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Genetic population structure and recruitment patterns of three sympatric shallow-water penaeid prawns in Ungwana Bay, Kenya, with implication for fisheries management

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Abstract. Penaeid prawns in Ungwana Bay, Kenya, are heavily exploited by artisanal fishers and industrial bottom trawlers. Human activities in mangrove and estuarine areas may affect prawn nursery habitats and influence juvenile recruitment to fished areas, therefore it was important to investigate recruitment patterns in the bay. To test the hypotheses that single genetic stocks exist, we utilised a combination of mtDNA sequence and microsatellite data. Three dominant sympatric species, *Penaeus monodon*, *Fenneropenaeus indicus* and *Metapenaeus monoceros* were targeted. Sample sites were chosen to represent the bulk of fishery activities, and included estuarine juveniles and offshore adults. An exceptionally high mtDNA haplotype diversity, coupled with low nucleotide diversity was observed for all three species and there was no genetic differentiation among sampling sites. Genetic panmixia was confirmed by the microsatellite analyses of *P. monodon*. Juveniles that recruit to adult populations in Ungwana Bay most likely originate from local estuaries, and conservation of the prawn nursery habitats along the edges of the bay is advocated. Each of the three species represents a single management unit, and the identification of spatial management strategies to mitigate resource-user conflicts should rather consider other ecological and socio-economic factors than the genetic delineation of stocks.

Additional keywords: *Fenneropenaeus indicus*, fisheries management, *Metapenaeus monoceros*, microsatellite, mtDNA, *Penaeus monodon*.

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Introduction

Ungwana Bay in Kenya is an economically-important, species-rich ecosystem in the tropical Western Indian Ocean, where many crustacean, mollusk, fish, shark and ray species are exploited by fisheries (Fulanda *et al.* 2011; Munga *et al.* 2012). An artisanal prawn fishery in this region dates back to the 9th Century, coinciding with the rise of the East African Indian Ocean trade that linked this coast to Arabia, Persia and India (Fulanda 2003). The artisanal fishery exploits the estuaries and nearshore areas of the bay using traditional and more recent fishing gears, including cast nets, beach seines and prawn seines (Munga *et al.* 2013). Ungwana Bay also supports an industrial bottom trawl fishery, active since the early 1970s (Fulanda *et al.* 2011; Munga *et al.* 2012). Bottom trawlers operate mainly beyond 3 nautical miles (nm) from the shore (formerly 5 nm; Government of Kenya 2010), and use polypropylene trawl nets with 50–55 and <40 mm diamond mesh sizes at the body and cod-end, respectively. The trawl fishery catches at least five species of penaeid prawns: *Fenneropenaeus indicus* H. Milne Edwards 1837, *Penaeus monodon* Fabricius 1798, *Metapenaeus monoceros* Fabricius 1798, *Penaeus semisulcatus* De Haan 1844 and *Marsupenaeus japonicus* Bate 1888.

Resource-user conflicts between artisanal and industrial bottom trawl fisheries in Ungwana Bay date back several decades, and are exacerbated by arbitrary partitioning of fishing grounds among sectors, weakly defined harvest strategies and declining prawn catches (Fennessy *et al.* 2004; Fulanda *et al.* 2009, 2011). Other factors that cause conflict are entangling of fishing gears by trawl nets, and the perception that trawlers catch and discard the finfish species that support artisanal fisheries. Escalations in conflict led to a commercial trawl ban in 2006 (Munga *et al.* 2012). Although the trawl fishery has since

resumed, the spatial and seasonal management strategies for Ungwana Bay are presently under review.

On average, prawn landings reported for Ungwana Bay consist of *F. indicus* (55–70% of landings), *M. monoceros* (10–15%) and *P. monodon* (<10%) (Fulanda *et al.* 2011). These three species are also regarded as some of the most economically important decapod crustaceans globally (Dall *et al.* 1990; Pérez Farfante and Kensley, 1997). *Penaeus monodon* and *F. indicus* inhabit the shallow continental shelves of the Indo-West Pacific, whereas *M. monoceros* occurs in the Indo-West Pacific and also in the Eastern Atlantic (Dall *et al.* 1990). The life cycles of these three species depend on both marine and estuarine environments. Adult females release their eggs in offshore waters, where they hatch into planktonic larvae. After several moults, post–larvae enter coastal and estuarine nursery areas, where they grow into benthic juveniles, which then migrate out of the estuaries to recruit to adult populations on offshore mudbanks (Dall *et al.* 1990). Juvenile *M. monoceros* appears to be a habitat generalist, able to live in muddy, sandy, seagrass and mangrove habitats, whereas *P. monodon* and *F. indicus* are restricted to sandy and/or muddy areas (de Freitas 1986; Macia 2004). Previous studies of prawns in Kenyan waters focused on their distribution and abundance (Osore 1992; Wakwabi and Jaccarini 1993; Mwaluma 2002; Mwaluma *et al.* 2010; Munga *et al.* 2013), stock assessments and population dynamics (Mwatha 2002), and fisheries and management (Fulanda *et al.* 2009, 2011; Munga *et al.* 2012). Distinct prawn species composition and abundance patterns occur near the outflows of the Tana (shallower, more turbid; dominated by *F. indicus*) and Sabaki (deeper, less turbid; dominated by *P. semisulcatus*) rivers, and abundance increased at both sites during the South-east monsoon (SEM) season (Munga *et al.* 2013). These patterns may suggest species-specific nursery areas for juveniles of the studied species.

Nevertheless, marine species with dispersive larvae often comprise an admixture of juveniles and adults from different sources, suggesting that recruits may originate from local nurseries, and/or from multiple sources (Roberts 1997; Mora and Sale 2002). Larval dispersal patterns can be influenced by many factors, including life history characteristics (Matthee *et al.* 2007; Pelc *et al.* 2009; Sivasundar and Palumbi 2010; Faurby and Barber 2012), ocean currents and physical or hydrographical barriers (Williams and Benzie 1998; Gopal *et al.* 2006; von der Heyden *et al.* 2011; Groeneveld *et al.* 2012) or environmental cues (e.g. sharp salinity gradients, deep waters, circular currents or eddies; Gilg and Hilbish 2003). Oceanographic features that facilitate larval dispersal may not be permanent, depending on geological or climatic changes, but where barriers persist for long enough, they may give rise to genetically structured populations (McMillen-Jackson and Bert 2003; Teske *et al.* 2007; Sivasundar and Palumbi 2010). Although the effective population sizes of prawns is putatively large, *P. monodon* in the South West Indian Ocean region demonstrated significant genetic differentiation among western Madagascar populations and those from Kenya and Tanzania (Duda and Palumbi 1999; You *et al.* 2008). Conversely, no genetic structure was found among *P. monodon* populations in South Africa, Mozambique and Madagascar (Benzie *et al.* 2002). These populations occur along a 4000 km stretch of the east African coast, with ~500 km separating Madagascar from Mozambique at the closest point.

Fisheries management in the marine environment can be enhanced by the application of molecular tools to aid with the delineation of stocks or management units, which are important for defining spatial fisheries management strategies (Coyle 1998; Schwartz *et al.* 2007; Palsbøll *et al.* 2007; Waples *et al.* 2008; Dudgeon *et al.* 2012). The objectives of this study were to determine whether finer-scale population genetic analyses of *F. indicus*, *P. monodon* and *M. monoceros* in Ungwana Bay should be considered in the development of spatial management strategies, using stock delineation, and whether recruitment patterns (nursery habitats) in the bay could be discerned. These objectives were deemed to be particularly important, because of continued resource-user conflicts between the artisanal and industrial trawl fisheries, and because human activities in mangrove and estuarine areas may affect prawn nursery habitats.

To address the management questions pertaining to penaeid prawns in Ungwana Bay, we specifically targeted the control region of the mtDNA and also used species specific microsatellites to confirm the pattern in *P. monodon*. The data were analysed to answer a key question particularly relevant to fisheries in the region, namely whether juvenile and adult prawns on offshore banks in Ungwana Bay (i.e. bottom trawl fishing grounds) are genetically similar to those in local estuaries and nursery habitats (artisanal fishing grounds). Whereas genetically divergent populations would provide a basis for spatially structured management of prawn resources in Ungwana Bay, the absence of genetic structure in the bay (no stock delineation) will bring other considerations into play when management strategies are developed. These may include socio-economic factors, the conservation of nursery habitats to ensure adequate recruitment to adult populations, and species-specific prediction of the effects of various harvest strategies on prawn populations.

Materials and methods

Study area

The Malindi-Ungwana Bay complex in Kenya (known as Ungwana Bay) combines the larger Ungwana Bay extending from Ras Shaka in the north to Ras Ngomeni in the south, and the smaller neighbouring Malindi Bay, that extends further south from Ras Ngomeni to Malindi town (Fig. 1). The bay extends along a coastal stretch of ~210 km, and the fishing grounds cover an estimated 35 300 km^2 (Iversen *et al.* 1984; Fulanda 2003; Mwatha 2005). Fringing reefs and occasional rocky outcrops limit the area for trawling, which is officially restricted to beyond 3 nautical miles (nm) offshore. The continental shelf is 15–60 km wide, and two large rivers drain into the bay, the Sabaki River to the south near Malindi, and the Tana River at Kipini, near the northern boundary of the study area. Like the rest of the East African coast, the bay experiences a humid tropical climate with two distinct seasons: the dry North-east monsoon (NEM) season between October and March, and the wet SEM season between April and September. These seasons greatly influence the productivity of the marine and coastal fisheries (McClanahan 1988; Kitheka *et al.* 2005). For instance, the numbers of active fishers and marine fishery catches in Kenya are higher during the NEM than during the SEM, when adverse weather limits artisanal fishing activities

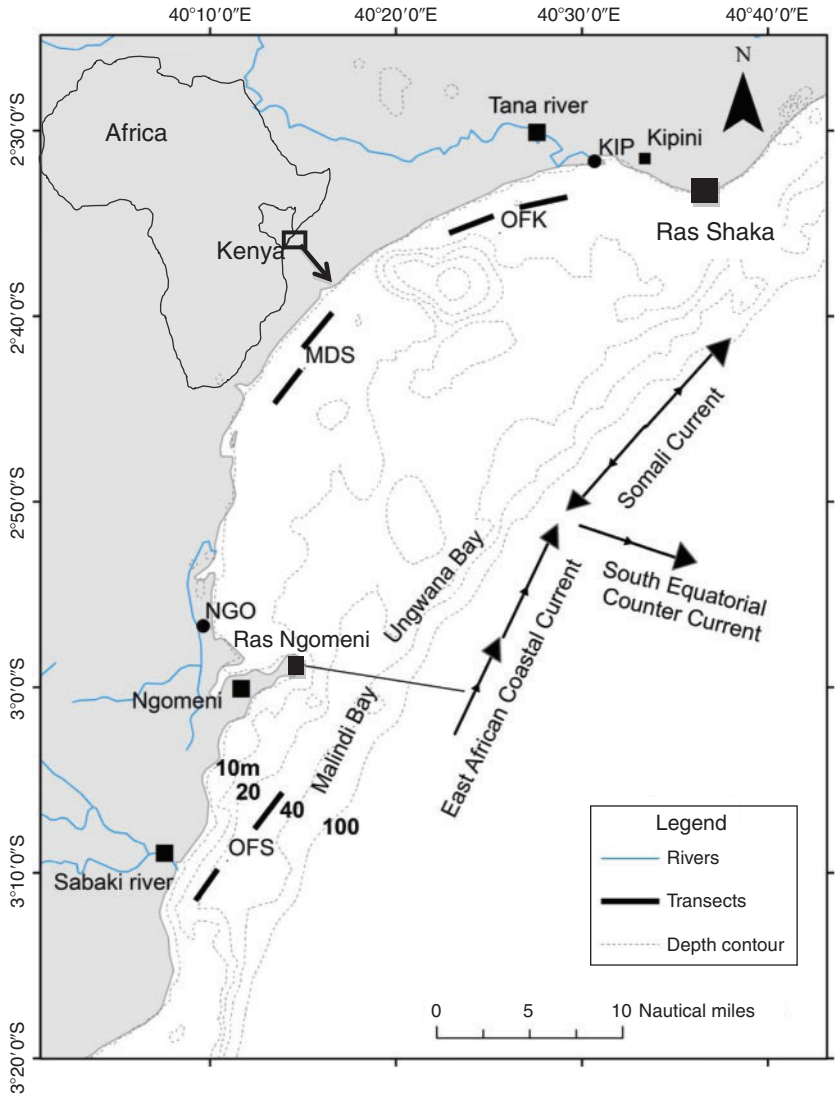

Fig. 1. Five sampling localities from where prawn samples of *P. monodon*, *F. indicus* and *M. monoceros* were obtained. Abbreviations represent Ngomeni (NGO), Kipini (KIP), mid station (MDS), offshore of Ngomeni (OFS) and offshore of Kipini (OFK). Arrows indicate the prevailing flow directions of the Somali Current, East Africa Coastal Current and South Equatorial Counter Current in the vicinity of Ungwana Bay.

(Fulanda *et al.* 2009). Water movements in Ungwana Bay and beyond are influenced by the northerly flowing East African Coastal Current, and the seasonally-reversing Somali Current. The latter current aligns its flow direction northwards with the SEM wind direction (McClanahan 1988), but flows southerly during the NEM. The area where the Somali and East Africa Coastal Currents converge marks the beginning of the offshore South Equatorial Counter Current.

Sample collection

Sampling localities were chosen to represent the Ungwana Bay prawn populations that support both artisanal and commercial trawl fisheries, and to include juvenile and adult cohorts that are presumably connected to each other through larval dispersal processes and migrations. A total of five sites were sampled (Table 1). From north to south they were: Kipini (KIP) at the mouth of the Tana River; offshore of Kipini (OFK); mid station (MDS) about halfway between Kipini and Ngomeni; a nearshore station just north of Ngomeni (NGO); and offshore of the mouth of the Sabaki River near Sabaki town (OFS) (Fig. 1). Prawns collected from KIP and NGO were mainly juveniles from nearshore waters, captured by local artisanal fishers during 2010, whereas mostly adults were collected from MDS, OFS and OFK during a prawn trawl survey undertaken in 2011. A total of 30 specimens per species were collected from each of the five localities.

DNA extraction, PCR and sequencing

Total genomic DNA was extracted from muscle tissues preserved in ethanol (96%) using the Wizard® SV Genomic DNA Extraction Kit (Promega, Madison, WI, USA) and stored at

Table 1. Geographic coordinates from where genetic prawn samples were obtained

Locality	Abbreviation	Transects	Geographic coordinates (latitudes and longitudes)			
			Start_lat. (S)	End_lat. (S)	Start_long. (E)	End_long. (E)
Kipini	KIP	–	02°31′688″	–	040°31′388″	–
Ngomeni	NGO	–	02°59′994″	–	040°10′588″	–
Mid station	MDS	1–2	02°44′708″	02°42′862″	040°13′456″	40°14′882″
‘	‘	1–3	02°39′802″	02°41′709″	040°16′571″	40°14′989″
Offshore of Kipini	OFK	1–5	02°34′591″	02°35′513″	040°25′25″	40°22′862″
‘	‘	1–6	02°33′577″	02°34′138″	040°29′208″	40°26′644″
Offshore of Sabaki	OFS	1–1	03°11′078″	03°10′914″	040°08′502″	40°08′601″
‘	‘	3–3	03°11′′488″	03°90′747″	040°10′943″	40°12′154″

−20°C before further analysis. PCR amplification of the mtDNA control region of *P. monodon* was performed using the species-specific primer pair PmCON–2F and PmCON–2IR and similar conditions as described in You *et al.* (2008). For *F. indicus* and *M. monoceros* the universal primers for penaeid prawns were used (Chu *et al.* 2003; McMillen-Jackson and Bert 2003) and the thermal profile for the latter two species was one cycle of 3 min at 95°C, 35 cycles of 50 s at 95°C, 60 s at 48°C, 90 s at 72°C, and one cycle of 5 min at 72°C. PCR products were gel purified and the reverse strand was sequenced using the BigDye terminator chemistry (Applied Biosystems) and analysed on an ABI 3100 automated sequencer. Additionally, a subset of *P. monodon* DNA samples was also amplified and sequenced using the universal primers. The latter was done to gain additional confidence in the authenticity of the *P. monodon* control region sequences generated in this study (see Walther *et al.* 2011).

Mitochondrial DNA data analysis

The program SEQUENCHER *v.*4.8 (Gene Codes, Corp., Ann Arbor, Michigan) was used to edit all sequences, which were then aligned using ClustalW (Thompson *et al.* 1994) as implemented in MEGA ver. 5 (Tamura *et al.* 2011). Data for each species was treated separately and analyses were also performed for separate sampling localities. Genetic diversity estimates [i.e. the number of polymorphic sites (*s*), number of haplotypes (*Nh*), haplotype diversity (*h*) and nucleotide diversity (π)] were obtained from ARLEQUIN ver. 3.11 (Excoffier *et al.* 2005). The same software was used to calculate pairwise Φ_{ST} statistics and to perform an Analysis of Molecular Variance (AMOVA). Significance levels were obtained through a nonparametric procedure with 10 000 permutations (Excoffier *et al.* 1992). The sequential Bonferroni correction (Rice 1989) was used to adjust α values. Evolutionary divergence between sequences was estimated from the uncorrected *p*–distance method using the bootstrap approach (10 000 replications) in MEGA ver. 5 (Tamura *et al.* 2011). We determined evolutionary relationships among haplotypes using a statistical parsimony network (Templeton *et al.* 1992) constructed by TCS ver. 1.21 (Clement *et al.* 2000), enforcing a 95% connection limit.

Microsatellite genotyping and analyses

Ten polymorphic di–nucleotide microsatellite loci developed for *P. monodon* (Brooker *et al.* 2000; Pan *et al.* 2004) were amplified for this species. Microsatellite loci were grouped into three panels for multiplex PCR amplifications. This grouping relied on fluorescent dyes, published allelic size ranges and annealing temperatures. Panel 1 included loci PM09 (GenBank accession number AF068826), PM25 (AF068827), PM27 (AF068828) and PM2345 (AY500860). Panel 2 consisted of loci PM138 (AY500853), PM3854 (AY500863) and PM1713 (AY500858) and Panel 3 comprised PM580 (AY500856), PM3945 (AY500864) and PM4018 (AY500865). Multiplex PCR amplification was carried out in a 10 µL reaction volume containing 1 µL (5–50 ng) of template DNA, 6 µL of Qiagen multiplex PCR (Qiagen) master mix, 2 µL of ddPCR H_2O and 1 µL of primer mix (0.2 µM final concentration for each primer). The annealing temperature was 57.3°C for panels 1 and 3, and 58.0°C for panel 2, and the thermal profile followed that of Pan *et al.* (2004). The internal size standard Genescan^{TM} 500Liz (Applied Biosystems) was used to score allele sizes on the ABI PRISM 3730 Genetic Analyzer (Applied Biosystems). Microsatellite alleles were scored using GeneMapper^{TM} software ver. 3.7 (Applied Biosystems). Individuals that had ambiguous peaks were reamplified and scored more than once and ~20% of all individuals were randomly chosen for reamplification and genotyped to confirm the initial results.

Microsatellite analyses were performed for each sampling locality separately and for the combined collection. Genotypic linkage disequilibrium (LD) between pairs of loci was determined using FSTAT ver. 2.9.3 (Goudet 2002). Sequential Bonferroni correction (Rice 1989) was used to adjust *p* values for multiple tests. Deviations from Hardy–Weinberg Equilibrium (HWE) were assessed in GENEPOP ver. 4.1 (Rousset 2008), where the Wright (1949) inbreeding coefficient (F_{IS}) with heterozygosity deficit was used as the option. Genotyping errors which normally take the form of null alleles, stuttering and large allele dropouts were investigated using MICROCHECKER ver. 2.2.3 (van Oosterhout *et al.* 2004). When null alleles were suspected, their frequencies were estimated using the van Oosterhout (van Oosterhout *et al.* 2006) and sequential Bonferroni methods (Rice 1989). Genetic diversity summary statistics were obtained using MICROSATELLITE TOOLKIT (Park 2001). Allelic richness (AR) was obtained from FSTAT ver. 2.9.3 (Goudet 2002), using the rarefaction method (Petit *et al.* 1998).

Population differentiation was assessed using ARLEQUIN ver. 3.11 (Excoffier *et al.* 2005) where pairwise R_{ST} values were used to test the null hypothesis of panmixia. Significance levels were obtained using the exact test of population differentiation

Table 2. Genetic diversity summary statistics of *P. monodon*, *F. indicus* and *M. monoceros* drawn from 5 sampling localities
Sample size (*n*), polymorphic sites (*s*), number of haplotypes (*Nh*), haplotype diversity (*h*) and nucleotide diversity (π). Abbreviations correspond to those in Table 1

Spp.	Station	Genetic diversity indices				
		n	*s*	*Nh*	*h*	π
P. monodon	KIP	28	65	28	1.0000 ± 0.0095	0.0139 ± 0.0070
	NGO	24	55	24	1.0000 ± 0.0120	0.0134 ± 0.0070
	MDS	28	70	28	1.0000 ± 0.0095	0.0163 ± 0.0090
	OFK	27	64	26	0.9972 ± 0.0111	0.0150 ± 0.0080
	OFS	22	67	22	1.0000 ± 0.0137	0.0153 ± 0.0080
	Total	129	120	126	0.9996 ± 0.0010	0.0147 ± 0.0076
F. indicus	KIP	25	92	25	1.0000 ± 0.0113	0.0161 ± 0.0083
	NGO	24	69	24	1.0000 ± 0.0120	0.0153 ± 0.0080
	MDS	15	53	15	1.0000 ± 0.0243	0.0149 ± 0.0080
	OFK	17	42	17	1.0000 ± 0.0202	0.0123 ± 0.0066
	OFS	15	51	15	1.0000 ± 0.0243	0.0143 ± 0.0077
	Total	96	159	95	0.9998 ± 0.0015	0.0147 ± 0.0020
M. monoceros	KIP	22	30	20	0.9870 ± 0.0201	0.0094 ± 0.0051
	NGO	15	37	15	1.0000 ± 0.0243	0.0111 ± 0.0061
	MDS	7	16	6	0.9524 ± 0.0955	0.0090 ± 0.0055
	OFK	10	50	8	0.9333 ± 0.0773	0.0165 ± 0.0092
	OFS	17	32	16	0.9926 ± 0.0230	0.0099 ± 0.0055
	Total	71	91	61	0.9815 ± 0.0110	0.0109 ± 0.0057

(Raymond and Rousset 1995). To determine the number of homogenous genetic clusters (*K*), the program STRUCTURE ver. 2.3 (Pritchard *et al.* 2000) was used. The admixture model (Pritchard *et al.* 2000) was used in combination with the correlated allele frequencies model (Falush *et al.* 2003). We used a burnin length of 1 000 000 and 10 000 Markov Chain Monte Carlo (MCMC) iterations, and sequential independent runs were performed with values of *K* ranging from 1 to 5. STRUCTURE does not automatically give the correct number of possible *K* values present in the dataset (Kalinowski 2011), and therefore the *ad hoc* guidelines suggested by the STRUCTURE ver. 2.3 manual and the more formal procedures of Evanno *et al.* (2005) were used.

Results

Fenneropenaeus indicus

Analyses of 791 base pairs of 96 *F. indicus* specimens resulted in 95 haplotypes, (GenBank accession numbers KC590224–KC590318). Nucleotide frequencies had a strong bias towards A/T as typically expected for mtDNA data (A = 37.96%, *t* = 42.68%, C = 9.84% and G = 9.52%). High haplotype and lower nucleotide diversity values were observed at each of the five localities and this pattern was concordant with the combined dataset (Table 2). The within species uncorrected sequence divergences between haplotypes (± s.e.) ranged from 0.1% ± 0.1% to 7.1% ± 0.9% (mean = 1.48% ± 0.2%). Pairwise Φ_{ST} values were not significant among localities (Φ_{ST} 0.00000–0.00313, $P > 0.05$) and AMOVA supported the complete absence of genetic differentiation ($\Phi_{ST} = 0, p > 0.05$). The distribution of haplotypes derived from juvenile and adult individuals as indicated by the TCS networks showed no geographic or maturity patterns (Figs 2, 3), but three divergent haplotypes were not connected to the main network.

Metapenaeus monoceros

Analyses of 774 base pairs of 71 *M. monoceros* specimens resulted in 61 haplotypes, (GenBank accession numbers KC591951–KC592011). As reported for *F. indicus* (above) the nucleotide frequencies also conformed to the expected ratio for mtDNA (A = 40.67%, *t* = 43.33%, C = 7.97% and G = 8.03%). The genetic diversities estimated for *M. monoceros* were nearly identical to those found for *F. indicus* (Table 2). The within species uncorrected sequence divergence ranged from 0.1% ± 0.1% to 4.7% ± 0.7% (mean = 1.1% ± 0.18%). One significant pairwise Φ_{ST} value between NGO and OFK samples was observed ($\Phi_{ST} = 0.088$, $p < 0.002$), although the overall AMOVA analyses did not support differentiation ($\Phi_{ST} = 0.016$, $p > 0.05$). The TCS networks for *M. monoceros* were similar to *F. indicus* above (i.e. no geographic structure; Figs 2, 3).

Penaeus monodon

Chromatograms obtained from the same individuals using the primer pairs PmCON–2F/PmCON–2IR and DLA/DLB did not show any sign of double reads (evidence for co–amplification of pseudo genes and/or paralogous genes; see Walther *et al.* 2011) and were identical. The nucleotide frequencies for *P. monodon* (A = 39.55%, *t* = 39.46%, C = 11.61% and G = 9.38%) were also congruent with the other two species mentioned above, further supporting the mtDNA origin of the data. Analyses of 570 base pairs of 129 specimens resulted in 126 haplotypes (GenBank accession numbers KC590098–KC590223). Genetic diversity estimates were once again comparable to those obtained for the other two species (Table 2). Uncorrected sequence divergences between haplotypes ranged from 0.2% ± 0.2% to 3.3% ± 0.7% (mean: 1.49% ± 0.18%). All pairwise Φ_{ST} values were not significant (Φ_{ST} 0.0000–0.00367, $P > 0.05$) and the AMOVA showed no evidence of genetic differentiation among localities ($\Phi_{ST} = 0$, $p > 0.05$). The TCS networks showed no geographic or maturity patterns (Fig. 2 and 3).

Six out of the 10 polymorphic loci for *P. monodon* were successfully amplified for this species. All six loci indicated significant deviations from Hardy–Weinberg Equilibrium (HWE) when samples were combined (Table 3). Twenty-four out of 30 cases showed significant HWE deviations. The significant deviations from HWE were all supported by a positive and significant inbreeding coefficient F_{IS} suggesting heterozygote deficiency. MICROCHECKER analysis suggested the presence of null alleles at each of the six loci and their estimated frequencies were 0.1119 for PM25, 0.0489 for PM27, 0.1438 for PM580, 0.103 for PM3854, 0.1652 for PM3945 and 0.1761 for PM4018. We did not detect any two loci with significant genotypic linkage disequilibrium, thus each locus represents a genetically independent marker. All loci, except PM4018, were highly polymorphic as indicated by high values of allelic richness (AR) and expected heterozygosity (Table 3). The pairwise R_{ST} values were not significant ($R_{ST} = 0.000$–0.0222, $p > 0.05$). STRUCTURE analysis, in combination with the more formal algorithms (Evanno *et al.* 2005), suggested the presence of a single genetic population

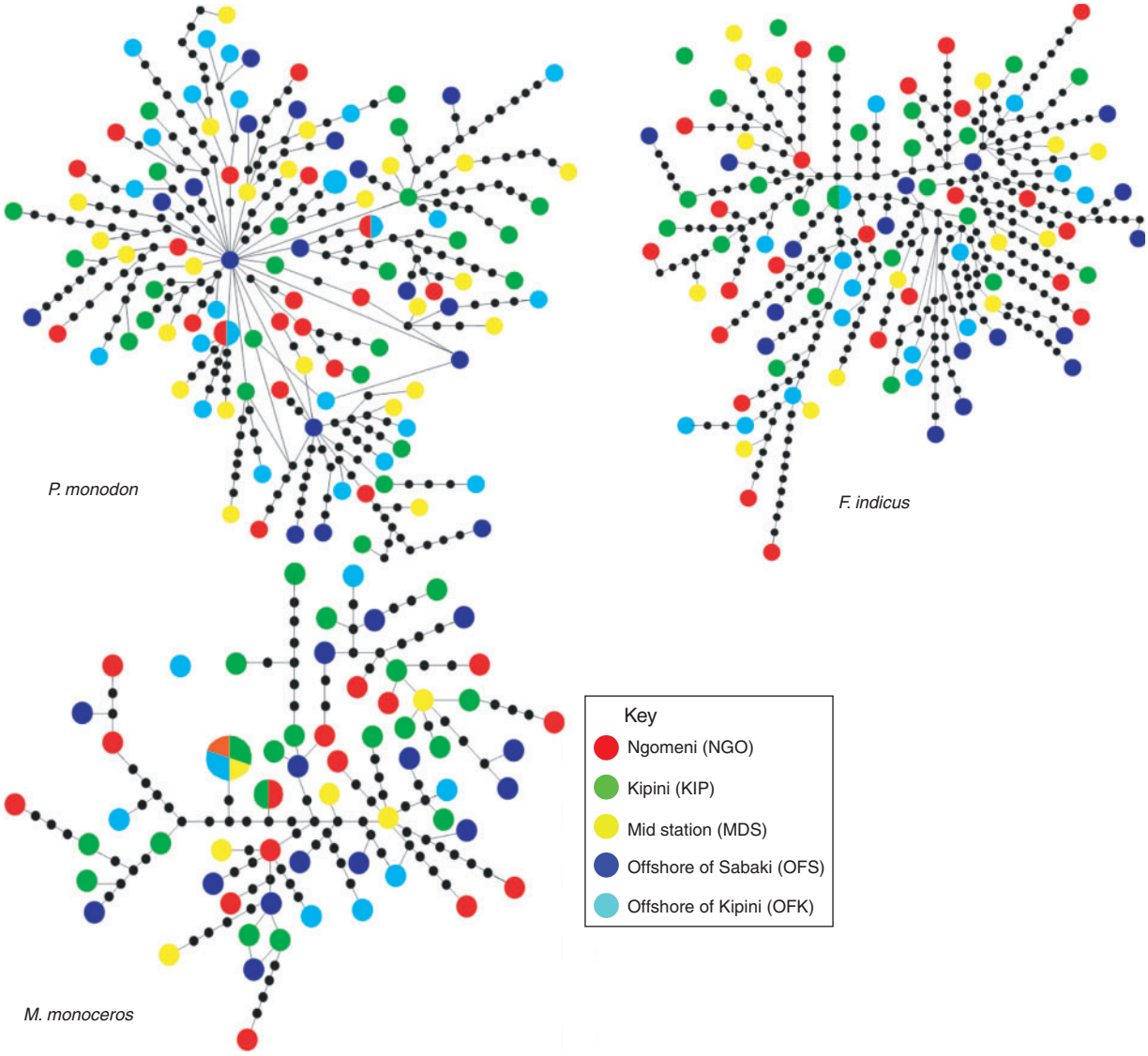

Fig. 2. Statistical parsimony network for *P. monodon*, *F. indicus* and *M. monoceros*. Control region mtDNA haplotypes are proportionally represented by coloured circles. Colour represents geographical localities from where haplotypes were sampled. Intermediate haplotypes (i.e. black circles) represent unsampled or extinct haplotypes. A black line connecting haplotypes represents one mutational step.

($K = 1$; Fig. 4), which confirms the results from the mitochondrial DNA analysis.

Discussion

The most important management finding that resulted from this study is the complete absence of geographic mtDNA structure within the three prawn species in Ungwana Bay. This was further confirmed by the microsatellite analyses of *P. monodon* indicating a single group. This finding is not unique for Ungwana Bay, and is supported by studies on parrot fish *Scarus ghobban* (Visram *et al.* 2010), and the mangrove crabs *Neosarmatium meinerti* (Ragionieri *et al.* 2010) and *Perisesarma guttatum* (Silva *et al.* 2010) in the same region. These species and the three penaeids under study all rely on larval dispersal driven by water movements, and the absence of genetic structure therefore suggests a lack of past geographic barriers to gene flow in Ungwana Bay. It thus appears that the pelagic larvae of several taxa, including fish and crustaceans, are mixed throughout the bay, where water movements are facilitated by river outflow, tidal exchange and monsoonal winds, coupled with ocean currents (McClanahan 1988; Kitheka *et al.* 2005). The differences in distribution range and habitat preference among the fauna of the region therefore do not appear to influence genetic differentiation patterns at a local scale in Ungwana Bay.

The absence of any genetic differentiation suggest that most recruitment to the offshore populations in Ungwana Bay could very well originate from local estuaries and nearshore sampling areas, thus highlighting the importance of these local nursery habitats to prawn fisheries in the bay. Unfortunately the sampling regime in the present study did not allow us to test for recruitment from other sources also, but the few haplotypes found that could not be connected to the networks pose an

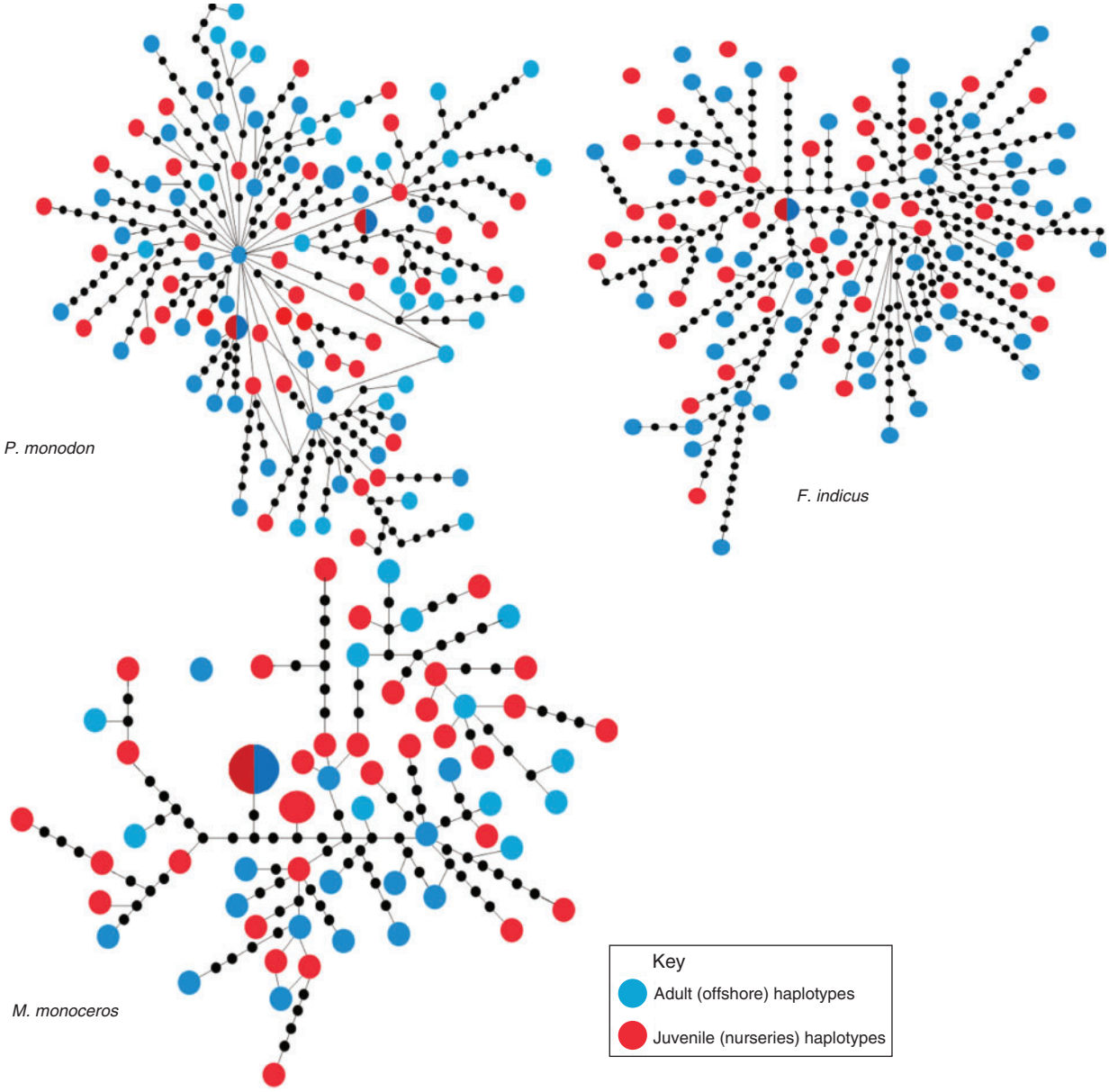

Fig. 3. Statistical parsimony network for *P. monodon*, *F. indicus* and *M. monoceros* showing evolutionary relationships of haplotypes distributed among adult and juvenile individuals. Haplotypes are proportionally represented by coloured circles. Colour represents maturity stages (juvenile/adults). Intermediate haplotypes (i.e. black circles) represent unsampled or extinct haplotypes. A black line connecting haplotypes represents one mutational step.

interesting hypothesis for future testing. Although these unconnected haplotypes may signal under-sampling (inadvertent failure to sample intermediate haplotypes; Chen *et al.* 2010) or sympatric speciation (Barluenga *et al.* 2006), we believe it is most likely an indication of some recruitment from distant sources, probably facilitated by alongshore currents and few physical barriers to gene flow. This hypothesis is supported by previous studies on marine crustaceans that have shown extensive regional dispersion along the East African coast (Duda and Palumbi 1999; You *et al.* 2008; Ragionieri *et al.* 2010).

The analysis of the mitochondrial DNA control region indicated exceptionally high haplotype diversity for all three species at each of the five sampling sites, and for all sites combined (*P. monodon,* $h = 0.9972–1.0000$; *F. indicus*, $h = 1.0000$; *M. monoceros*, $h = 0.9333–1.0000$). This result was congruent with previous studies on several penaeid species, including *P. monodon* from the Indo-West Pacific ($h = 0.969–1.000$; You *et al.* 2008), *Farfantepenaeus duorarum* from the south-eastern United States ($h = 1.000$; McMillen-Jackson and Bert 2004) and *Fenneropenaeus chinensis* from seas of northern China ($h = 0.9500–0.9900$; Kong *et al.* 2010). The microsatellite analysis confirmed the high genetic diversity of *P. monodon*, on the basis of heterozygosity ($He = 0.886–0.907$) and allelic richness ($AR = 14.333–24.833$). In a previous study, Waqairatu

Table 3. Genetic characteristics of six nuclear microsatellite loci for *P. monodon* samples obtained from five sampling localities
Abbreviations for sampling locations correspond to those in Table 1. *NA* = number of alleles, *AR* = allelic richness, *HO* = observed heterozygosity, *He* = unbiased expected heterozygosity, F_{IS} inbreeding coefficient (Bold F_{IS} indicate significant departure from HWE)

Locus		Sampling localities					
		KIP ($n = 21$)	NGO ($n = 20$)	MDS ($n = 22$)	OFK ($n = 20$)	OFS ($n = 20$)	Total ($n = 103$)
PM25	*NA*	17	14	14	16	17	20
	AR	16.710	14.000	13.622	16.000	17.000	15.119
	HO	0.857	0.650	0.636	0.700	0.750	0.718
	He	0.942	0.894	0.919	0.923	0.932	0.929
	F_{IS}	**0.092**	**0.278**	**0.312**	**0.246**	**0.199**	**0.225**
PM27	*NA*	18	19	15	20	19	24
	AR	17.660	19.000	14.786	20.000	19.000	17.245
	HO	0.714	0.850	0.864	0.850	0.950	0.845
	He	0.942	0.937	0.938	0.954	0.94	0.938
	F_{IS}	**0.246**	0.095	0.081	0.111	−0.011	**0.106**
PM580	*NA*	16	17	15	15	15	29
	AR	15.617	17.000	14.617	15.000	15.000	16.222
	HO	0.667	0.700	0.636	0.700	0.600	0.660
	He	0.916	0.933	0.932	0.922	0.906	0.926
	F_{IS}	**0.277**	**0.255**	**0.323**	**0.245**	**0.344**	**0.289**
PM3854	*NA*	24	18	24	22	12	34
	AR	23.373	18.000	22.617	22.000	12.000	20.262
	HO	0.762	0.800	0.727	0.750	0.750	0.757
	He	0.966	0.942	0.961	0.958	0.910	0.957
	F_{IS}	**0.216**	**0.154**	**0.247**	**0.221**	**0.180**	**0.206**
PM3945	*NA*	18	16	14	19	17	32
	AR	17.613	16.000	13.797	19.000	17.000	18.645
	HO	0.476	0.650	0.546	0.700	0.800	0.631
	He	0.934	0.946	0.923	0.953	0.949	0.946
	F_{IS}	**0.496**	**0.319**	**0.415**	**0.270**	**0.160**	**0.335**
PM4018	*NA*	7	4	6	6	6	10
	AR	6.95	4.000	5.727	6.000	6.000	5.983
	HO	0.381	0.650	0.318	0.350	0.550	0.447
	He	0.743	0.676	0.651	0.641	0.676	0.687
	F_{IS}	**0.494**	0.039	**0.517**	**0.460**	0.190	**0.348**
AR		24.833	14.667	14.667	16.333	14.333	24.833
HO/locality		0.643	0.717	0.621	0.675	0.733	0.676
He/locality		0.907	0.888	0.887	0.892	0.886	0.897
F_{IS}		**0.297**	**0.197**	**0.305**	**0.248**	**0.176**	**0.246**

et al. (2012) also found high heterozygosity (*He* = 0.82–0.91) in *P. monodon* from the Indo-West Pacific. Interestingly, the significant deviation from HWE indicated by heterozygote deficiency has also previously been shown for *P. monodon* (Brooker *et al.* 2000; Pan *et al.* 2004; You *et al.* 2008; Waqairatu *et al.* 2012), and is often found in other marine invertebrates and fish (Raymond *et al.* 1997; Huang *et al.* 2000; Hoarau *et al.* 2002; Addison and Hart 2005; Morin *et al.* 2009).

The high genetic diversity of the three penaeid species may be the result of the inferred large effective population sizes (also see Ovenden *et al.* 2007; Leffler *et al.* 2012), and/or high mutation rates at mitochondrial (Palumbi and Benzie 1991; Baldwin *et al.* 1998; McMillen-Jackson and Bert 2003) and nuclear microsatellite DNA (Chakraborty *et al.* 1997). The large effective population sizes suggest that mutation–random drift equilibrium acts to retain the high genetic diversity levels (see Kimura and Crow 1964; Kimura 1983). There was no genetic evidence of inbreeding or a severe bottleneck due to overfishing in the present study; both of these effects have been associated with smaller effective population sizes (Allendorf *et al.* 2008; Leffler *et al.* 2012). Lower effective population sizes may lead to a faster rate of loss of variation due to drift (Charlesworth 2009), which was not evident in this study.

Penaeus monodon, *F. indicus* and *M. monoceros* in Ungwana Bay exhibit some differences in habitat preference, abundance and distribution patterns (Dall *et al.* 1990; Macia 2004; Munga *et al.* 2013), but they likely share similar mechanisms of dispersal and recruitment between estuaries and offshore

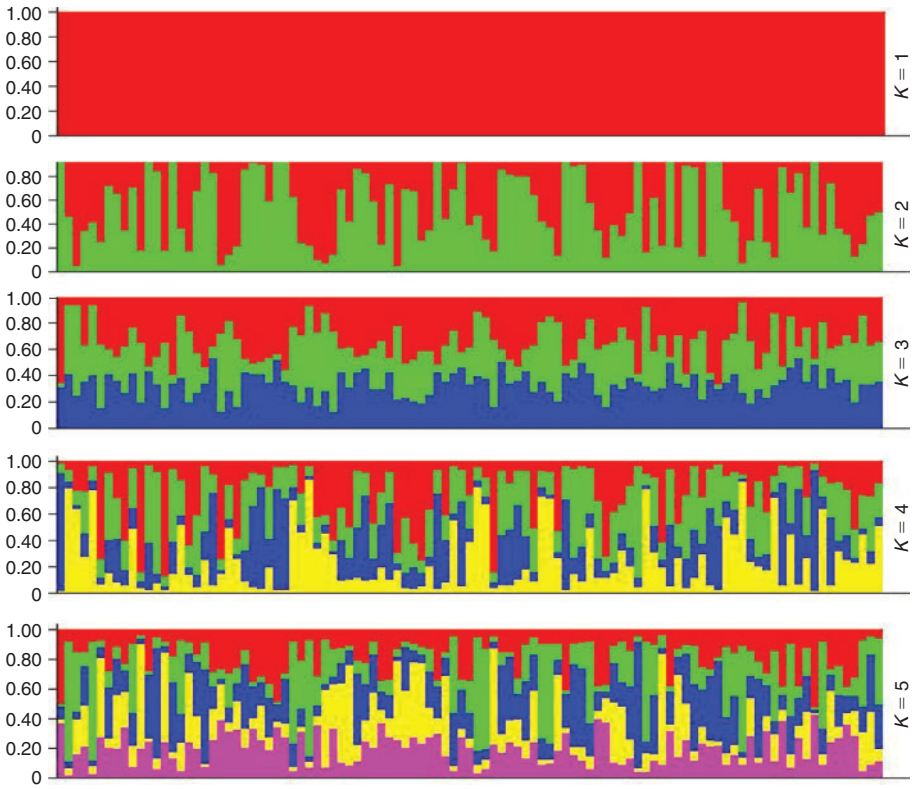

Fig. 4. Results from the *P. monodon* STRUCTURE analysis (performed using six microsatellite loci) showing genetic population clusters ranging from $K = 1$ to $K = 5$. Each colour represents a single inferred genetic cluster irrespective of the geographic origin of samples. Each individual is represented by a vertical bar. The numbers and proportions of colours (ranging from 0 to 5) contained in each individual indicates the extent of genetic admixture of that individual.

habitats. Comparable levels of mtDNA genetic diversity can therefore be explained by a combination of similar life–history patterns, rates of molecular evolution and effective population sizes. Nevertheless, *M. monoceros* exhibited a slightly lower haplotype diversity than the other two species, despite its more generalist habitat preferences and higher abundance in the bay than *P. monodon.* Possible explanations could be that *M. monoceros* might have experienced a more severe reduction in numbers due to fishing, or alternatively, its effective population size may be smaller than for the other two species (for example see Ramos-Onsins *et al.* 2004; Piganeau and Eyre-Walker 2009). Furthermore, Kumar *et al.* (2012) suggest that the rate of mitochondrial evolution is not uniform among penaeid species and it is therefore possible that the mutation rate of *M. monoceros* is somewhat lower than in the other two species.

Our analyses could not reject the hypothesis that nearly all prawn recruits into Ungwana Bay originate from nearby estuaries. It, however, could not estimate the relative contributions of each of the two estuaries to the offshore population in the bay. On the basis of this, it is important that the estuaries be recognised as potential nursery habitats that support both artisanal and commercial prawn fisheries. Nursery areas in Kenya are thus important components of the fishery and should be conserved and managed to maintain a sustainable industry. Factors such as habitat degradation through the discharge of untreated wastes or chemicals, clearance of mangrove habitats for human settlements, and the building of ports, harbours or upstream dams that may affect river discharge can all affect juvenile habitats and alter prawn recruitment patterns (see Turpie and Lamberth 2010). Even though the high genetic diversity of prawns in Ungwana Bay implies that they may be able to adapt to environmental change, the importance of the local prawn stocks to long-term human needs in the region need to be considered.

From a fisheries management perspective, the genetically panmictic prawn populations in Ungwana Bay do not support the spatial partitioning of the bay into artisanal (shore to 3 nm from the coast) and industrial trawl (>3 nm from the coast) fisheries areas. Both fisheries target the same stocks with a single gene pool in each species. Indicators other than genetic delineation should therefore be used to support fisheries management strategies, and particularly the spatial partitioning of fishing grounds among artisanal and industrial fishing sectors. These may be based more appropriately on other ecological factors (species composition, distribution and abundance patterns; see Munga *et al.* 2013), conservation of prawn nursery areas in estuaries and nearshore waters (see above), and socio–economic criteria such as historical fishing practices (Fulanda *et al.* 2009).

At a regional level, *F. indicus*, *M. monoceros* and *P. monodon* support numerous other artisanal and commercial trawl fisheries in the South West Indian Ocean, in Tanzania, Mozambique,

South Africa and Madagascar (van der Elst *et al.* 2009). The question therefore remains whether the prawn populations supporting these fisheries are genetically panmictic at a regional level? Some information suggests panmixia along the East African coast, but structure between coastal and island populations in the South West Indian Ocean has been suggested for *P. monodon, N. meinerti* and *P. guttatum* (Duda and Palumbi 1999; You *et al.* 2008; Ragionieri *et al.* 2010; Silva *et al.* 2010). Nevertheless, fisheries have been managed individually by the countries in which the resources occur, and consequently a variety of management policies and methods exist at present (see FAO 2006). Should stocks be genetically panmictic at regional level, it would provide impetus for the harmonization of management strategies for shallow-water prawn fisheries in the South West Indian Ocean.

To conclude, three things are now clear to fisheries managers responsible for prawn fisheries in Ungwana Bay: a) that the three most abundant prawn species are genetically panmictic at the scale important to management of fisheries in the bay; b) that nearly all of the prawn recruits could originate from the estuaries and mangrove swamps in the bay, thus highlighting the importance of conserving these nursery habitats; and c) that all three species have high genetic diversity, suggesting that the respective gene-pools are probably resilient towards change.

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