Chapter 4

Fast adaptive responses under natural conditions in the premaxilla of Lake Victoria cichlids

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Abstract

Rapid morphological changes in response to fluctuating natural environments are a common phenomenon in species that show adaptive radiation. Most of these changes are attributed to evolutionary responses to directional selection although the potential role of phenotypic plasticity has recently gained the interest of evolutionary biologists. The dramatic ecological changes in Lake Victoria provide a unique opportunity to study environmental effects on cichlid fish morphology. The present study shows how several haplochromine cichlids changed their premaxilla (upper jaw) during the past 30 years, presumably as an adaptation to a changed diet. Directly after the diet change towards larger and faster prey, the premaxilla changed in a way that is in agreement with a more food manipulating feeding style. One out of four species showed a clear correlation of rapid change in premaxilla traits with a change in diet. These responses could be due to rapid genetic change or phenotypic plasticity, for which there is ample evidence in cichlid fish structures associated with food capture and processing. Either way, our findings indicate a potential for extremely fast adaptive responses to environmental fluctuations, which not only contributed to the rapid adaptive radiation of haplochromine cichlids but also speaks to their ability to cope with environmental changes.

Introduction

Adaptive radiation is considered to be caused by divergent natural selection as a result of environmental differences and competition (Schluter 2000). In many model organisms like Galapagos finches, Hawaiian honeycreepers and East-African cichlids, one of the major causes of adaptive radiation is the diversification of the feeding apparatus (Kocher 2004). Research on this and other ecological causes has mainly focused on the forces of selection on certain traits rather than the phenomenon of phenotypic plasticity (Schluter 2000; Rundle & Nosil 2005; Pfennig *et al.* 2010). Phenotypic plasticity is defined as the environmentally-sensitive production of alternative phenotypes by a given genotype (DeWitt & Scheiner 2004).

Recently, plasticity has gained more attention and is thought to play an underappreciated role in speciation and adaptive radiation (Pfennig *et al.* 2010). Nonetheless, most of the reported fast morphological changes in species confronted with changing environments (e.g. Galapagos finches, three-spined stickle backs, Bahamian *Anolis* lizards) are attributed to responses to directional natural selection on the morphological characters (Grant & Grant 1995; Losos *et al.* 1997; Reznick *et al.* 1997; Aguirre & Bell 2012). In addition, experimental evolution studies have shown that responses to selection can occur in relatively few generations (reviewed in Kawecki *et al.* 2012). For example, Reznick *et al.* (1990) found that under natural conditions, Trinidadian guppies evolved different life-history traits due to differential predation within 11 years (30-60 generations). Two field populations of *Daphnia* differentiated in genetic composition due to parasite infection within only 15 generations (Zbinden *et al.* 2008). In cichlids, however, the mechanisms responsible for the observed fast morphological changes under natural conditions remain obscure (Van Rijssel & Witte 2013 [Chapter 3 in this thesis]).

The Lake Victoria cichlids, which probably represent the fastest adaptive radiation on earth (Schluter 2000), met severe environmental and ecological changes during the past 30 years. This makes them an ideal model to test for environmental influences on morphology. In the 1980s, the introduced Nile perch boomed in the lake (Goudswaard *et al.* 2008). Concurrently, eutrophication resulted in lower dissolved oxygen levels and a turbidity increase (Seehausen *et al.* 1997a; Hecky et al. 2010; Chapter 5 in this thesis). These changes contributed to the decline in the population size and number of species of haplochromine cichlids (Witte *et al.* 2000, 2007, 2013). At the same time, the relative abundance of large-bodied calanoids in the copepod-dominated zooplankton decreased (Wanink *et al.* 2002), while macroinvertebrates such as insects, molluscs and shrimps, and the small cyprinid fish *Rastrineobola argentea* (dagaa) increased in abundance (Kaufman 1992; Wanink 1999; Goudswaard *et al.* 2006; Table 4.1). During the 1990s, some haplochromine species, predominantly detritivores and zooplanktivores, recovered (Witte *et al.* 2007). They changed their diet towards larger and more robust prey such as macroinvertebrates and small fishes (Van Oijen & Witte 1996; Katunzi *et al.* 2003; Kishe-Machumu *et al.* 2008). Van Rijssel & Witte (2013) [Chapter 3 in this thesis] found that cheek depth increased in these haplochromines during the 1990s (through phenotypic plasticity or/and natural selection), probably to facilitate processing of the larger prey.

Table 4.1 Abundance of zooplanktivorous haplochromines and some of their prey types in the Mwanza Gulf through time.

Year	Zooplanktivores (n/10 min)	Large zooplankters (% calanoids in copepods)	Shrimps (n/10 min)	Fish (dagaa) (n/10min)
1973	-	25.0	-	-
1974	-	25.0	-	-
1979	122.1	-	0	-
1981	188	-	-	111
1982	-	-	0	270
1983	-	8.1	0	36
1984	45.9	-	-	50
1985	-	-	-	865
1986	41.8	-	-	1,048
1987	-	-	436	1,301
1988	0.7	8.4	200,000	929
1989	-	9.6	-	1,185
1991	5.0	-	-	-
1992	-	-	100,000	-
1994	24.6	-	-	-
2001	141.4	5.8	200,000	961
2002	-	-	400,000	119
2005	447.0	-	-	-
2006	660.3	-	1,300	47
2008	165.7	-	9,500	568

Abundances of zooplanktivores (Kishe-Machumu 2012) and shrimps (Goudswaard *et al.* 2006; J. H. Wanink, unpublished data; M. A. Kishe-Machumu, unpublished data) are based on daytime bottom trawling at the HEST research transect (Witte *et al.* 1992a). Nightly surface trawls at the principal sampling station G of the HEST transect were used to estimate the abundance of adult dagaa (Wanink 1998; J. H. Wanink, unpublished data; M. A. Kishe-Machumu, unpublished data). Relative abundances of calanoids are based on daytime sampling of the bottom layer or the whole water column (recalculated from Wanink *et al.* 2002; J. H. Wanink, unpublished data). Yearly averages are given for zooplanktivores, calanoids and dagaa, and yearly maxima for shrimps.

Phenotypic plasticity is a common phenomenon in cichlids. Laboratory experiments have confirmed the ability of many cichlid species to change the jaw apparatus in response to different diets (Hoogerhoud 1986; Meyer 1987; Wimberger 1991; Huysseune 1995; Stauffer & Van Snik Gray 2004; Muschick *et al.* 2011). Most of these studies focused on the pharyngeal jaw apparatus, though some showed phenotypically plastic responses in the upper jaw (premaxilla) to different food types (Witte 1984; Meyer 1987; Wimberger 1991; Bouton *et al.* 2002a).

Three feeding styles are seen in cichlids; inertial suction, ram feeding and manipulation (Liem 1980). Manipulation includes a broad range of feeding behaviours with the actual use of oral teeth during, for example, gripping and biting. Earlier studies revealed that suction-feeding cichlids generally have a premaxilla with a longer ascending (asc.) arm and an angle (β) between asc. and dentigerous (dent.) arm smaller than 90°. The reverse held for fish that "bite" or scrape food from a substrate (Otten 1983; Witte 1984; Bouton *et al.* 2002a). The above mentioned plasticity studies also showed that the premaxilla of the cichlids that used a more biting-like feeding style had, amongst others, a less acute angle β and a shorter asc. arm (Witte 1984; Meyer 1987; Wimberger 1991; Bouton *et al.* 2002a).

In this study, we compared premaxilla morphology and diet in four different Lake Victoria cichlids over a 33 year period; two zooplanktivores *Haplochromis (Yssichromis) laparogramma* (*lap*) and *H. (Y.) pyrrhocephalus* (*pyr*), a zooplankti/insectivore *H. tanaos* (*tan*) and a mollusci/detritivore *Platytaeniodus degeni* (*deg*). As the exact feeding mode of the studied cichlids on their new prey types is unknown, and because the diversity of the diet has increased, we could only make tentative predictions. Assuming that larger and more robust prey will involve a more biting-like feeding style, we expected the premaxilla to have a shorter asc. arm and a smaller angle β, as has been found in the plasticity experiments described above.

Materials and methods

Fish collection

Fishes were collected during the years 1978-2011, in the northern part of the Mwanza Gulf, Lake Victoria, Tanzania. In total, 450 adult males of four species (an average of 12 specimens per species per year, at sampling intervals of approximately three years) were selected from the specimens used in Van Rijssel & Witte (2013); see also Chapter 3 and Table 4.2 in this thesis.

Table 4.2 Catch locations and number of specimens per species per year.

Year	*H. laparogramma*	N	*H. pyrrhocephalus*	N	*H. tanaos*	N	*P. degeni*	N
1978	Transect	8	Transect	13	BB, NB	13	BB, J, NB	14
1981	G, Transect	14	G	13	BB	12	BB, J, NB	12
1984	G	14	G	13			BB	9
1985	G	30*						
1987	G	14	Luanso Bay	13			BB, Transect	4
1990	Luanso Bay	14						
1991	J, P	14	E, J, P	12				
1993	G, H, I	13	H, I, J	13	I, J, K	4		
1999	Transect	6	Transect	16				
2001	G	12	G	14	J, BB	16		
2002	J	14	J	14			J	12
2006	F-J	13	G	13	E	16	E,F,J	13
2011	F-J	13	F, G	15	J	13	F,J,K	13
Total		**149**		**149**		**74**		**77**

E-J, stations on the transect; P, Python Island-Nyamatala Island; BB, Butimba Bay; NB, Nyegezi Bay; Entrance, Entrance of the Mwanza Gulf; Transect, unknown station along the transect. The location of Python Island, Nyamatala Island and Luanso Bay are indicated on maps found in Bouton *et al.* (2002b), Witte *et al.* (1992b) and Goldschmidt *et al.* (1993), respectively. *Additional specimens used to check teeth coverage only.

Fishes and diet samples were divided into three different periods; (1) the pristine period (1978–1984), which is considered as the period before the environmental and diet changes (2) the perturbed period (1987–2002), which is the period of severe environmental changes and observed diet changes (3) the recovery period (2006–2011), in which the environmental changes are considered less severe compared to the previous period (Van Rijssel & Witte 2013 [Chapter 3 in this thesis]). The three periods differ somewhat from the periods described in van Rijssel & Witte (2013) [Chapter 3 in this thesis] as in this study, the periods are also based on dietary contents of the fishes, instead of on environmental changes alone.

We obtained volume percentages of stomach and intestine contents for all four species. For *lap*, a selection of the specimens used for the premaxilla morphology was made. For *deg* we only had fish available from the pristine and recovery period (Table 4.3). For *pyr* and *tan* we used data from the dietary studies of van Oijen & Witte (1996), Katunzi *et al.* (2003) and Kishe-Machumu (2012).

Table 4.3 Origins and number of fish used per period for the diet analysis, N is given between brackets.

	Pristine period	Perturbed period	Recovery period
H. laparogramma	1978-1984 (7)[1]*	1987-2001 (31) [1]*	2006 (8) [1]*
H. pyrrhocephalus	1977-1982 (32) [2,3]	1999-2001 (13) [2]	2005-2006 (48) [3]
H. tanaos	1977-1981 (34) [3,4]	1993 (10) [4]*	2005-2006 (31) [3]
P. degeni	1979-1982 (22) [1]	-	2005-2006 (22) [1]

[1] This study; [2] Katunzi *et al.* 2003; [3] Kishe-Machumu 2012; [4] Van Oijen & Witte 1996
* Same fish used as for the premaxilla morphology.

Diet analysis

Volume percentages of stomach and intestine contents of all four species were averaged and analyzed following the procedure described in Kishe-Machumu *et al.* (2008). Our method differs in that volume percentages were corrected for empty stomach and intestines. The prey types were classified in three size categories based on their smallest diameter: small (zooplankton, phytoplankton, detritus and ostracods <0.5mm), intermediate (midge larvae and pupae and insect remains, up to c. 2mm) and large (fish, shrimps, molluscs and leeches >2mm, Katunzi *et al.* 2003; Kishe-Machumu *et al.* 2008).

Premaxilla morphology

The right premaxilla from every fish was dissected, cleaned and preserved in 70% ethanol. The lateral side of each premaxilla was photographed with a digital camera (Nikon Digital Sight DS-Fi1) mounted on a microscope (Nikon SMZ800) with a reference scale.

Based on homologous structures, eight landmarks (LM) were placed on each photograph using TpsDig2 version 2.15 (Rohlf 2001). Eight morphological characteristics were derived from these landmarks; the asc. arm length, the dent. arm length, angle β, teeth coverage, the number of teeth (teeth nr), tooth length and tooth shape.

Two reference lines were drawn to measure angle β between the asc. arm (LM 1-3) and the dent. arm (LM 2-5, Witte 1984). In the dent. arm, this line was fitted through the dentigerous area (~LM 3-4). In the asc. arm, the line runs through the tip of the asc. spine

(LM 1) and touches the caudal incurvation (Witte 1984). Landmark 7 was determined by a reference line which starts at landmark 5 and touches the most rostral point of the upper side of the dent. arm. Landmark 8 was determined by a reference line which starts at landmark 6 and follows the direction of the caudal side of the asc. arm, touching the cavity at its most rostral point.

For calculating the percentage of the dent. arm that is covered with teeth (teeth coverage), the length between landmark 3 and 4 was measured and divided by the total length of the dent. arm, which was then multiplied by 100. Missing teeth were included by counting empty sockets and carefully checking the presence of minute teeth or empty sockets on the rostral and caudal end of the dentigerous area of the premaxilla (Barel *et al.* 1977). The number of teeth was determined by counting from the photograph.

Tooth length was measured of five teeth which were distributed evenly over the total number of teeth. The total number of teeth was divided by four, and the resulting number of teeth was used as the interval at which teeth were measured. The length of the teeth was measured from the implantation to the tip of the teeth and the mean was calculated. All measurements were conducted in TPSDig2 version 2.15 or by calculating the distance between landmark coordinates in Excel 2007.

The tooth shape of the five measured teeth were described as unicuspid, weakly bicuspid, bicuspid and tricuspid following Barel *et al.* (1977). The number of teeth per shape-aspect was scored for each specimen.

The teeth coverage was measured because in contrast to most other Lake Victoria haplochromines, the zooplanktivores *lap* and *pyr* have the caudal ¼ to $^1/_3$ of the premaxillary dentigerous arm edentulous (toothless), which was one of the autapomorphic features used to define the genus *Yssichromis* (Greenwood 1980).

Geometric morphometrics were performed using MorphoJ version 1.05a (Klingenberg 2011) following Van Rijssel and Witte (2013) [Chapter 3 in this thesis]. All four species showed a significant effect of centroid size on premaxilla shape ($p < 0.05$). Therefore, all analyses were conducted on the residuals of the multivariate regression. For multiple and pairwise group comparison between years, a Canonical Variate Analysis (CVA) and Discriminant Function Analysis (DFA) were used respectively. The average premaxilla shape (consensus) of each group of the DFA was visualized by applying an outline to the shape differences which were exaggerated three-fold for better visualization.

Statistical analysis

The volume percentages of prey sizes per period were compared for each species separately with a Mann Whitney U-test. For *lap*, a Spearman correlation test between prey size and the morphological characters was conducted. For all four species, a general linear model (GLM) with standard length (SL) as covariate and year as independent factor was applied to test if the morphological characters of the premaxilla changed through time following Van Rijssel and Witte (2013) [Chapter 3 in this thesis]. All residuals of the GLMs were normally distributed ($P > 0.05$, Shapiro-Wilk test). The P-values of all tests were corrected with a sequential Bonferroni test. All statistical tests were performed with SPSS version 20.

Results

Ecomorphological changes during the perturbed period

For all four species, the diet consisted mainly (65%-91%) of small prey during the pristine period. During the perturbed period, there was a significant decrease of small prey and a significant increase of intermediate and large prey for *lap*, *pyr* and *tan*. The same pattern was observed for *deg* in the recovery period ($P < 0.05$; Figure 4.1; Table 4.4). The diet during the perturbed period consisted mainly of intermediate and large prey such as insects, shrimps, fish, molluscs and leeches (Appendix Table 4.1).

Table 4.4 *P*-values of the Mann Whitney U-test between prey sizes and periods. Significant *P*-values after sequential Bonferroni correction are depicted in bold.

		Period	
H. laparogramma	**Pristine vs Perturbed**	**Pristine vs Recovery**	**Perturbed vs Recovery**
Small	**0.003**	0.694	**0.007**
Intermediate	**< 0.001**	0.513	**< 0.001**
Large	**0.005**	0.368	**0.025**
H. pyrrhocephalus			
Small	**0.020**	**0.008**	0.526
Intermediate	**0.012**	0.475	**0.024**
Large	**< 0.001**	**< 0.001**	0.405
H. tanaos			
Small	**0.002**	**0.001**	**0.043**
Intermediate	**0.007**	0.300	0.109
Large	**0.008**	**< 0.001**	0.063
P. degeni			
Small		**< 0.001**	
Intermediate		0.128	
Large		**0.007**	

The four studied species showed significant premaxilla shape changes after the pristine period (DFA, $P < 0.001$; Figure 4.2). The asc. arm length decreased for *lap*, *pyr* and *tan* during the perturbed period and for *deg* during the recovery period ($P < 0.001$; Figure 4.3A, B). The dent. arm length increased for *pyr* and *lap* during the perturbed period and for *tan* and *deg* during the recovery period ($P < 0.01$; Figure 4.3C). The angle β decreased for *lap* during the perturbed period, while β increased for *deg* during the recovery period ($P < 0.001$; Figure 4.3D). For *pyr*, there were significant differences between year in angle β ($P < 0.001$), though no clear pattern could be recognized.

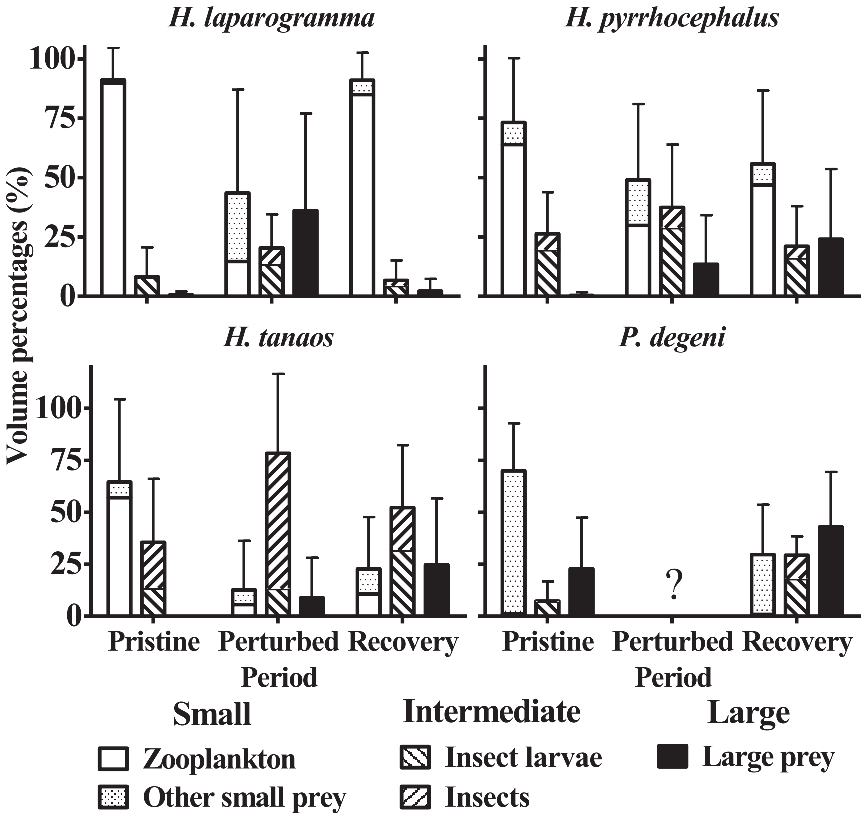

Figure 4.1 Volume percentages with standard deviation of prey sizes of the four studied species in the pristine, perturbed and recovery period. Zooplankton and other small prey; and insect larvae and insects are combined in stacked bars.

The zooplanktivores *lap* and *pyr* both showed a significant increase in teeth coverage and teeth nr in the perturbed period ($P < 0.01$), whereas *tan* and *deg* showed a slight decrease of these characters during the recovery period ($P < 0.05$, teeth nr *deg* $P = 0.06$; Figure 4.3E). The average tooth length and number of unicuspid teeth (Figure 4.3F) increased in *deg* in the recovery period ($P < 0.001$; Figure 4.4, Table 4.5). The tooth shape of *lap*, *pyr* and *tan* did not change significantly and consisted predominantly of bicuspids and some tricuspids (*pyr* and *lap*) and bicuspids/unicuspids (*tan*).

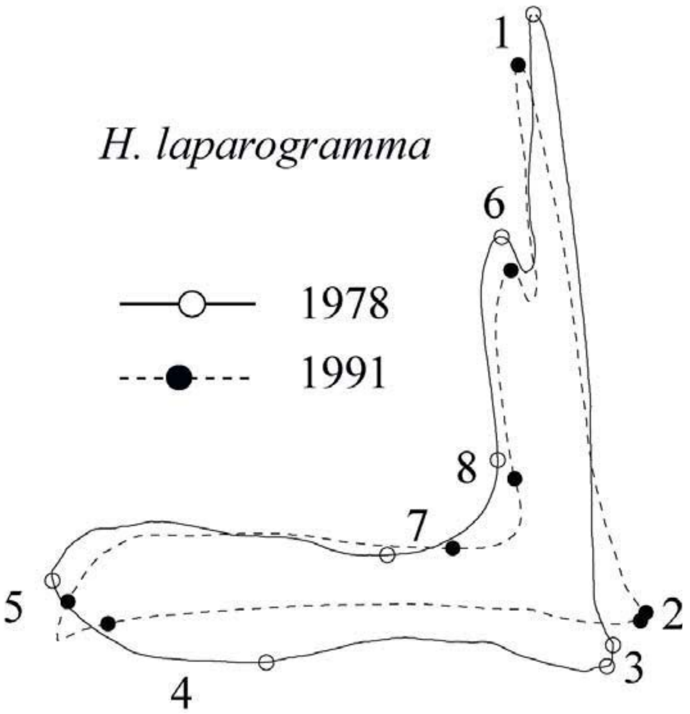

Figure 4.2 Example of premaxilla changes found in the four species illustrated by the outline of *H. laparogramma* derived from the DFA. The continuous line with blank dots represents the year 1978. The dashed line with filled dots represents the year 1991. Differences are exaggerated three-fold for better visualization.

Ecomorphological changes in the recovery period

During the recovery period, the two zooplanktivores showed a reversal (for *pyr* only partly) in diet towards that of the pristine period; there was an increase of small prey and a decrease of intermediate and large prey compared to the perturbed period for *lap* ($P < 0.05$) and a similar trend was found for *pyr* (though only significant for intermediate prey; Figure 4.1; Table 4.4). Concomitant with the (partly) diet reversal, in both species the shape of the premaxilla returned to the form of the pristine period (DFA, $P > 0.05$). The changes in asc. arm (not for *pyr*), dent. arm, teeth coverage and teeth nr reversed between 1999 and 2011 (Figure 4.3A, C, E).

The diet of *tan* and *deg* did not show a reversal towards smaller prey; the amount of large prey remained relatively high (Figure 4.1; $P < 0.05$) and, concurrently, their premaxilla morphology did not show a reversal either.

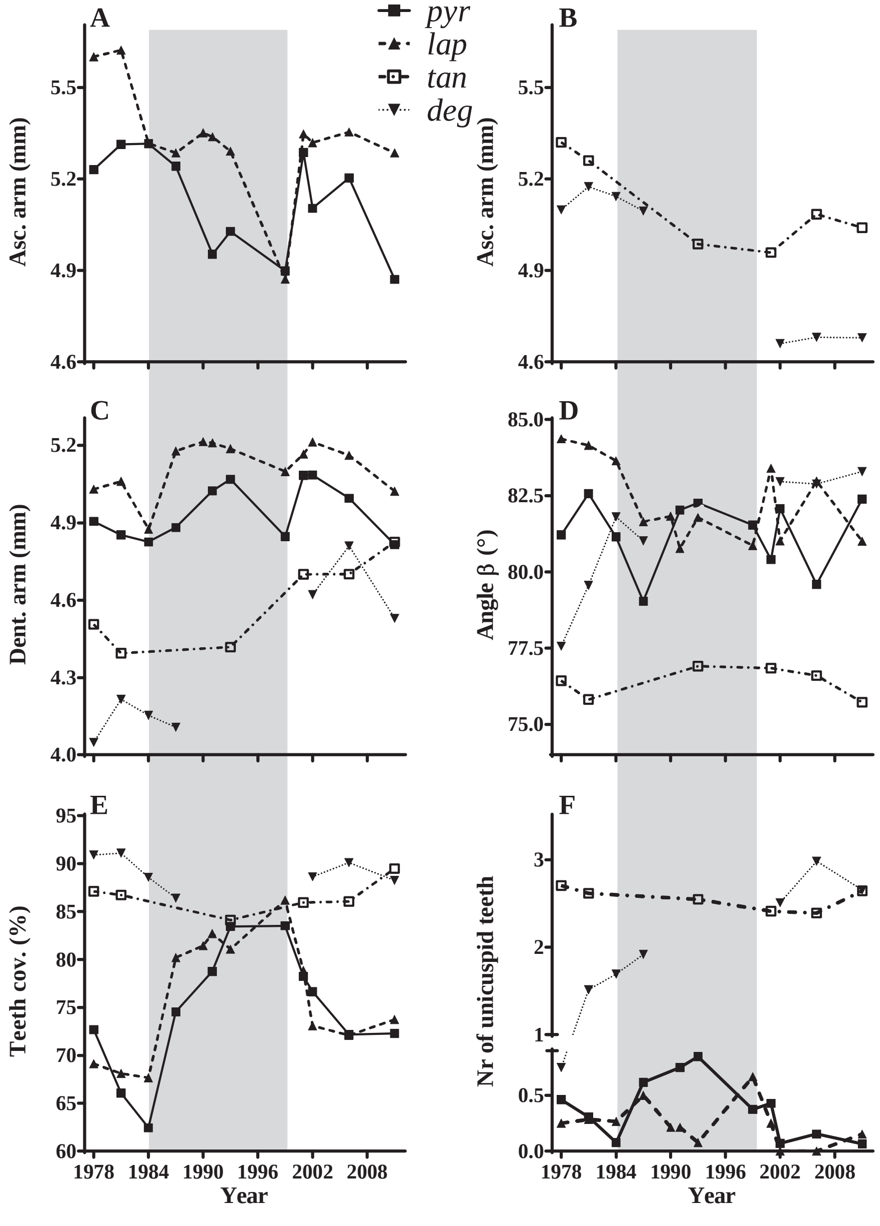

Figure 4.3 Estimated marginal means through time for all four species of A, B: ascending arm, C: dentigerous arm, D: angle β, E: teeth coverage and F: number of unicuspid teeth through time of all four species. Estimated marginal means of the ascending arm are illustrated in two panels for better visualization. The grey shade represents the perturbed period.

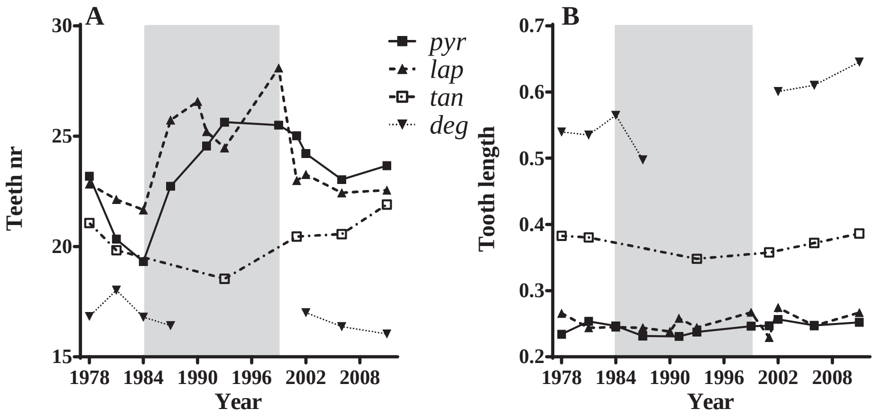

Figure 4.4 Estimated marginal means of A: teeth nr and B: tooth length through time of all four species. The grey shade represents the perturbed period.

Table 4.5 *P*-values of the effect of year from the GLM per species with SL as covariate.

	Asc. arm	Dent. arm	Angle β	Teeth coverage	Teeth nr	Teeth length	Nr of unicuspid teeth
H. laparogramma	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.177	0.167
H. pyrrhocephalus	**<0.001**	**0.009**	**<0.001**	**<0.001**	**<0.001**	0.093	**0.008**
H. tanaos	**<0.001**	**<0.001**	0.516	**0.004**	**0.002**	0.045	0.982
P. degeni	**<0.001**	**<0.001**	**<0.001**	**0.02**	0.064	**<0.001**	**<0.001**

Significant *P*-values after sequential Bonferroni correction are depicted in bold. *P*-values of the effect SL for all GLMs were all <0.05, except for angle β for which the covariate SL was excluded from the GLM.

Correlation between diet and premaxilla morphology

For *lap*, significant correlations between large prey proportion and the premaxilla characters dent. arm, angle β and teeth coverage were found ($P < 0.05$; Figure 4.5). Correlations for small prey with these characters were found as well, although these were not significant after sequential Bonferroni correction (Table 4.6).

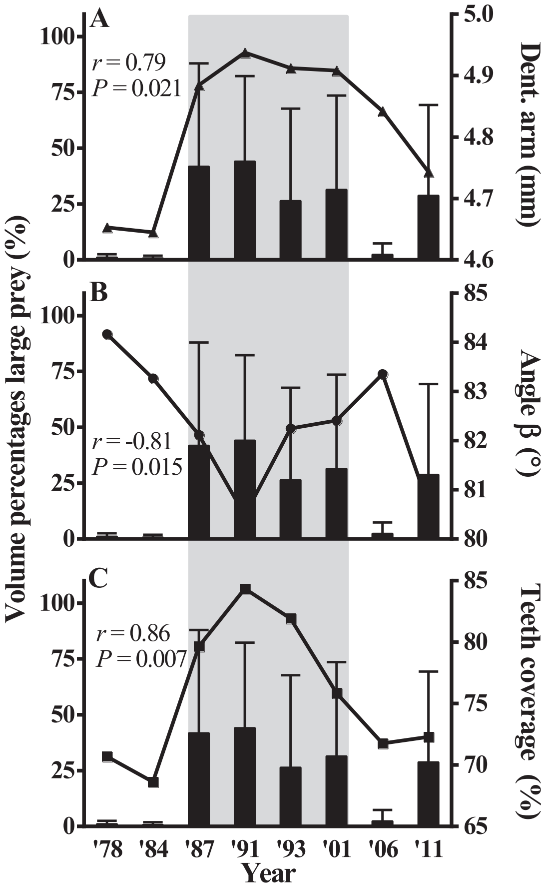

Figure 4.5 Volume percentages of large prey (bars, left axis) and estimated marginal means through time for *H. laparogramma* (solid lines, right axis) of A: dentigerous arm, B: angle β and C: teeth coverage. The coefficient (*r*) and the *P*-value of the Spearman correlation are given per morphological character. The grey shade represents the perturbed period.

Table 4.6 Correlation coefficients of the Spearman correlation between the estimated marginal means of the morphological characters and prey size of *H. laparogramma*. Significant *P*-values after sequential Bonferroni correction are depicted in bold.

Morphological character	Correlation small prey (*r*)	*P*	Correlation large prey (*r*)	*P*
Asc. arm	0.5	NS	-0.57	NS
Dent. arm	-0.76	0.028	0.79	**0.021**
Angle β	0.74	0.037	-0.81	**0.015**
Teeth coverage	-0.81	0.015	0.86	**0.007**

Discussion

Diet change and reversal

This study shows that, under natural conditions, the premaxilla in several cichlid species rapidly adjusts to, most likely, a change in diet (see below). Although there is some variability in the observed diet change, stable isotope analysis of the same specimens confirms the diet changes assessed here by stomach content analysis (Chapter 6 in this thesis). Three possible explanations for the shift towards larger prey in the perturbed period have been proposed by Kishe-Machumu *et al.* (2008). Firstly, the increased abundance of larger prey types (Table 4.1, Kaufman 1992; Wanink 1999; Goudswaard *et al.* 2006). Secondly, the dramatic decline in haplochromine species, resulting in competitive release. Thirdly, the decreased water transparency of the lake and decreased eye size of the resurgent cichlids (Van Rijssel & Witte 2013 [Chapter 3 in this thesis]). These smaller eyes, in combination with the lower visual resolution of some species (Van der Meer *et al.* 2012 [Chapter 2 in this thesis]), may have made it harder to distinguish small-sized prey types (e.g. zooplankton) in more turbid water.

Remarkably, in one of the two originally zooplanktivorous species (*lap*), the diet returned towards smaller prey during the recovery period while the other (*pyr*) showed only a partial reversal of the diet. Although some of the large prey were still abundant in the lake (Budeba & Cowx 2007; Kayanda *et al.* 2009; J. C. van Rijssel & F. Witte, personal observations; Table 4.1), the strong increase of haplochromines during the recovery period (Witte *et al.* 2007; Kishe-Machumu *et al.* 2012) likely has increased competition. Further, water transparency in the Mwanza Gulf in the recovery period has increased again compared to the perturbed period (Chapter 5 in this thesis). Finally, the eye size of the haplochromines studied increased again during the recovery period (Van Rijssel & Witte 2013 [Chapter 3 in this thesis]). Probably a combination of these three phenomena has resulted in the (partial) reversal of the diet in the two zooplanktivorous species. However, these explanations do not clarify why the two zooplanktivores included small prey in their diet again whereas the other two species did not.

Adaptive responses of premaxilla

The diet change from small zooplankton or detritus to larger and more robust prey (such as insects and shrimps) has most likely induced the observed changes in the premaxilla for three reasons. First, the observed changes in the premaxilla show large similarities with the premaxilla of cichlids that used a more biting-like feeding style in the phenotypic plasticity experiments (smaller asc. arm, larger angle β, Witte 1984; Meyer 1987; Wimberger 1991; Bouton *et al.* 2002a). Second, the observed changes in the premaxilla agree with the higher number (for the zooplanktivores) and a more unicuspid shape of tooth found in insectivorous and piscivorous cichlids (Witte & Van Oijen 1990). Third, the observed changes in the premaxilla have enlarged the gape width to accommodate the larger prey (larger dent. arm), as was found for the cheek depth of the same individuals (Van Rijssel & Witte 2013 [Chapter 3 in this thesis]).

All these changes suggest a feeding style that mainly involves manipulation (Liem 1980) of the larger and more robust prey with the use of the oral teeth. Apart from a shorter asc. arm, a larger angle β is considered to increase biting force (Otten 1983). Only *deg* showed a significant increase in the angle β. However, similar to the other three species, the angle β did hardly reach the range found for biters (83°-103°, Witte 1984). These findings suggest that the feeding style of these fish did not switch to extreme biting as is found in true oral mollusc shellers and epilithic algae scrapers.

The increase in teeth coverage in the zooplanktivores during the perturbed period is remarkable. In his generic revision of the haplochromines, Greenwood (1980) described the edentulous part of the premaxilla as a diagnostic character for the genus *Yssichromis*. However, our findings indicate that this is not, in fact, a diagnostic feature.

Possible mechanisms behind the observed morphological changes

The direct responses of the premaxilla to the dietary shifts in *lap* suggest a fast adaptation mechanism. Directional selection might be involved in the rapid morphological changes in the oral jaws (Albertson *et al.* 2003a). Albertson *et al.* (2003b) found that the oral jaw apparatus is controlled by relatively few genes and some parts of it by only one gene (e.g. tooth shape), which can result in an extremely quick response to selection.

However, the variation of morphological traits of the cichlid premaxilla between time periods in this study is generally low. For example, features like a completely covered dentigerous arm have only been encountered for the zooplanktivores in the perturbed period (1987-2002, N = 179) and never in the pristine period (1978-1984, N = 75) or the recovery period (2006-2011, N = 54). An additional 30 specimens of *lap* collected in 1985 all had an edentulous area. In addition, although not included in the analysis, females (also an average of 12 specimens per species per year) revealed similar shape changes as found for the males, and none of the individuals showed a completely covered dentigerous arm during the pristine or recovery period.

Nonetheless, despite the rarity of a completely covered dentigerous arm in these periods, the possibility of genetically based changes remains quite likely. The modest samples used in this study may not reflect the complete population which may harbour rare phenotypes that can increase in frequency by selection. Selection can act within a few generations on relatively little variation as has been shown for several taxa (including fish) in the field (Reznick *et al.* 1990; Rundle 2003; Zbinden *et al.* 2008), in the lab (Kawecki *et al.* 2012 and references therein) and in domesticated systems e.g. Hillman & Davies (1990) and Trut *et al.* (2009). Even when a phenotype does not occur within a population, it may emerge as a result of genetically based changes by selection on polygenic traits.

Phenotypic plasticity, on the other hand, has been found to play a major role in cichlid oral jaw morphology in laboratory experiments (Witte 1984; Meyer 1987; Wimberger 1991; Bouton *et al.* 2002a). Some of these studies showed a shorter ascending arm and a larger angle β of the premaxilla (Witte 1984; Wimberger 1991; Bouton *et al.* 2002a) as a phenotypic response to a feeding style involving manipulation of prey (especially biting). The results of these plasticity studies concur with the results found in this study.

Although a change in teeth number and shape through phenotypic plasticity is not uncommon in the cichlid pharyngeal jaw (Huysseune 1995; Smits *et al.* 1997) our study is the first reporting a higher number of teeth (no size decrease) in combination with a higher teeth coverage. It must be noted, however, that none of the plasticity studies used zooplanktivorous cichlids with an edentulous part on the dentigerous arm.

Phenotypic plasticity and genetically based changes are not mutually exclusive. In fact, natural selection can act on phenotypic plasticity. In addition, both forces can act on morphological characters simultaneously. As is supposed for the cichlid mandible, it might be that regions that come in contact with prey (dentition) evolve independently from regions involved in opening and closing of the premaxilla (Albertson *et al.* 2005; Parsons *et al.* 2012). In other words, phenotypic plasticity might act on premaxilla shape characters involved in prey uptake, while genetic changes might be involved in adjustment of other features, such as tooth morphology, that are used in prey processing.

Recent plasticity experiments in Lake Tanganyika cichlids, three-spined sticklebacks, and Trinidadian guppies have shown morphological responses resembling adaptive phenotypes found under natural conditions (Kerschbaumer *et al.* 2011; Torres-Dowdall *et al.* 2012; Wund *et al.* 2012). These studies all suggest that phenotypic plasticity might play an important role in the colonization of novel environments.

Although a first colonization of a newly-opened niche might be facilitated by phenotypic plasticity, it is expected that sustained selection on traits in the new environment is most likely to involve genetic changes. Theoretically, genetic evolution occurs in an environment in which selection is predictable, and can lead to evolution of mean trait values without the loss of phenotypic plasticity (Gomulkiewicz & Kirkpatrick 1992; Gavrilets & Scheiner 1993). For instance in the fruit fly *Drosophila melanogaster*, an artificial selection experiment demonstrated directional selection on larger body size at lower temperatures without the loss of phenotypic plasticity of the trait (Partridge *et al.* 1994). A quantitative trait analysis has related genetic variation with body size along a latitudinal cline, potentially related to temperature (Gockel *et al.* 2002) and modern sequencing techniques have alluded to which pathways and processes are involved in evolution along a cline (Fabian *et al.* 2012). In the field, fruit flies at lower temperatures had an increased body size, which is consistent with the response to experimental evolution lines (James & Partridge 1995).

Similarly, in our example of phenotypic change of the jaw characteristics, the cichlid fish have been first exposed to a radical change in the environment. After this, for at least a decade the cichlids have been living in an environment in which the diet was altered, but in a constant manner. Since genetic evolution has taken place for morphology in other species at this time scale, we suggest that it is likely that these phenotypic changes have been partly accommodated by underlying genetic changes in the studied fish, especially as the generation time of these cichlids is about 1 year only. However, as in the example of body size in *Drosophila*, experimental (field) studies on trait plasticity and selection with the use of genetic analysis of traits (epigenetics, quantitative trait analysis), will enhance our understanding of their role in adaptive radiation in these cichlid species.

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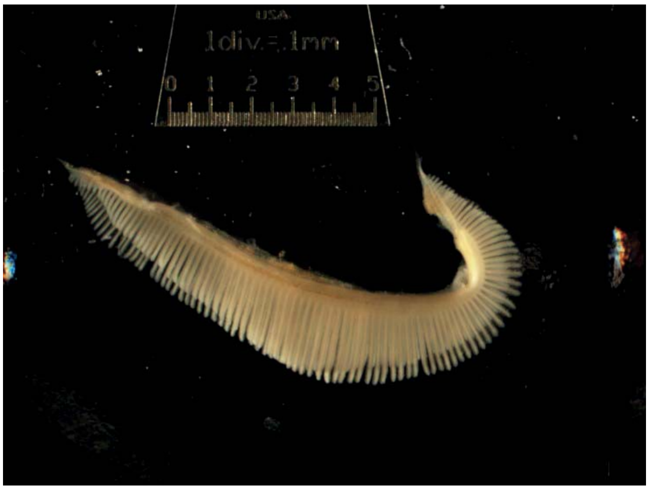
Appendix Table 4.1 Volume percentages of different prey types per period per species.

	H. laparogramma			*H. pyrrhocephalus*			*H. tanaos*			*P. degeni*	
	N=9	N=31	N=8	N= 32	N=13	N=48	N=47	N=10	N=31	N=22	N=22
	1977-1984	**1987-2001**	**2006**	**1977-82**	**1999-2001**	**2005-06**	**1977-81**	**1993**	**2005-06**	**1979-82**	**2005-06**
Detritus	0.3	17.6	0.0	4.2	12.6	4.6	2.9	0.8	4.9	60.6	19.6
Phytoplankton	1.2	8.5	4.9	3.6	4.8	1.1	1.4	0.0	0.0	7.7	3.7
Zooplankton	85.6	9.9	84.2	63.9	29.9	46.9	57.1	5.7	10.7	1.3	0.9
Ostracods	0.0	0.3	0.0	0.0	0.8	1.7	7.7	6.1	7.1	0.4	3.4
Chironomids	10.5[1]	14.4[1]	4.1[1]	2.1	16.5	5.3	4.6	0.0	30.5	7.2[1]	18.1[1]
Chaoborus				15.6	12.3	10.9	24.3	13.4	1.3		
Insects	0.0	7.2	2.7	6.8	8.5	4.8	0.0	64.6	20.4	0.1	11.2
Molluscs	0.0	0.0	0.0	0.0	11.4	0.6	0.0	0.0	0.5	22.8	27.8
Shrimps	0.0	28.7	0.0	0.0	1.5	6.4	0.0	0.0	13.6	0.0	6.6
Fish	0.9	7.1	2.5	0.4	0.6	15.0	0.0	8.9	10.8	0.0	0.0
Others	1.6	6.3	1.5	3.5	1.1	2.7	2.2	0.5	0.1	0.0	8.7[2]

[1] Difference between chironomid and *Chaoborus* larvae could not be distinguished; [2] Consists of leeches.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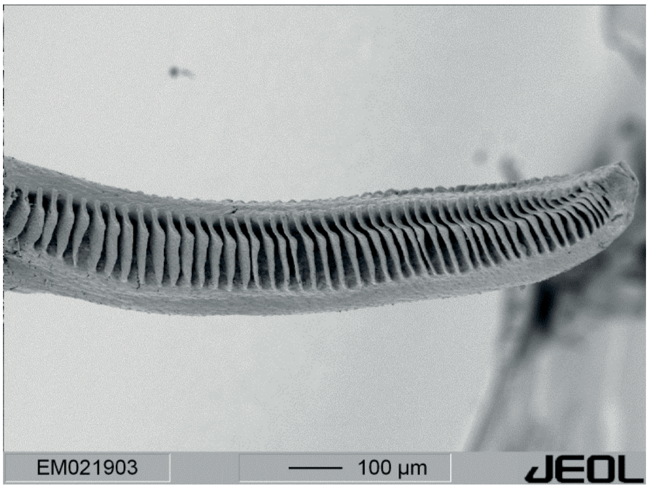
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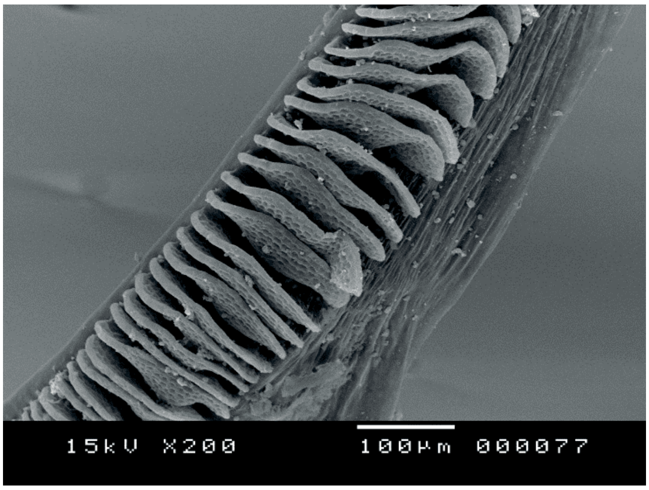
100 µm
JEOL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