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# Genetic population structure of the endemic fourline wrasse (*Larabicus quadrilineatus*) suggests limited larval dispersal distances in the Red Sea

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## Abstract

The connectivity among marine populations is determined by the dispersal capabilities of adults as well as their eggs and larvae. Dispersal distances and directions have a profound effect on gene flow and genetic differentiation within species. Genetic homogeneity over large areas is a common feature of coral reef fishes and can reflect high dispersal capability resulting in high levels of gene flow. If fish larvae return to their parental reef, gene flow would be restricted and genetic differentiation could occur. *Larabicus quadrilineatus* (Labridae) is considered as an endemic fish species of the Red Sea and Gulf of Aden. The juveniles of this species are cleaner fish that feed on ectoparasites of other fishes. Here, we investigated the genetic population structure and gene flow in *L. quadrilineatus* among five locations in the Red Sea to infer connectivity among them. To estimate genetic diversity, we analysed 369 bp of 237 mitochondrial DNA control region sequences. Haplotype and nucleotide diversities were higher in the southern than in the northern Red Sea. Analysis of molecular variance (AMOVA) detected the highest significant genetic variation between northern and central/southern populations ( $\Phi_{CT} = 0.01$ ;  $P < 0.001$ ). Migration analysis revealed a several fold higher northward than southward migration, which could be explained by oceanographic conditions and spawning season. Even though the  $\Phi_{ST}$  value of 0.01 is rather low and implies a long larval dispersal distance, estimates based on the isolation-by-distance model show a very low mean larval dispersal distance (0.44–5.1 km) compared to other studies. In order to enable a sustainable ornamental fishery on the four-line wrasse, the results of this study suggest that populations in the northern and southern Red Sea should be managed separately as two different stocks. The rather low larval dispersal distance of about 5 km needs to be considered in the design of marine protected areas to enable connectivity and self-seeding.

*Keywords:* aquarium trade, Arabian Peninsula, conservation, effective migrants, isolation by distance, neutrality test, population expansion

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## Introduction

The connectivity among marine populations is determined by the dispersal capabilities of adults as well as their eggs and larvae. Fishes on coral reefs have a life history with two totally different phases: larvae of virtually all species are planktonic (Leis 1991), whereas adults are relatively strongly site-attached and sedentary (Sale 1980). Dispersal

distances and directions have a profound effect on gene flow and genetic differentiation within species. Genetic homogeneity over large areas is a common feature of coral reef fishes and can reflect high dispersal capabilities resulting in high levels of gene flow (Planes *et al.* 1993; Doherty *et al.* 1995; Shulman & Birmingham 1995; Bernardi *et al.* 2001). If fish larvae return to their parental reef, gene flow would be restricted and genetic differentiation could occur (Planes *et al.* 1996; Planes *et al.* 1998). However, the scale of dispersal might be limited by fish behaviour or oceanographic factors even in fishes that have long pelagic

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larval durations (Sponaugle *et al.* 2002). On the one hand, studies on late pelagic stages of coral reef fishes have demonstrated that they can swim against strong currents (Leis & Carson-Ewart 1997). On the other hand, gyres can cause retention of larvae at their parental reef (Johannes 1978; Swearer *et al.* 1999).

Connectivity of populations can be inferred by investigating the genetic population structure with molecular markers, such as the mitochondrial control region. The control region is a suitable marker for such studies (Avice *et al.* 1987; Parker *et al.* 1998), because it has a much higher mutation rate than any of the mitochondrial genes (Lee *et al.* 1995).

However, only a few studies used genetic tools to infer gene flow in the Red Sea and therefore connectivity among populations in this region is virtually unknown. Most of these studies are restricted to the northern Red Sea and Gulf of Aqaba, including fishes (Hassan *et al.* 2003; Kochzius & Blohm 2005) and a stony coral (Maier *et al.* 2005). Only one study compares northern and southern Red Sea populations of a eulittoral bivalve (Shefer *et al.* 2004) at the African coastline.

The fourline wrasse, *Larabicus quadrilineatus* (Labridae), occurs on coral reefs and is considered as an endemic fish species of the Red Sea and Gulf of Aden. The juveniles of this species are cleaner fish that feed on ectoparasites and mucus of other fishes, whereas adults appear to feed mainly on coral polyps (Randall 1983). Several studies supported the view that the interaction between cleaner fish and its client is a mutual relationship and plays a very important ecological role. Studies by Grutter (1999) and Bshary (2003) found a significant reduction of client fish after the experimental removal of cleaner fish. In particular, Bshary (2003) conducted a field experiment on the impact of the cleaner wrasse *Labroides dimidiatus* on reef fish distribution in the Red Sea at Ras Mohammed National Park, Egypt. He showed significant decline in fish diversity after 4–20 months of cleaner fish removal, and inferred that cleaner fish are a key organism for local reef fish diversity.

This study aims to investigate connectivity among populations of the ecologically important endemic fourline wrasse *L. quadrilineatus* along the entire Red Sea coastline of the Arabian Peninsula by determining the levels of genetic differentiation and estimating the amount of gene flow among its populations. This covers almost the whole species range. Such data on migration rates will uncover the degree of connectivity among populations in the Red Sea and will give insights in dispersal and evolutionary processes in the Red Sea.

## Materials and methods

Tissues and fin clips of 237 individuals from five locations in the Red Sea (Fig. 1) were collected in November and December 2003 either by scuba diving or by obtaining

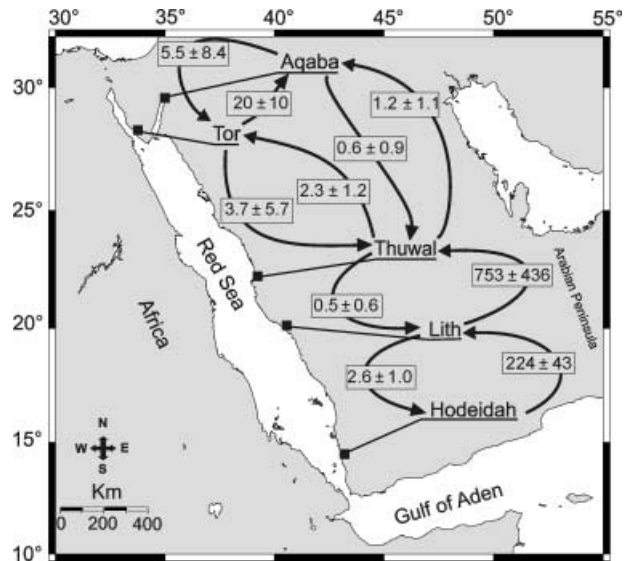


Fig. 1 Map of the Red Sea showing collections sites and the mean number of effective migrants  $\pm$  standard deviation of *Larabicus quadrilineatus* among these sites. The results are mean values of six runs with the program MIGRATE.

them from local ornamental fishermen. Samples were placed immediately in absolute ethanol and were stored in the laboratory at  $-75^{\circ}\text{C}$ .

DNA was extracted with Chelex® as described in Söller *et al.* (2000). A fragment of 369 bp from the 5'-end of the mitochondrial control region was amplified with the universal primer CR-A and the specific primer XAN-DL-R (Fauvelot *et al.* 2003). Each polymerase chain reaction (PCR) with a volume of 50  $\mu\text{L}$  contained 10 mM Tris-HCl (pH 9), 50 mM KCl, 2 mM  $\text{MgCl}_2$ , 0.2 mM each dNTP, 0.2  $\mu\text{M}$  each primer, 2 U *Taq* polymerase and 2  $\mu\text{L}$  of the DNA template. The following temperature profile was used for the PCR: hot start at  $95^{\circ}\text{C}$  for 5 min followed by 35 cycles of  $94^{\circ}\text{C}$  (45 s),  $50^{\circ}\text{C}$  (45 s),  $72^{\circ}\text{C}$  (60 s), and a final extension at  $72^{\circ}\text{C}$  for 5 min.

PCR products were purified with the QIAquick PCR purification kit (QIAGEN). Sequencing of both strands was performed with the same primers used for PCR and an ABI PRISM 310 automated sequencer following the manufacturer's protocol (Applied Biosystems). The two sequenced strands were aligned with the programme SEQUENCE NAVIGATOR (version 1.0.1; Applied Biosystems) and confirmed by eye. A multiple alignment of all sequences was performed with the programme CLUSTAL W (Thompson *et al.* 1994) as implemented in BIOEDIT (version 7.0.4.1; Hall 1999).

The number of haplotypes and polymorphic sites was derived using the programme COLLAPSE (version 1.2; available at <http://darwin.uvigo.es>) and verified by DNASP (version 4.10.3; Rozas *et al.* 2003). Mean nucleotide and haplotype diversities were calculated using ARLEQUIN (version 3.0.1; Schneider *et al.* 2000).

A haplotype network was constructed using the median-joining network method as implemented in NETWORK (version 4.1.1.1 available at [www.fluorous-engineering.com](http://www.fluorous-engineering.com); Bandelt *et al.* 1999). This method begins by combining the minimum spanning trees (MST) within a single network [minimum spanning network (MSN)] using an analogous algorithm to that proposed by Excoffier & Smouse (1994). Then, median vectors (which represent missing intermediate haplotypes) are added to the network using the parsimony criterion.

Tajima's  $D$ -test (Tajima 1989) and Fu's  $F_s$  test (Fu 1997) with 10 000 permutations as implemented in ARLEQUIN (version 3.01; Schneider *et al.* 2000) were used to test the hypothesis of neutrality of the marker. Negative Tajima's  $D$ -values can indicate selection, but also population bottlenecks or population expansions (Tajima 1989). We used mismatch distributions to evaluate the hypothesis of recent population growth with 99 999 permutations as implemented in ARLEQUIN 3.0.1 (Rogers & Harpending 1992). This distribution is commonly unimodal in populations that have passed through a recent demographic expansion, and is multimodal in stable populations.

Collection sites were regarded as populations and the hypothesis of panmixing was tested using AMOVA (Excoffier *et al.* 1992), as well as the exact test of population differentiation (10 000 permutations; Raymond & Rousset 1995). Alternative groupings between populations were tested using AMOVA to find the highest significant grouping. AMOVA is similar to other approaches based on the analysis of variance of gene frequencies, but it takes into account the number of mutations between molecular haplotypes. The significance of  $\Phi$  values was tested using a nonparametric permutation procedure with 10 000 permutations. The exact test of population differentiation evaluates the hypothesis of a random distribution of different haplotypes among pairs of populations.

The amount of connectivity among the collection sites was measured by estimating (i) the number of effective migrants among populations using the programme MIGRATE, and (ii) the mean larval dispersal distance based on the slope of the relationship of genetic and geographical distances. Based on the linear distribution of collection sites along the Red Sea coast of the Arabian Peninsula we hypothesized a stepping-stone model for estimating migration rates only between adjacent collection sites. Because of the detected isolation by distance in *Larabicus quadrilineatus*, the slope of the regression of  $F_{ST}/(1 - F_{ST})$  to geographical distance was considered appropriate to estimate the mean larval dispersal distance (Rousset 1997).

Migration among collection sites was evaluated using the programme MIGRATE (version 2.0.3; Beerli & Felsenstein 1999, 2001; Beerli 2005). MIGRATE estimates migration rates  $M$ , and effective population sizes  $\Theta$ . The effective number of migrants among collection sites ( $\gamma$ ) was calculated as follows:  $\gamma = \Theta M$ . The programme MIGRATE uses a maximum-

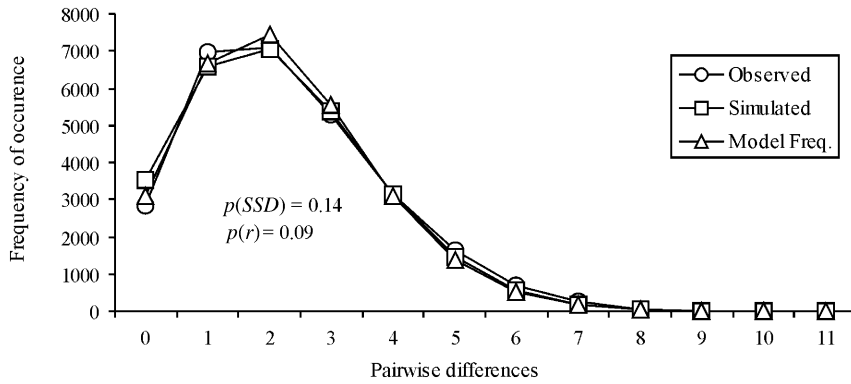
likelihood framework based on the coalescence theory and investigates possible genealogies with migration events using a Markov chain Monte Carlo approach (Beerli & Felsenstein 2001). A transition/transversion ratio of 3.93 and the following base frequencies were incorporated in the analysis: A = 0.32, C = 0.21, G = 0.16, T = 0.31. These parameters were obtained with the programme MODELTEST (version 3.7; Posada & Crandall 1998). We performed six independent runs, each run with 10 short chains of 5000 steps and 3 long chains of 50 000 steps with a sampling increment of 100. In addition to that, heating was used for 4 chains.

The relationship between genetic and geographical distances among populations was examined using reduced Major Axis (RMA) regression. Correlation of pairwise genetic distances ( $F_{ST}/1 - F_{ST}$ ; Rousset 1997) and geographical distances among populations was assessed using the Mantel test (Mantel 1967) as implemented in the isolation-by-distance web service (IBDWS version 2.6; Bohonak 2002; <http://phage.sdsu.edu/%7Ejensen/>) with 30 000 permutation to test significance. Mean larval dispersal distance was estimated from the slope of the relationship of genetic and geographical distances. The slope of the regression of  $F_{ST}/(1 - F_{ST})$  to geographical distance is related to the inverse of  $4D\sigma^2$ , where  $D$  refers to the density of individuals and  $\sigma^2$  refers to the variance of parental position relative to offspring position (Rousset 1997). By substituting values of slope and appropriate adult densities ( $D$ ), estimates of  $\sigma^2$  are obtained. Adult densities were taken from Khalaf & Kochzius (2002). Assuming that dispersal follows a symmetrical two-sided exponential distribution centred at zero, estimate of  $\sigma^2$  may then be related to average deviation from the mean (standard deviation or mean dispersal distance) as  $\sigma^2 = 2d^2$ , where  $d$  refers to the mean dispersal distance.

## Results

Sequences of a 369-bp fragment of the mtDNA control region were obtained for 237 individuals sampled from five locations. Fifty-seven polymorphic sites revealed 91 haplotypes. Three haplotypes were shared among all populations, eight were shared by two or three populations, and all others were either unique to Aqaba (10), Tor (18), Thuwal (11), Lith (19), or Hodeidah (22). Haplotype diversity ranged from 0.80 to 0.95, nucleotide diversity from 0.38% to 0.82%, and the mean number of nucleotide differences ranged from 1.4 to 3.0 (Table 1).

AMOVA analysis rejected the null hypothesis that the studied populations are homogeneous in the Red Sea ( $\Phi_{ST} = 0.01$ ;  $P = 0.01$ ; Table 2). The great majority of variation was within (98.99%) rather than among populations (1.01%). Alternative groupings between populations with AMOVA showed the highest significant differentiation when we arranged populations in two groups: northern



**Fig. 2** Mismatch distribution of mtDNA sequences of *Larabicus quadrilineatus*. The curves represent the frequency distribution of pairwise differences.

**Table 1** Mitochondrial DNA control region sequences variation of *Larabicus quadrilineatus* populations in the Red Sea and their genetic diversity indices

Sample site	<i>n</i>	No. of haplotypes	No. of variable sites	Haplotype diversity <i>h</i> ± SD	Nucleotide diversity $\pi$ % ± SD	Mean number of pairwise nucleotide differences ± SD
Aqaba	52	16	16	0.80 ± 0.05	0.38 ± 0.27	1.4 ± 0.87
Tor	48	26	21	0.92 ± 0.03	0.64 ± 0.40	2.3 ± 1.30
Thuwal	46	18	18	0.86 ± 0.03	0.54 ± 0.35	1.9 ± 1.10
Lith	39	25	25	0.95 ± 0.02	0.82 ± 0.48	3.0 ± 1.60
Hodeidah	52	32	30	0.95 ± 0.02	0.79 ± 0.47	2.8 ± 1.50

**Table 2** Hierarchical analysis of molecular variance (AMOVA) of mtDNA control region sequences in *Larabicus quadrilineatus* from the Red Sea. \*0.05 ≥ *P* ≥ 0.01, \*\*0.01 > *P* ≥ 0.001, \*\*\**P* < 0.001

Region groupings	$\Phi_{ST}$	$\Phi_{CT}$	% variance among groups
No grouping	0.010**	—	1.01
(Aqaba) (Tor, Thuwal, Lith, Hodeidah)	0.008	0.003	-0.34
<b>(Aqaba, Tor) (Thuwal, Lith, Hodeidah)</b>	<b>0.015</b>	<b>0.012***</b>	<b>1.18</b>
(Aqaba, Tor, Thuwal) (Lith, Hodeidah)	0.014	0.011**	1.11
(Aqaba, Tor, Thuwal, Lith) (Hodeidah)	0.007	0.005	-0.50
(Aqaba, Tor) (Thuwal) (Lith, Hodeidah)	0.012	0.011**	1.07
(Aqaba, Tor) (Thuwal, Lith) (Hodeidah)	0.011	0.005	0.47

(Aqaba and Tor) and central/southern (Thuwal, Lith and Hodeidah; Table 2) Red Sea. The exact test of population differentiation was significant (*P* < 0.05) between the northern and central/southern Red Sea, but not within these groups (*P* > 0.05).

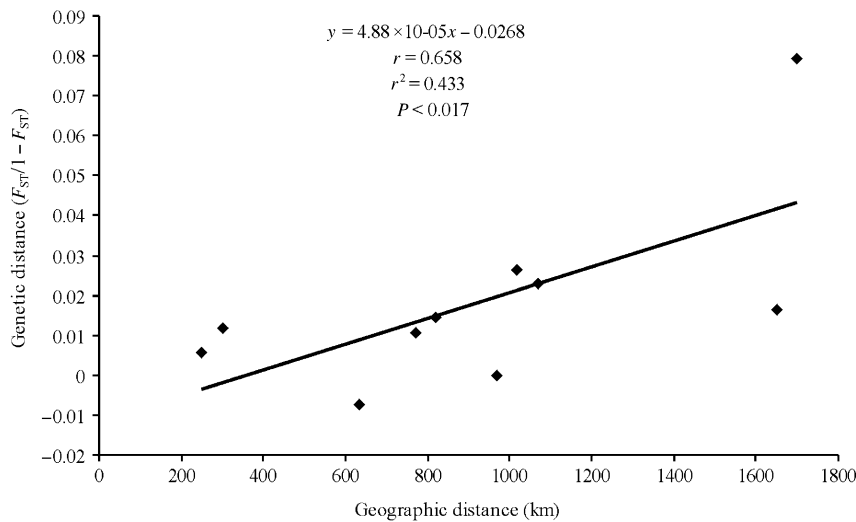
The median-joining network (not shown) of the 91 haplotypes contained many loops and was therefore not very informative. Three of the haplotypes were shared among all populations and represented 43% of all haplotypes. The proportion of haplotype 1 decreases from North to South, whereas the proportion of haplotype 3 increases.

The negative and significant values of Tajima's *D* and Fu's *F<sub>s</sub>* tests rejected the selective neutrality hypothesis and implied selection, population bottleneck or population

expansion. Mismatch distribution was unimodal, which is consistent with the model of sudden population expansion from a small number of fish (Fig. 2). Both sum of square deviations (SSD) and raggedness index (*r*) tests suggested that the observed distribution curves do not significantly differ from the simulated distribution curves under a model of demographic expansion (Table 3).

Results of MIGRATE analysis indicated a several fold higher northward than southward migration (Fig. 1). The effective population sizes are higher in the southern Red Sea than in the northern Red Sea (Table 4).

According to the Mantel test (*r* = 0.66; *P* = 0.017), the relationship between genetic and geographical distances was significantly correlated (Fig. 3). The mean larval



**Fig. 3** Relationship between genetic vs. geographical distances in *Larabicus quadrilineatus* using reduced major axis (RMA) regression.

**Table 3** Neutrality tests and the estimated parameters of mismatch distribution for populations of *Larabicus quadrilineatus* from the Red Sea.  $D$ , Tajima's  $D$ ;  $p(D)$ ,  $P$  value for  $D$ ;  $F$ , Fu's  $F_s$ ;  $p(F)$ ,  $P$  value for  $F_s$ ; Mismatch, mean and standard deviation of mismatch distribution;  $p(SSD)$ ,  $P$  value of sum of square deviations ( $SSD$ ) between the observed and the expected mismatch;  $p(r)$ ,  $P$  value of the raggedness index of the observed distribution. Some values are missing because the least squares procedures to fit the model mismatch distribution and the observed distribution did not converge after 1800 steps. Significant  $P$  values are marked with \*

Sampling site	$D$	$p(D)$	$F$	$p(F)$	Mismatch	$p(SSD)$	$p(r)$
Aqaba	-1.870	0.011*	-11.883	< 0.001*	1.39 ± 1.55	—	—
Tor	-1.505	0.045*	-24.953	< 0.001*	2.34 ± 1.90	—	—
Thuwal	-1.521	0.043*	-12.294	< 0.001*	1.96 ± 2.09	0.351	0.024*
Lith	-1.568	0.038*	-22.191	< 0.001*	2.98 ± 2.94	0.685	0.333
Hodeidah	-1.889	0.010*	-26.481	< 0.001*	2.85 ± 2.82	—	—
Aqaba and Tor	-1.911	0.006*	-27.610	< 0.001*	1.86 ± 1.88	—	—
Thuwal, Lith and Hodeidah	-1.986	0.003*	-26.746	< 0.001*	2.59 ± 2.74	0.363	0.265
All	-2.142	0.001*	-26.808	< 0.001*	2.29 ± 2.45	0.137	0.085

dispersal distance was estimated from the slope of the RMA regression of genetic and geographical distances. Dispersal distances based on a slope of 0.0488 per 1000 km and on adult densities of 100, 120, 500 and 13 320 adults  $\text{km}^{-2}$  are 5.1, 4.6, 2.3, and 0.44 km per generation, respectively.

## Discussion

### Genetic population structure

This study on *Larabicus quadrilineatus* revealed significant levels of genetic differentiation among populations. The used marker, a fragment of the mitochondrial control region, obtained 91 haplotypes from 237 individuals. All collection sites, including Aqaba and Hodeidah (1700-km distance), were dominated by three ancestral haplotypes.

The detected genetic differentiation between the northern (Aqaba and Tor) and central/southern (Thuwal, Lith and Hodeidah) Red Sea is inferred from the significant

**Table 4** Proportions of theta values (effective population sizes) of *Larabicus quadrilineatus* from five populations in the Red Sea. Theta values ( $\Theta$ ) were estimated using the programme MIGRATE

	$\Theta$	Proportion (%)
Aqaba	0.000413	4.5
Tor	0.000436	4.7
Thuwal	0.000082	1.0
Lith	0.001937	21.0
Hodeidah	0.006348	68.8

estimate of  $\Phi$  values and the significant pairwise variances. This genetic structure is supported by the MIGRATE analysis, which revealed a higher number of migrants within the northern (Aqaba and Tor) and within the central/southern (Thuwal and Lith; Lith and Hodeidah) Red Sea, compared to the number of migrants between North and South. The genetic differentiation observed between the northern and

central/southern regions is congruent to findings in differences of fish communities in the Red Sea at about 20 N°. This is due to ecological differences between the northern and southern Red Sea, such as an increase of turbidity and decrease of coral variety and reef development in the South (Roberts *et al.* 1992; Sheppard *et al.* 1992).

The lack of population structure within northern region of the Red Sea coincides with studies on fishes by Kochzius & Blohm (2005) and Hassan *et al.* (2003), which suggest high gene flow and panmixing in the Gulf of Aqaba and northern Red Sea.

#### Historical demography

The median-joining network did not reveal any geographical cluster. All haplotypes of all populations were scattered throughout the network. This lack of structure is likely due to incomplete separation of haplotypes between the northern and central/southern Red Sea caused by migration among these populations or a population expansion. Tajima's *D* and Fu's *F<sub>s</sub>* tests rejected the neutrality hypothesis for all collection sites. These tests cannot distinguish between selection and changes in population size. We performed Roger's test to detect departure from the null hypothesis of sudden demographic expansion (Rogers & Harpending 1992). Our data showed a unimodal distribution of pairwise differences that indicates a recent population expansion. Therefore, a sudden demographic expansion rather than selection seems to be the reason for the rejection of the neutrality tests.

This pattern of a shallow population structure and indication of a sudden population expansion could be explained by the demographic history of *L. quadrilineatus*. Palaeoceanographic studies revealed that during the Pleistocene, water exchange between the Red Sea and Indian Ocean at the shallow sill of Bab-el-Mandab was restricted (Siddall *et al.* 2003) or even interrupted (Braithwaite 1987). However, the extent of environmental changes in the Red Sea is still controversial. Some authors assume hypersalinity that killed most marine life (Sheppard *et al.* 1992) or even conditions comparable with the present day Dead Sea (Braithwaite 1987), while others suggest survival of the fauna (Goren 1986; Klausewitz 1989; Rohling *et al.* 1998). However, in any case the population size of organisms in the Red Sea was reduced and a population expansion either took place from a reduced surviving population in the Red Sea or by recolonization from the Gulf of Aden, which is proposed as a refuge of the Red Sea fauna during the glacials (Klausewitz 1989).

The estimated effective population sizes were higher in the southern Red Sea (Lith and Hodeidah) than northern Red Sea (Aqaba and Tor) (Table 4). This difference might be caused by the northward directed recolonization from the Gulf of Aden after the last sea-level lowstand (Klausewitz 1989).

#### Direction of gene flow and mean larval dispersal distance

The estimated effective number of migrants among the collection sites using the programme MIGRATE was several fold higher to the North than to the South. This pattern of migration in the Red Sea seems to be influenced by a combination of two factors: currents and spawning season. In summer (April to October), the currents are directed to the North, with a deflection to the Arabian Peninsula. In contrast, during winter (November to March) currents are southward directed and show a deflection to the African coast (Morcos 1970). There are no published studies about the spawning season of *L. quadrilineatus*, but several studies indicate that the spawning season of fishes in the Red Sea is during the summer months (Wahbeh & Ajiad 1985; Froukh 2001; Zekeria 2003). This finding shows that oceanographic conditions and timing of spawning are important factors for determining the direction of dispersal in a marine organism.

Even though the  $\Phi_{ST}$  value of 0.01 is rather low and implies a long larval dispersal distance, estimates based on the isolation-by-distance model show a very low mean larval dispersal distance (0.44–5.1 km) compared to other studies. Buonaccorsi *et al.* (2004) estimated 10 km as a larval dispersal distance per generation of the grass rockfish (*Sebastes rastrelliger*) from the Californian coastline based on 100 individuals km<sup>-2</sup>. Based on a two-dimensional stepping-stone model and assuming a population size of 500 individuals km<sup>-2</sup>, Kinlan & Gaines (2003) estimated mean dispersal distances from genetic isolation-by-distance slopes. Their estimates of dispersal for 28 fish ranged from few kilometres (2.3 km for *Axoclinus nigricaudus*; Tripterygiidae) to several hundred kilometres (527 km for *Sciaenops ocellatus*; Sciaenidae). The majority of fish species have a mean dispersal distance of more than 20 km.

The discrepancy between low  $\Phi_{ST}$  value and low mean larval dispersal distance in *L. quadrilineatus* could be explained by the historical demography of the species, causing a shallow genetic structure due to population expansion. This shows that larval dispersal distances on ecological timescales can be much smaller than on evolutionary timescales (Palumbi 2003).

#### Implications for conservation

Cleaner fishes are very popular and are therefore collected for marine ornamental trade in high numbers. *Larabicus quadrilineatus* is exported by several companies in Egypt, Saudi Arabia, Yemen and Djibouti to Europe and North America. It is one of the top 10 fishes exported from Saudi Arabia (Wood 2001; Khalaf & Ali 2005). Because of the role of cleaner fishes in maintaining the health and diversity of their clients, concerns have been raised about the impact

of removing large quantities of cleaner fishes on the populations of their clients and reef health in general (Wood 2001). Khalaf & Ali (2005) recommended a regular monitoring of *L. quadrilineatus* to prevent overexploitation.

Marine protected areas (MPA) are considered to be an appropriate tool to prevent overexploitation and to ensure the sustainable use of living marine resources. It is proposed that MPAs should be arranged in a network and that the spatial distribution should match the dispersal capabilities of the species to be protected (Palumbi 2003).

The estimated larval dispersal distance for *L. quadrilineatus* suggests a space of less than 4.6 km among marine protected areas to interconnect them by larval dispersal and to enable larvae to settle in protected areas. Gladstone *et al.* (2003) proposed a regional network of 12 MPAs, eight in the Red Sea and four in the Gulf of Aden. The size of two MPAs in the Red Sea was undefined, one in Jordan has a length of 7 km, one in Sudan has an area of 12 km<sup>2</sup> and the remaining MPAs range in their sizes between 300 km<sup>2</sup> and 3310 km<sup>2</sup>. The network of MPAs proposed by Gladstone *et al.* (2003) cannot connect populations of *L. quadrilineatus* on an ecological timescale in the Red Sea and Gulf of Aden, because a maximum larval dispersal distance of about 5 km cannot facilitate exchange between these distant locations. However, seven of the established or proposed MPAs are large enough to enable self-seeding in *L. quadrilineatus*.

In order to enable a sustainable ornamental fishery on the fourline wrasse, the results of this study suggest that populations in the northern and southern Red Sea should be managed separately as two different stocks. The rather low larval dispersal distance of about 5 km needs to be considered in the design of MPAs to enable connectivity and self-seeding. On the one hand, a network of small MPAs could be arranged along the coastline with a maximum distance of about 5 km to enable connectivity among them. Currently, only the network of MPAs along the coasts of Egypt, Israel, and Jordan in the Gulf of Aqaba (Kochzius 2002), and to some extent along the Egyptian coast of the northern Red Sea proper fulfil this requirement. On the other hand, a number of large MPAs with a minimum size of 5 km in diameter are self-seeding and can serve as a source of recruits for areas where ornamental fishery takes place. However, only seven declared MPAs in the Red Sea and Gulf of Aden reported by Gladstone *et al.* (2003) are large enough to enable self-seeding. Further studies on other fishes and invertebrates from more locations are needed to draw a generalised picture of connectivity among populations in the Red Sea in order to design a functioning network of MPAs.

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This study is part of Tawfiq Froukh's PhD thesis investigating the population genetics of ornamental fish in the Red Sea. T. Froukh holds a scholarship from the German Academic Exchange Service (DAAD) and is conducting his PhD in the department of biotechnology and molecular genetics at the University of Bremen. Marc Kochzius is assistant lecturer in the same department. His research interests are population genetics, phylogeography and molecular systematics of marine fishes and invertebrates, as well as the development of DNA micro arrays for the identification of marine organisms.

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