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Chapter 3

Adaptive responses in resurgent Lake Victoria cichlids over the past 30 years

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Abstract

Textbook examples of adaptive radiation such as the Galapagos finches and the East-African cichlids form a subject of major interest in evolutionary biology. Many of these species often show rapid morphological changes in response to a perturbed environment. The dramatic environmental changes in Lake Victoria during the past three decades, e.g. Nile perch predation and eutrophication, provide a unique opportunity to study environmental effects on cichlid morphology. Preliminary research has revealed that the lake's haplochromines tend to be extremely plastic and sensitive to these environmental changes. So far, long-term ecomorphological studies at short-term intervals are extremely rare. In this study, we examined morphological changes over a 30 year period in six haplochromine species. Geometric morphometric analyses at intervals of approximately three years revealed adaptive responses. Three out of four resurgent haplochromines had a smaller head surface/caudal peduncle area (HS/CPA) ratio during the upsurge of the predatory Nile perch. During the same period, all four resurgent species had a larger cheek depth and a smaller eye size. The smaller HS/CPA ratio and larger cheek depth are likely to be adaptive responses to a high predation pressure and a diet shift to larger prey. The smaller eye size seems to be the result of a trade off between the eyes and other morphological structures in the smaller head of these species. Interestingly, the direction of the morphological changes was different between the four resurgent cichlid species and two species that became extremely rare or even may have gone extinct. The HS/CPA ratio increased in the extinct species whereas it decreased in the resurgent species. This study suggests that predation is a major driver of these morphological changes, which may be mediated by either phenotypic plasticity or adaptive changes.

Introduction

Adaptive radiation of species has attracted the interest of many evolutionary biologists resulting in multiple studies on this speciation process in vertebrates (Streelman & Danley 2003). Well known examples of adaptive radiation are the Galapagos finches, *Anolis* lizards and the African cichlids. These and other model organisms (like the peppered moth and the three-spined stickleback), often show rapid morphological changes through selection by (human-induced) alteration of their environment (Clarke *et al.* 1985; Grant & Grant 1995; Losos *et al.* 1997; Reznick *et al.* 1997; Bell *et al.* 2004; Aguirre & Bell 2012). However, with the exception of studies on the Galapagos finches (Grant *et al.* 2004; Grant & Grant 2006) and three-spined sticklebacks (Aguirre & Bell 2012), there are no long-term ecomorphological studies where samples have been taken at short time-intervals. By focusing on the ecomorphology of species at short time intervals, we can pinpoint the changes in morphology (including reversals and stasis) possibly induced by environmental changes (Gingerich 1983; Hendry & Kinnison 1999; Gingerich 2001; Reznick & Ghalambor 2001; Hairston *et al.* 2005).

Lake Victoria cichlids have experienced major environmental changes for almost 30 years, and this makes them ideal model species to study environmentally-induced morphological changes. In the 1950s the predatory Nile perch was introduced, and its population boomed in the 1980s (Ogutu-Ohwayo 1990; Pringle 2005; Goudswaard *et al.* 2008). Concurrently, eutrophication through increasing human population densities and inappropriate agricultural practices resulted in increased phytoplankton blooms, especially of cyanobacteria (Hecky 1993; Mugidde 1993; Verschuren *et al.* 2002). This caused the lake water to become murky and low in dissolved oxygen (DO; Hecky *et al.* 1994; Seehausen *et al.* 1997a; Chapter 5 in this thesis). Along with these dramatic changes, a greater abundance of shrimps, molluscs, insects and small cyprinid fish (*Rastrineobola argentea*) were observed (Kaufman 1992; Wanink 1999; Goudswaard *et al.* 2006; Chapter 4 in this thesis). These environmental changes had a major impact on cichlid diversity and resulted in a 40% decline in the number of species (Witte *et al.* 1992a, 2000; Seehausen *et al.* 1997a).

During the 1990s, when intense fishing caused the Nile perch population to decline, some haplochromine species were able to recover (Witte *et al.* 2007, 2013). These resurgent species shifted their diet during the environmental changes from zooplankton and detritus to larger and more robust prey items (Van Oijen & Witte 1996; Katunzi *et al.* 2003; Kishe-Machumu *et al.* 2008; Chapter 4 in this thesis). One of them was *Haplochromis (Yssichromis) pyrrhocephalus*, now the most common zooplanktivorous haplochromine in the Mwanza Gulf (Witte *et al.* 2000; Kishe-Machumu 2012). This species adjusted its head morphology in response to the low DO levels and the changed diet by enlarging its gills and increasing the crushing muscles of the pharyngeal jaw apparatus (Witte *et al.* 2008).

Major environmental and ecological changes which can initiate rapid morphological responses in fish include, amongst others, increase of predation, eutrophication (causing turbidity and low DO levels), and a diet shift (e.g. Wootton 1998; Bittner *et al.* 2010; Langerhans 2010). A common morphological response to increased predation in fishes is a decreased head surface and an increased caudal peduncle area. A literature review revealed 16 different species that have a smaller rostral region and a larger caudal region at high-predation localities or treatments (Langerhans 2010). However, a decrease in the size of a fish head may limit the size of structures in the head, such as the eyes (Barel *et al.* 1989).

For most fish species, vision is a major source of sensory information (Guthrie & Muntz 1993). Increasing turbidity of freshwater lakes due to eutrophication is becoming a common phenomenon and results in a loss of fish biodiversity (Seehausen *et al.* 1997a; Taylor *et al.* 2006; Witte *et al.* 2013). For a fish, one way to cope with increasing turbidity, is to increase the cone size in the eye in order to capture the decreased incoming light (Van der Meer & Anker 1984). The enlargement of the cones will involve an increase in eye size, given that the density of the cones remains unchanged.

Changes in vision can be the cause or result of a diet shift in fish (Hairston & Li 1982; Li *et al.* 1985; Hobson 1991; Wainwright & Bellwood 2002). In addition to vision, gape width and buccal cavity size are limiting factors for a fish's diet (Werner 1974; Wainwright & Richard 1995; Carroll *et al.* 2004). Therefore, a shift to larger prey is likely to increase the buccal cavity and with it, cheek depth (Witte *et al.* 2008). Cheek depth, eye size and head profile are interrelated as was shown by Barel *et al.* (1989).

Cichlid morphology shows phenotypically plastic responses to changes in diet, light conditions and DO levels in the lab (Meyer 1987; Witte *et al.* 1990; Van der Meer 1993; Smits *et al.* 1997; Chapman *et al.* 2000; Bouton *et al.* 2002a; Stauffer & Van Snik Gray 2004; Rutjes *et al.* 2009; Crispo & Chapman 2010a, b) as well as in the field (Smits *et al.* 1996; Chapman *et al.* 2000; Crispo & Chapman 2010a, b). As Witte *et al.* (2008) noted, it is not yet clear whether the morphological changes found in *H. pyrrhocephalus* are the result of phenotypic plasticity or genetic changes. Moreover, it still has to be established that the observed morphological changes have indeed led to enhanced chances of survival in the changed environment, i.e. that they constitute adaptive responses. When morphological responses would occur in the same direction in several Lake Victoria haplochromine species, it becomes more likely that these responses are adaptive.

By studying morphological changes that have occurred in concert with environmental changes, this is the first study comparing morphological changes over a time span of 30 years at about three year time intervals in six different cichlid species; five zooplanktivores, *Haplochromis (Yssichromis) laparogramma* (*lap*), *H. (Y.) pyrrhocephalus* (*pyr*), *H. (Y.) heusinkveldi* (*heus*), *H. tanaos* (*tan*), *H. piceatus* (*pic*) and a mollusci-/detritivore *Platytaeniodus degeni* (*deg*). Four of these species have successfully recovered (*pyr*, *lap*, *tan* and *deg*) and two are extremely rare or extinct (*pic* and *heus*; Witte *et al.* 2000, 2007).

We expected adjustments in body shape and morphology of the resurgent species in relation to the environmental changes. Predictable adaptive responses to the environmental changes in external body shape, are i) a smaller head surface (HS) / caudal peduncle area (CPA) ratio to facilitate burst swimming in order to escape predation by Nile perch; ii) a larger buccal cavity (represented by cheek depth) to facilitate swallowing larger prey; iii) an increase in eye size to harbour larger, and consequently more light sensitive cones, to cope with the decreased light conditions. Moreover, in case of adaptive responses, we expected the resurgent species to change in similar directions, whereas we predicted the extinct species not to change, or to change in a different morphological direction.

Materials and methods

Fish collection

Fishes were collected during the years 1978-2006, in the northern part of the Mwanza Gulf, Lake Victoria, Tanzania. Fish samples were divided into three different periods; 1) the pristine period (1978-1981), which is considered as the period before the severe environmental changes; 2) the perturbed period (1984-1999), which is during the severe environmental changes; 3) the recovery period (2001-2006), in which the environmental changes are less severe compared to the previous period (Table 3.1). Most fish were caught with bottom trawls along a 5 km transect at a depth range of 6-14m (Figure 1.1, for remaining catch locations see (Witte *et al.* 1992b; Bouton *et al.* 2002b; Goldschmidt *et al.* 1993) and were taxonomically classified in the field. Fishes were fixed and preserved in 4% formaldehyde solution (buffered with borax) and, after shipment to Leiden, transferred to 70% ethanol. The specimens were stored in the Naturalis Biodiversity Center, Leiden. For the present study, we selected fish from multiple year groups that differed by approximately three years. The sex of each specimen was determined by examination of the gonads in the lab. In total, 1,019 adult fish [standard length (SL) 44.1-86.3 mm] of six species were selected (Table 3.2).

Table 3.1 Periods of environmental changes.

Years*	1978-1981	1984-1999	2001-2006
Period	Pristine period	Perturbed period	Recovery period
Environment	No Nile perch[1], high number of cichlids[2], no hypoxic conditions[3], diet of studied cichlids consists of small prey[4]	Nile perch boom[1], low number of cichlids[2], increased hypoxia[3], diet shift towards larger prey[4]	Lower Nile perch numbers than in perturbed period[5], high number of cichlids[2], less severe hypoxia than in perturbed period[6], diet shift towards smaller prey[7]

* Years represent the years of sample collection.[1] Goudswaard *et al.* (2008); [2] Witte *et al.* (2013); [3] Hecky *et al.* (1994), Chapter 5 in this thesis; [4] Van Oijen & Witte (1996), Katunzi *et al.* (2003), Kishe-Machumu *et al.* (2008), Chapter 4 in this thesis; [5] Matsuishi *et al.* (2006), Mkumbo *et al.* (2007), Kayanda *et al.* (2009); [6] Sitoki *et al.* (2010); [7] Kishe-Machumu 2012, Chapter 4 in this thesis.

Geometric morphometry

To ensure reliable measurements we selected the most straight specimens that had not been deformed during preservation and transport; slightly bent fish were pinned down and straightened. Based on homologous structures that are also used for taxonomic measurements e.g. Barel *et al.* (1977), 21 landmarks (LM) were placed by a single researcher (JCVR) with a waterproof marker pen (Figure 3.1). The left side of each fish was, together with a reference scale, photographed with a Canon EOS 450D digital photo camera and a Sigma 70 mm macro lens. The landmarks on the photographs were digitized in TpsDig version 2.15 (Rohlf 2001). PAST version 2.05 (Hammer *et al.* 2001) was used to check for landmark displacement errors and aberrant specimens.

Geometric morphometrics were performed using MorphoJ version 1.02g (Klingenberg 2011) following Klingenberg *et al.* (2003). To avoid the effect of sexual dimorphism, males

Table 3.2 Catch locations per species subdivided in years with N for males and females resp. between brackets.

Year	*pyr*	N	*lap*	N	*tan*	N	*deg*	N	*heus*	N	*pic*	N
1978	T	(13/13)	T	(8/15)	BB,NB	(17/15)	BB,J,NB	(15/12)	T	(13/13)	E/F	(14/14)
1981	G	(13/13)	G,T	(14/16)	BB	(15/13)	BB,J,NB,G	(12/14)	G	(14/12)	E/F	(16/12)
1984	G	(13/13)	G	(15/11)			BB	(10/17)	G	(15/11)	E/F	(14/14)
1985									G	(21/8)	E/F	(13/16)
1987	L	(13/13)	G,Ent	(14/14)			BB,T,L,Ent	(4/3)				
1990			L	(14/13)								
1991	E,J,P	(12/14)	J,P	(14/13)								
1993	H,I,J	(13/13)	G,H,I	(13/14)	I,J,K	(4/5)						
1999	T	(19/3)	T	(6/2)								
2001	G	(14/14)	G	(12/13)	J,BB	(16/10)						
2002	J	(14/14)	J	(14/13)			J	(13/13)				
2006	G	(13/13)	F-J	(13/14)	E	(16/12)	J,E,F	(13/13)				
Total		137/123		137/138		68/55		73/68		63/44		57/56

E-J, stations on the transect; P, Python Island-Nyamatala Island; BB, Butimba Bay; NB, Nyegezi Bay; L, Luanso Bay; Ent, Entrance of the Mwanza Gulf; T, 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.

and females were analyzed separately. A procrustes superimposition was applied to extract shape co-ordinates. This method translates the shape to a common origin, scales to unit centroid size and rotates to minimize the procrustes distance between landmarks (Rohlf 1999; Zelditch *et al.* 2004; Mitteroecker & Gunz 2009). An allometry correction was applied to all data, which were divided in subgroups (e.g. species or year) by performing a multivariate regression of the procrustes coordinates on the centroid size (Monteiro 1999; Klingenberg *et al.* 2003). For multiple group comparison between years, a Canonical Variate Analysis (CVA) was used to calculate body shape differences (variation). The Discriminant Function Analysis (DFA) was used for pairwise group comparison between years (pooled for all years). Each analysis was accompanied with a permutation test (10,000 ×) to test for significance. To visualize body shape differences between groups, the thin plate spline technique was applied. This technique interpolates between landmarks and landmark displacements (e.g. Zelditch *et al.* 2004). The average body 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.

Allometry correction and effects of catch location

To check if body shape differences were predominantly caused by a time effect, we tested these shape differences for the effects of centroid size (allometry) and catch location. The limited number of specimens resulted in non-overlapping SLs in some cases (e.g. *lap* 1981 and *lap* 2006). Nonetheless, the effect of centroid size, although significant, accounts for a maximum of only 6.6% (while CV 1 and CV 2 explain at least 51.3%) of the shape changes and had no major effect on the analysis. It should be noted that, although an allometry correction was applied, group comparison with no overlap in SL (e.g. *lap* 1981 and *lap* 2006) may include an allometry effect which cannot be accounted for. Yet, groups with complete overlap in SL did show significant changes (e.g. *pyr* 1993 and *pyr* 2006) which can only be attributed to a time effect.

Next to centroid size, catch location might also potentially influence body shape. Van Oijen & Witte (1996) described intraspecific variation between *H. tanaos* specimens caught at two sand patches in the Butimba Bay, about 500m apart and separated by a mud bottom. In this study, not all species were from exactly the same catch location; we therefore conducted CVAs with catch location as classifier. These analyses gave no clustering per catch location. Rather, they resulted in almost the same classification in morphospace as did the classification by year alone (data points of different catch locations in one year were still clustered by year instead of by catch location) indicating no significant influence of catch location in the dataset.

Morphological character measurements

Morphological characters that might be influenced by environmental parameters were measured by calculating the distance between the coordinates of the different landmarks in Excel 2007. For each fish, SL, body depth, head length (HL), an estimation of head surface (HS) by considering the head as a triangular shape (LM 8,14,21), eye length, eye depth, cheek depth, caudal peduncle depth and an estimation of the caudal peduncle area (CPA) by considering the tail as rectangular shape (LM 15, 16, 18, 19) were measured and a simplified HS/CPA ratio was calculated (Figure 3.1).

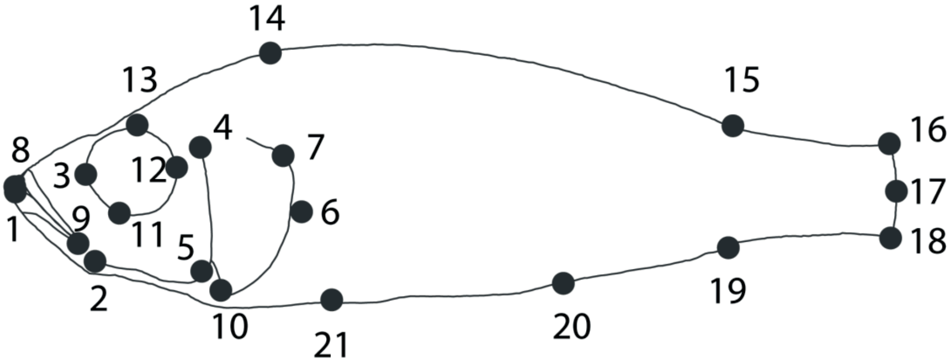

Figure 3.1 Location and description of 21 homologous landmarks used in this study 1: dorsal corner of lower jaw symphysis, 2: quadrate head centre, 3: preorbital process, 4: suspensorial lateral line foramen 1, 5: suspensorial lateral line foramen 4, 6: upper insertion of pectoral fin, 7: caudal/dorsal extremity of the operculum, 8: rostral tip of snout, 9: caudal extremity of the gape, 10: the crevice between the operculum and interoperculum, 11: orbital margin between lachrymal and infra orbital, 12: postorbital process,13: neurocranial lateral line foramen 3, 14: rostral insertion of the dorsal fin, 15: caudal insertion of the dorsal fin, 16 and 18: upper and lower insertion of caudal fin, 17: middle of border line between caudal peduncle and caudal fin, 19 and 20: rostral and caudal insertion of the anal fin, 21: rostral insertion of the pelvic fin. Description of morphological of characters used in this study: standard length (SL, 8-17), body depth (14-21), head length (HL, 7-8), an estimation of the head surface (HS, 8, 14, 21), eye length (2-3), eye depth (11-13), cheek depth (2-11), caudal peduncle depth (15-19) and an estimation of the caudal peduncle area (CPA, 15-16-18-19).

Statistical analysis

A general linear model (GLM) with SL or HL as covariate and year as independent factor was applied to test for the effect of time on the morphological characters. In this selective model, dependent variables, independent factors and their interactions were inserted. With the use of Multivariate Analyses of Covariance (MANCOVA), non-significant interactions were removed stepwise from each model; thereafter non-significant independent factors were removed stepwise. Significant interactions (int.act.) between SL and year were plotted to determine the effect of each factor. Estimated marginal means of all dependent variables were used to plot morphological character changes in time. We assumed that our dataset was representative for the natural population of haplochromines and expected a normal distribution of morphological characters. Almost all residuals of each of the 360 GLMs were normally distributed (*P* > 0.05, Shapiro-Wilk test, Shapiro and Wilk 1965). Six non-normally distributed residuals were found. Nonetheless, we included in these residuals in the GLMs as the observed values did not show large deviation from the expected normal distribution in the Q-Q plots. The *P*-values of the GLM were corrected with a sequential Bonferroni test (Posch & Futschik 2008). To test SL changed over time, an analysis of variance (ANOVA) was used. A linear regression with the morphological characters as dependent and the SL as independent factor was performed. The residuals of this regression were used to test for randomness in the dataset with a Runs test (with mean as cut point). All statistical tests on the morphological characters were performed with SPSS version 16.

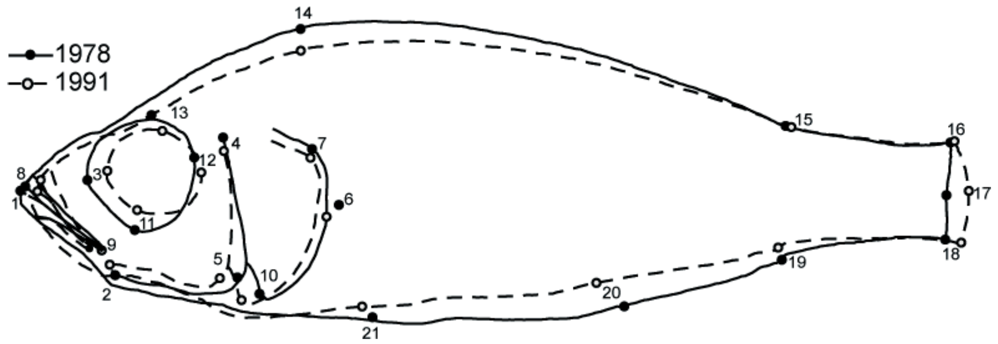

Figure 3.2 Body shape outline of *pyr* males obtained from the DFA. The continuous lines and the black landmarks represent the consensus of the year 1978. The dashed line and white landmarks represent the consensus of the year 1991. Differences were enlarged three times for better visualization.

Results

Shape changes in resurgent species, pristine vs. perturbed period

Both sexes of all six species showed significant body shape changes between the pristine and the perturbed period (pairwise comparison between years, $P < 0.05$, Figures 3.2, 3.3, 3.4) and a reduction of SL ($P < 0.01$; Figure 3.4). The most pronounced changes occurred during the perturbed period (Figures 3.3, 3.4; Appendix Tables 3.1, 3.2). Besides the effect of year, there was a significant effect of the covariates SL and HL for each GLM ($P < 0.001$). Males and females showed changes in the same direction. As males showed more pronounced changes than females (Appendix Table 3.3), primarily the results of males are discussed.

As hypothesized for the two resurgent species *pyr* and *deg*, the head surface/caudal peduncle area (HS/CPA) ratio decreased significantly in the perturbed period ($P < 0.05$). The resurgent species *lap* showed a similar trend although the decrease of the HS/CPA ratio was not significant. In contrast with our expectations, the other resurgent species, *tan*, increased its HS/CPA ratio in the perturbed period ($P = 0.049$; Figure 3.4), mainly by increasing its body depth (Appendix Figure 3.1).

All four resurgent species showed similar other morphological changes in time. The cheek depth (with SL and HL as covariates) increased significantly in the perturbed period for *pyr*, *lap* and *deg* ($P < 0.05$, Figure 3.4; Appendix Table 3.4). Against our expectations, the eye depth and length showed a similar inverse trend, ($P < 0.05$; Figure 3.4; Appendix Figure 3.1). In the resurgent species *tan*, eye depth ($P = 0.001$), eye length and cheek depth showed the same trend as in the other resurgent species, though changes in eye length were not significant and a significant interaction with SL was found for cheek depth (int.act. $P = 0.016$; Figure 3.4; Appendix Figure 3.1, table 3.4).

The changes in HS/CPA ratio for *pyr* were non-random ($P < 0.05$), but not for the other resurgent species. The changes in body depth, cheek depth, eye depth and eye length were non-random for all four resurgent species ($P < 0.05$).

Shape changes in the extinct species, pristine vs. perturbed period

There were some morphological changes that went into a different direction in the resurgent species compared to the extinct species. The extinct species *heus* showed an increase in the HS/CPA ratio during the perturbed period ($P = 0.002$; Figure 3.4), while it decreased for three out of the four resurgent species (*pyr*, *deg* and *lap*). In the resurgent species, the caudal peduncle area remained the same, or even increased in the deep bodied *deg* females ($P = 0.003$), whereas this character decreased in the extinct species *pic* and *heus* ($P = 0.012$ and int.act. with SL $P = 0.063$ respectively), mainly due to the decrease of the caudal peduncle depth ($P < 0.001$ and $P = 0.001$ respectively; Appendix Figure 3.1).

In addition, the extinct species showed an increase in eye length during the perturbed period ($P < 0.05$, Appendix Figure 3.1), whereas three out of four of the resurgent species showed a significant decrease of this morphological character in the perturbed period.

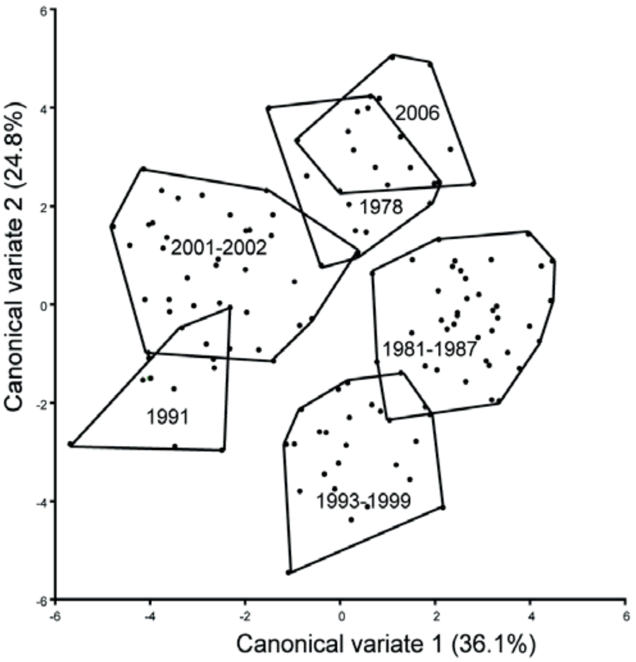

Figure 3.3 Plot of *pyr* males from 1978-2006 obtained from the CVA. Each dot represents the body shape of one specimen. CV 1 and CV 2 explain together 60.9% of the total body shape variation in years.

Reversal in morphology in resurgent species, perturbed vs. recovery period

During the recovery period, in three out of the four resurgent species (*pyr*, *tan* and *lap*), overall body shape changed back into the form of the pristine period ($P < 0.05$; Figures 3.3, 3.5). Although *lap* showed a similar body shape alteration as *tan* and *pyr*, the 2006-form still differed slightly from the 1978-form by having a somewhat smaller body depth, smaller caudal peduncle area and a slightly larger eye length and depth (Procrustes distance 0.0163, $P = 0.028$).

Morphological characters that showed a reversal of changes during the recovery period in *pyr*, *lap* and *tan* were the HS/CPA ratio, cheek depth, eye depth and eye length ($P < 0.05$; Figure 3.4; Appendix Figure 3.1). The species *deg* showed the same trend for the HS/CPA ratio and eye length but not for cheek depth and eye depth.

Discussion

Just like peppered moths, Galapagos finches, Bahamian lizards, Trinidadian guppies and three-spined sticklebacks (Clarke *et al.* 1985; Grant & Grant, 1995; Losos *et al.* 1997; Reznick *et al.* 1997; Bell *et al.* 2004; Aguirre & Bell 2012), Lake Victoria haplochromines showed morphological changes within a decade. As in Galapagos finches, the most pronounced changes occurred during, and just after, major fluctuations in the environment.

Adaptations to Nile perch predation

Nile perch predation was associated with the major morphological changes found in this study. The decrease of SL in all six species has been reported before for some of these species, as well as a decrease in size at first maturity (Witte *et al.* 1995). In addition to intensive trawling in the 1970s and early 1980s with mesh sizes that selectively caught the larger individuals (Witte *et al.* 1995), these size changes could be the result of Nile perch predation, which also may have been size selective (Witte *et al.* 1992b).

Conforming with our expectations, Nile perch predation likely resulted in a smaller HS/CPA ratio during the perturbed period in three out of four resurgent species (*pyr*, *deg* and *lap*, although not significant for *lap*). Additional data from another successful resurgent species, the detritivore *H.* "paropius-like" showed a decreased HS/CPA ratio in specimens of 2006 compared to specimens from 1977-84 (respectively 3.41 and 4.90, measured from average body outline; C. van Geest, J. C. van Rijssel, F. Witte, unpublished data). A smaller HS/CPA ratio has shown to be beneficial for mosquitofish (Langerhans *et al.* 2004; Langerhans 2009), three-spined sticklebacks (Hendry *et al.* 2011) and guppies (Hendry *et al.* 2006) at high-predations sites, to enhance burst swimming speed, which is essential for predator escape performance (Blake 2004). As in the above species, the smaller HS/CPA ratio found in the present study is in line with a response to increased predation by predatory fishes.

Surprisingly, the fourth resurgent species, *tan*, showed an increase in the HS/CPA-ratio. As *tan* is the most slender haplochromine in the lake and the average width and volume of its head is much smaller than that of the other species (J.C. van Rijssel, unpublished data), we speculate that it might have been forced to increase its head surface (and thus HS/CPA-ratio) because of possible trade-offs (see below) with for instance a larger gill size. Despite the increase of the HS/CPA-ratio of the resurgent *tan*, the ratio remained in the same range as that of the other resurgent zooplanktivores (Figure 3.4).

Nile perch predation did not have the same effect on the two extinct or poorly recovering species. These species showed caudal peduncle area- and eye size- changes in the opposite direction of those of the resurgent species. This resulted in, amongst other things, a larger HS/CPA ratio for *pic* and *heus*. Supplementary data for another poorly recovering zooplanktivore, *H.* "argens" showed an increase of the HS/CPA ratio as well, from 2.75 in 1979 to 3.75 in the 2000s (measured from average body outline, Table 3.3). Although we lack an explanation for these changes in the opposite direction, negative impacts of an increased HS/CPA ratio may include impaired burst swimming speed. During the last decade, morphology-performance mapping has received increased attention in fish literature and it has been suggested that morphology-performance related changes can determine an individual's fitness (Hendry *et al.* 2011). Our data support the suggestion that the ability to change the morphology in a performance enhancing direction may be beneficial to species survival.

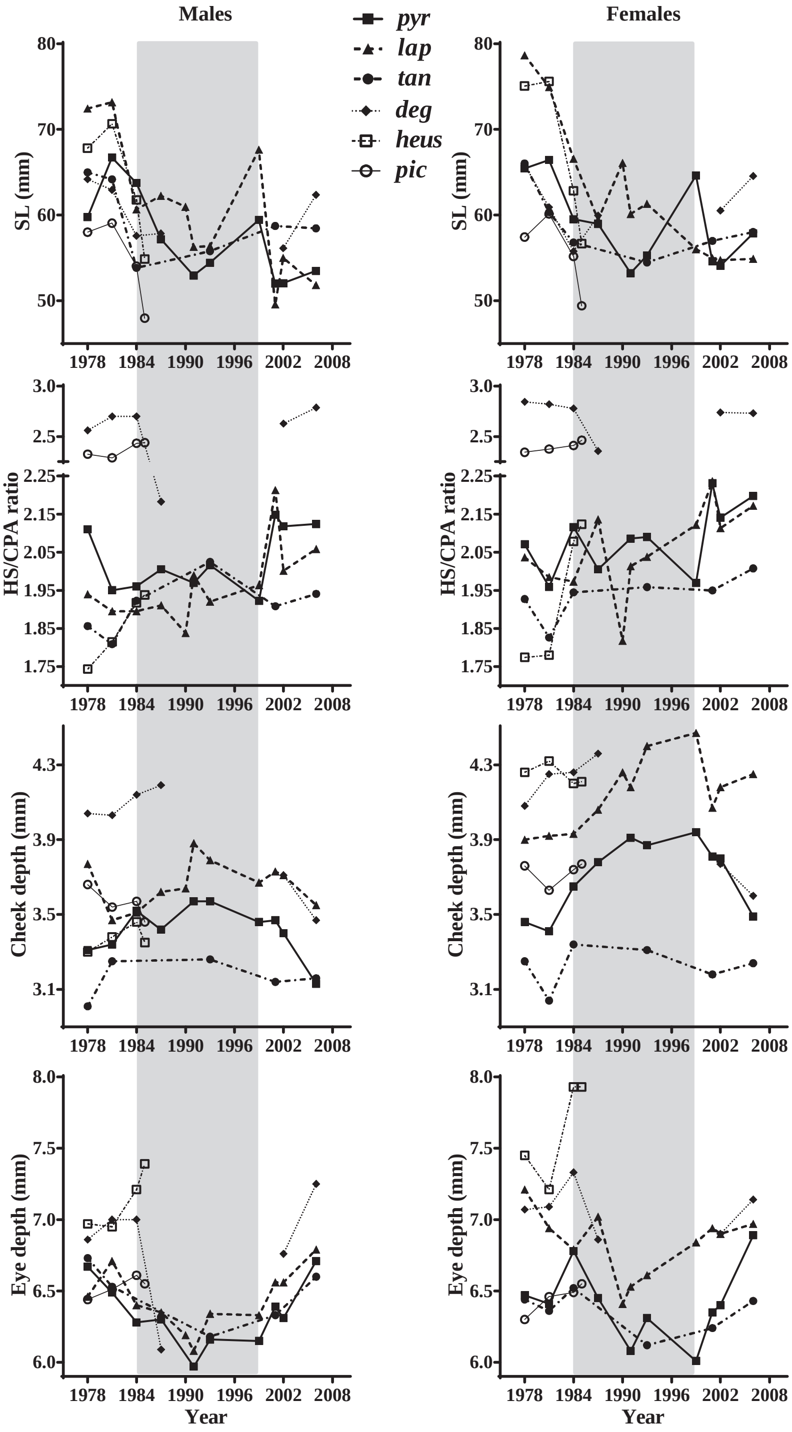
Males
Females
pyr
lap
tan
deg
heus
pic
SL (mm)
80
70
60
50
HS/CPA ratio
3.0
2.5
2.25
2.15
2.05
1.95
1.85
1.75
Cheek depth (mm)
4.3
3.9
3.5
3.1
Eye depth (mm)
8.0
7.5
7.0
6.5
6.0
1978
1984
1990
1996
2002
2008
Year

Figure 3.4 Plots of the estimated marginal means of the GLM of all species (plots of SL show standard means). Each line represents the morphological character changes in time per species with SL as covariate. Filled symbols represent resurgent species, blank symbols represent extinct species. The grey shade represents the period when major ecological and morphological changes occurred. Plots of estimated marginal means with HL as covariate are not shown as they did not differ much from those with SL as covariate.

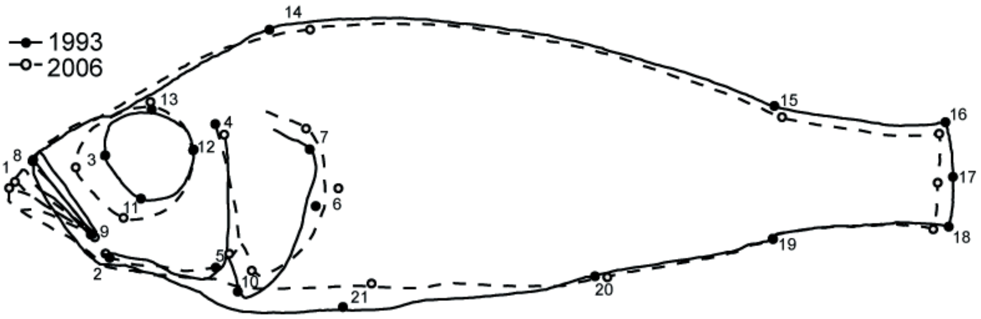

Figure 3.5 Body shape outline of *pyr* males obtained from the DFA. The continuous lines and the black landmarks represent the consensus of the year 1993. The dashed line and white landmarks represent the consensus of the year 2006. Differences were enlarged three times for better visualization.

Adaptations to larger prey with an inevitable trade-off

As a possible adaptation to larger prey consumed during the perturbed period (Van Oijen & Witte 1996; Katunzi *et al.* 2003; Kishe-Machumu 2012; Chapter 4 in this thesis), the present study found an increase in cheek depth in all four resurgent species. The increase in cheek depth could also have affected the eye size, as cheek depth and eye size are negatively correlated (Barel *et al.* 1989). The latter is likely to be the case as the eye size of all four resurgent species showed a decrease in the perturbed period (both with respect to SL and HL). This decrease has been reported for *pyr* by Witte *et al.* (2008) as a possible trade-off for the accommodation of the larger gills. Most likely, both the larger cheek depth and larger gills in a smaller head have resulted in smaller eyes of these haplochromines. A study of the retinas of *pyr* and *tan* suggest that the reduction of eye size only had an influence on the blue light-sensitivity (single cones) and the visual resolution (for *pyr*), but not on the photopic sensitivity of the double cones. So, although the eyes did not increase in size, these species may have found a way to cope with the turbid environment by changing their retinal features (Van der Meer *et al.* 2012 [Chapter 2 in this thesis]).

Table 3.3 Direction of morphological changes in all species.

	Resurgent zooplanktivores (3)		Resurgent *P. degeni* (1)		Extinct/rare zooplanktivores (2)
Character	1970s-1990s	1990s-2000s	1970s-1980s	1980s-2000s	1970s-1980s
HS / CPA ratio	2↓, 1=	↑	↓[a]	↑	↑[b]
Cheek depth	↑	↓	↑	↓	=
Eye size	↓	↑	↓	↑	↑

Numbers represent the number of species changed, no numbers indicate changes in all species, ↑, increase; ↓, decrease; =, no change. [a] Including data of the detritivore *H.* "paropius-like". [b] Including data of the zooplanktivore *H.* "argens".

Reversal of morphology in resurgent species

During the recovery period, the environmental conditions in the lake were less harsh than in the perturbation period. Firstly, the Nile perch biomass during the recovery period was well below the level of the perturbed period (Matsuishi *et al.* 2006; Mkumbo *et al.* 2007; Kayanda *et al.* 2009). Secondly, some zooplanktivores (including *H. pyrrhocephalus* and *H. laparogramma*) have (partly) shifted their diet back to that of the pristine period, including again more zooplankton (Kishe-Machumu 2012; Chapter 4 in this thesis). Thirdly, water transparency in the Mwanza Gulf in 2006 has increased again compared to the late 1980s (Chapter 4 in this thesis). Fourthly, the lake-wide oxygen levels have increased (Sitoki *et al.* 2010; Chapter 4 in this thesis). It is likely that all these changes have resulted in the reversal of the body shape, the HS/CPA ratio, cheek depth and eye size in the studied species during the recovery period.

Haplochromines are not the only organisms showing this reversal in morphology. Peppered moths, Galapagos finches, Bahamian lizards and three-spined sticklebacks all showed reversed morphological changes after restoration of the perturbed environment through fluctuating directional selection (Clarke *et al.* 1985; Grant & Grant 2006; Losos *et al.* 2006; Kitano *et al.* 2008), hybridization (Taylor *et al.* 2006), or by a form of reverse or relaxed selection (Teotonio & Rose 2001). So far, we lack sufficient (genetic) data to confirm if reverse selection acted on the morphological characters of the resurgent haplochromines as well.

Possible mechanisms behind the morphological changes

Phenotypic plasticity has been found to play a major role in body shape changes of African cichlids (Crispo & Chapman 2010a) and many other morphological traits (Chapman *et al.* 2000; Bouton *et al.* 2002a; Rutjes *et al.* 2009). Crispo & Chapman (2010a) found larger gills driven by hypoxia, which had an influence on body shape (deeper heads and shorter bodies), and similar results were found in the cyprinid *Barbus neumayeri* (Langerhans *et al.* 2007). A review of phenotypic changes in wild animal populations indicated that human induced environmental changes are an important source of phenotypically plastic responses (Hendry *et al.* 2008). However, haplochromine numbers were extremely low during the end of the 1980s and early 1990s (Witte *et al.* 1992a; Witte *et al.* 2013) and a strong selection pressure might have acted on these fishes. The morphological characters measured in this study show enough variation on which natural selection could have acted. Fast evolution of morphological traits by natural selection is not uncommon in vertebrates which show adaptive radiation (Grant & Grant 2006; Losos *et al.* 2006; Kitano *et al.* 2008). Unfortunately, so far, we lack the genetic data to confirm whether the rapid morphological changes are the result of phenotypic plasticity or natural selection.

Conclusions

From this study we conclude that rapid changes of morphological traits and their reversal occurred in Lake Victoria cichlids within a couple of decades. Predation appears to be a major driver of these changes. Natural selection and phenotypic plasticity or a combination of these two mechanisms are likely to be responsible for the contemporary morphological changes. Ongoing morphological and genetic studies, in combination with plasticity experiments in the laboratory, will provide us with a more detailed insight in the adaptive radiation of these fast evolving species.

Acknowledgements

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Appendix Table 3.1 Multiple group comparison procrustes distances of males per species between years. Significant procrustes distances (sequential Bonferroni corrected) are depicted in bold.

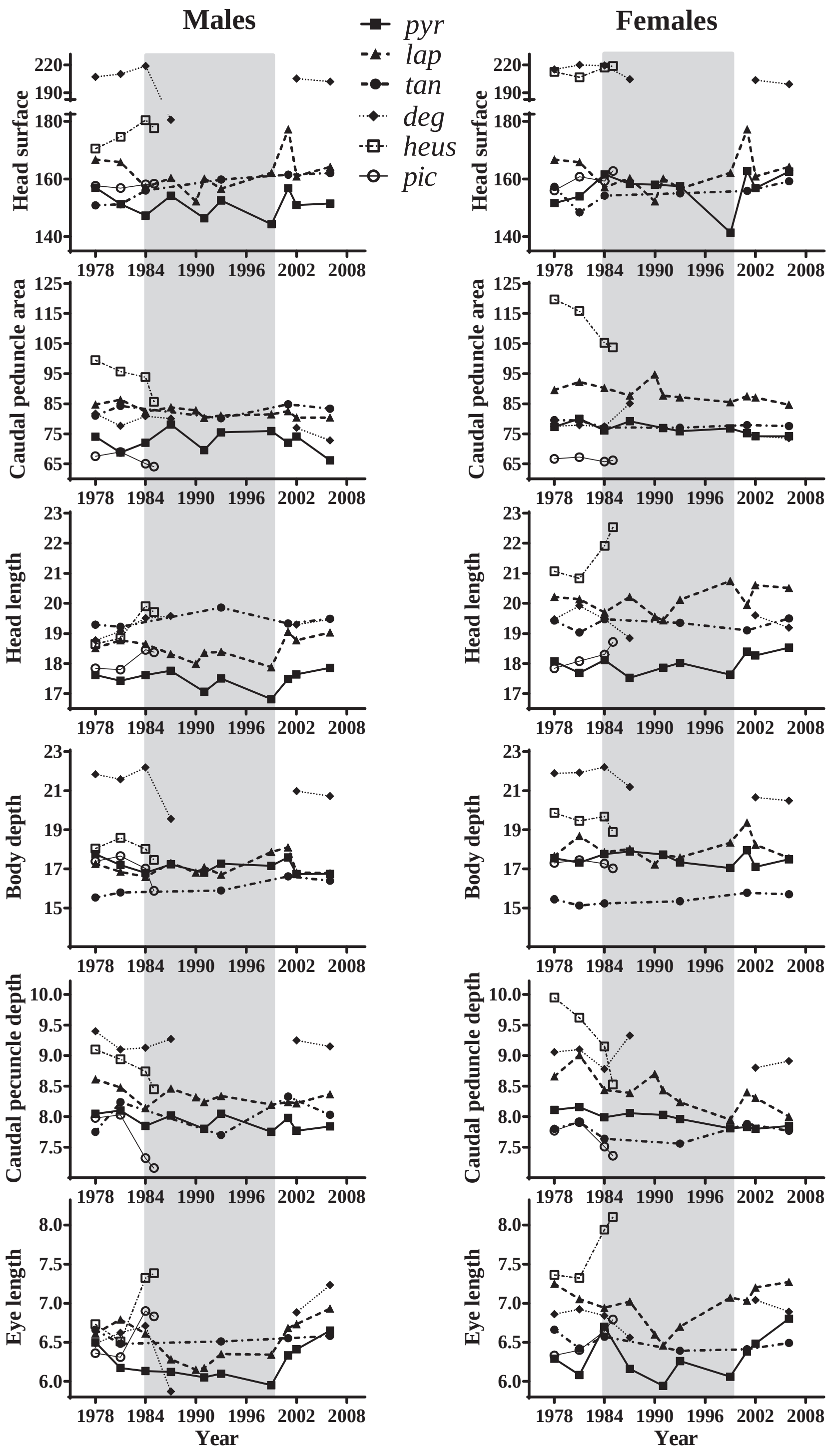
Males		**1978**	**1981**	**1984**	**1987**	**1990**	**1991**	**1993**	**1999**	**2001**	**2002**
pyr	**1981**	**0.0160**									
	1984	**0.0208**	0.0124								
	1987	**0.0161**	0.0146	**0.0196**							
	1991	**0.0177**	0.0129	**0.0162**	**0.0163**						
	1993	**0.0195**	**0.0145**	**0.0185**	**0.0153**		**0.0127**				
	1999	**0.0189**	**0.0163**	**0.0242**	**0.0175**		**0.0129**	**0.0184**			
	2001	0.0129	**0.0180**	**0.0217**	0.0135		**0.0164**	**0.0168**	**0.0178**		
	2002	0.0120	**0.0177**	**0.0194**	**0.0156**		**0.0142**	**0.0193**	**0.0186**	**0.0124**	
	2006	0.0115	**0.0203**	**0.0228**	**0.0176**		**0.0201**	**0.0232**	**0.0210**	**0.0144**	0.0093
lap	**1981**	0.0110									
	1984	0.0143	0.0125								
	1987	**0.0175**	**0.0181**	**0.013**							
	1990	**0.0226**	**0.0219**	**0.0145**	0.0094						
	1991	0.0168	**0.0180**	**0.0130**	0.0108	0.012					
	1993	0.0143	**0.0164**	0.0109	0.010	0.0114	0.0084				
	1999	**0.0266**	**0.0275**	**0.0225**	**0.0152**	**0.0178**	**0.0187**	**0.0199**			
	2001	**0.0179**	**0.0204**	**0.0185**	**0.0152**	**0.0216**	**0.0168**	**0.0178**	**0.0203**		
	2002	0.0111	**0.0136**	**0.0145**	**0.0196**	**0.023**	**0.0161**	**0.0139**	**0.0298**	**0.0195**	
	2006	**0.0152**	**0.0137**	**0.0163**	**0.0185**	**0.0221**	**0.0194**	**0.0158**	**0.0263**	**0.0195**	**0.0150**
tan	**1981**	0.0077									
	1993	**0.0249**	**0.0239**								
	2001	**0.0166**	**0.0150**					**0.0200**			
	2006	**0.0134**	**0.0117**					**0.0238**		0.0092	
deg	**1981**	**0.0153**									
	1984	0.0145	**0.0178**								
	1987	**0.0270**	**0.0305**	**0.0351**							
	2002	**0.0190**	**0.0197**	**0.0274**	**0.0288**						
	2006	**0.0215**	**0.0213**	**0.0283**	**0.0338**						**0.0163**
heus	**1981**	0.0089									
	1984	**0.0171**	**0.0165**								
	1985	**0.0163**	**0.0151**	0.0112							
pic	**1981**	0.0077									
	1984	**0.0169**	**0.0181**								
	1985	**0.0173**	**0.0187**	0.0073							

Appendix Table 3.2 Multiple group comparison procrustes distances of females per species between years. Significant procrustes distances (sequential Bonferroni corrected) are depicted in bold.

Females		**1978**	**1981**	**1984**	**1987**	**1990**	**1991**	**1993**	**1999**	**2001**	**2002**
pyr	**1981**	0.012									
	1984	0.0138	0.0111								
	1987	**0.0173**	0.0115	**0.0138**							
	1991	**0.0165**	**0.0147**	**0.0172**	**0.0126**						
	1993	0.0152	0.0125	0.0134	0.0125		0.0092				
	1999	0.0222	**0.0261**	0.0248	**0.0242**		**0.0257**	0.0229			
	2001	0.0136	**0.0159**	**0.0151**	**0.0209**		**0.0186**	**0.0175**	**0.0254**		
	2002	**0.0178**	**0.0169**	**0.0172**	**0.0180**		0.0125	0.0128	**0.0274**	**0.0194**	
	2006	0.0119	**0.0164**	0.0143	**0.0203**		**0.0203**	**0.0178**	**0.0223**	0.0107	**0.0178**
lap	**1981**	0.0067									
	1984	**0.0183**	**0.0177**								
	1987	**0.0206**	**0.0208**	**0.0146**							
	1990	**0.0306**	**0.0295**	**0.0160**	**0.0211**						
	1991	**0.0172**	**0.0154**	0.0145	**0.0189**	**0.0225**					
	1993	**0.0147**	**0.0155**	**0.0169**	**0.0205**	**0.0277**	**0.0144**				
	1999	0.0236	**0.0264**	0.0269	**0.0273**	**0.0368**	**0.0285**	0.0168			
	2001	**0.0202**	**0.0187**	**0.0185**	**0.015**	**0.0266**	**0.0152**	**0.0210**	**0.0309**		
	2002	**0.0165**	**0.019**	**0.0245**	**0.0253**	**0.0359**	**0.025**	**0.0139**	0.0133	**0.0265**	
	2006	**0.0187**	**0.0205**	**0.0200**	**0.0164**	**0.0289**	**0.0218**	**0.0148**	0.0161	**0.0227**	**0.0145**
tan	**1981**	**0.0147**									
	1993	0.0122	0.0117								
	2001	**0.0176**	0.0124					0.0129			
	2006	0.0104	**0.0182**					0.0138		**0.0150**	
deg	**1981**	0.0072									
	1984	0.0141	0.0104								
	1987	0.0275	0.0298	0.0243							
	2002	**0.0180**	**0.0205**	**0.0258**	**0.0345**						
	2006	**0.0242**	**0.0285**	**0.0294**	**0.0302**						**0.0221**
heus	**1981**	0.008									
	1984	**0.0148**	0.0115								
	1985	**0.0201**	**0.0162**	**0.0114**							
pic	**1981**	0.0122									
	1984	**0.0175**	0.0143								
	1985	0.0160	0.0153	0.0128							

Appendix Table 3.3 Pairwise group comparison *P*-values and procrustes distances (PD) of males and females per species. Significant procrustes distances (sequential Bonferroni corrected) and *P*-values are depicted in bold.

		Males			**Females**		
	Comparison	***P***	**PD**	**N**	***P***	**PD**	**N**
pyr	**1978-1981**	**0.0307**	**0.0161**	26 (13-13)	0.1581	0.0133	26 (13-13)
	1978-1984	**0.0025**	**0.0209**	26 (13-13)	0.1837	0.013	26 (13-13)
	1978-1987	**0.0259**	**0.0164**	26 (13-13)	**0.0193**	**0.0172**	26 (13-13)
	1978-1991	**0.0014**	**0.0181**	25 (13-12)	0.0935	0.0139	27 (13-14)
	1978-1993	**0.0001**	**0.0191**	26 (13-13)	**0.0103**	0.0206	26 (13-13)
	1978-1999	**0.0003**	**0.0189**	32 (13-19)	0.1276	0.0241	16 (13-3)
	1978-2001	0.0966	0.0122	27 (13-14)	0.0703	0.0146	27 (13-14)
	1978-2002	0.0574	0.0126	27 (13-14)	0.0763	0.0146	27 (13-14)
	1978-2006	0.1183	0.0121	26 (13-13)	0.246	0.0115	26 (13-13)
lap	**1978-1981**	0.4747	0.011	22 (8-14)	0.6582	0.0075	31 (15-16)
	1978-1984	0.1971	0.0135	23 (8-15)	**0.0005**	**0.0217**	26 (15-11)
	1978-1987	**0.0272**	**0.0165**	22 (8-14)	**<.0001**	**0.0229**	29 (15-14)
	1978-1990	**0.0076**	**0.0217**	22 (8-14)	**<.0001**	**0.0341**	28 (15-13)
	1978-1991	0.0791	0.016	22 (8-14)	**0.0009**	**0.0208**	28 (15-13)
	1978-1993	0.2663	0.0132	21 (8-13)	**0.0131**	**0.0152**	29 (15-14)
	1978-1999	**0.0124**	**0.0261**	14 (8-6)	0.306	0.0212	17 (15-2)
	1978-2001	**0.0464**	**0.0169**	20 (8-12)	**0.0003**	**0.0233**	28 (15-13)
	1978-2002	0.3954	0.0115	22 (8-14)	**0.0106**	**0.0148**	28 (15-13)
	1978-2006	**0.018**	**0.0162**	21 (8-13)	**0.0006**	**0.0189**	29 (15-14)
tan	**1978-1981**	0.2372	0.0085	32 (17-15)	0.1345	0.0106	28 (15-13)
	1978-1984	0.5712	0.015	19 (17-2)	0.484	0.0114	19 (15-4)
	1978-1993	**0.0258**	**0.0008**	21 (17-4)	0.5967	0.0096	20 (15-5)
	1978-2001	**0.0001**	**0.0164**	33 (17-16)	**0.0053**	**0.0147**	25 (15-10)
	1978-2006	**0.0036**	**0.0131**	33 (17-16)	0.1016	0.0114	27 (15-12)
deg	**1978-1981**	**0.0220**	**0.0153**	28 (15-12)	0.5396	0.0111	26 (12-14)
	1978-1984	0.1137	0.0138	25 (15-10)	0.0723	0.0153	29(12-17)
	1978-1986	**0.003**	**0.0275**	19 (15-4)	**0.0256**	**0.0275**	16 (12-3)
	1978-2002	**<.0001**	**0.0197**	28 (15-13)	**0.0137**	**0.0198**	25 (12-13)
	1978-2006	**0.0001**	**0.0217**	28 (15-13)	**<.0001**	**0.0243**	25 (12-13)
heus	**1978-1981**	0.5084	0.0082	27(13-14)	0.165	0.0127	25 (13-12)
	1978-1984	**0.001**	**0.0169**	28 (13-15)	**0.0047**	**0.0202**	24 (13-11)
	1978-1985	**<.0001**	**0.0177**	34 (13-21)	**0.0004**	**0.0304**	21(13-8)
pic	**1978-1981**	0.45	0.0087	30 (14-16)	0.4177	0.0083	26 (14-12)
	1978-1984	**0.0059**	**0.0183**	28 (14-14)	**0.0022**	**0.0156**	28 (14-14)
	1978-1985	**0.0031**	**0.019**	27 (14-13)	**<.0001**	**0.022**	30 (14-16)


Males
Females
pyr
lap
tan
deg
heus
pic
Head surface
Caudal peduncle area
Head length
Body depth
Caudal pecuncle depth
Caudal peduncle depth
Eye length
Year
220
190
180
160
140
125
115
105
95
85
75
65
23
22
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19
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10.0
9.5
9.0
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8.0
7.5
7.0
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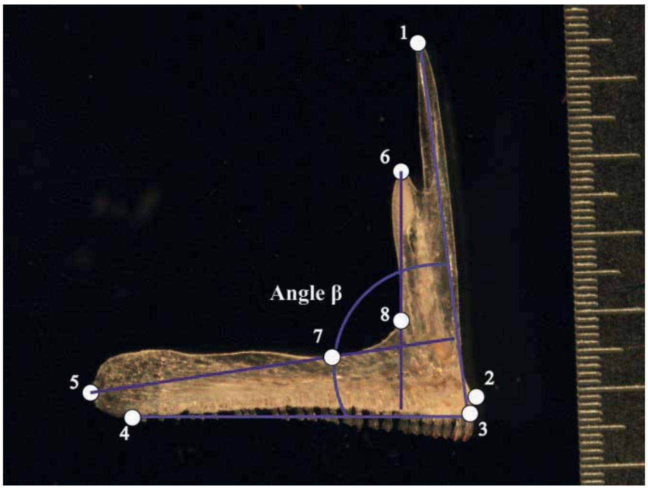
1978
1984
1990
1996
2002
2008

Appendix Figure 3.1 Plots of the estimated marginal means of the GLM of all species. Each line represents the morphological character changes in time per species with SL as covariate. The grey shade represents the period when major ecological and morphological changes occurred. Plots of estimated marginal means with HL as covariate are not shown as they did not differ much from those depicted in this figure.

Appendix Table 3.4. *P*-values of the effect of year from the GLM per species subdivided in sex with SL and HL as covariates.

		Cov	**SL**	**HS/CPA**	**Eye depth**	**Eye length**	**Cheek depth**	**Body depth**	**Head length**	**Head surface**	**Caudal peduncle area**	**Caudal peduncle depth**
pyr	**M**	**SL**	**<0.001**	**0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.003**	**int.act.**	**0.01**
		HL	X	X	int.act.	**<0.001**	**<0.001**	**<0.001**	X	X	X	X
	F	**SL**	**<0.001**	**0.007**	int.act.	**int.act.**	**0.002**	**<0.001**	**0.009**	**int.act.**	0.054	0.217
		HL	X	X	**<0.001**	**<0.001**	**<0.001**	**<0.001**	X	X	X	X
lap	**M**	**SL**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.004**	**<0.001**	**0.004**	**<0.001**	0.232	0.148
		HL	X	X	**<0.001**	**<0.001**	**<0.001**	**<0.001**	X	X	X	X
	F	**SL**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.002**	**int.act.**	**0.005**	**0.001**	**0.004**	**<0.001**
		HL	X	X	**<0.001**	**<0.001**	**<0.001**	**int.act.**	X	X	X	X
tan	**M**	**SL**	**0.006**	0.049	**0.001**	0.65	**int.act.**	**<0.001**	0.471	**0.003**	0.059	**<0.001**
		HL	X	X	**<0.001**	0.161	**int.act.**	**int.act.**	X	X	X	X
	F	**SL**	**0.001**	0.067	0.126	0.412	0.174	0.082	0.35	**0.005**	0.791	0.354
		HL	X	X	0.205	0.48	0.72	0.35	X	X	X	X
deg	**M**	**SL**	**0.01**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**int.act.**	**<0.001**	**<0.001**	0.652
		HL	X	X	**0.001**	**<0.001**	**<0.001**	**<0.001**	X	X	X	X
	F	**SL**	**<0.001**	0.062	0.036	**int.act.**	**<0.001**	**<0.001**	0.276	**0.002**	**0.003**	0.046
		HL	X	X	**0.03**	0.287	**<0.001**	**0.003**	X	X	X	X
heus	**M**	**SL**	**<0.001**	**0.002**	0.014	**<0.001**	0.244	**0.005**	int.act.	0.093	int.act.	**0.001**
		HL	X	X	0.181	**<0.001**	**0.002**	**<0.001**	X	X	X	X
	F	**SL**	**<0.001**	**0.001**	**0.008**	**0.01**	0.901	0.048	0.028	0.436	**<0.001**	**<0.001**
		HL	X	X	0.28	0.154	**0.048**	**<0.001**	X	X	X	X
pic	**M**	**SL**	**<0.001**	0.12	0.612	**0.001**	0.463	**int.act.**	0.064	0.985	0.012	**<0.001**
		HL	X	X	0.339	**0.007**	0.052	**int.act.**	X	X	X	X
	F	**SL**	**<0.001**	0.411	0.061	**0.003**	0.632	0.303	**0.008**	0.242	0.821	**<0.001**
		HL	X	X	0.46	0.046	0.089	**<0.001**	X	X	X	X

Significant *P*-values after sequential Bonferroni correction are depicted in bold. *P*-values of the effect of both covariates (SL & HL) were for all GLMs <0.001.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Angle β
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