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Genetic erosion in the snail *Littoraria subvittata* (Reid, 1986) due to mangrove deforestation

Alex Nehemia[1,2], Filip Huyghe[1] and Marc Kochzius[1]

[1]*Department of Biology, Ecology and Biodiversity, Marine Biology, Vrije Universiteit Brussel (VUB), Pleinlaan 2, 1050 Brussels, Belgium; and*
[2]*Department of Biological Sciences, Sokoine University of Agriculture, PO Box 3038, Morogoro, Tanzania*

Correspondence: A. Nehemia; e-mail: alexander_nehemiah@yahoo.co.uk

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ABSTRACT

In tropical coastal ecosystems mangrove forests are important as feeding, spawning, breeding and nursery grounds for many marine species. High human population pressure in coastal areas has led to the loss and deterioration of mangrove habitats. Solar salt production can affect these habitats along the East African coast. Littorinid snails live on mangrove trees, forming an important component of the mangrove ecosystem and have been used as bioindicators of environmental health and community stress. *Littoraria subvittata* is the most abundant littorinid species in mangroves along the East African coast. Partial mitochondrial cytochrome oxidase subunit 1 (COI) gene sequences of 298 individuals were used to assess the impact of mangrove deforestation at salt ponds on the genetic diversity and structuring of *L. subvittata* populations, as well as to infer the demographic history of the species. Nucleotide and haplotype diversities were found to be lower in samples from mangroves at salt ponds than in samples from natural mangroves. The mean nucleotide diversity was 0.049 ± 0.036% and 0.115 ± 0.068% in mangroves at salt ponds and natural mangroves, respectively. The mean haplotype diversity was 0.23 ± 0.14 and 0.50 ± 0.14 in mangroves at salt ponds and natural mangroves, respectively. Analysis of molecular variance (AMOVA) detected a significant population structure (Φ_{st} = 0.049; $P < 0.0001$) for the combined populations. Hierarchical AMOVA detected a significant population genetic structure only between populations from mangroves at salt ponds and natural mangroves (Φ_{ct} = 0.022; $P < 0.05$), but not between any other groupings. Populations from natural mangrove sites showed a significant genetic structure (Φ_{st} = 0.054, $P < 0.0001$), while populations from sites at salt ponds could not be differentiated (Φ_{st} = −0.0026, $P = 0.64$). Reduced effective population size was observed in most samples from mangrove sites at salt ponds compared with natural mangrove. The direction of migrants was mostly from salt ponds to natural mangroves. These results show that salt ponds have a negative impact on the genetic diversity of *L. subvittata* populations and modify the population's genetic structure.

INTRODUCTION

Mangrove forests provide a habitat for a diverse fauna with a complex food web (Nagelkerken *et al.*, 2008). High human population pressure in coastal areas has led to the conversion of substantial mangrove areas into sites designated for human use, such as infrastructure, aquaculture and salt production (FAO, 2007; Walters *et al.*, 2008). Globally, mangrove forests have been reduced by about 35% since the 1980s. Each year, 2.1% of the existing mangrove area is lost worldwide and most of this loss is due to anthropogenic pressure (Valiela, Bowen & York, 2001). The estimated loss of mangroves between 1990 and 2010 alone was 500,000 ha (FAO, 2010). The East African region lost about 8% of its forests between 1980 and 2005, and salt farming is one of the main factors leading to this loss (Spalding, Kainuma & Collins, 2010).

The clear-cutting of mangroves for salt production is therefore one of the most serious threats to mangrove ecosystems (Masalu, 2000; Kairo et *al.*, 2001; Mazda et *al.*, 2002; Wang *et al.*, 2003; ISME, 2013). Along the coast, most salt is produced in solar ponds situated in mangrove areas, although it would be possible to relocate them outside the mangroves on bare land (Semesi, 1992). The consequences of mangrove forest loss include sediment destabilization, alteration of hydrology, forest fragmentation, habitat loss for fauna, species extinction and reduced carbon sequestration, leading to increased climate change (Harper *et al.*, 2007).

Despite these serious threats, only limited information about the effects of salt production in mangroves on animal populations and communities is available. Low numbers of mangrove trees and trees with small diameters have been recorded in mangrove forests used for salt production as compared with natural sites, indicating low recruitment of saplings (Liingilie *et al.*, 2015). Dykes constructed parallel to the coastline for salt farming prevent free flow of water locally. This causes a buildup of water during rains and

hence severe floods in the area (Ocholla *et al.*, 2013). Nothing, however, is known about the effect of salt production in mangroves on the genetic diversity and population structure of macroinvertebrates associated with these forests.

Littorinid snails are a common and important component of the mangrove ecosystem (Torres *et al.*, 2008). Within the food web, they are herbivores and/or detritivores, contributing to ecosystem functioning (Nagelkerken *et al.*, 2008). They have been used as biomonitors to assess environmental contamination (De Wolf *et al.*, 2001; De Wolf & Rashid, 2008). *Littoraria subvittata* is a member of the subgenus *Littorinopsis* that is ovoviviparous, brooding eggs in the mantle cavity for a few days, but releasing planktotrophic veliger larvae (Reid, 1986; Reid, Dyal & Williams, 2010). Along the East African coast, it is the most abundant member of this subgenus in mangroves (Torres *et al.*, 2008), which makes it a good candidate for use in investigating the effects of deforestation on the genetic diversity of mangrove fauna.

In coastal habitats with human influence, lower abundances of snails have been reported compared with those seen in natural habitats. This is caused by increased predation risk, disease and extreme environmental factors (Beasley *et al.*, 2005). Wastewater inflow was reported to cause lower abundances of gastropods in East African coastal areas (Cannicci *et al.*, 2009). Clear-cutting and selective logging of *Avicennia germinans* in the southern Caribbean resulted in decreased abundances of the gastropod *Neritina virginea*, which is likely associated with microclimate alteration, increased insolation, induced canopy gaps and the promotion of predation (Amortegui-Tores, Taborda-Marine & Blanco, 2013)

Ecological factors such as food availability and habitat quality may impact larval dispersal capabilities, which influence intrapopulation genetic variation and gene flow (Nanninga & Berumen, 2014). Gene flow among and within populations is an important part of the maintenance of species integrity and genetic diversity, and can be affected by both natural and anthropogenic factors (Banks *et al.*, 2013). Altered genetic diversity can influence the fitness of populations, decreasing the capability of species to adapt to environmental change (Keller & Waller, 2002; Auld & Relyea, 2010). The analysis of mitochondrial DNA (mtDNA) sequence data is a powerful tool for tracing the recent evolutionary history of marine gastropods in terms of bottlenecks, population expansion or contraction, and founder effects (Crandall *et al.*, 2007; Duda & Lee, 2009; Madeira *et al.*, 2012; Haupt, Micheli & Palumbi, 2013).

In this study, *L. subvittata* is used as a model species to assess the influence of deforestation due to salt farming on the genetic diversity and population structure of marine invertebrates. It is hypothesized that (1) genetic diversity among *L. subvittata* decreases under the influence of salt farming; (2) salt farming causes bottleneck effects or inbreeding and (3) limits connectivity among populations.

MATERIAL AND METHODS

Sampling

Samples were collected along the coast of the Tanzanian mainland (five sites) and Zanzibar Islands (two sites) (Fig. 1A). At each location, at least 20 specimens were collected from mangrove forests at salt ponds and from natural, relatively unaffected mangroves. The distance between the two sample sites at each location was 4–18 km (Table 1). All salt ponds had actively produced salt for many years, some since 1920 (Liingilie *et al.*, 2015). The only exception was Unguja (Makoba), where salt production in mangroves had been banned by governmental regulation (Mmochi *et al.*, 2001). *Littoraria subvittata* individuals were randomly picked from mangrove leaves and preserved in 99% ethanol. In all forests at salt ponds, there was evidence of alteration by humans in the

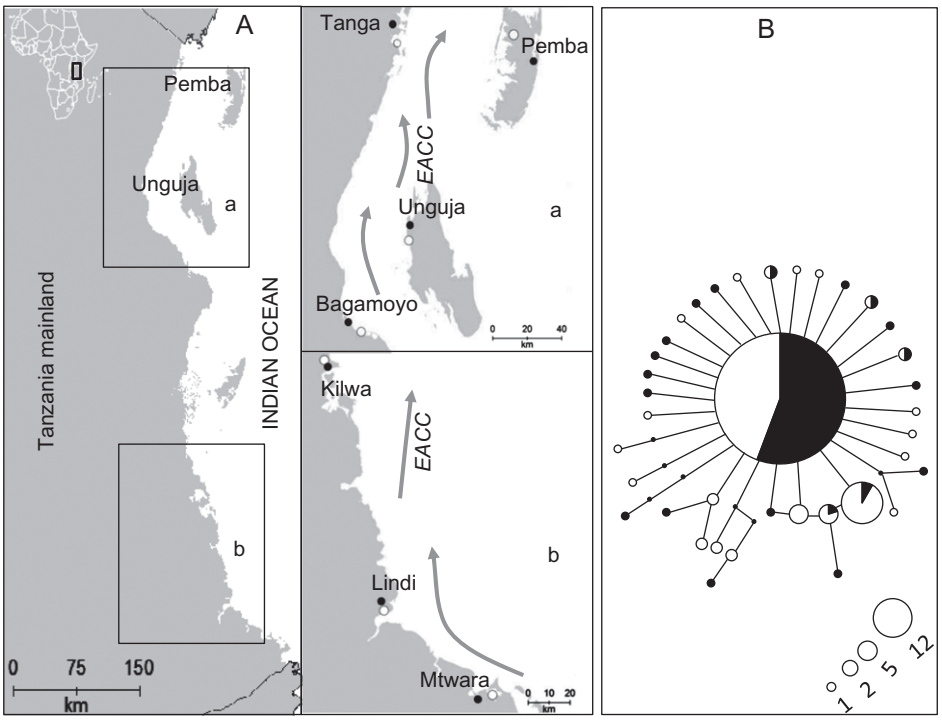

Figure 1. A. Map showing the sample sites for *Littoraria subvittata* in the western Indian Ocean. Black circles represent mangrove at salt ponds and open circles natural mangroves. The direction of the dominant East African Coast Current (EACC) is indicated by grey arrows. **B**. Haplotype network of partial cytochrome oxidase subunit 1 sequences. Proportion of specimens from sites at salt ponds is shown in black, and for those from natural mangroves in white. Size of circles corresponds to number of individuals as indicated in bottom-right corner. Largest, central circle represents 237 individuals. Lines between circles represent one mutational step and dots on lines indicate additional mutational steps.

Table 1. Description of sample sites for *Littoraria subvittata* in the western Indian Ocean (see Fig. 1).

Sample area	Salt-pond site	Code	Sample size	Natural mangrove site	Code	Sample size	Distance between natural mangrove and salt-pond sites (km)
Tanga	Mpirani	TS	27	Lumbachia	TN	21	10.96
Bagamoyo	Nunge	BS	23	Kaole	BN	21	11.20
Kilwa	Makubuli	KS	19	Timaki	KN	20	3.65
Lindi	Mbanja	LS	19	Mbanula	LN	21	4.47
Mtwara	Kilimahewa	MS	18	Ng'wale	MN	21	7.24
Pemba	Wete	PS	21	Chakechake	PN	21	8.29
Unguja	Makoba	US	23	Fujoni	UN	23	17.62

form of the clearing and selective logging of mangrove trees, as well as the construction of dykes and water channels.

Extraction and amplification of mtDNA

Genomic DNA was extracted from 298 *L. subvittata* individuals, 150 from salt-pond sites and 148 from natural sites. The E.Z.N.A.® Tissue DNA Kit (Omega Bio-Tek, California, USA) was used to isolate DNA from 5–10 mg of foot tissue, following the manufacturer's protocol. The universal primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') were used to amplify a part of the cytochrome oxidase subunit 1 (COI) gene (Folmer *et al.*, 1994). PCR was performed in a BIORAD T100™ thermocyler. A total volume of 50 µl per reaction was used, containing 3 µl of DNA extract, 1 mM PCR buffer, 0.2 µM forward primer, 0.2 µM reverse primer, 0.5 mg/ml of BSA, 3 mM $MgCl_2$, 0.2 mM dNTPs and 1 U Taq polymerase. The PCR temperature profile was as follows: initial denaturation for 5 min at 94 °C, followed by 35 cycles of 94 °C for 60 s, 41 °C for 1.5 min and 72 °C for 1 min, then a final extension for 5 min at 72 °C. The extracted DNA and PCR products were visualized by staining with GelRed during gel electrophoresis in a 2% TBE agarose gel for quality control. PCR products were sequenced with an ABI 3770XL automated sequencer (Applied Biosystems, Foster City, USA).

Sequence analysis and statistical tests

The sequences were edited using the software Chromaspro v. 1.5 (Technelysium). The program Squint Alignment Editor v. 1.02 was used to translate the DNA sequences into an amino-acid sequence in order to ensure that a functional gene was sequenced. The local alignment search tool (BLAST) at GenBank was used to verify the correct species identity. The online program FaBox v. 1.41 was used to collapse the sequences into haplotypes. CLUSTAL W (Thompson, Higgins & Gibson, 1994) as implemented in the software MEGA 6 (Tamura *et al.*, 2013) was utilized for the multiple alignment. Haplotype and nucleotide diversities (Nei & Jin, 1989), as well as pairwise Φ_{st} values between populations, were calculated with the software Arlequin v. 3.5.2.2 (Excoffier & Lischer, 2010).

Analysis of molecular variance (AMOVA) (Excoffier, Smouse & Quattro, 1992) was used to test for differentiation among and within populations. Hierarchical AMOVA was performed to test for the influences of salt farming and geography on population genetic structure in the dataset. We grouped the populations for AMOVA as follows. First we compared salt-pond and natural sites, independent of location (A). Then we compared populations among all the sites, pooling together affected (salt pond) and natural sites (B). We also compared North Tanzania with South Tanzania (C, D) and between opposite coasts (Tanzania mainland and Zanzibar Archipelago) (E).

The program TCS v. 1.21 (Clement, Posada & Crandall, 2000) was used to calculate a haplotype network. Tajimas's *D* test (Tajima, 1989) and Fu's *F*s test (Fu, 1997) were conducted using Arlequin to confirm the selective neutrality of the marker. The same software was used to analyse the mismatch distribution of the sum of squared deviations (SSD; Rogers & Harpending, 1992) and Harpending's raggedness index under a sudden population-expansion model (Rogers, 1995). The probability of the heterogeneity of haplotype distribution among subpopulations from mangroves at salt ponds and natural mangroves at each site was estimated *via* contingency χ^2 tables, using a Monte Carlo simulation as implemented in the software R: package coin v. 1.1-2 (Hothorn *et al.*, 2008). The simulated χ^2 with a *P*-value was based on 2,000 replicates.

The program MIGRATE v. 3.11.6 (Beerli & Felsenstein, 2001) was used to estimate the effective female population size and the migration rate between subpopulations collected from salt ponds and natural mangroves sites. This was performed by first conducting three preliminary replicate runs. Each run consisted of one long chain, 1,000,000 sampled parameter values and 100,000 recorded steps, with a burn-in of 10,000 and uniform prior distribution in order to estimate the boundaries Θ ($2N_{ef}\mu$) and M (m/μ) (where N_{ef} = effective population size of females; μ = mutation rate; m = immigration rate). The final run consisted of a long chain of 40,000,000 sampled parameter values and 400,000 recorded steps, with a burn-in of 50,000, a sequential prior distribution and multiple Markov chains, which consisted of four chains with a static heating scheme and start temperatures of 1.00, 1.50, 3.00 and 1,000,000.00. To estimate the migration rate, the formula $N_{ef}m_i = 0.5\ X\Theta i \times M_{j \rightarrow i}$ was used, where i is the receiving site and j is the source site (Keeney *et al.*, 2009).

RESULTS

Genetic diversity

The alignment of 298 sequences was 681 bp long. The sequences are deposited at the European Nucleotide Archive (ENA; accession numbers LT548620–LT548917). A total of 38 haplotypes were identified, of which 29 (71%) were private (Table 2). The dominant haplotype (h1) accounted for 79% of all individuals and was present in all 14 populations. The second most frequent haplotype was found in 12 individuals, while the third and fourth most frequently occurring haplotypes (h10 and h11) were found in five individuals each. The highest number of private haplotypes was recorded in the natural mangrove site at Tanga (7). Only six (16%) haplotypes were shared between the salt-pond and natural sites.

The overall nucleotide and haplotype diversity values for populations from mangrove at salt ponds were 0.05% and 0.23, respectively, while in natural mangroves these values were 0.11% and 0.5, respectively. These genetic indices, however, varied considerably among populations, with nucleotide diversity ranging from 0 to 0.09% and haplotype diversity ranging from 0 to 0.39 in mangroves at salt ponds (Fig. 2A, B). Nucleotide diversity in populations at natural mangrove sites ranged from 0.04 to 0.16%,

Table 2. Haplotype distribution based on COI sequences in *Littoraria subvittata* from natural mangroves and mangroves at salt ponds.

Haplotypes	Codes															
	TN	TS	BN	BS	KN	KS	LN	LS	MN	MS	PN	PS	UN	US	NhN	NhS
h1	14	22	18	19	12	19	12	15	17	17	14	18	17	21	106	131
h2				1												1
h3				1												1
h4				1												1
h5				1												1
h6			1												1	
h7			1												1	
h8			1		1									1	2	
h9					1										1	
h10	1				3		1								5	
h11					3		1	1							4	1
h12	1							1							1	1
h13								1								1
h14	2						5	1	2				2		9	3
h15							1								1	
h16							1								1	
h17										1						1
h18									1						1	
h19									1						1	
h20	1											1			1	1
h21												1				1
h22												1				1
h23											1					1
h24											2				2	
h25											2				2	
h26											1				1	
h27											1				1	
h28		1														1
h29		1														1
h30		1														1
h31		2														2
h32	1														1	
h33	1														1	
h34														1		1
h35													1		1	
h36													1		1	
h37													1		1	
h38													1		1	

Abbreviations: NhN and NhS are total number of haplotypes for natural mangroves and mangroves at salt ponds, respectively; for site codes, see Table 1.

and haplotype diversity ranged from 0.27 to 0.64 (Fig. 2). The lowest nucleotide and haplotype diversities were found in the salt-pond site at Kilwa, where all of the 19 sequenced individuals shared the same haplotype. The four populations with the highest nucleotide and haplotype diversities were all from natural sites (Pemba, Tanga, Kilwa and Lindi). These four populations also contained the highest number of private haplotypes. The three populations with the lowest nucleotide and haplotype diversities were all from salt-pond sites (Kilwa, Mtwara and Unguja) (Fig. 2). Both nucleotide and haplotype diversities were higher at natural sites than at salt-pond sites in six of the seven stations. Only at Bagamoyo were nucleotide and haplotype diversities slightly higher in salt ponds than in the natural mangroves.

The pairwise comparison *via* contingency chi-square tables revealed significant differentiation between most of the subpopulations (TN-TS: $\chi^2 = 38.9$, $P > 0.05$; BN-BS: $\chi^2 = 38.4$, $P < 0.05$; KN-KS: $\chi^2 = 38.0$, $P < 0.05$; LN-LS: $\chi^2 = 48.0$, $P < 0.01$; MN-MS: $\chi^2 = 38.1$, $P > 0.05$; PN-PS: $\chi^2 = 38.5$, $P < 0.05$ and UN-US: $\chi^2 = 38.3$, $P < 0.05$; for site name abbreviations, see Table 1).

The pairwise comparison used to estimate the effective female population size revealed reduced effective population size in most samples from mangrove sites at salt ponds. The direction of migrants was mostly from salt ponds to natural mangroves (Table 3).

Genetic population structure and mismatch distribution analysis

The haplotype network displayed very little genetic variation, without any evidence of population structure (Fig. 1B). It had a star-like structure, with many private or sparsely occurring haplotypes differing by only one mutational step from a dominant, central haplotype, which is an indication of recent population expansion. The AMOVA among all populations, on the contrary, did indicate a significant population structure ($\Phi_{st} = 0.049$; $P < 0.001$). Only a hierarchical AMOVA, pooling natural mangroves against salt-pond sites, resulted in a fixation index

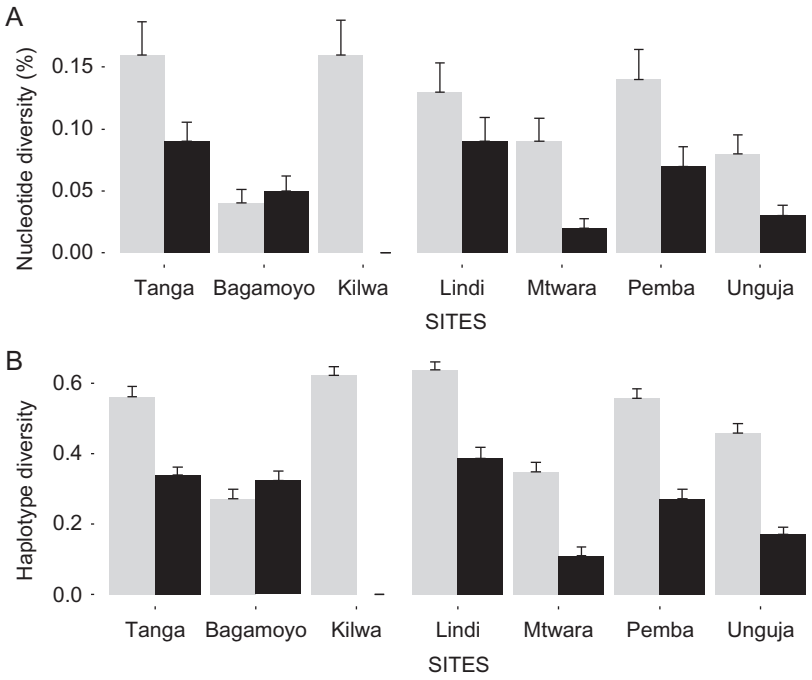

Figure 2. Nucleotide diversity (**A**) and haplotype diversity (**B**) of populations of *Littoraria subvittata* from natural mangroves (grey bars) and mangroves at salt ponds (black bars). Error bars are standard error of the mean.

Table 3. Bayesian estimates of boundaries (Θ) and pairwise migration (m) for *Littoraria subvittata* populations.

Codes	Θ		m		
	Mean	(2.5%, 97.5%)	Direction	Mean	(2.5%, 97.5%)
TN	0.0062	(0.0017, 0.0123)	TN → TS	146.8	(0.0, 381.3)
TS	0.0030	(0.0008, 0.0057)	TN ← TS	221.2	(0.0, 528.5)
BN	0.0032	(0.0008, 0.0063)	BN → BS	137.6	(0.0, 369.1)
BS	0.0038	(0.0010, 0.0073)	BN ← BS	131.6	(0.0, 355.7)
KN	0.0047	(0.0012, 0.0096)	KN → KS	111.7	(0.0, 315.7)
KS	0.0013	(0.0000, 0.0029)	KN ← KS	184.9	(0.0, 457.1)
LN	0.0040	(0.0011, 0.0078)	LN → LS	288.2	(0.5, 601.1)
LS	0.0046	(0.0008, 0.0100)	LN ← LS	137.0	(0.0, 404.8)
MN	0.0043	(0.0011, 0.0086)	MN → MS	118.0	(0.0, 430.4)
MS	0.0018	(0.0002, 0.0039)	MN ← MS	601.1	(0.0, 404.8)
PN	0.0046	(0.0012, 0.0091)	PN → PS	123.0	(0.0, 346.7)
PS	0.0037	(0.0009, 0.0073)	PN ← PS	151.6	(0.0, 404.3)
UN	0.0045	(0.0012, 0.0086)	UN → US	121.6	(0.0, 334.9)
US	0.0024	(0.0005, 0.0048)	UN ← US	152.6	(0.0, 400.5)

Values are means with 95% confidence intervals. For site codes, see Table 1.

significantly different from zero ($\Phi_{ct} = 0.022$; $P < 0.05$; Table 4). Among populations from natural sites, a significant population structure was detected ($\Phi_{st} = 0.057$; $P < 0.001$), while among populations from salt ponds no significant structure could be found ($\Phi_{st} = -0.0026$). Genetic differentiation among salt-pond and natural populations was confirmed by pairwise comparison. Ten out of the 14 pairwise Φ_{st} values that were significant were found between salt-pond and natural populations, and the four remaining significant values were found between the natural population at Pemba and the natural populations at Tanga, Kilwa, Lindi and Unguja (Table 5).

The mismatch distribution analysis did not reject the hypothesis of sudden demographic expansion, because all populations from both salt-pond sites and natural sites exhibited a unimodal mismatch distribution pattern (Fig. 3). On the one hand, all salt-pond populations, except for that from Mtwara, exhibited significantly negative values for Tajima's D and Fu's Fs. On the other hand, all populations from natural sites had Tajima's D values that were significantly different from zero, with the exception of those from Kilwa, Lindi and Pemba. Only the populations from natural sites at Kilwa and Mtwara did not have values that were significantly different from zero in Fu's Fs test (Table 6). For the overall population, neither the SSD nor Harpending's raggedness indices rejected the hypothesis of demographic expansion under a sudden expansion model, even when the salt-pond and natural populations were analysed separately. For the salt-pond populations, the

overall Tajima's *D* was significantly different from zero ($P < 0.05$), but this was not true for the populations from natural sites. The overall Fu's *F*s value was not significant for either type of site.

DISCUSSION

Genetic diversity

The overall genetic diversity levels that were observed in this study were low compared with similar values published for other marine mangrove gastropods from the Indian Ocean (Crandall *et al.*, 2007; Madeira *et al.*, 2012; Silva *et al.*, 2013). However, similarly low levels of genetic diversity were observed in the crab *Uca annulipes* (Silva, Mesquita & Paula, 2010b). The authors of this last study suggested that this was due to low effective population size caused by a bottleneck or founder event, followed by a population expansion. In our study, the higher haplotype diversity as compared with nucleotide diversity may be explained by an excessive number of private haplotypes that only differ by a single mutational step from a central haplotype.

Genetic diversity, in terms of both nucleotide and haplotype diversities, is important for population resilience, because it provides the capacity to cope with environmental changes and therefore enhances fitness (Faulks, Gilligan & Beheregaray, 2011). Genetic diversity was significantly lower in populations from mangrove sites at salt ponds than in populations from natural sites. The estimate of the difference in heterogeneity of haplotype distribution revealed the presence of variations between most samples from mangroves at salt ponds and natural mangroves. Various aquatic and terrestrial studies on population genetic variation and structure in disturbed habitats have reported low genetic diversity within the species studied in these habitats and have linked this to habitat loss or fragmentation (Grant & Bowen, 1998; Habel & Zachos, 2013; Chen, Du & He, 2015; Jm *et al.*, 2015). The results of this study confirm these earlier findings, indicating that habitat disturbances caused by human activities, such as salt farming, may have a negative impact on genetic diversity in populations of mangrove-associated invertebrates. The results from Unguja, where salt production stopped several years ago, did not differ from the others in this respect, indicating that recovery through gene flow from other sites does not necessarily take place rapidly enough to offset the effects of human-induced population reductions. All individuals from the salt ponds in Kilwa shared the same haplotype, suggesting that deforestation due to salt production activities promotes a decline in genetic diversity. In general, we found that salt-pond populations had a reduced effective population size as compared with natural populations. Lower genetic diversity has been observed as a factor compromising reproductive fitness in affected populations, increasing the risk of local extinction (Markert *et al.*, 2010). More migrants were observed from salt ponds towards natural mangroves than *vice versa*. The reduced effective female population size in salt-pond populations may help to account for the lower level of genetic diversity observed, yet we found higher migration from salt ponds towards natural sites despite this lower effective female population size. However, the habitats for snails influenced by human activities have been suggested to be associated with higher predation risk, diseases and extreme environmental factors (Beasley *et al.*, 2005). These elements, coupled with loss of mangroves in the area, may have contributed to the poor recruitment of larvae in the salt ponds compared with a higher recruitment rate of migrants towards natural sites, which would then explain the observed migration pattern.

Table 4. Hierarchical analysis of molecular variance based on COI sequences from *Littoraria subvittata*.

Groupings	Φ_{ct}	*P*
A		
(TN, BN, KN, LN, MN, PN, UN) *vs* (TS, BS, KS, LS, MS, PS, US)	0.0215	0.017
B		
(TN, TS) *vs* (BN, BS) *vs* (KN, KS) *vs* (LN, LS) *vs* (MN, MS) *vs* (PN, PS) *vs* (UN, US)	−0.0029	0.499
C		
(TN, BN) *vs* (KN, LN, MN)	0.0074	0.351
D		
(TS, BS) *vs* (KS, LS, MS)	0.0031	0.788
E		
(TN, TS, BN, BS, KN, KS, LN, LS, MN, MS) *vs* (PN, PS, UN, US)	0.00621	0.190

Groupings: A, populations from natural mangroves *vs* mangroves at salt ponds; B, samples from natural mangroves and salt ponds at nearby sites pooled; C, populations from northern *vs* southern sites (natural mangrove sites from Tanzania mainland); D, population from northern *vs* southern sites (salt-pond sites from Tanzania mainland); E, Tanzanian mainland *vs* Zanzibar. For site codes, see Table 1.

Table 5. Pairwise Φ_{st} values based on COI sequences for populations of *Littoraria subvittata* from natural mangroves and mangroves at salt ponds.

Codes	TN	TS	BN	BS	KN	KS	LN	LS	MN	MS	PN	PS	UN	US
TN	0.000													
TS	0.030	0.000												
BN	0.047	0.072*	0.000											
BS	0.045	0.004	−0.000	0.000										
KN	0.037	0.111*	0.117	0.107*	0.000									
KS	0.051	−0.005	−0.005	−0.009	0.141	0.000								
LN	−0.009	0.101*	0.114	0.107*	−0.013	0.136	0.000							
LS	−0.015	−0.014	0.002	−0.002	0.051	0.000	0.044	0.000						
MN	−0.013	0.028	0.030	0.026	0.042	0.003	−0.000	−0.009	0.000					
MS	0.044	0.028	−0.003	−0.006	0.126	0.003	0.120*	−0.002	−0.009	0.000				
PN	0.078*	0.072*	0.079	0.078	0.126*	−0.091	0.123*	0.061	0.078	0.080	0.000			
PS	0.025	−0.002	0.000	0.001	0.108	−0.005	0.095	−0.008	0.025	−0.005	0.066	0.000		
UN	0.007	0.012	0.008	0.009	0.086	0.005	0.043	−0.008	−0.011	0.004	0.074*	0.008	0.000	
US	0.055	0.004	−0.017	−0.000	0.139*	−0.009	0.131*	−0.006	0.037	−0.003	0.091*	0.002	0.011	0.000

*$P < 0.05$, after sequential Bonferroni correction. For site codes, see Table 1.

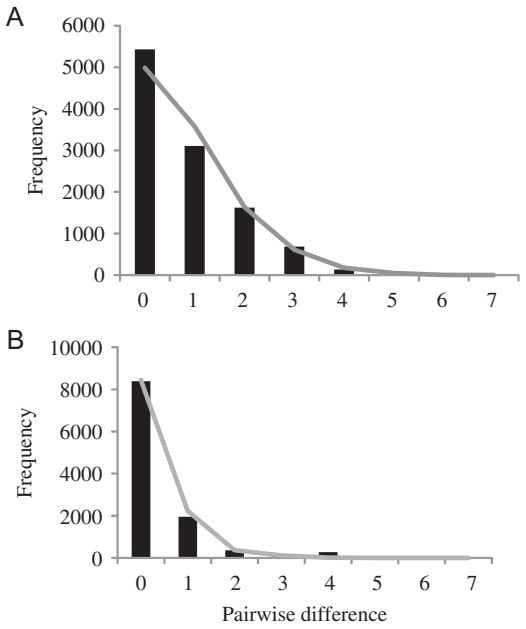

Figure 3. The observed (bars) and expected (line) mismatch distributions of COI sequences for *Littoraria subvittata* under the sudden expansion model. **A.** Populations from natural mangroves. **B.** Populations from mangroves at salt ponds.

Table 6. Mismatch distribution indices based on COI sequences for populations of *Littoraria subvittata*.

Codes	Mismatch distribution analysis				Tajima' *D* test		Fu's F_s test	
	SSD	P_{SSD}	HRI	P_{HRI}	Tajima's *D*	*P*	*Fs*	*P*
TN	0.00565	0.73	0.0676	0.83	−1.7156	0.027	−3.1633	0.008
TS	0.00807	0.35	0.2311	0.68	−2.0521	0.005	−2.1672	0.031
BN	0.00493	0.47	0.2814	0.55	−1.7268	0.020	−2.8198	0.001
BS	0.23108	0.14	0.2181	0.18	−1.8811	0.009	−3.8963	0.000
KN	0.00058	0.98	0.0348	1.00	−1.0942	0.138	−0.8469	0.262
KS	NA	NA	NA	NA	NA	NA	NA	NA
LN	0.00458	0.59	0.0907	0.41	−1.0725	0.171	−2.5234	0.012
LS	0.00889	0.61	0.1936	0.68	−2.0462	0.005	−2.3993	0.005
MN	0.00840	0.45	0.2539	0.53	−1.6302	0.027	−0.9731	0.134
MS	0.00010	0.43	0.6173	0.70	−1.1647	0.141	−0.7943	0.090
PN	0.02061	0.49	0.1157	0.69	−1.3652	0.082	−2.3609	0.027
PS	0.02626	0.16	0.4469	0.57	−1.9814	0.005	−1.6297	0.053
UN	0.00717	0.29	0.1432	0.52	−1.8209	0.018	−4.2616	0.000
US	0.00094	0.43	0.4672	0.66	−1.5150	0.049	−2.0272	0.008

Abbreviations: SSD, sum of squared deviations; HRI, Harpending's raggedness index; for site codes, see Table 1.

Population genetic differentiation and mismatch distribution analysis

The lack of a clear phylogeographic structure in the haplotype network analysis is consistent with results obtained in other marine invertebrates in the western Indian Ocean and indicates a high degree of genetic connectivity among populations as a result of extensive gene flow (Silva *et al.*, 2010a, b2013; Madeira *et al.*, 2012). The East Africa Coast Current (EACC), which consistently flows from south to north (Fig. 1), may be responsible for this high level of gene flow among coastal populations by transporting dispersing eggs and larvae over long distances. Despite this lack of geographic differentiation among populations, however, our results show significant population differentiation on another level. Populations from mangroves at salt ponds were genetically differentiated from populations from more natural mangrove sites. One important factor explaining the difference between populations from salt ponds and those from natural mangroves was the relatively low number of shared haplotypes between these two types of habitats, which was partly caused by the reduced number of haplotypes in mangroves at salt ponds. This result may be explained by low effective population size observed in mangroves at salt ponds as compared with natural mangroves.

More importantly, the lack of genetic diversity in salt-pond populations and the differentiation of these populations from populations from more natural sites may be caused by bottleneck effects, which are enhanced by salt faming activities. Bottleneck effects can result in population expansions from a small effective maternal population, which can promote inbreeding and genetic erosion. The analysis of mismatch distribution revealed a skewed distribution for all the populations combined, as well as when the samples from salt-pond and natural sites were analysed separately (Fig. 3). This may be related to a recent bottleneck and sudden population-expansion event. Tajima's *D* test for all populations combined, as well as for populations from salt-pond sites, indicated a significantly negative value, but this was not true for natural populations. For both types of populations, the values of the SSD and Harpending's raggedness test support the hypothesis of population expansion, but the unimodal nature of the curve is much more pronounced in the salt-pond sites (Fig. 3B). This indicates that the populations close to salt ponds may have suffered from more severe and more recent bottleneck events than the populations taken from natural sites.

A study of the population genetic structure of the marine coral *Acropora tenuis* found genetic structuring between southern Tanzania, northern Tanzania plus Kenya, and Zanzibar (van der Ven *et al.*, 2015). In contrast, our analysis did not detect any geographical genetic structuring for the species studied, even when all sites were analysed together. The only genetic structure detected was between populations at natural mangroves and salt ponds. Salt farming in the area and consequent mangrove degradation is likely to explain the pattern of population genetic structure and differentiation among the populations studied.

Habitat alteration or destruction has a potential impact on populations through the substantial reduction of effective population size and genetic variability (Weber, 2004). The loss of genes in these populations can increase the effect of genetic drift, leading to genetic differentiation from unaffected populations (Vandergast *et al.*, 2006; Dibattista, 2008). For example, the low genetic diversity of the endangered crab *Uca arcuata* on Okinawa may be caused by genetic drift in small populations, in combination with low incoming gene flow from other populations (Aoki *et al.*, 2008). Human disturbances of the habitats of terrestrial animals have also been found to be a cause of low genetic variation of populations (e.g. Ernest *et al.*, 2003; Epps *et al.*, 2005; Riley *et al.*, 2006).

The populations sampled from salt-pond sites and natural sites were only separated from one another by a few kilometres, which is a distance that is likely to be crossed by individual larvae dispersing from one population to another. Furthermore, the geographic distance between the populations was not correlated with genetic differentiation, so a geographical barrier to dispersal does not seem to be present. Rather, genetic differentiation between populations is likely to be caused by recent human-induced bottlenecks that have affected salt-pond populations more than natural populations. The construction of salt ponds in mangroves involves the disruption of water flow by constructing bund walls for the ponds and water channels that bring water from the sea. Such hydrological interruptions affect the normal functioning of the mangrove ecosystem (Crook *et al.*, 2015) and may have contributed to decreased genetic diversity in populations of *L. subvittata* at these sites. The repeated logging of mangroves for salt-pond construction, firewood and housing construction may also have reduced

the effective population size of this species in the area due to the creation of local habitats that are not suitable for the recruitment of larvae. The extent of larval dispersal is determined by complex interactions, including oceanographic regimes and habitat quality (Villamor, Constantini & Abbiati, 2014). Selective logging may have caused reduced microhabitat complexity regarding trees and pneumatophores and resulted in an unsuitable environment for larvae and adults, which depend on them for refuge, food and development. This may also be responsible for the migration pattern observed in this study. It should, however, be noted that *L. subvittata* may not be an obligate mangrove associate, because it can also be found on other hard substrates (Reid, 1986).

Population bottlenecks for marine organisms involved in complex life cycles have been linked to unsuitable environments and predation, which results in mortality during the larval recruitment stage (Leggett & Deblois, 1994; Nanninga & Berumen, 2014). Habitat modification involving selective logging has also been associated with a reduced abundance of snails (Amortegui-Tores, Taborda-Marine & Blanco, 2013).

Although the mitochondrial COI gene is of proven use in detecting population structure in invertebrates (e.g. Silva, Mesquita & Paula, 2010a), the information obtained from mtDNA alone is not always enough to infer population structure completely, due to its haploid and uniparental inheritance (Teske *et al.*, 2011). It would be interesting for further study to use larger samples and multiple and more sensitive markers, to test whether the pattern of genetic structure obtained in this study is confirmed. More sensitive molecular markers, such as microsatellites, have higher power to detect traces of more recent fluctuations in gene flow compared with mtDNA.

CONCLUSION

One important factor to consider when assessing genetic population differentiation is genetic erosion at certain sampling sites caused by human disturbances. Salt works, like other human activities in mangrove ecosystems, may have a significant impact on both fauna and flora and therefore pose a threat to the longterm existence and productivity of these ecosystems. Our results indicate that destructive human activities, such as salt-pond construction, can cause a reduction of genetic diversity in mangrove-associated invertebrates *via* reduced effective female population size and may even be a threat to the survival of these populations. These results should encourage coastal resource managers to halt the further destruction of mangroves for salt evaporation pans and other purposes, in order to control removal or overexploitation of this ecologically important resource.

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