.

Roberts, C. M., 1997. Connectivity and management of Caribbean coral reefs. Science 278: 1454–1457.

Saitou, N. & M. Nei, 1987. The neighbour-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4: 406–425.

Schmitt, R. J. & S. J. Holbrook, 2002. Correlates of spatial variation in settlement of two tropical damselfishes. Marine and Freshwater Research 53: 329–337.

Shulman, M. J. & E. Bermingham, 1995. Early life histories, ocean currents, and the population genetics of Caribbean reef fishes. Evolution 49: 897–910.

Simpson, S. D., M. G. Meekan, R. D. McCauley & A. Jeffs, 2004. Attraction of settlement-stage coral reef fishes to reef noise. Marine Ecology Progress Series 276: 263–268.

Stepien, C. A., J. E. Randall & R. H. Rosenblatt, 1994. Genetic and morphological divergence of a circumtropical complex of goatfishes: *Mulloidichthys vanicolensis, M. dentatus*, and *M. martinicus*. Pacific Science 48: 44–56.

Swearer, S. E., J. E. Caselle, D. W. Lea & R. R. Warner, 1999. Larval retention and recruitment in an island population of a coral-reef fish. Nature 402: 799–802.

Swofford, D. L., 2002. PAUP*: phylogenetic analysis using parsimony (and other methods). Version 4.0 Beta. Sinauer Associates, Sunderland, Massachusetts, USA.

Taylor, M. S. & M. E. Hellberg, 2003. Genetic evidence for local retention of pelagic larvae in a Caribbean reef fish. Science 299: 107–109.

van Herwerden, L., J. Benzie & C. Davies, 2003. Microsatellite variation and population genetic structure of the red throat emperor on the Great Barrier Reef. Journal of Fish Biology 62: 987–999.

Vos, P., R. Hogers, M. Bleeker, et al., 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Research 23: 4407–4414.

Warner, R. R., 1997. Evolutionary ecology: how to reconcile pelagic dispersal with local adaptation. Coral Reefs 16: S115–S120.

Wright, S., 1931. Evolution in Mendelian populations. Genetics 16: 97–159.

Yeh, F. C. & T. J. B. Boyle, 1999. POPGENE Version 1.31. Microsoft Window-based Freeware for Population Genetic Analysis. Available at http://www.ualberta.ca/~fyeh/.

Zatcoff, M. S., A. O. Ball & G. R. Sedberry, 2004. Population genetic analysis of red grouper, *Epinephelus morio*, and scamp, *Mycteroperca phenax*, from the southeastern US Atlantic and Gulf of Mexico. Marine Biology 144: 769–777.