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USING OF GARDENING AS AN APPLICABLE TOOL FOR ACTIVE REEF RESTORATION IN TANZANIA

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Using of the gardening concept as an applicable tool for active reef restoration in Tanzania

By Nsajigwa Emmanuel Mbije

Abstract

The global massive decline of coral-reefs ecosystem, caused by anthropogenic and natural disturbances, has been recorded in the last three decades. In response, several approaches, some of which were guided by different points of views, have discussed a variety of methodologies for the recovery of reef ecosystems worldwide. One of these approaches is based on the coral gardening concept, and employs a two-steps tactic for active restoration: (1) culturing of small coral fragments in mid-water nurseries until they reach appropriate sizes for transplantation, and (2) transplantation of farmed coral colonies into denuded reef areas. The tool-box of this active restoration concept has been studied here in Tanzania, aiming at devising appropriate restoration protocols in face of rapidly declining reef areas in the East Africa reef system. Two mid-water nurseries were established in September 2007 at Chumbe Island in Zanzibar and Chole Bay in Mafia Island, each holding 10,000 fragments from six species(*Acropora formosa, A. hemprichi, A. nasuta, Millepora* sp., *Pocillopora verrucosa, Porites cylindrica*), each represented by three genotypes. The results, following a period of nine-months nursery phase, revealed interspecific significant differences in survival and growth rates for the acroporid species, *Pocillopora verrucosa* and *Millepora* sp., which showed improved outcomes when compared with *Porites cylindrica.* In both sites, *Millepora* suffered no mortality while other species exhibited low mortality ranging between 3% and 24% (per coral genotype) in Zanzibar and between 13 and 44% in Mafia Island. Coral species in Zanzibar nursery performed better in growth rates than those in Mafia; however, farmed corals in both sites were ready for transplantation within this short period of nine months. In total, 14,022 nursery farmed coral colonies were transplanted; 6912 in Changuu Zanzibar and 7110 in Kitutia, Mafia. In each site, we randomly set up 12 plots ($36m^2$ each), of which three were transplanted with a mix of three *Acropora* sp. (Treatment 1, T1), three with a mix of all six scleractinian species (T2), and six served as controls. Within one month of transplantation, an outbreak of *Acanthaster planci* in Changuu caused mortality at 50%. One year survival of transplants in T1 and T2 at Kitutia reached 66.4% and 62.5% respectively, significantly higher than at Changuu; an outcome recorded through species-by-species comparisons for four species (*P. verrucosa, P. cylindrica, A. muricata, A. nasuta).* One year following transplantation, no significant difference was documented in ecological volumes (EV)

between T1 and T2 a result which is in contrast to the among species comparisons in T1, at each site. A within treatment one-way ANOSIM comparison for fish assemblages performed between the first and last three months of the transplantation year (Kitutia reef) revealed strong separation (T1, Global R = 0.743, P <0.001; T2, R = 0.445, P < 0.001 and T3, R = 0.694, P < 0.001) while the same treatment revealed weak separation at Changuu site T1 (R = 0.035, P >0.262) and T2 plots (R = 0.119, P < 0.043). Similarly, one-way ANOSIM done on the initial and last 3-month periods on invertebrates' community composition (at all sites, except T1 of Changuu reef), showed no significant difference for communities composition between the initial period and end of the sampling period. Furthermore, The genetic diversity and population genetic structures of *Drupella cornus* populations from six localities in the northern Gulf of Eilat (GOE) and five localities in Tanzania (269 individuals) were investigated using mitochondrial cytochrome c oxidase subunit I (COI) gene sequences. Overall, 108 haplotypes, 47 in GOE and 61 in Tanzania were revealed, with similar calculated haplotype diversity for all *D. cornus* populations within each location (0.9 +/- 0.00025 and 0.903 +/- 0.00078, respectively). Only one haplotype was shared between the GOE and Tanzanian populations. Network analysis for the 108 COI haplotypes displayed two major clades, separated by nine mutations. Bayesian analyses of population structures revealed two clusters highly correlated with the collecting region. Analysis of molecular variance showed 73% of the molecular variance for all *Drupella* populations is a result of the differences among regions. Within regions, most of the molecular variance is based on within population differences, 89% north vs. south in Tanzania and 98%, Israel vs. Jordan in GOE. Fu's and Tajima's D values for all populations were negative, suggesting that the *Drupella* populations in GOE and Tanzania underwent population expansion or purifying selection. Conclusively, with the cost for establishing the nursery standing at 0.1 US$ per fragment and that of transplantation at US$ 0.19 per colony, the results indicated that large quantities of coral colonies can be generated and transplanted in damaged reefs at relatively low cost. Cumulatively, field results and economic evaluations showed that transplantation of nursery-grown colonies might uphold critical ecosystem functions, while successfully used in reversing coral reefs' phase shifts states. Furthermore, a study on population genetic of *D. cornus* demonstrates that the distance between the two regions, GOE and Tanzania, provides for the observed differences in genetic structuring. Furthermore, the existing oceanic dispersal patterns governs the observed within regions population genetics variations.

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Preface and research questions

This dissertation addresses issues of coral reef management in the face of their rapid decline due to both natural and human disturbances, with a strong emphasis on active coral reef restoration and population genetics of one of the recent major coral stressors, *Drupella cornus*. I employed the two-step coral gardening concept and followed the genetic diversity and population genetic structure of *Drupella cornus*, using mitochondrial cytochrome c oxidase subunit I (COI) gene sequences. The major aim of this study was to evaluate the efficiency of the tool-box of protocols/methodologies for low cost coral management strategies in Tanzania with an eye to other developing countries. Specific aims:

1. Can we employ a mid-water nursery, under Tanzanian specific environmental conditions, for different coral species?
2. What is the natural variation in terms of growth and survivorship between different genotypes of the same species when farmed/transplanted under same conditions?
3. Are the colonial morphological forms representing nursery related traits for survivorship and growth rates?
4. Which ecological engineering concepts can be applied?
5. What is the level of connectivity between different populations of *Drupella cornus* in Tanzania?
6. How do local communities perceive the coral reef decline situation?

I declare that specific study aims were all met and the information provided in this thesis is entirely a product of my own work based on them.

1.0 Scientific background

1.1. Coral restoration

The world has in recent times witnessed very massive coral reefs decline. The major causes for this decline are anthropogenic in nature (Hughes *et al.*, 2003; Bellwood *et al.*, 2004) which are further enhanced and complicated by severe natural disturbances such as storms (Connell, 1997; Crabbe *et al.*, 2008; Garrison and Ward, 2008), crown-of-thorn outbreaks (Kenneth, 1994; Nicholas *et al.*, 2004) and large scale coral bleaching events (Williams et al., 2001; Hoegh-Guldberg *et al.*, 2004), reflecting global change impacts. One of these massive bleaching events, encompassing the whole Western Indo-Pacific region (March to May 1998) resulted into immense regional coral deaths, including the reefs along Tanzanian coastline (Wilkinson *et al.*, 1999; Lindahl *et al.*, 2001; Garpe and Öhman, 2003). The destruction by these bleaching events was severe and many reefs have ever since remained bare, probably due to prevention of recruitment resulting from the appearance of macro-algae beds and/or absence of seeding populations that may have been wiped out (Lindahl *et al.*, 2001).

The decline prompted the initiation of various novel management and restoration initiatives, which in the beginning were mostly passive. One of the common passive approaches, also practiced in many reef areas, was the establishment of protected marine areas and no-take zones for promoting natural recovery (Roberts *et al.*, 2001). Despite these efforts, the decline continued unabated, an indication that the approach has failed to achieve the intended results (Rinkevich, 2005a). This general failure led to promotion of other working hypothesis for reef restoration worldwide that, however, resulted in imperfect results (Harriott and Fisk, 1988; Gleason and Wellington, 1993; Naveh, 1994; Christensen *et al.*, 1996; Hobbs and Norton, 1996; Bowden-Kerby, 1997; Dobson *et al.*, 1997; van Treeck and Schuhmacher, 1997; Edwards and Clark, 1998; Franklin *et al.*, 1998; Smith and Hughes, 1999; Putz, 2000; Bowden-Kerby, 2001; Bruckner and Bruckner, 2001; Lindahl, 2003; Rinkevich, 2005a,b, 2006, 2008). Additionally, many of these attempts employed techniques that were either labor intensive or too expensive to be applied in developing countries where most reefs are found (Edwards and Clark, 1998; Allison et al., 2003). Following this, a number of researches issued that were aimed at developing suitable low cost restoration protocols. One of these includes a coral reef

restoration concept, deriving its rationale from well-established scientific field of terrestrial forestation named "coral gardening concept" (Rinkevich, 1995).

1.2 Coral restoration through application of gardening concept

In the coral gardening approach, like corresponding terrestrial silviculture protocols, a two-steps approach is applied. In terrestrial ecosystems, silviculture is a cultural practice involved in controlling the establishment, growth, health and quality of trees or forest to meet intended objectives. The same conservation approach has been put in practice in the management of denuded coral reef ecosystem (Rinkevich, 1995). In this approach, small sized coral fragments are first reared in a protected nursery then upon reaching suitable sizes are transplanted into denuded areas. In comparison to the harmful practice of harvesting corals for direct transplantation from donor reef areas, the establishment of coral nurseries, containing local species and genets that are managed in a sustainable manner, eliminates the need for the extraction of valuable coral material for transplantation (Rinkevich, 1995, 2000; Epstein and Rinkevich, 2001; Epstein *et al.*, 2001). A nursery phase is very important as it provides a relaxed, non-competitive environment for the coral fragments to grow at a bigger size thus ensuring better survivorship and growth before being transplanted. Through transplantation of nursery-grown fragments, besides promotion of diversity, it also helps in preventing genet and species extinctions in degrading sites. The processes is archived through selecting some coral species, which in most case, formed part of the reef biota before denudation and later on farm them in nurseries before being transplanted (Mbije *et al.*, 2010, 2013). This coral reef restoration technique besides improving coral diversity it also helps in diversifying of genetic of coral (Rinkevich, 1995, 2000, 2002, 2005a, 2006, 2008; Epstein and Rinkevich, 2001; Soong and Chen, 2003; Rinkevich *et al.*, 2005; Shafir *et al.*, 2003, 2006a, b; Shafir and Rinkevich, 2005, 2008; Amar and Rinkevich, 2007; Shaish *et al.*, 2008, 2010a,b; Levi *et al.*, 2010; Mbije *et al.*, 2010, 2013).

1.3 Genetics population structure

The gastropod *Drupella cornus* is known to voraciously prey and cause heavy damage on corals (Moyer *et al.*, 1982; Turner, 1994; Al-Horani *et al.*, 2011). Although there is an unusual example where a usually non-preferred coral is eaten (Cumming and McCorry, 1998), this predator with a very wide biogeographic distribution feeds exclusively on corals (Moran, 1986; Turner, 1994; McClanahan, 1997; Morton and Blackmore, 2009;

Shafir *et al.*, 2008). The species shows a strong tendency towards preference of microhabitat according to its different life stages; while juveniles are mostly found hiding cryptically in braching coral forms, adult are ubiquitously found in several substrate and coral growth forms (Forde, 1992; Schoepf *et al.*, 2010). Although the gastropod occurs as a resident species in many tropical reefs in low numbers (McClanahan and Muthiga, 1992) it recently has been shown to make intermittent outbreaks (Riegl and Velimirov, 1994; McClanahan, 1994; McClanahan *et al.*, 1997; Antonius and Riegl (1998), which inflict massive damage to reefs (Turner, 1992b; Turner 1994; Cumming and McCorry, 1998). There are few studies to establish the causes of these outbreaks but many hypotheses tend to associate it with increased frequency in coral injuries although there could be others factors such as removal of unspecialized potential predators such as lethrinids (emperors) and ballistids (triggerfish) (McClanahan, 1994; Armstrong, 2009).

Drupella cornus has a relatively long life expectancy of up to 8 years (Wilson 1992; Williams *et al.*, 2011) with larvae spending a planktonic life for about 60 days (Turner, 1992a). The long planktonic larval life has effects on species distribution and therefore can influence genetic flow and divergence (Nishida and Lucas, 1988; Ayre and Dufty 1994; Ayre *et al.*, 1997; Ayre and Hughes, 2000). Likewise, population genetics study has indicated that *Drupella cornus* show high tendency towards exhibiting high local patchiness with low level subdivision between distant cohorts (Holborn *et al.*, 1994). Despite the increased interest in *Drupella cornus* around the world, still there little information that is related to its distribution and genetic connectivity in many reefs. Among others, studying genetic population differentiation are crucial in conservation biology (Wright, 1977; Michalakis and Excoffier, 1996; Frankham and Ralls, 1998; Saccheri *et al.*, 1998; Eldridge *et al.*, 1999; Higgins and Lynch 2001).

2.0 Data collection

Data collection hinged on four main axes: farming of corals in mid water nurseries, coral transplantation and monitoring, *Drupella cornus* population genetic connectivity, and local community interactions with coral reefs.

2.1 Nursery construction

Assembling the nurseries was adapted from Shaish *et al.* (2008), modified to suspended, midwater constructions at 4 m at both sites. Nurseries of rectangular modular tables ('nursery base' modules of 6 m^2 each) were made of 2" PVC pipes, 6 m long, that were joined end-to-end by 1m PVC pipes. Each nursery was made of 10 suspended tables, each tied to the bottom by four cement blocks, 50kg each, and suspended by 6 plastic containers (buoys) of 5 litres each, previously used to carry cooking oil.

Three coral genotypes (growing at least 20 m from each other) from each one of six species, four branching (*Acropora formosa, A. hemprichi, A. nasuta, Millepora* sp.) and two submassive (*Pocillopora verrucosa, Porites cylindrica*), were collected from reefs situated 12-13 km from the nursery site in Chumbe and 3 km from Chole Bay. Using a chisel and hammer, whole donor colonies were detached from the substrates, immersed in 20 litres plastic containers filled with fresh seawater and transported immediately to nursery sites where they were cut into 1.5 to 3 cm long fragments by electrician's wire cutters providing appropriate sizes to fit into pre-cut 7 cm long domestic hosepipes. Sampled donor colonies provided 50 (*Porites cylindrica*) to 100 (*Acropora hemprichi*) fragments. We prepared about 700 hosepipes pieces, 7 cm long each, from a 50 m hosepipe roll, commonly found in hardware shops. Each hosepipe was then trimmed on one side to make a sharpened end for easy insertion into 0.5' mesh size fishing nets (1 m2) that were stretched over 0.5' PVC frames. Each frame carried 169 hosepipes arranged in 13 × 13 straight lines. Nets were obtained from a stock of confiscated nets at Mafia Marine Park acquired through an exchange program that promoted the use of larger fishing net-gear eyes. Freshly prepared small coral fragments were manually inserted into the hosepipes pieces while working on a boat with local fishermen. Immediately after filling up each individual frame, it was immersed in water and tied to the nursery by cable ties.

The nurseries were monitored once a month for a duration of nine months, as per Shafir *et al.*, (2006a,b). In each session, we documented the status of all coral fragments (missing, dead, partial death, alive, bleaching). Ramets (30 fragments for each genotype in each site, i.e., 90 ramets per species, per site) were digitally photographed (Cannon Digital US90IS) twice at day zero, just before and after immersion, and each month, from the side and from

above. Detailed growth patterns were taken by a Vernier calliper, which involved measuring height, length and width of the developing fragment. The average diameter (d) of each ramet (d) was calculated as: d = (l+w)/2. Results are presented as means with standard error. An ecological volume index was established for each fragment by approximating the initial and developing structures to the shape of a cylinder, with volume V = πr2h (r = (l+w)/4 (Rinkevich and Loya, 1983), best expressed the general architecture of developing colonies. Following the exponential growth rates of the colonies at small sizes, their growth rate constant (K) per day for ecological volumes (E) measurements were calculated by the formula $E_t = E_0 e^{kt}$, providing $k = (\ln E_t/E_o)/t$ (t=time, in days, 0-values at the beginning of the experiment; Shafir *et al.*, 2006b). Statistical analyses were performed by a SPSS 16 2007 data editor. The preliminary assumption was the existence of normal distribution and therefore T Test and One Way ANOVA tests were performed. If data were not normally distributed, we employed non-parametric tests like Mann–Whitney U Test and Kruskal–Wallis Test.

2.2 Coral transplantation

2.2.1 Selection of coral species

We used one-year old nursery reared scleractinian colonies from six species: *Acropora muricata*, *A. nasuta*, *A. hemprichi*, *Pocillopora verrucosa*, *Porites cylindrica* and *Millepora* sp. (Mbije *et al.*, 2010). The transplantation sites at Changuu and Kitutia (about 15 and 23 km respectively from nurseries) were located at the same depths as the original site from which the nursery materials originated and consisted of substrates with consolidated dead coral fragments, loose gravel and a few live corals. Farmed colonies were carefully removed from the nursery by means of carpentry scissors, their bases cleaned from settling sedentary organisms, immersed in marked, large water-filled plastic bins, and loaded onto a boat, before being transferred to the transplantation sites. At each transplantation site, we haphazardly established 12 plots (each $36m^2$) that were clearly marked for further inspection; six of which were transplanted with nursery reared coral colonies. We followed a pre-set design comprised of three treatments (T1-T3): three plots were transplanted with a mix of three *Acropora* spp. (T1), three plots transplanted with a mix of all six coral species (T2) and six plots, designated within non-transplanted areas, served as controls (T3). Each transplanted plot was further partitioned into nine sub-plots, each $4m^2$. Eight sub-plots within each plot were transplanted with coral colonies (some

were populated with colonies of the same coral genotype, with various genotypes of the same species, or different coral species) at 16cm distance from each other, while the central $4m^2$ sub-plot was left unattached, to elucidate possible impacts of surrounding transplanted plots. Each $4m^2$ sub-plot was populated with 144 coral colonies, 1152 colonies per a $36m^2$ plot. In total, 6912 coral colonies were out planted in Changuu reef at Zanzibar and 7110 in Kitutia reef at Mafia. Although the design and fragment spacing was the same in both sites, we transplanted extra colonies in Kitutia reef and these were not part of the monitored plots.

A large proportion of the substratum at Kitutia reef is composed of consolidated dead coral branches, mostly of *Acropora* spp., while at Changuu reef the substratum is composed of hard rock with attached dead coral branches. Two transplant attachment techniques were tested, (a) plugging the short pieces of the hosepipes carrying corals directly between the firmed attached dead coral branches (4123 and 5324 transplants in Changuu and Kitutia respectively, and (b) plugging the short pieces of hosepipes into holes drilled in the substrates (2789 and 1786 transplants in Changuu and Kitutia respectively). General-purpose epoxy compound (M-seal®) reinforced the attachment of loose transplants to the substratum.

2.2.2 Monitoring and data analysis

Once a month, a team of three divers monitored the transplantation plots and transplants for bleached colonies, detached transplants, survival rates, corals' size measurements, fish surveys, and recruitment of selected reef associated invertebrates. Every three months, we assessed size-measurements of transplants in the field by measuring height, width and length of each colony with plastic callipers. From these measurements, the colonies' ecological volumes (EV) in cm^3 were calculated as $EV = \pi*H*([W+L]/4)$ (Rinkevich and Loya, 1983).

Fish surveys involved identifying and counting all adult individuals found to occupy each 3D setting (plot and water column around and above the fragments); all fishes > 3cm long were identified to the lowest taxon possible. Censuses were performed by slow-motion scuba diving that allowed counting fish populations without disturbing the animals. This exercise was repeated in each plot of the three treatments and fish censuses were

conducted between 1000H and 1500H and completed within the same week (the exercise was repeated twice for each plot). Analyses were performed on fish abundance, species richness and species composition. While fish abundance refers to the number of fish of a certain species, species richness is the total number of fish species, and species composition refers to the relative abundance of fish species per treatment/plot. Censuses of large invertebrates were carried out under the same protocol, with a single deviation, observing their presence within crevices, in the substrates, between coral branches, underneath coral colonies and on hard substrate.

2.2.3 Coral recruitment

From the start of the transplantation experiment, we closely monitored the central bare sub-plots within the plots for coral settlement. Because of the lengthy underwater time required for this procedure, we were forced to conduct our monitoring at low spring tide (mean springtide at 3.3m and maximum at 4.0m).We applied an extensive underwater search procedure that included fanning away sediments while searching for recruits. Since most coral recruits are very small at settlement and their growth rate is slow (Wallace and Bull 1981), we concentrated on established recruits of size ranging between 0.5 and 2.0cm, also performed in order to minimise confusion or mistakes in coral species identification. Identification of coral recruits to the lowest possible taxon was done with the aid of the key plates found in English *et al.* (1994), Wilkinson (1994) and Babcock *et al.* (2003) that were laminated to suit underwater work. Results were summarised as average numbers per species/treatment/site.

Average colonies' sizes, mean percentages of survival, detachment and bleaching were calculated for each sub-plot/treatment/monitoring date. Results were analysed by using an SPSS 16 2007 data editor. We employed t-test when comparing between sites and one way-ANOVA for comparison between treatments. Prior to the analysis, all data were subjected to Levene's (1960) test to check for assumption of homogeneity of variances and whenever necessary, the data were square root transformed. Similarly, the analysis in fish and invertebrate assemblage structure within each treatment, among treatment and between sites, were analysed using either one way or two way crossed ANOSIM (on density data) based on Bray-Curtis similarity measures (Clarke, 1993). To reduce the weight of dominant values (mostly from schooling taxa), the data were square root

transformed. Non-metric multidimensional scaling (nMDS) ordinations were used to visualise the patterns of similarities among sites.

2.3 *D. cornus* population genetics study

2.3.1 Specimen collection and DNA extraction

Drupella cornus specimen were haphazardly collected from alive coral colonies in shallow reefs (between 1 to 12 m) at the Gulf of Eilat, northern Red Sea (Israel - five sites; Jordan - three sites) and from five Tanzanian reefs (Table 1, Fig. 2; total of 269 specimens). The animals were shipped to the laboratory in seawater-filled containers. Genomic DNA was commenced by cracking each snail shell to allow the removal (by industrial razor blades) of the animal's pedal area. Each individual foot was cut in two, each part homogenized separately in 200 µl of lysis buffer (0.25 M Trisborat pH 8.2, 0.1 EDTA, 2% SDS, 0.1M NaCl mixed with 40 µl of sodium per-chlorite ($NaClO_4$), following Graham (1978). Equal volumes of phenol/chloroform/isoamyl alcohol (25:24:1) were added, mixed by vortex and centrifuged for 10 min at 14,000 g, 4°C. The aqueous phase was further extracted with chloroform/isoamyl alcohol (24:1). The DNA was precipitated with absolute ethanol, washed with 70% ethanol, dried and re-suspended in water.

2.3.2 COI amplification

The COI gene fragments of *Drupella cornus* samples were amplified according to Folmer *et al.* (1994) using the COI marine invertebrates' universal primers (HCO2198r, 5'TAAACTTCAGGGTGACCAAAAAATCA3' and LCO1490f, 5'GGTCAACAAATCATAAAGATATTGG 3'). Two µl of diluted DNA (1:50) from each sample were added to a reaction mixture containing 5 µM of each primers and DreamTaq™ DNA polymerase (Green PCR Master Mix 2×; Fermentas) in a total solution volume of 50 µl. Reaction conditions were as followed: 74°C for 10 sec and 95°C for 5 min followed by 35 cycles of 95°C for 1 min, 45°C or 1 min and 72°C for 1 min and an additional elongation step of 72°C for 10 min. The PCR products were screened on 1.3% agarose gel and then, were sent for direct sequencing (Macrogen Inc, South Korea) using the same universal primers from both sides.

2.3.3 Sequence Analysis

The Drupella cornus origin of each sequence was verified through BLAST searches against Genbank (http://blast.ncbi.nlm.nih.gov/Blast.cgi). All sequences which had high homology to D. cornus (more the 96% of identity), were aligned and corrected using BioEdit software (Hall, 1999). Sequences, whose forward and reverse sequencing match less than 1% were deemed good quality sequences and used for further analysis. Haplotype diversity analyses were performed using GeneAlex 6.5 (Peakall and Smouse, 2006) and DNAsp 5.10 (Librado and Rozas, 2009). Tajima's D and Fu's Fs neutrality tests were calculated per population with DNAsp 5.10. Neighbour joining and Maximum likelihood trees were constructed with ClustalX (Thompson *et al.*, 1997) and Tapoli V2.5 software. Trees were drawn by MEGA5 (Tamura *et al.*, 2007). Bayesian clustering for population structure was studied using BAPS 6.0 (Corander *et al.*, 2008). Network analysis was performed by NETWORK 4.6.1.1 (Clement *et al.*, 2000). Nei genetic distance for all Drupella populations was calculated with GeneAlex 6.5. Neighbor joining phylogenetic tree for all population was drawn by online T-REX Program at the web site: http://www.trex.uqam.ca/index.php?action=trex&menuD=1&method=2. D. cornus sequences FR853820, FR853819, FR853818, FR853817, FR853843, FR853842, FR853841, FR853830, FR853826 and FR853825 (from Guam, Hawaii, Japan, New Caledonia, Philippines and Vanuatu; Indo Pacific; Claremont *et al.*, 2011) from the Gene Bank were used for comparison in the COI phylogram.

2.4 Effects of community interaction with coral reefs in Tanzania

2.4. Field data collection

Household surveys were conducted in three villages, Kivinje, Songo songo and Songo Mnara Purposive sampling was used to select the study villages based on accessibility and position. Kivinje was chosen as representing a relatively developed urban area, Songo songo as representing islands and Songo Mnara as representing isolated fishing villages, common along Kilwa coastline, away of large markets urban areas. The village registrars from the three villages were used as a sampling frame. Ninety households, representing 10 % of the total households for the three villages, were randomly selected for this survey. A questionnaire was administered to participants for data collections. The questionnaire included questions on type of fishing gears used, fishing methods, types of fishing grounds, content and quantities of fish catches, quantities sold and revenue from their sale,

total household income, percentage contribution of fishing products to total income, trends in fishing comparing period before and after 1997/98 massive coral bleaching, fishing constraints, fishing trends and climate change effects. In addition, direct observations were conducted in various locations to appreciate types of fishing vessels, fishing gears, contents of catches at fish markets, crops grown and general life styles of the communities in the three selected villages.

Focus group discussions were held with villages officials, district natural personnel and agriculture officers to get their views on trends of fishing, caught fish species and their contribution in revenue earning, patterns in agriculture as well as management challenges of resources.Secondary data were obtained by reviewing literature on Kilwa district's coral reefs, mangrove, sea grass and fishery. The documents reviewed included Mangrove Management Project report (TCMP, 2009); Fisheries Regulation Act (URT, 2005); Guideline for Collaborative Management of Marine Resources of Kilwa District; Collaborative Fisheries Management (CFM) monitoring plans for Rufiji, Mafia, Kilwa Fishing communities and the Annual District Fisheries Reports (URT, 2005) .

3.0 Publications

3.1 Testing the First Phase of the 'Gardening Concept' as an Applicable Tool in Restoring Denuded Reefs in Tanzania. Ecological Engineering (2010) 36: 713-721

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Abstract

Studies on coral reef restoration through a two steps coral gardening protocol have lately proved to be a viable solution for future reef restoration. This involves a first step of gardening small colonies in mid-water nurseries and the second step, their transplantation, upon reaching suitable size, onto the pre-surveyed damaged areas. We established in September 2007 two mid-water nurseries, each holding 10,000 fragments measuring 2 cm average initial size, at four meter depths (high tide) in Zanzibar and Mafia Islands, Tanzania. Each nursery comprised six species, each of which was represented by three genotypes. During nine months, we followed developments by analyzing and comparing survivorship and growth rates of fragments between the different nurseries, species and genotypes. A significant difference between species survival and growth rates was observed in acroporid species, in *Pocillopora verrucosa* and *Millepora* sp., which showed better success than *Porites cylindrica.* In both sites, *Millepora* suffered no mortality and other species exhibited low mortality ranging (per coral genotype) between 3% and 24% in Zanzibar (most cases below 10%) and between 13 and 44% (mostly below 25%) in Mafia Island. Most of fragments' mortality occurred during the first two nursery months. Coral species in Zanzibar nursery also performed better in growth rates than those in Mafia, but in both sites, farmed corals were ready for transplantation just nine months after the nursery was set-up. Economic evaluations involved in the overall nursery set and the results indicated that the coral gardening approach could be used in Tanzania to generate large quantities of coral colonies for restoration of damaged reefs at relatively low cost.

Key words; Zanzibar, Mafia Island, mid-water nurseries, coral gardening

1. Introduction

The culprits responsible for the unprecedented worldwide coral reefs decline are primarily anthropogenic in nature (Hughes *et al.*, 2003; Bell *et al.*, 2006), and their impacts are enhanced by major natural disturbances such as storms (Connell, 1997; Crabbe *et al.*, 2008; Garrison and Ward, 2008), crown-of-thorn outbreaks (Kenneth, 1994; Nicholas *et al.*, 2004) and massive coral bleaching events (Williams *et al.*, 2001; Hoegh-*Guldeberg et al.*, 2007). Tanzanian fringing and patch reefs, stretching along about 800 km of continental shelf, have been, with no exception, most severely hit by anthropogenic activities and natural catastrophes with significant proportion of the reef structures damaged beyond the power for natural repair (Lindahl *et al.*, 2003). In Tanzania, reef system comprises a very significant natural resource, on which coastal fishing communities depend entirely for their livelihood (Darwall and Guard, 2000; Muhando *et al.*, 2002; Mbije, 2001; Mbije *et al.*, 2002). In fact, 70% of artisanal fisheries of Tanzania come from the coral reef ecosystems (Ngoile and Horrill, 1993; Jiddawi and Öhman, 2002), supplying 90% of the animal protein consumed and the primary source of income for the people (Wagner, 2004). Tanzanian coral reefs are also a major tourist attraction. Coastal tourism based on coral reefs brings foreign currency into the country and provides livelihoods for coastal people through employment and other services. With these products and services provided by Tanzanian reefs, any sign of habitat destruction, before documenting major biodiversity devastation and extensive species losses, would be sufficient cause for alarm, to promote immediate and imperative mitigation measures. This tenet is further backed by the continues regression of Tanzanian reefs, a decade following a massive bleaching events (Mohammed *et al.*, 2000; N. Mbije, pers. obs.), which, while instigating active conservation schemes and several rehabilitation programs, has yielded no obvious success (Franklin *et al.*, 1998; Lindalh, 1998, 2002, 2003). This failure to promote a working hypothesis for Tanzanian reef restoration resembled worldwide attempts that resulted in unsatisfactory, sometimes conflicting, outcomes (Harriott and Fisk, 1988; Gleason and Wellington, 1993; Bowden-Kerby, 1997; Edwards and Clark, 1998; Bruckner and Bruckner, 2001; Rinkevich, 2005a,b, 2006, 2008). Additionally, many of these attempts employed techniques that were either labor intensive or too expensive to be applied in a developing country like Tanzania.

To overcome these obstacles, a two-step restoration operation termed as the 'gardening concept' (Rinkevich, 1995, 2000, 2005a, 2006, 2008; Epstein *et al.*, 2001; Epstein and

Rinkevich, 2001) was introduced and weighed against traditional conservation acts. This concept incorporates stock farming of small coral fragments in mid-water nurseries (step 'a') which, upon reaching suitable sizes, are transplanted onto denuded reef areas (step 'b'). This notion for active reef restoration had been tested and proved to be successful in studies employed in the northern Gulf of Eilat, Red sea (Rinkevich, 2006, 2008; Shafir *et al.*, 2006a,b) and recently in other localities worldwide, including the USA (Herlan and Lirman, 2008), the Philippines (Shaish *et al.*, 2008) and Thailand (Putchim *et al.*, 2008). Furthermore, the gardening technique has been found to be the least laborious, the least costly and with a high yield of survivor and growth rates (Rinkevich, 2006; Shafir *et al.*, 2006a,b; Amar and Rinkevich, 2007; Shafir and Rinkevich, 2008; Shaish *et al.*, 2008). Different localities worldwide represented site-specific conditions and unalike working considerations (Shafir *et al.*, 2006a; Shaish *et al.*, 2008). This calls for general standardization of the basic protocols, particularly, the development of effective and approved-to-use regional specific protocols that would transfer restoration activities into a unified conceptual and practical scheme.

The present study, the first gardening project initiated in East Africa, aims at testing the applicability and efficiency of the concept for restoring reefs in Tanzania damaged by human activities and the deadly 1997/98 SST warming event. We concentrated on step 'a' of the gardening concept, farming coral colonies in underwater floating nurseries.

2. Materials and Methods

2.1 Study sites

The study was carried out at Chumbe Island in Zanzibar (6°16' N 39°18' E) and at Mafia Island Marine Park (7040' N, 40040' E), about 120 km away (Fig. 1). The reefs in both sites had undergone various forms of damage (Wagner, 2004), one of them being the 1998 global coral bleaching event (Souter *et al.*, 2000; Lindahl *et al.*, 2001). Chumbe Island was selected as the nursery site because it offers rangers' protection whereas the coral fragments were collected about 12 km to the north, at Changuu Island. In Mafia, Chole Bay was the selected site for the nursery and coral fragments were collected within the same bay from corals of opportunity. Both selected sites have been managed as protected reef areas whereby the former is a coral park leased and run by a private company and the later is a marine park. Chumbe Island has a shallow fringing reef on the western side, with more than 120 described corals species, from a depth of 3 m at the northern tip to 10 m (at

high spring tide) in the middle of the Island. This reef is one of the healthiest and most diverse in Tanzania. Further south are scattered coral heads of *Porites lobata* interspersed within sea grasses. The eastern side of Chumbe Island is made of shallow water sandbanks, with beds of seagrass and some solitary small coral heads in deeper parts. Chole Bay, Mafia, is located in the deep channel that brings water from high seas into the bay with very strong currents at spring tides. The bay has several isolated patchy reefs; the most significant is Msumbiji reef with high coral diversity. In both sites, coral nurseries were placed at above 10 m depth substrates in high spring tides. In both nurseries, farming of the same Tanzanian species at the same depth (4 m) was evaluated, providing a great impetus to compare impacts of local conditions. The whole operation was strongly supported by local fishermen who agreed to cooperate in the nurseries' preparation and construction at small wage. Nurseries' constructions were guided by suggestions and recommendations offered by the literature (South *et al.*, 2001; Fox *et al.*, 2005; Lindahl, 2003; Shafir *et al.*, 2006a,b; Edwards and Gomez, 2007; Shafir and Rinkevich, 2008; Shaish *et al.*, 2008). Basically, the nurseries were situated in mid-water, above the substrate to avoid sedimentation; far from the reef to avoid coralivorous and fishermen impacts; in an area sheltered from storms and wave actions but sufficiently shallow to supply good light conditions for fast growth and easy maintenance (Shafir and Rinkevich, 2006a,b).

2.2 Nursery construction

Assembling the nurseries was adapted from Shaish *et al.*, (2008), modified to suspended, midwater constructions at four m depth (mean water of spring tides; tidal amplitudes are 3.5 m on average). Nurseries of rectangular modular tables ('nursery base' modules of 6 m^2 each) were made of 2" PVC pipes, 6 m long, that were joined end-to-end by 1m PVC pipes. Each nursery was made of 10 suspended tables, each tied to the bottom by four cement blocks, 50kg each, and suspended by 6 plastic containers (buoys) of 5 litres each, previously used to carry cooking oil.

Three coral genotypes (growing at least 20 m from each other) from each of six species, four being branching (*Acropora formosa, A. hemprichi, A. nasuta, Millepora* sp.) and two submassive (*Pocillopora verrucosa, Porites cylindrica*), were collected from reefs situated at 12-13 km from the nursery sites in Chumbe and at 3 km in Chole Bay (Table 1). Using a chisel and hammer, whole donor colonies were detached from the substrates, immersed in 20 litres plastic containers filled with fresh seawater and transported immediately to

nursery sites where they were cut into 1.5 to 3 cm long fragments by electrician's wire cutters providing appropriate sizes to fit into pre-cut 7 cm long domestic hosepipes (Fig. 2e, f). Sampled donor colonies provided 50 (*Porites cylindrica*) to 100 (*Acropora hemprichi*) fragments. We prepared about 700 hosepipes pieces, 7 cm long each, from a 50 m hosepipe roll, commonly found in hardware shops. Each hosepipe was then trimmed on one side to make a sharpened end for easy insertion into 0.5' mesh size fishing nets (1 m2) that were stretched over 0.5' PVC frames (Fig. 2b-d). Each frame carried 169 hosepipes arranged in 13 × 13 straight lines. Nets were obtained from a stock of confiscated nets at Mafia Marine Park (Fig. 2a) acquired through an exchange program that promoted the use of larger fishing net-gear eyes. Freshly prepared small coral fragments were manually inserted into the hosepipes pieces while working on a boat with local fishermen (Fig. 2e). Immediately after filling up each individual frame, it was immersed in water and tied to the nursery by cable ties.

2.3 Monitoring and data analysis

The nurseries were monitored once a month for a duration of nine months, as per Shafir *et al.*, (2006a,b). In each session, we documented the status of all coral fragments (missing, dead, partial death, alive, bleaching). Ramets (30 fragments for each genotype in each site, i.e., 90 ramets per species, per site) were digitally photographed (Cannon Digital US90IS) twice at day zero, just before and after immersion, and each month, from the side and from above. Detailed growth patterns were taken by a Vernier calliper, which involved measuring height, length and width of the developing fragment. The average diameter (d) of each ramet (d) was calculated as: d = (l+w)/2. Results are presented as means with standard error. An ecological volume index was established for each fragment by approximating the initial and developing structures to the shape of a cylinder, with volume V=πr2h (r= (l+w)/4 (Rinkevich and Loya, 1983), best expressed the general architecture of developing colonies. Following the exponential growth rates of the colonies at small sizes, their growth rate constant (K) per day for ecological volumes (E) measurements were calculated by the formula $E_t=E_0e^{kt}$, providing $k=(\ln E_t/E_o)/t$ (t=time, in days, 0-values at the beginning of the experiment; Shafir *et al.*, 2006b).

Statistical analyses were performed by a SPSS 16 2007 data editor. The preliminary assumption was the existence of normal distribution and therefore T Test and One Way ANOVA tests were performed. If data were not normally distributed, we employed non-parametric tests like Mann–Whitney U Test and Kruskal–Wallis Test.

3. Results

The two mid water nurseries in Zanzibar and Mafia were established during July-September 2007, housing altogether 21,600 coral fragments cut from six most common coral species in the Tanzanian reefs. Assembling nurseries and filling them with coral fragments were performed by six fishermen in each site, two 'nursery base' modules per day, totalling 5 working days per site. Construction of coral carrying $1m^2$ quadrates, including mending nets (Fig. 2a-c), took about two hours per quadrate; totalling 240 person-hours (1.5 person/months per site for 120 quadrates). Donor corals originated from various locations (Table 1) and likewish Shafir *et al.* (2006a, b), each worker produced 100-120 fragments/hour, in addition to 15 min for inserting the hosepipe carrying fragments onto each net frame (Fig. 2d-e). A 10,000 fragments nursery construction (Fig. 2f), per site, was performed by six persons in one month and totally cost about $1080 (Table 2) with a firm nursery bed and construction, reusable for consecutive sets of farmed corals.

3.1 Coral fragment survivorship

We monitored coral survivorship, bleaching and detachment on a monthly basis. Apart from natural causes (fish bites, predation by coralivorous organisms, competition with fouling organisms), fishermen activities and nocturnal poaching have destroyed 12 of the $1m^2$ units in both sites, causing death to farmed fragments. In Chumbe, we recorded fishing-caused deaths in *Pocillopora verrucos* (n=32), *Porites cylindrica* (102), *Acropora hemprichi* (19), *A. formosa* (11), and *A. nasuta* (24). Fishing-caused mortalities in Mafia influenced farmed *Porites cylindrica* (n=123), *A. nasuta* (101) and *Pocillopora verrucosa* (21). Subtracting these figures from the mortality toll revealed very high survivorship rates of all genotypes and coral species farmed. *Millepora* suffered no mortality at all in both sites and other species exhibited low mortalities ranging (per coral genotype) between 3% and24% in Zanzibar (most cases below 10%) and between 13% and 44% (mostly below 25%) in Mafia Island (Table 3). With regard to fragment detachment, in the first two months, few detached fragments were found on the net and were re-fixed to their original site, so no loss from detachment had been recorded. It had been recorded that during the early nursery phases, all acroporid and *Porites cylindrica* fragments, in both nurseries, produced copious quantities of mucus that, in many, was the first sign of partial

fragment death, caused by the stress inflicted during fragmentation and transportation, performed by untrained labor. However, the majority of these partially deteriorating fragments recovered, regenerated lost tissues and revealed the regular species-specific growth rates. At Chole Bay site, sedentary organisms including algae, ascidians and sponges that covered the nets and the supporting PVC frames heavily fouled the nursery. This necessitated extra maintenance.

Fragments of the same six coral species (albeit different genotypes) were farmed in both nurseries and most of the mortality occurred in the first two nursery months. In Chumbe, fragment mortalities peaked up during the first 30-60 days from initiation (through species-specific patterns) and then, no mortality was documented (Fig. 3a). At Chole Bay (Fig. 3b), mortality continued for 2-3 more months before ceasing after 120-150 nursery days, revealing cumulatively higher mortality rates than those recorded in Chumbe (t-test, $P < 0.05$). Observing species-by-species mortalities, we recorded lower survivorship of *Acropora formosa* and *A. nasuta* in the Mafia site as compared to Chumbe (t test; $P< 0.05$). The rest four species (*A. hemprichi*, *Porites cylindrica*, *Pocillopora verrucosa* and *Millepora* sp.) showed no significant differences in survivorship between the nurseries (t test; $P > 0.05$). Generally, the state of the health of all surviving coral colonies was good during the whole 270 nursery days (Fig. 4), corals showed no bleaching and exhibited fast growth (Figs. 4, 5a-c).

3.2 Coral fragment growth

We used small coral fragments as source material. The average height of fragments for the species reared in the nursery ranged between 1.7±0.3 and 2.3±0.4 for *Acropora hemprichi* and 2.3±0.4 for *A. nasuta* in Zanzibar and 1.5±0.3 to 2.1±0.5 for *Millepora* sp. in Mafia (Table 4a,b). We followed closely the growth rates of 540 colonies/site (90 per species). Analyses revealed that on the average, colony height nearly doubled after 153 days, tripled after 270 days (Table 4a, b). In the Chumbe nursery, colony height increased 1.8-2.8 times after 153 days for *Acropora nasuta* and *Pocillopora verrucosa* and tripled and quadrupled, respectively, after 270 days (Table 4). Similarly, colonial ecological volumes (EV) increased in the first 153 days from 10 times for *A. nasuta* and *Porites cylindrica* to 15 times for *A. formosa*. After 270 days the increase in EV was between 37 to 130 times from initial values for *Porites cylindrica* and *Millepora* sp., respectively, with *A. nasuta, A. formosa, A. hempichii* and *Pocillopora verrucosa* showing intermediate values between these, all resulting in colonies with sizes amenable for transplantation (Table 4a, Fig. 4).

Developing fragments at Chole Bay in Mafia revealed same trends in height and ecological volume increases (Table 4b). On the average, colonial heights doubled during the first 153 days for all species and tripled at day 270, except for *A. formosa*, which increased 6 times. EV values for the first 153 days increased between 8 times (*Pocillopora verrucosa*) and 13 times (*A. hemprichi*). In both sites, the changes in ecological volume increase for all species varied greatly but generally was slower during the first five months (Feb. 2008), with rapid increase thereafter (Fig. 5a).

No significance differences were found between the initial ecological volumes of coral genotypes within each species at Chumbe site in Zanzibar ($P > 0.05$; One-Way ANOVA). The same test performed after 270 days in the nursery showed significant difference in ecological volumes in two species, *Acropora nasuta* and *Porites cylindrica* ($P < 0.05$; One-Way ANOVA). At Mafia nursery, differences in initial ecological volumes were recorded only among the genotypes in *Acropora nasuta* and *Porites cylindrica* ($P > 0.05$; One-Way ANOVA). When the test was repeated after 270 days, it showed no significant difference in ecological volumes observed ($P < 0.05$; One way Anova). Similarly, when the same test was done to compare the same species between the two nurseries, it showed no significant difference ($P < 0.05$; One-Way ANOVA).

4. Discussion

This study demonstrates that farming of small sized coral fragments in a mid-water nursery is a practical instrument for producing, in less than one year, large quantities of coral colonies amenable for restoration of denuded reefs in Tanzania; establishing a source material for inhabiting other nurseries or for any other needs. Using the developed methodology for reef restoration, we make use of simple and cheap materials, easily accessible in local shops. This study, therefore, joins the conclusions of a burgeoning number of recent studies, all testing the 'active restoration' concept (Rinkevich, 1995, 2005, 2006, 2008; Lindahl, 2003; Soong and Chen, 2003; Okamoto *et al.*, 2005; Omori *et al.*, 2007, 2008; Omori, 2005; Shafir *et al.*, 2006a,b; Putchim *et al.*, 2008; Shafir and Rinkevich, 2008; Shaish *et al.*, 2008: Lirman *et al.* 2010). Reef restoration measures, as reflected in the literature above and the results of the present study, incorporate economic considerations, providing a well-established platform for decision-making authorities; where, when, and how much efforts should be invested in restoration measures at any specific reef site (Rinkevich, 2008).

This study tested farming of two major coral architectural forms, branching and submassive species. While all species studies revealed high survivorship rates, the branching forms, as expected, showed faster growth rate as compared to submassive forms. The excellent performance of the *Millepora* fragments (100% survivorship) as well the high survivor rates of *Pocillopora verrucosa* and *Acropora hemprichi* attest to the potentiality of using these species in restoration, as first coral species to be farmed in nurseries around Tanzania and other East Africa sites.

Similar to the findings by Shafir *et al.* (2006a,b) in The Gulf of Eilat, Red Sea, the growth rates of farmed colonies was high in both sites studied and farmed coral colonies were ready for transplantation just nine months after nursery set-up. In Shafir *et al.* (2006a,b) the observed fast growth rates of fragments in the nurseries was attributed to nutrients from adjacent fish farm cages. Conversely, the two Tanzanian nurseries in Zanzibar and Mafia Islands were located in areas of high tidal currents, which might have been responsible for promoting fast growth rates through aeration and possible supply of nutrients from distant places.

Most of the previous studies done in Tanzania on coral restoration through transplantation (Franklin *et al.* 1998; Lindahl, 1998, 2003; Wagner *et al.*, 2001) did not take into consideration the establishment of reliable and yet non-destructive sources of coral fragments and the active involvement of local communities. Only Wagner *et al.* (2001) integrated neighbouring local communities as key stakeholders in coral restoration in Dar es Salaam Marine Reserve area. In this study, we incorporated volunteers who were ready to work at low wage from surrounding villages in Mafia and some beach boys in Zanzibar. Likewise, the future of large-scale coral reefs restoration of damaged areas in the Western Indian Ocean and other developing countries is possible only through applying relatively cheap and easily adaptable techniques with voluntary involving surrounding local communities for sustainability.

Our results from the mid-water nurseries at Chumbe site in Zanzibar and Chole Bay in Mafia have indicated that coral species can be easily cultured in this nursery type to obtained large quantities of material, which can instantly be used. Studies by Rinkevich (2005a, 2000, 2006, 2008) emphasized the importance of adequate preparations through selecting appropriate protected sites for nurseries, the right materials and cultivation protocols of multi-species under the same nursery conditions. Since many reefs in Tanzania have remained severely damaged after the 1997/98 El-Nino event and the decades of intermittent anthropogenic disturbances, restoration measures like this, that take into account multi-species transplantation, are urgently needed to increase habitat complexity for reef dwelling organisms, helping in conserving biodiversity. In addition, integrating local communities has proven to be essential for the preparation of large numbers of fragments and the overall set up of the nursery. Much remains to be learnt about adaptation of various other species to nursery condition for the proper restoration of Tanzanian reefs.

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Figure legend

Figure 1. Map of the coast of Tanzania (a) and Tanzania (Africa insert), showing Chumbe nursery site in Zanzibar (b) and Chole Bay site in Mafia Island (c).

Figure 2. Nursery construction in Tanzania: (a) stock of confiscated nets at Mafia Island Marine Park. Nets are made of natural fibre, (b) construction of 1×1 m PVC frames, (c) sewing nets into the PVC frames and plugging 7 cm long hosepipes, (d) ready to-go frames, (e) insertion of coral fragments into hosepipes, (f) the underwater suspended nursery at day of establishment.

Figure 3. Mortality figures for the different coral species farmed at (a) Zanzibar nursery; (b) Mafia nursery.

Figure 4. Examples of nursery reared coral fragments on day 1 and after 270 days (1 and 2, respectively): (a1, a2) *A. hemprichi*, (b1, b2) *Millepora* sp., (c1, c2) *Pocillopora verrucosa.*

Figure 5. Growth parameters of nursery reared coral species. (a) Changes in average ecological volumes, (b) increase in fragments' average heights and (c) increase in fragment's average width over time per species after five months (February 2008) and nine months (July 2008).

Fig. 1

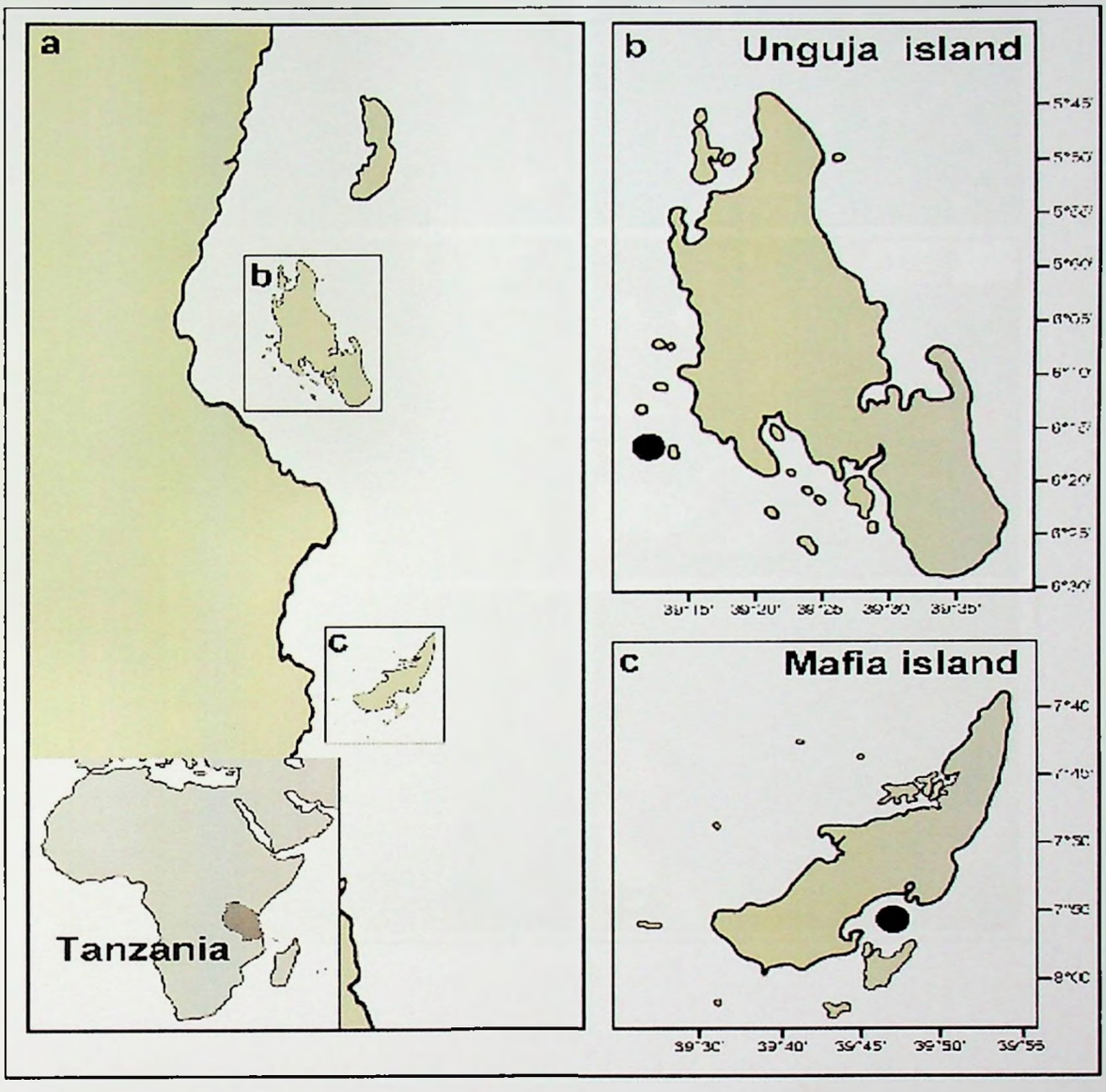

Fig. 2

Fig. 3

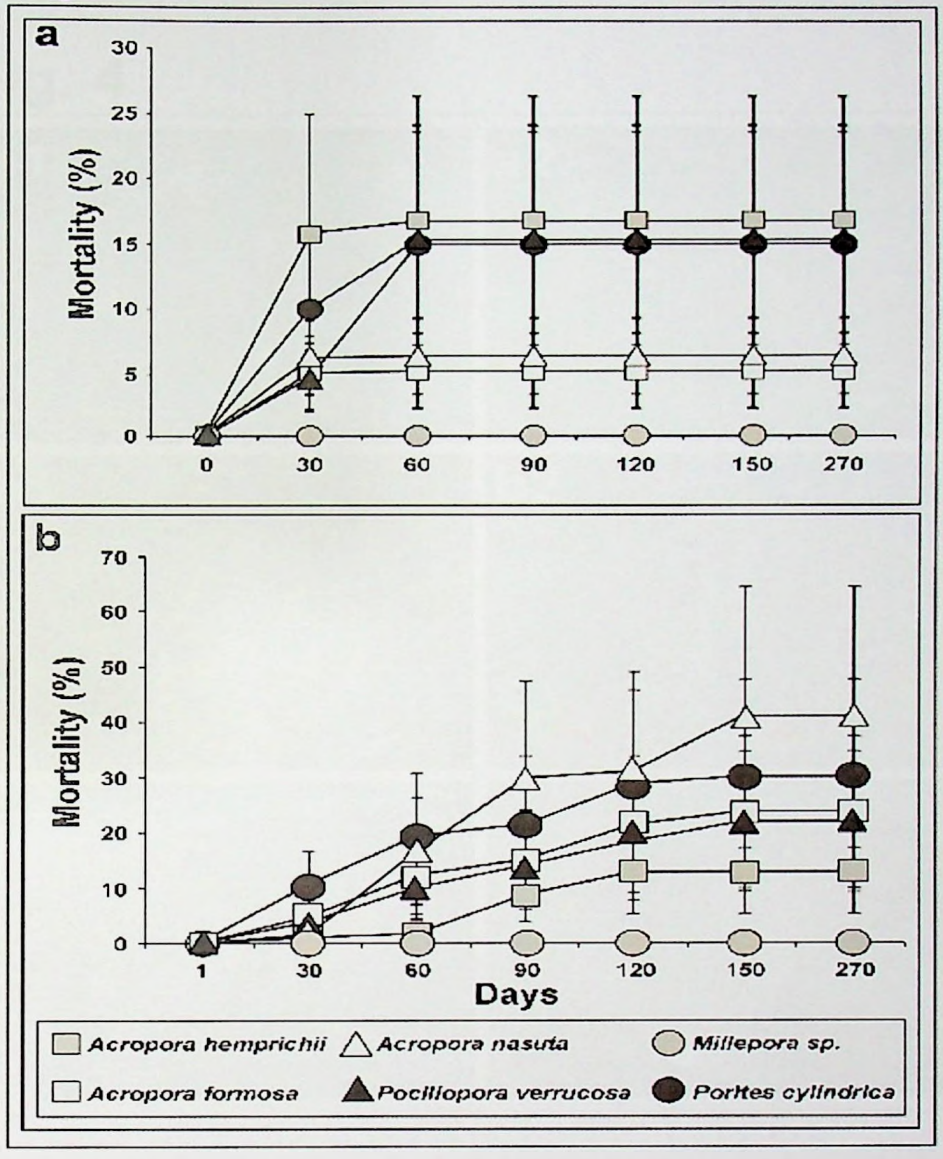
a
Mortality (%)
30
25
20
15
10
5
0
0
30
60
90
120
150
270
b
Mortality (%)
70
60
50
40
30
20
10
0
1
30
60
90
120
150
270
Days
Acropora hemprichii
Acropora nasuta
Millepora sp.
Acropora formosa
Pocillopora verrucosa
Porites cylindrica

Fig. 4

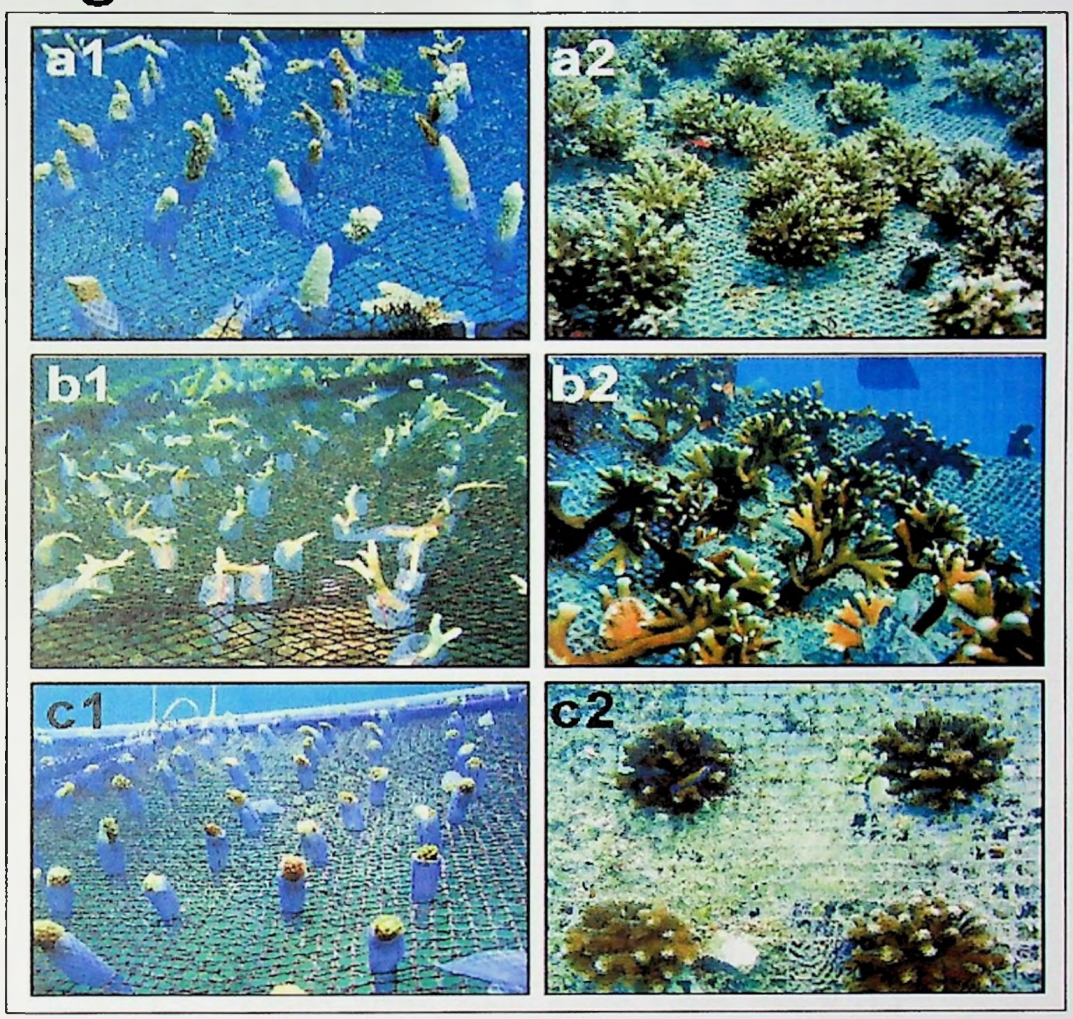

Fig. 5

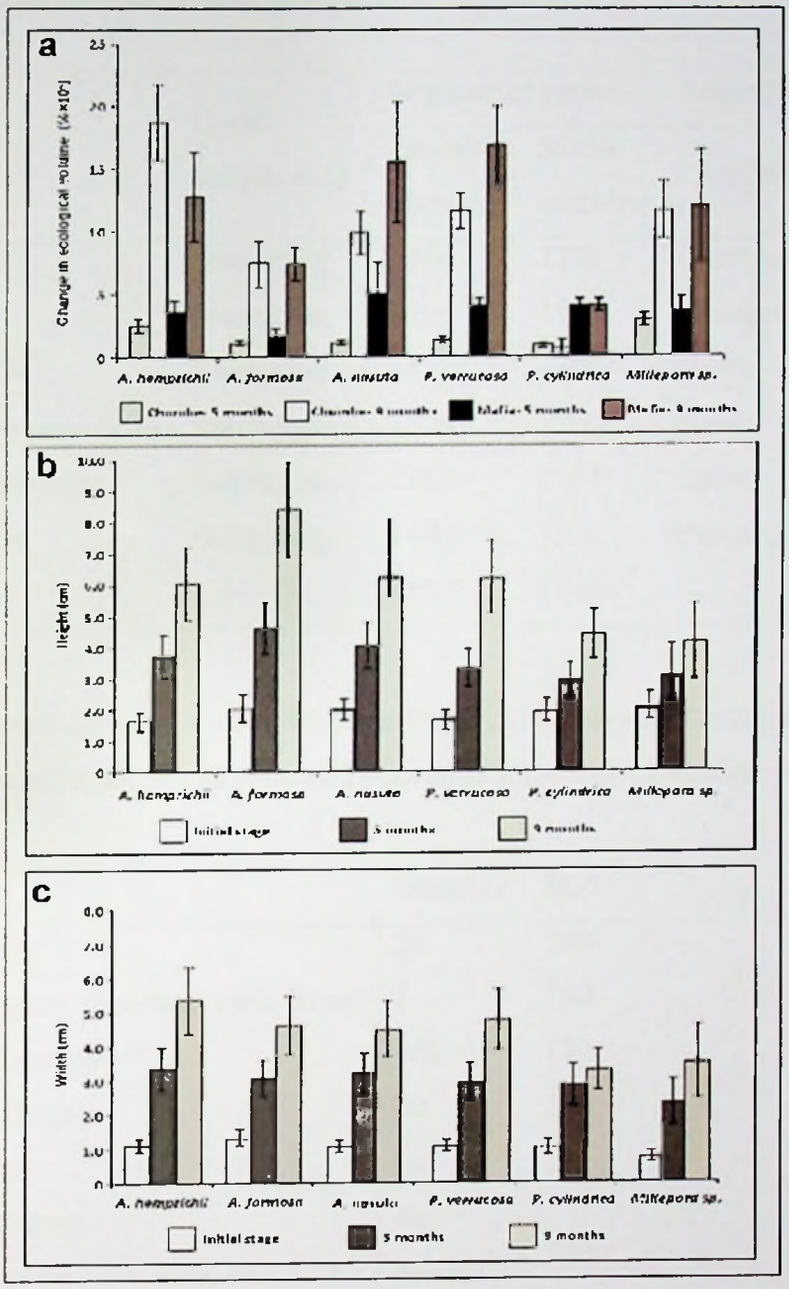
a
Change in ecological volume (%x10²)
A. hemprichii
A. formosa
A. insulsa
P. verrucosa
P. cylindrica
Millepora sp.
b
Height (cm)
A. hemprichii
A. formosa
A. insulsa
P. verrucosa
P. cylindrica
Millepora sp.
Initial stage
5 months
9 months
c
Width (cm)
A. hemprichii
A. formosa
A. insulsa
P. verrucosa
P. cylindrica
Millepora sp.
Initial stage
5 months
9 months

Table 1. Coral species farmed in Tanzanian nurseries

Species	Coral morphology	Number of ramets		Collection site	
		Zanzibar nursery	Mafia nursery	Zanzibar	Mafia
A. formosa	Branching	1296	1728	Changuu	CholeBay
A. nasuta	Branching	1296	1728	Changuu	Chole Bay
A. hemprichi	Branching	1872	2304	Bawe	Chole Bay
Pocillopora verrucosa	Sub massive	1296	2304	Bawe	Chole Bay
Porites cylindrica	Sub massive	2304	2304	Bawe	Chole Bay
Millepora sp.	Branching	1440	1728	Pange reef	Chole Bay
Total		**9504**	**12096**		

Table 2. Detailed cost for a 10,000 coral fragment nursery in Tanzania (1 $US to 1300 Tanzanian Shilings). The confiscated nets used were free-of-charge.

Items	Quantity	$US
PVC pipes*	20	200
Nylon ropes* (bundles, each 50 m)	6	150
Plastic containers*	108	120
Cement (bags)	20	280
Cable ties	40	180
Miscellaneous (PVC connections, glue, etc)	As needed	150
Total		**1080**

* Reusable materials may reduce this cost

Table 3: Corals' status after 9-months farming period in the Tanzanian coral nurseries

Coral species/genotype		Zanzibar			Mafia		
Species	Genotype	Net numbers *	Dead	Survivorship (%)	Net numbers *	Dead	Survivorship (%)
A. formosa	A	419	4	97.0	400	176	69.4
	B	402	30	93.1	460	116	79.9
	C	401	31	93.2	458	118	79.5
A. nasuta	A	413	19	96.3	372	204	64.6
	B	401	31	93.0	320	256	55.6
	C	412	20	95.1	328	248	56.9
A. hemprichi	A	507	69	88.0	556	212	72.4
	B	468	98	88.0	669	99	87.1
	C	456	120	79.2	570	198	74.5
Pocillopora verrucosa	A	380	52	88.0	205	83	71.2
	B	391	41	90.3	234	54	81.2
	C	330	102	76.4	234	54	81.2
Porites cylindrica	A	595	110	84.7	768	191	75.1
	B	655	65	91.0	427	341	55.6
	C	575	145	79.9	602	166	78.4
Millepora sp.	A	576	0	100	576	0	100
	B	576	0	100	576	0	100
	C	576	0	100	576	0	100

(*= Net numbers, after reducing loss caused by fisherman vandalism).

Table 4. Growth parameters of farmed corals at 0 153 and 270 nursery days. (A) Chumbe, Zanzibar; (B) Chole Bay, Mafia (EV= Ecological Volume; GRC= Growth Rate Constant; H= Height; W= Width)

Coral species	Day	Measurements (cm)			Size augumentation			GRC (%/d)
A		H	W	EV (cm^3)	H	W	EV (cm^3)	
A. hemprichi	0	1.7± 0.3	1.0± 0.2	1.8± 1.0	--	--	-	--
	153	3.7±0.7	2.0±0.4	23.4 ±4.3	2.1±6.4	3.9±3.9	13.5±11.7	2.0
	270	6.4±1.2	3.9±0.7	189.1±34.9	3.7±4.1	3.3±3.3	108.4±325.2	2.0
A. formosa	0	2.3±0.4	1.4±0.3	3.8±0.7	--	--	--	--
	153	4.8±1.0	3.0±0.5	35.4±6.5	2.9±1.8	2.5±1.1	15.90±3.0	1.5
	270	7.2±1.3	5.2±1.0	154.5±21.8	3.6±2.0	2.8±2.0	75.73±42.2	1.3
A. nasuta	0	2.3±0.4	1.2±0.2	3.01±0.6	--	--	--	--
	153	4.0±0.8	2.9±0.5	29.2±5.3	1.8±1.3	3.5±2.5	10.7±7.3	1.6
	270	7.0±1.3	5.2±1.0	157.8±28.8	3.4±1.4	2.9±1.4	93.3±27.2	1.4
Pocillopora	0	1.8±0.3	1.3±0.2	2.6±0.5	--	--	--	--
verrucosa	153	3.5±0.7	3.0±0.6	28.4±5.2	2.0±1.5	2.8±2.8	11.6±15.4	1.6
	270	7.9±1.5	6.0±1.1	229.9± 42.0	4.9±1.9	3.6±1.3	117.1±21.6	1.5
Porites	0	2.0±0.4	1±0.2	2.6±0.5	--	--	--	--
cylindrica	153	3.1±0.6	2.9±0.5	28±0.9	1.1±0.6	1.6±2.2	10.2±12.8	1.4
	270	4.7±0.9	4.1±0.8	64.7±11.8	2.5±3.6	2.1±3.6	37.7±17.3	1.1
Millepora sp.	0	2.2±0.4	1.1±0.2	1.9±0.4	--	--	--	--
	153	4.0±0.7	3.5±0.5	40.7±8.6	2.3±1.3	6.0±3.3	43.6±24.1	2.1
	270	6.0±1.2	5.4±1.0	146.7±26.8	3.3±1.8	3.0±1.7	130.0±71.8	1.5
B								
	0	1.5±0.3	1.3± 0.2	2.3± 0.4	--	--	--	--
A. hemprichi	153	3.8±0.7	3.1±0.6	32.0±5.9	2.5±1.6	2.6±2.0	14.0±1.3	2.5
	270	5.6±1.1	5.3±0.9	112.2±20.6	3.8±1.9	3.3±1.9	49.0±33.4	0.9
	0	1.8±0.3	1.5±0.3	3.5±0.6	--	--	--	--
A. formosa	153	4.4±1.0	2.8±0.7	31.1±5.7	2.5±2.7	1.8±2.8	9.3±10.7	2.3
	270	9.7±1.8	4.4±1.6	161.1±29.5	5.6±4.8	3.1±2.8	48.3±47.5	1.1
	0	1.6±0.5	1.4±0.3	2.6±0.9	--	--	--	--
A .nasuta	153	4.6±0.9	3.0±0.7	34.1±11.2	2.2±2.2	2.0±2.6	13.0±13.2	2.5
	270	5.8±1.4	4.9±1.2	136.1±25.3	3.6±3.2	4.0±2.8	42.9±41.4	1.0
Pocillopora	0	1.5±0.3	0.9±0.2	2.1±0.4	--	--	--	--
verrucosa	153	3.1±0.6	2.4±0.4	14.2±3.8	2.0±1.3	2.8±1.7	8.2±2.8	2.2

Porites cylindrica	270	4.5±0.9	3.9±0.7	58.3±16.2	3.0±1.4	2.9±1.4	31.5±8.6	1.0
	0	1.9±0.4	1.2±0.3	2.8±1.0	--	--	--	--
	153	2.6±0.6	2.1±0.5	10.4±3.4	1.4±1.4	2.1±2.1	3.7±2.1	1.6
	270	2.9±0.8	2.4±1.5	18.8±6.4	2.1±1.8	1.5±1.5	8.7±1.5	0.5
Millepora sp.	0	2.0±0.5	1.0±0.4	2.3±1.0	--	--	--	--
	153	4.1±1.1	2.6±0.7	22.1±5.2	2.0±1.7	2.7±2.1	9.50±6.7	2.4
	270	4.8±1.3	4.5±1.2	83.9±18.7	2.4±2.1	2.4±2.0	36.11±22.7	1.0

3.2 A first endeavour in restoring denuded, post-bleached reefs in Tanzania. Estuarine, Coastal and Shelf Science (2013) 128: 41-51

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Abstract

The worldwide decline in coral reefs has prompted a search for effective restoration protocols. We transplanted 6912 and 7110 corals (*Acropora muricata*, *A. nasuta*, *A. hemprichi*, *Pocillopora verrucosa*, *Porites cylindrica*, *Millepora* sp.) in Changuu, Zanzibar and Kitutia, Mafia, Tanzanian reefs that suffered in 1998 from a massive coral bleaching incident, causing a wide spread coral death. No sign for natural recovery has been recorded thereafter. In each site, we randomly set up 12 plots (36m^2 each), of which three were transplanted with a mix of three *Acropora* spp. (Treatment 1, T1), three with a mix of all six scleractinian species (T2), and six served as controls. Within one month of transplantation, an outbreak of *Acanthaster planci* in Changuu caused mortality at 50%. One year survival of transplants in T1 and T2 at Kitutia reached 66.4% and 62.5% respectively, significantly higher than at Changuu; an outcome recorded through species-by-species comparisons on four species only (*P. verrucosa, P. cylindrica*, *A. muricata, A. nasuta)*. After one year no significant difference was documented in ecological volumes (EV) between T1 and T2 in stark contrast to the among species comparisons in T1, at each site. A within treatment one-way ANOSIM comparison for fish assemblage structures performed between the first and last three months of the transplantation year (Kitutia reef) revealed strong separation (T1, Global $R = 0.743$, $P < 0.001$; T2, $R = 0.445$, $P < 0.001$ and T3, $R = 0.694$, $P < 0.001$) while the same treatment revealed weak separation at Changuu site T1 ($R = 0.035$, $P > 0.262$) and T2 plots ($R = 0.119$, $P < 0.043$). Similarly, one-way ANOSIM done on the initial and last 3-month periods on invertebrates' community composition (at all sites, except T1 of Changuu reef), showed no significant difference between community composition at both ends of the sampling period. Altogether, transplantation cost (US$0.19/colony) suggested that large scale transplantation is economically viable. Cumulatively, field results and economic evaluations showed that transplantation of nursery-grown colonies might uphold critical ecosystem functions while used in reversing phase shift states in coral reefs.

1. Introduction

Coral reefs worldwide are facing continued decline due to a variety of natural and anthropogenic instigations (Hoegh-Guldberg, 2004; Edward and Gomez, 2007). While in the past, coral reefs managed to recover from perturbations in a short time (Edward and Gomez, 2007; Dudgeon *et al.*, 2010) through coral fragmentation (Lewis, 1991) or larval recruitment (Nzali *et al.*, 1998), worldwide reef recovery trends and processes have recently becomes lower because of several causes most important being substrate instability (Wells and Alcala, 1987) and larval depletion (Quinn and Kojis, 2006), altogether reducing ecosystem resilience; leading to ecological phase shifts in which coral reefs become algal- dominated systems (Hughes *et al.*, 2007). Responding to reef ecosystems' decline, several approaches made suggestions for novel applications in order to rehabilitate reef services (Lesica and Allendorf, 1999; Köhlin and Ostwald, 2001; Bruckner and Bruckner, 2001; Bowden-Kerby, 2001; Ortiz-Prosper *et al.*, 2001; Lindahl, 2000, 2002; Franklin et al., 1998; Bongiorni *et al.*, 2011; Guest *et al.*, 2011; Horoszowski-Friedman *et al.*, 2011; Linden and Rinkevich, 2011; Muko and Iwasa, 2011). The declaration by the Convention on Biological Diversity that restoration of terrestrial, inland water and marine ecosystems is inevitable in order to restore ecosystem functioning and ecosystem services (Normile, 2010) is a strong evidence of global support in ecological restoration efforts. Accordingly, there have been many localized attempts in various parts of the world to design appropriate active restoration protocols especially in denuded reef areas. One such attempt is the 'reef gardening' tenet (Rinkevich, 2005, 2006, 2008), a two steps restoration operation which has been tested in various reefs worldwide (Amar and Rinkevich, 2007; Bowden-Kerby, 1997; Shafir and Rinkevich, 2008, 2010; Shaish *et al.*, 2010a, b; Putchim *et al.*, 2009; Levi *et al.*, 2010; Mbije *et al.*, 2010; Bongiorni *et al.*, 2011; Linden and Rinkevich, 2011). This tenet incorporates stock farming of small coral fragments in mid-water floating nurseries which, upon reaching suitable sizes, are transplanted onto denuded reef areas. A major conclusion emerged from the above studies is that the application of appropriate active restoration protocols may enhance reef recovery (Rinkevich, 2005, 2006, 2008). This is supported by experimental manipulations showing that improved live corals coverage and their structural complexity influence significantly the recovery of reef fish communities (Garpe and Ohman, 2007; Cabaitan *et al.*, 2008; Ferse,

2009). Coral gardening studies (Lindahl, 2003; Soong and Chen, 2003; Rinkevich, 2005, 2006, 2008; Amar and Rinkevich, 2007; Chou *et al.*, 2009; Putchim *et al.*, 2009; Shafir and Rinkevich, 2008, 2010; Shaish *et al.*, 2008, 2010a,b; Omori and Iwao, 2009; Edwards, 2010; Ferse, 2010; Iawo *et al.*, 2010; Levi *et al.*, 2010; Lirman *et al.*, 2010; Mbije *et al.*, 2010; Bongiorni *et al.*, 2011; Guest *et al.*, 2011; Horoszowski-Friedman *et al.*, 2011; Linden and Rinkevich, 2011; Muko and Iwasa, 2011) provided results showing that coral reef restoration can be applied successfully and efficiently at very low costs.

Like in other countries (Baticados, 2004; Wells, 2009), a significant reef area in Tanzania faced an advanced degradation state due to decades of intermittent anthropogenic disturbances, destructive fishing and current proliferation of touristic activities in Mafia Island, Zanzibar and Pangani beaches (Wagner, 2005). Although most of anthropogenic impacts have relatively been controlled, much of the reef system in Tanzania has remained severely damaged in the last 15 years ensuing the1997/1998 El-Niño that caused a massive coral bleaching incident, followed by a wide spread coral death (Lindahl *et al.*, 2001; Mbije *et al.*, 2010), with no signs for natural recovery. The combined effects of anthropogenic activities and the 1998 coral-bleaching incident are therefore accountable for the continued pressure and demise of the large shallow water reefs in Tanzania (Lindahl *et al.*, 2001). Such a grave situation requires the intervention of scaled-up management and the application of appropriate active restoration protocols (Rinkevich, 2005, 2006, 2008; Mbije *et al.*, 2010).

In response to the persistent decline of the coral reefs in East Africa, an experimental study based on the 'gardening concept' was done in two Tanzania sites, Changuu reef in Zanzibar and Kitutia reef in Mafia (Mbije *et al.*, 2010). We (Mbije *et al.*, 2010) already documented that the coral gardening approach could be used in Tanzania to generate large quantities of coral colonies for restoration of damaged reefs at relatively low cost. Following that, the major aim of this study was to test the applicability and efficiency of the second step of the 'gardening tenet', the successful transplanting of nursery reared coral colonies. This was performed by monitoring transplants' survival/bleaching, colonial ecological volumes, coral recruitment and transplantation impacts on reef fish and community structures of reef dwelling invertebrates. The underlying principle used in this study is based on the understanding that reefs' biological and physical features influence structures of reef

communities (Cabaitan *et al.*, 2008). Furthermore, in order to assess the economic applicability of our approach, we also analysed overall costs associated with transplantation.

2. Material and Methods

2.1 Study location

The study was carried out in Changuu reef of Unguja Island in Zanzibar (6°16' N 39°18' E) and Kitutia reef in Mafia Island Marine Park (7°40' N, 4°40' E), about 120 km apart (Fig. 1a, b, c). Changuu Island has a shallow fringing reef on the northern side (3 to 10m at high spring tide) and a shallow south side dominated by sea grass mats. The dominant coral genus in Tanzanian reefs is *Acropora* (Richmond, 2002), which is evident in the extensive mass of dead *Acropora* branches, especially at Kitutia reef, that accumulated after the unprecedented 1997/1998 mass bleaching followed by extensive mortality (Lindahl *et al.*, 2001). Similarly, dead coral fragments of mixed species dominate Changuu reef (Mbije, personal observation). The devastated areas in the two reefs have shown no signs of recovery in the last 13 years, with dead coral fragments overgrown by extensive algal mats.

2.2 Coral transplantation

We used one-year old nursery reared scleractinian colonies from six species: *Acropora muricata*, *A. nasuta*, *A. hemprichi*, *Pocillopora verrucosa*, *Porites cylindrica* and *Millepora* sp. (Mbije *et al.*, 2010). The transplantation sites at Changuu and Kitutia (about 15 and 23 km respectively from nurseries) were located at the same depths as the original site from which the nursery materials originated and consisted of substrates with consolidated dead coral fragments, loose gravel and a few live corals. Farmed colonies were carefully removed from the nursery by means of carpentry scissors, their bases cleaned from settling sedentary organisms, immersed in marked, large water-filled plastic bins, and loaded onto a boat, before being transferred to the transplantation sites. At each transplantation site, we haphazardly established 12 plots (each $36m^2$) that were clearly marked for further inspection; six of which were transplanted with nursery reared coral colonies. We followed a pre-set design comprised of three treatments (T1-T3): three plots were transplanted with a mix of three *Acropora* spp. (T1), three plots transplanted with a mix of all six coral species (T2) (Appendix A) and six plots, designated within non-transplanted areas, served as controls (T3). Each transplanted plot was further partitioned into nine sub-plots, each $4m^2$ (Fig. 2a). Eight sub-plots within

each plot were transplanted with coral colonies (some were populated with colonies of the same coral genotype, with various genotypes of the same species, or different coral species) at 16cm distance from each other, while the central $4m^2$ sub-plot was left unattached, to elucidate possible impacts of surrounding transplanted plots (Fig.2a). Each $4m^2$ sub-plot was populated with 144 coral colonies, 1152 colonies per a $36m^2$ plot (Fig. 2a-c). In total, 6912 coral colonies were out planted in Changuu reef at Zanzibar and 7110 in Kitutia reef at Mafia. Although the design and fragment spacing was the same in both sites, we transplanted extra colonies in Kitutia reef and these were not part of the monitored plots.

A large proportion of the substratum at Kitutia reef is composed of consolidated dead coral branches, mostly of *Acropora* spp., while at Changuu reef the substratum is composed of hard rock with attached dead coral branches. Two transplant attachment techniques were tested, (a) plugging the short pieces of the hosepipes carrying corals directly between the firmed attached dead coral branches (4123 and 5324 transplants in Changuu and Kitutia respectively, and (b) plugging the short pieces of hosepipes into holes drilled in the substrates (2789 and 1786 transplants in Changuu and Kitutia respectively). General-purpose epoxy compound (M-seal®) reinforced the attachment of loose transplants to the substratum.

2.3 Monitoring and data analysis

Once a year, a team of three divers monitored the transplantation plots and transplants for bleached colonies, detached transplants, survival rates, corals' size measurements, fish surveys, and recruitment of selected reef associated invertebrates. Every three months, we assessed size-measurements of transplants in the field by measuring height, width and length of each colony with plastic callipers. From these measurements, the colonies' ecological volumes (EV) in cm^3 were calculated as $EV=\pi*H*([W+L]/4)$ (Rinkevich and Loya, 1983).

Fish surveys involved identifying and counting all adult individuals found to occupy each 3D setting (plot and water column around and above the fragments); all fishes > 3cm long were identified to the lowest taxon possible. Censuses were performed by slow-motion scuba diving that allowed counting fish populations without disturbing the animals. This exercise was repeated in each plot of the three treatments and fish censuses were conducted between 1000H and 1500H and completed within the same week (the exercise was repeated twice for each plot). Values for fish abundance, species richness and species composition were

analysed. While fish abundance refers to the number of fish of a certain species, species richness is the total number of fish species, and species composition refers to the relative abundance of fish species per treatment/plot. Censuses of large invertebrates were carried out under the same protocol, with a single deviation, observing their presence within crevices, in the substrates, between coral branches, underneath coral colonies and on hard substrate. Invertebrate abundance and species richness were used in the analysis.

2.4 Coral recruitment

From the start of the transplantation experiment, we closely monitored the central bare sub-plots within the plots for coral settlement. Because of the lengthy underwater time required for this procedure, we were forced to conduct our monitoring at low spring tide (mean springtide at 3.3m and maximum at 4.0m).We applied an extensive underwater search procedure that included fanning away sediments while searching for recruits. Since most coral recruits are very small at settlement and their growth rate is slow (Wallace *et al.*, 1981), we concentrated on established recruits of size ranging between 0.5 and 2.0cm. This was also performed in order to minimise confusion or mistakes in coral species identification. Identification of coral recruits to the lowest possible taxon was done with the aid of the key plates found in English and Wilkinson (1994) and Babcock *et al.* (2003) that were laminated to suit underwater work. Results were summarised as average numbers per species/treatment/site.

Average colonies' sizes, mean percentages of survival, detachment and bleaching were calculated for each sub-plot/treatment/monitoring date. Results were analysed by using an SPSS 16 2007 data editor. We employed t-test when comparing between sites and one way-ANOVA for comparison between treatments. Prior to the analysis, all data were subjected to Levene's (1960) test to check for assumption of homogeneity of variances and whenever necessary, the data were square root transformed. Similarly, the analysis in fish and invertebrate assemblage structure within each treatment, among treatment and between sites, were analysed using either one way or two way crossed ANOSIM (on density data) based on Bray-Curtis similarity measures (Clarke, 1993). To reduce the weight of dominant values (mostly from schooling taxa), the data were square root transformed. Non-metric

multidimensional scaling (nMDS) ordinations were used to visualise the patterns of similarities among sites.

3. Results

3.1 Economic evaluation of coral transplantation

We out planted 14, 022 coral colonies in both sites as follows: *A. muricata* 3464, *A. hemprichi* 2879, *A. nasuta* 2087, *P. verrucosa* 2095, *Millepora* sp. 2090 and *P. cylindrica* 1780. In each dive, the time taken to fix transplants in rubble was longer than in drilled holes, though the latter consumed more diving tanks. Taking these two facts into account, the actual costs for each type of attachment was similar, US$0.14 per colony or US$2020 for all 14,022 coral colonies (Table 1), which is a minimal number compared to the benefits derived from transplantation in terms ofecological functions and tourism amenities transplants provide. In addition, such low costs can also be applied even in developing countries where resources are scarce.

3.2 Coral detachment, survival and bleaching

Most transplants remained firmly attached to the substrates in the first month post transplantation, with only few detachments recorded in the dead coral fragment mixed species plots. These include 199 (4.8%) colonies in Changuu and 247 (4.6%) in Kitutia. Detached colonies were reattached to their original places by underwater epoxy. However, one month post transplantation, an unexpected outbreak of the crown-of-thorns starfish, *Acanthaster planci*, was documented at Changuu site in T1 and T2 but not in T3 (control plots), or in adjacent natural reef areas. Almost half of the Changuu transplants were lost to predation (Fig. 3a). No single *Acanthaster* specimen was seen during a preliminary survey carried out on the entire reef when the decision on transplantation sites was made. In total, more than 180 *Acanthaster* specimens were handpicked, with up to 30 individuals per plot (with the aid of volunteer fishermen) and buried on the beach. Following this predation, almost no further mortality was observed in the next 10 months (one-year survival of 52 and 51% in T1 and T2 plots respectively (Fig. 3a). At Kitutia reef, Mafia, about 25% of initial coral fragments were removed through the activities of fishermen. One year survival reached 66.4 % and 62.5%in T1 and T2 respectively, significantly higher than in Changuu ($p < 0.05$; t test). Species-by-species comparisons revealed lower survival of *Pocillopora verrucosa, Porites cylindrica*,

Acropora muricata and *Acropora nasuta*, in the Changuu site as compared to Mafia ($p<0.05$; t-test). *A. hemprichi* and *Millepora* sp. showed no significant differences in survival between the two sites ($p > 0.05$; t test).

In both sites, high levels of bleaching events were recorded one-month post transplantation (Fig. 3b). At Changuu, T1 and T2 plots showed 16% and 22% bleaching respectively. No significant difference in bleaching was recorded between plots that were transplanted with *Acropora* species (T1; $p > 0.05$; one-way ANOVA) but bleaching differed significantly between T2 plots that were transplanted with mixed species ($p < 0.05$; one-way ANOVA). In Kitutia reef, bleaching was 11% and 16% for T1 and T2, respectively (Fig. 3b). No significant difference in bleaching was recorded between *Acropora* species plot in T1 and between mixed species plot in T2 ($p > 0.05$; one-way ANOVA). In both sites, the most resilient species were *Millepora* sp. and *Acropora hemprichi*, with only 76 (0.54%) and 67 (0.48%) bleached colonies, respectively, while *Acropora muricata* (n=302, 23.6%), *Pocillopora verrucosa* (n=211, 32.1%), *Porites cylindrica* (n=192, 37.2%) and *Acropora nasuta* (n=188, 18.5%) suffered from higher bleaching than the other species (n = number of bleached fragments). Among the severely bleached coral species were those transplanted by plugging directly between the dead coral fragments. These include *Acropora muricata* (n = 65, 20%), *Pocillopora verrucosa* (N = 72, 34%), *Porites cylindrica* (n = 79, 41%), and *A. Nasuta* (N = 103, 55%). Bleaching did not result in significant transplants' mortality as 95% of bleached colonies recovered within two months after transplantation.

A second bleaching event occurred from February to April 2009 (Fig. 3b), when for several weeks, water temperatures rose to 32.1°C and 31.8°C in Changuu and Kitutia respectively, causing wide spread bleaching in many reefs of Tanzania (http://www.cordioea.org/bleachingalert, 2009). On average, at Changuu site we counted seven (0.7%) bleached *Galaxea fascicularis* colonies/plot (including the six control sub-plots), 19 or 0.78% *Pocillopora verrucosa*, 21 or 2.8% *Porites cylindrica* and five *Fungia* sp., while at Kitutia reef only two bleached coral species were recorded, *Acropora muricata* (n = 31, 6.2% and *Pocillopora verrucosa* (n = 11, 7.2%) in the three mixed plots. Bleaching was 100% in *Galaxea fascicularis* and *Pocillopora verrucosa* colonies with *Porites cylindrica* colonies turned pale for one month.

Acropora muricata and *Acropora nasuta*, in the Changuu site as compared to Mafia ($p<0.05$; t-test). *A. hemprichi* and *Millepora* sp. showed no significant differences in survival between the two sites ($p > 0.05$; t test).

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3.3 Transplants growth rates

We closely followed the growth of the same 180 representative and marked coral colonies per treatment, once a month (i.e., 360 per site for both attachment protocols). The data for each treatment in each site were analysed separately for comparison purposes. It was found that while the colonial ecological volumes (EV) increased slightly after 90 and 120 days, EV nearly tripled after 180 days and quadrupled after 270 days (Fig. 4). In Changuu reef, *Acropora nasuta* EV in plots T1 and T2 increased 12 and 17 fold by day 360 respectively. *A. Muricata* EV increased 6 and 7 times in T1 and T2 respectively, and *A. Hemprichi* increased 6 and 4 times, respectively (Fig. 4a). *P. verrucosa* and *P. cylindrica* EV increased seven and five times in T1 and T2, respectively, and *Millepora* sp. EV increased only four times in the first year after transplantation (Fig.4a). A significant difference in EV increase was recorded between transplanted *A. nasuta, A. muricata* and *A. hemprichi* within T1 ($p < 0.05$; one-way ANOVA). However, among *Acropora* species, in the same period, no significant difference was observed between T 1 and T2 plots ($p > 0.05$; one-way ANOVA).

Similar patterns of EV increase were recorded at Kitutia reef in Mafia (Fig. 4b). The EV values in the first three and six months increased slightly but tripled after 270 days in all species and increased ten times after 360 days. After 360 days, EV of *A. Nasuta* increased 11 times in T1 and T2 plots. The EV values for *A. muricata* increased three and seven times after 270 days and six and twelve times after 360 days in T1 andT2 plots, respectively. Similarly, the EV in *Acropora hemprichi* increased three and two times after 270 days and five and six times after 360 in T1 and T2 plots, respectively. We recorded 4 fold EV increase in *P. verrucosa* and 3 fold increase in *Millepora* sp. after 360 days in T2 where as *P. Cylindrica* EV multiplied 12 times after 360 days. In Kitutia reef, a significant difference in EV increase was observed between *A. muricata*, *A. nasuta* and *A. Hemprichi* in T1 ($p < 0.05$; one-way ANOVA). EV increase comparisons for T1 and T2 plots revealed no significant difference for the above three coral species ($p > 0.05$; one-way ANOVA).

3.4 Impacts of transplantation on fish communities

Throughout the experimental duration, a total of seven species were observed at Changuu reef and 29 fish species at Kitutia reef, appearing in all three treatments/sites (Appendix B). A within treatment one-way ANOSIM comparison for fish assemblage structures performed

between the first and last three months of the transplantation year (Kitutia reef) revealed a strong separation (T1, Global R = 0.743, P <0.001; T2, R = 0.445, P < 0.001 and T3, R = 0.694, P < 0.001). At Changuu site, a within treatment one-way ANOSIM comparison of fish assemblage structures between the first and last three months of the experiment, revealed no difference in the T1 plots, and a very weak one in the T2 treatment while T3 showed no significant difference (R = 0.035, P >0.262, R = 0.119, P < 0.043, R = - 0.052, P > 0.750 respectively). These patterns are illustrated in the nMDS ordination plots (Fig. 5).

3.5 Invertebrate composition and abundance

In both sites, Echinoidea and Asteroidea were the two major invertebrate groups observed in T1, T2 and T3 plots with some individuals from one species of class Gastropoda (Appendix C). A within treatment one-way ANOSIM comparison of invertebrates assemblage structures between the first and last three months of the transplantation year at Kitutia reef revealed weak separations for T1 plots (R = 0.014, P > 0.322) and weaker for T2 (R = -0.067, P > 0.881) andT3 (R = -0.026, P > 0.552). In contrast, at Changuu reef, the difference between community compositions in T1 between the first and last three months of the transplantation appeared strong, while it was less in T2 and T3 (R = 0.55, P < 0.001,R = 0.104, P > 0.10 and R = 0.426, P > 0.001 respectively). These patterns are illustrated in the nMDS ordination plots (Fig. 6).

3.6 Effects of transplantation on central empty blocks.

During the first two months after transplantation, no coral recruitment was recorded in all 12 central T1, T2, and T3 bare sub-plots in both sites. However, as from the third month there was a continuous recruitment of corals in T1 and T2 central empty sub-plot, less recruitment in Kitutia empty T3 sub-plot, and no recruitment in Changuu empty T3 sub-plot (Fig. 7a, b; therefore, no sub-Fig. 7a3 is provided).

Three species of *Acropora* (*A. hemprichi*, *A. Nasuta* and *A. muricata*) dominated coral recruitment in Changuu T1 central sub-plot after 12 months (Fig. 7a). *A. hemprichi*, *A. muricata* and *A. Nasuta* reached densities of 1.4, 1.25 and 1.2 recruits/m^2, respectively, showing no significant difference (p> 0.05; one-way ANOVA). We recorded recruits belonging to the other species transplanted (*Millepora* sp. 0.4/m^2; *Pocillopora verrucosa*0.4m/m^2, and *Porites cylindrica*, 0.2/m^2) and of species that were not part of this

experiment (*Pocillopora damicornis*, 0.88/m^2, *Galaxea fascicularis*, 0.75/m^2 and *Porites rus*, 1.0/m^2). In T2, the most common recruited species in the bare sub-plot was *Millepora* sp. (1.6 individuals/m^2; Fig. 7b), followed by *Acropora muricata* (1.25/m^2), *Acropora nasuta* (1.1/m^2), *Acropora hemprichi* and *Pocillopora verrucosa* (each 1.0/m2). Comparison of recruits' densities betweenthe three *Acropora* species in T1and T2 showed no significant difference ($p > 0.05$; one way ANOVA).

Acropora species were the most common recruits in Kitutia central sub-plot. In T1, *Acropora muri*cata showed the highest figure (2.0 individuals/m^2) followed by *Acropora hemprichi* (1.75/m^2), *Acropora nasuta* and *Pocillopora verrucosa* (each 1.50/m^2), and *Millepora* sp. (0.75/m^2), with no significant difference in densities among them ($p > 0.05$; one-way ANOVA). There was no record for *Porites cylindrica* recruits in this treatment. In T2, *Acropora nasuta* was the most common (2.0 individuals/m^2) followed by *Acropora muricata* (1.75/m^2), *Acropora hemprichi* and *Millepora* sp. (each, 1.5/m^2), *Pocillopora verrucosa* (1.0/m^2) and *Porites cylindrica* (0.25/m^2), showing no significant difference among them ($p > 0.05$; one way ANOVA). At this site, we recorded lower level of hard corals recruitment in central bare sub-plot of T3 as compared to T1 and T2.Species identified were *Pocillopora damicornis* (0.7/m^2), *Porites rus* (0.75/m^2), *Acropora humilis* (0.50/m^2) and several othercoral species that were too small and grouped together as unidentified species (2.0/m^2).

4. Discussion

This study is one among many others aiming at developing appropriate methodologies and site specific restoration protocols for denuded reef areas around the world. Here we recorded in both studied sites high survival and growth rates of transplanted coral colonies, coupled with increased densities of fishes and recruitment of corals with time. Additionally, there was no significant difference in transplant detachment rates for the two attachment protocols used. The branching *Acropora* species showed the highest growth rates (as in other studies; Yap *et al.*, 1992), a characteristic feature for coral species employing fragmentation as a major asexual reproduction mode in their life history patterns (Bothwell, 1982; Highsmith, 1982). The observed fast growth rates of transplanted species may be attributed to the use of large sized fragments in transplantation (Highsmith, 1982; Bowden-Kerby, 2001; Lindahl *et al.*, 2001). In contrast, *Millepora* sp. and *Pocillopora verrucosa* that were growing equally fast to

Acropora species in nurseries (Mbije *et al.*, 2010) showed slower growth rates after transplantation, while still high enough for natural reef conditions. These results provide further evidence for the argument that active coral restoration initiatives may bring back denuded coral reef areas to their original state (Lindahl, 2003; Rinkevich, 2005, 2006, 2008). Concomitantly, while some studies indicated that coral reef restoration through transplantation can be costly costs (Edwards and Gomez, 2007; Edwards, 2010), our results indicate that a large scale coral transplantation can also be done in developing countries at low costs with the available resources, less than one tenth of the costs as evaluated earlier.

Similar to recent documentation of environmental impacts on reef restoration (Shaish *et al.*, 2010a), the Tanzanian coral transplants experienced two catastrophes, *Acanthaster planci* infestation and a major coral bleaching event. The unexpected infestation by crown-of-thorns starfish at Changuus' reef, immediately after colony transplantation, which was not documented in adjacent natural reef areas, may have been related to the response of resident organisms to newly arrived transplants. Similar results from transplantation experiments in the Red Sea (Horoszowski, personal communication, 2010) and South East Asia (Shaish *et al.*, 2010a) showed that new transplants are prone to attacks by resident fish and corallivorous invertebrates that may eventually kill them. Starfish infestation impacted all coral species but the most affected species were *Pocillopora verrucosa*, *Porites cylindrica*, *Acropora muricata* and *Acropora nasuta*. Whereas Harriott *et al.* (2003) discussed different theories for *Acanthaster planci* outbreaks, determining the reason for the specific outbreak at the transplantation sites is beyond the scope of this study. Damage to coral colonies by bleaching of transplants was the second major stressor, further documented in the studied Indo Pacific reef sites (Shaish *et al.*, 2008, 2010; Mbije *et al.*, 2010). The most prominent bleaching episode during the first year after transplantation was part of the wide-spread bleaching event developing in the Western Indian Ocean (http://www.cordioea.org/bleachingalert, 2009). Generally, no significant mortality of transplants was associated with this phenomenon. However, Mafia Island and neighbouring areas of Kilwa, are among the remaining pristine fishing grounds of Tanzanian reefs and therefore highly prone to anthropogenic pressure. Some groups of fishermen have been invading the reefs, especially at night, and gain access to abundant coral reef fisheries resources (Wagner, 2004). The uprooting of transplanted

Acropora species in nurseries (Mbije *et al.*, 2010) showed slower growth rates after transplantation, while still high enough for natural reef conditions. These results provide further evidence for the argument that active coral restoration initiatives may bring back denuded coral reef areas to their original state (Lindahl, 2003; Rinkevich, 2005, 2006, 2008). Concomitantly, while some studies indicated that coral reef restoration through transplantation can be costly costs (Edwards and Gomez, 2007; Edwards, 2010), our results indicate that a large scale coral transplantation can also be done in developing countries at low costs with the available resources, less than one tenth of the costs as evaluated earlier.

Similar to recent documentation of environmental impacts on reef restoration (Shaish *et al.*, 2010a), the Tanzanian coral transplants experienced two catastrophes, *Acanthaster planci* infestation and a major coral bleaching event. The unexpected infestation by crown-of-thorns starfish at Changuus' reef, immediately after colony transplantation, which was not documented in adjacent natural reef areas, may have been related to the response of resident organisms to newly arrived transplants. Similar results from transplantation experiments in the Red Sea (Horoszowski, personal communication, 2010) and South East Asia (Shaish *et al.*, 2010a) showed that new transplants are prone to attacks by resident fish and corallivorous invertebrates that may eventually kill them. Starfish infestation impacted all coral species but the most affected species were *Pocillopora verrucosa*, *Porites cylindrica*, *Acropora muricata* and *Acropora nasuta*. Whereas Harriott *et al.* (2003) discussed different theories for *Acanthaster planci* outbreaks, determining the reason for the specific outbreak at the transplantation sites is beyond the scope of this study. Damage to coral colonies by bleaching of transplants was the second major stressor, further documented in the studied Indo Pacific reef sites (Shaish *et al.*, 2008, 2010; Mbije *et al.*, 2010). The most prominent bleaching episode during the first year after transplantation was part of the wide-spread bleaching event developing in the Western Indian Ocean (http://www.cordioea.org/bleachingalert, 2009). Generally, no significant mortality of transplants was associated with this phenomenon. However, Mafia Island and neighbouring areas of Kilwa, are among the remaining pristine fishing grounds of Tanzanian reefs and therefore highly prone to anthropogenic pressure. Some groups of fishermen have been invading the reefs, especially at night, and gain access to abundant coral reef fisheries resources (Wagner, 2004). The uprooting of transplanted

fragments was probably a result of drag net activities that stopped after the case was reported to the park authority.

When comparing the initial and the last three months of the research for each treatment, we observed a significant difference in fish abundance for all three treatments in Kitutia reef but not in Changuu reef. In contrast, we observed no significant spatial variability for invertebrates at Kitutia reef between and within treatments, as opposed to Changuu reef where this was true only in T1 plots. Although we documented unclear patterns in invertebrate composition over time, the outcomes of this study further suggested that the primary reasons for the gradual variability in fish community composition within treatments for each site, over time, were the improved live coral cover and habitat structure caused by the transplantation as reported by Roberts and Olmond, (1987) and Fowler *et al.*, (1992). The above findings have been further supported by Cabaitan *et al.* (2008) and Ferse (2009) studies on the impacts of coral colonies on associated fish/invertebrates biota. This suggests that large-scale reef restoration projects may catalyse an increase in reef communities' species richness, abundance and composition; thus supporting faster attainment of original states.

Central bare sub-plots comparisons for initial and last three months revealed increased numbers of new spat belonging to the transplanted species, similarly to an earlier study on coral recruitment following coral transplantation (*Acropora muricata* in Mafia; Lindahl, 1998). There is a possibility for larval contribution from some on-site transplanted colonies during the studied period (Horoszowski-Friedman *et al.*, 2011). However, the dominance of recruits from the three broadcasting *Acropora* species in all transplantation plots might indicate that the presence of the transplants *per se* may have acted as a cue for metamorphosing larvae (e.g., reef sounds; Vermeij *et al.*, 2010) as changes in currents or fish communities.

While this study provides the first insight into reef restoration through application of the two-step restoration protocol in East Africa, large-scale restoration projects that are urgently needed in Tanzania may require involvement of local communities (Wagner, 2004; Rinkevich, 2008; Mbije *et al.*, 2010). Moreover, since many reefs in Tanzania have remained severely damaged after the 1997/98 El-Niño incident and after decades of anthropogenic disturbances including destructive fishing practices, large scale restoration measures, that take

into account multi-species transplantation, are urgently needed to increase habitat complexity for reef dwelling organisms and for enhanced coral recruitment, altogether helping in conserving biodiversity. With the initiation of diverse studies globally, all attempting to address low cost applications in reef restoration (Clark and Edwards, 1995; Edwards and Clark, 1998; Bowden-Kerby, 2001; Lindahl, 2003; Fox, 2004; Raymundo *et al.*, 2007; Garrison and Ward, 2008; Forrester, 2012), a ubiquitous approach is envisaged. On the other hand, special consideration should be given to associated social, economic and cultural themes (Christensen *et al.*, 1996) that may vary between different reef sites. For example, the decision as to which and how much habitat should be restored may require discussions that involve reef stakeholders and, most importantly, local communities. Successful restorations are those that consider reef restoration methodologies appropriately adapted to local socio-economic limitations. Therefore, the future of large-scale coral reefs restoration of damaged areas in the Western Indian Ocean, as in other developing countries, is possible through applying relatively cheap and easily adaptable techniques with manpower involving surrounding local communities for sustainability.

Acknowledgements

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Figure legend

Figure 1. Map of Tanzanian coast (a) and Tanzania (Africa insert), showing (b) Changuu and (c) Kitutia transplantation sites in Zanzibar and Mafia Islands, respectively.

Figure. 2. Coral transplantation, (a) A scheme of 36 m^2 transplantation plots with eight $4m^2$lots with coral transplants and one $4m^2$ bare lot at the middle for *Acropora* and mixed species lots. The enlarged mixed species lot reveals corals spaced at 16 cm from each other, (b) coral transplants *in situ*, immediately after transplantation; *Millepora* sp., 4 m depth at

Changuu reef and (c) three months after transplantation; *Millepora* sp. and *Pocillopora verrucosa* 4 m depth at Kitutia reef.

Figure. 3. One year outcomes for transplants in Treatments 1 and 2 at Changuu in Zanzibar and Kitutia in Mafia. (a) Accumulated mean mortalities; (b) Mean bleaching levels. Error bar represent +/- standard error.

Figure. 4. Mean (±SE) ecological volumes of transplanted coral species; (a) Changuu and (b) Kitutia reefs. 1 - 2 refer toT1 and T2, respectively.

Figure. 5. Non-metric multidimensional scaling (nMDS) ordination of fish assemblage structure separated within treatments separation (A- F) based on fish density data of initial and last three months of transplantation year. The lots are based on Bray-Curtis similarities index using square root-transformed data of fish density.

Figure. 6. Non-metric multidimensional scaling (nMDS) ordination of large invertebrate assemblage structure within treatments separation (AeF) based on invertebrate density data at the experimental starting point and the last three months (10e12 m after transplantation). Analyses performed by BrayeCurtis similarities index using square root-transformed data of invertebrate density.

Figure. 7. Mean numbers of recruits in central bare lots. (a) Changuu reef in Zanzibar and (b) Kitutia reef in Mafia. 1–3 refer to treatments 1 - 3.

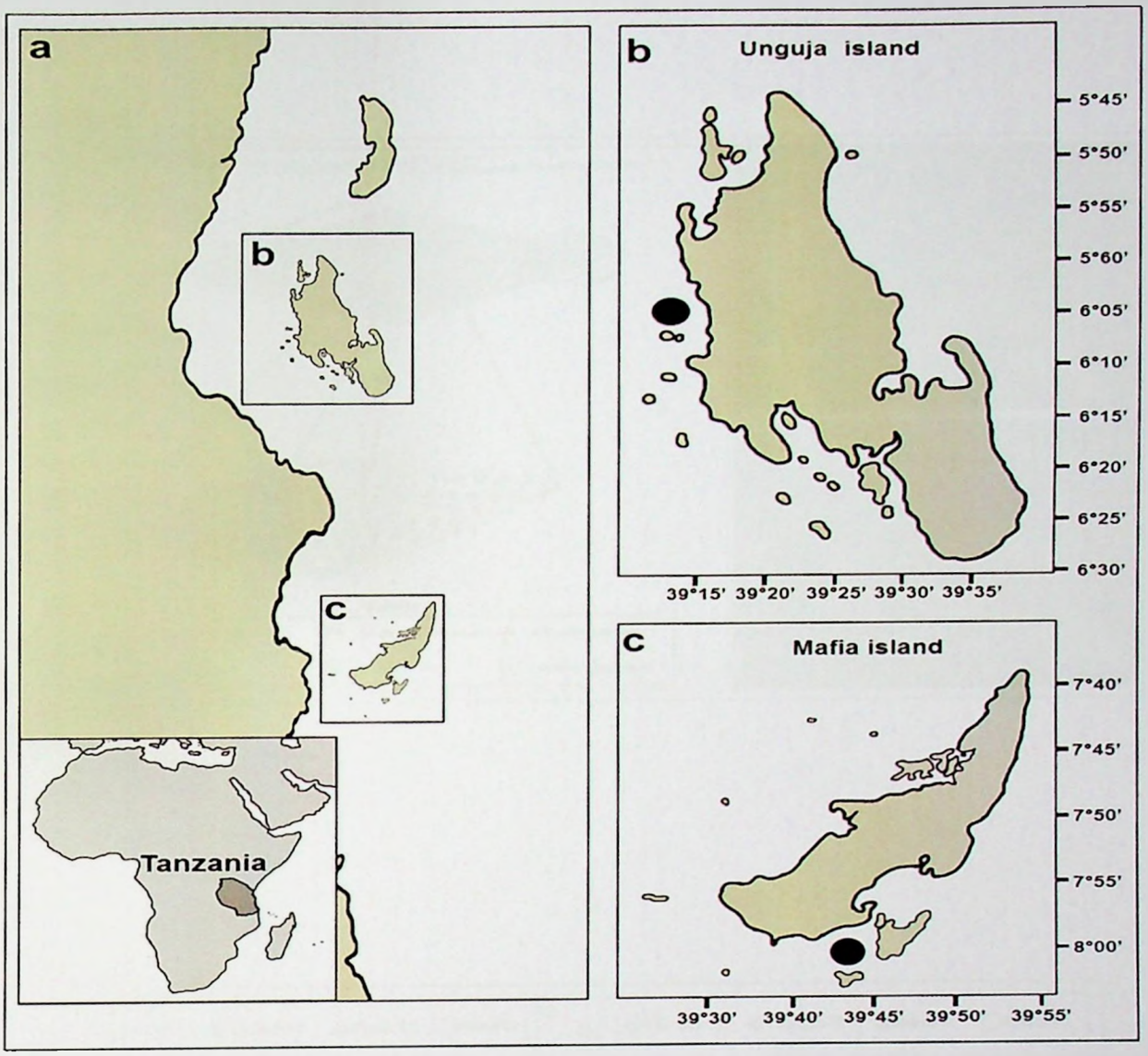

a
b
c
Tanzania
b
Unguja island
5°45'
5°50'
5°55'
5°60'
6°05'
6°10'
6°15'
6°20'
6°25'
6°30'
39°15' 39°20' 39°25' 39°30' 39°35'
c
Mafia island
7°40'
7°45'
7°50'
7°55'
8°00'
39°30' 39°40' 39°45' 39°50' 39°55'

Fig. 2

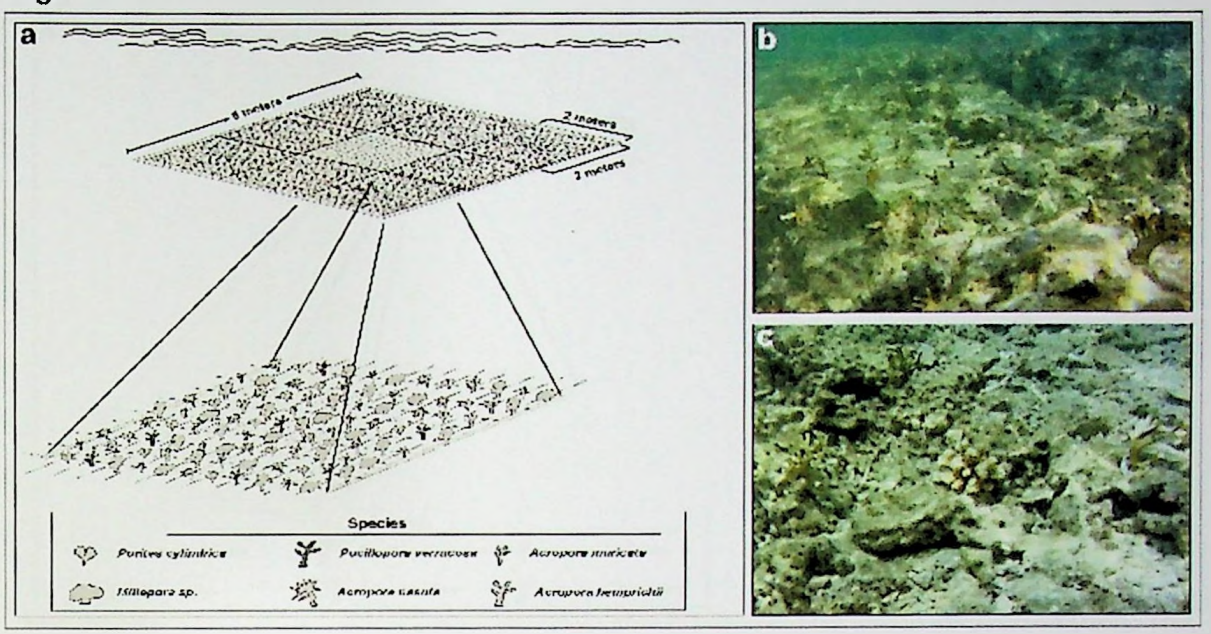
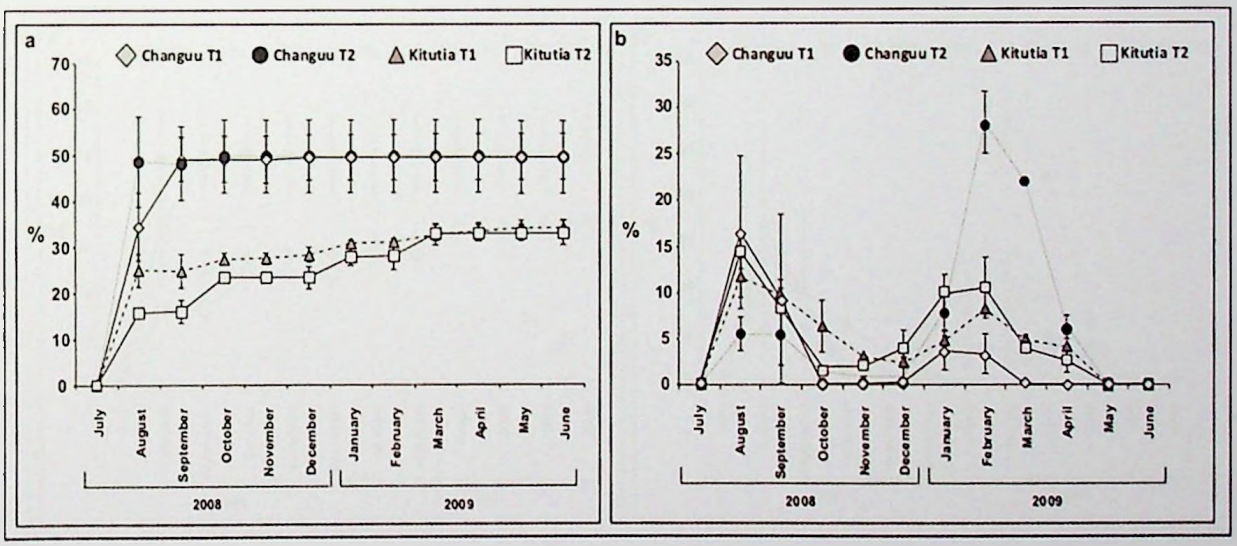

a
b
c
Species
Porites cylindrica
Pocillopora verrucosa
Acropora muricata
Millepora sp.
Acropora hemprichii

Fig. 3


a
b
Changuu T1
Changuu T2
Kitutia T1
Kitutia T2
%
July
August
September
October
November
December
January
February
March
April
May
June
2008
2009

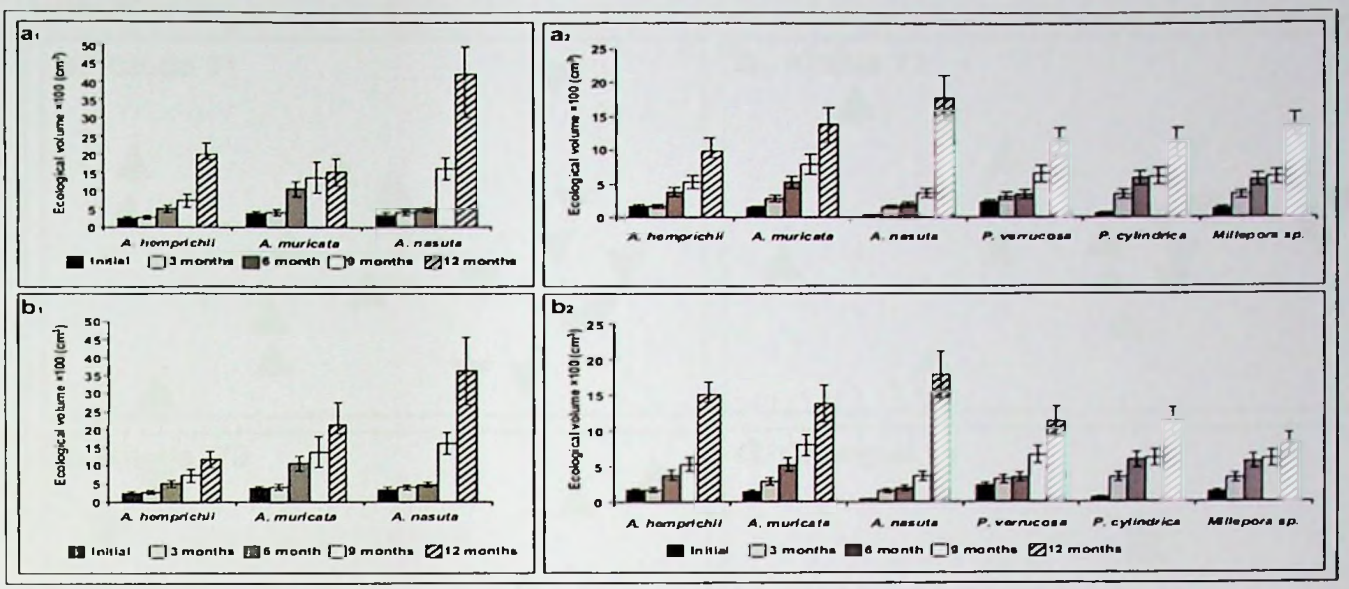
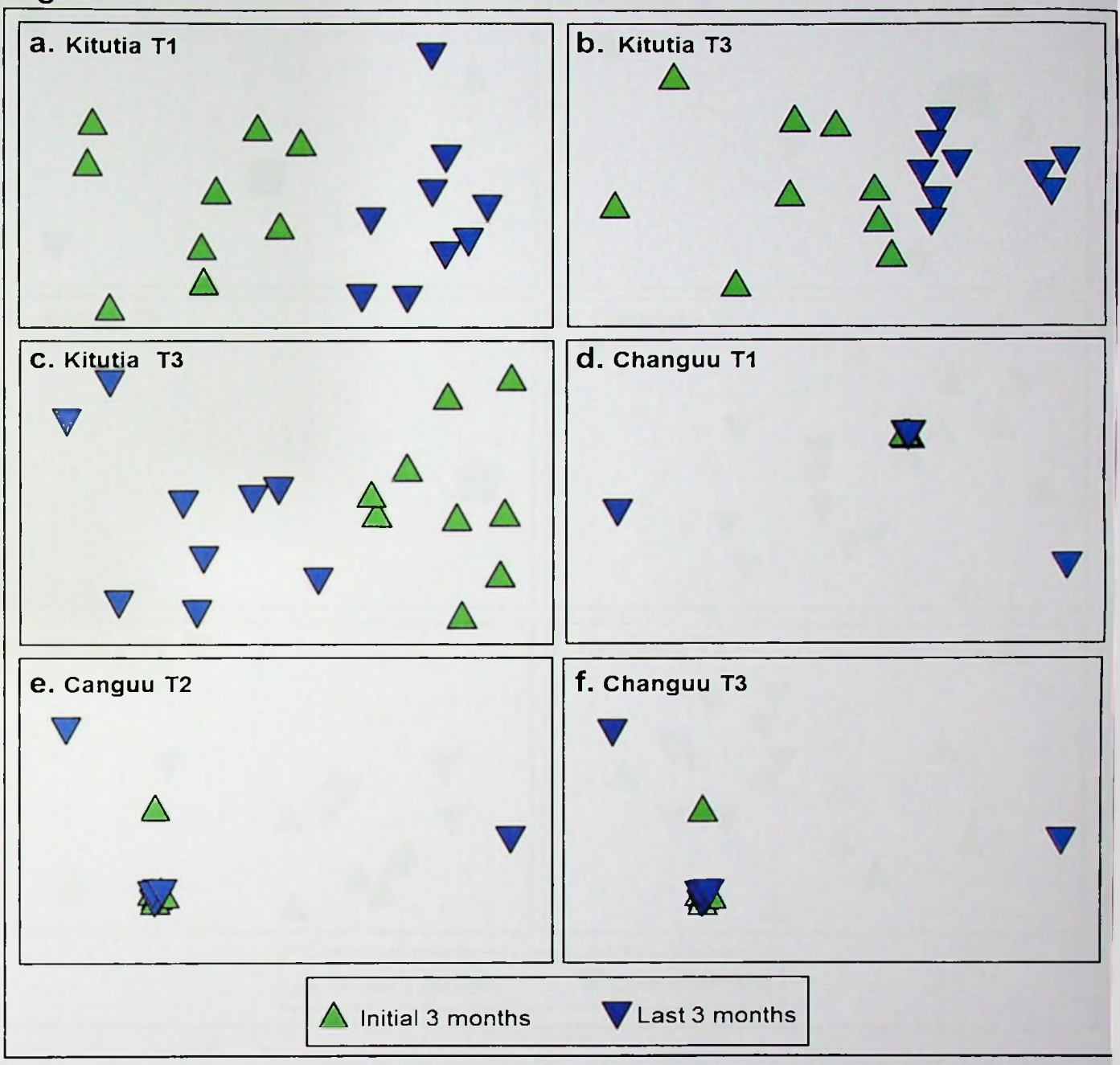
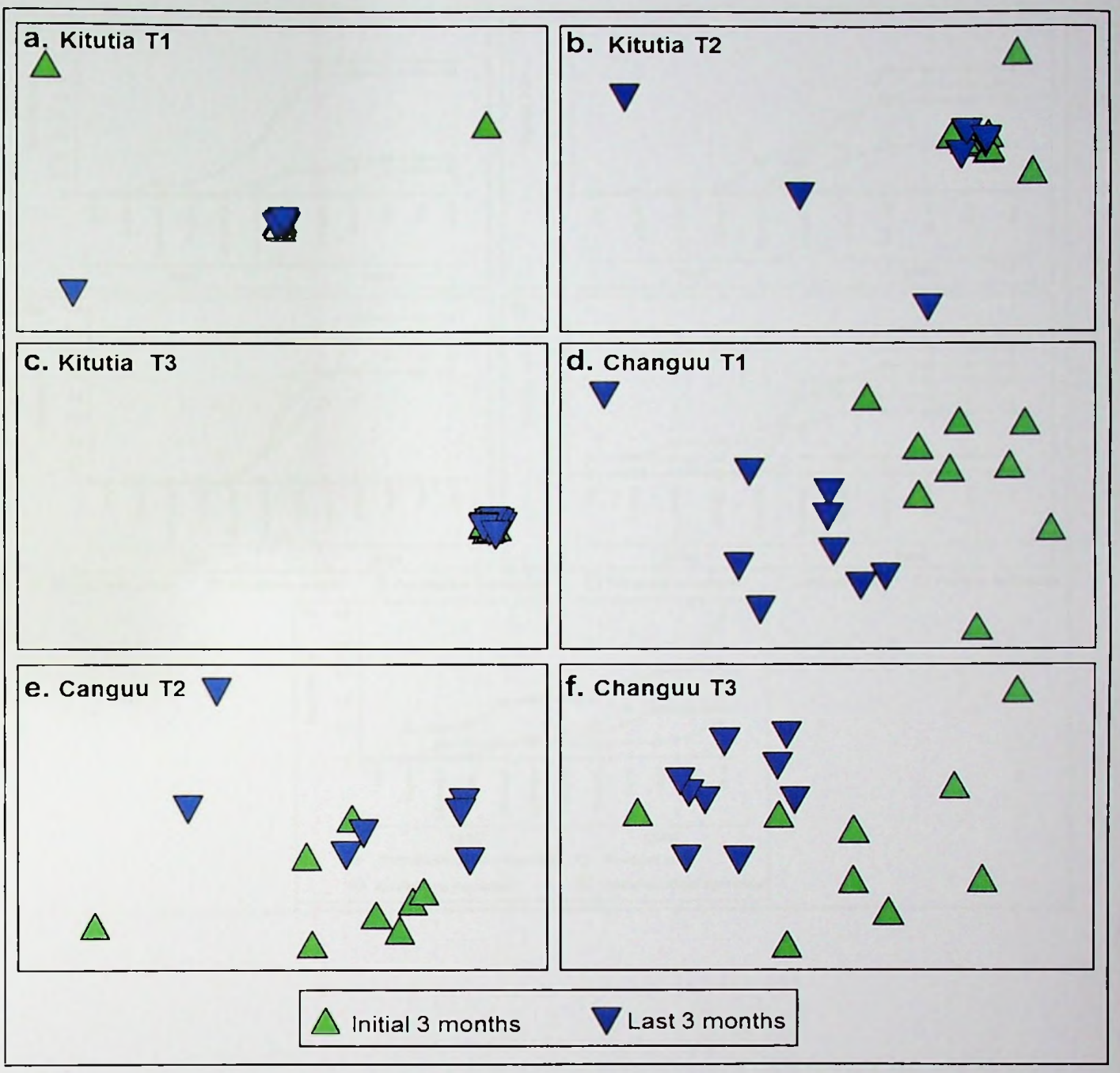

a1
Ecological volume ×100 (cm³)
A. hemprichii
A. muricata
A. nasuta
Initial
3 months
6 month
9 months
12 months
a2
Ecological volume ×100 (cm³)
A. hemprichii
A. muricata
A. nasuta
P. verrucosa
P. cylindrica
Millepora sp.
b1
Ecological volume ×100 (cm³)
A. hemprichii
A. muricata
A. nasuta
Initial
3 months
6 month
9 months
12 months
b2
Ecological volume ×100 (cm³)
A. hemprichii
A. muricata
A. nasuta
P. verrucosa
P. cylindrica
Millepora sp.
Initial
3 months
6 month
9 months
12 months

Fig. 5


a. Kitutia T1
b. Kitutia T3
c. Kitutia T3
d. Changuu T1
e. Canguu T2
f. Changuu T3
Initial 3 months
Last 3 months


a. Kitutia T1
b. Kitutia T2
c. Kitutia T3
d. Changuu T1
e. Canguu T2
f. Changuu T3
Initial 3 months
Last 3 months

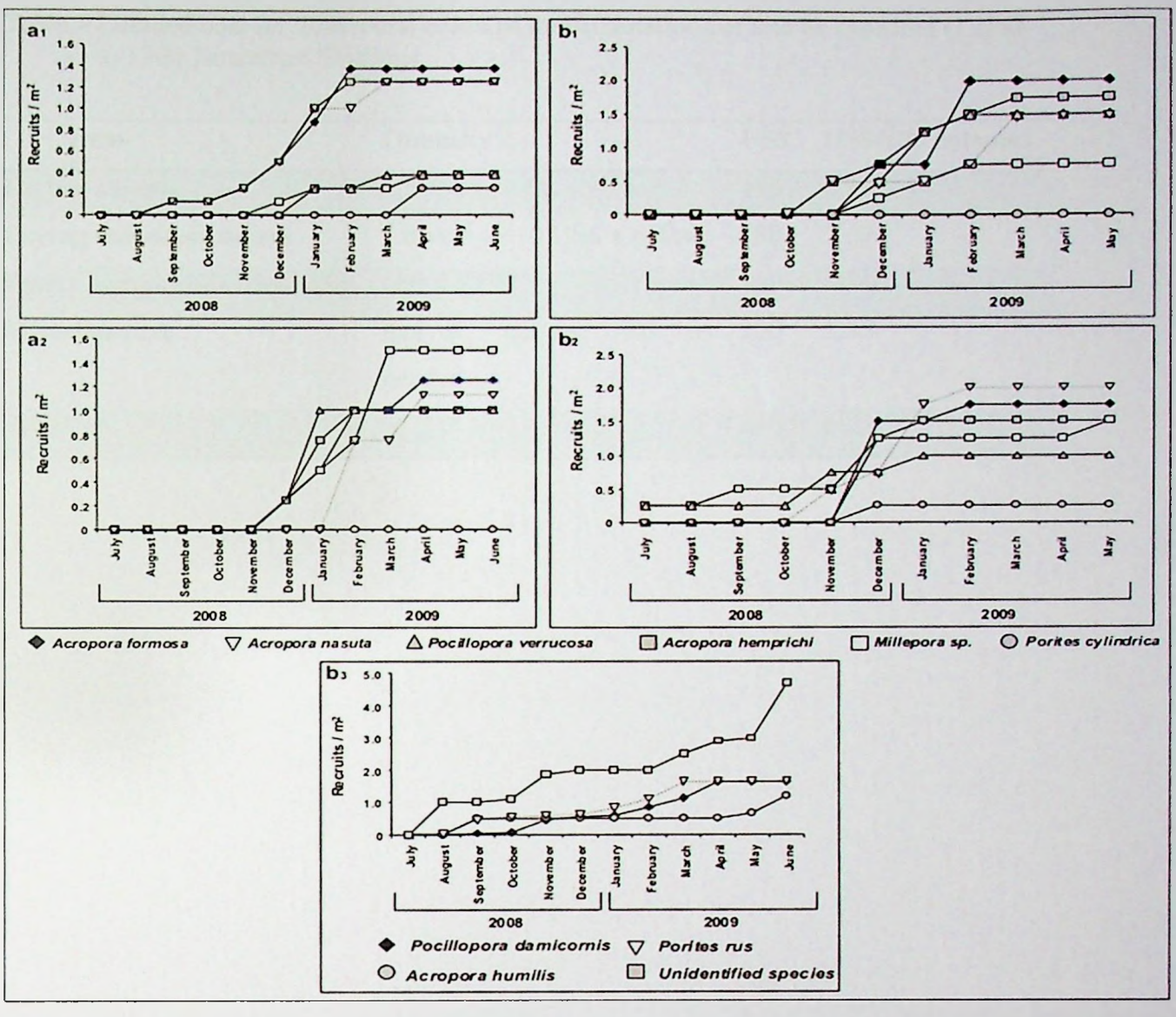

a1
b1
a2
b2
b3
Recruits / m²
July
August
September
October
November
December
January
February
March
April
May
June
2008
2009
Acropora formosa
Acropora nasuta
Pocillopora verrucosa
Acropora hemprichi
Millepora sp.
Porites cylindrica
Pocillopora damicornis
Porites rus
Acropora humilis
Unidentified species

Table 1 Detailed cost for 7000 coral colonies transplantation per site in Tanzania (1 US$ to 1300 Tanzanian Shillings

Item	Quantity	US$	US$/100 colonies
Hiring a boat	80 US$ per day x 6 days	480	6.85
Paying volunteer divers	3 divers @ 10 US$ x 6 days	180	2.57
Epoxy compounds M-seal®)	100 x 5 US$	500	7.14
Miscellaneous	Knives, cutters, etc as needed	200	2.85
Total		1360	19.41

Appendix 1. Experimental design for transplanted coral colonies for each site; (A) Treatment 1 and (B) Treatment 2.T1 = *Acropora* species: *A. muricata. A. hemprichi and A. nasuta* and T2 =All species: *Acropora nasuta, A. hemprichi, A. muricata, Millepora sp., Pocillopora verrucosa and Porites cyndrica.*

A

Treatment 1			**Distribution of coral fragments within plots (each 4m^2)**		
			Plot No.	**Corals transplanted**	**Species**
Transplantation (36m^2)	lot	1	1	Genome 1	*Acropora muricata*
			2	Genome 2	*Acroporahemprichii*
			3	Genome 3	*Acroporanasuta*
			4	Mixed genomes	*Acropora muricata*
			5	Mixed species	3 *Acropora* species
			6	Mixed species	3 *Acropora* species
			7	Mixed species	3 *Acropora* species
			8	Mixed genomes	*Acroporahemprichii*
Transplantation (36m^2)	lot	2	1	Mixed genomes	*Acroporanasuta*
			2	Genome 2	*Acropora muricata*
			3	Genome 1	*Acroporahemprichii*
			4	Mixed species	3 *Acropora* species
			5	Mixed genomes	*Acroporanasuta*
			6	Mixed species	3 *Acropora* species
			7	Genome 3	*Acropora muricata*
			8	Mixed species	3 *Acropora* species
Transplantation (36m^2)	lot	3	1	Mixed species	3 *Acropora* species
			2	Mixed species	3 *Acropora* species
			3	Mixed species	3 *Acropora* species
			4	Mixed species	3 *Acropora* species
			5	Mixed species	3 *Acropora*species
			6	Mixed species	3 *Acropora* species
			7	Mixed species	3 *Acropora* species
			8	Mixed species	3 *Acropora* species

B

Treatment 2	**Distribution of coral fragments within lots (each $4m^2$)**		
Transplantation for each of lots 1, 2 and($36m^2$)	Plot 1	Mixed species	All species
	plot 2	Mixed species	All species
	Plot 3	Mixed species	All species
	Plot 4	Mixed species	All species
	Plot 5	Mixed species	All species
	Plot 6	Mixed species	All species
	Plot 7	Mixed species	All species
	Plot 8	Mixed species	All species

Appendix 2: Major taxa of recorded fish species at Kitutia and Changuu reefs.

Family	Species	Site recorded
Pomacentridae	*Chromis dimidiata*	Kitutia and Changuu
	C. ternatensis	Kitutia
	Plectroglyphidodon lacrymatus	Kitutia
	Dascyllus trimaculatus	Kitutia and Changuu
	Cirrhilabru sexquisitus	Kitutia
	Chrisiptera unimaculata	Kitutia
	Stegastes nigricans	Kitutia and Changuu
	Abdefduf sexfasciatus	Kitutia and Changuu
	Chromis viridis	Kitutia
	Abdefduf sparoides	Kitutia
	Dascyllus aruanus	Kitutia
Labridae	*Halichoeres cosmetus*	Kitutia
	Gomphosus caeruleus	Kitutia
	Labrichthys unilineatus	Kitutia
	Pseudocheilinus hexataenia	Kitutia and Changuu
	Thalassoma amblycephalum	Kitutia
	T. hebraicum	Kitutia and Changuu
Chaetodontidae	*Chaetodon auriga*	Kitutia
	C. trifacsiatus	Kitutia
Scaridae	*Scarus sordidus*	Kitutia and Changuu
	Leptoscurus vaigiensis	Kitutia
Haemulidae	*Plectorhincus gaterinus*	Kitutia
Caesionidae	*Caesio caerulaureus*	Kitutia
Lutjanidae	*Lutjanus fluviflamma*	Kitutia
	Lutjanus kasmira	Kitutia
	Lutjanus bohar	Kitutia
Lethrinadae	*Lethrinus harak*	Kitutia
	Lethrinus nebulosus	Kitutia
Holocentridae	*Myripristis murdjan*	Kitutia

Appendix 3: Major taxa of recorded invertebrate species at Kitutia and Changuu reefs

Class	Species	Site recorded
Echinoidea	*Diadema setosum*	Kitutia and Changuu
	Diadema savignyi	Kitutia and Changuu
	Echinometra mathai	Kitutia and Changuu
	Echinothrix diadema	Kitutia and Changuu
Asteroidea	*Acanthaster planci*	Kitutia
	Culcita schmideliana	Kitutia
	Linckia guildingi	Changuu
	Pentaceraster mammillatus	Kitutia and Changuu
	Pentaceraster tuberculatus	Kitutia and Changuu
	Protoreaster lincki	Changuu
	Synapta maculata	Kitutia
	Tripneustes gratilla	Kitutia
Gastropoda	*Cypreaea tigris*	Kitutia

3.3 Anthropogenic impacts on coral reefs and their effect on fishery of Kilwa district, Tanzania. Ethiopian Journal of Environmental Studies and Management (2013) 6 (5): 443-452.

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Key words: coral reefs, Kilwa, Tanzania, dynamite fishing

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Abstracts

Tanzanian fishing coastal communities live on fishing activities as one their major economic activities, practicing fishing on shallow coral reefs areas whereby about 70% of fishery is artisanal. Improper use and overexploitation of fishery resources have resulted in damaging the coral reefs and the subsequent low quality catches. This study aimed at examining the impacts of coral reef fishery decline on rural livelihoods with an emphasis on food insecurity, alternative capabilities and activities on coastal communities of Kilwa district, Tanzania. Data collection methodology included household questionnaire survey, key informant interviews, participant observation and photographing. The survey was based on a sample of 90 households, randomly selected from three villages. The findings attest for a gradual reduction in fish catches over time, brought about by natural and anthropogenic impacts. Overfishing, use of illegal and destructive fishing methods, as well as extreme weather conditions, all threaten the sustainability of marine resources, particularly coral reef fishery that constitutes an important source of food and livelihood. Following the results and as a way of lessen the current pressure on marine resources and diversifying livelihood capabilities we recommend introduction of mariculture and modern farming technologies especially on green vegetables on farms that can potentially be irrigated. The study further recommends establishment of a marine protected area and; in addition, the need to promote educational programs on environmental and resources uses as well as application of active restoration protocols for damaged coral reefs.

Key words: Songo songo, Kivinje, Songo mnara, livelihoods, coral bleaching

Introduction

A third of Tanzania's population live at coastal areas occupying 22% of the 881,289 km^2 of mainland Tanzania (NBS, 1999). Besides practicing subsistent agriculture, they rely on exploitation of natural resources, fishing, being most important (Jiddawi, 1997). The largely non- mechanised agricultural activities are seasonal and strongly influenced by rain patterns, involving mostly women, (Mwichande, 2001). In contrast, fishing activities are conducted throughout the year acting a major source of food that supplies 90% of consumed animal protein and the perpetual, year-round source of income (Wagner, 2005). Most fishery activities are practiced within a narrow range of shallow water along the coastal areas that include sea grass beds, mangrove and coral reefs (Jiddawi and Öhman, 2002; Wagner, 2004). Fisheries in coral reefs involve harvesting of fish and shellfish for food and collection of merchandise for the curio and aquarium trades. On the other hand, reef associated tourism brings foreign currency into the country, establishing improved livelihoods for coastal people through employment and costs of services (Andersson, 1998).

Conversely, in recent times reefs have been declining at an alarming rate thus threatening fisheries integrity of the coastal communities. It is estimated that global coral reef decline associate with ocean acidification, warming, pollution and overfishing has reached 125, 000 square kilometres (Hoegh-Guldberg and Bruno, 2010) and continues to increase. The decline due to overfishing and using bad fishing practices are common in developing world including Tanzania (Guard and Massaiganah 1997; Wilkinson, 2004; Souter and Linden, 2000; Mbije *et al.*, 2002; 2010). Furthermore, the current climate changes associated with unpredictability of rains for agriculture have forced local communities to either intensify fishing or migrate for alternative livelihood within or outside their native areas (Mwamsojo, 2000). Following the above, the main objective of this study was to understand the scope and magnitude of human induced coral reef fisheries decline in Kilwa District, Tanzania and its implication on local livelihoods, followed by suggesting strategies for solutions to improve the welfare of the fishing communities.

Methodology

Description of the Study Site

This study was conducted in three coastal fishing villages namely; Kilwa Kivinje, Songo Mnara and Songo songo found in Kilwa district located in south-eastern region of Lindi region in Tanzania (Figure 1). Songo Mnara is located on the southern part of Kilwa coastline whereas Kilwa Kivinje is centrally located. Songo songo is an island within the vast Kilwa archipelago. The major economic activity in these villages is fishing, supported by agriculture at subsistent level (Ngoile *et al.*, 2001; Mwichande, 2001). Besides terrestrial vegetation that form continuum of the mostly *Acacia* sp. traversing into hinterland, there are extensive mangrove forests covering 22, 438.7 ha, from Mohoro Bay at the border of Rufiji district to Mzungu Bay bordering Lindi district (Semesi *et al.*, 1991). These mangrove form important breeding and nursery grounds for both marine and terrestrial fauna, including some commercially important species such as sardines (*Rastrelliger kanagurta*), catfishes (Ariidae), milkfish (*Chanos chanos*), goatfish (*Mullidae*), *apogionidae*, *clupeidae*, crabs and molluscs (Semesi *et al.*, 1991).

Fig. 1

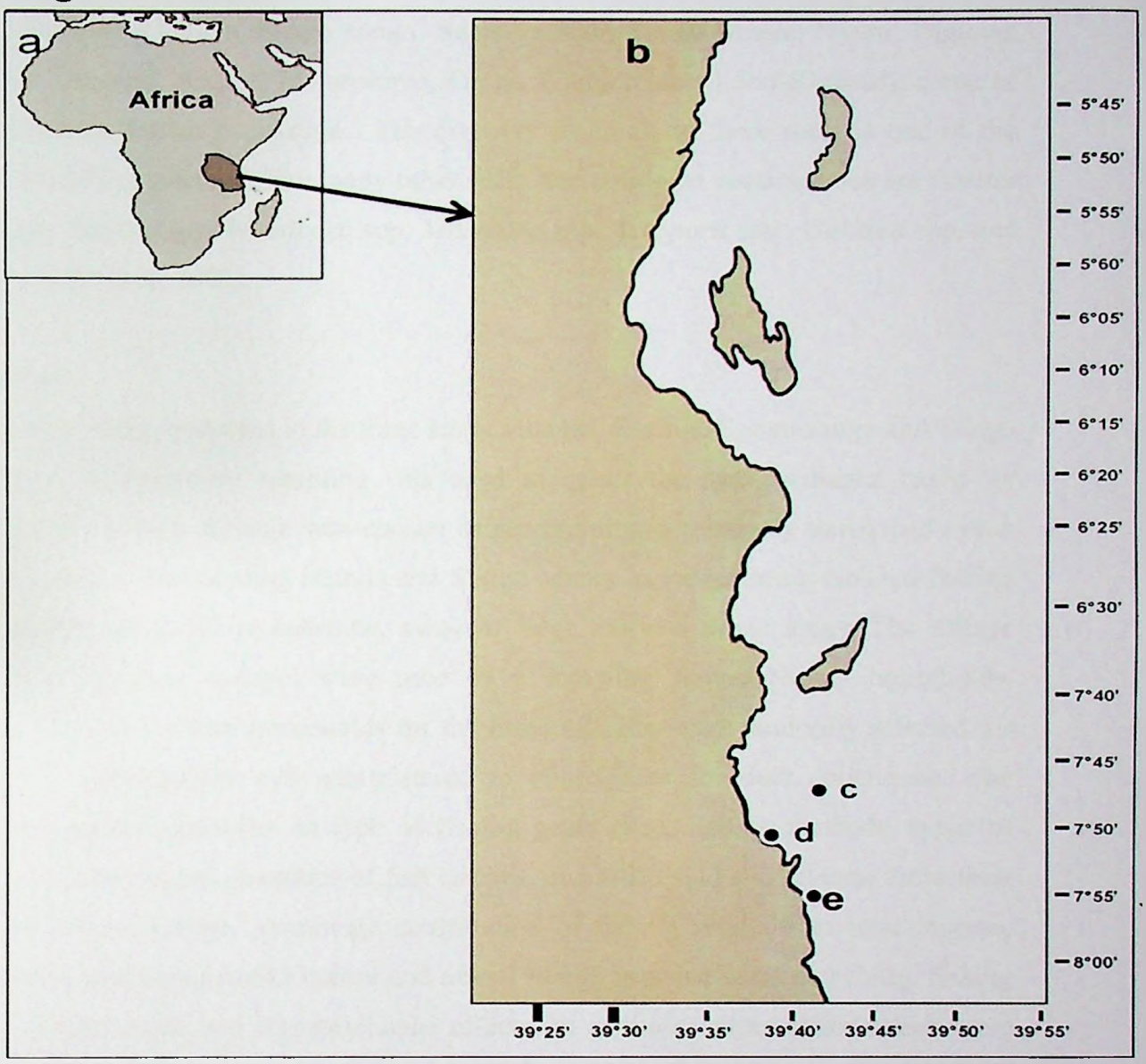

Figure 1. Map of Tanzania showing (a) Tanzania (Africa insert), (b) Tanzanian coastline (c, d, e) Songo songo, Kilwa Kivinje and Songo Mnara study sites, respectively.

Coral reefs of Kilwa are mostly fringing, forming complex tropical shallow water ecosystems with high biodiversity (Wagner, 2004). These reefs are found within a complex island archipelago (including Ukuza, Songo songo, Sanje ya Kati, Songo Mnara, Nyuni, Fanjove, Limbi, Kilwa Kisiwani, Amana, Mwanakayo, Fungu Wango, Mpovi and Kiswasi); some of them inhabited by human population. The diversity of corals in these reefs is one of the highest in East Africa whereby, like many other reefs, the dominant coral species are *Porites* spp, *Favia* spp, *Pavona* spp, *Montipora* spp, *Millepora* spp, *Acropora* spp., *Galaxea* spp. and *Favites* spp (Mbije *et al*, 2002).

Data collection

Household survey was conducted in the three study villages, Kivinje, Songo songo and Songo Mnara (Figure 1). Purposive sampling was used to select the study villages based on accessibility and position. Kivinje was chosen as representing a relatively developed urban area, Songo songo as representing islands and Songo Mnara as representing isolated fishing villages, common along Kilwa coastline, away of large markets urban areas. The village registrars from the three villages were used as a sampling frame. Ninety households, representing 10 % of the total households for the three villages, were randomly selected for this survey. A questionnaire was administered to participants for data collections. The questionnaire included questions on type of fishing gears used, fishing methods, types of fishing grounds, content and quantities of fish catches, quantities sold and revenue from their sale, total household income, percentage contribution of fishing products to total income, trends in fishing comparing period before and after 1997/98 massive coral bleaching, fishing constraints, fishing trends and climate change effects. In addition, direct observations were conducted in various locations to appreciate types of fishing vessels, fishing gears, contents of catches at fish markets, crops grown and general life styles of the communities in the three selected villages.

Focus group discussions were held with villages officials , district natural personnel and agriculture officers to get their views on trends of fishing, caught fish species and their contribution in revenue earning, patterns in agriculture as well as management challenges of

resources.Secondary data were obtained by reviewing literature on Kilwa district's coral reefs, mangrove, sea grass and fishery. The documents reviewed included Mangrove Management Project report (MMP, 2003); Fisheries Regulation Act (URT, 2005); Guideline for Collaborative Management of Marine Resources of Kilwa District (WWF-RUMAKI, 2008); Collaborative Fisheries Management (CFM) monitoring plans for Rufiji, Mafia, Kilwa Fishing communities (WWF, 2008) and the Annual District Fisheries Reports (2001-2009) .

Data analysis

Statistical Package for Social Sciences (SPSS) was used to analyze the primary quantitative data collected by using a questionnaire whereas content analysis was used for analyzing qualitative data obtained from the unstructured interviews, focus group discussion, documents review and observation. The qualitative data was sorted and categorized into meaningful themes in keeping with the objectives of this study.

Results and Discussion

Demographic characteristics of respondents

The majority (93.3%) of the respondents were males with only 6.7% females, two thirds of these (66.7%) being native to their respective villages (Table 1). The majority (90%) of non-natives (n=81) immigrated from Mtwara and other parts of Lindi regions, mainly for the purpose of looking for alternative livelihoods. The abundance of natural resources available within the district, availability of fertile land and the reliability of rains were cited as factors for immigration (Mwichande, 2001).

Table 1: Respondents' place of birth

Villages	Native (%)	Immigrant (%)
Songosongo	25.6	7.8
KilwaKivinje	22.2	11.1
SongoMnara	18.9	14.4
Total	70.0	30.0

Before the construction of the bridge across Rufiji River and the tarmac road from Dar es Salaam to Mtwara, Kilwa was the Tanzanian southernmost district that was easily reached mainly through the seasonal road by crossing Rufiji river at Ndundu ferry. This contributed in making the district more vibrant economically thus attracting immigrants. The various goods shipped from this area to northern markets of Tanzania, mainly Dar es Salaam and Zanzibar includes forestry, fishery and some agricultural products (District Fisheries Statistics (2009)).

Poverty and its indicators in the study villages

We used 'poverty' as the major characteristic to describe households' socio-economic status in relation to access or use of natural resources. Poverty was attributed to five indicators: (i) household food, (ii) income insecurity (iii) household assets and material life styles (iv) household literacy, and (v) health wellbeing. With guidance from village leaders we defined three wealth groups, poor, rich, and very rich. In order to relate between poverty in individual villages and ownership of fishing assets, data on fishing gear and fishing vessels were cross-tabulated against the data on economic status of the households (Table 2).

Table 2: Types and number of fishing gears possessed by fishermen in the three villages

	Item	Total	SongoSongo			KilwaKivinje			SongoMnara		
			Poor	Average	rich	Poor	Average	Rich	Poor	Average	Rich
1	Seine nets	Count	22	21	20	19	34	18	6	8	13
		%	**34.9**	**33.3**	**31.7**	**26.7**	**47.8**	**25.3**	**22.2**	**29.6**	**48.1**
2	Shark nets	Count	54	84	20	43	60	62	3	11	13
		%	**34.1**	**53.1**	**12.6**	**26.1**	**36.4**	**37.5**	**11.1**	**40.7**	**48.1**
3	Hand lines	Count	460	50	0	550	0	27	200	10	18
		%	**90.1**	**8.9**	**0**	**95.3**	**0**	**4.7**	**87.7**	**4.2**	**7.8**
4	Ring nets	Count	0	23	15	0	15	20	0	0	0
		%	**0**	**60.5**	**39.5**	**0**	**42.8**	**57.2**	**0**	**0**	**0**
5	Fencing	Count	8	0	0	5	0	0	38	5	10
		%	**100**	**0**	**0**	**100**	**0**	**0**	**88.3**	**11.7**	**0**
6	Dugout canoes	Count	41	52	0	34	15	40	26	42	30
		%	**44.0**	**56.0**	**0**	**38.2**	**16.8**	**45**	**26.5**	**42.8**	**30.6**
7	Sail boats	Count	7	6	7	2	5	14	0	1	2
		%	**35**	**30**	**35**	**9**	**23.8**	**66.6**	**0**	**33.3**	**66.6**
8	Outboard Engines	Count	0	20	22	0	14	22	0	0	0
		%	**0**	**47.6**	**52.3**	**0**	**38.8**	**62.2**	**0**	**0**	**0**

Fishing gears that required heavy capital investment (starting from 3 million Tanzanian shillings) were few (row 4 and 8) when compared to among the more popular hand lines and fencing used by the low income individuals (Table 2: rows 3 and 5). Thus outboard engines were owned by a few individuals while the canoes and sail boats were shared between the low income and the rich (rows 6 and 7). The canoes are easily made out from mangroves that readily available in the area. Considering types of fishing gear as poverty indicator, comparison across villages revealed that poverty was more prevalent in Songo Mnara where fishermen did not have a single outboard engine, or any ringnets but possessed several low cost fishing gears. Studies by Francis *et al.* (2001) and Wagner (2004) indicate that one of the main underlining root causes of degradation in marine environments in Tanzania is poverty. Correspondingly, some of the lower quality gears are responsible for marine environment degradation through intensification of fishing in the same areas thus leading to over-exploitation.

Major livelihoods strategies in the study villages

In the study area, most respondents (93.3%) in the surveyed villages are engaged with fishing activities (Figure 2). Further analysis of the three villages revealed high variation in income from fishing activities (Table 3).

Table 3: Percentage income derived from fishing activities

Villages	Don't know	0-25%	26-50%	51-75%	Above 75%
Songosongo	14.4	24.3	15.1	15.2	30.1
KilwaKivinje	27.7	25.5	10.1	16.6	20.1
SongoMnara	24.5	15.6	25.2	17.8	17.0

Generally, the majority of the surveyed participants relied on fishing as their main income where Songo Songo Island was more dependent on fishing compared to the Kilwa Kivinje and Songo Mnara (Table 3). This could be related to the geography of the villages in which Songosongo is and Island lying about about 12 km off the coastline from Kilwa Kivinje where

option for other activities than fishing is limited. Further analysis of data revealed that, 68.9% of the households preferred using coral reef areas as their main fishing grounds, whereas 31.1% preferred using non-reef areas (Table 4).

Table 4: Percentage preference of the fishing grounds

Villages	**Percentages**	
	Reef Areas	**Non Reef Areas**
Songosongo	18.8	14.4
Kilwa Kivinje	21.1	12.2
Songo Mnara	28.9	4.4
Total	**68.9**	**31.1**

This information underscores the importance of coral reefs to fishery of Tanzania and confirms findings by Jiddawi (1997) and Wagner (2004) that majority of fishing communities along the coastline of Tanzania rely on coral reefs as their main fishing grounds. The existence of extensive formation of reefs within the Kilwa Archipelago and their convenient accessibility in most part provides a broad spectrum of areas upon which fishing is practised. Focused group discussion revealed that large schools of tuna species (*Katsuwonus pelamis*, *Thunnus thynnus* and *Thunus albacores*) and swordfish (*Xiphias gladius*) are regularly spotted in several islands within the Kilwa archipelago. The small sized pelagic species are also captured at night through the use of hand operated chandeliers (Figure 4b). Further household survey indicated that besides fishing, participants practiced other income generating activities, crop farming (60%), petty business (27.7%), with small contributions from other activities (Figure 2).

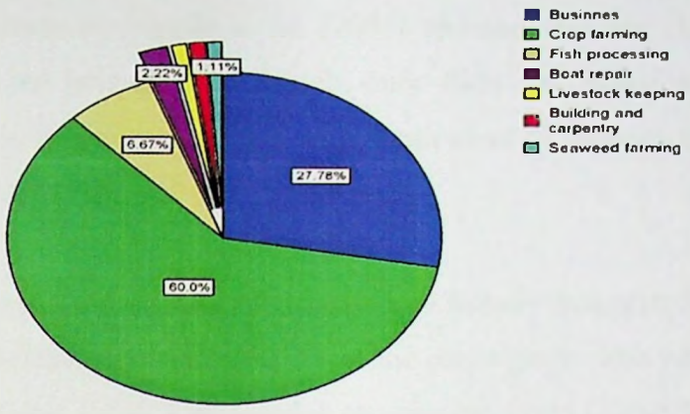

Figure 2: The respondents' alternative livelihood strategies

Kilwa district has about 8,863 km^2 of the potential arable land and agriculture employs more than 90% of people contributing 70% to the District's GDP (Ngoile *et al.*, 2001). During focussed group discussion (FGD) session with local leaders at Kilwa Kivinje and Songo Mnara it was revealed that, in response to increased demands, many households had recently increased the number of crops cultivated. Traditional crops included sim sim (*Sesamum indicum*), cashewnuts (*Anacardium occidentale*), rice (*Oryza sativa*), maize (*Zea may*), cassava (*Manihot esculenta*) and green vegetables (amaranth) but recently sweet potatoes and

a variety of legumes were adopted. Apparently, the discussions also revealed that in recent years agricultural production has been severely hampered by the unpredictability of rains. A study by Boko *et al.* (2007) asserts that rains patterns like this usually results in serious food insecurity and destabilisation of social networks, hindering eradication of poverty. On the other hand, a study by Ngoile *et al.* (2001) showed that the District has about 433.2km^2 potential area for irrigation of which only 8km^2 is being irrigated. This means that intensification of irrigation activities with application of modern farming methods may boost agricultural productivity.

Coral reef degradation and its relationship to fishery integrity in Kilwa

During the household interviews, 82.2% of the participants who were fishermen indicated that the fish catches have steadily declined in the past ten years (Table 5).

Table 5: Respondents' view (%) on trend in fish catch over the past 10 years

Villages	Do not know	Increasing	Decreasing	The same
Songosongo	4.4	0	25.6	3.3
KilwaKivinje	1.1	1.1	26.7	4.4
SongoMnara	1.1	1.1	30.0	1.1
Total	**6.6**	**2.2**	**82.3**	**8.8**

According to the interviews, commercial fish species that contribute significantly to their total gains but recently show rapid decline include goatfish (*Mullidae*), angelfish (*Pomacanthidae*), surgeonfish (*Acanthuridae*), wrasses (*Scaridae*) and butterfly (*Chaetodintiae*). When asked the reason for this decline, 35.6% of the participants attributed it to bad fishing practices that include use of dynamite, small mesh size nets and seine nets; 12.2% put a blamed on the climate changes; and 8.9% said it was a combination of the above (Figure 5).

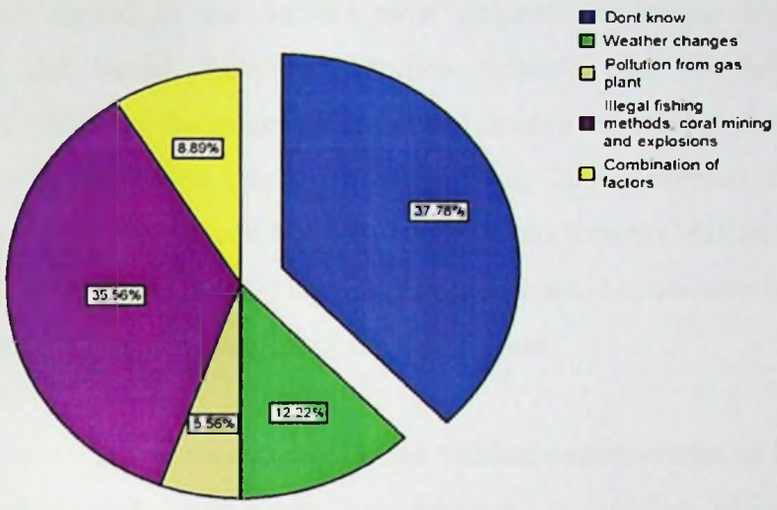

Figure 5: Respondents' view on reasons for the decrease in fisheries catch over the last 10 years

Three factors that promote bad fishing and over-utilization of marine resources were ranked. These were: (i) increased number of fishermen; (ii) Bad and destructive fishing practices; (iii) encroachment of foreign vessel (Table 4). Most of interviewees consider the influx of short term fishermen from other parts of Tanzania to be the biggest threats to the sustenance of their fisheries and livelihood general. When asked about other causes of fishery decline in the area, climate change was ranked as the second most culprits for killing corals destabilizing ecosystems where fish breed. Likewise, studies indicate that the higher sea surface temperature of 1997/1998 and the associated massive death of coral reefs have been shown to have influenced the decline in fish catches (Lindahl *et al.*, 2001). Overall, the decline in fish catches is becoming more widespread both in Tanzania and Kenya (McClanahan *et al.*, 1999; McClanahan, 1997; Garpe *et al.*, 2003) resulting in major socio-economic hardships for poor coastal communities, as a result of declining fishing income

Coping strategies and their consequences to the fishing communities of Kilwa

Development of alternative livelihoods has become a popular policy to uplift the socioeconomic status of small-scale fishers and to reduce fishing pressure on overexploited fisheries (Crawford, 2002). The interviewed households expressed mixed feelings with regards to diversification of livelihood strategies. Of the total households interviewed, 51.1% wished to increase alternative livelihood options, whereas 31.0% thought better fishing gear will solve the problem. Some 14.4 % wanted to move to other remote fishing sites (Table 6).

Table 6: Respondents' views on how to counteract low gain from fisheries

	Percentages		
Villages	Changes to other fishing sites	Change in fishing gear	Diversify alternative livelihood strategies
Songosongo	6.7	7.8	18.9
KilwaKivinje	6.7	10.0	16.7
SongoMnara	1.1	16.7	15.6
Total	**14.5**	**34.4**	**51.1**

Although it may be difficulty in a short term, to control damages within the reef and other marine areas because of their vast size and lack of funds, it is however, high time to think of reducing the pressure by introducing alternative viable livelihoods. Generally there are two main objectives for promotion of alternative livelihood. The first is to raise the economic standard of living of the communities and the second is to reduce resource extraction efforts (Smith, 1979). When asked what alternative livelihood strategies they propose in order to counteract the effects of falling fish catches, 36.67% of households suggested introduction of livestock farming while 15.56% said they would like to intensify agricultural activities and the same percentage suggested starting up private businesses (Figure 6).

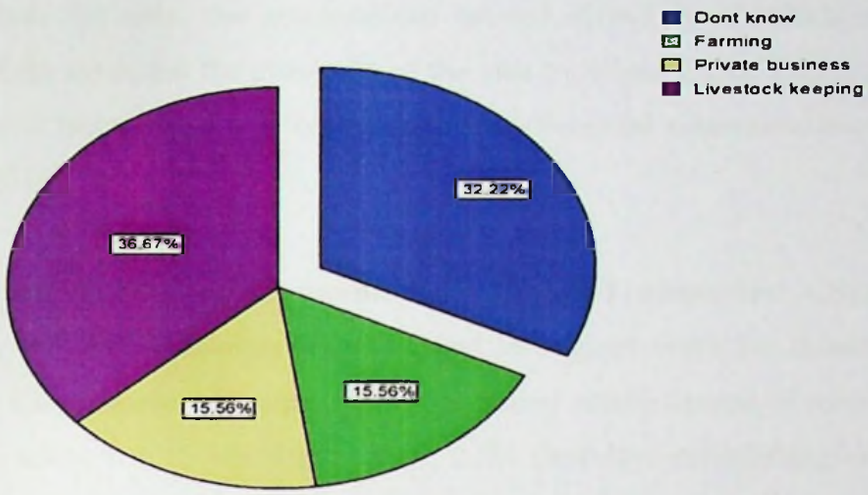

Figure 6: Respondents' suggested alternative livelihood to lessen pressure on marine resources

During focused group discussions, livestock keeping as the appropriate alternative livelihood strategy resurfaced frequently and was motivated by the increasing number of pastoralists that migrated into the area, mostly from Ihefu in Mbeya. According to them, before these pastoralists came into the area, the communities around Kilwa were reluctant to keep livestock because of the myth that the proximity of the area to Selous Game Reserve rendered the area vulnerable to tsetse fly, i.e., sleeping sickness (Personal communication; District Livestock Officer, 2010).

Coral Reef Conservation and Local communities Livelihood Trajectories: A Synthesis

During this survey, when the participants were asked to suggest ways for minimizing the rapidly diminishing Kilwa marine resources, 78.9% proposed establishment of core areas that would be closed to allow fish to breed and grow, 2.2% proposed establishing community based management plan, and 1.1% proposed acquiring modern fishing gear (Figure. 7).

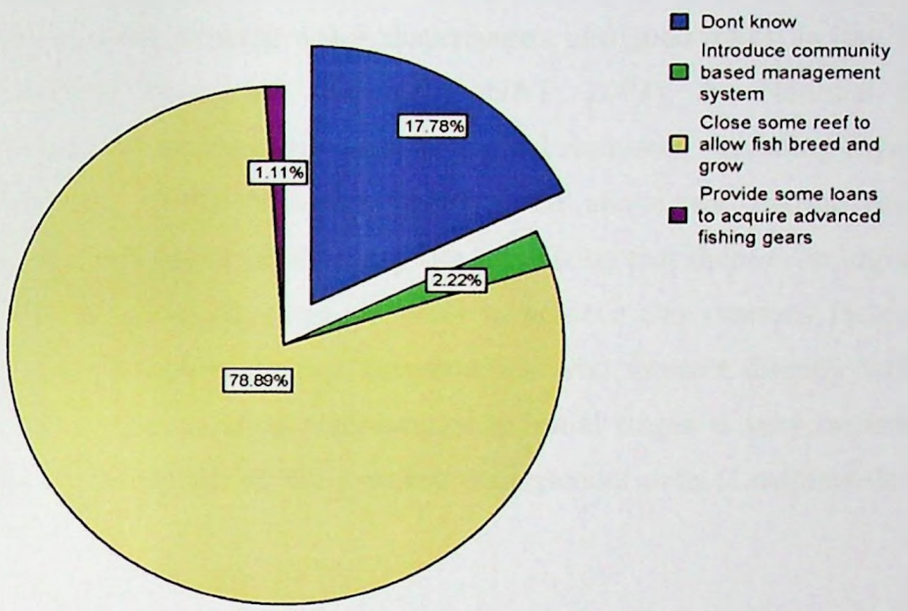

Figure 7: Respondents' proposed management initiatives for marine areas

A similar move to establish Mafia and Ruvuma Estuary Marine resulted in gradual decline of destructive fishing practices that had previous threatened wiping out marine resources in these areas (Lindahl *et al.*, 2001). Moreover, studies show that establishing conservation area is an appropriate approach in dealing with issues related to resource decline (McClanahan and Kaunda-Arara, 1996). Once a conservation area is established, in addition to creating resource use sustainability; it also empowers fishermen and other communities through improvement of social services schools, clean drinking water, dispensaries, and good roads. In line with the objectives of the Fisheries Policy of Tanzania (MNRT, 2003), the National Poverty Eradication Strategy intends to increase utilization of natural resources, including fisheries, to fight poverty (VP's Office, 1998). However, as observed above, the marine resources, including fisheries are diminishing at an alarming rate suggesting that there is an urgent need to develop mechanisms for salvaging them. In order to achieve this, various factors must come into play; the most important are the communities who interact directly with their resources on a daily basis. Incorporating communities at initial stages is very important for them to have a sense of ownership of the planned management area (Coughanowr *et al.*, 1995).

Conclusion and Recommendations

Various causes that range from human actions such as destructive fishing practices to natural acts such as climate change have already been affecting the coral reefs and many areas have died due to coral bleaching. This has had big impacts on many types of fish stocks and marine life, causing the stocks to decline. Unlike agriculture, products from the sea are available throughout the year. Additionally, because of fluctuations in prices of agricultural products and unreliability of rains, agriculture is less popular in most areas including those located on the coastline. The observed continued decline of fishery catches necessitates the need for diversification of local livelihoods. Introduction of modern farming, especially such crops are tomatoes, green vegetables, legumes and fruits that involve irrigation in areas close to water sources may serve as important source of incomes to many as reliable markets are guaranteed in Dar es Salaam and other neighboring major cities. Though not yet perfected, widespread introduction and promotion of mud-crab (*Scylla serrata*) and milkfish (*Chanos Chanos*)

farming specifically in mangrove may significantly contribute in diversifying livelihoods in the area. As a permanent solution to frequent coral reef degradation we recommend establishment of marine management area within the whole Kilwa coastline. Lastly, besides promotion of education programs on importance of reefs and the management techniques, we recommend application of low cost conservation practices such as restoration so as to reverse the badly denuded reefs to their original states.

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3.4 Population genetics parameters of the emerging corallivorous snail Drupella cornus in the northern Gulf of Eilat and Tanzanian coastlines based on mitochondrial COI gene sequences. Submitted: Journal of Experimental Marine Biology and Ecology

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ABSTRACT

The genetic diversity and population genetic structures of *Drupella cornus* populations from six localities in the northern Gulf of Eilat (GOE) and five localities in Tanzania (269 individuals) were investigated using mitochondrial cytochrome c oxidase subunit I (COI) gene sequences. Overall, 108 haplotypes, 47 in GOE and 61 in Tanzania were revealed, with similar calculated haplotype diversity for all *D. cornus* populations within each location (0.9 +/- 0.00025 and 0.903 +/- 0.00078, respectively). Only one haplotype was shared between the GOE and Tanzanian populations. Network analysis for the 108 COI haplotypes displayed two major clades, separated by nine mutations. Bayesian analyses of population structures revealed two clusters highly correlated with the collecting region. Analysis of molecular variance showed 73% of the molecular variance for all *Drupella* populations is a result of the differences among regions. Within regions, most of the molecular variance is based on within population differences, 89% north vs. south in Tanzania and 98%, Israel vs. Jordan in GOE. Fu's and Tajima's D values for all populations were negative, suggesting that the *Drupella* populations in GOE and Tanzania underwent population expansion or purifying selection. Based on the differences in genetic structuring within populations, the study strongly recommends application of conservation approaches that suit the description of the population in each region.

Introduction

The muricid gastropod *Drupella cornus* has been identified as one of the emergent diverse predator taxa that voraciously prey on reef building corals (Barco *et al.*, 2010), inflicting retardation and deaths on individual coral colonies (*Moyer et al.*, 1982; Turner, 1994; Vermeij and Carlson, 2000; Al-Horani *et al.*, 2011), trailed by significant damages to coral reefs worldwide (Moran, 1986; Turner, 1994; Johnson and Cumming, 1995; Cumming *et al.*, 1999; Rotjan and Lewis, 2008). While *D. cornus* juveniles are mostly found cryptically hiden among live branching coral forms (Forde, 1992), adults are known to be associated with substrates and several coral growth forms (Schoepf *et al.*, 2010). Studies also indicated that the snails are obligate corallivorous organisms, mostly preferring acroporids (Turner, 1994; Morton *et al.*, 2002; Shafir *et al.*, 2008; Schoepf *et al.*, 2010), showing strong tendency feeding specialization (Cumming and McCorry, 1998; Morton and Blackmore, 2009). Although snails habitually resides, in low numbers, in many tropical reefs as a benign partner of reef biota (McClanahan and Muthiga, 1992; Lane, 2011), it has recently been shown that this species makes precipitous increase in intermittent outbreaks, simultaneously, at various sites within its contemporary geographic distributions, overall inflicting massive damages to the reefs (Moyer *et al.* 1982, 1985; Boucher, 1986; Turner, 1992a, 1994; Riegl and Velimirov, 1994; McClanahan, 1994, 1997; Antonius and Riegl, 1998; Shafir *et al.*, 2008; Morton *et al.*, 2002; Cummings, 2009; Schoepf *et al.*, 2010 Al-Horani *et al.*, 2011), also leading to their decline (Turner, 1992b, 1994; Cumming and McCorry, 1998; Vermeij and Carlson, 2000). Few studies have attempted to establish causes and trajectories for these outbreaks and while the leading tenet links *Drupella* outbreaks with increased frequency in coral injuries (McClanahan, 1994; Armstrong, 2009) and diseases (Antonius and Riegl, 1997) it is also possible that various anthropogenic impacts, especially those associated with near shore eutrophication (Moyer *et al.*, 1982) and overfishing (Aronson, 1992), may lead to the development of snails' aggregations.

Drupella cornus has a relatively long life expectancy of up to 8 years under natural conditions (Wilson, 1992; Williams *et al.*, 2011) with a long planktonic larval life of about 60 days (Turner, 1992a). This influences genetic flow between remote populations and species

divergence (Nishida and Lucas, 1988; Ayre and Dufty 1994; Johnson, and Cumming, 1995, Ayre *et al.*, 1997; Ayre and Hughes, 2000). Likewise, *Drupella cornus* populations show high tendency towards high local patchiness with low-level subdivision between distant cohorts (Holborn *et al.*, 1994). Thus, despite the increased interest in the *Drupella cornus* global outbreaks and corallivory evolution, little is known on population genetics parameters and genetic connectivity, crucial in conservation biology (Yang *et al.*, 2013; Pengzhi *et al.*, 2013). Studies on genetic connectivity of various populations within marine environment have proved to be essential in designing appropriate management strategies (Torda *et al.*, 2013).

This study aims at providing initial insights into *D. cornus* population parameters and connectivity between and within reefs in Tanzania and in the northern Gulf of Eilat (GOE) with an eye to recent outbreaks of this species in these Indo Pacific reefs (Ayling and Ayling, 1987; Gur, 1988; Turner, 1994; McClanahan, 1994, 1997; Cumming, 1999; Kimura *et al.*, 2004; Shafir *et al.*, 2008; Mallon, 2010) and as a contribution to improved coral reefs management. Specifically, this study applies mitochondrial cytochrome c oxidase subunit I (COI) gene sequences to investigate *D. cornus* population genetic parameters in these two regions. A study by Mallon (2010) on reef corallivores of two reefs in Bawe and Chumbe, Zanzibar Island, established the presence of *D. cornus* with slight difference in abundance between the reefs. Furthermore, a survey across many reefs in Tanzania revealed that the species prefers the brownish coral species *Galaxea fascicularis* (personal observation). Likewise, in the northern GOE, previous studies established the occurrence of *D. cornus* populations along different ecological gradients (Gur, 1988; Zuschin *et al.*, 2001; Shafir et al., 2008) and their biology (Schoepf *et al.*, 2010; Al-Horani *et al.*, 2011) with no emphasis on genetic patterns and regional connectivity.

MATERIAL AND METHODS

Animal collection and DNA extraction

Drupella cornus specimen were haphazardly collected from alive coral colonies in shallow reefs (between 1 to 12 m) at the Gulf of Eilat (GOE), northern Red Sea (Israel - five sites; Jordan - three sites) and from five Tanzanian reefs (Table 1, Fig. 1a,b, c; total of 269

specimens). The animals were shipped to the laboratory in seawater-filled containers. Genomic DNA was commenced by cracking each snail shell to allow the removal (by industrial razor blades) of the animal's pedal area. Each individual foot was cut in two, each part homogenized separately in 200 µl of lysis buffer (0.25 M Trisborat pH 8.2, 0.1 EDTA, 2% SDS, 0.1M NaCl mixed with 40 µl of sodium per-chlorite ($NaClO_4$), following Graham (1978). Equal volumes of phenol/chloroform/isoamyl alcohol (25:24:1) were added, mixed by vortex and centrifuged for 10 min at 14,000 g, 4°C. The aqueous phase was further extracted with chloroform/isoamyl alcohol (24:1). The DNA was precipitated with absolute ethanol, washed with 70% ethanol, dried and re-suspended in water.

COI amplification

The COI gene fragments of *Drupella cornus* samples were amplified according to Folmer *et al.* (1994) using the COI marine invertebrates' universal primers (HCO2198r, 5'TAAACTTCAGGGTGACCAAAAAATCA3' and LCO1490f, 5'GGTCAACAAATCATAAAGATATTGG 3'). Two µl of diluted DNA (1:50) from each sample were added to a reaction mixture containing 5 µM of each primers and DreamTaq™ DNA polymerase (Green PCR Master Mix 2×; Fermentas) in a total solution volume of 50 µl. Reaction conditions were as followed: 74°C for 10 sec and 95°C for 5 min followed by 35 cycles of 95°C for 1 min, 45°C or 1 min and 72°C for 1 min and an additional elongation step of 72°C for 10 min. The PCR products were screened on 1.3% agarose gel and then, were sent for direct sequencing (Macrogen Inc, South Korea) using the same universal primers from both sides.

Sequence Analysis

The *Drupella cornus* origin of each sequence was verified through BLAST searches against Genbank (http://blast.ncbi.nlm.nih.gov/Blast.cgi). All sequences which had high homology to *D. cornus* (more the 96% of identity), were aligned and corrected using BioEdit software (Hall, 1999). Sequences, whose forward and reverse sequencing match less than 1% were deemed good quality sequences and used for further analysis. Haplotype diversity analyses were performed using GeneAlex 6.5 (Peakall & Smouse, 2006) and DNAsp 5.10 (Librado

and Rozas, 2009). Tajima's D and Fu's Fs neutrality tests were calculated per population with DNAsp 5.10. Neighbour joining and Maximum likelihood trees were constructed with ClustalX (Thompson *et al.*, 1997) and Tapoli V2.5 software. Trees were drawn by MEGA5 (Tamura *et al.*, 2007). Bayesian clustering for population structure was studied using BAPS 6.0 (Corander *et al.*, 2008). Network analysis was performed by NETWORK 4.6.1.1 (Clement *et al.*, 2000). Nei genetic distance for all *Drupella* populations was calculated with GeneAlex 6.5. Neighbor joining phylogenetic tree for all population was drawn by online T-REX Program at the web site: http://www.trex.uqam.ca/index.php?action=trex&menuD=1&method=2. *D. cornus* sequences FR853820, FR853819, FR853818, FR853817, FR853843, FR853842, FR853841, FR853830, FR853826 and FR853825 (from Guam, Hawaii, Japan, New Caledonia, Philippines and Vanuatu; Indo Pacific; Claremont *et al.*, 2011) were used for comparison in the COI phylogram.

RESULTS

Genetic diversity

Drupella cornus mitochondrial gene cytochrome oxidase I (COI) sequences were successfully obtained from 269 specimens collected in the GOE and Tanzanian sites (Fig.1 b, c). GOE samples from the Dekel beach, Kisosky beach and at the coral nursery (about 1km radius) were pooled and termed as Eilat north (Is_EN) and those from Taba and at the Inter-University Institute (IUI; about 1 km away) were pooled as Eilat south (Is_ES, ca. 7 km south to Is_EN). These two Israeli locations were compared with the three Jordanian sites (1.5-6 km apart from each other, 12.5 to 6 km away from Is_ES and Is_EN; Fig. 1c, Table 1) and with the five Tanzania locations (about 3,500 km south to GOE, 52 to 560 km between locations in Tanzania; Fig.1b, Table 1).

The final alignments of 606 bp for *D. cornus* COI gene revealed total of 108 haplotypes (Table 2). In GOE, of the total 47 haplotypes recorded here, 32 were assigned to the Jordanian populations and 20 in Israel, with 5 shared haplotypes (Fig. 1c). In Tanzania, 61 haplotypes were found (Fig. 1b). Only one haplotype (haplotype no 3) was shared between the Jordanian

populations in GOE (site Jr _AL, one specimen; Table 1 and Tanzania). The most common haplotype in GOE was haplotype No.1 (27/89 samples; 30.3%, found in all 5 major locations. The second common haplotype in GOE (haplotype No. 8) included six samples from all locations in Aqaba and Taba beach in Eilat, whereas all other haplotypes included only 1-3 samples, each. In Tanzania, four major haplotypes were assigned: haplotype No. 3 (see above); haplotype no. 48 with 21 samples (11.7% of all samples) collected form DSM, MF, TA and ZNZ; haplotype no. 51 with 20 samples (11.1%) from DSM, MF, MT and TA; and haplotype no. 60 with 10 samples (5.6%) collected from MF and TA. All other haplotypes included 1-5 samples, each (Fig. 1b).

Haplotype diversity calculated for all *D. cornus* samples from Tanzania and GOE altogether was high (0.943+/-0.00007, Table 2), with similar values obtained for haplotype diversity in each region (0.9 +/- 0.00025 and 0.903 +/- 0.00078; Tanzania and GOE, respectively). In GOE, there was no difference in haplotype diversity between the Israeli and the Jordanian populations (Table 2). Low values of haplotype diversity were found in two Tanzanian sites (DSM and ZNZ; Table 2).

Phylogenetic and cluster analyses

Maximum likelihood phylogenetic tree, including *Drupella cornus* sequences from the NCBI, resulted in three main branches (HKY+G+I as best-fit model). Two branches were associated with the two collecting regions (northern Gulf of Eilat and Tanzania) whereas the third branch contained the *D. cornus* sequences from the gene bank (Fig. 2). Only five specimens from GOE region (from sites AS, AL, AL, AL and Kis) were included in the Tanzanian regional branch and neither one of the east African samples was included in the Gulf of Eilat's branch. In order to confirm that COI samples that have been originated in this work are indeed *Drupella cornus* snails, two shells from each region were sent to an expert (Hendrik Klaas Mienis, Tel Aviv University, Israel) for traditional taxonomy authentication and all were identified as *Drupella cornus*. The sequence divergence between GOE and Tanzania was about 2% (less than 1% within each group) and about 5-6% sequence divergence when compared to the Genebank sequences. Based on these results, the *Drupella cornus* sequences from the Gene Bank were excluded from further analyses and comparisons.

Network analysis of all 108 COI haplotypes (Fig. 3a) revealed two major haplotype groups as per their collecting regions. Nine mutations separate between the two groups while only 1 to 3 mutations differ between haplotypes within each group (except for haplotypes 49 and 106 in Tanzania which were separated from the main haplotypes groups with 6 and 7 mutations, respectively). Four haplotypes (five samples) from GOE were included in the Tanzanian-region haplotype group.

Clustering all COI samples from the Gulf of Eilat and Tanzania using BAPS (Bayesian analysis of population structure; Fig. 3b) revealed two clusters highly correlating with the collecting region (Log (marginal likelihood) of optimal partition: -2078.3632, with probabilities for the number of clusters, n= 1). The GeneBank sequences were assigned to a separated third cluster (as in Fig. 2). Only five Tanzanian samples (same as in in the Maximum likelihood tree), presenting four haplotypes, were clustered together with the GOE samples (Fig. 2).

Analysis of molecular variance

Analysis of molecular variance (GenAlex 6.5, 999 permutations) for each location/region revealed that most of the molecular variance between *Drupella* populations recorded in this study is based on within population differences (Fig. 4), 89% north vs. south in Tanzania and 98% Israel vs. Jordan in the GOE . Only 3% of variance between the north and south of the Gulf of Eilat was due to the differences between the regions (97% within populations). The molecular variance between north and south Tanzanian regions was only 2% and among populations was 9%. High molecular variance revealed between Tanzania and the Gulf of Eilat (73%) whereas 25% attributed within populations and only 2% attributed among populations (Fig. 4).

Neutrality Tests and estimates of population expansion

Fu's Fu and Tajima's D values for most populations were significantly negative (Table 2). For pooled samples of all locations and for each region, Fu's Fu and Tajima's D values were all negative and significant (-111.697 and -1.9672 respectively for all, -50.327 and -2.2973 for the Gulf of Eilat and -79.762 and -2.408 for Tanzania). These results suggest that the

Drupella populations in GOE and Tanzania may experience population expansion or purifying (negative) selection.

DISCUSSION

Drupella cornus is one of the predators that have recently been presenting additional serious threats to the already existing stressed coral reefs. The earlier predator-prey (*D. cornus*-corals) ecological equilibrium has been deteriorated by the snails' population explosions, further augmented by the documented snails' outbreaks in different reefs worldwide (Moyer *et al.*, 1982, 1985; Boucher, 1986; Turner, 1992a, 1994; Riegl and Velimirov, 1994; McClanahan, 1994, 1997; Antonius & Riegl, 1998; Shafir *et al.*, 2008; Morton *et al.*, 2002; Cummings, 2009; Schoepf *et al.*, 2010 Al-Horani *et al.*, 2011;) also impacted by the voracious predation of *D. cornus* on corals (Morton *et al.*, 2002). *D. cornus* has been spotted intermittently in East Africa (McClanahan, 1994; McClanahan, 1997; Mallon 2010) and in the Gulf of Eilat (Gur, 1988: Riegl and Velimirov, 1994; Zuschin *et al.*, 2001; Shafir *et al.*, 2008; Schoepf *et al.*, 2010; Al-Horani *et al.*, 2011), causing further reef degradation. Despite this recorded threat of *D. cornus* to coral reefs and the observed population outbreaks, there have been no studies on its population genetics

Data collected from five sites in Tanzania and five sites in GOE revealed populations characterized by highly diverse haplotypes. While the high number of haplotypes in Tanzania can be explained by the high level of gene flow, by the wide geographical range of this study leading to geographical isolation (hundreds of kilometres between any two sampling sites) and by the high diversity of studied habitats, extending from varying fringing and barrier reefs across 1400 km of shoreline, it is not the case in GOE region, where the study was performed along 11 km of shoreline. Furthermore, of the 47 haplotypes found in GOE, only five were common to Jordan and Israel (only one haplotype was shared between GOE and Tanzania), revealing in the GOE, highly local COI diversity and possible low connectivity along short distances, in spite of the about two months long planktonic larval stage (Turner, 1992a). On the other hand, a study in the Great Barrier Reef, Australia (Holborn *et al.*, 1994), indicated mixed genetic structure in which there was low allelic diversity with increase geographical distance, an indication of a high degree of planktonic dispersal while at local level there was

high degree of heterogeneity. In nature, the faunistic connectivity within and between geographical regions is mostly associated with the mechanisms of larval dispersal (Carpenter *et al.*, 2011) that is influenced by the prevailing local factors, such as currents and tidal movements that can have significant influence on population genetic structure of marine organisms (White *et al.*, 2009). The ocean currents play significant role in structuring marine populations. The Agulhas currents, for example, moving over large area of East African (Bryden *et al.*, 2003), are potentially influencing the observed *D. cornus* population structuring. Furthermore, the high haplotype and nucleotide diversity in both sites is also an indication of fast adaptation and specialisation for local conditions in the different studied habitats. In addition, factors such as human interaction with the reefs, habitation and migration (Xue *et al.*, 2006) that are beyond the scope of this study, could further impact patterns in the observed population diversities.

Analyses revealed that populations in each region (Tanzania and GOE) clustered together, separately between regions. A further comparison between regions revealed distant phylogenetical connections, separated by nine mutations, suggesting low gene flow between the two regions (Yang *et al.*, 2013). According to Kimura (1953) and Kimura and Weiss (1964); the dispersal probability declines with distance, thus minimizing chances of genes mixing, creation of dissimilarities between populations.

The high genetic distance observed between Tanzania and GOE as one branch separated from the genebank is a cause for discussion. The divergence between the sequences from the Gulf of Eilat and Tanzania (2%) is within the range of variation for the same species classification (Hebert *et al.*, 2003a, b) whereas the divergence between those sequences and the *Drupella* sequences from the NCBI exceed this value (5-6%), a suggestion that the NCBI sequence may represent a close but different species.

At local level, there was significant genetic structuring of *D. cornus*. For example, in Tanzania, Tanga is closely related to Mafia as Zanzibar is to Dar es Salaam but Mtwara, at the southern end of the country, remained distinct. Similarly, in GOE, the IUI site was closer to Aqaba south than Aqaba south was to the other two Aqaba sites. Likewise, analysis of molecular variance revealed that significant portion of the molecular variance of all the

Drupella population is a result of the differences among regions. Smaller portion of the molecular variance is a result of the differences within the populations.

The haplotype network analysis revealed a star-like phylogeny, implying recent population expansion succeeding a population bottleneck. The Tajima's D test, Fu's Fs analysis indicated significant negative values thus suggesting that populations may have experienced evolutionary population expansion or purifying selection. Based on this development, we conclude that the *D. cornus* populations in both regions are highly structured genetically, a factor that may lead to adaptation and expansion. Similarly, the differences in genetic structuring within population, suggest application of conservation approaches that address each region and population separately.

The findings from this study using mtDNA COI in *D. cornus* identified high genetic diversity among populations and significant genetic differentiation between regions. The high genetic diversity and differentiation indicates that subdivisions exist among the *D. cornus* populations in GOE and Tanzania thus calling for application of different management efforts for effective conservation. Furthermore, we propose the application of multiple genetic marker system (Gruenthal *et al.*, 2007), thus presenting a clearer picture for *D. cornus* population structures.

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Figure legends

Figure 1: Maps of the collecting sites (a) and distribution of *D. cornus* COI haplotypes (b) Tanzania and (c) Northern Gulf of Eilat (Table 1 for reference and site abbreviations). Each color in the pies represents a different haplotype.

Figure 2: Maximum likelihood tree of all available COI *Drupella cornus* sequences from northern Gulf of Eilat (green, n=89), Tanzania (red, n-180) and *Drupella* COI sequences from the GeneBank (black, n= 12).

Figure 3: (a) Network analysis of all 108 COI haplotypes. Green colors are for GOE samples and red for Tanzanian samples. Circle sizes reflect the number of haplotypes. Black circles represent sites for missing haplotypes (median vectors). Numbers in red represent the location of a mutation along the sequence. (b) STRUCTURE analysis of the 269 *D. cornus* individuals sampled from Tanzania (red) and the Gulf of Eilat (green).

Figure 4: Results of the analysis of molecular variance (AMOVA) for the *D. cornus* populations from the Gulf of Eilat and Tanzania.

Fig. 1

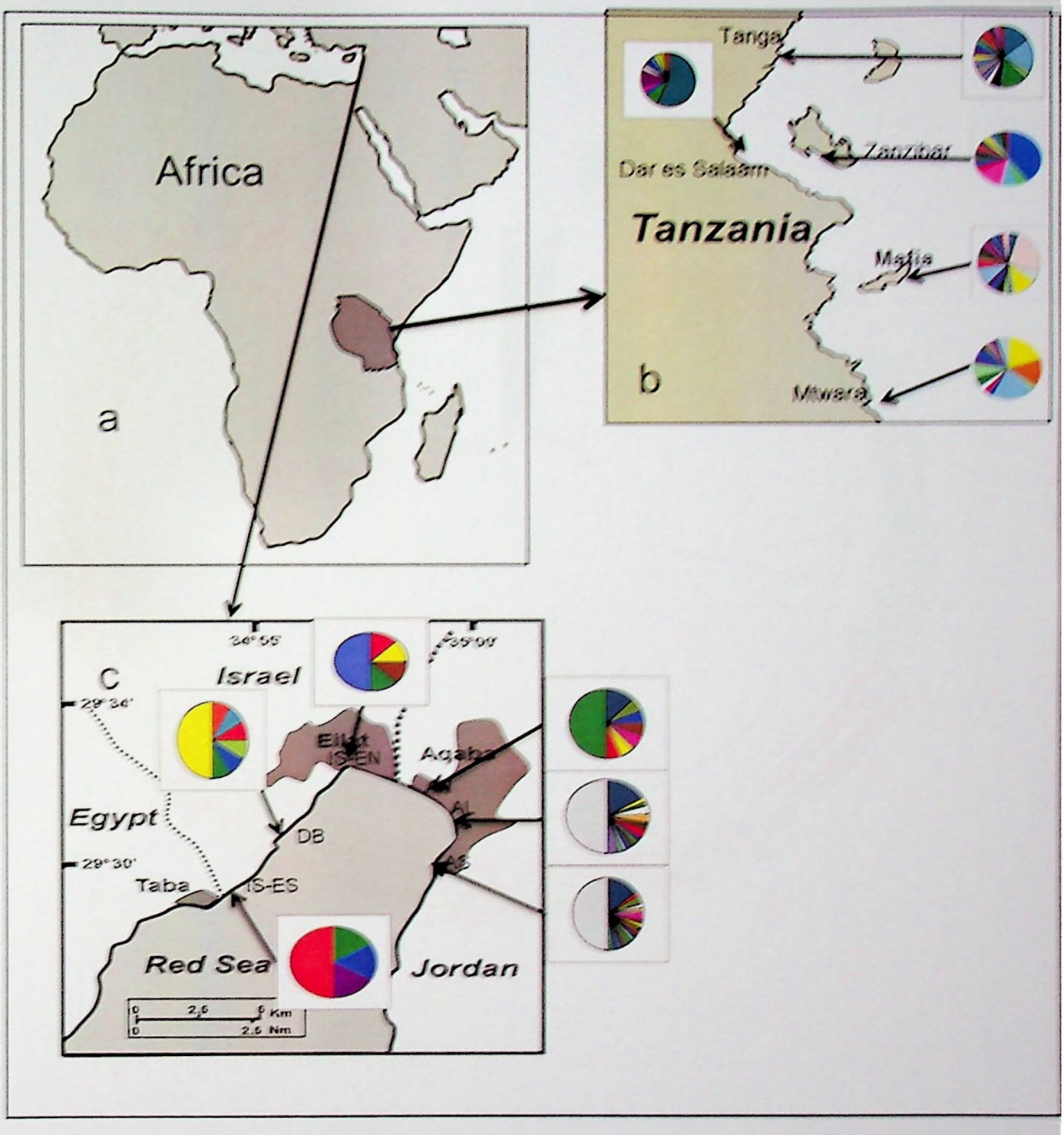

Africa
a
Tanga
Zanzibar
Dar es Salaam
Tanzania
Mafia
Mtwara
b
C
Israel
Egypt
Taba
Red Sea
Jordan
DB
IS-ES
0 2.5 5 Km
0 2.5 Nm

Fig. 2

Fig. 3

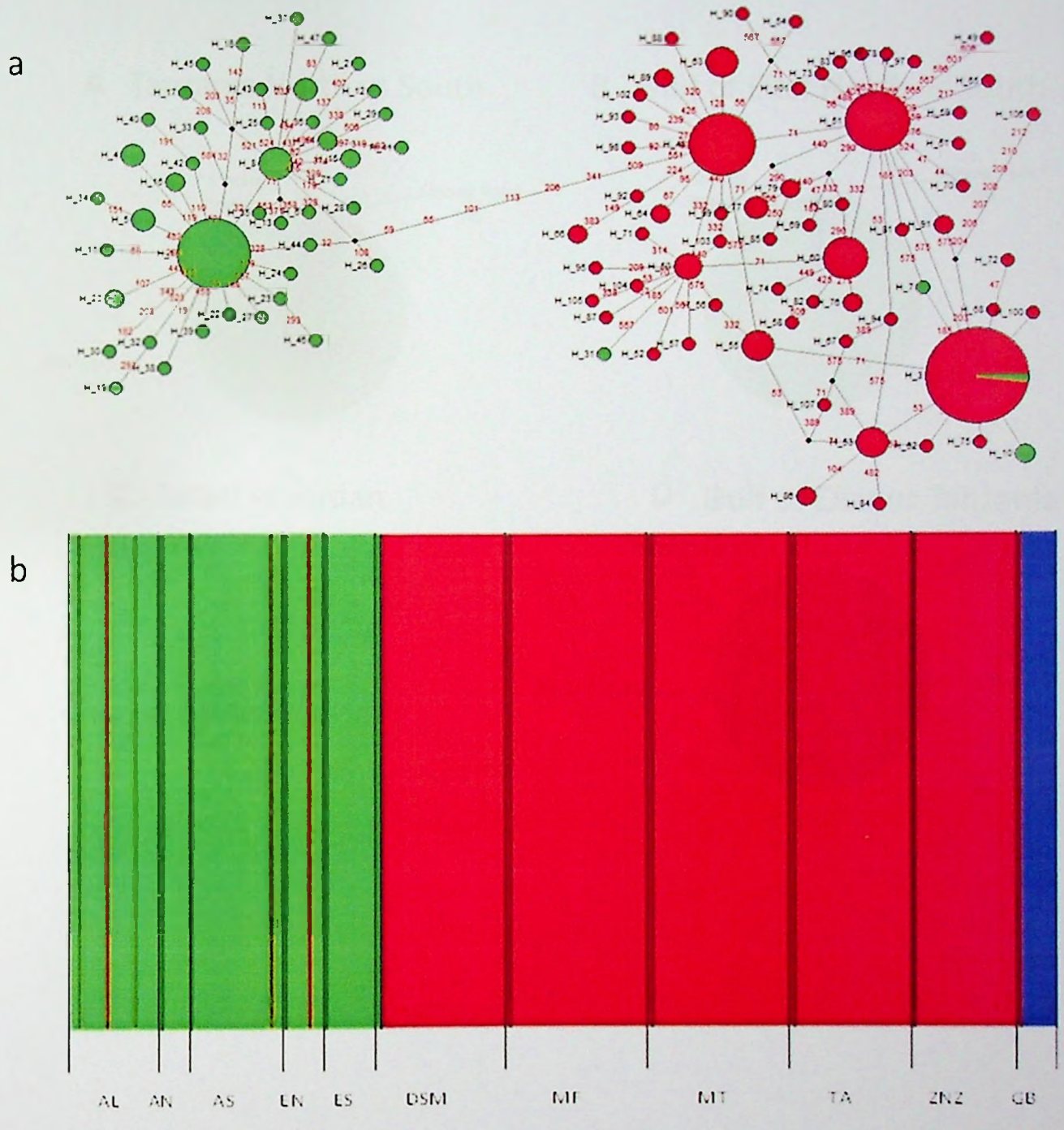
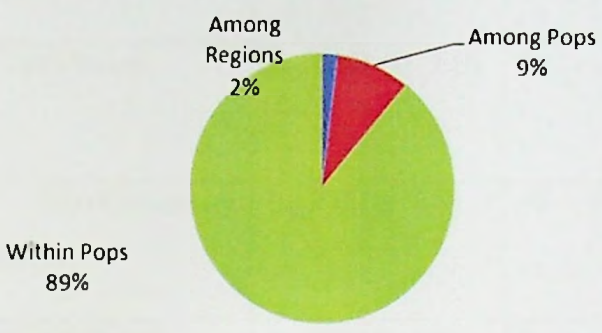


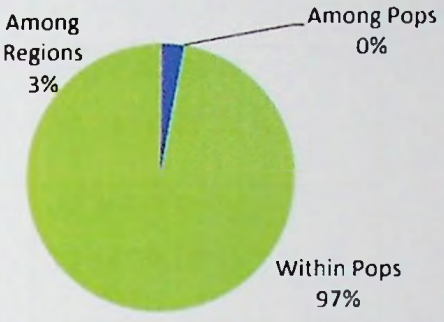
Fig. 4

Percentages of Molecular Variance

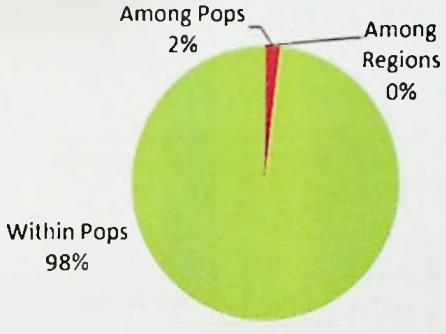
A Tanzania North vs South



B Gulf of Eilat North vs South



C Israel vs Jordan



D Gulf of Eilat vs Tanzania

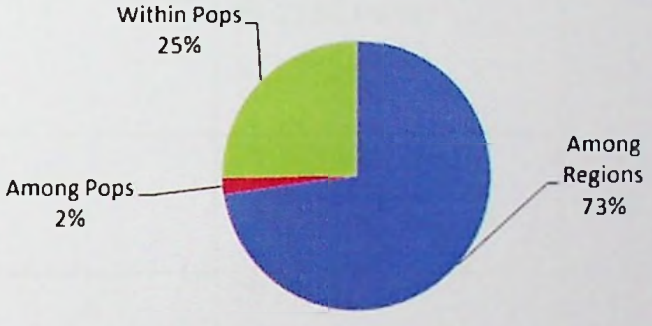


Table 1. ***Drupella cornus:*** **Sites, GPS coordinates and the number of specimen collected for COI analyses in the studied areas (Is= Israel; Jr= Jordan; Tz = Tanzania; ES = Eilat south; EN= Eilat north). See maps on Fig. 1 for sites orientations.**

Location		No of Specimens *	GPS coordinates
Name	**Abbrv.**		
Is. Taba beach	Is_TAB (Is_ES)	3	29°29'32.08"N 34°54'14.77"E
Is. InterUniversity Institute	Is_IUI (Is_ES)	12	29°30'03.11"N 34°54'59.10"E
Is. Dekel beach	Is_DB (Is_EN)	6	29°32'22.60"N 34°56'50.52"E
Is. Kisoski beach	Is_KIS (Is_EN)	2	29°32'50.00"N 34°57'13.56"E
Is. Coral nursery	Is_NUR (Is_EN)	4	29°32'32.76"N 34°58'21.78"E
Jr. Aqaba Marine lab	Jr _AL	27	29°27'58.0"N 34°58'31.85"E
Jr. Aqaba North	Jr _AN	8	29°28'50.5"N 34°58'51.7"E
Jr. Aqaba South	Jr _AS	27	29°25'54.5"N 34°58'37.5"E
Tz. Zanzibar	Tz_ZNZ	30	6°16'39.67"S

			39^{0}10'32.20"E,
Tz. Mafia	Tz_MF	40	8^{0}1'1.87"S 39^{0}45'6.16"E,
Tz. Mtwara	Tz_MT	40	10^{0}13'38.06"S 40^{0}9'45.35"E,
Tz. Dar es Salaam	Tz_DSM	37	6^{0}39'33.76"S 39^{0}14'52.45"E,
Tz. Tanga	Tz_TA	33	5^{0}9'13.74"S 39^{0}6'53.84"E,

* *D. cornus* sequences in good quality

Table 2: Population genetic parameters of *D. cornus* from Tanzania and the Northern Red Sea, based on COI (N, sample size; S, The number of segregating sites; Hap,The number of haplotypes; Hd, Haplotype diversity and its sampling variance (VarHD, Nei, 1987); pi, nucleotide diversity; TajimaD, Tajima's D (Tajima, 1989), and statistical significance; FuFs, Fu's Fs (Fu, 1997)and statistical significance.

Population	N	S	Hap	Hd	VarHd	Pi	TajimaD	FuFs
D. cornus (All)	269	109	108	0.943	0.00007	0.01109	-1.9672*	-111.7**
Jordan	62	48	32	0.9	0.0011	0.00601	-2.1829**	-24.781**
Israel	27	34	20	0.92	0.00228	0.00562	-2.2808**	-15.584**
Eilat_gulf	89	60	47	0.903	0.00078	0.00586	-2.2973**	-50.327**
Tanzania	180	65	61	0.9	0.00025	0.00416	-2.408**	-79.762**
JR_AL	27	27	15	0.886	0.00287	0.0072	-1.3777	-4.815*
JR_AN	8	8	7	0.964	0.00596	0.00407	-0.9735	-3.848**

JR_AS	26	31	17	0.908	0.00251	0.00513	-2.3464**	-10.929**
IS_EN	12	23	11	0.985	0.00162	0.00785	-1.6639#	-5.755**
IS_ES	15	14	10	0.857	0.00813	0.00374	-1.8772*	-5.492**
TZ_DSM	37	13	11	0.67	0.00702	0.00284	-1.6823#	-4.377*
TZ_MF	40	26	19	0.915	0.00088	0.00455	-1.9303*	-11.269**
TZ_MT	40	16	17	0.903	0.0007	0.00364	-1.3319	-10.419**
TZ_MA	33	20	18	0.93	0.00065	0.00377	-1.836*	-13.467**
TZ_ZNZ	30	20	14	0.83	0.004	0.00428	-1.693#	-6.425**
D_cornus_GB	10	9	5	0.756	0.01678	0.00392	-1.106	-0.209

**P<0.01, *p<0.05, # p < 0.1

4.0 Summary and Discussion

4.1. Farming of coral fragments in nurseries

Several denuded reefs were pre surveyed in Tanzania for possibility of testing the farming of coral species for growth rates, survivorship, attachment to the substratum and re-engineering of denuded reef areas retrospectively. The final decision resulted in picking up Mafia Island Marine Park in Tanzania mainland as well as Chumbe and Changuu Islands in Zanzibar as they have representation of the reefs in terms of denudation and coral diversity to the rest of reefs in Tanzania (Mohammed *et al.*, 2000). Similarly, the sites were selected on the basis of convenience and security as others were either far away from equipment supply sources or in very remote areas where follow-up would have been difficult. In each site, I established two coral nurseries for farming six common coral species namely; *Acropora muricata*, *A. nasuta*, *A. hemprichi*, *Pocillopora verrucosa*, *Porites cylindrica*, *Millepora* sp and followed their performance in pre-designed transplantation plots for one year (Mbije *et al.*, 2010; 2013). During the establishment of the nurseries special consideration on environment factors and adaptability of corals to local conditions were made (Yap et al., 1992; Edwards and Clark, 1998; South *et al.*, 2001; Fox *et al.*, 2005; Lindahl, 2003; Edward and Gomez, 2007; Shafir *et al.*, 2006a,b; Shaish *et al.*, 2008, 2010).

During the coral nursery stage, I observed a good development of coral fragments in both sites (Mbije *et al.*, 2010; 2013). As in other studies (Harriott and Fisk, 1988; Clark and Edwards, 1998; van Treeck and Schuhmacher, 1997; Yap *et al.*, 1998; Bowden-Kerby *et al.*, 2001; Shaish *et al.*, 2008a, 2010) all species studies in both nurseries revealed high survivorship rates, the branching forms, as expected, showed faster growth rate as compared to submassive forms. The growth rates of nursery farmed colonies was high in both sites studied so these colonies were ready for transplantation just nine months after nursery set-up, a reflection of the findings by Shafir *et al.*, (2006a, b) in The Gulf of Eilat. In Shafir *et al.* (2006a, b) the observed fast growth rates of fragments in the nurseries was attributed to nutrients from adjacent fish farm cages. Conversely, the two Tanzanian nurseries in Zanzibar and Mafia Islands were located in areas of high tidal currents, which might have been

responsible for promoting fast growth rates through aeration and possible supply of nutrients from distant places.

The farming of coral fragments in mid-water nurseries provided a prospect for reef restoration in East African reefs, as previous studies (Franklin *et al.*, 1998; Lindahl, 1998, 2003; Wagner *et al.*, 2001) failed because of the involved high labour costs and application of destructive practices (Rinkevich, 2005a). This also conform to other finding where similar efforts have signalled the possibility for large scale production of healthy coral fragments for restoring large denuded areas (Harriott and Fisk, 1988; Clark and Edwards,1998; Bowden-Kerby *et al.*, 2001; Shafir *et al.*, 2006a, b; Shaish *et al.*, 2008a, 2010). Similar studies that failed to take off relied only on promotion of natural recruitment through submerging various artificial substrates (Bowden-Kerby, 1997, 2001; van Treeck and Schuhmacher, 1997; Nzali *et al.*, 1998). The major weakness of using artificial substrates is associated with introduction of alien structures into the sea which may be deemed as pollutants and may change seascapes. The current protocol, besides production of large number of healthy coral fragments for transplantation, it completely elimates possibilities for introduction of any alien structures in marine environments.

While this mid-water coral farming provides light on the future of coral reef restoration, several questions still remains on its applicability for large denuded reefs. Since the farming of this nature is still at its infancy level (Shaish *et al.*, 2008), especially to Tanzanian community, extensive research is required to warrant its applicability especially in some areas where the management reefs is difficulty due to their remoteness. Furthermore, the fact Tanzania reefs are among the most diverse in terms of species (Mbije *et al.*, 2002) a longer time is required to study issues related to fragments' sizes, combination of species and where to place the nurseries as well as designating transplantation areas.

4.2 Coral transplantation

I selected areas that were denuded during the past coral bleaching events, i.e., 1997/98. In Zanzibar, a large portion of Changuu reef on the northern part and also some part of Kitutia reef in Mafia, were among the significantly affected areas in Tanzania (Mohammed *et al.*, 2000). I recorded a significant change in coral communities' abundance and diversity after the transplantation of all nursery reared coral fragments in the selected two sites. In this experiment, the branching forms, i.e., *Acropora*, *Millepora* and *Pocillopora* species grew very fast as compared to the submissive forms of *Porites,* similar to other other studies (Yap et al., 1992; Lindahl, 2001; Wagner *et al.*, 2001; Shaish *et al.*, 2010). During this time, I observed a significant coral recruitment within the designated transplantation plots. The recruits number and abundance differed between plots but the *Acroporids* were the most significantly abundant. Although coral larvae may have come from the surviving coral species around my transplantation plots (Horoszowski-Friedman *et al.*, 2011), the dominance of recruits from the three broadcasting *Acropora* species may haveacted as a cue for metamorphosing larvae to settle (e.g., reef sounds; Vermeij *et al.*, 2010). Furthermore, there was a significant recruitment of fish and invertebrates within the transplanted plots, an evidence for the argument that active coral restoration initiatives may bring back denuded coral reef areas to their original state (Abelson and Shlesinger, 2002; Rinkevich, 2005, 2006, 2008; Cabaitan *et al.*, 2008; Ferse, 2009). The presence of large numbers of new spat belonging to both the transplanted species and others not transplanted within the radius of experimental plots and also increase in fish and invertebrates (Mbije *et al.*, 2013) strongly support the selection of the transplanted species as potential keystone species (Byers *et al.*, 2006). Concurrently, the central goal of coral transplantation is not only to establish viable and persistent coral community but also to enhance other benthic organisms and fish assemblages (Cabaitan *et al.*, 2008; Jaap, 2000). Likewise, restoration measures that take into account multi-species transplantation result into increased habitat complexity for reef dwelling organisms at the same time helping in conserving biodiversity.

Despite these successes, I had to overcome some unexpected ecological challenges; most important included an infestation by *Acanthaster planci* immediately after transplantation that

was followed by a major coral bleaching event (Mbije *et al.*, 2013). This is similar to a study by Shaish et al. (2010) and, whereas there was no immediately solution to bleaching event, with assistance from tourists and personnel from nearby dive centres, we collected all the COTs and buried them on the sand in the nearby beach. This infestation, observed at Changuus' reef, immediately after colony transplantation may have been related to the response of resident organisms to newly arrived transplants. Similarly, results from transplantation experiments in the Red Sea (Horoszowski, personal communication, 2010) and South East Asia (Shaish *et al.*, 2010) showed that new transplants are prone to attacks by resident fish and corallivorous invertebrates/fish that may eventually kill them. The impact by the starfish infestation was wide spread to all coral species but the most affected species were *Pocillopora verrucosa*, *Porites cylindrica*, *Acropora muricata* and *Acropora nasuta*. Damage to coral colonies by bleaching of transplants was the second major stressor, further documented in the studied Indo Pacific reef sites (Shaish *et al.*, 2008, 2010; Mbije *et al.*, 2010). The most prominent bleaching episode during the first year after transplantation was part of the wide-spread bleaching event developing in the Western Indian Ocean. As was the case during this period, bleaching of transplants showed species specific patterns (Lindahl et al., 2003; Yap, 2004; Shaish *et al.*, 2010). Generally, no significant mortality of transplants was associated with this phenomenon.

The occurrence of stressors such invasion by the crown-of-thorn starfish and coral bleaching during transplantation as observed above is a serious challenge that require attention before designating any large scale coral transplantation project (Shaish *et al*, 2010a, Morrison and Lindell 2011; Muko and Iwasa, 2011). Although it may not be possible to predict the variation of environment parameters during transplantation, care must be taken to avoid starting farming of colonies in plots during summer time (Yap 1992; Yap *et al.*, 1998).

4.3 Costs involved in coral reef restoration

While some studies indicated that coral reef restoration through transplantation can be costly costs (Edwards and Gomez, 2007; Edwards and Wells, 2010), the results from this study indicate that large scale coral transplantation can also be done in developing countries at low costs with the available resources, less than one tenth of the costs as evaluated earlier. With

the cost for establishing the nursery standing at 1080 US$ per 10,000 fragments, i.e., 0.1 US$ (equivalent to 160 Tanzania Shillings) per fragment and that of transplantation at US$ 0.19 (equivalent to 340 Tanzanian Shillings per colony, the results indicated that large quantities of coral colonies can be generated and transplanted in damaged reefs at relatively low cost. Cumulatively, field results and economic evaluations showed that transplantation of nursery-grown colonies might uphold critical ecosystem functions, while successfully used in reversing coral reefs' phase shifts states.

With the advent of similar studies across the world that attempts to address application of low cost approaches in reef restoration (Forsman *et al.*, 2006; Shafir *et al.*, 2003, 2006a, b; Shafir and Rinkevich, 2005, 2008; Amar and Rinkevich, 2007; Raymundo et al., 2007; Garrison and Ward, 2008; Shafir and Rinkevich, 2008; Shaish *et al.*, 2008, 2010a, 2010b; Levi *et al.*, 2010; Mbije *et al.*, 2010; 2013), further experimentations of the same may lead to development of one that globally accepted. The ultimate purpose is to have lowest cost possible yet having maximal effects on key reef recovery parameters.

4.4 Community involvement in coral reef restoration

While this study provides the first insight into reef restoration through application of the two-step restoration protocol in East Africa, large-scale restoration projects that are urgently needed in Tanzania, may require direct involvement and participation of local communities (Wagner, 2004; Rinkevich, 2008; Mbije *et al.*, 2010). This means, in order to achieve a highly successful production of many fragments for restoration in a short time, the active involvement of local communities is very important (Mbije *et al.*, 2010). The extent of denuded reefs along the coastline is so huge that without community involvement the whole exercise may not be meaningful. On the other hand, unlike the past restoration efforts in Tanzania (Franklin *et al.*, 1998; Lindahl, 1998, 2003; Wagner *et al.*, 2001) the current one experienced direct community interaction through tempering of the nurseries thus prompting hiring of guards for a few months. Accordingly, the community involvement, besides providing abundant manpower for making potentially large nurseries for production of large quantities of fragments for restoration, it also creates sense of ownership thus minimizes unwarranted vandalism of the nurseries (Mbije *et al.*, 2010). In other studies, the local

community participation has demonstrated to be effective in conservation and restoration initiatives around the world (Ferrer *et al.*, 1996; Meñez *et al.*, 1998; Meñez *et al.*, 2012). Thus during making decisions to make restoration, special consideration should be given to associated social, economic and cultural themes (Christensen *et al.*, 1996). For example, the decision as to which and how much habitat should be restored may require discussions that involve reef stakeholders and, most importantly, local communities. Successful restorations are those that consider reef restoration methodologies appropriately adapted to local socio-economic limitations.

In addition to these restoration initiatives, efforts to stop direct human related coral damage through dynamite and other means are underway across Tanzania. Foremost and very effective approach is through education (Mbije and Rinkevich, 2013).Various marine conservation organisation in the country have team up in a move that have seen massive conservation campaigns along the whole of coastline (TCMP, 2009). While this is underway, in a parallel move, there have been several endeavors to address the issues of coral reef damage through involving communities. Some of these have dwelt into understanding the cultural and economic history of communities that lead to coral reef environment degradation (Mbije and Rinkevich, 2013) as in other places (Sutinen and Kuperan, 1999; Sumaila *et al.*, 2006). Based on this, besides the already known natural causes, arthopogenic actions such as illegal fishing and application of dynamites have been cited as the major reason for the degradation in Tanzania (Mbije and Rinkevich, 2013). Apparently, these practices are mainly conducted by a few, greedy members of the fishing society whom control has proved difficulty due to absence of appropriate management mechanism for the reefs. As a way of combating this, a large percentage of community (78.9%) in Kilwa propose establishment of core areas that would be closed to allow fish to breed and grow (Mbije and Rinkevich, 2013). This emanates from the fact that closure of areas that have previously been badly damaged by bad fishing practices in some reefs of Tanzania resulted in gradual recovery of reef as well as increase of fish species number and sizes (TCMP 2009). Similarly, in the neighbouring country, Kenya, the establishment of conservation areas has proved to be effective in addressing marine resource decline issues (McClanahan and Shafir, 1990; McClanahan *et al.*,

1997; McClanahan 1998). Concomitantly, well managed reef is vital to improvement of marine organisms' diversity which in turn has vast multiplier effects; among them increased fish catches and raising tourism. This may in a long term lead to poverty alleaviation among Tanzanian living along the coastline.

4.5 Population genetic structure of *Drupella cornus*

Drupella cornus is one of the predators that have recently been presenting serious threats to the already existing stressed coral reefs. The maintained ecological predator-prey (*D. cornus*-corals) equilibrium has been deteriorated by the snails' population explosions, following by the documented outbreaks in different reefs worldwide (Moyer *et al.*, 1982, 1985; Boucher, 1986; Turner, 1992a, 1994; Riegl and Velimirov, 1994; McClanahan, 1994, 1997; Antonius and Riegl, 1998; Shafir *et al.*, 2008; Morton *et al.*, 2002; Cummings, 2009; Schoepf *et al.*, 2010 Al-Horani *et al.*, 2011) and by the voracious predation of *D. cornus* on corals (Morton *et al.*, 2002). In addition, this species has been spotted intermittently in East Africa (McClanahan, 1994; McClanahan, 1997; Mallon 2010) and in the Gulf of Eilat (Gur, 1988: Riegl and Velimirov, 1994; Zuschin *et al.*, 2001; Shafir *et al.*, 2008; Schoepf *et al.*, 2010; Al-Horani *et al.*, 2011), causing further reef degradation. Despite this recorded threat of *D. cornus* to coral reefs and the observed population outbreaks, there have been no studies on its population genetics.

Data collected from five sites in Tanzania and five sites in GOE revealed populations characterized by highly diverse haplotypes. While the high number of haplotypes in Tanzania can be explained by the wide geographical range of this study and the high diversity of studied habitats resulting, extending from varying fringing and barrier reefs across 1400 km of shoreline, it is not the case in GOE region, where the study was performed along 11 km of shoreline. Furthermore, of the 47 haplotypes found in GOE, only five were common to Jordan and Israel (only one haplotype was shared between GOE and Tanzania), revealing in the GOE, highly local COI diversity and possible low connectivity along short distances. On the other hand, a study in the Great Barrier Reef, Australia (Holborn *et al.*, 1994), indicated mixed genetic structure in which there was low allelic diversity with increase geographical distance, an indication of a high degree of planktonic dispersal while at local level there was high degree of heterogeneity. The later has been associated with stages of D. cornus outbreaks

(Holborn *et al.*, 1994). In nature, the faunistic connectivity within and between geographical regions is mostly associated with the mechanisms of larval dispersal (Carpenter *et al.*, 2011). The high level of gene flow and geographical isolation of the studied populations, especially in Tanzania could explain the observed high genetic differentiation among populations. Similarly, the prevailing local factors such as currents or tidal movement can have significant influence on population genetic structure of marine organisms (White *et al.*, 2009). The ocean currents play significant role in structuring marine populations. The Agulhas currents, for example, moving over large area of East African (Bryden *et al.*, 2003), are potentially responsible for the observed *D. cornus* population structuring. Furthermore, the high haplotype and nucleotide diversity in both sites is an indication of low level of disturbance to the species leading to adaptation and specialisation in the different studied habitats. In addition, factors such as human interaction with the reefs, habitation and migration (Xue *et al.*, 2006) that are beyond the scope of this study, could explain patterns in the observed diversity.

Further analysis of Maximum Likelihood phylogenetic tree of all samples with Drupella sequences from the NCBI produced the results revealing that populations in each region (Tanzania and GOE) clustered together, separately between regions. A further comparison between regions revealed distant phylogenetical connections, separated by nine mutations, suggesting low gene flow between the two populations (Yang *et al.*, 2013). According to Kimura (1953) and Kimura and Weiss (1964); the dispersal probability declines with distance, thus minimizing chances of genes mixing, creation of dissimilarities between populations. The high genetic distance observed between Tanzania and GOE as one branch and the genebank genetic data is a cause for discussion. The divergence between the sequences from the Gulf of Eilat and Tanzania (2%) is within the range of variation for the same species classification (Hebert *et al.*, 2003a, b) whereas the divergence between those sequences and the *Drupella* sequences from the NCBI exceed this value (5-6%).

At local level, there was significant genetic structuring of *D. cornus*. For example, in Tanzania Tanga is closely related to Mafia as Zanzibar is to Dar es Salaam but Mtwara, at the southern end of the country, remained distinct. Similarly, in GOE, the IUI site was closer to Aqaba south than Aqaba south was to the other two Aqaba sites. Likewise, analysis of

molecular variance revealed that significant portion of the molecular variance of all the Drupella population is a result of the differences among regions. Smaller portion of the molecular variance is a result of the differences within the populations.

The haplotype network analysis revealed a star-like phylogeny, implying recent population expansion succeeding a population bottleneck. The Tajima's D test, Fu's Fs analysis indicated significant negative values thus suggesting that populations may have experienced evolutionary population expansion or purifying selection. Based on this development, we conclude that the *D. cornus* populations in both regions are highly structured genetically, a factor that may lead to adaptation and expansion. Similarly, the differences in genetic structuring within population, suggest application of conservation approaches that address each region and population separately.

The findings from this study using mtDNA COI in *D. cornus* identified high genetic diversity among populations and significant genetic differentiation between regions. The high genetic diversity and differentiation indicates that subdivisions exist among the *D. cornus* populations in GOE and Tanzania thus calling for application of different management efforts for effective conservation. Furthermore, we propose application of multiple genetic marker systems (Gruenthal *et al.*, 2007) that describe different parts of the genome, thus presenting a clear picture for effectively understand the population structure in *D. cornus*.

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שימוש במושג הגינון ככלי ישים לשיקום שונית פעיל בטנזניה

מגיש Nsajigwa עמנואל Mbije

תקציר

ההידרדרות המסיבית של המערכת האקולוגית של שוניות האלמוגים בעולם, הנגרמת על ידי הפרעות אנתרופוגניות וטבעיות, תועדה בשלושת העשורים האחרונים . כתוצאה מכך, פותחו מס' גישות לשיקום שוניות אלמוגים בעלות השקפה שונה זו מזו ובעלות מתודולוגיות שונות להבראת המערכת האקולוגית בשוניות אלמוגים ברחבי העולם. אחת מהגישות הללו מבוססת על עקרון הלקוח מתחום היערנות של גינון האלמוגים, גישה זו של **שיקום פעיל** הינה בת שני שלבים: **שלב ראשון** גידול/תירבות של שברי אלמוגים קטנים במשתלות תת ימיות (mid-water) עד הגיעתם לגודל מתאים המאפשר שתילתם בשונית **ושלב שני** הכולל שתילת מושבות האלמוגים באזורי שוניות רצויים.

שימוש בעקרון שיקום פעיל זה נחקר בטנזניה, במטרה להכין פרוטוקול שיקום מתאים נוכח מהירות הידרדרות השונית באזורי שוניות אלמוגים במזרח אפריקה.

שתי משתלות תת ימיות, הוקמו בספטמבר 2007 האחת באי Chumbe שבזנזיבר והשניה - ב Chole Bay שבאי Mafia, כל אחת הכילה 10,000 מקטעי מושבות אלמוגים משישה מינים (*Acropora formosa, A. hemprichi, A. nasuta, Millepora sp., Pocillopora verrucosa, porites cylindrica)*, כאשר כל מין אופיין בשלושה גנוטיפים שונים זה מזה.

בתום תקופה של תשעה חודשים- שלב הגדילה במשתלה, נמצאו הבדלים תוך מיניים (interspecific) משמעותיים בשרידות ובשיעורי הגדילה של מיני *Acroporid*, *Pocillopora* verrucosa ו-*sp.* *Millepora* ואשר הראו שרידות גבוהה יותר בהשוואה ל-*Porites cylindrica*. בשני האתרים, אלמוגי ה-*Millepora* לא סבלו מתמותה כלל וכן במינים אחרים התמותה היתה נמוכה ונעה בין 3% ל-24% (פר גנוטיפ אלמוגים) בזנזיבר, ובין 13% ל- 44% באי Mafia. מושבות האלמוגים במשתלה בזנזיבר הראו שיעורי גדילה טובים מאלו שבאי Mafia וכל האלמוגים שגודלו בשני האתרים היו מוכנים לשתילה בתום תקופה קצרה לאחר תשעה חודשים. בסך הכל, 14,022 אלמוגי משתלה הועברו לשונית; מהם 6,912ב- Changuu שבזנזיבר ו-7,110ב- Kitutia באי מאפיה. בכל אחד מהאתרים הוקמו באופן אקראי 12 חלקות (36מ"ר כל אחת). תוכנית השתילה היתה כלהלן: בשלושה אתרים נשתלו במשולב שלושה מיני *Acropora sp.* (טיפול 1 , T1),