Chapter 5: Morphometric, genetic and ecological comparison of two important demersal species along a gradient from the South West Arm to Nkhata Bay

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Introduction

The state of the demersal trawling fisheries in Lake Malawi has been monitored since the development of this industry in 1968 (Tweddle & Magasa 1989). Various authors have reported the consequences of trawling activities on fish communities, such as the profound size structure modifications, the decreased occurrence of some species and the disappearance of some others in the SEA, and drew the attention to the associated dangers for biodiversity conservation (Turner 1977a, 1977b, Turner 1995, Turner et al. 1995). However, demersal trawling only occurs in a restricted area in the southern part of the lake, representing less than five percent of its total surface. A recent report from the Fisheries Department revealed that most of the exploited species in the SE and SW Arms also occur in the non exploited trawling grounds between Dormira Bay and Nkhata Bay (Banda & Tómasson 1996, Tómasson & Banda 1996). When considering both fisheries management and biodiversity conservation issues, a logical question arises: are the species that have disappeared from the catches in the SEA really endangered if they also occur in other areas of the Lake? Although some of these species are supposed to occur only in restricted parts of the lake (Turner 1996), their degree of stenotopy is unknown and a decreasing stock might be repopulated from other geographical areas. However, a single species present in geographically distant parts of the lake might be composed of a single widespread population, or of different populations (or "stocks" in fisheries language). For the conservation of biodiversity as well as for the fisheries management, it appears crucial to know whether a species is represented by a single population distributed all over the lake, or by different populations with distinctive morphometric, genetic and life-history characteristics. If the threatened species of the southern arms were to be composed of a single widespread population, their disappearance from the exploited trawling areas would not represent an irreversible threat for the biodiversity. On the other hand, if they were distinct populations with different morphometric, genetic and life-history characteristics, their disappearance would lead to an irreversible loss of biodiversity. The starting hypothesis for this study is that shallow-water species have more chances to encounter physical barriers to their movements and therefore are more likely to be structured in distinct populations than deep-water species. A study was undertaken in collaboration with the taxonomists of the project, to compare the morphometrics, the genetics and some life-history traits of two species (a shallow-water and a deep-water species) from four different locations between the SWA and Nkhata Bay (Figure P1). This would allow us to assess whether they are part of a single widespread population or of distinct populations and consequently whether eventual differences are related to geographical distance and depth.

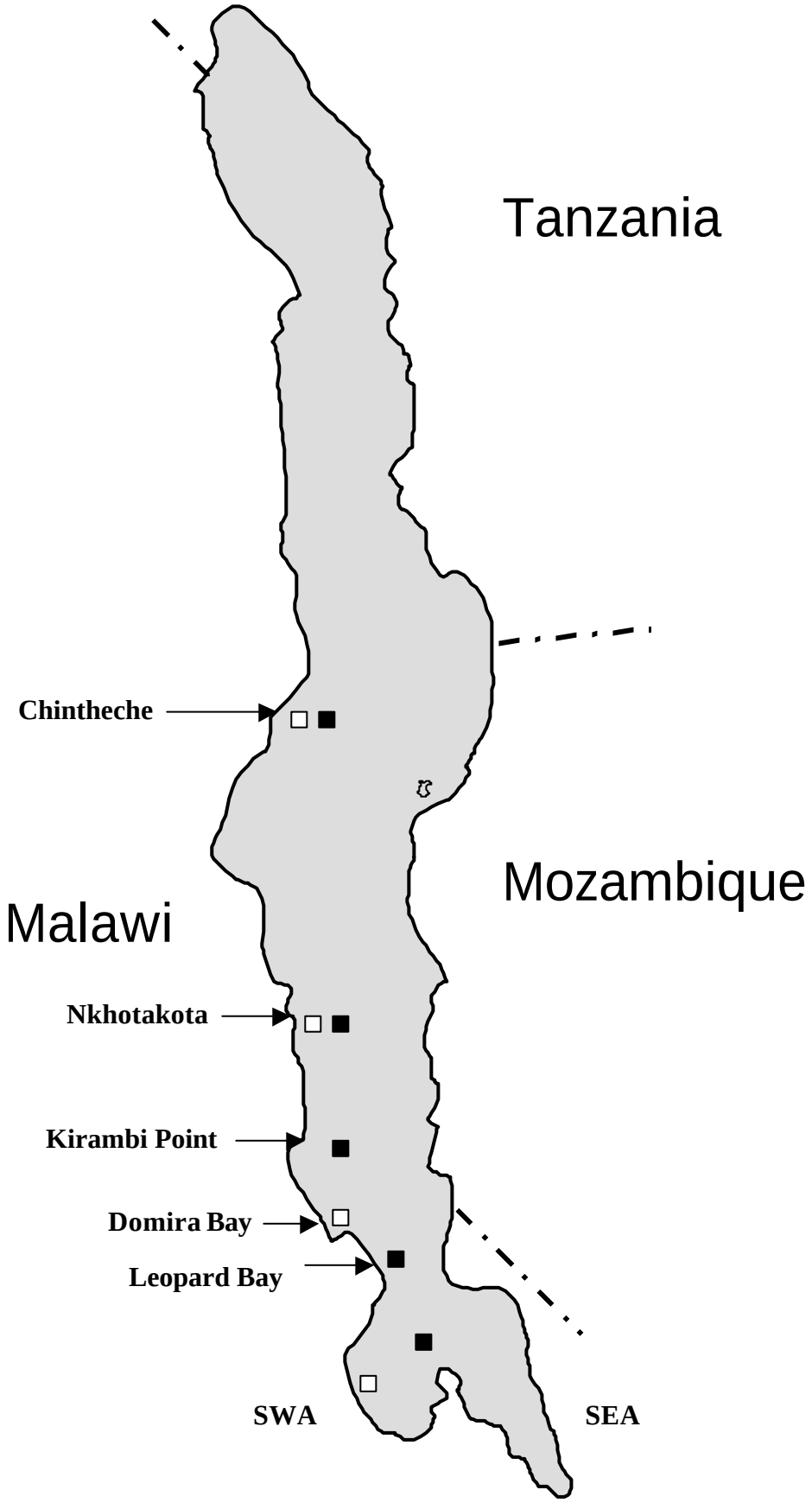

Figure P1. Map of Lake Malawi showing the sampling sites for *Mylochromis anaphyrmus* (white squares) and *Lethrinops gossei* (black squares) populations comparison.

Material and Methods

The shallow-water species, *Mylochromis anaphyrmus*, was sampled in the north of the SWA (where the monthly survey was done), Dormira Bay, Nkhotakota and Chinteche in February 1999. *M. anaphyrmus* was chosen for this study as a representative of the shallow water demersal community. It was one of the target species for the life-history studies within the project. Reasons for this choice are the fact that this species is relatively easily to identify, and common within its distribution range. Eccles & Trewavas (1989) reported that *M. anaphyrmus* is very common over sandy substrates, in waters of 15 to 35 meters depth in the southern part of the lake. They added that it was also reported from Nkhotakota. Konings (1995) stated that *M. anaphyrmus* is endemic to the southern and western parts of the lake. Turner (1996) reported that this species was present in 42 out of 57 experimental trawl catches between 18 and 72 m depth in the South-East Arm where it is often one of the most abundant species. Banda & Tómasson (1996) reported it in the SW and SE Arms as well as from Domira Bay to Nkhata Bay.
The deep-water species, *Lethrinops gossei* was chosen for the same reasons. It is an abundant species in deep water and was one of the target species for life-history studies within the project.. Eccles & Trewavas (1989) reported that it dominates the benthic community at depths of 92-130 m in the SEA. Turner (1996) stated it was one of the dominant species at depths of 90 m or more in the SEA. He also reported what appeared to be a female *L. gossei* caught off Karonga, in the far north of the lake. Banda & Tómasson (1996) reported it in the SW and SE Arms as well as from Domira Bay to Nkhata Bay. It was sampled during the same February 1999 cruise in the north of the SWA, where the monthly survey was done, off Leopard Bay, off Kiramby Point, in Nkhotakota and Chinteche (Figure P1).

It was planned that at each location, 30 specimens of each species were to be collected for morphometric analyses, 75 to 100 specimens for genetics and 100 to 200 specimens for life history traits analysis. *L. gossei* proved to be rare in Chinteche area between Bandawe Point and Sanga Point so that only 35 specimens were caught as a whole.

Morphometric analysis

At the time we were writing this report, the morphometric analysis of *L. gossei* was not yet finished so that only the analysis for *M. anaphyrmus* will be presented here.
Ideally, for each locality 15 specimens of each sex, all of similar size, should have been preserved. For some localities this could not be done. Hence, the results and analyses are based on a lower number of specimens. No female specimens were preserved from Nkhotakota. On all specimens 23 measurements and 17 counts were taken following Snoeks (1994). Two techniques were used to explore and analyse the metric data: Principal Component Analysis (PCA) on the log-transformed measurements and Mann-Whitney U-Tests on the relative measurements (percentages).
List of abbreviations used:
LacD : lachrymal depth ; SnL : snout length ; LJL : lower jaw length ; ChD : cheek depth ; EyeD : eye diameter ; IOW : interorbital width ; HW : head width ; HL : head length ; SL : standard length ; BD : body depth ; DFB : dorsal fin base ; AFB : anal fin base ; PrD : predorsal distance ; PrP : prepectoral distance ; PrV : preventral distance ; PrA : preanal distance ; CPL caudal peduncle length ; CPD caudal peduncle depth ; PhJL : pharyngeal jaw length ; PhJW : pharyngeal jaw width ; DeArL ; dentigerous area length (pharyngeal jaw) ; DeArW : dentigerous area width ; UJT : number of outer teeth in the upper oral jaw ; LJT : number of outer teeth in the lower oral jaw.

Genetic analysis

Microsatellites variability for the two species has been investigated using primers already defined by Rico *et al.* (1993,1996) and Zardoya *et al.* (1996). These primers have been already tested across a panel of very diverse fish species and successfully amplified in non-source species due to a high level of conservation of the flanking regions of these microsatellites. Eight different loci have been tried (Table P1). Numerous amplification conditions were used for each pair of primers with variations in the concentration of $MgCl_2$ and of the annealing temperature.

For locus GMO 2 we always observed two bands of respectively 250 and 290 bp in both species. Locus GMO 132 gave also two bands of 50 and 300 bp. At loci GMO 145 and CIER 51, we did not obtain any band. A 310 bp band was observed for *M. anaphyrmus* at locus CIER 62 and a 250 bp band for both species at locus TMO M25.

Only locus TMO M5 and TMO M 27 gave, for both species, bands varying in size from 300 to 400 bp for TMO M5 and from 230 to 250 bp for TMO M27.

PCR conditions were as follows: DNA was amplified (94°C 60 s, 48°C 60s, 72°C 60s) for 35 cycles in 20 µl volumes (1 x polymerase buffer, 1.5 mM $MgCl_2$, 0.4 mM of each dNTP, 75 ng of each primer, and 1 unit of Taq Promega).

The genetic population structure has been statistically described using Wright (1969) "F statistics" indices with Weir & Cokerham (1984) formulas. Fis measure the deficit of heterozygous due to non-random mating in a (sub)population while Fst measure the loss of heterozygosity due to the subdivision of the sample in two or more populations.

Isolation by distance was tested using Mantel's test (Mantel 1967). Mantel's test consists of a comparison of two matrices (here Fst versus geographical distances). This test determines if there is a correlation between the two matrices. The Mantel coefficient Z is calculated from the real data and then the data are permuted to obtain pseudo matrices and the corresponding pseudo Z' values. The various Z' values obtained are compared to the Z values. If Z is statistically different of all Z', then the two matrices are correlated.

All coefficient and statistical analysis have been done using GENETIX programme (Belkir et al. 1996)

Life history traits analysis

For both species, length-weight relationships, percentage of ripe females, and fecundity were compared between populations. As it needs to be estimated during the peak of the breeding season, which may vary at each site, size at maturity was not compared between populations. Determination of life history traits was done as described in Chapter 2.

Comparison of the percentage of ripe females between populations was carried out using a Kruskal-Wallis one way ANOVA on ranks (Sherrer 1984).

As for most of the fish species, length-weight relationship among populations of the two species were characterised by the following equation: $W = a.L^b$. Direct comparison of populations using the maximum likelihood method (Tomassone et al. 1993) was not possible because regression residuals increased with length. Length and weights were then logarithmically transformed (ln), which lead to a linear relationship between length and weight. Therefore, estimation of differences between populations was investigated by comparing regression lines between length and weight. The regressions were compared by an analysis of covariance (Scherrer 1984) followed by a 2×2 comparison method. First, slopes were compared, and populations whose slopes were not significantly different were then

compared for intercepts. As the type I error increases when more than two populations are compared pairwise (Scherrer 1984), a probability α' was calculated so that the overall α (α = 0.05 in our case) was maintained over the k(k-1)/2 comparisons. The new α' was calculated by the following formula: $\alpha' = 1-(1-\alpha)^{2/(k(k-1))}$.
Rather than comparing regressions between fecundity and body weight over a narrow weight range, we compared relative fecundity (fecundity per kg of body weight). Over the weight ranges studied, no correlation was found between relative fecundity and body weight, as it may happen in cichlids (Legendre 1992). Relative fecundity was compared using one way ANOVA followed by Tukey's all pairwise multiple comparison test (Scherrer 1984).

Table P1. Microsatellites loci and primers tested.

Locus	Primers	references
GMO 2	F ccctcagattcaaatgaagga	Rico *et al*., 1993
	R gtgtgagatgactgtgtcg	Rico *et al*., 1996
GMO 132	F ggaacccattggattcaggc	
	R cgaaaggacgagccaataac	
GMO 145	F gcattgtaggaacaacaattaac	
	R gtgcatgtgctcattatagc	
CIER 51	F gccaaaacactgacgaggtga	
	R tttgcgcaagcttcaggatga	
CIER 62	F ggtgctgtcacttttggccac	
	R aactctgctggtcgccactcc	
TMO M5	F gctcaatattctcagctgacgca	Zardoya *et al*., 1996
	R aga aca gcg ctg gct atg aaa agg t	
TMO M25	F ctgcagtggcacatcaagaatgagcagcggt	
	R caagaacctttcaagtcattttg	
TMO M27	F aggcaggcaattaccttgatgtt	
	R tactaactctgaaagaacctgtgat	

Results and Discussion

Morphometric analysis

Principal Component Analysis.

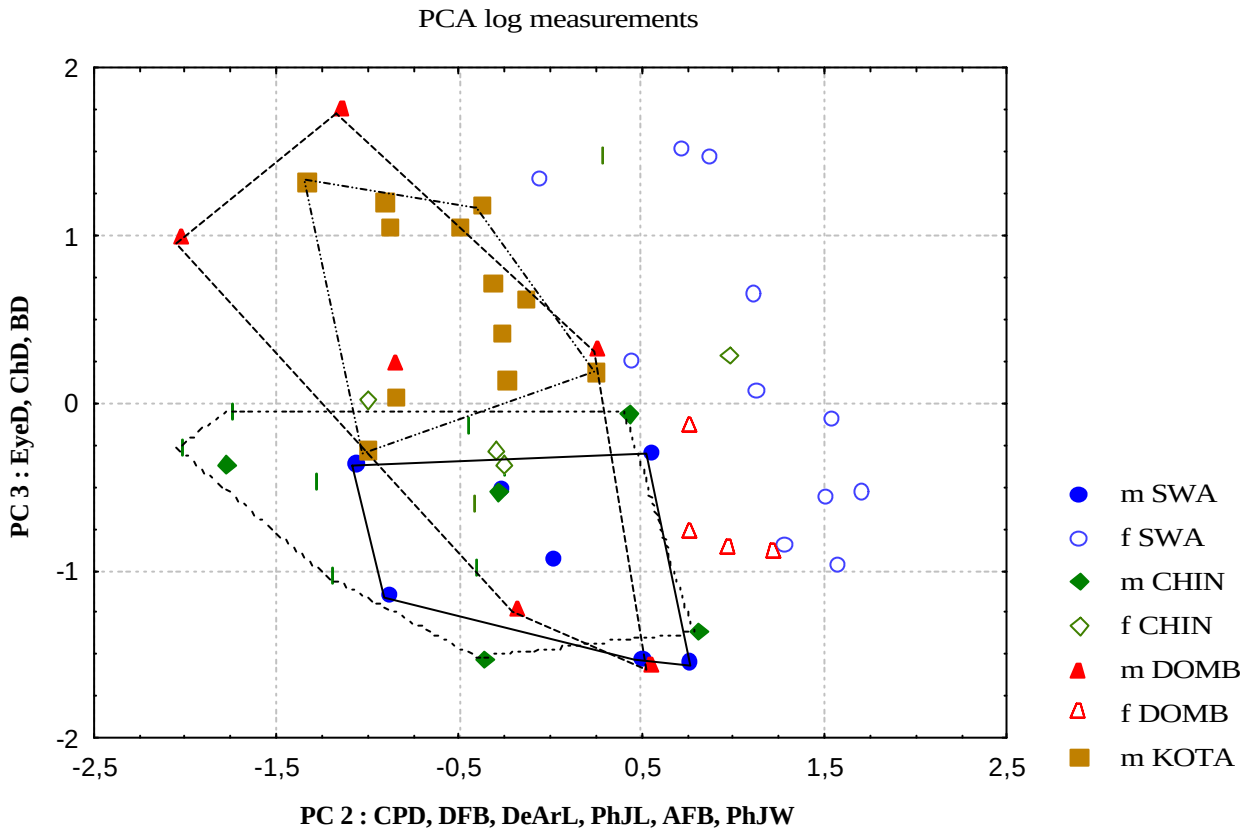

Figure P2. PCA of the log-transformed measurements, all specimens included (n=60).

A first PCA (Figure P2) on all specimens showed that morphological differences between males and females are generally larger than the differences observed between the populations. Male and female specimens are partly separated on PC2; female specimens are shifted towards the positive, male specimens towards the negative side of PC2. Polygons are drawn for the males only. All further analyses were therefore based on either males or females alone.

In this plot SWA and CHIN males are relatively well separated on PC3 from the KOTA males. The DOMB males overlap with all other populations on PC3. This result is surprising since the South West Arm and Chinteche are the most distant localities sampled and we would have expected differences to be most prominent between these two populations. The third component is mainly defined by eye diameter and cheek depth (both measurements are obviously strongly correlated). This surprising result is probably due to the fact that all specimens were included in this analysis. The larger morphological differences that are found between sexes are mixed and analysed together with the inter population morphological differences and may therefore 'blur' the latter ones. Therefore another PCA was done,

including only male specimens (Figure P3). The plot of the specimens on the second and third principal component shows a large overlap between almost all populations. Some distinction appears between the SWA and KOTA males on PC2. The SWA males are mainly on the positive part of PC2, the KOTA males are shifted towards the negative part of PC2.

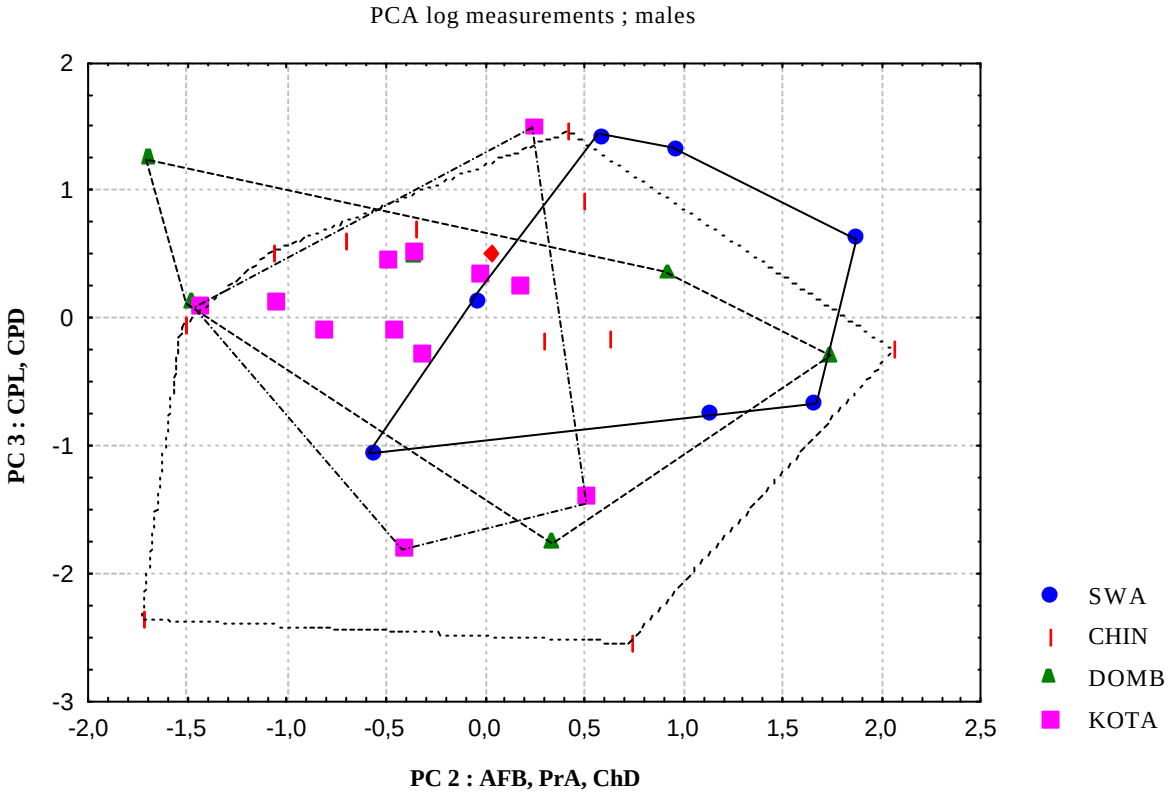

Figure P3. PCA of the log-transformed measurements, male specimens (n=37).

The factor loadings from this analysis indicated that the third component was almost entirely defined by a single character, the caudal peduncle length. After verification of the measurements, it appeared that one of the males collected in Chinteche was aberrant for this character. However, the measurement was checked and found to be correct. Consequently, the third principal component is unreliable to distinguish the populations (Figure P3).

We therefore added another plot using the second and fourth principal component (Figure P4). On PC4, the CHIN males are relatively well separated from the males of the other populations. One CHIN specimen was aberrant and scored high on PC4 (Figure P4: specimen marked with arrow). This was again the same aberrant male with the high CPL value.

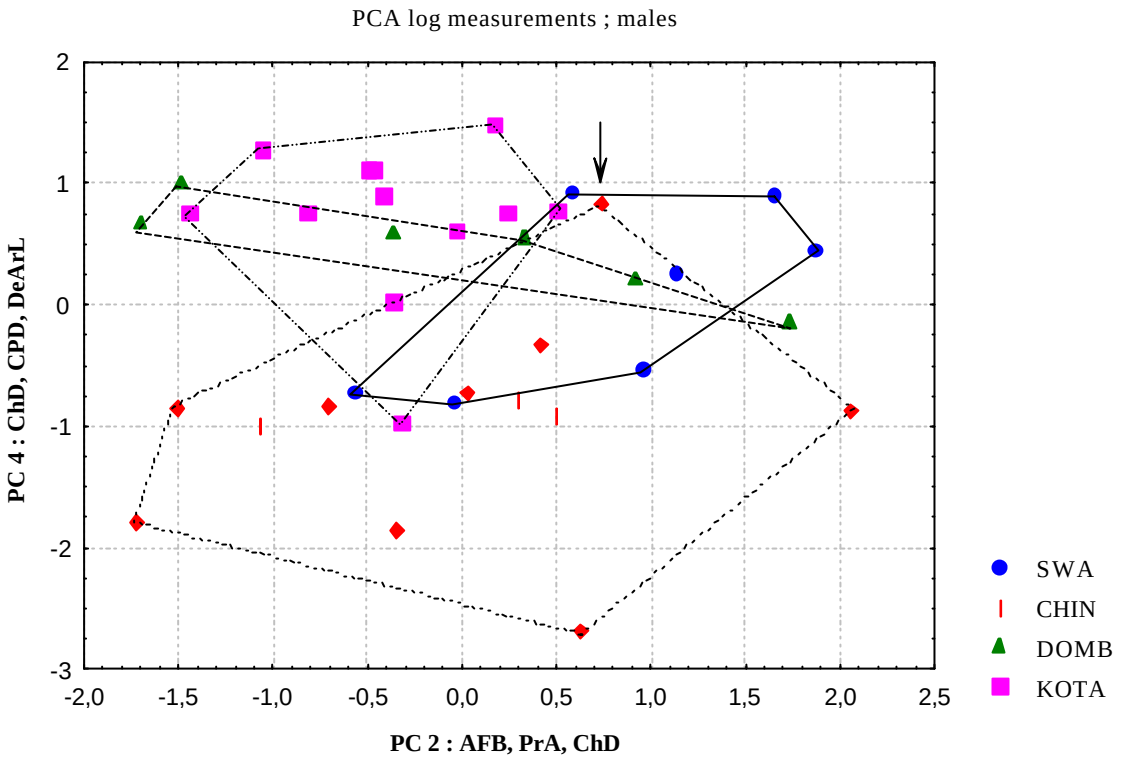

Figure P4. PCA of the log-transformed measurements, male specimens (n=37).

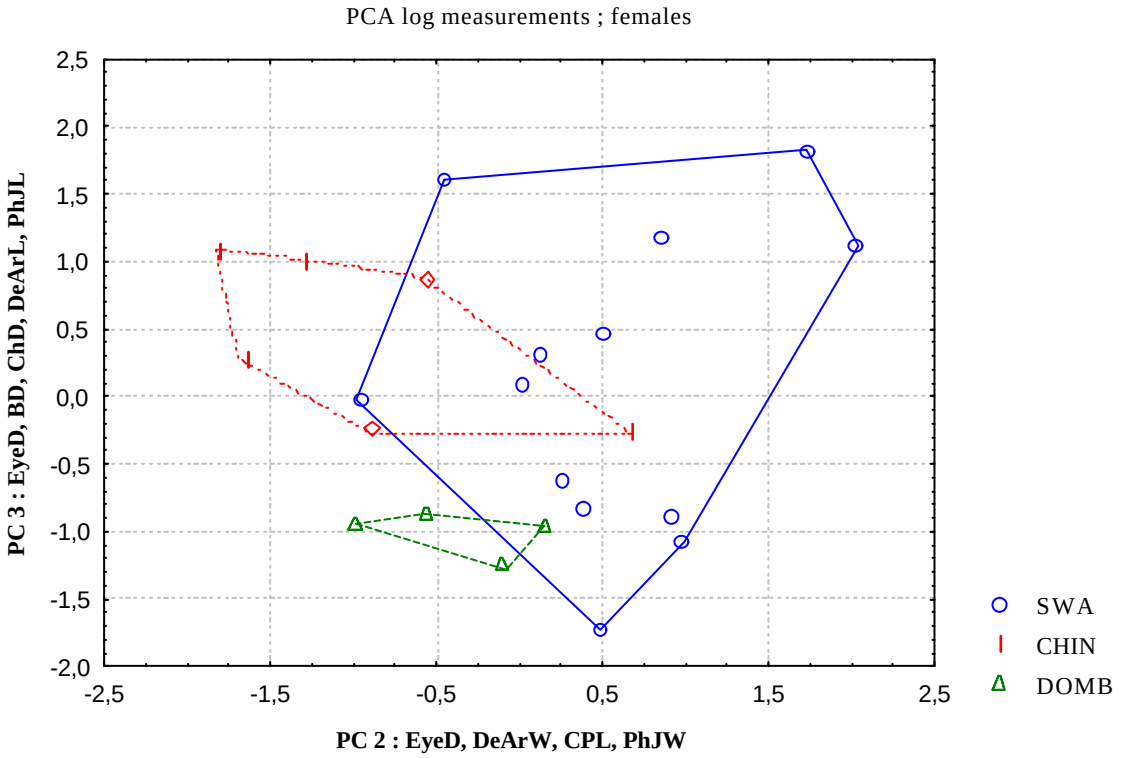

Figure P5. PCA of the log-transformed measurements, female specimens (n=23).

A PCA on the log-transformed measurements for the female specimens was done as well (Figure P5). Here we see that the SWA population is morphologically more diverse than

the other two (there are obviously more observations from the SWA which explains part of the higher variability) and overlaps with both other populations. On PC3 the CHIN females are neatly separated from the DOMB females. Factor loadings on PC3 indicate that they mainly differ in eye diameter and body depth.

Further PC analyses were made to compare all populations two-by-two. These analyses did not show clear or better results than the previous PC analyses. Large overlaps were observed for almost all PCA analyses when only two populations were included.

Mann-Whitney U-Test.

The Mann-Whitney U-test was used to analyse morphological differences between all populations. Data used for these analyses are the relative measurements (percentages).
We used only specimens of the same sex for these tests. To avoid allometric inferences, specimens were selected on the basis of their standard length for each test between two populations, so that the p-value for the standard length was close to 0.5 or larger. So, for each test a different subset of specimens was used, and in most cases not all specimens from a given locality were included in the analyses. A first test was done including male specimens from all populations.

Table P2 gives an overview of the results of the analysis between all four populations.

Table P2. Comparison of all four populations using the Mann-Whitney U-Test. Above diagonal the number of characters for which significant differences were found, p values as follows : * indicates p < 0,05; ** p < 0.005; *** p < 0.0005. Below diagonal p value for SL and number of specimens from each population used for the comparison. Characters for which significant differences were found are given and discussed in the text.

	SWA	DOMB	KOTA	CHIN
SWA		2 *	3 * 1 **	4 * 3 **
DOMB	p=0.52 for SL n SWA 19 n DOMB 10		1 * 1 **	3 * 1 **
KOTA	p=0.86 for SL n SWA 9 n KOTA 9	p=0.56 for SL n DOMB 8 n KOTA 9		1 ** 1 ***
CHIN	p=0.46 for SL n SWA 18 n CHIN 16	p=0.93 n DOMB 6 n CHIN 12	p=0.52 n KOTA 10 n CHIN 14	

The number of characters that differ between populations increases with increasing distance between the populations. In addition, the morphological differences found between populations become more significant with increasing distance.

The characters that were found to be significantly different between the four populations are:

SWA-DOMB.	* : EyeD/HL and DeArW/PhJW
SWA-KOTA.	* : HL/SL, PrD/SL and DeArW/PhJW
	** : PhJL/HL.
SWA-CHIN.	* : LacD/HL, SnL/HL, LJL/HL and CPD/SL
	** : HL/SL, PrD/SL and PhJL/HL
DOMB-KOTA.	* : EyeD/HL
	** : DeArW/PhJW
DOMB-CHIN.	* : LJL/HL, ChD/HL, IOW/HL and DeArW/PhJW
KOTA-CHIN.	** : LacD/HL
	*** : ChD/HL

The table and short overview show that all populations differ from each other for at least two characters. Some populations differ in a particular character from all or most other populations. For the dentigerous area width of the pharyngeal jaw the SWA population is significantly different from the DOMB and KOTA populations but not from the CHIN population. Moreover, for this character the DOMB population differs from all other populations. For the eye diameter a significant difference was observed between the DOMB population and the SWA and KOTA, but again not when compared to the CHIN population. The CHIN population is furthermore significantly different from the SWA and KOTA populations but not from the DOMB Population for the lachrymal depth. No consistent differences were found between the Nkhotakota population and the three other populations.

Differences between females from three populations were analysed as well. Unfortunately three out of four female specimens from the DOMB population were significantly larger than the females from the SWA and CHIN populations. So, no comparison between DOMB and the two other could be made. As a result, only the SWA and CHIN populations could be compared. Seven SWA and five CHIN female specimens were used in the analysis, the p value for SL was 0.68. We found only two significant differences ($p<0.05$ but >0.005) for the ChD/HL and DeArL/PhJL.

Meristics

For only three characters (the number of outer teeth in the oral jaws and the number of dorsal fin spines) significant differences were found between the populations.
Plots of the number of outer teeth in the upper and lower oral jaws are given. Specimens are categorised by locality and sex. Polygons are only given for male specimens (Figures P6 and P7).
Both graphs show that female specimens cannot be reliably distinguished on the basis of this character. The SWA females overlap with all other populations for the number of outer teeth both in upper and lower jaws. The only difference was noted for the outer teeth in the upper jaw, the female DOMB specimens have a higher number than the female CHIN specimens (52-58 vs 43-52).

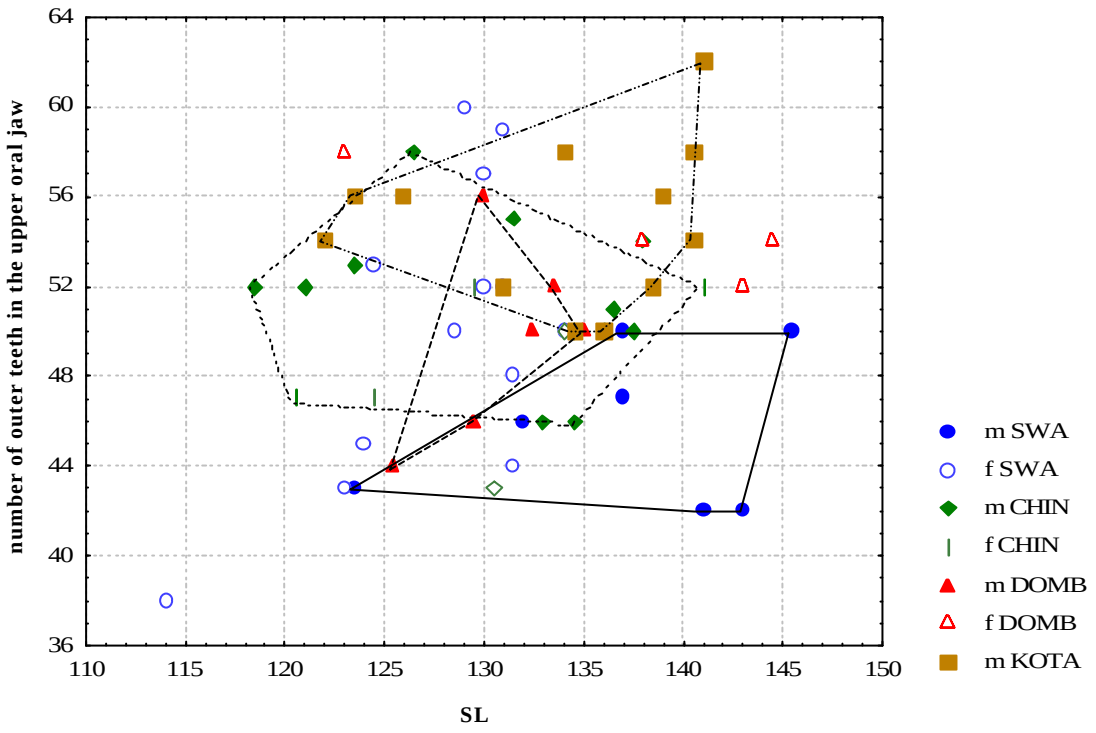

Figure P6. Plot of the number of outer teeth in the upper oral jaw vs SL, male specimens marked with polygons (n=60).

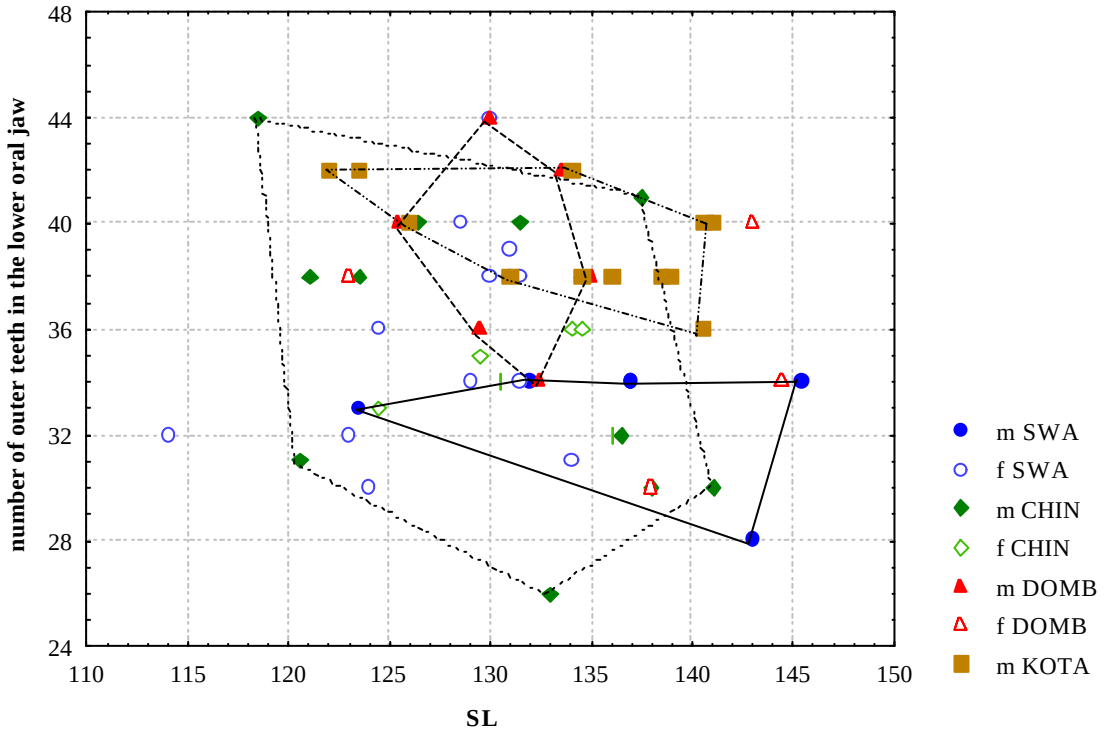

Figure P7. Plot of the number of outer teeth in the lower oral jaw vs SL, male specimens marked with polygons (n=60).

For the male specimens, the differences on teeth number are clearer. The SWA males are clearly different from the KOTA males, in having a lower number of outer teeth (42-50 vs 50-62 UJT and 28-40 vs 36-42 LJT respectively). For the outer teeth number in the upper oral jaw, the range of both other populations (DOMB and CHIN) is intermediate. For the outer teeth number in the lower jaw, the DOMB population has a relatively large range that overlaps completely with the KOTA population and is different from the SWA population. The CHIN males have the largest range and overlap with all other populations.

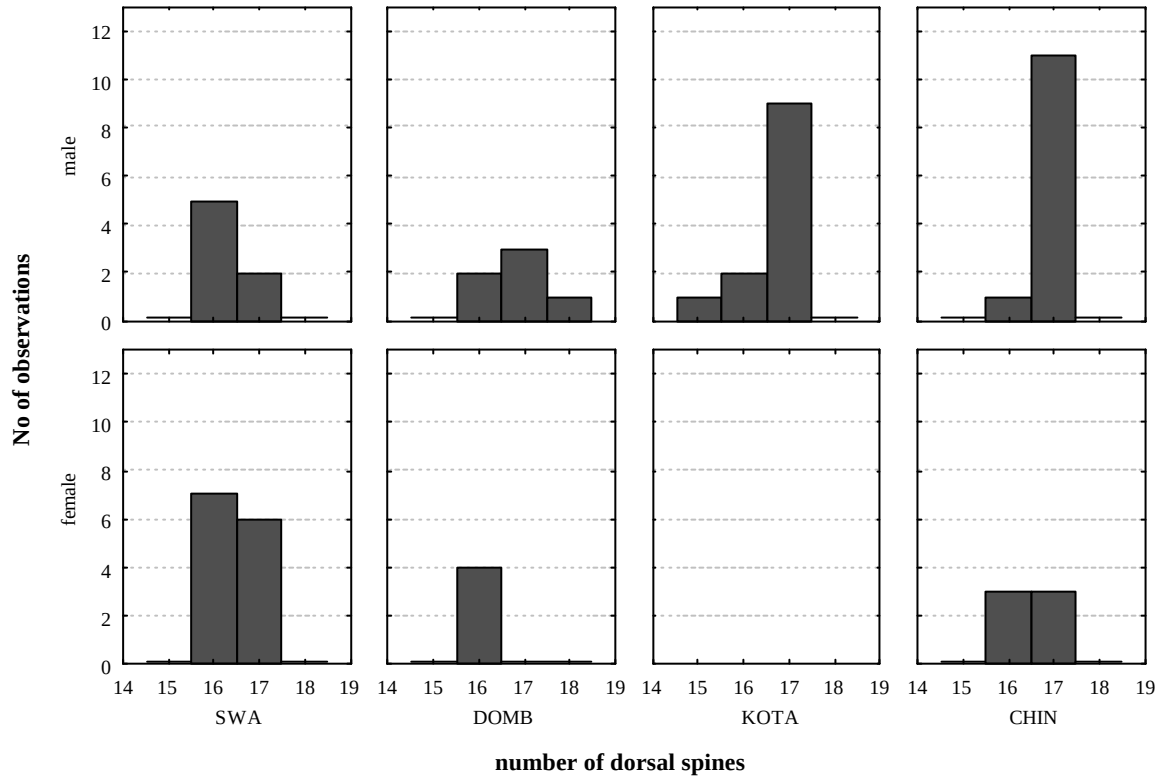

Figure P8. Histogram with the number of dorsal spines, categorised by sex and locality. (n=60).

The histogram of the number of dorsal fin spines (Figure P8) shows that in males there was a slight increase in spine number along the south-north axis. This pattern was not visible in females. In the SWA population, the modal number of dorsal spines is 16 for males (about 2/3 16, 1/3 17); for the DOMB males both numbers are about equal; for the KOTA and particularly the CHIN males the majority have 17 dorsal fin spines.

These results show the presence of small but significant morphological differences between all *M. anaphyrmus* populations examined. The amount of difference is clearly linked to geographical distance.

Table P3. Allelic frequencies observed in the four populations of *M. anaphyrmus*: South West Arm (SW), Domira Bay (DO), Nkhotakota (NK) and Chinteche (CH).

	SW	DO	NK	CH
Locus TMO M5				
(N)	21	15	14	4
295	0.00	0.00	0.00	0.25
313	0.05	0.00	0.00	0.00
315	0.00	0.13	0.00	0.00
317	0.02	0.10	0.00	0.00
321	0.05	0.00	0.04	0.00
323	0.43	0.20	0.00	0.00
327	0.00	0.03	0.00	0.50
329	0.12	0.03	0.04	0.25
331	0.00	0.13	0.14	0.00
333	0.00	0.03	0.21	0.00
335	0.17	0.07	0.29	0.00
337	0.02	0.17	0.21	0.00
339	0.00	0.00	0.07	0.00
353	0.02	0.00	0.00	0.00
361	0.05	0.00	0.00	0.00
363	0.00	0.07	0.00	0.00
365	0.02	0.00	0.00	0.00
367	0.02	0.00	0.00	0.00
369	0.02	0.03	0.00	0.00
H	0.79	0.90	0.83	0.71
H obs.	0.48	0.67	0.50	0.50
Locus TMO M27				
(N)	17	30	15	21
236	0.00	0.02	0.00	0.05
238	0.74	0.55	0.87	0.45
240	0.00	0.08	0.03	0.05
242	0.26	0.23	0.10	0.45
244	0.00	0.12	0.00	0.00
H	0.40	0.63	0.25	0.60
H obs.	0.29	0.60	0.13	0.67
H obs.	0.39	0.63	0.32	0.58
A	7.00	8.00	5.00	3.50

Genetic analysis

Allelic frequencies obtained are summarised in Table P3 and P4 for *Mylochromis anaphyrmus* and *Lethrinops gossei,* respectively.

Mylochromis anaphyrmus

Four populations were investigated: South West Arm (SWA), Domira Bay (DOMB), Nkhotakota (KOTA) and Chinteche (CHIN). 19 alleles were observed at locus TMO M5 and 5 at locus TMO M27. Fis and Fst values are summarised in Table P5 and Table P6, respectively.

Table P5. Fis Values in *M. anaphyrmus* populations for both TMO M5 and TMO M27 loci. Statistically significant values are underlined.

Locus	TMO M5	TMO M27
South West Arm	0.3976	0.2727
Domira Bay	0.2689	0.0526
Nkhotokota	0.4052	0.4667
Chinteche	0.323	-0.1133

At locus TMO M5, three out of four Fis values were statistically significant indicating that there was an excess of homozygous. Only the Chinteche sample did not exhibit such homozygous excess but it was likely due to the low sample size (4). No significant values have been observed for locus TMO M27.

Table P6. Fst Values in *M. anaphyrmus* populations for all loci combined, TMO M5 alone and TMO M27 alone. Statistically significant values are underlined.

All loci	DOMB	KOTA	CHIN
SWA	0.038	0.1057	0.1563
DOMB	_	0.0639	0.0968
KOTA		_	0.2018
Locus TMO M5	**DOMB**	**KOTA**	**CHIN**
SWA	0.047	0.1348	0.1984
DOMB	_	0.0453	0.1341
KOTA		_	0.1851
Locus TMO M27	**DOMB**	**KOTA**	**CHIN**
SWA	0.035	0.0257	0.0824
DOMB	_	0.0947	0.0378
KOTA		_	0.2304

Table P4. Allelic frequencies observed in the five populations of *L. gossei*: South West Arm (SW), Leopard Bay (LB), Kiramby Point (KP), Nkhotakota (NK) and Chinteche (CH).

	SW	LB	KP	NK	CH
Locus TMO M5					
(N)	17	19	2	11	3
237	0.00	0.00	0.00	0.05	0.00
293	0.00	0.00	0.00	0.14	0.00
299	0.00	0.00	0.00	0.18	0.00
317	0.00	0.03	0.00	0.00	0.00
319	0.00	0.03	0.00	0.00	0.00
321	0.00	0.00	0.00	0.23	0.00
323	0.00	0.00	0.50	0.00	0.00
325	0.00	0.05	0.00	0.00	0.50
327	0.09	0.05	0.25	0.05	0.00
329	0.03	0.00	0.00	0.00	0.00
331	0.06	0.18	0.25	0.00	0.00
333	0.03	0.00	0.00	0.00	0.00
335	0.12	0.16	0.00	0.00	0.17
337	0.09	0.00	0.00	0.00	0.00
339	0.03	0.13	0.00	0.00	0.33
341	0.09	0.03	0.00	0.00	0.00
343	0.03	0.05	0.00	0.00	0.00
345	0.06	0.08	0.00	0.00	0.00
347	0.03	0.00	0.00	0.00	0.00
349	0.00	0.03	0.00	0.00	0.00
351	0.00	0.03	0.00	0.00	0.00
353	0.00	0.05	0.00	0.00	0.00
355	0.12	0.00	0.00	0.23	0.00
357	0.06	0.03	0.00	0.00	0.00
359	0.00	0.03	0.00	0.00	0.00
361	0.00	0.00	0.00	0.14	0.00
365	0.03	0.03	0.00	0.00	0.00
369	0.12	0.00	0.00	0.00	0.00
373	0.00	0.03	0.00	0.00	0.00
379	0.03	0.00	0.00	0.00	0.00
H	0.94	0.92	0.83	0.86	0.73
Hobs.	0.88	0.63	0.50	0.64	0.33
Locus TMO M27					
(N)	27	29	24	27	6
219	0.04	0.00	0.00	0.00	0.00
225	0.02	0.00	0.00	0.00	0.00
229	0.00	0.00	0.04	0.00	0.00
232	0.00	0.02	0.00	0.00	0.00
236	0.00	0.00	0.08	0.04	0.00
238	0.78	0.86	0.56	0.81	0.83
240	0.02	0.05	0.00	0.00	0.00
242	0.15	0.07	0.29	0.15	0.17
244	0.00	0.00	0.02	0.00	0.00
Hn.b.	0.38	0.25	0.60	0.32	0.30
Hobs.	0.26	0.24	0.54	0.22	0.33
Hobs	0.57	0.44	0.52	0.43	0.33
A	10.5	10.5	4.0	5.0	2.5

When using the two loci, all the populations were differentiated with statistically significant Fst values. All the populations but one were differentiated with locus TMO M5 alone and all but two with locus TMO M27 alone.

Mantel test (using Fst and geographical distances matrices) obtained with locus TMO M5 indicated a positive correlation between Fst and the geographical distances between populations (no Z' value superior to Z) (Table P7).

Table P7. Mantel matrices for Fst values (upper matrices) using all loci, TMO M5 alone or TMO M27 alone, and for geographical distances between *M. anaphyrmus* populations (lower matrices)

All loci	SWA	DOMB	KOTA	CHIN
SWA		0.038	0.1057	0.1563
DOMB	60	_	0.0639	0.0968
KOTA	135	75	_	0.2018
CHIN	275	215	140	_
Z=226.81, 24 permutations, 5 values ≥ Z				

Locus TMO M5	SWA	DOMB	KOTA	CHIN
SWA		0.047	0.1348	0.1984
DOMB	60	_	0.0453	0.1341
KOTA	135	75	_	0.1851
CHIN	275	215	140	_
Z=267.44, 24 permutations, 0 values ≥ Z				

Locus TMO M27	SWA	DOMB	KOTA	CHIN
SWA		0.035	0.0257	0.0824
DOMB	60	_	0.0947	0.0378
KOTA	135	75	_	0.2304
CHIN	275	215	140	_
Z=151.43, 24 permutations, 16 values ≥ Z				

Lethrinops gossei

Five populations were investigated: South West Arm (SWA), Leopard Bay (LB) Kiramby Point (KP), Nkhotakota (KOTA) and Chinteche (CHIN). 30 alleles have been observed at locus TMO M5 and 9 at locus TMO M27.
Fis and Fst values are summarised in Table P8 and Table P9, respectively.

Table P8. Fis Values in *L. gossei* populations for both TMO M5 and TMO M27 loci. Statistically significant values are underlined.

Locus	TMO M5	TMO M27
South West Arm	0.0698	0.318
Leopard Bay	0.3229	0.0485
Kiramby Point	NA	0.120
Nkhotokota	0.2708	0.3067
Chinteche	NA	-0.1111

Significant Fis values were observed at Leopard Bay and Nkhotakota for locus TMO M5 and at South West Arm for locus TMO M27.

Table P9. Fst values in *L. gossei* populations for all loci, TMO M5 alone and TMO M27 alone. Statistically significant values are underlined.

All loci	LB	KP	KOTA	CHIN
SWA	0.0092	0.0485	0.0403	0.0595
LB	_	0.0514	0.0727	0.0175
KP		_	0.0886	0.07133
KOTA		_		0.1080
Locus TMO M5	LB	KP	KOTA	CHIN
SWA	0.0108	0.0462	0.0608	0.0999
LB	_	0.0061	0.0932	0.0294
KP			0.0983	0.0938
KOTA			_	0.1584
Locus TMO M27	LB	KP	KOTA	CHIN
SWA	0.0046	0.0540	-0.0178	-0.0600
LB	_	0.1404	0.0012	-0.0263
KP		_	0.0693	0.0321
KOTA			_	-0.0636

Fst values were statistically significant between KOTA and SWA, KOTA and LB and between SWA and CHIN, mainly due to locus TMO M5.

All Mantel tests did not indicate any correlation between Fst and geographical distances matrices for *L. gossei* populations (Table P10**)**.

Table P10. Mantel matrices for Fst values (upper matrices) using all loci, TMO M5 alone or TMO M27 alone, and for geographical distances between *L. gossei* populations (lower matrices).

All locus	SWA	LB	KP	KOTA	CHIN
SWA	_	0.009	0.049	0.040	0.060
LB	31	_	0.051	0.073	0.018
KP	63	32	_	0.089	0.071
KOTA	123	92	60	_	0108
CHIN	243	212	180	120	_
Z =131.82, 120 permutations, 75 values ≥ Z					

Locus TMO M5	SWA	LB	KP	KOTA	CHIN
SWA	_	0.011	0.046	0.061	0.100
LB	31	_	0.006	0.093	0.029
KP	63	32	_	0.098	0.094
KOTA	123	92	60	_	0.158
CHIN	243	212	180	120	_
Z =183.51, 120 permutations, 39 values ≥ Z					

Locus TMO M27	SWA	LB	KP	KOTA	CHIN
SWA	_	0.005	0.054	-0.018	-0.060
LB	31	_	0.140	0.001	-0.026
KP	63	32	_	0.069	0.032
KOTA	123	92	60	_	-0.064
CHIN	243	212	180	120	_
Z =-23.82, 120 permutations, 117 values ≥ Z					

Despite the high number of specimens analysed at each location for each species, some populations are represented by a very low number of specimens due to technical problems. Both loci are polymorph and it is evident that the polymorphism of low sampled populations is underestimated (for every locus and species, the number of alleles increases with the sample size). Nevertheless, these data can be analysed keeping in mind that the results only indicate tendencies and have to be confirmed with more specimens and even more loci.

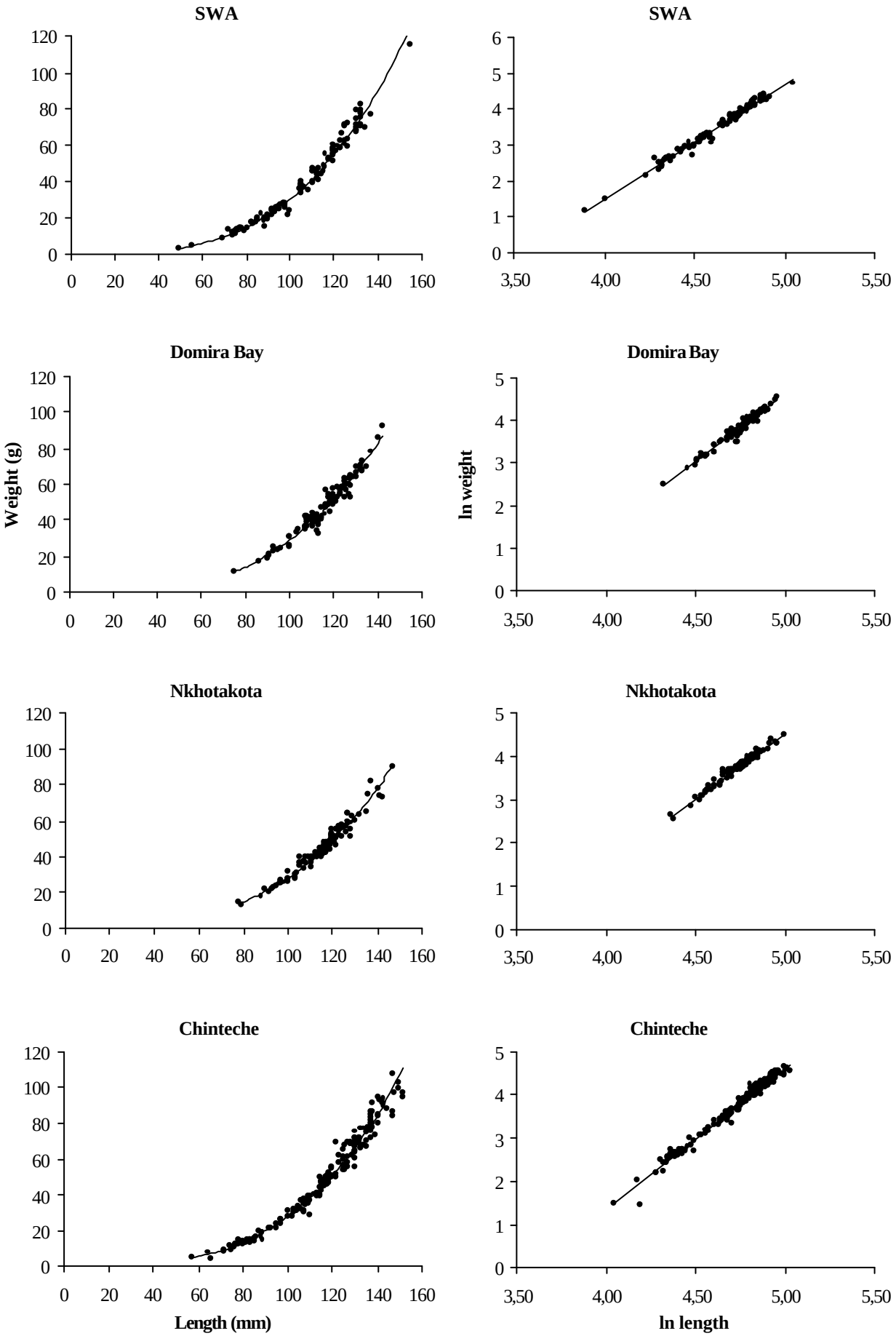

Figure P9. Length-weight relationships for *Mylochromis anaphyrmus* females at four locations in Lake Malawi.

In both species we found some significant Fis values. It is very likely that, to some extent, a non-random mating exists. Under the neutral model, heterozygous deficits are expected either when there is a mixture of different reproductive units inside a sample ("classical" Wahlund effect) or when there is family structuring or inbreeding within this sample. Typically when two or more populations are mixed in a sample, linkage desequilibrium (non randomly association between alleles of different loci) is observed as well as heterozygous deficits. Linkage desequilibrium can also be caused by random drift in small size population or by strong selection, two hypotheses that can be rejected here. For both species and loci, no linkage desequilibrium has been detected, hence local inbreeding might explain the observed results.
We also have to pay attention to possible null alleles, which can create artificial heterozygous deficits: in this case also, no linkage desequilibrium was observed.
Statistically significant Fst values were observed in both species for both loci and populations of *M. anaphyrmus* were more differentiated than those of *L. gossei.* It is then possible that the different populations of both species are genetically differentiated.
The results of the Mantel test indicated a correlation between Fst values and the geographical distances between all pairs of populations in *M. anaphyrmus*. This could correspond to an isolation by distance model, a model in which populations exchange few migrants, mainly with close populations.

Life history traits analysis

Mylochromis anaphyrmus:

At every sample site, fish were collected in February 99, which corresponded with the beginning of the breeding season in the SWA. The timing of breeding season for the species is unknown in Domira Bay, Nkhotakota and Chinteche. However, the percentages of ripe females were not significantly different among sites (One way ANOVA on ranks Kruskal-Wallis, H=2.916, 3 df, p=0.405): 6% in SWA, 4% in Domira Bay, 9% in Nkhotakota and 6% in Chinteche.

Length-weight relationships at each location are given in Table P11. Comparison of length-weight relationships after Log transformation (Figure P9) revealed significant differences among populations ($F_{3,535}$=6.145, p<0.0001). Multiple comparison procedure indicated that every population differed from the others, either by slope or intercept differences, except Domira Bay and Chinteche (Table P12).

Table P11. Comparison of *Mylochromis anaphyrmus* populations. Total number of fish (N), length (L)_weight (W) relationships, determination coefficient (R^2), number of fish used for fecundity calculation (n), mean relative fecundity (MRF) ± SEM.

Location	N	Relationships	R^2	n	MRF
SWA	126	$W = 0.00001 \times L^{3.2353}$ $\ln(W) = 3.2373 \times \ln(L) - 11.4997$	0.984	7	2391 ± 94
Domira Bay	107	$W = 0.00001 \times L^{3.1446}$ $\ln(W) = 3.1446 \times \ln(L) - 11.1180$	0.957	3	3469 ± 273
Nkhotakota	122	$W = 0.00003 \times L^{3.0204}$ $\ln(W) = 3.0204 \times \ln(L) - 10.5690$	0.971	11	2494 ± 121
Chinteche	185	$W = 0.000007 \times L^{3.2946}$ $\ln(W) = 3.2946 \times \ln(L) - 11.8430$	0.984	11	2281 ± 77

Table P12. Comparison of length-weight relationships (ln transformed) among populations of *Mylochromis anaphyrmus*. ns : non significant, *: significant slope differences ($p<0.05$), #: significant intercept differences ($p<0.05$).

	Domira Bay	Nkhotakota	Chinteche
SWA	#	*	#
Domira Bay		#	ns
Nkhotakota			*

Relative fecundity (Table P11) was also significantly different among populations ($F=9.88$, $p<0.001$), though only the Domira Bay population differed from all the others (Table P13). Despite the low number of observations for the Domira Bay population, all three fish were above 3000 eggs per kg, which only one female reached in all the other populations pooled.

Table P13. Comparison of relative fecundity among populations of *Mylochromis anaphyrmus*. ns : non significant, *: significant differences ($p<0.05$).

	Domira Bay	Nkhotakota	Chinteche
SWA	*	ns	ns
Domira Bay		*	*
Nkhotakota			ns

Lethrinops gossei:

As for *M. anaphyrmus*, fish from every location were collected in February 1999, which corresponded to the peak of breeding activity for this species in the SWA. The percentage of ripe females differed significantly among populations ($H=66.866$, 4 df, $p<0.001$): 24% in SWA, 0% in Leopard Bay, 12% at Kiramby Point, 10% in Nkhotakota and 31% in Chinteche. SWA population differed from all the other populations except Chinteche, and Leopard Bay differed from all the others as well (Table P14). The absence of significant differences between the Chinteche population and the others is caused by the low number of fish analysed for Chinteche (13).

Table P14. Comparison of percentages of ripe females among populations of *Lethrinops gossei*. ns : non significant, *: significant differences ($p<0.05$).

	Leopard Bay	Kiramby Point	Nkhotakota	Chinteche
SWA	*	*	*	ns
Leopard Bay		*	*	*
Kiramby Point			ns	ns
Nkhotakota				ns

Length-weight relationships at each location are given in Table P15.

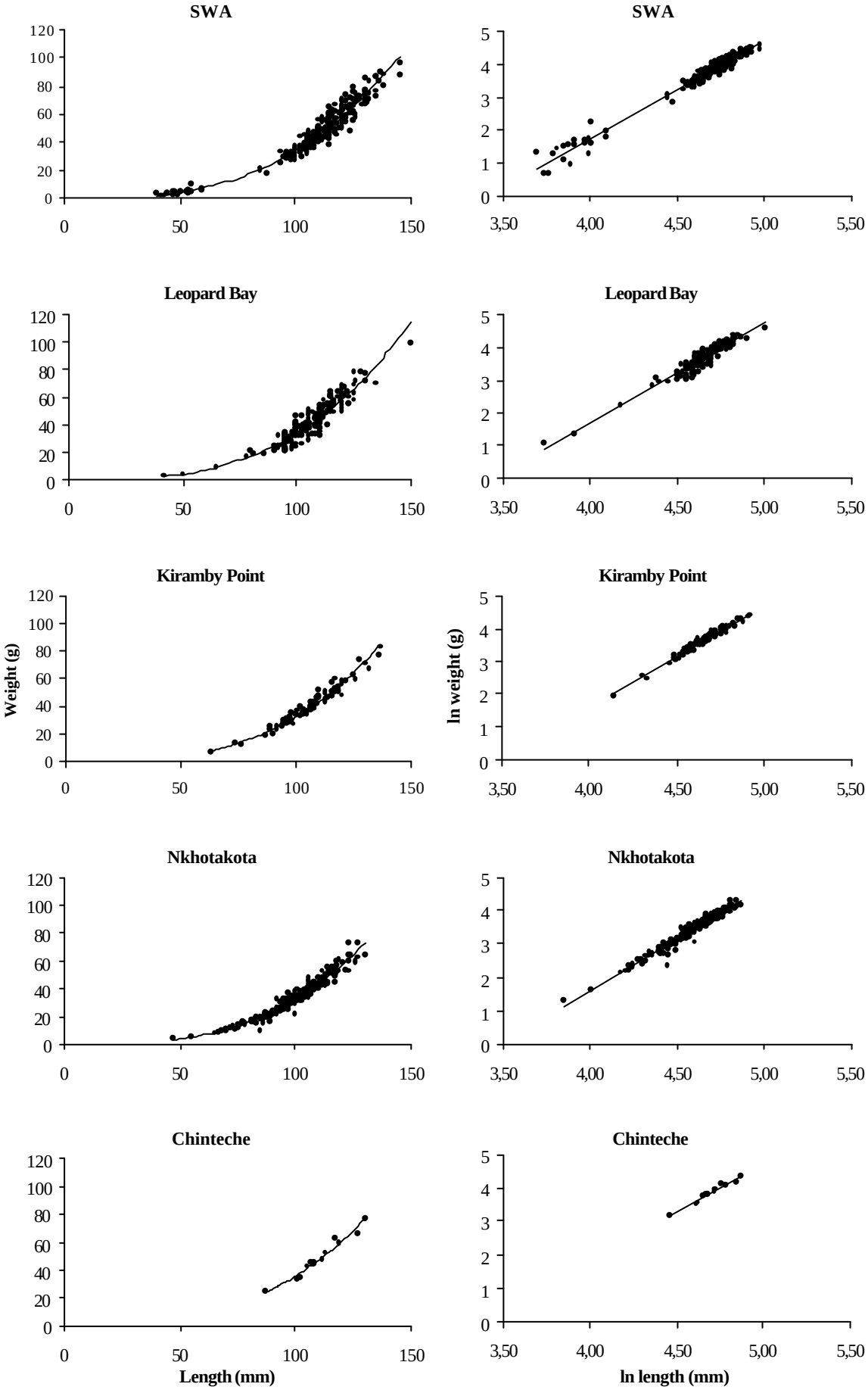

Figure P10. Length-weight relationships for *Lethrinops gossei* females at five locations in Lake Malawi.

Table P15. Comparison of *Lethrinops gossei* populations. Total number of fish (N), length (L)_weight (W) relationships, determination coefficient (R^2), number of fish used for fecundity calculation (n), mean relative fecundity (MRF) ± SEM.

Location	N	Relationships	R^2	n	MRF
SWA	287	$W = 0.00004 \times L^{2.9598}$ $\ln(W) = 2.9598 \times \ln(L) - 10.107$	0.971	67	1999 ± 46
Leopard Bay	192	$W = 0.00003 \times L^{3.0107}$ $\ln(W) = 3.0441 \times \ln(L) - 10.502$	0.866	0	
Kiramby Point	89	$W = 0.00002 \times L^{3.0749}$ $\ln(W) = 3.0749 \times \ln(L) - 10.687$	0.960	11	2204 ± 103
Nkhotakota	281	$W = 0.00002 \times L^{3.1267}$ $\ln(W) = 3.1267 \times \ln(L) - 10.933$	0.959	27	2054 ± 57
Chinteche	13	$W = 0.00005 \times L^{2.9097}$ $\ln(W) = 2.9097 \times \ln(L) - 9.8257$	0.967	4	1823 ± 85

Comparison of length-weight relationships after Log transformation (Figure P10) revealed significant differences among populations ($F_{4,857}$=2.505, p=0.041) even when Chinteche was removed from analysis ($F_{3,845}$=3.291, p=0.02). Multiple comparison procedure showed either slope or intercept differences between SWA and Kiramby Point, SWA and Nkhotakota, Leopard bay and Nkhotakota, Kiramby Point and Chinteche, Nkhotakota and Chinteche (Table P16).

Table P16. Comparison of length-weight relationships (ln transformed) among populations of *Lethrinops gossei*. ns : non significant, *: significant slope differences (p<0.05), #: significant intercept differences (p<0.05).

	Leopard Bay	Kiramby Point	Nkhotakota	Chinteche
SWA	ns	#	*	ns
Leopard Bay		ns	#	ns
Kiramby Point			ns	#
Nkhotakota				#

The number of eggs per kg body weight (relative fecundity Table P15) produced by females did not differ among populations for *L. gossei* (F=1.589, p=0.196).

Except for the percentage of ripe females, which differed significantly among *L. gossei* populations only, differences among populations were more intense (see significance levels of statistic tests) and more numerous for *M. anaphyrmus* than for *L. gossei.* Indeed, there was no fecundity difference among populations for *L. gossei*, and the significance level for length-weight relationships differences was just below 5% (p=0.041) while it was highly significant between *M. anaphyrmus* populations (p<0.0001). The intensity of ecological differences among populations seemed higher for *M. anaphyrmus* than for *L. gossei.* Unlike for morphometric and genetic analysis, differences of life history traits among populations were apparently not related to geographical distance.

Conclusions

Owing to technical problems in the genetic analysis resulting in a low sample number and time constraints preventing the analysis of morphometric data in time for *L. gossei*, the results of this study are not as complete and elaborated as we wished they would be, and further analysis are still needed. However, despite these hitches, the innovative combination of the genetic, morphometric and ecological approaches proved very complementary and informative. All three approaches resulted in matching results and strong tendencies can already be drawn.

For *M. anaphyrmus*, highly significant genetic, morphometric and ecological differences were found among the four populations analysed. Morphometric and genetic differences were linked to geographical distance between the populations.

For *L. gossei*, genetic and ecological differences were also observed among the populations studied, although these differences were less significant and abundant than for *M. anaphyrmus*. Also, the observed differences were not related to geographic isolation.

These results indicate that, from the South West Arm to Nkhata Bay, both *M. anaphyrmus* and *L. gossei* are not constituted of one large uniform stock but of several distinct populations separated by restricted gene flow, which should be considered as different stocks for fisheries management. They also suggest that, as expected, the degree of population differentiation was stronger (highest) for the shallow water species (*M. anaphyrmus*) than for the deep water one (*L. gossei*), which encounter much less physical barriers to its movements and therefore to the gene flows among individuals from distant geographic areas. This hypothesis is supported by the recent demonstration that no genetic difference were found among any of the populations of *Diplotaxodon limnothrissa* (Turner et al. 1999), which as a pelagic species (Allison et al. 1996, Thompson et al. 1996, Turner et al. 1999) does not encounter any barrier to its movements.