Adaptive responses to environmental changes in Lake Victoria cichlids

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Adaptive responses to environmental changes in Lake Victoria cichlids

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Voor Frans

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Chapter 1

General introduction and thesis outline

Adaptations to changing environments

Classical evolutionary theory (Darwin 1859) states that species change through natural selection by adapting behavioural, physiological or morphological traits to the environment and pass these on to the next generations. Darwin (1859) considered the rate of evolution to be very slow, because he derived his expectations from the fossil record. In recent years, however, a number of studies have shown that the rate of evolution can be many orders of magnitude faster than that inferred from the fossil record (Reznick & Ghalambor 2001). Several examples are known where natural selection acts on morphological characters of birds, fish and lizards resulting in adaptations to the environment within a decade or even a year (Grant & Grant 1995; Reznick *et al.* 1997; Losos *et al.* 2006; Aguirre & Bell 2012).

For instance during a period of drought in the 1970s, Grant & Grant (1995) found that large Galapagos ground finches with deep beaks survived better than small ground finches with small beaks because large-hard seeds became more abundant than small-soft seeds. During a later drought in the 1980s, selection on beak traits acted in the opposite direction, as small-soft seeds became more abundant. For Bahamian lizards, it was found that selection favoured lizards with longer legs to escape from invaded predatory lizards. After six months, when the prey lizards were driven to more arboreal areas by the predatory lizard, selection favoured smaller legs, as they are better suited for movement on the irregular tree surfaces (Losos *et al.* 2006). These studies show how natural selection can act on morphological characters in a remarkably short time period.

Why study cichlids?

The studies mentioned above used organisms that have undergone adaptive radiation. According to Schluter (2000): "Adaptive radiation is the evolution of ecological and phenotypic diversity within a rapidly multiplying lineage. It involves the differentiation of a single ancestor into an array of species that inhabit a variety of environments and that differ in the morphological and physiological traits used to exploit those environments". The wide species array is one of the major reasons why many biologists are interested in species that have undergone adaptive radiation and use them as a model to study evolution. Classical examples of extensive adaptive radiation are the Galapagos (Darwin's) finches and the East-African cichlids (Teleostei, Perciformes). The cichlids of the African Great Lakes (Lake Tanganyika, Lake Malawi and Lake Victoria) all show stunning species diversifications which are reflected in 12-16 different trophic groups or tribes (Fryer & Iles 1972; Witte & Van Oijen 1990) and 250-700 species per lake (Turner *et al.* 2001).

Lake Tanganyika is the oldest and deepest lake with an estimated origin of 9-12 million years ago (MYA; Cohen *et al.* 1993), a maximum depth of 1,500m (Bootsma & Hecky 1993) and an assemblage of around 250 cichlid species. Lake Malawi is a little younger because its basin started to develop 8.6 MYA and deepwater conditions developed only 4.5 MYA (Delvaux 1995). Furthermore, Lake Malawi has a maximum depth of 700m and holds about 700 cichlid species. Lake Victoria is the youngest of the three lakes with an estimated age of 100,000-400,000 years (Meyer *et al.* 1990; Johnson *et al.* 1996; Verheyen *et al.* 2003), a maximum depth of only 70m and an assemblage of more than 500 cichlid species (Witte *et al.* 2007). There is even evidence that Lake Victoria dried up completely about 14,600 years ago (Johnson *et al.* 1996; Stager & Johnson 2008), implying that the evolution of the Lake Victoria species flock has been much faster than the estimated 100,000-400,000 years, although this fast evolution has been heavily debated (Fryer 2004). Regardless of whether the lake indeed dried out completely or not, its young age still means that the Lake Victoria cichlids are the fastest example of adaptive vertebrate radiation known (Schluter 2000), which makes them of great interest from an evolutionary point of view.

Lake Victoria and its haplochromine cichlids

Lake Victoria is the largest tropical lake in the world (Fryer & Iles 1972). It has a surface area of 68,800 km^2 and an average depth of 40m (Stager & Johnson 2008). It borders three East African countries; Tanzania, Uganda and Kenya (Figure 1.1). More than 30 million people live around the lake, and at least 1.2 million of these people are directly dependent on the lake's fisheries (Matsuishi *et al.* 2006) — one of the most productive inland fisheries of the world (Ntiba *et al.* 2001). In addition to its 500 or more cichlid species, Lake Victoria was home to 46 other species of teleost (Greenwood 1974; Van Oijen 1995; Witte *et al.* 2007).

Greenwood was the first biologist who studied the Lake Victoria cichlids both in the field as in museums during the 1950s and 1960s. Greenwood studied mainly the northern part of the lake and described (partly with others) 49 new haplochromine species. Later studies led to the division of these haplochromine species into 15 trophic groups including detritivores, phytoplanktivores, algae grazers, molluscivores, zooplanktivores, insectivores, prawn-eaters, crab-eaters, piscivores, paedophages, scale-eaters and parasite eaters (Greenwood 1974; Witte & Van Oijen 1990).

During the 1970s, the Haplochromis Ecology Survey Team (HEST) in collaboration with the Tanzania Fisheries Research Institute (TAFIRI) started a survey in the southern part of the Lake especially focussing on a relatively small research transect in the Mwanza Gulf (Figure 1.1). The Mwanza Gulf is a 60 km long, relatively narrow (5 km width on average) gulf with a depth varying from 2-25m (Goudswaard *et al.* 2002). The research transect is situated in the northern part of the Gulf and extends from Butimba Bay to Kissenda Bay. It has six sampling stations (E-J) with a mud bottom and these range in depth from 6-14m. In Butimba Bay itself, four additional sampling stations are defined (A-D), with A and B (2-4m in depth) having a sandy bottom and C and D (4-6m) having a mud bottom (Witte 1981, Figure 1.1). It was on this research transect that HEST members studied the haplochromine cichlid diversity and recorded more than 72 different species, before severe environmental changes occurred in the lake, as I shall discuss in detail below. This research transect is the only part of Lake Victoria which has been extensively studied both prior to, and during, that period of environmental changes.

The data and fish collections from the HEST therefore provide a unique opportunity to study the effects of environmental perturbations on the ecomorphology of haplochromines. This way, rates of morphological changes and potential effects of selection could be studied in a large "natural" experiment. These kind of long-term ecomorphological studies with short time intervals are extremely rare and have been conducted so far for Galapagos finches (Grant *et al.* 2004; Grant & Grant 2006) and three-spined sticklebacks (Aguirre & Bell 2012) only.

Environmental changes in Lake Victoria

The increased demands for fish, due to the growing human populations around the lake, had a strong impact on fish catches during the first half of the last century (Balirwa *et al*. 2003). Several popular food fishes such as the Singidia tilapia *Oreochromis esculentus* (Graham 1928) and the cyprinid *Labeo victorianus* (Ningu) Boulenger, 1901 had already declined in numbers in the 1950s due to heavy fishing. To improve the declining catches, several tilapia species and the Nile perch *Lates niloticus* (Linnaeus, 1758) were introduced into the lake in the 1950s (Welcomme 1988; Pringle 2005; Goudswaard *et al.* 2008).

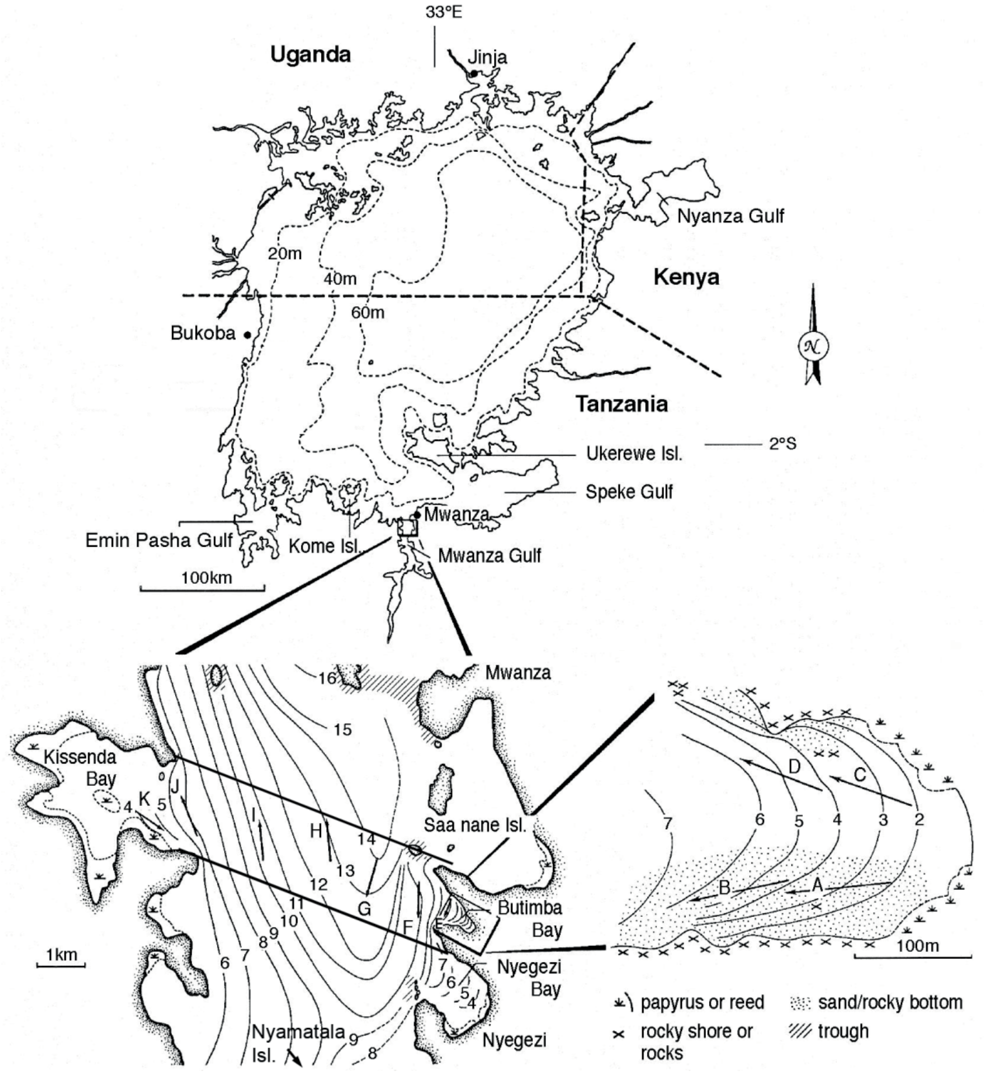


Figure 1.1 Map of Lake Victoria and the northern part of the Mwanza Gulf with the research transect (Station E-J) and the Butimba Bay with station A-D depicted. Numbers indicate depth in meters.

At the end of the 1960s, a lake-wide trawl survey estimated a standing stock of about 600,000t of haplochromines (80% of the demersal fish stock, Kudhongania & Cordone 1974). This finding led to the setting up in 1976 of a fishery program that used bottom trawling to supply a recently installed fishmeal factory. With about 10-15t of haplochromines a day converted into animal fodder, signs of intense fishing were already being reported in the Mwanza Gulf in the late 1970s (Witte & Goudswaard 1985). During the 1980s, there was a huge increase in the population of Nile perch (Pringle 2005; Goudswaard *et al.* 2008) and this boom coincided with a dramatic decrease of haplochromine numbers and species (Witte *et al.* 1992a).

At the same time, severe eutrophication (the enrichment of bodies of water by inorganic plant nutrients e.g. nitrate and phosphate, Lawrence *et al.* 1998) and algal blooms were reported throughout Lake Victoria (Ochumba & Kibaara 1989; Hecky 1993). The eutrophication of the lake already started in the 1920s and 1930s and increased due to enhanced agricultural activity including shoreline deforestation during the 1980s (Hecky 1993; Verschuren *et al.* 2002). Deforestation, which increased soil erosion around the lake, is thought to have contributed to the nutrient influx (Verschuren *et al.* 2002). On the other hand, climatic variability is suggested to have enhanced eutrophication as well (Kolding *et al.* 2008; Hecky *et al.* 2010).

The phytoplankton abundance increased and its composition was altered by a shift from diatoms such as *Aulacoseira* (*Melosira*) to mainly cyanobacteria (blue-green algae) such as *Microcystis* and *Anabaena* (Ochumba & Kibaara 1989; Hecky 1993; Kling *et al.* 2001; Verschuren *et al.* 2002). The nutrient influx, phytoplankton increase and thermal stratification resulted in a decrease of water transparency (Mugidde 1993; Seehausen *et al.* 1997a).

At the same time, levels of dissolved oxygen (DO) were found to be reduced, presumably as a result of the thermal stratification and the decomposition of the increased algal biomass (Hecky *et al.* 1994, 2010; Wanink *et al.* 2001). The low DO levels led to large numbers of dying fish, as reported in several studies (Ochumba & Kibaara 1989; Kaufman 1992; Wanink *et al.* 2001; Goudswaard *et al.* 2011).

Concurrently, especially in the 1990s, the invasive water hyacinth *Eichhornia crassipes* (Martias) Solms, 1883 showed an enormous increase in abundance throughout the lake (Williams *et al.* 2005). During the late 1990s, the infestation was brought to a halt, probably by a combination of the introduction of South American weevils (*Neochetina eichhorniae* Warner, 1970 and *N. bruchi* Hustache, 1926) and the increased water motion caused by El Niño (Williams *et al.* 2005; Williams *et al.* 2007; Wilson *et al.* 2007).

All the environmental changes combined also resulted in increased densities of macroinvertebrates including insects, molluscs and the shrimp *Caridina nilotica* (Roux 1833), and of the small cyprinid fish *Rastrineobola argentea* (Pellegrin 1904) locally known as dagaa (Kaufman 1992; Wanink 1999; Goudswaard *et al.* 2006). In addition, small-bodied predatory cyclopoid copepods increased in abundance relative to the large-bodied herbivorous calanoids and cladocerans (Wanink *et al.* 2002).

Effect of environmental changes on haplochromines

The predatory Nile perch is has a preference for haplochromines (Kishe-Machumu *et al.* 2012) and is suggested to be partly responsible for the extinction of 40% of the haplochromine species (Witte *et al.* 1992a, 2007). The murky waters are also likely to have contributed to the extinctions (Witte *et al.* 1992a; Seehausen *et al.* 1997a). The mate choice of Lake Victoria cichlids is based on male colouration, and they show strong assortative mating which results in sexual isolation. The decreased water transparency interfered with the colour perception of the fishes. As the decreased water transparency hampered differentiation of both colours and colour vision, benefits of assortative mating became smaller and females start selecting for other traits than colour. Because the number of potential mates decreased concurrently, hybridization between species occurred. The result was a loss of cichlid biodiversity (Seehausen *et al.* 1997a).

During the 1990s, intense fishing resulted in a decline in numbers of Nile perch (Matsuishi *et al.* 2006; Mkumbo *et al.* 2007; Kayanda *et al.* 2009). At the same time, populations of some haplochromine species, mainly detritivores and zooplanktivores, recovered (Seehausen *et al.* 1997b; Witte *et al.* 2000, 2007). Species of both trophic groups shifted their diet to the more abundant macroinvertebrates such as insects, molluscs and shrimps and to small fishes (juveniles of dagaa) (Van Oijen & Witte 1996; Katunzi *et al.* 2003; Kishe-Machumu *et al.* 2008). In addition to these dietary changes, some species also showed adaptive morphological responses to the changed environment. For example, Witte *et al.* (2008) found that the zooplanktivore *Haplochromis* (*Yssichromis*) *pyrrhocephalus* Witte & Witte-Maas 1987 showed an increased gill surface, presumably as a response to the low DO levels. They also reported an increase of a pharyngeal jaw crushing muscle, the *musculus levator posterior*, probably reflecting an adaptive response to the larger and more robust prey. Their study actually laid the foundations for this thesis.

The aim of the thesis

The HEST collected haplochromines since the 1970s (before the severe environmental changes that affected Lake Victoria). These fishes are now stored in the Naturalis Biodiversity Center (which holds about 125,000 cichlid specimens). This collection represents a unique opportunity to study the effects of the environmental changes on the ecomorphology of the haplochromine cichlids. The specimens and environmental variables were collected on an almost yearly basis enabling detection of morphological changes as soon as they occurred in the cichlid populations.

The main objectives of this thesis were (i) to discover whether or not four recovered species showed morphological adaptive responses to the environmental changes; and (ii) if they do, what mechanism (see below) lays behind this response.

As has been described in the first section of this introduction, several species are known to adapt rapidly under certain selection regimes. Cichlids show a high degree of phenotypic plasticity, a phenomenon which has been described as "the environmentally sensitive production of alternative phenotypes by given genotypes" (DeWitt & Scheiner 2004). Several haplochromine species show plastic responses in body shape (Crispo & Chapman 2010a), head volume (Rutjes *et al.* 2009), gill surface (Chapman *et al.* 2000; Rutjes *et al.* 2009), pharyngeal jaw apparatus (Hoogerhoud 1986; Huysseune *et al.* 1994, 1995; Smits *et al.* 1996, 1997; Muschick *et al.* 2011), premaxilla (upper jaw, Witte 1984; Meyer 1987; Wimberger 1991; Bouton *et al.* 2002a) and eye properties (Van der Meer 1993). In addition to selection and phenotypic plasticity, recent and ongoing hybridization might influence cichlid morphology.

For this thesis, four recovered haplochromine species collected from 1977-2011, and two haplochromine species collected from 1978-1985 and thought to be now extinct, were selected. The resurgent species were: two closely-related zooplanktivorous species; *H.* (*Y.*) *pyrrhocephalus* and *H.* (*Y.*) *laparogramma* Greenwood & Gee, 1969; one zooplankti/insectivorous species; *H. tanaos* Van Oijen & Witte, 1996 and one mollusci/detritivorous species; *Platytaeniodus degeni* Boulenger, 1906. The species that are thought to be extinct and have not been caught on the research transect since 1986 were *H. (Y.) heusinkveldi* Witte & Witte-Maas, 1987 and *H. piceatus* Greenwood & Gee, 1969.

For the purpose of revealing ecological causes and developmental mechanisms of morphological changes, this thesis addresses the following research questions:

(1) Do all four recovered species show morphological changes over time?
If so, it is likely that the changing environment has influenced the morphology of all of these four species, like in *H. pyrrhocephalus*. A lack of morphological changes would indicate either that there is no need to adjust to the changed environment or that species are not able to adjust.

(2) Over what time scale did the morphological changes take place?
By answering this question, insight will be provided into the mechanism behind the morphological changes (see below, question 5). Firstly, genetically based morphological changes will be slower and are likely to appear more gradually over time than plastic responses. Secondly, a wider trait variation in the old populations (before the environmental changes) than in the modern ones (after the environmental changes) might indicate stronger natural selection than before or a potential bottleneck effect. Thirdly, genetic introgression through hybridization may be traced by comparing traits of resurgent species, as morphological convergence between recovered species might indicate hybridization.

(3) Can the morphological changes be linked to environmental changes?
As most the severe environmental changes peaked in the same time period (1984-87), unravelling the exact timing of environmental, ecological and morphological changes may reveal causes and effects of these changes. In addition, by comparing morphological responses of resurgent and extinct species, more insight may be provided into how some species adapted and survived while others maladapted and became extinct.

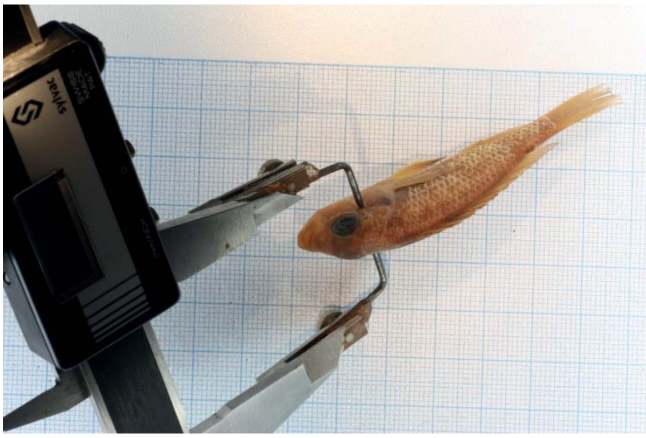
(4) Are the morphological changes adaptive?
If the morphological changes are adaptive, they should in principle enhance the inclusive fitness of the fishes in the changed environment. Alternatively, changes could be random and thus neutral or even maladaptive. If, however, the pattern of morphological changes is the same in several species, it becomes more plausible to suggest that they are relevant for an enhanced exploitation of the changed environment. Again, a comparison between resurgent and extinct species might shed light on this matter.

(5) Are the morphological changes due to phenotypic plasticity or to genetic changes?
Gradual or sudden morphological trait shifts, trait variation, interspecific trait comparison and genetic information all can provide information on the mechanism(s) behind observed morphological changes (natural selection, phenotypic plasticity, hybridization or a combination of these mechanisms). For instance, the absence of genetic changes might imply an important role for phenotypic plasticity in the morphological changes.

Thesis outline

This thesis consists of seven chapters. The introduction (**Chapter 1**) is followed by five research chapters and a 7th chapter that summarizes and discusses the results and provides future perspectives.

Chapter 2 describes morphological changes in the eyes of two haplochromine cichlid species and examines how these changes could represent adaptations to increased water turbidity, to larger prey, or to both. **Chapter 3** describes changes in the body shape of four resurgent cichlid species, and examines the timescale over which these changes occurred. It also discusses the hypothesis that these changes could be adaptations. In addition, a comparison is made with the body shape of extinct species. **Chapter 4** investigates whether any of the four resurgent haplochromines shifted their diet to larger and more robust prey; and, if they did, what was the effect of this shift on the premaxilla (upper jaw) of these fishes. As both the diet and premaxilla of the same fish are studied in this chapter, direct correlations can be examined. **Chapter 5** studies whether climatic variability might have influenced gill morphology in the four resurgent haplochromine species. It also examines whether the eutrophication of the lake was caused by anthropogenic perturbations alone, or whether climatic changes might also have played a role. **Chapter 6** explores stable isotope signatures in formalin-preserved haplochromine tissues and whether or not these reflect the observed dietary changes which are based on stomach content analysis. The possibilities that stable isotopes might reflect increased primary production, and thus eutrophication, are discussed. **Chapter 7** summarizes the research chapters, puts their results in a broader perspective, and discusses future outlooks.



Chapter 2

Photopic adaptations to a changing environment in two Lake Victoria cichlids

Henny J. van der Meer, Jacco C. van Rijssel, Leon C. Wagenaar & Frans Witte

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Abstract

During the past 30 years, Lake Victoria cichlid fishes have encountered severe environmental and ecological changes including an introduced predator and other prey types. Furthermore, increased eutrophication led to reduced water transparency and shifted the spectral composition of underwater light to longer wavelengths. Here, collections of two cichlid species, *Haplochromis pyrrhocephalus* and *Haplochromis tanaos*, from before and after the environmental changes, were compared with respect to their photopic resolution and sensitivity. Eyes of both species were dissected and retinal features were measured from tangential sections. In both species the eyes became smaller, independently of body size. This decrease possibly occurred to make space for other structures in the head that increased in size. In *H. pyrrhocephalus,* a significantly lower resolution was found. However, despite the smaller eyes, the size and thus photon catching ability of the double cones, remained unchanged. In the modern populations of *H. tanaos*, the double cone size increased in relation to eye size, so that the photon catching ability of the smaller modern fishes remained the same. However, no significant decrease in resolution was found. Shortwave sensitivity was found to be lower in both modern populations, because of reduction or complete absence of single cones. Our results imply that these resurgent zooplanktivores are capable of adapting their eye morphology to the changed environmental conditions without losing crucial aspects used for survival and reproduction.

Introduction

Since the 1980s, Lake Victoria's endemic haplochromine cichlids have had to cope with extreme environmental changes in the lake. Nile perch were introduced into the lake and their numbers boomed, resulting in a decline in abundance of the haplochromines (Ogutu-Ohwayo 1990; Witte *et al.* 1992a; Goudswaard *et al.* 2008). At the same time, eutrophication increased, resulting in lower dissolved oxygen (DO) levels and poorer light conditions (Muggide 1993; Hecky *et al.* 1994; Seehausen *et al.* 1997a; Chapter 5 in this thesis). The increased light absorption by dissolved and dispersed organic matter resulted in reduced illumination and less penetration of short-wavelength blue light (Seehausen *et al.* 2008). The multiple stressors mentioned above contributed to the decline of the haplochromines (Kaufman 1992; Witte *et al.* 1992a; Seehausen *et al.* 1997a; Hecky *et al.* 2010). However, after a decline of the Nile perch population in the 1990s, a number of haplochromine species reappeared in the Mwanza Gulf of Lake Victoria. This occurred despite the fact that predation pressure by the remaining Nile perch was still high, DO concentrations were still low, and the light conditions were still poor (Witte *et al.* 2000, 2007). Two of the resurging species were the zooplanktivores *Haplochromis (Yssichromis) pyrrhocephalus* and *Haplochromis tanaos.* In the 1970s, *H. pyrrhocephalus* co-existed with some other zooplanktivores in the open waters of the Mwanza Gulf. It was mainly found near the bottom, at depths of 8-14m during the day, and nearer to the surface at night (Goldschmidt *et al.* 1990). *Haplochromis tanaos* mainly occupied shallow sand bottoms at depths of less than 6m in bays of the Mwanza Gulf (Van Oijen & Witte 1996).

After its resurgence, *H. pyrrhocephalus,* extended its habitat into regions of only 4 m deep (Kishe-Machumu 2012) and became the most common haplochromine cichlid of the Mwanza Gulf (Witte *et al.* 2000). *Haplochromis tanaos* extended its habitat to deeper (13 m) mud bottoms and also became one of the more common species (Van Oijen & Witte 1996; Seehausen *et al.* 1997b; Kishe-Machumu 2012). In addition, both species shifted their diet from zooplankton to insects and other larger and more robust invertebrates (Van Oijen & Witte 1996; Katunzi *et al.* 2003; Kishe-Machumu *et al.* 2008; Chapter 4 in this thesis).

Major morphological changes were observed in the resurgent *H. pyrrhocephalus*. An increase in the surface area of the gills seemed to be an adaptation to the lower DO concentrations (Witte *et al.* 2008). A decrease in head size, which could be an adaptation for escaping Nile perch predation, was also observed (Chapman *et al.* 2008; Witte *et al.* 2008; Van Rijssel & Witte 2013 [Chapter 3 in this thesis]). More difficult to explain was a decrease in eye size, despite the decreased light conditions. Witte *et al.* (2008) suggested that the smaller eye size might be due to a trade-off with the increased space needed to accommodate larger gills and a larger buccal cavity depth in a smaller head. They suggested that the structure of the retina should be studied to see whether a decrease in eye size would negatively influence the visual capacities of the fish.

There are many ways to compensate for the above-mentioned decrease in eye size in a turbid environment. Recent studies have shown that opsin gene expression plays a major role in cichlid eye adaptation and speciation in turbid environments (Carleton *et al.* 2005; Seehausen *et al.* 2008; Hofmann *et al.* 2009, 2010; Maan & Seehausen 2010). In addition, behavioural changes in response to turbidity can contribute to the persistence of cichlid species (Gray *et al.* 2011; Gray *et al.* 2012). However, this is beyond the scope of the present study, where we concentrate on morphological changes.

To cope with the environmental changes, the haplochromines, which are visual predators (Fryer & Iles 1972), would require an increased photopic sensitivity. This would mean an enlargement of the cones at the cost of their planimetric and thus angular density (Van der Meer & Anker 1984). Enlargement of the cones is a common feature in growing cichlid eyes (Van der Meer 1993, 1994). So, the reduced eye-size found in the modern population of *H. pyrrhocephalus* was unexpected in relation to the assumed visual demands. Studies of the retina of *H. pyrrhocephalus* from before the environmental changes (old populations) however, revealed exceptionally large long-wavelength-sensitive (LWS) double cones (Van der Meer *et al.* 1995). In addition, Van der Meer & Bowmaker (1995) showed that the spectral sensitivity of this species covered significantly longer wavelengths than in other haplochromines investigated. This suggested a "pre-adaptation" to the new light conditions which may have contributed to the successful recovery of *H. pyrrhocephalus*.

A decrease in eye-size does not necessarily imply a reduction of photopic sensitivity, since the photon catching ability (PhCA) depends on cone-size and not on eye size (Van der Meer & Anker 1984). An increased PhCA (due to increased cone size) compensates for the reduced visibility of nearby objects (predator, prey or congener). The higher the PhCA, the sooner a fish can respond, either by approaching or escaping the object of detection. Following retinal studies on haplochromines by Van der Meer *et al*. (1995), such detection is most profitable in a lateral direction. Therefore, the largest cones were expected to be located in the medial and rostral regions of the retina because the eyes are slightly directed forward. Detail discrimination, e.g. for manipulation of food particles, is determined by resolution, which depends on the angular density of the photopic units (single and double cones, Van der Meer & Anker 1984). Therefore, the highest angular density of LWS double cones (which are red-green sensitive) was expected in the caudal periphery creating a detailed image of objects directly in front of the snout.

Since blue sensitivity is assumed to have become redundant in the changed spectral environment, we expected a reduction in the size and number of single cones as they are known to contain the SWS photopigments (Van der Meer & Bowmaker 1995). We also expected to find a square mosaic of double and (small) single cones in the old population of *H. tanaos*. This pattern was also found in *H. (Ptyochromis) fischeri* (formerly *H. sauvagei*), that used to coexist with *H. tanaos* in the shallow sand habitat. A reduction in the number and size of single cones may provide more room for double cones in a regular cone mosaic (Van der Meer 1992). Therefore, we expected a modest enlargement of the double cones in the modern population of *H. tanaos* just as in blue-light-deprived specimens of *H. fischeri* (Van der Meer 1993). To investigate if the photopic sensitivity and resolution of the modern populations (collected between 1991 and 2001) of *H. pyrrhocephalus* and *H. tanaos* showed adaptive responses to the new environment, we compared their retinal morphology with those of the old populations (collected between 1977 and 1981).

Materials and methods

Adult specimens of both *H. pyrrhocephalus* and *H. tanaos* were collected with bottom trawls in the northern part of the Mwanza Gulf of Lake Victoria over the period 1977-2001. The fish were initially fixed and preserved in 4% formalin (buffered with borax) and afterwards transferred to 70% ethanol in the Naturalis Biodiversity Center, Leiden, for long-term storage. In total, 22 specimens of *H. pyrrhocephalus* and 12 specimens of *H. tanaos* were selected from the period of 1977-1981 and 22 specimens of *H. pyrrhocephalus* and 13 specimens of *H. tanaos* were selected from the period 1991-2001. As far as the available material permitted, adult fish of equal sizes were selected from the different periods. The standard lengths (SL) of these specimens were measured *sensu* Barel *et al.* (1977). Specimens of *H. pyrrhocephalus* used in the study of Witte *et al.* (2008) were included in this study as well.

The eyes were isolated by dissection and the lens-radius (r) was measured either by using an eye-piece micrometer mounted on a binocular microscope, or from digital photographs (the two techniques gave identical results). Due to damage of the retina, not all eyes were suitable for sectioning. Therefore, we selected 20 specimens of old populations (13 *H. pyrrhocephalus* and 7 *H. tanaos*) and 22 specimens of modern populations (16 *H. pyrrhocephalus* and 6 *H. tanaos*) for further analysis (Appendix Table 2.1, 2.2). Whole eyes were dehydrated and embedded in paraplast® using the position of the falciform process as a means of orientation. Semi-thin (5µm) tangential sections were made on a microtome with a steel knife using a graduated location-device (Van der Meer & Anker, 1986) to determine the original position of the sections. The falciform process, which is always located caudo-ventrally, was used as a reference. Accordingly, data were collected from 12 corresponding retinal areas, *viz.* the medial (4) and peripheral (8) regions of the dorsal, rostral, ventral and caudal areas (Figure 2.1).

All sections were stained with hematoxylin and eosin to obtain sufficient contrast between inter- and intra-cellular spaces. The sections were photographed and stored as TIF (Tagged Image File format) files. With the use of Image Tool 1.28 (*H. tanaos*) and ImageJ 1.44p (*H. pyrrhocephalus*) the mean size of the double cones (S_d; based on five double cones) and their areal density (D_d; number per retinal area in three locations; counting was conducted within a field of 10^3 μm^2) were measured and stored. S_d was actually the area of the cross-section through the semi-combined ellipsoid of a double cone and was considered to be a measure of photopic sensitivity (Van der Meer & Anker 1984). The angular density of the double cones (H_d; number per degree of visual angle), a measure of retinal resolution, was calculated, using:

$$H_d = (2.5 \cdot r \cdot \pi \cdot 360^{-1})^2 \cdot \pi \cdot D_d$$ (Van der Meer *et al.* 1995)

The size and angular density of the double cones in the twelve regions were registered for each fish. Measurements on similar retinal locations allowed us to compare individual specimens by mean values. Also the maximum regional values of size (S_{max}) and angular density (H_{max}) were registered for each specimen, as well as their location.

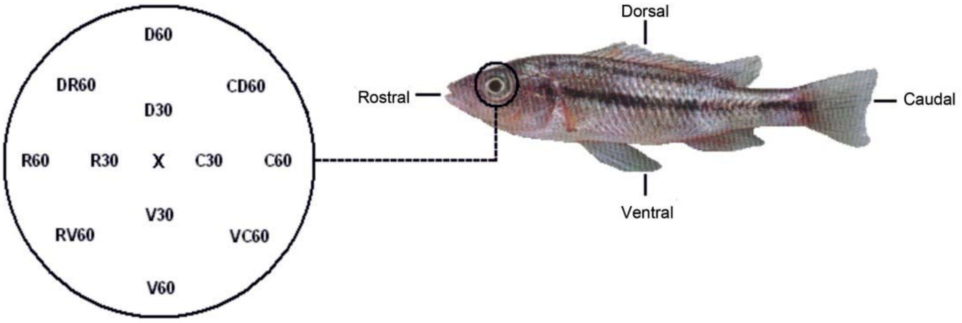

Figure 2.1 The twelve retinal locations sampled in both species (picture of *H. tanaos*). X, centre of retina; C, caudal; D, dorsal; R, rostral; V, ventral; 30, 30°; 60, 60°.

As not all areal densities of each retina could be measured for *H. tanaos*, missing values were interpolated with the use of known mean densities of the double cones of the other individuals of the same group. By calculating mean relative densities (per retinal position) for other individuals and revaluating those according to the other retinal positions, an absolute measure of the unknown areal density was found. Ten missing values for mean density, out of a total of 168 known values, were calculated, assuming an equal retinal distribution amongst individuals.

The size of the inner segments of the single cones S_s was measured in the regions where they were observed. In these regions the relation between the number of single and double cones (s/d) was registered. As in a perfect square mosaic s/d = 0.5, this value was referred to as 100% and the measured single cone occupancy was expressed accordingly. The mean value of S_s in each specimen was derived from the observed single cones, if any, in the entire retina. The mean value of s/d and the percentage of single cone occupancy was derived from the measurements in all twelve regions, including the ones where single cones were absent.

An Analysis of Variance (ANOVA) was used to test if there was a difference in SL among the selected samples. Since both cone-size and angular density increase during growth, SL and r were chosen as covariates for the General Linear Model (GLM), with the population period (old or modern) as independent factor, to test for the effect of period on the morphological characters. Dependent variables, independent factors and the interactions between them were inserted in this selective model. With the use of Multivariate Analyses of Covariance (MANCOVA), non-significant interactions were removed stepwise from each model and estimated marginal means (EMMs, the means of the morphological characters corrected for the used covariate) were calculated. Significant interactions between SL and population period were plotted to determine the effect of each factor. The unstandardized residuals of each GLM were used to test for normality with the Shapiro-Wilk test. *P*-values of the GLM were corrected with a sequential Bonferroni test. All statistical tests on the morphological characters were performed with SPSS version 16.

Results

H. pyrrhocephalus

The mean SL of the modern *H. pyrrhocephalus* studied (61.4 mm) was slightly, but significantly smaller (4.4 %) than that of the specimens of the old population (64.2 mm; $P = 0.025$). The lens-radius of the modern population ($r = 1.21$ mm) was also significantly smaller compared to that of the old population ($r = 1.46$ mm; $P < 0.001$; Table 2.1). Due to a constant ratio between lens diameter and eye diameter (Matthiessen's ratio; Otten, 1981) the lens radius serves as a measure for eye size. The relation between r and SL (Figure 2.2; Table 2.1) showed consistently smaller lenses (17.1 %) in similar sized specimens of the modern population, indicating a smaller eye-size compared to the old population.

In seven out of 13 specimens (54%) of the old population, small single cones were sporadically observed and were randomly allocated within the retinal regions (Figure 2.3A). In the modern population, except for a few single cones in one specimen, the inner segments or ellipsoids of single cones were never observed (Figure 2.3B). In eight out of 16 specimens (50%) of the modern population, relics of single cones (possibly the remains of their nuclei), were sporadically observed in several regions (Appendix Table 2.1). In the retinas of both the old and modern populations, the double cones were more or less irregularly arranged in rows (Figures 2.3A, B). In both populations, the highest densities of double cones were predominantly found in the caudal periphery, whereas the largest cones were mostly measured in the rostral part of the retina (Appendix Table 2.1).

The H_d and H_{max} (the latter in the caudal periphery) have significantly decreased in the modern population (by 28% and 33%, respectively, $P < 0.001$, Figure 2.2; Table 2.1). Both the S_d and S_{max} of modern specimens with a small standard length ($SL \leq 60$ mm) tend to be larger compared to those of the old population (by 15% and 17% respectively). This occurred while S_d and S_{max} of larger specimens ($SL > 60$ mm) had decreased in the modern population (by 13% and 19%, respectively), which altogether results in a significant interaction (Figure 2.4). Estimated marginal means (EMMs) of S_d and S_{max} differed only slightly between the old and modern populations (Table 2.1; Figure 2.4). Concerning H_d and H_{max} in relation to r, there was no significant effect of period, but there was a significant effect of r as covariate (Table 2.1). Both S_d and S_{max} showed a significant interaction, their EMMs differing only slightly (Table 2.1, Figure 2.5).

H. tanaos

The mean SL of the modern *H. tanaos* (64.7 mm) studied did not differ significantly from that of the specimens of the old population (66.3 mm; $P = 0.289$). The r of the modern population of *H. tanaos* was 1.10 mm. This value is 9.0% smaller ($P < 0.001$) than that of the old population (1.21 mm; Figure 2.2; Table 2.2). The single cone size (S_s) of the modern population was significantly smaller than in the old population ($P < 0.001$, with SL and r as covariate; Figure 2.4, 2.5). The mean single cone occupancy decreased from 88% to 56% ($P < 0.001$, with SL as covariate) which affected the ratio between single cones and double cones (s/d). There was a significant decrease of s/d from 0.44 in the old population to 0.29 in the modern population ($P < 0.001$, Table 2.2).

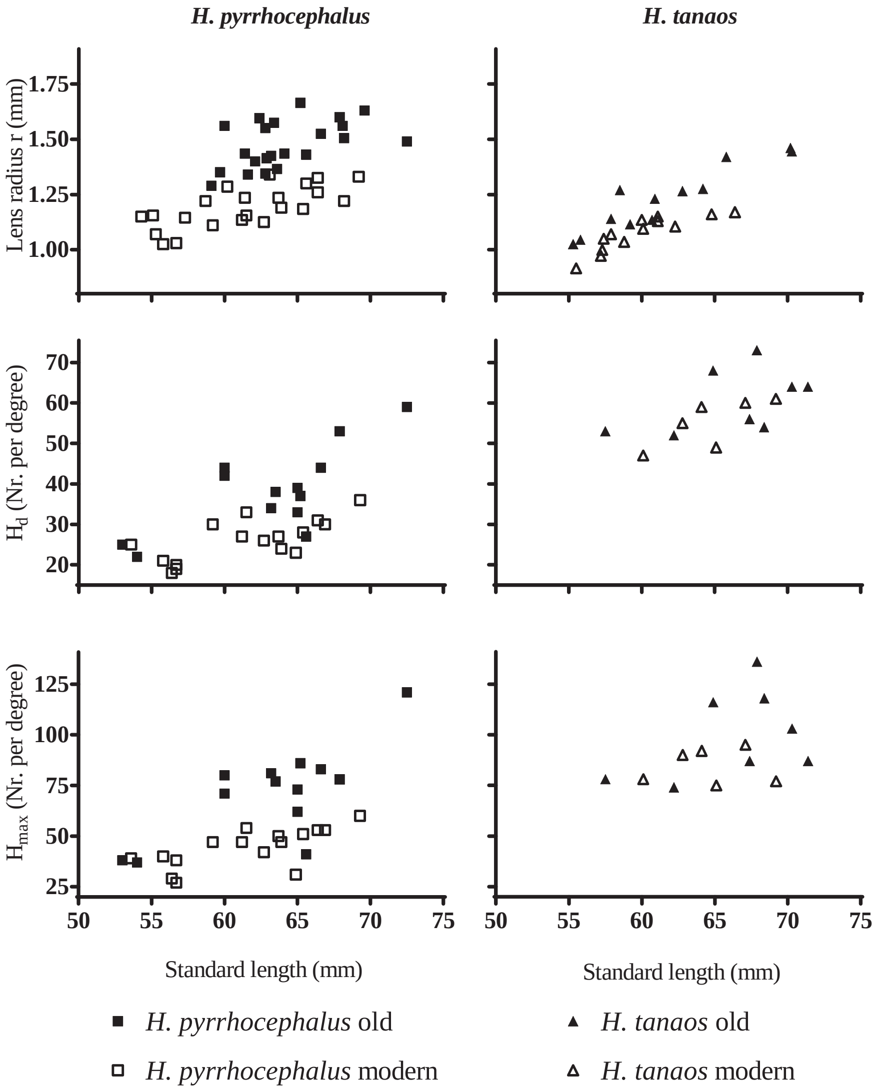

Figure 2.2 Plots of r, H_d, H_{max}, as a function of SL.

The loss of single cones did not coincide with a changed configuration from a square pattern into a row pattern (Figure 2.3C, D). Square patterns with small or absent single cones were usually transformed into diamond patterns (intermediate between square and row patterns, Van der Meer 1992). A clear row pattern was found only on a few occasions in the modern population and only in rostral areas. In both populations, the highest densities of double cones were found predominantly in the dorsal periphery. In the old population, the largest cones were usually observed in the rostral regions of the retina. In the modern population, the largest cones were not strictly confined to a specific region although they were mainly observed in the medial parts (Appendix Table 2.2).

Table 2.1 Results of the GLM analyses on the morphological characters of *H. pyrrhocephalus* with SL and lens radius (r) as covariates. Significant interactions are shown in italics, significant values after sequential Bonferroni corrections are shown in bold.

H. pyrrhocephalus			Cov SL					Cov r				
Character	Pop	N	Mean	Difference (%)	*P* Population	*P* SL	*P* Pop * SL	Mean	Difference (%)	*P* Population	*P* r	*P* Pop * SL
Lens-radius	old	22	1.46									
(r, mm)	modern	22	1.21	**-17.1**	**0.000**	**0.000**	NS	X	X	X	X	X
Angular density	old	13	33.6					31.3				
(H_d, Nr. per degree)	modern	16	24.1	**-28.3**	**0.000**	**0.000**	NS	31.8	1.6	NS	**0.000**	NS
Max. angular density	old	13	68.8					56.3				
(H_{max}, Nr. per degree)	modern	16	46.4	**-32.6**	**0.000**	**0.000**	NS	56.5	0.4	NS	**0.000**	NS
Double cone size	old	13	*44.3*					*41*				
(S_d, μm^2)	modern	16	*42.4*	*-4.3*	*0.028*	*NS*	*0.024*	*39.4*	*-3.9*	*0.029*	*NS*	*0.035*
Max. double cone size	old	13	*70.1*					*63.8*				
(S_{max}, μm^2)	modern	16	*64.7*	*-7.7*	*0.001*	*NS*	*0.001*	*59*	*-7.5*	*0.009*	*NS*	*0.01*
Single cone size	old	7	12.2									
(S_s, μm^2)	modern	1	8									
Ret. Occ.	old	13	7									
(%)	modern	16	4									
Ratio single cones -	old	13	0.04									
double cones (s/d)	modern	16	0									

Means are estimated marginal means derived from the GLM. Pop, population.

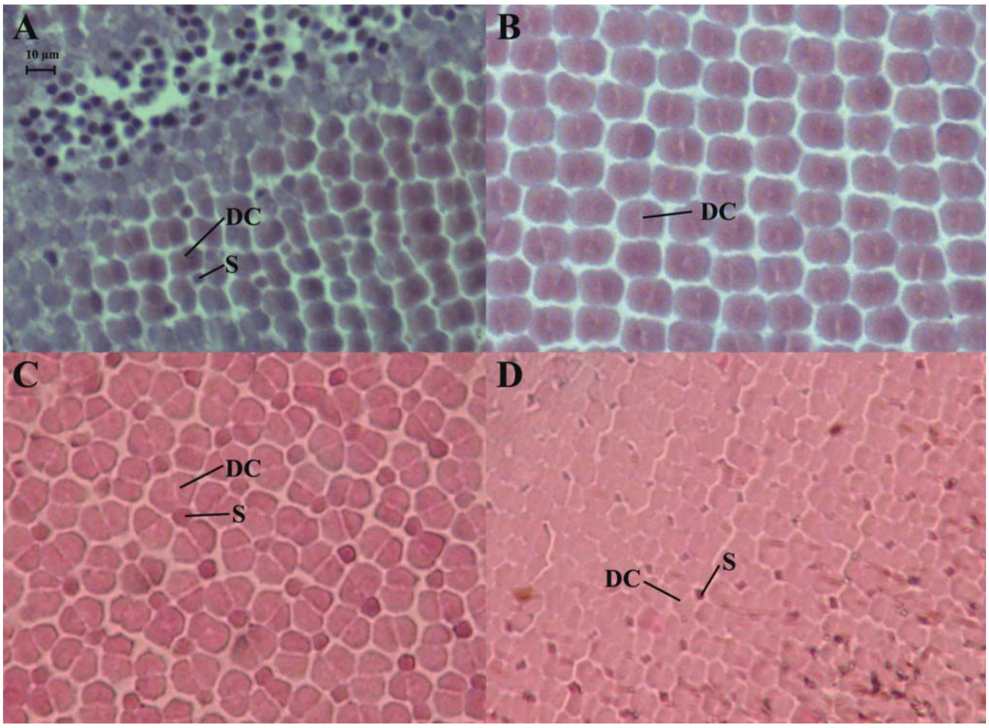

Figure 2.3 Double cone patterns; DC, double cone; S, single cone. A: Row pattern of double cones with presence of some single cones in caudal region of *H. pyrrhocephalus* from 1978 (spec. nr 316-131). B: Row pattern of double cones with no single cones present in the rostral-ventral region in *H. pyrrhocephalus* from 2001 (316-205). C: Square pattern of double cones around single cones in the rostral region in *H. tanaos* from 1978 (320-09). D: Diamond pattern of double cones around tiny single cones in the rostral-ventral region in *H. tanaos* from 2001 (320-15).

The H_d and H_{max} (in the dorsal periphery) of the double cones did not show a significant decrease with SL and r as covariate (Table 2.2; Figures 2.2, 2.5). With SL as covariate, S_d showed a significant interaction, with EMMs of the modern population being slightly larger. No significant difference was found for the S_{max} (Figure 2.4; Table 2.2). The S_d and S_{max} were significantly larger in the modern population with r as covariate. However, after Bonferroni correction, the difference for S_{max} was no longer significant (Table 2.2).

Discussion

In the resurging populations of *H. pyrrhocephalus* and *H. tanaos*, lens size, and thus eye size, decreased, possibly to permit changes in head morphology for other functions than vision (e.g. Witte *et al.* 2008). Both species showed a lower resolution and a decreased blue SWS light sensitivity. In addition, despite the smaller eyes, *H. tanaos* showed an increase of their photopic sensitivity. These changes are in accordance with the increased turbidity, the larger prey types included in the diet of both modern species and the shift to greater depths in *H. tanaos*.

Cone size comparison

There is some discrepancy regarding the double cone size of the old *H. pyrrhocephalus* population from this study, and that of the sample used by Van der Meer *et al*. (1995). The average double cone size in the old population of *H. pyrrhocephalus* from the latter study was larger (52 μm^2) than that of the old population used in the present study (45.5 μm^2, both not corrected for SL). This difference may have several explanations. Retinal rods and cones exchange places under the influence of the environmental light by contraction or elongation of their myoids (light- and dark-adaptation; Ali 1975). The specimens used in the earlier publication were super-exposed to light when caught. This exposure makes sure they were completely light-adapted, *i.e.* the cone myoids were maximally contracted which compressed the ellipsoids towards the outer limiting membrane. This may have been less intense in the light-adapted specimens used in the present study. Moreover, the number of samples from one retina in Van der Meer *et al*. (1995) was much larger (over 30 regions) than in the present study. This affects the balance between the periphery and the centre of the retina for the benefit of the latter, where also the larger cones are located. Furthermore, the fish specimens (and their eyes) used in the study by Van der Meer *et al*. (1995) were stored in 10% formalin (buffered with borax), and were only exposed to alcohol during a relatively short dehydration step. By contrast, the specimens used in the present study were stored in alcohol for many years, and this may have caused more shrinkage of retinal tissue. However, it should be stressed that in the present study, the techniques used for both old and modern fish were identical, thus making the samples comparable.

Eye size

The observed reduction in eye-size in the modern population of *H. pyrrhocephalus* (Witte *et al.* 2008) was confirmed by the present data on lens size in *H. pyrrhocephalus* and *H. tanaos*. The reduction of eye size can be explained by the changed environment. Environmental conditions, especially low dissolved oxygen levels, have a major influence on body shape and cause an increase in gill surface area (Chapman *et al.* 2000; Rutjes *et al.* 2009; Crispo & Chapman 2010a). The first two studies found, under lab conditions, larger gills in a *larger* head. By contrast, Witte *et al*. (2008) found larger gills in a *smaller* head in wild modern *H. pyrrhocephalus*. The smaller head may have been caused by the increased predation pressure of Nile perch (Chapman *et al.* 2008; Van Rijssel & Witte 2013 [Chapter 3 in this thesis]). Moreover, Witte *et al.* (2008) suggested that the observed larger buccal cavity depth (cheek depth) could be relevant for eating larger prey items. Consequently, it is likely that the smaller eyes are caused by predation pressure, diet change and hypoxia. Thus, the smaller eyes may act as a trade-off for larger gills and a larger buccal cavity in smaller heads (Witte *et al.* 2008). However, Gray *et al*. (2011, 2012) found that behavioural changes are also important in the survival of cichlids in a turbid environment. Furthermore, female sticklebacks rely more on olfactory than on visual cues in turbid waters when choosing a mate (Heuschele *et al.* 2009), which might be the case for cichlids too. These studies suggest that there may be multiple strategies to cope with a turbid environment.

Angular density

As visual resolution primarily depends on the number of receptors per visual angle, the lower angular density in the modern populations indicates a lower resolution in the modern populations, compared to the old population. Since cichlids are known to be visual feeders (Fryer & Iles 1972), the lower visual resolution is in agreement with the diet shift of *H. pyrrhocephalus* and *H. tanaos* from zooplankton to larger prey types (Van Oijen & Witte 1996; Katunzi *et al.* 2003; Kishe-Machumu 2012; Chapter 4 in this thesis). Differences between old and modern populations in angular density in relation to eye size of both species were caused by the smaller retina.

SWS single cones

The reduction of the SWS single cones in both species agrees with the stronger absorbance of shortwave light due to eutrophication of the environment (Seehausen *et al.* 2003) and also with the shift of *H. tanaos* to deeper water over mud bottoms. Apparently, there was no "need" anymore for single cones because the short wavelengths are absorbed by the turbid water. The absence of functional elements of single cones in the studied sections of *H. pyrrhocephalus* does not imply their complete disappearance, as indicated by the occasional nuclei observed in the sections.

A recent study on cone opsin expression in Lake Malawi cichlids revealed that phyto/zooplanktivores had higher SWS opsin gene expression than species feeding on fish or benthic invertebrates (Hofmann *et al.* 2009). However, SWS opsin gene sequence of some Lake Victoria cichlid species show hardly any variability between species, in contrast with LWS opsin gene sequence (Carleton *et al.* 2005). These LWS opsin genes have shown to have a high differentiation rate between two sympatric *Pundamilia* phenotypes in association with water clarity (Seehausen *et al.* 2008). This difference resulted in longer LWS pigments for the deep water red species compared to the shallow water blue species. Multiple studies suggest that the variation in the expression of opsin genes might be adaptive and driven by variation in ambient light (Carleton *et al.* 2005; Seehausen *et al.* 2008; Hofmann *et al.* 2009; Maan & Seehausen 2010). In addition, Hofmann *et al.* (2010) found evidence suggesting sensory plasticity played a role in cichlid diversifications in Lake Malawi. Selection on and/or plasticity of opsin genes might also have resulted in the reduction or absence of single cones found in the present study. Unfortunately, our sample did not allow us to study opsin gene expression as fish were preserved in formalin.

Furthermore, it cannot be ruled out that in the modern populations, the reduction of single cones was the result of elongation of their myoids (as in dark-adaptation, Ali 1975) In this scenario, myoid elongation screens the single cones from incoming light and reduces their function. Such a reversible phenotypic phenomenon, however, is not supported by earlier studies of the retina in *H. pyrrhocephalus* raised in brightly illuminated tanks for several generations (Van der Meer & Bowmaker 1995).

Table 2.2 Results of the GLM analyses on the morphological characters of *H. tanaos* with SL and lens radius (r) as covariates. Significant interactions are shown in italics, significant values after sequential Bonferroni corrections are shown in bold.

H. tanaos			Cov SL					Cov r				
Character	Pop	N	Mean	Difference (%)	*P* Population	*P* SL	*P* Pop * SL	Mean	Difference (%)	*P* Population	*P* r	*P* Pop * SL
Lens-radius	old	12	1.21									
(r, mm)	modern	13	1.10	**-9.1**	**0.000**	**0.000**	**NS**	X	X	X	X	X
Angular density	old	8	59.9					57.6				
(H_d, Nr. per degree)	modern	6	56	-6.5	NS	0.045	NS	59.1	2.6	NS	**0.01**	NS
Max. angular density	old	8	98.9					94.4				
(H_{max}, Nr. per degree)	modern	6	85.8	-13.2	NS	NS	NS	91.8	-2.8	NS	0.072	NS
Double cone size	old	8	*27.4*					26.2				
(S_d, μm^2)	modern	6	*29.5*	*7.7*	*0.029*	*0.034*	*0.035*	31.7	**21.0**	**0.001**	**0.000**	NS
Max. double cone size	old	8	39.3					37.8				
(S_{max}, μm^2)	modern	6	43.3	10.2	NS	NS	NS	45.3	19.8	0.019	**0.018**	NS
Single cone size	old	8	7					6.6				
(S_s, μm^2)	modern	6	4.2	**-40.0**	**0.000**	0.014	NS	4.7	**-28.8**	**0.001**	**0.001**	NS
Ret. Occ.	old	7	88.5					*87.9*				
(%)	modern	6	55.8	**-36.9**	**0.000**	NS	NS	*40.8*	*-53.6*	*NS*	*0.050*	*0.038*
Ratio single cones -	old	7	0.44					*0.44*				
double cones (s/d)	modern	6	0.29	**-34.1**	**0.000**	NS	NS	*0.22*	*-50.0*	*0.047*	*0.028*	*0.021*

Means are estimated marginal means derived from the GLM. Pop, population.

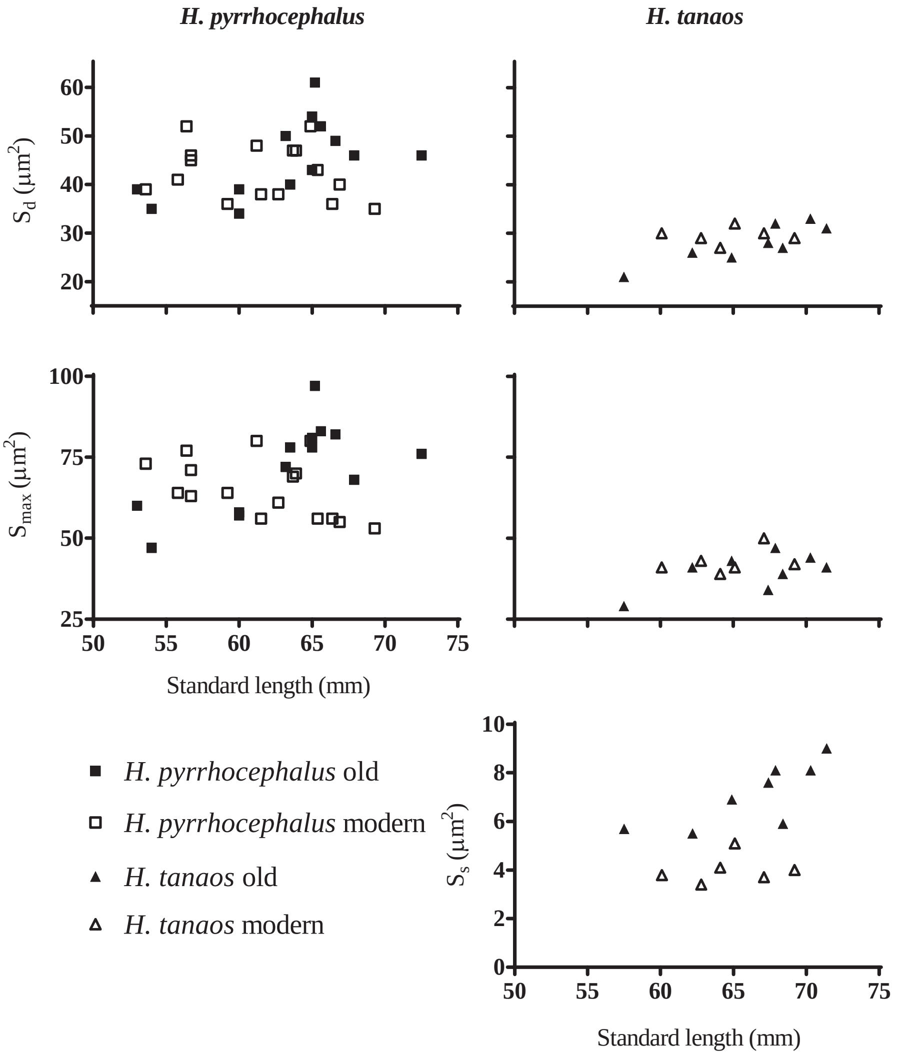

Figure 2.4 Plots of S_d, S_{max} and S_s as a function of SL.

LWS double cones

Even though the eyes became smaller in the modern populations of both species, the double cones remained of a similar size in the adult fish, or even increased for modern *H. tanaos* in relation to the smaller lens-radius. Consequently, the presumed photopic sensitivity did not decrease. The increase in double cone size in *H. tanaos* is likely facilitated by the reduction in size, and decrease in number, of single cones relative to eye-size.

The larger size of double cones in the smaller eyes of the modern population of *H. tanaos* suggests a shift in retinal growth from addition of cone cells to stretching of cone cells. *Haplochromis pyrrhocephalus* shows no increase of photon catching ability (by enlargement of its double cones) as an adaptive adjustment to the decreased light conditions. We can confirm that the already large cones and LWS photopigments of this species (also observed by Van der Meer & Bowmaker 1995) might be a "pre-adaptation" to the turbid environment.

The observed maximum double cone size in the medial and rostral regions of the retinas of both species, and the maximum angular density in the caudal periphery of *H. pyrrhocephalus*, are both in accordance with our expectations (see the introduction of this chapter). They seem to be characteristic for pelagic zooplanktivorous fish (Browman *et al.* 1990). The high dorsal resolution in *H. tanaos* (dorsal location of the maximum angular density in both the old and modern populations), suggests a detailed scanning of the bottom. This would categorise this species as a bottom-dweller as was also suggested by Van Oijen & Witte (1996) because *H. tanaos* was never caught in surface trawls. The observed retinal findings agree with the relation between retinal cell topography and feeding behaviour in other fishes (Shand *et al.* 2000).

Mechanisms behind retinal changes

The retinal changes in the modern populations of both species may have been the result of phenotypic plasticity as observed in shortwave light-deprived specimens of *H. fischeri* in laboratory experiments (Van der Meer 1993). But there are several other possibilities.

Based on mitochondrial DNA, Mzighani *et al.* (2010) suggested that in the relatively murky Mwanza Gulf, modern *H. pyrrhocephalus* hybridises with *H. laparogramma*, in contrast to three other locations with clearer water. If the modern specimens of *H. pyrrhocephalus* from the Mwanza Gulf were hybrids, this may have influenced the size of their double cones. As larger double cones were expected in the modern population of *H. pyrrhocephalus* and the double cones of *H. laparogramma* with the same SL were smaller (before the environmental changes, adult *H. laparogramma* were larger than adult *H. pyrrhocephalus*; Van der Meer *et al*. 1995), hybridization might have resulted in relatively smaller double cone sizes for *H. pyrrhocephalus*.

In addition to phenotypic plasticity and hybridization, natural selection might have played a role in the observed retinal changes as was suggested for several opsin genes (Carleton *et al.* 2005; Seehausen *et al.* 2008; Hofmann *et al.* 2009; Maan & Seehausen 2010).

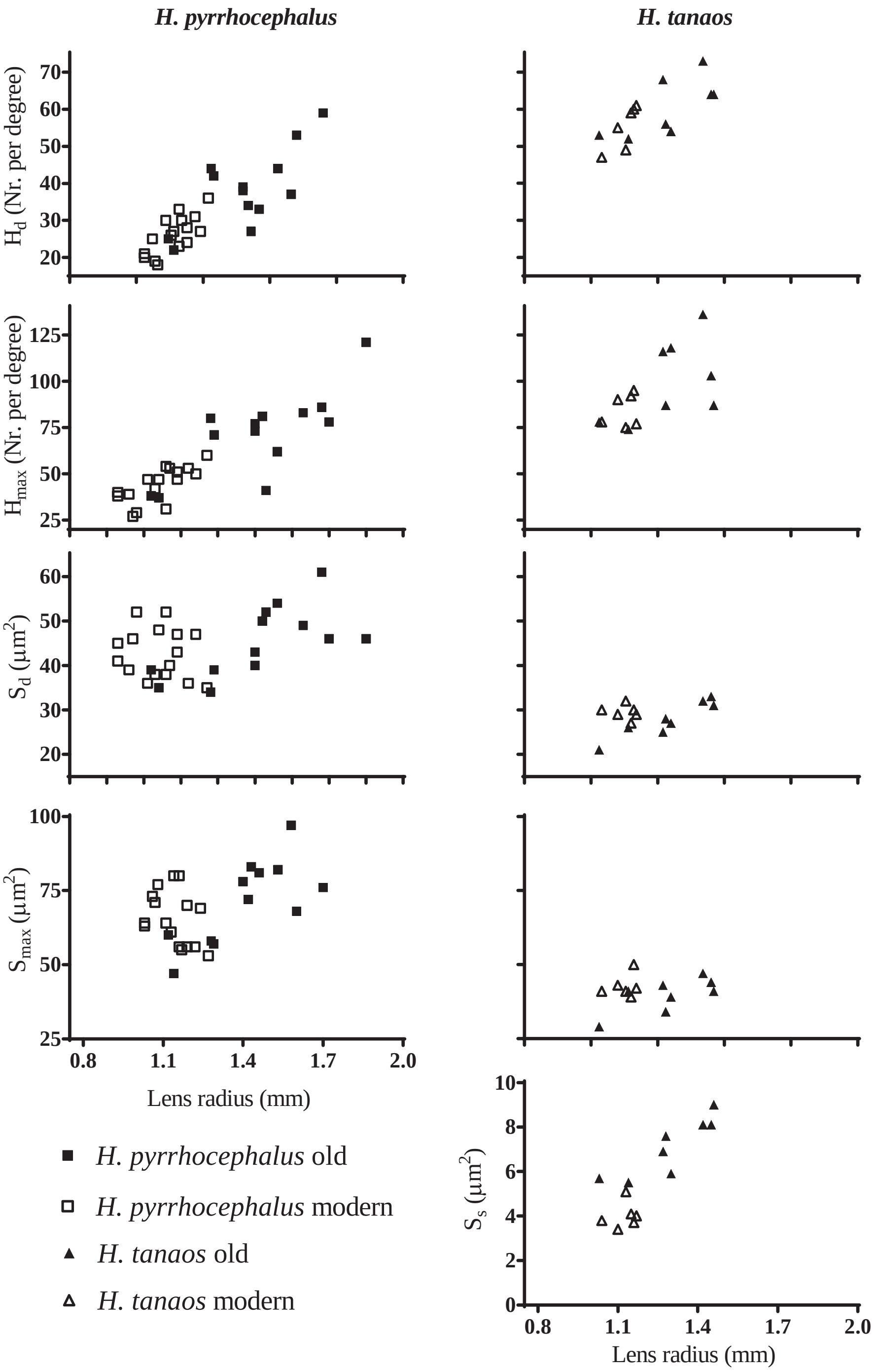

Figure 2.5 Plots of H_d, H_{max}, S_d, S_{max} and S_s as a function of r.

Morphological and ecological convergence

In addition to the reduction of single cones, both species show other changes in the same direction (morphological convergence). For example, the mean difference in lens size (and thus eye size) between the old populations of both species was almost twice as large (17.1%) as that between the modern populations (9.1%). There was also some convergence for cone size between both species as the difference became slightly smaller for the modern populations (30.1%) compared to the difference between the old populations (38.1%). The morphological convergences agree with the overlap in diet and habitat found for the resurgent populations of both species. Moreover, body shape convergence was found between *H. pyrrhocephalus* and *H. laparogramma* (J. C. van Rijssel & F. Witte unpublished data); the latter species shifted its habitat and diet towards that of *H. pyrrhocephalus* (Witte *et al.* 1995; Chapter 4 in this thesis).

Conclusions

Haplochromis pyrrhocephalus and *H. tanaos* have shown apparently adaptive ecological responses including a probable reallocation of internal head structures (Witte *et al.* 2008). In addition, retinal features have changed in such a way that only the resolution, and not the sensitivity for the ambient wavelengths in the modern environment, was negatively affected. Whether the morphological and retinal adjustments found in this study are the result of response to selection, phenotypic plasticity or hybridization remains unclear. Nonetheless, these presumably adaptive responses might have contributed to the recovery of these two zooplanktivores while other species went extinct.

Acknowledgements

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Appendix Table 2.1 General data of *H. pyrrhocephalus* specimens used in this study.

H. pyrrhocephalus

Period	Number	Catch	SL (mm)	r (mm)	H_d (Nr. per degree)	H_{max} (Nr. per degree)	S_d (μm^2)	S_{max} (μm^2)	S_s (μm^2)	Ret. Occ. (%)	s/d	Loc. H_{max}	Loc. S_{max}
Old	316-121	1977	72.5	1.7	59	121	46	76		0	0	C60/CV60	R60
Old	316-100	1981	65	1.46	33	62	54	81	9.1	18	0.9	C60	R30
Old	316-131	1978	66.6	1.53	44	83	49	82	3.1	4	0.02	CV60	R60
Old	316-126	1978	65.2	1.58	37	86	61	97		0	0	C60	R30
Old	316-105	1981	65	1.4	39	73	43	78	5.3	28	0.14	CV60	D30
Old	316-?	1980	53	1.12	25	38	39	60	19.6	4	0.02	C60	R30
Old	316-?	1980	54	1.14	22	37	35	47		0	0	CV60	R30
Old	316-96	1981	60	1.29	42	71	39	57	20.4	2	0.01	C60	RV60
Old	316-98	1981	63.5	1.4	38	77	40	78	6.6	34	0.17	CV60	R30
Old	316-127	1978	65.6	1.43	27	41	52	83		0	0	C60	R30
Old	316-130	1977	67.9	1.6	53	78	46	68		0	0	C60	R30
Old	316-?	1980	60	1.28	44	80	34	58	21.2	1	0.005	C60	R30
Old	316-128	1978	63.2	1.42	34	81	50	72		0	0	C60	R30
Modern	316-132	1993	56.4	1.08	18	29	52	77		0	0	C60	R30
Modern	316-133	1993	56.7	1.07	19	27	46	71	<1	2	0.01	C60	RD60
Modern	316-134	1993	55.8	1.03	21	40	41	64		0	0	V60	D30
Modern	316-135	1993	53.6	1.06	25	39	39	73		0	0	RV60	R30
Modern	316-136	1993	56.7	1.03	20	38	45	63		0	0	C60	RD60
Modern	316-144	1991	64.9	1.16	23	31	52	80		0	0	C60	R60
Modern	316-147	1999	66.9	1.17	30	53	40	55		0	0	CD60	R30
Modern	316-149	1999	66.4	1.22	31	53	36	56	8	6	0.03	C30	V60
Modern	316-150	1999	69.3	1.27	36	60	35	53	<1	2	0.01	D30	RV60
Modern	316-205	2001	61.2	1.14	27	47	48	80	<1	1	0.005	CV60	V30
Modern	316-196	2001	59.2	1.11	30	47	36	64	<1	12	0.06	V60	R30
Modern	316-192	2001	63.7	1.24	27	50	47	69	<1	28	0.14	CD60	RV60
Modern	316-189	2001	63.9	1.19	24	47	47	70	<1	9	0.04	CD60	R60
Modern	316-154	1999	62.7	1.13	26	42	38	61		0	0	CD/CV60	RD60
Modern	316-152	1999	65.4	1.19	28	51	43	56	<1	9	0.04	C60	D60
Modern	316-151	1999	61.5	1.16	33	54	38	56		0	0	C60	R60

Ret. Occ. = Retinal occupation of single cones. Loc. H_{max} = Location of the H_{max}, Loc. S_{max} = Location of the S_{max}, s/d = ratio single cones / double cones. The <1 in single cone size stands for the absence of functional components, in spite of observed nuclei.

Appendix Table 2.2 General data of *H. tanaos* specimens used in this study.

H. tanaos

Period	Number	Catch	SL (mm)	r (mm)	H_d (Nr. per degree)	H_{max} (Nr. per degree)	S_d (µm^2)	S_{max} (µm^2)	S_s (µm^2)	Ret. Occ. (%)	s/d	Loc. H_{max}	Loc. S_{max}
Old	320-5	1978	67.4	1.28	56	87	28	34	7.6	90	0.45	D60	C30
Old	320-6	1978	70.3	1.45	64	103	33	44	8.1	86	0.43	D30	RD60
Old	320-7	1978	71.4	1.46	64	87	31	41	9	92	0.46	CD60	R60
Old	320-8	1978	62.2	1.14	52	74	26	41	5.5	88	0.44	D60	R30
Old	320-9	1978	67.9	1.42	73	136	32	47	8.1	88	0.44	D60	R60
Old	320-10	1978	64.9	1.27	68	116	25	43	6.9	88	0.44	D30	R30
Old	320-11	1978	57.5	1.03	53	78	21	29	5.7	86	0.43	CD60	R60
Old	320-12	1978	68.4	1.3	54	118	27	39	5.9			CD60	R60
Modern	320-13	2001	62.8	1.1	55	90	29	43	3.4	54	0.27	D60	R60
Modern	320-14	2001	65.1	1.13	49	75	32	41	5.1	46	0.23	D60	V30
Modern	320-15	2001	69.2	1.17	61	77	29	42	4	48	0.24	D60	C30
Modern	320-16	2001	67.1	1.16	60	95	30	50	3.7	64	0.32	D60	C30
Modern	320-17	2001	64.1	1.15	59	92	27	39	4.1	48	0.29	C60	C30
Modern	320-20	2001	60.1	1.04	47	78	30	41	3.8	76	0.38	CD60	V30

Ret. Occ. = Retinal occupation of single cones. Loc. H_{max} = Location of the H_{max}, Loc. S_{max} = Location of the S_{max}, s/d = ratio single cones / double cones. The retina section of specimen W320-012 was not suitable for determination of the retinal occupation and the s/d ratio.


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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).

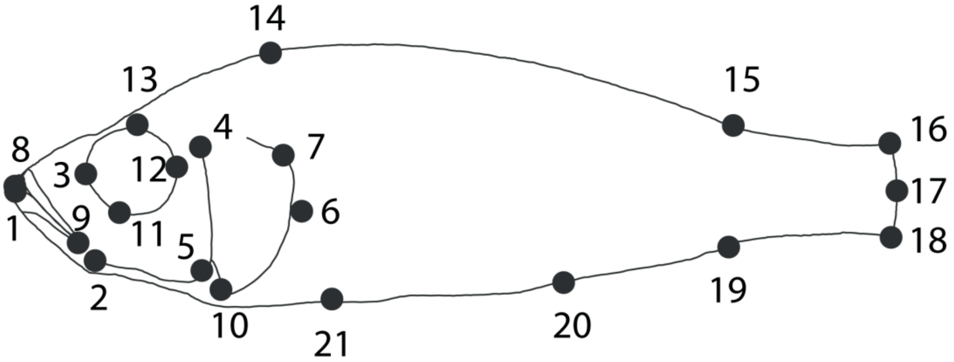

Figure 3.1 Location and description of 21 homologous landmarks used in this study 1: dorsal corner of lower jaw symphysis, 2: quadrate head centre, 3: preorbital process, 4: suspensorial lateral line foramen 1, 5: suspensorial lateral line foramen 4, 6: upper insertion of pectoral fin, 7: caudal/dorsal 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.

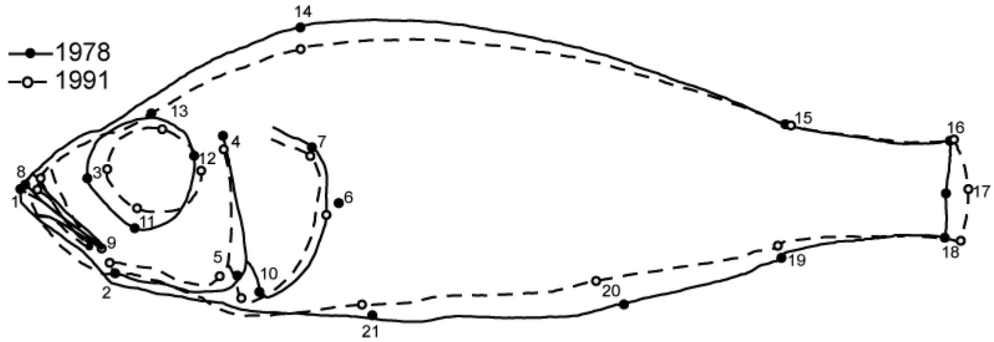

Figure 3.2 Body shape outline of *pyr* males obtained from the DFA. The continuous lines and the black landmarks represent the consensus of the year 1978. The dashed line and white landmarks represent the consensus of the year 1991. Differences were enlarged three times for better visualization.

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.

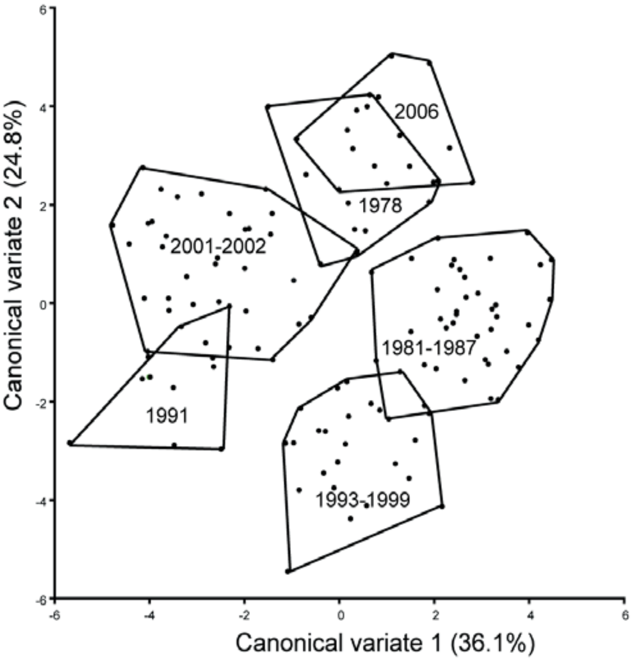

Figure 3.3 Plot of *pyr* males from 1978-2006 obtained from the CVA. Each dot represents the body shape of one specimen. CV 1 and CV 2 explain together 60.9% of the total body shape variation in years.

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.

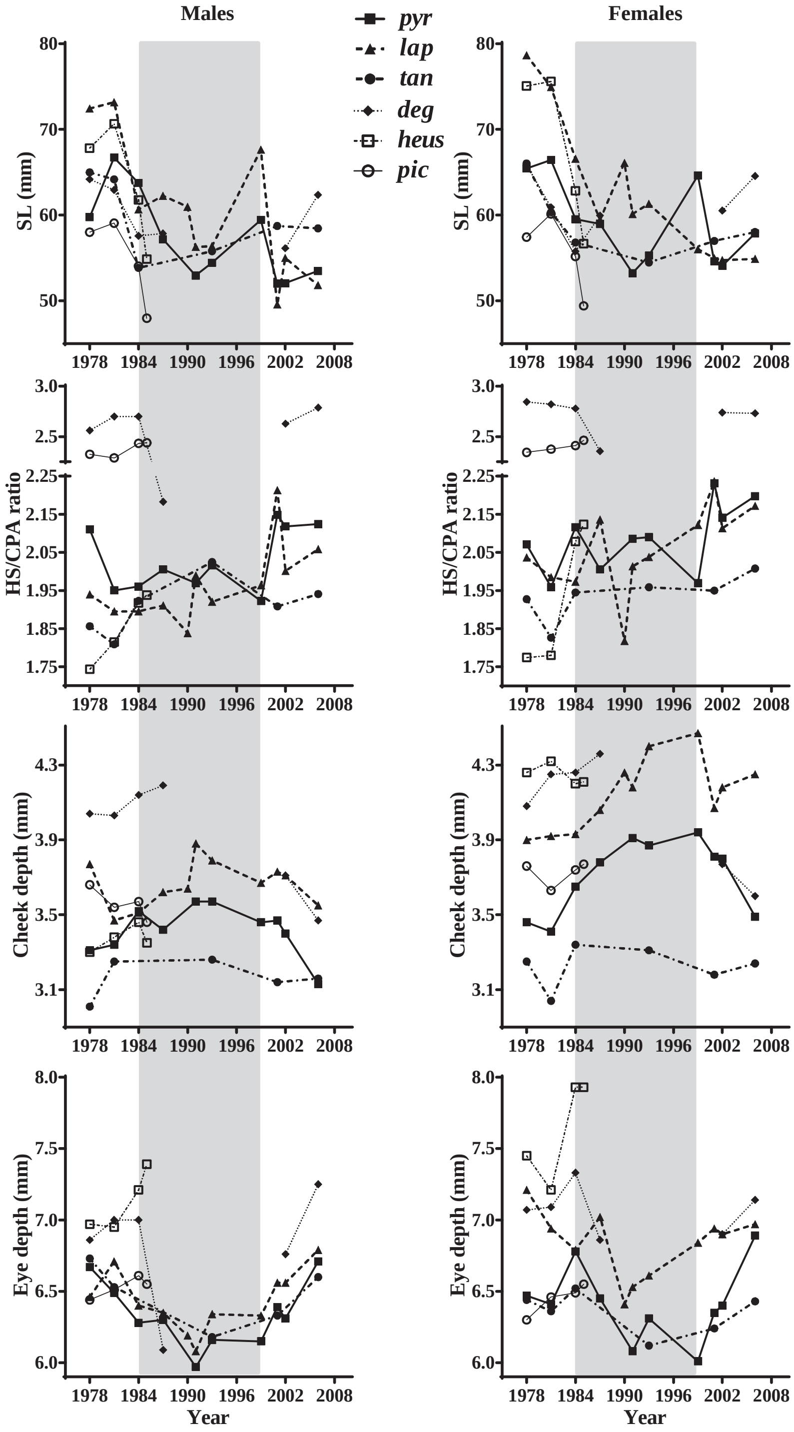
Males
Females
pyr
lap
tan
deg
heus
pic
SL (mm)
HS/CPA ratio
Cheek depth (mm)
Eye depth (mm)
Year
1978
1984
1990
1996
2002
2008
80
70
60
50
3.0
2.5
2.25
2.15
2.05
1.95
1.85
1.75
4.3
3.9
3.5
3.1
8.0
7.5
7.0
6.5
6.0

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.

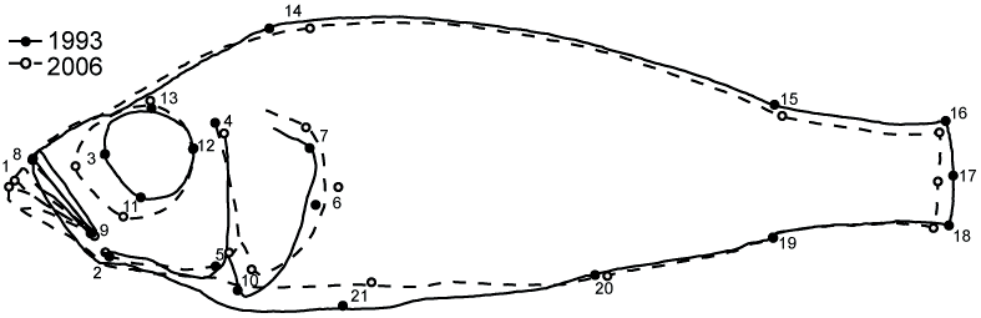

Figure 3.5 Body shape outline of *pyr* males obtained from the DFA. The continuous lines and the black landmarks represent the consensus of the year 1993. The dashed line and white landmarks represent the consensus of the year 2006. Differences were enlarged three times for better visualization.

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.

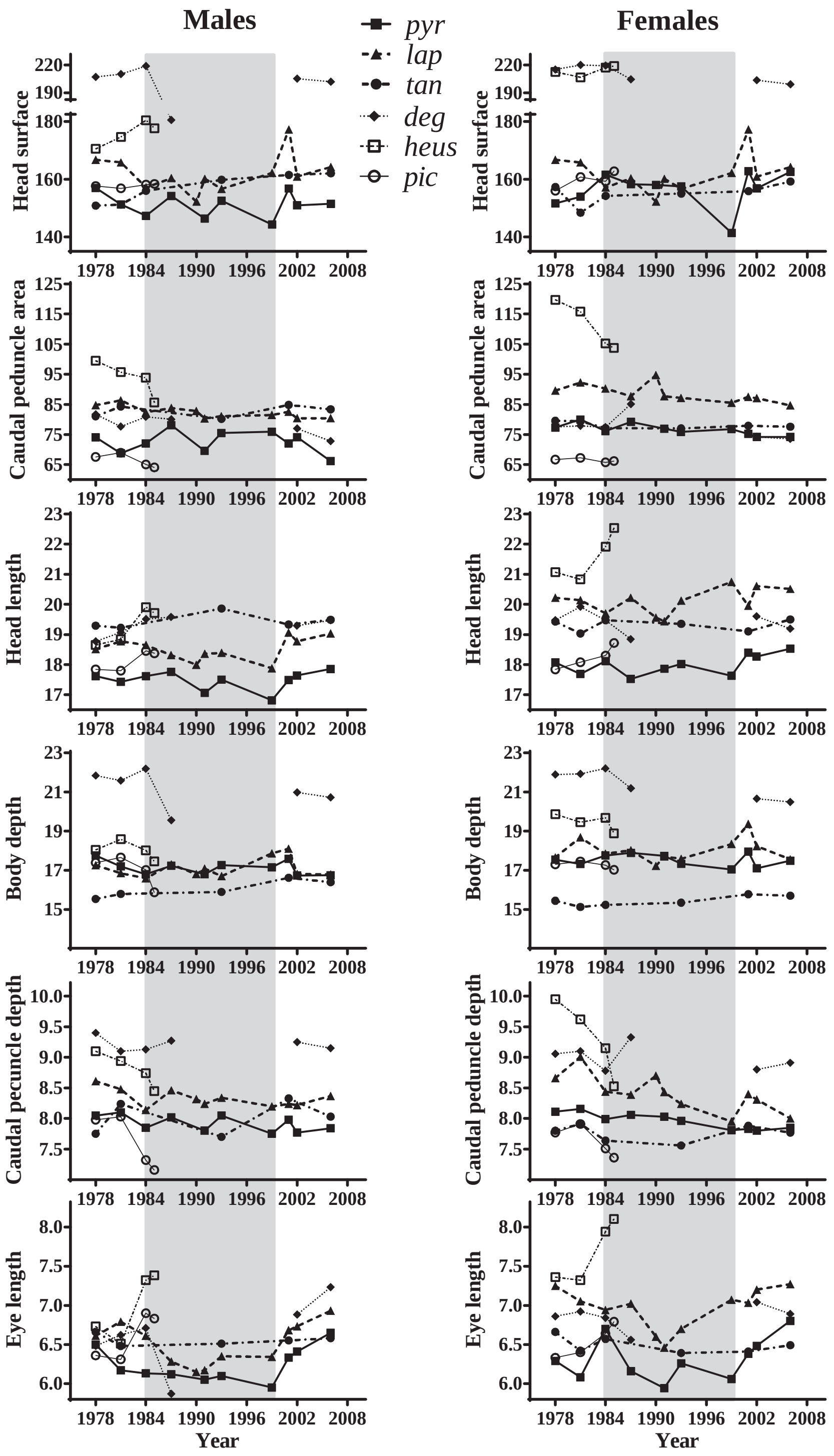
Males		**1978**	**1981**	**1984**	**1987**	**1990**	**1991**	**1993**	**1999**	**2001**	**2002**
pyr	**1981**	**0.0160**									
	1984	**0.0208**	0.0124								
	1987	**0.0161**	0.0146	**0.0196**							
	1991	**0.0177**	0.0129	**0.0162**	**0.0163**						
	1993	**0.0195**	**0.0145**	**0.0185**	**0.0153**		**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)

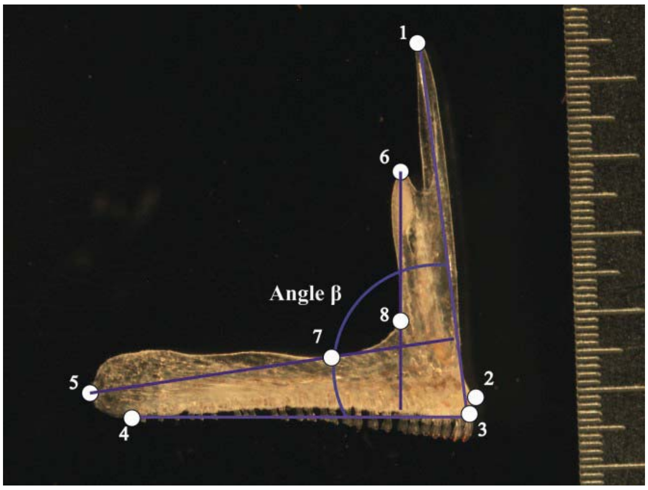

Males
Females
pyr
lap
tan
deg
heus
pic
Head surface
Caudal peduncle area
Head length
Body depth
Caudal pecuncle depth
Caudal peduncle depth
Eye length
Year
1978
1984
1990
1996
2002
2008
220
190
180
160
140
125
115
105
95
85
75
65
23
22
21
20
19
18
17
15
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0

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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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.

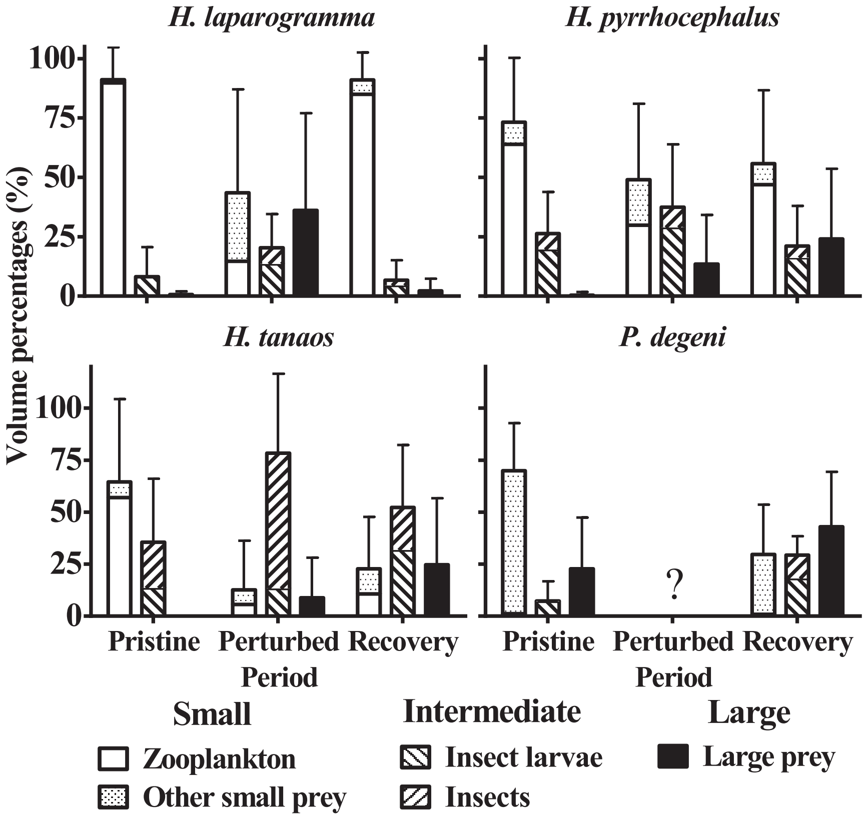

Figure 4.1 Volume percentages with standard deviation of prey sizes of the four studied species in the pristine, perturbed and recovery period. Zooplankton and other small prey; and insect larvae and insects are combined in stacked bars.

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*).

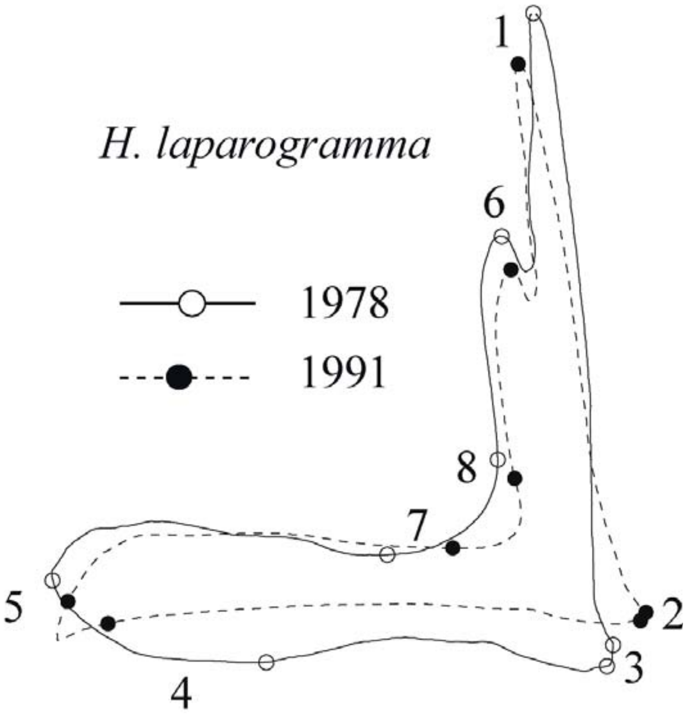

Figure 4.2 Example of premaxilla changes found in the four species illustrated by the outline of *H. laparogramma* derived from the DFA. The continuous line with blank dots represents the year 1978. The dashed line with filled dots represents the year 1991. Differences are exaggerated three-fold for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

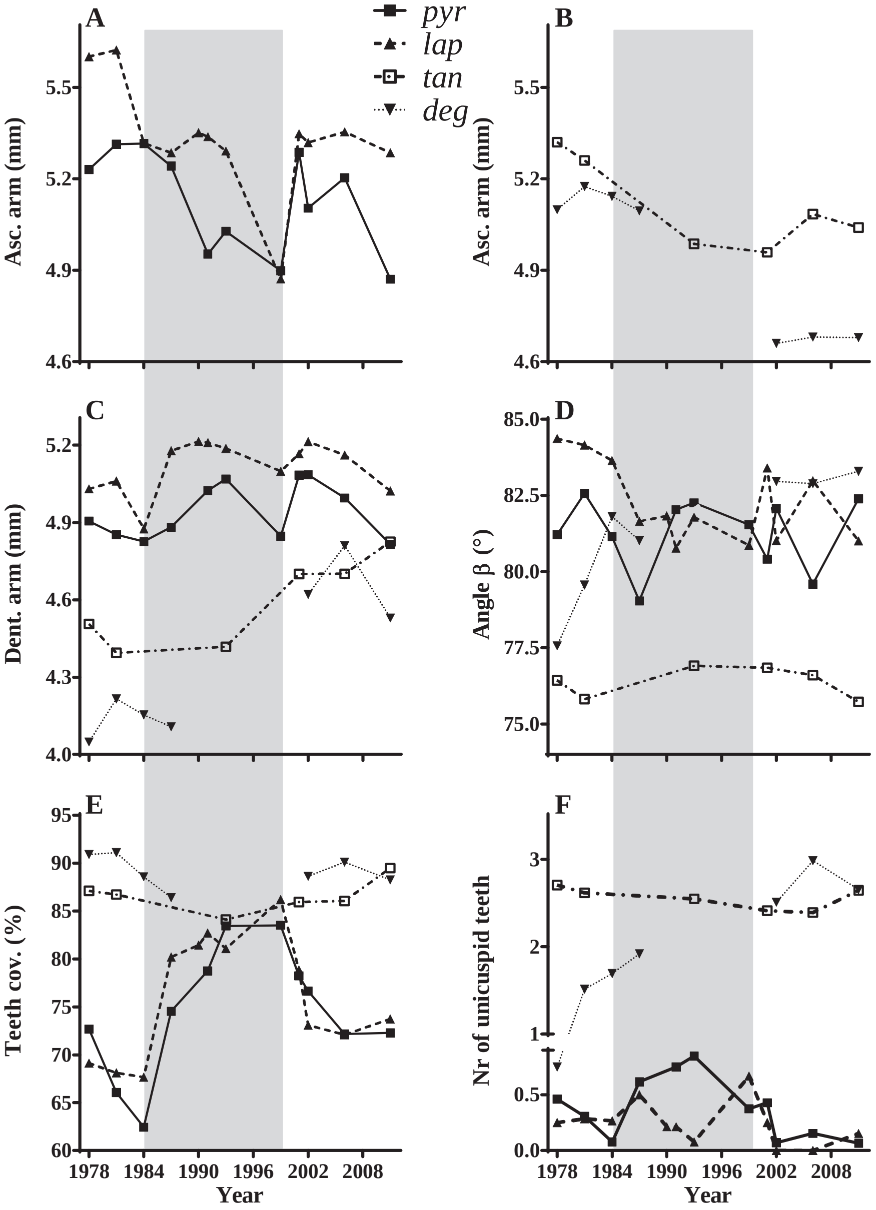

Figure 4.3 Estimated marginal means through time for all four species of A, B: ascending arm, C: dentigerous arm, D: angle β, E: teeth coverage and F: number of unicuspid teeth through time of all four species. Estimated marginal means of the ascending arm are illustrated in two panels for better visualization.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

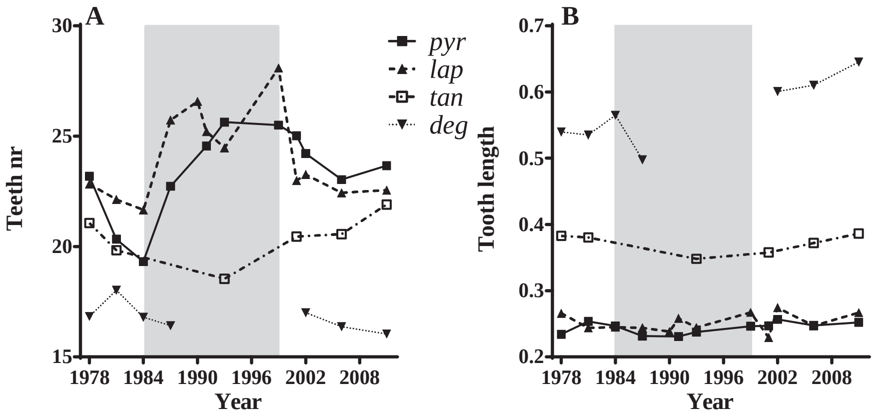

Figure 4.4 Estimated marginal means of A: teeth nr and B: tooth length through time of all four species. The grey shade represents the perturbed period.

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).

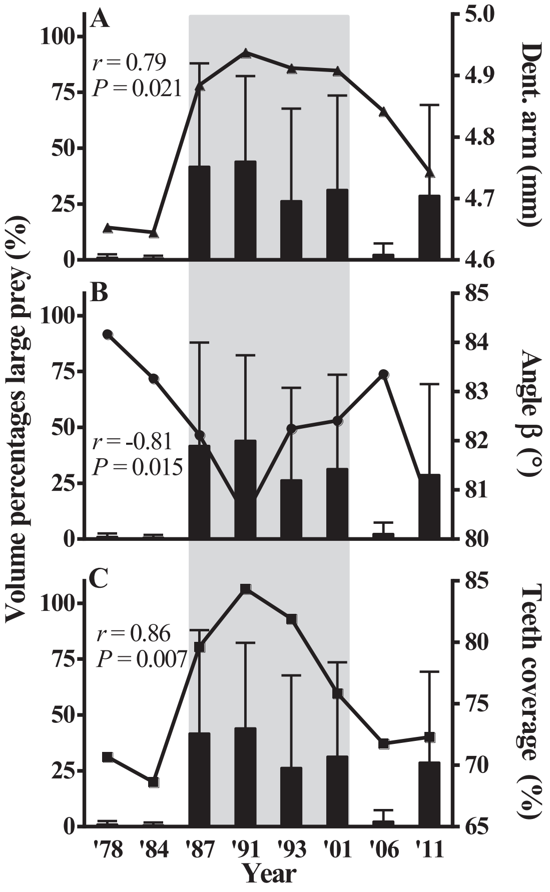

Figure 4.5 Volume percentages of large prey (bars, left axis) and estimated marginal means through time for *H. laparogramma* (solid lines, right axis) of A: dentigerous arm, B: angle β and C: teeth coverage. The coefficient (*r*) and the *P*-value of the Spearman correlation are given per morphological 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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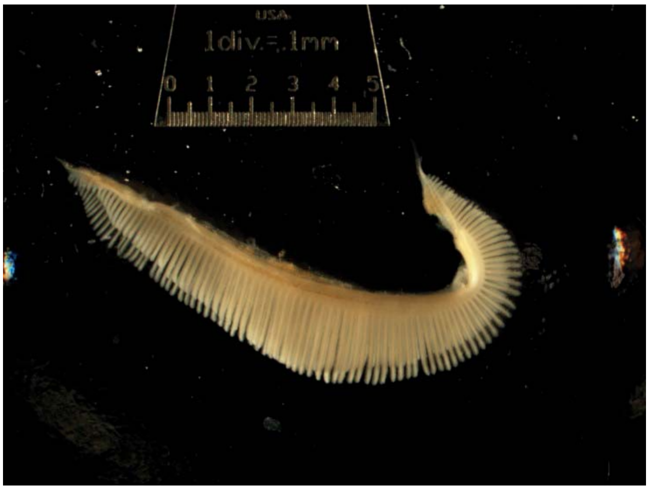
Appendix Table 4.1 Volume percentages of different prey types per period per species.

	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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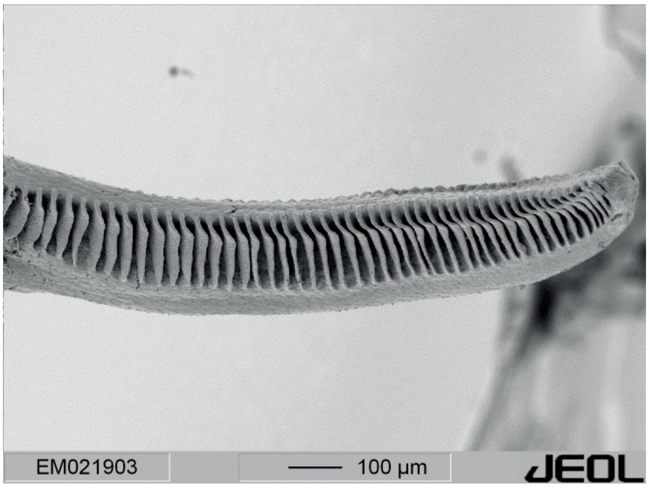
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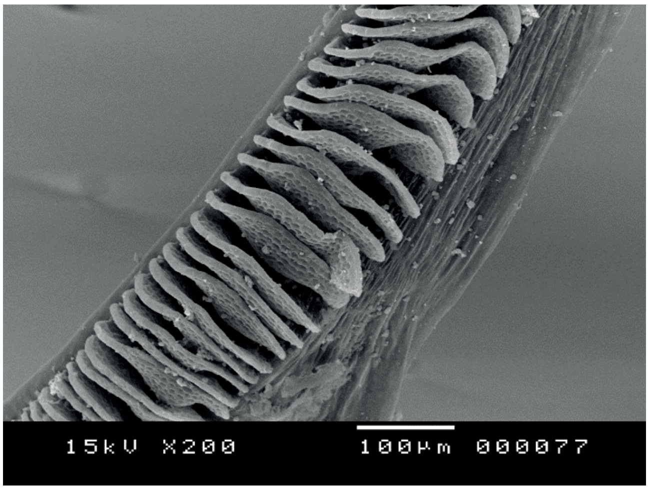
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Chapter 5

Climatic variability drives adaptive responses in the gills of Lake Victoria cichlids

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Abstract

Climate changes and global warming have severe consequences for aquatic ecosystems. In East Africa's Great Lakes, environmental perturbations are occurring as a result of global warming. Lake Victoria, famous for its stunning adaptive radiation of cichlids, has suffered from cultural eutrophication over the past decades. This eutrophication is thought to be partly responsible for the dramatically reduced cichlid biodiversity. However, environmental variability through climate-induced changes in mixing dynamics might also have contributed. To determine how both these changes have influenced the lake and its cichlids over the past 50 years, we gathered environmental and meteorological variables and related these to changes in gill surface of four cichlid species. We found a gradual increase of the maximum air temperature and a gradual decrease of lake water levels from the 1960s onwards. During the period of severe eutrophication (1980s), a change in wind direction in combination with reduced wind speeds and increased rainfall were observed. Concurrently, dissolved oxygen (DO) levels dropped as did water temperature and transparency. The gill surface in three out of the four cichlid species increased as a response to these environmental changes. During the 2000s, wind speed increased again to values higher than previously recorded. During the same time, an increase in DO levels, water transparency and water temperature and a decrease of cichlid gill surface were observed. Our results imply that climatic changes and especially wind speed and direction, and their variability, play a crucial role in tropical lake dynamics and might affect cichlid fish evolution. With continued eutrophication, we hypothesize that a sequential drop of wind speeds will be detrimental to Lake Victoria's unique ichthyofauna. Restrictions on anthropogenic nutrient inputs will be an important step to prevent future biodiversity crises in Lake Victoria and other African Great Lakes.

Introduction

Evidence that global warming can cause dramatic perturbations in freshwater ecosystems has rapidly accumulated over the past decade (Winder & Schindler 2004; Parmesan 2006; Woodward *et al.* 2010). In East-Africa, the warming of the Indian Ocean and the changes in the El Niño Southern Oscillation (ENSO) are believed to have resulted in an increase in air temperature and changes in rainfall (Nicholson & Kim 1997; Clark *et al.* 2003; Funk *et al.* 2008; Williams & Funk 2011). The effects of this warming on the physical properties of African Great Lakes have been documented (O'Reilly *et al.* 2003; Verburg *et al.* 2003; Lorke *et al.* 2004; Vollmer *et al.* 2005; Hecky *et al.* 2010; Tierney *et al.* 2010; Cozar *et al.* 2012). In Lake Tanganyika, the warming coincided with higher water temperatures (O'Reilly *et al.* 2003; Verburg *et al.* 2003) and lower wind speeds (O'Reilly *et al.* 2003) with inferred decreases in vertical mixing.

Periodic decreases in vertical mixing have been inferred for Lake Victoria as well during conditions of higher rainfall, reduced winds and higher relative humidity (Kolding *et al.* 2008; Hecky *et al.* 2010). The extent of vertical mixing is crucial for tropical lake dynamics and is an important driver of other environmental variables such as phytoplankton abundance, water transparency, and dissolved oxygen (DO) levels. These factors are important indicators of eutrophication. Eutrophication of freshwater lakes has become a worldwide issue in terms of biodiversity loss (Smith & Schindler 2009). The loss of species diversity can be inflicted in several ways, including demographic decline and introgressive hybridization. Though the latter can be beneficial for speciation during early adaptive radiation events (Seehausen 2004) it is more likely to result in loss of biodiversity through reversed speciation (Seehausen 2006). Evidence for reversed speciation through hybridization has been found in several freshwater fishes, including European whitefish, three-spined sticklebacks and East-African cichlids (Seehausen *et al.* 1997a; Taylor *et al.* 2006; Vonlanthen *et al.* 2012). Hybridization occurs as eutrophication hampers sexual selection by increasing the water turbidity (Seehausen *et al.* 1997a) or forces fish habitats and diets to overlap due to benthic oxygen depletion (Vonlanthen *et al.* 2012).

In Lake Victoria, several meteorological variables vary seasonally (Fish 1957; Talling 1966; Lehman 1998; Stager *et al.* 2009; Hecky *et al.* 2010). Stratification patterns have mostly been documented for the northern offshore waters. Stratification occurs after the southwest monsoon and has been attributed to reduced wind speeds (Talling & Lemoalle 1998; MacIntyre 2013). However, recent analyses using data from the National Center for Ecological Analysis and Synthesis indicate that stratification is due to flow of warmer water from the southern regions of the lake which experience greater rates of heating from September through March compared to other times of the year (S. MacIntyre, personal communication). This stratification results in lower DO levels near the bottom (Talling 1966; Hecky *et al.* 1994). During the stratified period, the phytoplankton biomass (which is light limited, Silsbe *et al.* 2006) tends to peak at inshore stations due to less vertical mixing and a high influx of nutrients from the land, created by heavy rains (Akiyama *et al.* 1977; Mugidde 1993; Cornelissen *et al.* 2013). These seasonal peaks are species dependent though, with some diatom species (e.g. *Melosira spp.*) dominating in the windy, mixing season (Talling 1966). Beginning at the end of the wet season, and in some years continuing in the dry season, the south-east trade winds cause the break-down of the stratification by internal wave motions and by increased evaporation and concomitant vertical mixing (Talling 1966; MacIntyre 2013; S. MacIntyre, personal communication). The vertical mixing influences nutrient and phytoplankton abundance that are reflected in a relatively high water transparency during the dry season. Recent variability in the seasonal patterns resulted in a change of these seasonal patterns and intensified the eutrophication of the lake (Wanink *et al.* 2001; Verschuren *et al.* 2002; Hecky *et al.* 2010).

The eutrophication of Lake Victoria has been attributed mainly to anthropogenic influences (Verschuren *et al.* 2002). However, recent studies imply that also climatic changes have contributed (Kolding *et al.* 2008; Hecky *et al.* 2010). The latter two studies suggest that the observed decrease in wind speeds during the 1970s and 1980s has enhanced the already inflicted eutrophication due to increased stability and persistence of seasonal stratification, which resulted in deoxygenation of the hypolimnion. During this time, the enhanced primary production and increase of cyanobacteria and algae resulted in a decrease in transparency and increased hypoxia (Seehausen *et al.* 1997a; Hecky *et al.* 2010). Together with the introduction of the piscivorous Nile perch, these dramatic ecological changes are thought to be responsible for the eradication of hundreds of endemic haplochromine cichlid species (Seehausen *et al.* 1997a; Witte *et al.* 2007).

Despite the severely changed environmental conditions, some haplochromines recovered (Witte *et al.* 2007; Kishe-Machumu 2012) and adjust morphologically to the altered environment in the Mwanza Gulf of Lake Victoria (Witte *et al.* 2008; Van der Meer *et al.* 2012 [Chapter 2 in this thesis]; Van Rijssel & Witte 2013 [Chapter 3 in this thesis]; Chapter 4 in this thesis). This is the site of a long term haplochromine monitoring study conducted by the Haplochromis Ecology Survey Team (HEST) since the 1970s. HEST found that, among other species, *Haplochromis (Yssichromis) pyrrhocephalus* increased its in gill surface by 64%, and suggested that this was a response to the low oxygen conditions (Wanink *et al.* 2001; Witte *et al.* 2008). Under laboratory conditions, several other cichlid species showed an increase in gill surface as a plastic response to low dissolved oxygen (DO) levels (Chapman *et al.* 2000; Rutjes 2006; Rutjes *et al.* 2009). The above-mentioned studies suggest that Lake Victoria cichlids are resilient to severe hypoxic conditions in the field as well as in the lab. It remains unclear, however, whether the morphological changes in the gills under natural conditions are the result of phenotypic plasticity or genetically based changes (in response to directional selection and/or resulting from hybridization (Chapman *et al.* 2000; Chapman *et al.* 2002; Witte *et al.* 2008).

The goal of this research was to study the effects of meteorological variables on the physical limnology of the Mwanza Gulf (Figure 1.1) and how these affect the gill morphology of four resurgent Lake Victoria haplochromines. We expected that climatic variability would have a major effect on the lake dynamics which influence environmental variables such as water transparency and DO levels. A decrease of these variables is expected to induce an increase of the gill surface of the four resurgent cichlid species (abbreviations of species in parentheses); *H.(Y.) pyrrhocephalus (pyr)*, *H. (Y.) laparogramma (lap)*, *H. tanaos (tan)* and *P. degeni (deg)*.

Materials and methods

Environmental variables

Monthly meteorological data was obtained from 1960-2012. We obtained data from the meteorological department measured at Mwanza Airport for minimum and maximum air temperatures (1960-2012), rainfall (1960-2012), wind speed (1972-2012) and wind direction (1977-2012). Wind speed and direction were measured during the day at 1200 h. Data for lake water levels (1965-2012) were obtained from the Lake Victoria Basin Water Office and were measured at a station located between Mwanza City and the village of Nyegezi (Figure 1.1). This station is located 1130 m above sea level which is extracted from the lake level measurements.

The Mwanza Gulf is a relatively long, narrow gulf which extends 60 km southward with an average width of 5km and which varies in depth from 1-2m in the southern part up to 20-25m at the entrance of the gulf (Figure 1.1; Goudswaard *et al.* 2002). Periodically, DO-levels, water transparency and the bottom and surface water temperatures (the difference between them was calculated) were measured monthly in 1973-74, 1980, 1983-85, 1986-87, 1988, 2002, 2006, 2008 and 2010-11. Some years were combined to obtain sufficient data for each period. Most of these measurements were conducted at station G (12-14m depth) of the research transect in Mwanza Gulf where most fishes used in this study were caught (see below; Figure 1.1). Daily DO levels were measured 1m above the bottom, together with water temperature, which was also measured at the surface. The surface and bottom temperature were subtracted from each other and this difference can be interpreted as an indicator of stratification. These measurements were made with a Yellow Springs Instrument (YSI) AQ model 57 while water transparency was measured with a Secchi disc (Table 5.1).

Table 5.1 Dissolved oxygen, bottom (1m above the bottom) and surface water temperature, and Secchi disc measurements at station G.

Year	**DO level**	**St. dev.**	**N**	**Bottom water temp.**	**St. dev.**	**Surface water temp.**	**St. dev.**	**N**	**Secchi depth**	**St. dev.**	**N**
1973-74	4.69	1.22	4	24.56	0.55	24.85	0.59	4	1.54	0.22	4
1980	4.41	1.52	8	25.13	0.52	26.51	0.83	8	2.48	0.22	4
1983-85	1.54	1.8	12	24.57	1.11	26.06	1.09	12	1.29	0.16	5
1986-87	1.18	0.78	4	23.63	0.31	24.4	0.60	9	1.13	0.12	8
1988	0.77	0.81	3	22.22	0.47	23.08	0.58	6	1.23	0.29	3
1995-96									1.38	0.23	3
2002	1.27	1.0	3	24.63	0.49	25.8	0.82	3	1.75	0.31	3
2006	3.4	1.7	2	25.0	0	25.47	0.46	3	2.0		1
2008	3.01	1.63	2	24.5	0.14	25.1	0.57	2	1.88	0.18	2
2010-11	4.62	0.95	4	25.3	0.69	26.09	1.53	3	1.23	0.15	4

Data from 1973-74 were obtained from Akiyama *et al.* (1977) who measured south of Nyamatala Island at a depth of 8m (five kilometers south of station G). To correct for the depth difference with station G, we compared the bottom water temperature, DO levels and

Secchi depth between station G and station E (also 8m depth) from the same time period. The average difference was subtracted or added from values reported by Akiyama *et al.* (1977). The data from 2010-11 were measured by SEDEC Wageningen UR, one kilometer south of station G (11m depth). We selected data from the wet season (February-April) with heavy rains but calm weather when thermal stratification and hypoxia occur in the lower part the water column in the open water of Lake Victoria (Talling 1966) as in the Mwanza Gulf (Akiyama *et al.* 1977; Witte & van Densen 1995; Wanink *et al.* 2001; Cornelissen *et al.* 2013). With lower visibility, lower DO levels and higher water temperatures, this seems to be the harshest period of the year for the cichlids.

Fish collection

Fishes were collected during the years 1981, 1984, 1987, 1991, 1993, 1999, 2002, 2006 and 2011, at the research transect in the northern part of the Mwanza Gulf, Lake Victoria, Tanzania. The species *pyr* and *lap* were mainly caught above mud bottoms at station G, while the species *deg* and *tan* were mainly caught at sand/mud bottom bays (Butimba and Kissenda, 4-8m depth) at opposite ends of the transect (Figure 1.1; Table 5.2).

We selected males only to avoid any effects of sexual dimorphism. In total, 214 adult fish of four species (an average of seven specimens per species per year) were selected for the gill morphology analysis. This is a subsample of the fish used in Van Rijssel & Witte (2013) [Chapter 3 in this thesis].

Table 5.2 Catch locations and number of specimens per species per year.

Year	***H. laparogramma***	**N**	***H. pyrrhocephalus***	**N**	***H. tanaos***	**N**	***P. degeni***	**N**
1978-1982	G, Transect	8	G	8	BB	8	BB, J	7
1984	G	8	G	8			BB	7
1987	G	8	Luanso Bay	7			BB, Transect	3
1991	J, P	8	J, P	8				
1993	G, H, I	8	H, I	8	I, J, K	4		
1999	Transect	6	Transect	8				
2001-2002	G	8	G	8	J, BB	8	J	7
2006	F-J	8	G	8	E	8	J	8
2011	F-J	8	F	7	J	8	F, J, K	6
Total		**70**		**70**		**36**		**38**

E-K, stations on the transect; P, Python Island-Nyamatala Island; BB, Butimba Bay; 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.

Gill morphology

The first and second gill arch on the right side of each fish were dissected and photographed with a digital camera (Nikon Digital Sight DS-FI1) mounted on a dissection microscope. Both sides (hemibranches) of the second gill arch were photographed with a reference scale. The length (L) and number (N) of the filaments were measured from these photographs. Four filaments equally divided over the medial hemibranch were selected for measuring the secondary lamellae (sec. lam.). From each side, ten sec. lam. were selected from the middle of the filament through scanning electron microscopy (SEM). The gill surface of the second gill arch was calculated following Witte *et al.* (2008):

$$A = N \times L \times d \times a,$$

where A is the gill surface of the second gill arch (mm^2), N is the total number of filaments on both hemibranches, L is the average filament length (mm), d is the average sec. lam. density (mm^{-1}) and a is the average sec. lam. surface (mm^2). The d was calculated by dividing 10 by the distance between the first and the tenth sec. lam resulting in the number of sec. lam. per mm. The a was calculated considering the sec. lam. as a triangle $a = l \times ½h \times 2$, where l is the sec. lam. length (mm) and h is the sec. lam. height (mm). Note that the surface area is multiplied by two because each sec. lam. has two functional sides. All measurements were conducted with ImageJ (1.47a).

Statistical analysis

Differences in environmental factors between years were tested with a One-way ANOVA unless the data were not normally distributed (tested with Shapiro-Wilk test). The latter was the case for minimum air temperature, wind speed, lake levels and the difference between surface and bottom water temperatures which were tested with a non-parametric Kruskal-Wallis test. A general linear model (GLM) with body volume (BV, measured by volumetric displacement) as covariate and year as independent factor was applied to test whether morphological characters of the gills differed in time following Van Rijssel & Witte (2013) [Chapter 3 in this thesis]. These data were log-transformed to achieve linearity, estimated marginal means (EMM) were anti-logged and plotted through time. A Pearson correlation test was used to see if the environmental factors were correlated with each other and with the EMM of the morphological characters. A Spearman correlation test was used for non-normally distributed data which was the case for minimum air temperature and bottom water temperature. The *P*-values of all tests were corrected with a sequential Bonferroni test. All statistical tests were performed with SPSS version 20.

Results

Environmental variables through time

The wind direction showed significant changes through time in the yearly dataset ($P = 0.003$, Figure 5.1). In the period 1978-1981, the wind roughly came from the west (253-266°) and from 1982 till 1994 mainly from the southwest (180-249°). Three years after the change in wind direction (1985), the wind speed dropped significantly (both in the yearly and periodical dataset, $P < 0.001$, Figures 5.1 & 5.2A). From 1985 onwards, the wind speed decreased and remained low until 1997, with the exception of a small peak in 1992-93 (Figure 5.2A). The wind direction changed back to mainly western winds from 1995 onwards. Three years later, in 1998, the wind speed increased again and during the 2000s reached speeds above those previously recorded (Figures 5.1 & 5.2A, B). Despite changes in wind speed and direction, rainfall did not change significantly through time (Figures 5.1 & 5.2A, B). However, there was an increase of rainfall in 1988 compared to the period 1983-85 ($P = 0.041$, not significant after sequential Bonferroni correction), which coincided with the change in wind direction and the decrease in wind speeds (Figure 5.2A, B). Even though there was no overall decrease in rainfall, the lake level decreased gradually over time with a steep decline during the early 2000s ($P < 0.001$; Figures 5.1 & 5.2B, C).

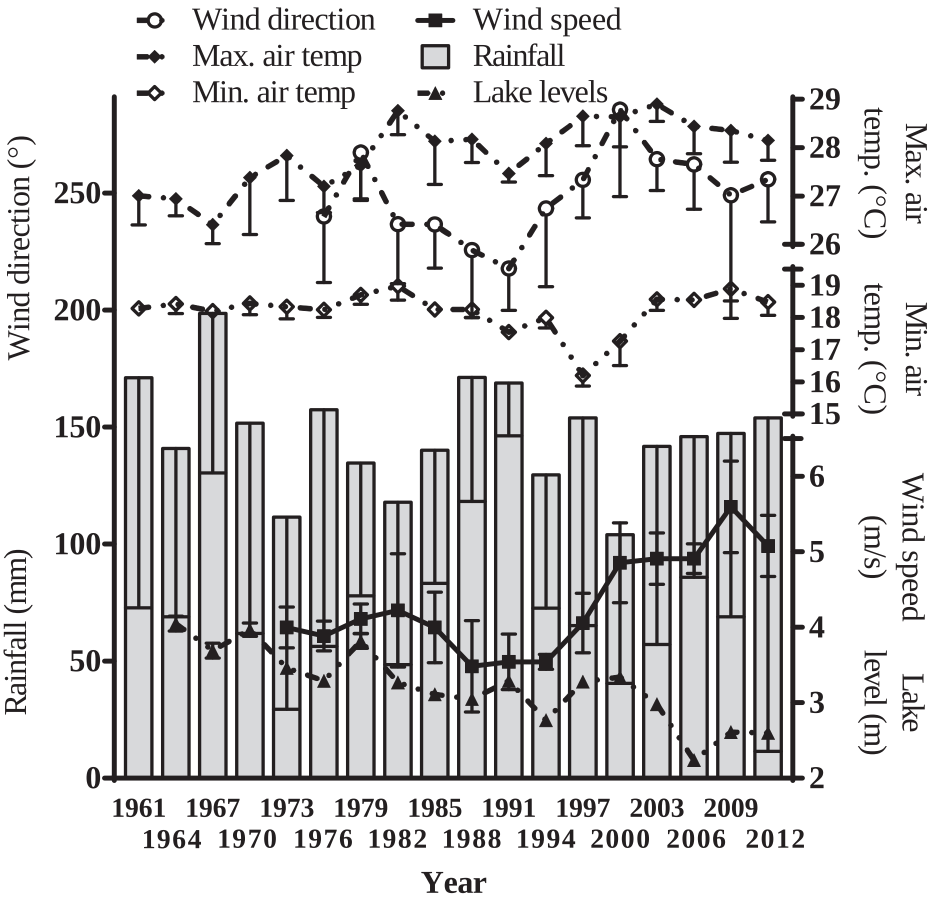

Figure 5.1 Meteorological variables and lake levels plotted through time measured at Mwanza airport and between Mwanza City and the village of Nyegezi respectively during the wet season (February-April). Lake levels are depicted as height values with the elevation of the Mwanza Gulf (1130m) extracted from them. For better visualisation, every three years were combined and averaged to one year with the middle year being depicted in the graph e.g. year 1961 represents years 1960, 1961 & 1962 etc. Depicted (lower end) standard deviations are averaged from yearly standard deviations.

The maximum air temperature increased significantly in 1980 compared to the 1960s-1970s (yearly dataset, $P < 0.001$, Figure 5.1). It remained high up till 2012 with the exception of a non-significant drop from 1985 till 1996 which also concurs with the period of major wind changes. The minimum air temperature remained stable during the 1960s and 1970s and

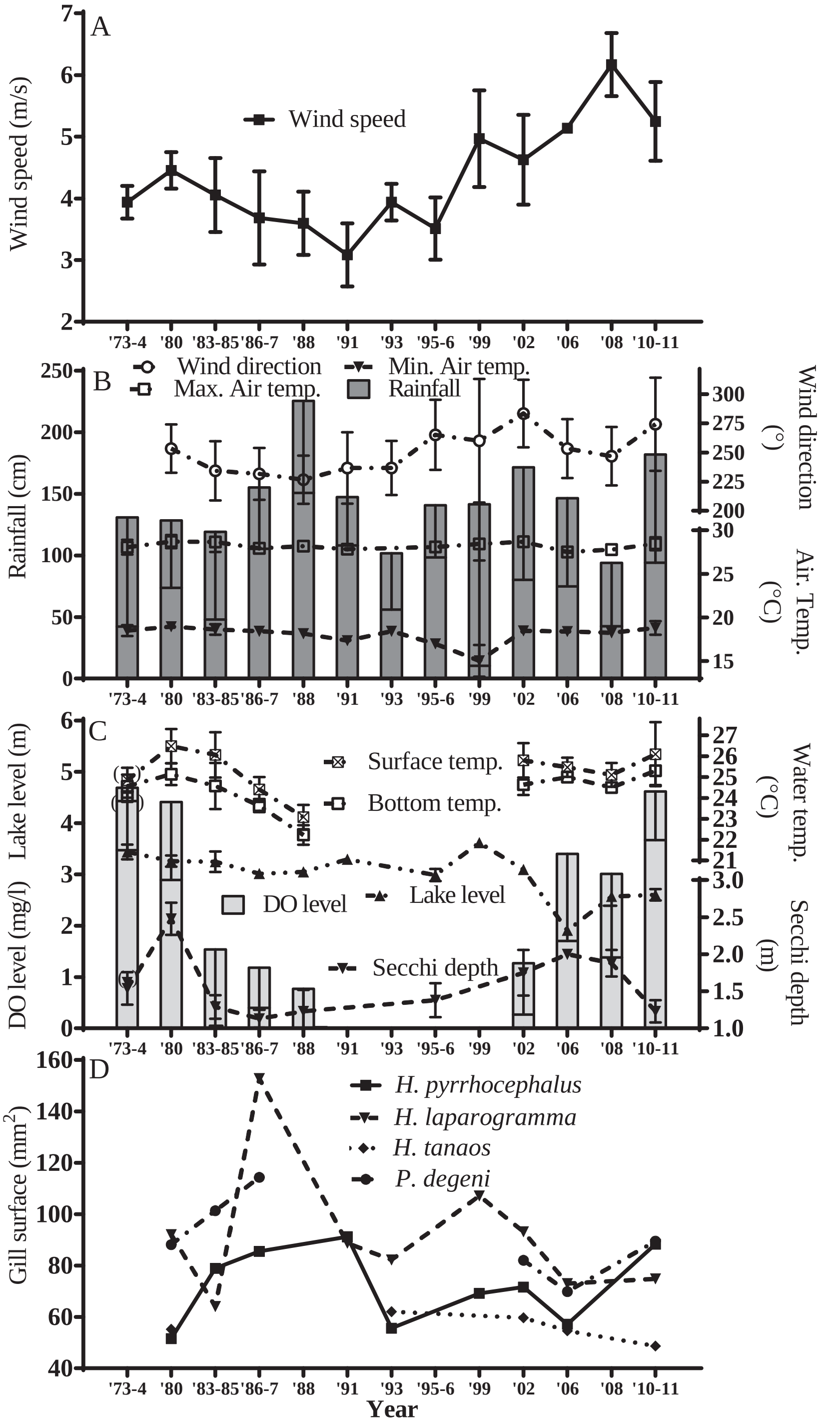
A
Wind speed
Wind speed (m/s)
2
3
4
5
6
7
'73-4 '80 '83-85 '86-7 '88 '91 '93 '95-6 '99 '02 '06 '08 '10-11
B
Wind direction
Max. Air temp.
Min. Air temp.
Rainfall
Rainfall (cm)
0
50
100
150
200
250
Wind direction (°)
200
225
250
275
300
Air. Temp. (°C)
15
20
25
30
C
Surface temp.
Bottom temp.
DO level
Lake level
Secchi depth
Lake level (m)
DO level (mg/l)
0
1
2
3
4
5
6
Water temp. (°C)
21
22
23
24
25
26
27
Secchi depth (m)
1.0
1.5
2.0
2.5
3.0
D
H. pyrrhocephalus
H. laparogramma
H. tanaos
P. degeni
Gill surface (mm^2)
40
60
80
100
120
140
160
Year

Figure 5.2 Environmental variables of the periodic dataset (A, B, C) and gill surfaces of four haplochromine species (D) plotted through time measured during the wet season (February-April). A: Wind speed, B: rainfall, minimum and maximum air temperature and wind direction, C: dissolved oxygen (DO) level, surface and bottom water temperature, Secchi depth and lake level, D: estimated marginal means of gill surfaces of four haplochromine species. Secchi depth, DO levels and water temperature have been measured at station G. Corrected values from Akiyama *et al.* (1977) are indicated in parentheses. Maximum air temperature and lake level are lacking for the year 1993. Dissolved oxygen levels and water temperature have not been measured in the period 1991-1999. From 1988 till 2001 there were no specimens available for *P. degeni* and from 1982-1992 no specimens were available for *H. tanaos*. Only lower end standard deviations are depicted for rainfall and DO levels.

then dropped gradually from 1980 onwards, reaching its lowest point in 1999 (both datasets, $P < 0.001$; Figure 5.1 & 5.2B). In 2000, the minimum air temperature increased again to the level of the 1960s and 1970s and remained at that level up till 2012.

Bottom and surface water temperature, Secchi depth and DO levels all show a significant drop during the mid and late 1980s compared to 1973-74 and 1980 ($P < 0.001$, Figure 5.2C) which concurs as well with the observed wind changes. Compared to 1973-74, the difference between bottom and surface temperature increased significantly in 1980 and 1983-1985 ($P < 0.01$) indicating stratification. The increased difference was maintained, albeit at a less pronounced level, from 1987 onwards and in 2006 the difference decreased ($P < 0.05$) to a similar level as in 1973-74. Although data from the 1990s are mostly missing, from 2002 onwards water temperature, Secchi depth and DO levels increased again reaching the same level as they were in 1973-74 and 1980 ($P < 0.001$). Secchi depth, however, showed again a significant drop in 2011 ($P < 0.001$).

Gill morphology through time

In the mid and late 1980s when DO levels decreased, three out of four species (*pyr, lap* and *deg*) showed a significant increase in gill surface ($P < 0.01$, Figure 5.2D) with a significant effect of the covariate BV ($P < 0.001$, Appendix Table 5.1). The increase was mainly due to a significant increase in filament length, sec. lam. surface (*pyr* and *lap*) and sec. lam. density (*deg*, Appendix Figure 5.1). The gill surface of the three species decreased significantly during the 2000s when DO levels had increased again ($P < 0.05$, Figure 5.2C, D). In 2011, however, there was once more a significant increase in gill surface for *pyr* and *deg* while DO levels were high ($P < 0.05$).

Correlations in yearly dataset

Wind speed shows a significant positive correlation with wind direction (r = 0.396, P = 0.017), which means that wind speed is higher when the wind is coming from the west and lower when the wind is coming from the south and southwest. Wind speed also shows significant correlations with minimum air temperature (r = 0.380, P = 0.014), maximum air temperature (r = 0.352, P = 0.032), rainfall (r = -0.353, P = 0.023) and lake level (r = -0.386, P = 0.011, Table 5.3). However, only the correlation with lake level was significant after sequential Bonferroni correction. Wind direction showed an almost significant negative correlation with rainfall ($r = -0.314$, $P = 0.062$), which would mean that there may be more rainfall with wind coming from the south and southwest and less rainfall with wind coming from the west. Rainfall also shows a significant negative correlation with the maximum air temperature ($r = -0.347$, $P = 0.015$), which is partly caused by the increased cloud cover reducing solar radiation with increased rainfall. The maximum air temperature shows an almost significant positive correlation with minimum air temperature ($r = 0.253$, $P = 0.079$) and a significant negative correlation with lake level ($r = -0.386$, $P = 0.011$, Table 5.3).

Table 5.3 Correlations of environmental parameters from the yearly dataset. Significant correlations are indicated in bold. Only the correlation between wind speed and lake level was significant after sequential Bonferroni correction.

Environmental variable	**Rainfall**	**Min. air temp.**	**Max. air temp.**	**Lake level**	**Wind direction**	**Wind speed**
Rainfall	X					
Min. air temp.	$r = 0.005$ $P = 0.973$	X				
Max. air temp.	**$r = -0.347$** **$P = 0.015$**	$r = 0.253$ $P = 0.079$	X			
Lake levels	$r = -0.02$ $P = 0.898$	$r = -0.138$ $P = 0.366$	**$r = -0.386$** **$P = 0.011$**	X		
Wind direction	$r = -0.314$ $P = 0.062$	$r = 0.078$ $P = 0.651$	$r = 0.200$ $P = 0.272$	$r = -0.060$ $P = 0.737$	X	
Wind speed	**$r = -0.353$** **$P = 0.023$**	**$r = 0.380$** **$P = 0.014$**	**$r = 0.352$** **$P = 0.032$**	**$r = -0.322$** **$P = 0.046$**	**$r = 0.396$** **$P = 0.017$**	X

Correlations in periodic dataset

The DO levels did not show a significant correlation with the gill surfaces of the four species (Appendix Table 5.2). Wind speed did show a negative correlation with the gill surface of *tan* (not significant, $r = -0.821$, $P = 0.088$) and *deg* ($r = -0.829$, $P = 0.041$). As found in the yearly dataset, wind speed also showed a positive correlation with wind direction, though not significant ($r = 0.569$, $P = 0.068$). The wind direction is also positively correlated with the bottom water temperature ($r = 0.826$, $P = 0.011$), which means that southern wind results in lower bottom water temperatures and western wind results in higher bottom water temperatures. These bottom water temperatures also show a positive correlation with minimum air temperature ($r = 0.733$, $P = 0.025$) and, although not significantly, with DO levels ($r = 0.650$, $P = 0.058$) and are negatively correlated with the gill surface of *tan* ($r = -0.949$, $P = 0.051$). Surface water temperatures showed strong positive correlations with minimum air temperature ($r = 0.817$, $P = 0.007$) and bottom water temperature ($r = 0.923$, $P < 0.001$). The difference between surface and bottom water temperature was positively correlated with the maximum air temperature ($r = 0.819$, $P = 0.007$). Secchi depth showed a strong negative correlation with the gill surface of *pyr* ($r = -0.967$, $P = 0.002$, Appendix Table 5.2).

Discussion

Environmental variables through time

This study shows how climatic variability can influence tropical lake dynamics on the relatively small scale of the Mwanza Gulf. Based on our results, we hypothesize that wind stress might be one of the major factors responsible for the observed environmental changes in the Mwanza Gulf (and Lake Victoria at large, Lehman 1998) and rapid morphological responses observed in the Lake Victoria cichlids.

During the 1980s, the wind changed to a more southwest direction in combination with lower wind speeds. These lower wind speeds are likely to have resulted in reduced mixing of the water (Fish 1957; Talling 1966; Lehman 1998; Stager *et al.* 2009; Hecky *et al.* 2010; MacIntyre 2013). This reduced mixing would have resulted in thermal stratification which in turn led to lower DO levels and lower water temperature. The change in wind speed and direction coincided with increased rainfall. Both the lower wind speed and higher rainfall are likely to have influenced the water transparency by reduced mixing and increased nutrient and sediment influx from the shores and watershed (Cornelissen *et al.* 2013).

In addition, as suggested by Hecky *et al.* (2010), the lower wind speeds created optimal conditions for buoyant phytoplankton taxa (e.g. cyanobacteria) compared to rapidly sinking taxa such as diatoms (Reynolds 2006) which may have reinforced the resulting decline in Secchi disc transparency. The increase of cyanobacteria has contributed to the decrease in water transparency and DO levels in deeper waters as well (Verschuren *et al.* 2002; Hecky *et al.* 2010).

Despite no overall change in rainfall over the period of record, the lake level decreased gradually over time reaching similar values as those observed in 1961-1962 (Yin & Nicholson 1998). This decrease is, based on our data, likely due to increased evaporation as a result of increased maximum temperatures. Next to the observed climatic changes, human management also regulates lake levels (Yin & Nicholson 1998). Especially the sharp lake level decline during the 2000s can for a large part be attributed to the operation regime, initiated in 1999, of the expanded hydropower facility in Uganda, the Kiira Dam (Swenson & Wahr 2009).

The cichlids in Lake Victoria have withstood substantial climatic changes for at least 15,000 years (Johnson *et al.* 1996), and are apparently able to cope with such fluctuations. However, recent anthropogenic perturbations are likely to have exacerbated the effects of climatic changes which together resulted in eutrophication of the lake (Verschuren *et al.* 2002; Hecky *et al.* 2010). Lake Victoria is regarded to be in a new, relatively stable state (Hecky *et al.* 2010). However, so far, phosphorous loadings continue to rise and signs of improvement of lake conditions are derived from environmental variables (e.g. increased oxygen levels and water transparency) which are symptoms of eutrophication rather than improvement in rising nutrient concentrations (Hecky *et al.* 2010; Sitoki *et al.* 2010). The reduction of phosphorous input is considered to be the only successfully proven method for reducing eutrophication (Schindler 2012). However, in highly eutrophic situations such as Lake Victoria, phytoplankton abundance as well as deep water oxygen conditions are under hydrodynamic control and not driven by nutrients alone (Silsbe *et al.* 2006), though phytoplankton abundance in the Mwanza Gulf might still partly be nutrient limited (Cornelissen *et al.* 2013).

In the case of the Mwanza Gulf, the wind speed increased during the 2000s with levels higher than recorded in the past 40 years. These high wind speeds are likely to have increased evaporation rates which probably have resulted in complete vertical mixing (Talling 1966; MacIntyre 2013). This mixing seems to be responsible for the improvement of environmental variables (higher DO levels, generally higher Secchi depth except for 2011) to their values from before severe eutrophication. Therefore, we predict that a future change in wind direction and a sequential drop in wind speeds would result in the recurrence of hypereutrophication with even harsher conditions for the haplochromine cichlids than experienced during the late 1980s (Kolding *et al.* 2008).

Although we base our predictions and conclusions on the Mwanza Gulf only, it might well be that the Mwanza Gulf is representative for many other gulfs and bays along the Lake Victoria shoreline which show similar signs of eutrophication (Ochumba & Kibaara 1989; Mugidde 1993; Gikuma-Njuru & Hecky 2005; Hecky *et al.* 2010; Ngupula *et al.* 2012). In fact, the enormous cichlid biodiversity is mainly determined from catches along the lake's shoreline, in and around the Mwanza Gulf as well as from several other gulfs and bays (Witte *et al.* 2007). These areas are not only important in terms of biodiversity but over two million people depend directly on Lake Victoria's fisheries which are mainly exploited along the shoreline (LVFO FMP 2, 2008). In addition, hypereutrophication might not be limited to the inshore gulfs and bays (Hecky *et al.* 2010) which would mean that the complete lake and the species therein will have to face the harsh environmental changes while they might not be able to cope with these conditions.

Adaptive responses of the cichlids

Unexpectedly, the DO levels did not correlate with the gill surfaces of the four studied species. The lack of significant correlations is probably due to shortcomings of the collected dataset. The environmental variables collected are presented as being static while many of these variables (e.g. Secchi depth, DO level) can vary substantially within a day. Despite the lack of correlation between gill surfaces and DO levels, certain predicted trends can be recognized especially during the 1970s and 1980s. A significant increase of gill surface was observed in three species when oxygen levels dropped in the 1980s (we lack sufficient data in the 1980s for the fourth species, *tan*). During the 2000s, the gill surfaces of these species decreased again while DO levels reached their former levels from before the severe eutrophication. Although the gill surfaces fluctuated during the 1990s and 2000s, they tend to stay beneath the high surface areas observed when hypoxic conditions were most severe (late 1980s). However in 2011, when water turbidity increased again, so did the gill surfaces of the species *pyr* and *deg*. The highly significant correlation of Secchi depth and the gill surface of *pyr* might reflect a causal relation. As the water transparency is largely controlled by the influx of sediment and increased cyanobacterial abundance, the increase in turbidity may make it more difficult for fish to extract oxygen from turbid water. In other words, the gill surface area might be sensitive to fouling by suspended material as has been found for fish gill cells by Campbell *et al.* (1997) and Galvez *et al.* (2008). So, as well as DO levels in the ambient water *per se*, suspended material (which is partly reflected in Secchi depth Cornelissen *et al.* 2013) might determine the need for larger gill surface areas.

The increase of gill surface as a response to hypoxic conditions is quite common in fish including several cyprinids (crucian carp, goldfish), and cichlids (Chapman *et al.* 2000; Sollid *et al.* 2003; Sollid *et al.* 2005; Rutjes *et al.* 2009). In these studies, the gill surface increased as a result of phenotypic plasticity induced by hypoxic conditions in the lab.

Differences between morphological responses under natural and laboratory conditions can shed more light on the mechanism behind these responses under natural conditions. Chapman *et al.* (2000) found that natural populations of the Lake Victoria cichlid *Pseudocrenilabrus multicolor victoriae* at low-oxygen sites had longer filaments and larger secondary lamellae, while fishes experimentally raised under hypoxia showed an increase in filament length and number only. They attributed this disparity in response to differences in selection pressure and morphological constraints. Plastic responses to hypoxia in the lab were also found for *H. pyrrhocephalus* (Rutjes 2006). Somewhat different from the findings of Chapman *et al.* (2000), these fishes increased their gill surface not only by space occupying means (longer filaments) but also by means not related to space occupation (larger secondary lamellae).

The current study showed that all three species had longer gill filaments and two species (*pyr* and *lap*) larger secondary lamellae, while *deg* had an increased density of the secondary lamellae. Since the head volume of these fish decreased or remained the same during the hypoxic period in the 1980s (J.C. van Rijssel, unpublished data), space occupying changes were expected to be limited by morphological constraints. On the other hand, the reduction in eye size (Witte *et al.* 2008; Van der Meer *et al.* 2012 [Chapter 2 in this thesis]; Van Rijssel & Witte 2013 [Chapter 3 in this thesis]), and the possible reduction of the muscle used for suction feeding (*musculus sternohyoideus*, Witte *et al.* 2008), might have acted as morphological trade-offs. Whether phenotypic plasticity or genetically based changes underlie the morphological changes remains unknown. As suggested by Chapman *et al.* (2000), it is likely that both mechanisms are involved in the observed responses.

Future effects of eutrophication on fish species

The Lake Victoria cichlids have shown to adjust to a variety of environmental changes (Witte *et al.* 2008; Van der Meer *et al.* 2012 [Chapter 2 in this thesis]; Van Rijssel & Witte 2013 [Chapter 3 in this thesis], Chapter 4 in this thesis). However, a state of hypereutrophication is likely to be detrimental for cichlid biodiversity for two major reasons. Firstly, hypereutrophication might result in a very hostile environment in which cichlids and other species cannot cope with the changed environment (Smith & Schindler 2009). The new environment might demand morphological, physiological or behavioural adaptations that cannot be achieved by the fish through either genetic changes or plasticity. Though cichlids have been observed to be very plastic in the lab (Meyer 1987; Chapman *et al.* 2000; Stauffer & Van Snik Gray 2004; Rutjes 2006; Rutjes *et al.* 2009; Muschick *et al.* 2011) there are limits to this plasticity which might be reached through the changed environment. Secondly, hypereutrophication is likely to co-occur with low water transparency and low oxygen levels which has been shown to reduce fish biodiversity by hybridization (Seehausen *et al.* 1997a; Taylor *et al.* 2006; Vonlanthen *et al.* 2012).

Although the other African Great Lakes as a whole have not reached the level of eutrophication of Lake Victoria (Bootsma & Hecky 1993; Hecky 1993), eutrophication has been observed in some regions of these lakes too (Chale 2003; Hecky *et al.* 2003; Otu *et al.* 2011). The increased eutrophication poses a major threat to biodiversity and to the people depending for their income or food supply on the fisheries of these lakes. As the African population continues to expand, especially in areas surrounding the African Great Lakes (UNEP 2008), anthropogenic influences are likely to increase eutrophication by cumulative nutrient loading. In combination with climatic fluctuations such as reduced winds and increased rainfall which favour eutrophication, in time, these lakes, or regions within these lakes, may undergo similar losses of biodiversity as observed in Lake Victoria. For these reasons, we consider restrictions on anthropogenic nutrient inputs into the lakes as the most important task for ecosystem management. In addition, it is imperative that environmental variables such as nutrient loadings, chlorophyll, Secchi depths and DO levels will be monitored on a regular basis along with continued collection of meteorological data. By adequately monitoring these variables, we can improve our understanding of the effects of eutrophication on biodiversity and, with reductions in nutrient loading, moderate biodiversity losses in Lake Victoria and prevent biodiversity crises in other African Great Lakes.

Acknowledgements

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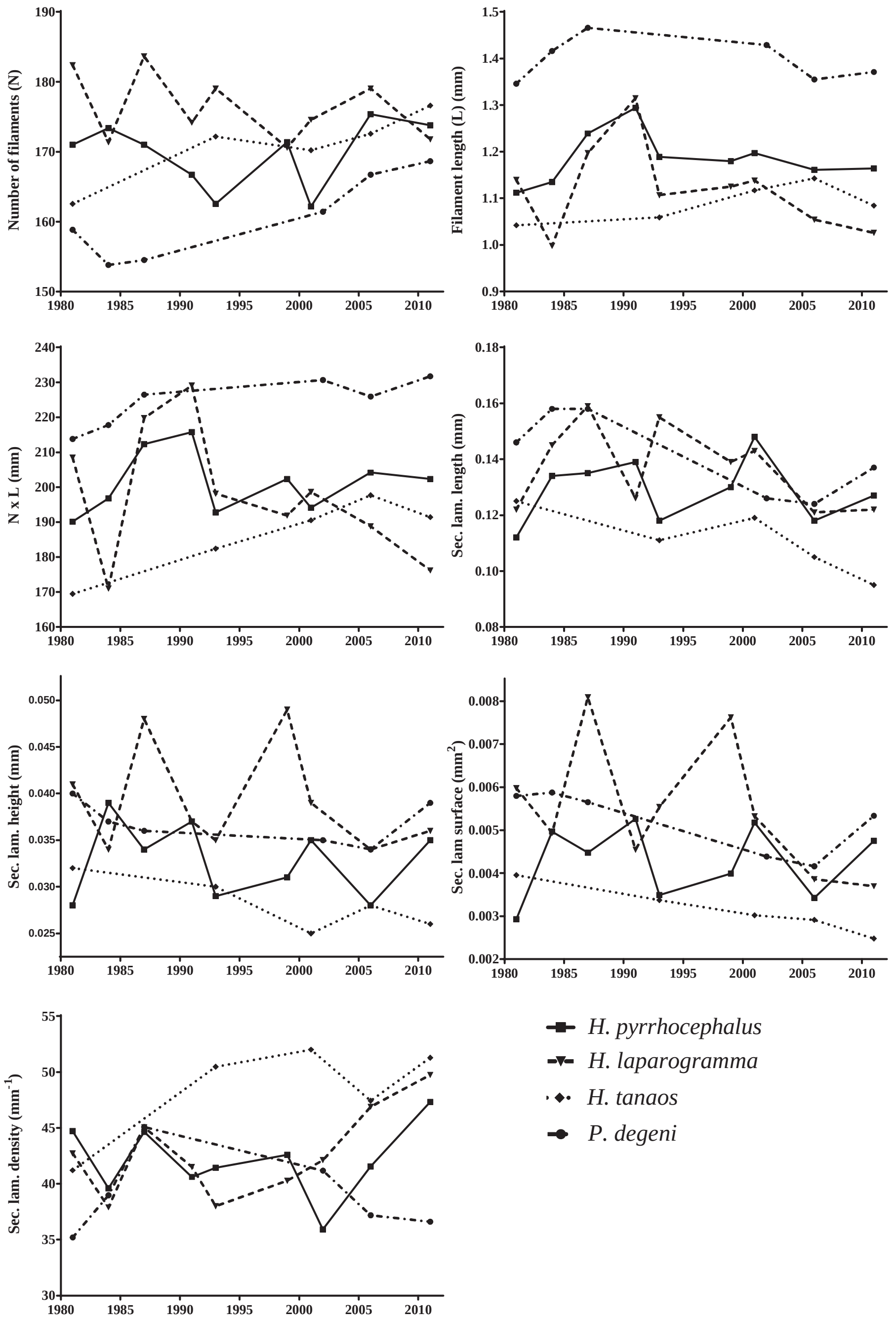
Appendix Table 5.1 Results of the GLM on morphological gill parameters. Significant *P*-values after sequential Bonferroni correction are depicted in bold. BV, body volume.

Species	Factor Covariate	Number of filaments (N)	Filament length (L)	N x L	Sec. lam. length	Sec. lam. height	Sec. lam. surface	Density	Gill surface
H. pyrrhocephalus	P year	**0.002**	**0.001**	**0.015**	**<0.001**	**0.004**	**<0.001**	**<0.001**	**<0.001**
	P BV	**<0.001**	**<0.001**	**<0.001**	0.003	0.297	0.042	0.160	**<0.001**
H. laparogramma	P year	**0.008**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	P BV	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.911	0.068	0.212	**<0.001**
H. tanaos	P year	**0.010**	**0.025**	**0.011**	**<0.001**	0.003	**<0.001**	**<0.001**	0.194
	P BV	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.032**	**<0.001**
P. degeni	P year	0.018	0.096	0.725	0.011	0.154	**0.004**	**<0.001**	**0.008**
	P BV	0.055	**<0.001**	**<0.001**	**<0.001**	0.817	**0.005**	0.204	**<0.001**



Appendix Figure 5.1 Estimated marginal means of morphological gill characters through time of four species where N is filament number and L is filament length.

Appendix Table 5.2 Correlations (r) of environmental parameters and gill surfaces from the periodic dataset. Significant correlations (after sequential Bonferroni correction) are indicated in bold.

Environmental variable / gill surface		Rainfall	Min. air temp.	Max. air temp.	Wind direction	Wind speed	Bottom water temp.	Surface water temp.	Difference in water temp.	Secchi depth	DO level	Lake level	Gill surface *pyr*	Gill surface *lap*	Gill surface *tan*	Gill surface *deg*
	r															
Rainfall	*P*	X														
Min. air	r	-0.126														
temp.	*P*	0.697	X													
Max. air	r	0.126	0.582													
temp.	*P*	0.711	0.060	X												
Wind	r	0.114	0.251	0.393												
direction	*P*	0.739	0.456	0.261	X											
Wind	r	-0.276	0.189	-0.043	0.569											
speed	*P*	0.385	0.556	0.900	0.068	X										
Bottom water	r	-0.033	0.733	0.400	0.826	0.600										
temp.	*P*	0.932	0.025	0.286	0.011	0.088	X									
Surface water	r	-0.506	**0.817**	0.470	0.610	0.425	**0.923**									
temp.	*P*	0.165	**0.007**	0.202	0.109	0.254	**< 0.001**	X								
Difference in	r	0.005	0.484	**0.819**	0.012	-0.226	0.063	0.441								
water temp.	*P*	0.990	0.187	**0.007**	0.978	0.558	0.873	0.235	X							
Secchi	r	-0.458	0.142	0.012	0.311	0.428	0.452	0.499	0.118							
depth	*P*	0.215	0.715	0.975	0.454	0.251	0.222	0.172	0.762	X						
DO	r	-0.364	0.600	-0.056	0.448	0.415	0.650	0.504	-0.346	0.451						
level	*P*	0.335	0.088	0.886	0.265	0.267	0.058	0.166	0.362	0.223	X					
Lake	r	-0.038	-0.418	0.553	-0.132	-0.522	-0.239	-0.053	0.418	-1.65	-0.136					
level	*P*	0.912	0.201	0.078	0.716	0.099	0.536	0.892	0.263	0.672	0.727	X				
Gill surface	r	0.550	-0.183	-0.107	-0.069	-0.349	-0.143	-0.404	-0.092	**-0.967**	-0.362	0.197				
pyr	*P*	0.125	0.637	0.800	0.861	0.357	0.787	0.427	0.863	**0.002**	0.481	0.641	X			
Gill surface	r	0.207	-0.300	-0.182	-0.199	-0.298	-0.257	-0.777	-0.221	-0.248	-0.419	0.290	0.236			
lap	*P*	0.295	0.433	0.666	0.608	0.437	0.623	0.069	0.674	0.636	0.409	0.485	0.540	X		
Gill surface	r	-0.606	-0.410	0.134	-0.349	-0.821	-0.949	-.264	0.378	0.460	-0.868	0.315	-0.554	0.483		
tan	*P*	0.278	0.493	0.866	0.565	0.088	0.051	0.736	0.622	0.540	0.132	0.685	0.333	0.410	X	
Gill surface	r	-0.148	0.200	0.182	-0.613	-0.829	-0.486	-0.451	0.275	-0.643	-0.455	0.612	0.657	0.625	-0.391	
deg	*P*	0.780	0.704	0.729	0.196	0.041	0.329	0.370	0.597	0.169	0.365	0.197	0.157	0.184	0.609	X

Chapter 6

Changing ecology of Lake Victoria cichlids and their environment: Evidence from C^{13} and N^{15} analyses

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Abstract

Eutrophication is an increasing global threat to freshwater ecosystems and the people depending on them for their livelihood. East Africa's Lake Victoria has suffered from severe eutrophication in the past decades which is partly responsible for the dramatic decline in haplochromine cichlid fish species diversity. Some zooplanktivorous and detritivorous haplochromine species recovered and shifted their diet to macroinvertebrates and small fishes. We used four formalin preserved cichlid species caught over the past 35 years to investigate whether stable isotopes of these fish are reflecting the dietary changes, habitat differences and if these isotopes can be used as indicators of eutrophication. We found that $\delta^{15}N$ signatures mainly reflected dietary shifts to larger prey in all four haplochromine species. We also observed shifts in $\delta^{13}C$ signatures (Suess corrected) that likely represent habitat differences and dietary changes. The $\delta^{13}C$ signatures tend to be heavier in haplochromines, Nile perch and *Rastrineobola argentea* caught from inshore stations compared to fish from offshore stations, indicating little horizontal dispersal of these fishes. In addition, a shift to remarkably heavy $\delta^{13}C$ signatures in 2011 was found for all four haplochromine species which might infer increased primary production and thus eutrophication although more research is needed to confirm this hypothesis. The observed temporal changes show that preserved specimens can be used to trace historic changes in fish ecology and the aquatic environment. This highlights the need for continued sampling as this information could be of essence for reconstructing and predicting the effects of environmental changes.

Introduction

Eutrophication is the enrichment of water bodies by inorganic plant nutrients (e.g. nitrate and phosphate, Lawrence *et al.* 1998). Eutrophication of freshwater ecosystems is increasingly common and is a major threat to biodiversity and to aquatic resource use by local human populations (Smith & Schindler 2009). Most eutrophication assessment methods identified increased primary production as the immediate biological response to nutrient enrichment (Ferreira *et al.* 2011); and consequently, primary productivity has been suggested to be a sensitive and accurate indicator of eutrophication (Paerl *et al.* 2003; Andersen *et al.* 2006; but see Smith 2007 and Garmendia *et al.* 2013 for exceptions). Increased primary productivity and nutrient enrichment generally result in the preferential removal and depletion of lighter ^{12}C leading to heavier $\delta^{13}C$ signatures in aquatic food chains (Schelske & Hodell 1991). Increased nitrogen pollution from runoff is reflected by heavier $\delta^{15}N$ signatures while a high N demand by primary producers can favour N-fixing cyanobacteria and consequently lighter $\delta^{15}N$ signatures (Peterson & Fry, 1987). Therefore, both carbon and nitrogen stable isotopes are sensitive to nutrient enrichment and increased primary productivity (Schelske & Hodell 1991; Cabana & Rasmussen 1996; Vander Zanden *et al.* 2005; Gu *et al.* 2006) and might therefore be useful indicators of eutrophication.

Besides being used as indicators of primary productivity and of changes in basal signatures in food webs, stable isotopes are commonly used to function as estimators of trophic position and carbon flow in aquatic ecosystems (Peterson & Fry 1987; Post 2002). The $\delta^{15}N$ signatures of consumers are typically enriched with 3-4‰ with each trophic level while the $\delta^{13}C$ signatures are similar or only slightly enriched ($\delta^{13}C$ <1‰) with each trophic level (Peterson & Fry 1987; Vander Zanden & Rasmussen 2001). Stable isotopes can also provide information on the habitat of aquatic species. In general, limnetic phytoplankton photosynthesis results in light $\delta^{13}C$ signatures whereas heavier $\delta^{13}C$ signatures are caused by benthic algae photosynthesizing within a boundary layer (France 1995; Hecky & Hesslein 1995). This phenomenon makes it possible to infer whether the prey of primary consumers has a benthic, littoral or limnetic origin (Hecky & Hesslein, 1995; Vander Zanden & Rasmussen 1999). Stable isotopes of primary consumers are also related to the habitat gradient, with light $\delta^{13}C$ and heavy $\delta^{15}N$ signatures in profundal habitats and vice versa in littoral habitats (Vander Zanden & Rasmussen 1999).

Lake Victoria has suffered from severe eutrophication in the past decades, and the shallow, inshore habitats especially have high algal biomasses and a high carbon demand by photosynthesis (Ramlal *et al.* 2001; Hecky *et al.* 2010). Based on paleolimnological analyses, changes in lower food web organisms began as early as the 1940s but accelerated dramatically through the 1960s and 1970s (Verschuren *et al.* 2002; Hecky *et al.* 2010). From the 1980s onwards, several studies showed increased nitrogen and phosphorous loadings in the lake, and these coincided with decreased water transparency and decreased oxygen levels (Mugidde 1993; Hecky *et al.* 1994, 2010; Seehausen *et al.* 1997a; Verschuren *et al.* 2002; Chapter 5 in this thesis). Most of the studies reporting eutrophication focussed on the northern part of the lake (Hecky 1993; Mugidde 1993; Mugidde *et al.* 2003); and because of the lack of regular and consistent measurements of biological productivity, paleolimnological analysis was used to provide more continuous analysis of historical changes in ecosystem. For the Mwanza Gulf, located in the southern part of the lake, even fewer data on productivity are available (Akiyama *et al.* 1977; Shayo *et al.* 2011; Cornelissen *et al.* 2013), although other environmental variables such as dissolved oxygen (DO) levels and Secchi depth data have been measured on a fairly regular basis in the last four decades (Chapter 5 in this thesis). In addition, the Lake Victoria biodiversity crisis has been well documented for the Mwanza Gulf from the 1970s onwards (Witte *et al.* 2007). In the 1980s, populations of the introduced Nile perch, *Lates niloticus*, boomed. Together with eutrophication, this boom resulted in a major decline of cichlid species because the Nile perch is predatory on cichlids (Witte *et al.* 1992a; Seehausen *et al.* 1997a; Goudswaard *et al.* 2008). During the 1990s, the population size of some cichlid species, especially zooplanktivores and detritivores, recovered (Seehausen *et al.* 1997b; Witte *et al.* 2007; Kishe-Machumu 2012) and shifted their diet towards macroinvertebrates and to fish (Van Oijen & Witte 1996; Katunzi *et al.* 2003; Kishe-Machumu *et al.* 2008; Chapter 4 in this thesis). Based on formalin-fixed, ethanol preserved cichlid specimens collected over the past 35 years, we demonstrated that the recovered species showed morphological changes that we hypothesise to be adaptive responses to the environmental changes (Witte *et al.* 2008; Van der Meer *et al.* 2012 [Chapter 2 in this thesis]; Van Rijssel & Witte 2013 [Chapter 3 in this thesis]; Chapter 4 in this thesis).

Here we use these same unique cichlid museum specimens caught at a three year time intervals from 1978 onwards, to test how the environmental and ecological changes might be reflected in the C and N stable isotopes of these fish and if they can be used as indicators of eutrophication. In addition, we investigated whether habitat and seasonal changes were reflected in these isotopes as not all fish were caught at the exact same location and period at the research transect. Kishe-Machumu (2012) showed that formalin fixation and ethanol storage had a small but consistent effect on the stable isotopes of cichlids. However, since all fish were preserved the same way, we assume that preservation effects will not influence our results.

For this study, we used two closely related zooplanktivorous species (we give abbreviations of the species names in parentheses); *Haplochromis pyrrhocephalus (pyr)*, *H. laparogramma (lap)*, the zooplankti/insectivorous species *H. tanaos (tan)* and the mollusci/detritivorous species *Platytaeniodus degeni (deg)*.

Dietary gut content analyses revealed that the species *pyr* and *lap* shifted their diet towards large macroinvertebrates such as insects, shrimps and molluscs, and to fishes during the 1990s. This diet partly changed back to zooplankton during the 2000s (Katunzi *et al.* 2003; Kishe-Machumu 2012; Chapter 4 in this thesis). The species *tan* and *deg* both showed the most pronounced diet changes towards macroinvertebrates and fish during the 2000s (Van Oijen & Witte 1996; Chapter 4 in this thesis).

There are no substantial changes over time in the sedimentary $\delta^{15}N$ of the lake based on results from three sediment cores from various locations (R. E. Hecky, unpublished data). Therefore, we expect the dietary changes to be reflected in the $\delta^{15}N$ signatures of the cichlids as was found by Kishe-Machumu (2012). However, the response of $\delta^{15}N$ in fish muscle could be more complex as shifts in basal signature in phytoplankton will be additive to possible shifts in diet to prey which might be lighter or heavier in $\delta^{15}N$. The eutrophication of the lake coincided with the increase of primary productivity, composition, and abundance of phytoplankton in the northern part (Hecky 1993; Verschuren *et al.* 2002) as well as in the southern part of the lake (Cornelissen *et al.* 2013). The shift from diatoms to cyanobacterial phytoplankton dominance was accompanied with an increase of 2‰ in the $\delta^{13}C$ (Suess corrected) of organic matter (Hecky *et al.* 2010). This probably occurred as the higher biomass of filamentous and colonial cyanobacteria raised the demand for CO_2 relative to availability in this soft water lake (Ramlal *et al.* 2001) and also may have decreased isotopic fractionation by boundary layer effects in the larger filamentous and colonial cyanobacteria (Hecky & Hesslein 1995). Therefore, we expect $\delta^{13}C$ signatures may have shifted towards heavier values in these cichlids even without shifts in their diets, especially in inshore habitats. In any case, we hypothesized that changes in environment and trophic behaviour may be evident in the fish isotopic composition for the historic collection of haplochromine fishes from Mwanza Gulf.

Materials and methods

Fish collection

Most fishes were collected from a research transect in the northern part of the Mwanza Gulf (6-14m) on the southern coast of Lake Victoria. Fishes were caught with a bottom trawler during the period 1978-2011. The species *pyr* and *lap* were mainly caught above mud at station G (12-14m) of the transect. Selected *pyr* specimens from 1987 were from Luanso Bay (Goldschmidt *et al.* 1993), a shallow bay (3-4m) 10 kilometres south of the transect, as no *pyr* specimens caught on the transect in 1987 were preserved. The species *tan* and *deg* were mainly caught at sand/mud bottoms (Butimba and Kissenda Bay) at the opposite ends of the transect (Figure 1.1). Fishes were fixed and preserved in 4% formaldehyde (buffered with borax) and after shipment to Leiden transferred to 70% ethanol and stored at the Naturalis Biodiversity Center. A total of 273 male specimens (eight fish per year per species on average) were selected from the years 1978, 1981, 1984, 1987, 1991, 1993, 1999, 2001-02, 2006, and 2011 which is a selection from the same specimens used by van Rijssel & Witte (2013); see also Chapter 3 and Table 6.1 in this thesis.

Table 6.1 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	5	Transect	5	BB, NB	5	BB	5
1981	G	5	G	5	BB	5	BB, K	5
1984	G	10	G	10			BB	10
1987	G	10	Luanso Bay	10			BB, Transect	4
1991	J, P	10	J, P	10				
1993	G, H, I	10	H, I	10	I, J, K	4		
1999	Transect	6	Transect	10				
2001-2002	G, J	10	G	10	J, BB	10	J	10
2006	F-J	10	G	10	E	10	J	10
2011	F, H-J, south of G	10	F	10	J	10	F, J, K	9
Total		**86**		**90**		**44**		**53**

E-K, stations on the transect; P, Python Island-Nyamatala Island; BB, Butimba Bay; NB, Nyegezi Bay; 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.

Stable isotope analysis

From each fish, the right side of the epaxial muscle located dorsal of the lateral line was dissected after removal of the skin. These muscle tissue samples were then freeze-dried for 72 hours and grounded into fine powder with a pestle in an Eppendorf tube. A subsample of 1.25 mg was placed into tin cups and shipped to the University of California Davis Stable Isotope Facility for analysis. Stable isotope analysis of ^{13}C and ^{15}N was carried out with a PDZ Europa Automated Nitrogen Carbon Analyzer-Gas Solids and Liquids (ANCA-GSL) elemental analyzer interfaced to a PDZ Europa 20-20 continuous flow Isotope Ratio Mass Spectrometer (IRMS). The $\delta^{13}C$ and $\delta^{15}N$ values were expressed relative to international reference standards V-PDB (Vienna PeeDee Belemnite) and air respectively. The difference (δ) in isotopic ratio between the sample and standards was calculated as:

$\delta^{13}C$ or $\delta^{15}N = (R_{sample} – R_{standard})/ (R_{standard}) \times 1000$
where $R= {}^{13}CO_2 / {}^{12}CO_2$ for $\delta^{13}C$ or $R= {}^{15}N_2 / {}^{14}N_2$ for $\delta^{15}N$
and values are expressed as ‰.

Glutamic acid, nylon and bovine liver which were similar in composition as the samples being used, were used as standards. These standards were previously calibrated against National Institute of Standard Technology (NIST) Standard Reference Materials such as International Atomic Energy Agency (IAEA-N1,-N2, -N3), USGS-40 (light carbon and nitrogen isotopes in L-glutamic acid) and USGS-41 (heavy carbon and nitrogen isotopes in L-glutamic acid).

6

Due to deforestation and fossil fuel burning which is naturally depleted in $\delta^{13}C$, atmospheric CO_2 levels have been increasing while $\delta^{13}C$ of CO_2 has declined, especially over the past 35 years (Francey *et al.* 1999). This decrease in $\delta^{13}C$ of atmospheric CO_2 due to anthropogenic perturbations is known as the Suess effect (Keeling 1979) and has been most severe as the present day is approached (Verburg 2007). As atmospheric and aquatic CO_2 equilibrate, it was necessary to apply a Suess correction in order to compare $\delta^{13}C$ signatures of fish collected over the last 35 years according to the following formula:

$$7.7738118 * 10^{-16} * Y^6 - 1.2222044 * 10^{-11} * Y^5 + 7.1612441 * 10^{-8} * Y^4 - 2.1017147 * 10^{-4} * Y^3 + 3.3316112 * 10^{-1} * Y^2 - 273.715025 * Y + 91703.261,$$

with Y as year since 1700, as recommended by Verburg (2007). The Suess correction was subtracted from $\delta^{13}C$ values of the years 1981-2011 with the smallest correction for 1981 (-0.07‰) and the largest correction for 2011 (-1.09‰).

Statistical analysis

Differences in stable isotopes over time were tested with a One-way ANOVA and *P*-values were corrected with a sequential Bonferroni test. To test if standard length (SL) influences the stable isotopes of the fish, a Pearson correlation test was used after testing for normality with a Shapiro-Wilk test. In three out of four species (*lap*, *pyr* & *tan*), only five significant correlations between $\delta^{13}C$ and SL were found within a year. There were no significant correlations between δ^{15}N and SL within years. Because there was no consistent trend and the correlations occurred in both positive and negative direction within a species, we decided not to correct for SL (Appendix Table 6.1). To test if the number of different catch locations per year influenced the variation in stable isotopes, we correlated this number with the standard deviation (st. dev.) of the stable isotopes per year. We applied the same method to test for seasonal effects by correlating the number of catch dates with standard variation of the stable isotopes. All statistical tests were performed with SPSS version 20.

Results

All four species showed significant changes over time in δ^{13}C ($P < 0.001$) and δ^{15}N ($P < 0.01$; Figures 6.1, 6.2). The two zooplanktivorous species (*pyr* and *lap*) showed shifts towards lighter δ^{13}C and heavier δ^{15}N during the 1990s compared to 1978 ($P < 0.05$). The species *tan* and *deg* both showed heavier δ^{15}N values in the 2000s and *deg* showed a shift towards heavy δ^{13}C values during that period. In 2011, three out of four species (*pyr*, *lap* and *tan*) shifted towards lighter δ^{15}N values ($P < 0.05$) while the δ^{13}C shifted to remarkably heavy values in all four species ($P < 0.001$; Figures 6.1, 6.2).

Haplochromis laparogramma

The δ^{13}C shifted to lighter values in 1981 compared to 1978 (though not significant, $P = 0.072$) and then in 1984 shifted back to heavier values ($P < 0.001$), similar to those of 1978. In 1987, there was again a shift towards lighter δ^{13}C values compared to 1984 ($P < 0.001$) which did not change significantly again until 1999. There was a significant shift towards heavier δ^{13}C values in the years 2006 and 2011 compared to all other years ($P < 0.001$, Figures 6.1A, 6.2A).

The δ^{15}N values increased in 1981 compared to 1978 ($P = 0.015$, not significant after sequential Bonferroni correction) but these values decreased again in 1984 ($P = 0.025$, not significant after sequential Bonferroni correction). The δ^{15}N values decreased even further in 1987 which gave the lowest δ^{15}N values compared to all other years ($P < 0.001$). All years in the period 1993-2006 showed significant higher δ^{15}N values compared to 1978 and 1984 ($P < 0.01$). The δ^{15}N values in the year 2011 did not differ from the δ^{15}N values in the period 1978-1984 (Figure 6.1A, 6.2A).

Haplochromis pyrrhocephalus

The δ^{13}C shifted towards lighter values in 1981 and 1984 ($P < 0.01$) and then shifted back to heavier values in 1987, comparable to those of 1978. It must be noted that specimens of *pyr* caught in 1987 came from the Luanso Bay, a shallow bay (3-4m) 10 kilometres south of the transect which might be the cause of the relatively heavy δ^{13}C values. During the period 1991-2001, there is again a significant shift towards lighter isotopes compared to 1984 ($P < 0.05$). In 2006, the δ^{13}C shifts to heavier values similar to those of 1984 and just as in *lap*, the δ^{13}C values of 2011 were the heaviest compared to all other years ($P < 0.001$, Figures 6.1B, 6.2B).

The δ^{15}N values increased in 1981 as well as in 1984 ($P < 0.001$) compared to 1978. As in *lap*, there was a decrease in 1987 in δ^{15}N values compared to 1984 ($P < 0.001$). The δ^{15}N values increased again in the period 1993-2006 compared to 1984, as was seen for *lap*. In 2011, the δ^{15}N values decreased to the level of 1981 and 1984 but δ^{15}N values were still significantly higher than in 1978 ($P < 0.001$, Figures 6.1B, 6.2B).

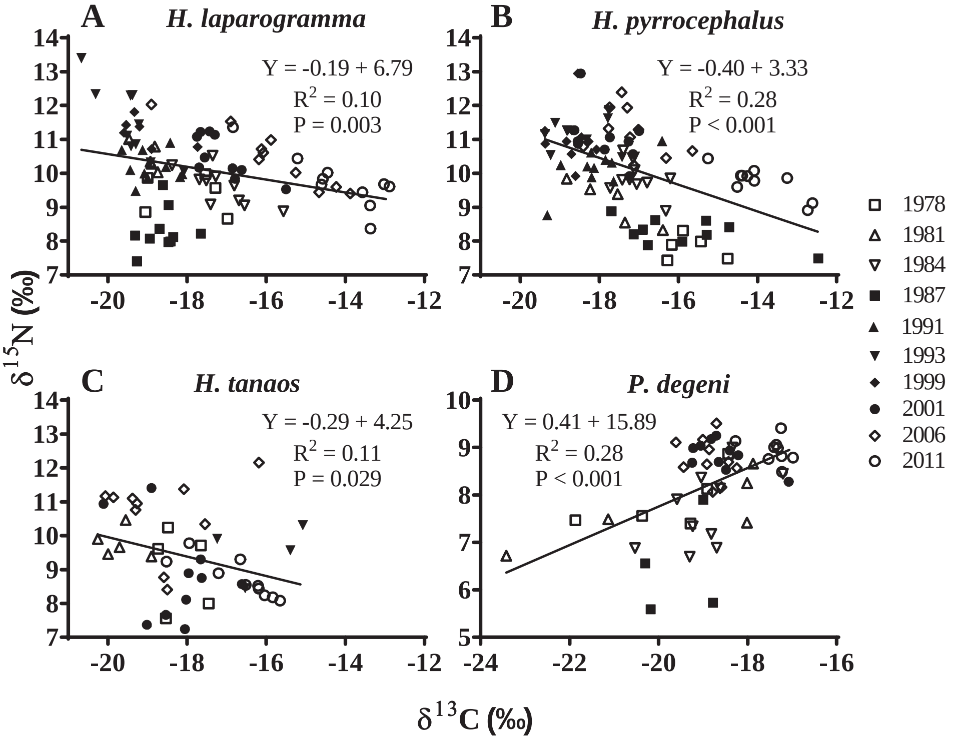

Figure 6.1 The Suess corrected δ^{13}C and δ^{15}N stable isotopes of the four cichlid species (A) *H. laparogramma*, (B) *H. pyrrhocephalus*, (C) *H. tanaos* and (D) *P. degeni* per year. Linear regression lines, their slopes, R-squared and *P*-values are depicted for each species as a whole.

Haplochromis tanaos

As seen in *lap* and *pyr*, the δ^{13}C shifted towards lighter values in 1981 ($P = 0.016$, not significant after sequential Bonferroni correction). In 1993 (no data available for 1984 and 1987), there is a shift to heavier δ^{13}C values compared to 1978 and 1981 ($P < 0.01$). In 2001 and 2006, there is a shift back to lighter δ^{13}C isotopes ($P < 0.001$) similar to those of 1978. As was found in *lap* and *pyr*, the δ^{13}C values of 2011 were the heaviest compared to all other years ($P < 0.01$) but do not differ significantly from δ^{13}C values in 1993 (Figures 6.1C, 6.2C). The δ^{15}N values only increased in 2006 and these were significantly higher than δ^{15}N values from 1978, 2001 and 2011 (Figures 6.1C, 6.2C).

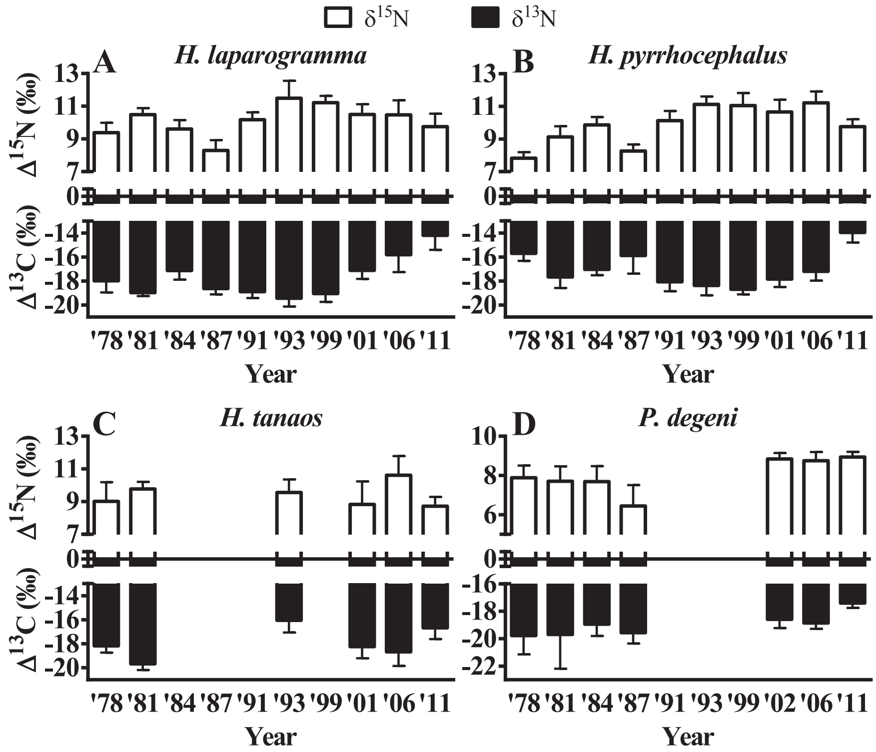

Figure 6.2. The average and standard deviation of Suess corrected δ^{13}C and δ^{15}N stable isotopes of the four cichlid species (A) *H. laparogramma*, (B) *H. pyrrhocephalus*, (C) *H. tanaos* and (D) *P. degeni* per year. The sample size of 1978 and 1981 of all species was 5, the sample size of all other years was 10, except for *H. laparogramma* 1999 (N = 6) and *H. tanaos* 1993 (N = 4).

Platytaeniodus degeni

The δ^{13}C values do not differ from each other during the period 1978-1987. There is a shift to heavier δ^{13}C from 1978 to 2002 ($P = 0.038$, not significant after Bonferroni correction). The δ^{13}C values of 2006 do not differ from other years except for 2011 which has, like in the other three species, the heaviest δ^{13}C values compared to all other years ($P < 0.05$, Figures 6.1D, 6.2D).

The δ^{15}N values do not differ in the years 1978-1984 and, as was found for *lap* and *pyr*, 1987 had the lowest δ^{15}N compared to all other years ($P < 0.01$). The years 2002-2011 did not differ from each other and all had significantly higher values compared to the years 1978-1987 ($P < 0.01$, Figures 6.1D, 6.2D).

Effect of catch location

Fish from multiple catch locations showed a higher within-year variation in $\delta^{13}C$ than fish caught in years with fewer catch locations (four different species combined, Spearman correlation, r = 0.422, $P = 0.014$). Since each catch location had a different depth, this means that fish caught in years with multiple catch locations were also caught from different depths. All four species showed positive (mostly non-significant) correlations between the number of catch locations per year and the st. dev. of $\delta^{13}C$. There was one significant correlation for *lap* (r = 0.851, $P = 0.002$) and an almost significant correlation for *deg* (r = 0.732, $P = 0.061$) between the number of catch locations per year and the st. dev. of $\delta^{13}C$ (Table 6.2).

The relation between the number of catch locations and the amount of within-year variation in $\delta^{15}N$ was less clear and showed no significant correlations. The number of catch locations per year showed a nearly significant positive correlation with the st. dev. of $\delta^{15}N$ for *lap* but a nearly significant negative correlation for *pyr* (Table 6.2).

Table 6.2 Pearson correlations per species between the number of catch locations, catch dates per year and the st. dev. of $\delta^{13}C$, $\delta^{15}N$. Significant values are depicted in bold.

Species	Number of catch locations / dates	N	$\delta^{13}C$		$\delta^{15}N$	
			r	***P***	**r**	***P***
H. laparogramma	Locations	10	**0.851**	**0.002**	0.597	0.068
	Dates	10	0.574	0.083	0.524	0.12
H. pyrrhocephalus	Locations*	10	0.055	0.879	-0.624	0.054
	Dates	10	-0.129	0.723	0.203	0.573
H. tanaos	Locations	6	0.147	0.781	0.514	0.297
	Dates	6	0.297	0.568	-0.037	0.945
P. degeni	Locations	7	0.732	0.061	0.285	0.536
	Dates	7	**0.770**	**0.043**	0.605	0.15

* Indicates Spearman correlations.

Effect of catch date

There was a significant positive correlation between the number of catch dates and the st. dev of $\delta^{13}C$ per year for *deg* (r = 0.77, $P = 0.043$) and an almost significant positive correlation for *lap* (r = 0.574, $P = 0.083$). There were no significant correlations between the st. dev. of $\delta^{15}N$ and the number of catch dates per year (Table 6.2).

Discussion

Stable isotope changes through time

This study shows how dietary shifts are reflected in the stable isotopes of formalin fixed Lake Victoria cichlids. The increase of $\delta^{15}N$ values through time of all four species concurs with the reported shift in diet to larger prey for all four species.

Although the species shifted their diet already in 1987 (Chapter 4 in this thesis), there was no increase but a decrease in $\delta^{15}N$ values in that year. Stomach and gut content analysis revealed that the diet of the zooplanktivores consisted for a large part of detritivorous shrimps and detritus (Chapter 4 in this thesis), which explains the low $\delta^{15}N$ values. Campbell *et al.* (2003a) reported that *Caridina* (shrimps) had substantially lower $\delta^{15}N$ and $\delta^{13}C$ than zooplankton in Napoleon Gulf in northern Lake Victoria which is in agreement with our results. Though stomach and gut contents were not analysed for *deg* in 1987, based on their low $\delta^{15}N$ values and the dramatic increase of shrimps in the Mwanza Gulf during that time (Goudswaard *et al.* 2006; Chapter 4 in this thesis), it is likely that this species had shifted to a diet similar to that of the zooplanktivores.

Based on stomach and gut content analysis, the species *tan* shifted its diet in 1993 from zooplankton and insects to mainly insects and fish. Although higher $\delta^{15}N$ values would be expected with a shift to larger prey, *tan* already had quite a high volume percentage of insects in their diet (8% chironomids, 5% *Chaoborus* larvae and 24% insects) before the environmental changes. Moreover, the aquatic insects and especially the decapod crustacean *Caridina* in Lake Victoria generally have lower $\delta^{15}N$ values than zooplankton, although there are exceptions among the insects (Campbell *et al.* 2003a; Ojwang *et al.* 2004) which might explain the lack of $\delta^{15}N$ increase in 1993 for *tan*. In 2006, *tan* included even more fish in their diet than in 1993 (Van Oijen & Witte 1996; Kishe-Machumu 2012; Chapter 4 in this thesis) which is reflected in the increase of $\delta^{15}N$ values as well.

More consumption of aquatic insects might also explain the lower $\delta^{15}N$ values of *pyr* compared to the closely related species *lap* from before the environmental changes. *Haplochromis laparogramma* was almost exclusively feeding on zooplankton during that time and *pyr* already included some chironomid larvae and insects next to their main prey zooplankton (Kishe-Machumu 2012; Chapter 4 in this thesis) which might have lowered their $\delta^{15}N$ values.

For one species (*lap*), we were able to perform a Pearson correlation test on the dietary contents with the stable isotopes from the same fish. However, none of the averaged volume percentages of the different food types (zooplankton, phytoplankton, detritus, insects, shrimps or fish) gave a significant correlation with $\delta^{13}C$ or $\delta^{15}N$ through time. The lack of correlation can be caused by three factors: 1) these fishes seem to be quite opportunistic regarding their food types. The studied species shifted their diet from mainly small prey (zooplankton/detritus) to a highly diverse diet containing multiple food types such as insects, fish, shrimps, detritus and phytoplankton at the time that large macroinvertebrate numbers increased in their environment (Chapter 4 in this thesis). These lower food web organisms show a high variability in their stable isotope signatures (Campbell *et al.* 2003a) which is reflected in the stable isotopes of the fish; 2) the stomach and gut contents only reflect what the fishes has been eating that day (or night) and do not always have to reflect fish's diet on the long term; 3) meteorological variability seems to be affecting the mixing depths of the Mwanza Gulf (Chapter 5 in this thesis) which have an effect on the $\delta^{13}C$ of particulate organic matter (POM) and fish and therefore interfere with stable isotope-food relationships. These three factors make direct dietary-stable isotope correlations hard to detect in these species.

Geographical variation

A larger number of catch locations correlated with a higher $\delta^{13}C$ variation. Unfortunately, the dataset we used did not allow us to detect a general trend in offshore and inshore isotopes (heavier $\delta^{13}C$ and lighter $\delta^{15}N$ values inshore vs. lighter $\delta^{13}C$ and heavier $\delta^{15}N$ offshore) as found by Hecky *et al.* (2010) and Mbabazi *et al.* (2010) in Lake Victoria and Lake Kyoga, respectively. However, these studies reported intra-lake variation on a large scale (from 1 to 150 km offshore) while our studied transect only covered 5km.

This intra-lake variation (Hecky *et al.* 2010) exhibits an inverse relationship between $\delta^{13}C$ and $\delta^{15}N$ for POM (Figure 6.3, Pearson correlation, r = -0.50, P = 0.002). This relationship shows that for every 1‰ increase in $\delta^{13}C$ (from offshore to inshore), the $\delta^{15}N$ decreases by 0.71‰ in POM. The species *pyr*, *lap* and *tan* seem to exhibit a similar trend with negative slopes of -0.40, -0.19 and -0.29 respectively (Figures 6.1A, B, C). But the slopes of these species are less steep than that of POM, and so geographic variation can only partly explain the shifts in stable isotope signatures if we assume that the relationship for POM lake-wide applies to Mwanza Gulf. The species *pyr* and *lap* did not extend their habitat to deeper water (as would be expected from the POM data and the decline of $\delta^{13}C$ in these species through the 1980s and 1990s) but rather they occupied shallower water (Seehausen *et al.* 1997b; Kishe-Machumu 2012). However, stomach and gut content analysis revealed a higher intake of chironomids, detritus and molluscs during the late 1980s and 1990s of both species (Katunzi *et al.* 2003; Kishe-Machumu, 2012; Chapter 4 in this thesis), indicating a more benthic feeding behaviour during this period. In contrast, the species *tan* did extend its habitat from shallow bays to deeper, open sublittoral areas. The species *deg* showed a positive slope (0.41, Figure 6.1D) which can only be explained by a shift to isotopically heavier prey as both $\delta^{13}C$ and $\delta^{15}N$ increase together from earlier to later years.

Evidence for geographic and habitat variation in stable isotope signatures was also found for the zooplanktivorous cyprinid *Rastrineobola argentea* (dagaa) and the carnivorous Nile perch as well (caught on the same research transect as our study, Table 6.3). Both *R. argentea* and the Nile perch showed consistently lighter $\delta^{13}C$ values at the offshore, 12-14m deep station G compared to the shallower stations E (6-8m) and J (4-6m), which lie at the opposite ends of the transect (Figure 1.1). Nile perch showed significantly lighter $\delta^{13}C$ values at the deeper station G (ANOVA, $P < 0.05$) compared to shallower stations E and J. The $\delta^{15}N$ values were also lighter at station G than at station E (P = 0.024) and J (P = 0.08) which cannot be explained by a shift in basal signatures expected from the POM relationship (Figure 6.3), but rather must imply feeding at a higher trophic level in Nile perch occupying shallower waters. Though the small sample size did not permit us to do statistics for *R. argentea*, $\delta^{13}C$ values show a pattern consistent with the pattern found for Nile perch with lighter $\delta^{13}C$ values at the deeper station G compared to heavier values at the shallower stations E and J. But unlike Nile perch, the $\delta^{15}N$ values were heavier for the deeper station G compared to the shallower station E and J (Table 6.3). *Rastrineobola argentea* isotopes show the same geographic trend on the relatively small research transect as the POM isotopes show on a lake-wide scale. The $\delta^{13}C$ values increase from station G to J with 3.8‰ while $\delta^{15}N$ decreases with 2.4‰. The ratio of these two is -0.63 which is similar to the slope of the POM isotopes (-0.71).

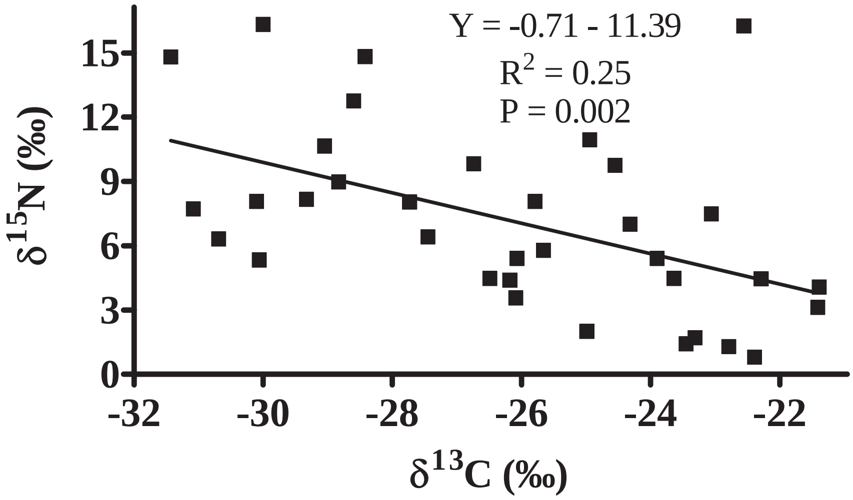

Figure 6.3 Stable isotopes of particulate organic matter (POM) collected from inshore and offshore stations along a transect from Mwanza in the south, to Port Bell in the north of Lake Victoria in October 1995; and from location V96-5MC in the middle of the lake; and from Bugaia Island in the northern part of the lake in 1995/96 (Campbell *et al.* 2003b; Hecky *et al.* 2010).

So for this species, differences between stable isotopes signatures seem to be an effect of basal signatures at base of food web per station. Nile perch isotopes, on the other hand, show an increase from station G to J of 3‰ for δ^{13}C and an increase of 1.8‰ for δ^{15}N which results in a slope of +0.6. Since this cannot be explained by changes in POM isotopes, these isotope data suggest that Nile perches are feeding on prey with a lighter isotopic composition at the deeper station G compared to the shallower stations J and E. The overall fairly low δ^{15}N values also imply that these small-sized Nile perches (<30 cm) are not entirely piscivorous yet and may feed on shrimps, insects as well as juvenile fishes (Kishe-Machumu *et al.* 2012). The relatively high δ^{15}N values of *R. argentea* suggest that these are mainly feeding on zooplankton and midge larvae (Wanink 1998).

Table 6.3 Stable isotope values of Nile perch and *R. argentea* caught at three different stations on the research transect from 25th-28th of February 2007. * Indicates significant difference of < 0.05 with values of station G (One-way ANOVA). TL, average total length.

		Nile perch				***R. argentea***			
Station	**Depth (m)**	**δ^{13}C**	**δ^{15}N**	**TL (cm)**	**n**	**δ^{13}C**	**δ^{15}N**	**TL (cm)**	**n**
J	4-6	-20.49*	8.79	19.7	4	-15.52	10.37	5.6	4
E	6-8	-21.21*	9.48*	18.9	4	-15.43	10.41	6	2
G	12-14	-23.54	6.96	15	4	-19.33	12.72	4.8	1

Although our cichlid dataset does not allow us to make within-year comparisons as depicted in Table 6.3, we suggest that a similar geographic variation in $\delta^{13}C$ isotopes might be present on such a small scale in cichlids as well. Specimens from the two closely related zooplanktivorous species *pyr* and *lap* from 1978 were caught all along the transect (specific station is unknown), while individuals of these species from 1981 were caught only at the deepest station of the transect, G (Table 6.1). Stomach and gut content analysis revealed that these fish fed mainly on zooplankton and that there was no within-species difference in volume percentages of this prey type before 1987 (Witte 1987; Chapter 4 in this thesis). This is why we consider the shift towards lighter $\delta^{13}C$ values of the two zooplanktivorous species in 1981 compared to 1978 (Figure 6.1a, b) more likely to be the result of geographic variation than a change in diet over time. The observed trend for lighter $\delta^{13}C$ values in deeper offshore water has been reported on a larger scale by Hecky *et al.* (2010). They attributed these lighter offshore $\delta^{13}C$ values to a lower offshore algal (cyanobacteria) productivity and biomass compared to inshore. Although our research transect is only 5km wide, the stable isotope data suggest that this relation might apply on a smaller scale to the Mwanza Gulf as well. This theory is supported by the findings of Kishe-Machumu *et al.* (submitted) who found heavier $\delta^{13}C$ values at the shallow station J compared to deeper stations in the Mwanza Gulf for two haplochromine cichlid species (including *H. pyrrhocephalus*).

This geographical variation in stable isotopes suggests also that the zooplanktivorous open water species used in this study have a limited dispersal between stations along the transect. It is known that many cichlid species are restricted by bottom types or depths but a virtual lack horizontal migration has not been reported for these open water species (Witte 1981; Witte *et al.* 2007). On the other hand, these fish have extended their habitat to shallower depths in the past decades indicating that there must be some horizontal migration but probably less than previously thought (Seehausen *et al.* 1997b; Kishe-Machumu 2012). More strikingly is the observation that, based on our limited data, Nile perch and *R. argentea* apparently show a similar habitat preference. These species are the most important commercial species in Lake Victoria since the 1980s (Ogutu-Ohwayo & Balirwa 2006; Tumwebaze *et al.* 2007). If these species are showing intra-specific habitat preference that results in reduced mobility, then this may have some major consequences for their fisheries and conservation aspects.

Seasonal variation

Primary producers are known to have within-year temporal variation in both $\delta^{13}C$ and $\delta^{15}N$ stable isotopes (Cabana & Rasmussen 1996; Post 2002). Enriched (heavy) $\delta^{13}C$ and decreased $\delta^{15}N$ values of primary producers and primary consumers have been reported during periods of stratification in temperate lakes, but to our knowledge not in tropical lakes (Quay *et al.* 1986; Zohary *et al.* 1994; Hodell & Schelske 1998; Caroni *et al.* 2012). In addition, larger consumers such as fish have long tissue turnover rates (months to years, Hesslein *et al.* 1993) and thus are their isotopic signatures representative of their diet for longer periods of time (Post 2002). This means that if there are seasonal differences in the lower food web, they will be hard to detect, especially with the dataset used in this study where we were limited to previously collected museum material.

The heavy $\delta^{13}C$ and light $\delta^{15}N$ values of 2011 found for *lap*, *pyr* and *tan* could be considered as being a seasonal effect as these fish were all caught during the warmer wet season when vertical stratification of the water column is more likely than in the cool dry season and this may lead to different availability of food resources. In contrast, comparison of these isotopic signatures from 2011 with stable isotope values from fishes caught during the wet season in the year 1999 shows that the latter actually had lighter $\delta^{13}C$ and higher $\delta^{15}N$ values. This leads us to believe that, based on our data, stable isotope signatures are a reflection of the fish's diet and location rather than season. In addition, so far, no seasonal variation in the diet of Lake Victoria cichlids has been reported (Van Oijen & Witte 1996; Katunzi *et al.* 2003; Kishe-Machumu *et al.* 2008; Kishe Machumu 2012; Chapter 4 in this thesis). Studies on seasonal variation of stable isotope signatures in Lake Victoria cichlids will provide definitive conclusions on this matter.

Signs of increased primary productivity?

Unexpectedly, the $\delta^{13}C$ values in the studied zooplanktivorous species shifted to lighter values during the 1990s where heavier values were expected due to increased demand for CO_2 and reduced isotopic fractionation resulting from the increased phytoplankton biomass (Hecky & Hesslein 1995; Hecky *et al.* 2010). However, during the 2000s and especially in 2011, there is a remarkable shift towards heavier $\delta^{13}C$ in all four species.

We hypothesize that this might be the result of increased primary productivity by phytoplankton and evidence for continued eutrophication of the lake. Recently, Cornelissen *et al.* (2013) found that phytoplankton productivity in 2009-2011 has increased in the Mwanza Gulf compared to the 1970s (Akiyama *et al.* 1977). The increase of primary productivity and a basal change of phytoplankton stable isotope signatures could be reflected in the $\delta^{13}C$ values of the fish when phytoplankton is (unintentionally) absorbed or ingested by the fish (or their prey), as has been found for several other fish species (especially during times of algal blooms, Christoffersen 1996; Smith *et al.* 2008). In case of the zooplanktivorous species (which again include mainly zooplankton in 2006 and 2011, Chapter 4 in this thesis), the preyed upon zooplankton (mainly copepods) should then feed upon cyanobacteria such as *Microcystis* and *Anabaena* and diatoms like *Nitzschia* which have replaced the original phytoplankton (mainly *Aulacoseira* [*Melosira*]) in the entire lake (Ochumba & Kibaara 1989; Hecky 1993; Kling *et al.* 2001; Verschuren *et al.* 2002) including the Mwanza Gulf (Sekadende *et al.* 2005; Cornelissen *et al.* 2013).

However, grazing experiments indicated that Lake Victoria's crustacean zooplankton (mainly cyclopoid copepods) do not control the cyanobacteria dominated phytoplankton biomass (Lehman & Branstrator 1993; Branstrator *et al.* 1998). In addition, other studies found cyanobacteria (*Microcystis*) to be toxic, nutritionally inadequate and suppressing feeding in copepods (Fulton & Paerl 1987; Demott & Moxter 1991; Demott *et al.* 1991).

On the other hand, there is a growing amount of evidence suggesting that copepods can grow and reproduce while feeding on toxic cyanobacteria (Koski *et al.* 2002; Reinikainen *et al.* 2002; Nascimento *et al.* 2008). In fact, several copepod species are known to (rapidly) adapt to increased cyanobacteria exposure enabling these zooplankters to feed upon the phytoplankton (Karjalainen *et al.* 2006; Colin & Dam 2007; Mariani *et al.* 2013).

Therefore, it is not improbable that the cyclopoid zooplankton (or cichlids) of the Mwanza Gulf partly feed upon the increased phytoplankton biomass that may have resulted in heavier $\delta^{13}C$ values in our fish. A recent stable isotope study on zooplankton caught in the Mwanza Gulf in the wet season of 2011 (same location and period as our fish) showed the same heavy $\delta^{13}C$ stable isotope values as for our fish (I. J. M. Cornelissen, unpubl. data), which supports the above mentioned hypothesis. Zooplankton grazing experiments on phytoplankton in the Mwanza Gulf would be needed to draw definitive conclusions.

Conclusions

Using a unique long term sampling data set, our study shows that stable isotope changes are reflecting dietary and habitat changes of four formalin fixed haplochromine species. In contrast, there does not seem to be a seasonal effect on the stable isotopes. Besides ecological changes, we suggest that the stable isotopes of these fishes might be reflecting variation in primary production and varying degrees of eutrophication over the last several decades. This would imply that these haplochromines could serve as indicators of eutrophication and could be used for eutrophication assessment methods. Our results also suggest that not only cichlids, but also Nile perch and dagaa seem to be quite stenotopic and might show less horizontal dispersal as previously thought.

The temporal variability of stable isotopes in these fishes shows that museum specimens can be used to trace historic changes in fish ecology and the aquatic environment. The reconstruction of the ecology and environment by stable isotope analysis might be applicable to other aquatic organisms as well. This highlights the need for continued sampling of fish and as well as other aquatic organisms important to fish feeding to reconstruct and predict environmental changes in aquatic ecosystems.

Acknowledgements

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Appendix Table 6.1 Pearson correlations per species and year between δ^{13}C, δ^{15}N and SL. Significant values are depicted in bold.

			δ^{13}C		δ^{15}N	
Species	**Year**	**N**	**r**	***P***	**r**	***P***
H. laparogramma	1978	5	-0.702	0.187	0.569	0.317
	1981	5	-0.420	0.482	0.848	0.069
	1984	10	**-0.672**	**0.033**	0.444	0.198
	1987	10	**-0.690**	**0.027**	-0.017	0.963
	1991	10	-0.570	0.085	-0.203	0.574
	1993	10	0.551	0.099	-0.448	0.194
	1999	6	-0.612	0.197	0.049	0.926
	2001	10	-0.139	0.702	0.275	0.442
	2006	10	0.019	0.959	-0.066	0.856
	2011	10	0.228	0.527	0.115	0.752
H. pyrrhocephalus	1978	5	-0.489	0.403	0.511	0.379
	1981	5	-0.323	0.596	-0.063	0.920
	1984	10	0.111	0.761	0.056	0.878
	1987	10	-0.273	0.446	-0.285	0.425
	1991	10	-0.511	0.131	-0.575	0.082
	1993	10	0.060	0.869	0.187	0.605
	1999	10	-0.427	0.219	-0.515	0.128
	2001	10	**-0.729**	**0.017**	-0.489	0.151
	2006	10	-0.125	0.731	0.018	0.960
	2011	10	0.371	0.291	-0.073	0.841
H. tanaos	1978	5	-0.607	0.278	0.663	0.222
	1981	5	0.707	0.182	0.350	0.564
	1993	4	-0.668	0.332	-0.729	0.271
	2001	10	0.345	0.329	0.546	0.103
	2006	10	**0.812**	**0.004**	0.336	0.342
	2011	10	**-0.668**	**0.035**	0.221	0.539
P. degeni	1978	5	0.251	0.684	-0.093	0.882
	1981	5	0.242	0.695	-0.361	0.550
	1984	10	0.267	0.456	0.202	0.576
	1987	4	-0.634	0.366	0.218	0.782
	2001	10	0.005	0.989	-0.299	0.402
	2006	10	0.303	0.394	0.202	0.575
	2011	10	-0.281	0.463	0.346	0.361


Date: 29-3-11
St + Nr.: J
Sp.: H. tan 1

Chapter 7

Synthesis

In this final chapter, I summarize the findings reported in this thesis, discuss their implications and discuss perspectives for future work on the topic.

Photopic adaptations to turbid waters and larger prey

Two goals of this thesis research were to study whether morphological changes took place and, if they did, on what time scale. In **Chapter 2** it was shown that the eyes of two Lake Victoria haplochromine cichlid species displayed morphological changes within two decades. Both species had smaller eyes, probably due to a trade-off with other morphological structures which increased in magnitude. Parameters such as cheek depth (**Chapter 3**) and the gill surface (**Chapter 5**) and possibly other structures related to feeding such as the pharyngeal crushing muscle (Witte *et al.* 2008; Van Rijssel *et al.* submitted) increased in size. The head size and/or volume of these fishes, however, remained the same or even decreased (**Chapter 3**; J. C. van Rijssel unpublished data). So, it seems that some morphological changes are not directly related to the environment but to morphological constraints.

One species, *H. pyrrhocephalus*, showed a decreased density of double cones when the water transparency was low, which implies diminished optical resolution of the eye. Double cones are long wavelength sensitive (LWS) and thus more sensitive to red and green light. However, despite the decrease in eye size, the size of these double cones remained the same. This means that the photopic sensitivity — the amount of light captured by the eye — remained the same. The other species, *H. tanaos*, actually showed changes consistent with an increase in the photopic sensitivity, namely having larger double cones. The single cones of both species were greatly reduced in number or even completely absent. Single cones are short wavelength sensitive (SWS) and capture mostly blue light, which is scarce in the murky waters of the Mwanza Gulf (**Chapter 5**). So, I hypothesise that, the morphological changes in the eye enabled the cichlids to cope with the decrease in water transparency.

Another goal of this thesis research was to investigate whether the morphological changes took place in relation to specific environmental variables. In the case of the morphological changes in the cichlid eyes, it is hard to pinpoint a single environmental or ecological variable. The fact that double cone size was maintained or increased is likely a response to the increased turbidity, as is the degradation or absence of single cones.

The loss of visual resolution might be due to a trade-off as well, because a smaller eye with increased cone sizes might have forced a decrease in double cone density, though this might be compensated by the loss of single cones. In addition, the role of the diet shift towards larger prey **(Chapter 4**) might be an important factor in the loss of optical resolution. It may be that dietary shift has enabled the eye to sacrifice high resolution (which is needed to detect small prey) in order to maintain photopic sensitivity (which is important for mate recognition and other activities).

However, it is not clear which factor is primary and which is secondary. Thus, the changes in eye resolution might have forced the fishes to switch to larger prey, as smaller prey are harder to detect with low eye resolution. Though this thesis does not provide a clear answer on this matter, it does show that these haplochromines already shifted their diet towards larger prey in 1987 while the zooplanktivorous species were mainly feeding on zooplankton until 1984 at least (**Chapter 4; Chapter 6**). The period 1986-87 is characterized by decreased oxygen levels (**Chapter 5**), increased water turbidity (**Chapter 5**) an increased number of large prey, mainly shrimps, and a decreased number of larger zooplankters (**Chapter 4**). Cichlids and teleosts in general can be quite opportunistic in their feeding behaviour. Therefore, in my view, the switch to larger prey as its abundance increased, was the primary effect, and the decrease in density of the double cones, and the consequent decrease in the resolution of the eye, followed secondarily.

Body shape change in response to predation

To determine the timescale over which the morphological changes took place, haplochromines caught over a period of 30 years with approximately a three year sampling interval were selected. Three out of four studied resurgent haplochromines showed a smaller head-to-tail ratio in nine years or even faster (**Chapter 3**). As multiple species from different trophic groups showed morphological changes in the same direction, these changes are likely to be adaptive.

There seems to be quite a clear connection between the number of Nile perch in the Mwanza Gulf on the one hand, and the body shape of the haplochromines on the other. As soon as Nile perch numbers increased in 1983 and 1984 (Goudswaard *et al.* 2008), the cichlids show a smaller head to tail ratio (**Chapter 3**). A smaller head and larger caudal area are essential for predator escape performance (Blake 2004). A smaller head to tail ratio has shown to be beneficial for predator escape performance in several fish species including mosquitofish (Langerhans *et al.* 2004; Langerhans 2009), three-spined sticklebacks (Hendry *et al.* 2011) and guppies (Hendry *et al.* 2006). In addition, a literature review showed that for 16 different fish species, smaller head-to-tail ratios were associated with high predation localities or treatments (Langerhans 2010).

Remarkably, when Nile perch numbers decreased during the 2000s (Matsuishi *et al.* 2006; Mkumbo *et al.* 2007; Kayanda *et al.* 2009) the body shape of the haplochromines returned to the previous form of the late 1970s (**Chapter 3**). The reversal of morphological characters after the reversal of environmental changes has been reported in other vertebrates. Thus, several species, including Galapagos finches, three-spined sticklebacks and Bahamian lizards show reversed morphological changes, probably due to natural selection, after the perturbed environment had returned to its initial state (Grant & Grant 2006; Losos *et al.* 2006; Kitano *et al.* 2008). It is therefore possible that the reversal of morphological changes in haplochromines are also due to genetic changes. An alternative possibility is that the flexibility of the reversal can be explained in terms of phenotypic plasticity, as body shape was found to be plastic in cichlids (Crispo & Chapman 2010a).

A species that is either extremely rare or extinct, *H. piceatus*, did not show any changes in the head to tail ratio, while another possibly extinct species *H. heusinkveldi* showed changes in the opposite direction from the examples above; an increased head to tail ratio (**Chapter 3**). So, it is possible that the lack of an adaptive morphological response to predation, or even a maladaptive response, might have played a role in the disappearance of these two *Haplochromis* species. However, both species were last caught in the period 1985-86 in the Mwanza Gulf. In the resurgent species, most pronounced body shape changes occurred during the late 1980s and 1990s when Nile perch numbers were highest

(Goudswaard et al. 2008). In fact, the most abundant zooplanktivore *H. pyrrhocephalus* only showed a decrease in head to tail ratio from 1987 onwards. So the two possibly extinct species might not have persisted due to other reasons than a lack of an adaptive morphological response to predation.

Upper jaw modifications in response to diet shifts

As soon as they shifted to larger and more robust prey, all four resurgent haplochromine species showed an upper jaw (premaxilla) that became adapted to a style of feeding more based on biting and food manipulation (**Chapter 4**). All four species showed a shorter ascending arm of the premaxilla which increases the bite force, and a larger dentigerous arm which increases the gape width. In the zooplanktivorous species *H. pyrrhocephalus* and *H. laparogramma*, the dentigerous area increased, and almost completely covered the dentigerous arm. Greenwood (1980) believed the large edentulous area on the premaxillae of the above species to be a unique and stable character and used it as a diagnostic character for *Yssichromis*. Our findings however indicate that this character is variable and therefore not suitable to be used as diagnostic generic character.

Though the actual food uptake mode of the cichlids on their new prey types is unknown, the fact that the haplochromines show similar responses makes it highly likely that the morphological changes of the premaxilla are adaptive. For one species, *H. laparogramma*, there was even a clear correlation between changes in the premaxilla and prey size.

The rate of morphological changes is quite fast as well. Three out of four species had an unchanged premaxilla in 1984 while they were still feeding on their original diet, but then showed changes in both their diet and premaxilla in 1987. This means that adaptive responses have occurred in three years or even less (**Chapter 4**).

These rapid adaptive responses must involve a fast adaptation mechanism. The oral jaw apparatus of cichlids is controlled by relatively few genes which can result in an extremely fast morphological response to selection (Albertson *et al.* 2003b). Just like in the body shape, a reversal of morphology was found in the premaxilla. During the 2000s, the premaxilla of *H. pyrrhocephalus* and *H. laparogramma* changed back to the same form from before their diet shift. This morphological reversal coincides with a (partly) reversal of their diet back to zooplankton. It is not clear whether these changes are due to heritable genetic changes and/or to phenotypic plasticity. Phenotypically plastic responses of the oral jaws have been observed in cichlids in the lab (Witte 1984; Meyer 1987; Wimberger 1991; Bouton *et al.* 2002a) and some of these plasticity studies showed similar responses to different feeding styles of the premaxilla as were found under natural conditions in this thesis (Witte 1984; Wimberger 1991; Bouton *et al.* 2002a).

The contribution of climatic variability to the changing lake environment

In addition to predation by Nile perch, eutrophication is thought to be a major contributor to the extinction of haplochromine cichlid species (Seehausen *et al.* 1997a). The eutrophication and its consequences are thought be mainly caused by poor agricultural practices and by soil erosion, both of which are linked to the increased human population around the lake (Verschuren *et al.* 2002). However, some studies suggest that climatic changes and, in particular, reduced wind speed, might have played a role in the eutrophication and stratification of the lake (Kolding *et al.* 2008; Hecky *et al.* 2010).

In **Chapter 5**, meteorological and environmental variables from the past 50 years were gathered and correlations within the datasets, and with data on haplochromine cichlid gill surface were found. The environmental data showed that the air temperature gradually increased and that the lake water levels decreased over the study period. Interestingly, during the 1980s, a change of wind direction and a drop in wind speed were observed. Concurrently, DO levels, water temperature and transparency also dropped. These changes are consistent with increased stratification. The gill surface of three out of four species increased concurrently with the environmental changes.

During the 2000s, the wind direction changed and the wind speed increased again to above levels previously recorded. At the same time, the DO levels increased, as did the water transparency and water temperature. The gill surface of the three cichlid species decreased again, possibly in response to the reversal of the environmental changes. So, it seems that the increased DO levels are a result of increased wind speeds which resulted in a upheaval of the stratification.

As eutrophication of the Lake and the Mwanza Gulf continues (Hecky *et al.* 2010; Cornelissen *et al.* 2013) the reversed environmental variables are not signs of improvement of the state of Lake Victoria, but rather an effect of climatic variability, especially variability of wind speed. Therefore, it is hypothesized that, if there is a sequential drop in wind speed in the future, environmental conditions will be even harsher for the haplochromine cichlids than they were in the 1980s. If the fish are not able to adapt to these even harsher conditions, a second biodiversity crisis might develop (**Chapter 5**).

Stable isotopes used as indicators of ecological changes

Stable isotopes are typically used to estimate trophic position and carbon flow in aquatic ecosystems (Peterson & Fry 1987; Post 2002). Since stable isotopes reflect the trophic group and source of the diet over months to years in fish (Hesslein *et al.* 1993) they provide an important addition to the more time-consuming gut and stomach content analyses which are essential for the assessment of prey diversity. A recent study on Lake Victoria haplochromine cichlids that have been preserved in formalin and stored in ethanol showed that these fish can be used for stable isotope analysis and that there is only a small but consistent effect of preservation on their stable isotopes (Kishe-Machumu 2012).

Chapter 6 shows that the stable isotopes of the four resurgent cichlid species changed accordingly with their diet change. All four species showed an increase of $\delta^{15}N$ at the time they shifted their diet to macroinvertebrates such as insects and to fish. In addition to reflecting dietary differences, the observed shifts in $\delta^{13}C$ probably also reflect habitat differences, with heavy $\delta^{13}C$ signatures found in the littoral habitat, and lighter $\delta^{13}C$ signatures found in the sublittoral habitat. A remarkable shift towards heavy $\delta^{13}C$ signatures was found in all four species in 2011. It might be that these shifts represent increased primary productivity by phytoplankton and thus increased eutrophication. However, more research on this topic is needed to determine whether this is indeed the case (**Chapter 6**).

Future directions

Some of the Lake Victoria haplochromine cichlid species have proven to be resilient to the environmental changes and are able to adapt to these in a remarkably fast way. However, increasing eutrophication or species introductions in Lake Victoria and other African Great Lakes might have devastating consequences for their biodiversity. If hypereutrophication were to occur in Lake Victoria, this would result in a very hostile environment with which the cichlids and other aquatic species might not be able to cope. In addition, increased eutrophication is likely to co-occur with decreased water transparency and DO levels which have been shown to reduce fish biodiversity by favouring hybridization (Seehausen *et al.* 1997a; Taylor *et al.* 2006; Vonlanthen *et al.* 2012). Therefore, is it essential that continued eutrophication is halted by limiting anthropogenic nutrient input. In addition, the results of this thesis emphasise that continued measurements and environmental data collection are imperative for our understanding of the lake's dynamics and ichthyofauna.

To answer the question of whether or not morphological changes in haplochromines are being caused by phenotypic plasticity or genetically based changes such as selection or hybridization, more genetic data on these fish will be needed. Unfortunately, all fish used in this study have been preserved in formalin which is known for inducing DNA-DNA, DNA-protein and protein–protein cross-links. This makes it extremely difficult to obtain amplifiable DNA larger than 200bp through conventional methods such as polymerase chain reaction (PCR) (Schander & Halanych 2003 and references therein).

Nonetheless, pilot studies in our lab using microsatellites showed that some of the fish used in this study contained amplifiable DNA which is promising for future work using more advanced techniques. Especially single nucleotide polymorphism (SNP) discovery shows high potential as only short fragments of 50-100bp are needed; for this reason, SNP analysis may be particularly useful for retrieving genetic information from these preserved fish. In addition, the genome of the Lake Victoria cichlid *Pundamilia nyererei* has been sequenced recently (http://cichlid.umd.edu/cichlidlabs/kocherlab/bouillabase.html). By analyzing different species from different trophic groups, the opportunity to discover morphological candidate SNPs will be provided. These candidate SNPs can be used to search for candidate genes involved in morphological adaptations.

Concluding remarks

The rate of appearance of the morphological changes found here is extremely fast (three years or less, **Chapter 4**) which implies a very fast adaptation mechanism. Both genetically based changes and phenotypic plasticity can act in such a fast way. Trait variation does not seem to be higher in the fish from before the environmental changes compared to fish during and after the severe environmental changes. Were this to be the case, it could be an indication of natural selection. However, the lack of change in variation certainly does not rule out genetically based changes because new phenotypes could, for instance, be the result of selection on polygenic traits (**Chapter 4**). Hybridization might have influenced the morphology of the fishes as well. In fact, convergence in morphology has been observed in the eyes between *H. pyrrhocephalus* and *H. tanaos* (**Chapter 2**), in the gills of all four resurgent species (**Chapter 5**) and in the body shape between *H. pyrrhocephalus* and *H. laparogramma* (J. C. van Rijssel, unpublished data). Although this study only used haplochromine species which were carefully identified in the field and re-identified in the lab to be sure no hybrids or look-alike species were included, an influence of hybridization on the morphological changes cannot be ruled out.

All the observed environmental changes; increased Nile perch predation, decreased DO-levels, decreased water transparency, and the increase of macroinvertebrates and fish in the environment as well as in the diet of the cichlids, are shown to covary with cichlid morphology (**Chapter 2-6**). The comparison of resurgent haplochromines with species that are thought to be extinct suggests that a lack of morphological change, or a morphological change in the opposite direction, might be detrimental for survival (**Chapter 3**).

Almost all the morphological changes found here are likely to enhance the inclusive fitness in the changed environment. The fact that the morphological changes occurred at the same time point, i.e. when the fish encountered the same environmental changes, also suggests that these morphological changes are actually adaptive responses to the changed environment.

Whether or not these adaptive responses are due to genetically based changes or phenotypic plasticity or (most likely) a combination of both remains obscure. Future research on the genetics of the same fish used in this study will possibly reveal the mechanism(s) behind the adaptive responses. Whatever the outcome, the fact that cichlids are able to adapt in such a quick way must have somehow contributed to their fast adaptive radiation. More knowledge on adaptation mechanisms will greatly improve our understanding of speciation and adaptive radiation of not only cichlids but also other famous radiations such as the Hawaiian honeycreepers and the Galapagos finches.

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Nederlandse samenvatting

Introductie (Hoofdstuk 1)

Darwin's evolutie theorie zegt dat soorten veranderen door natuurlijke selectie. Natuurlijke selectie houdt in dat organismen die beter aangepast zijn aan hun omgeving, meer kans hebben om te overleven en voor nakomelingen te zorgen dan minder goed aangepaste organismen. Hierdoor zal het type van het best aangepaste organisme beter overleven en steeds meer voorkomen in de populatie. De aanpassingen kunnen op allerlei niveaus liggen; en bijvoorbeeld morfologisch, fysiologisch of gedragsmatig zijn.

Darwin dacht dat de evolutie heel langzaam ging en dat het miljoenen jaren duurde voor er een nieuwe soort ontstaan was. Inmiddels zijn er verschillende studies die hebben aangetoond dat door natuurlijke selectie morfologische kenmerken, binnen een decennium, of zelfs binnen een jaar kunnen veranderen. Zo is bijvoorbeeld bij Darwinvinken aangetoond dat tijdens een periode van droogte in de jaren '70, grote vinken met diepe snavels beter overleefden dan kleinere vinken met kleinere snavels omdat grotere hardere zaden talrijker werden. Tijdens een latere droogte in de jaren '80, waren het juist de kleinere vinken met de kleinere snavels die beter overleefden ten opzichte van de grotere vinken met grotere snavels omdat de kleinere zachter zaden talrijker waren geworden.

Net als de Darwinvinken zijn de cichliden uit het Victoriameer een schoolvoorbeeld van adaptieve radiatie. Adaptieve radiatie is de diversificatie van soorten die van een gemeenschappelijke voorouder afstammen om verschillende ecologische niches te bezetten. De haplochromine cichliden uit het Victoriameer vertonen de snelste adaptieve radiatie onder de vertebraten, en worden daarom als modelorganisme gebruikt voor evolutionaire studies.

Het Victoriameer is 100.000-400.000 jaar geleden ontstaan. Sinds die tijd zijn er meer dan 500 verschillende soorten cichliden in het Victoriameer geëvolueerd. Er zijn zelfs studies die bewijs hebben gevonden dat het meer 15.000 jaar geleden helemaal droog heeft gestaan, wat betekent dat de soorten nog veel sneller zijn ontstaan. Het Victoriameer is het grootste tropische meer in de wereld en minstens 1,2 miljoen mensen zijn direct afhankelijk van visserij activiteiten in en rondom het meer. Naast meer dan 500 cichlidensoorten, kwamen er tot voor kort ook nog 46 andere vissoorten in het meer voor.

Omdat de bevolkingsgroei en de vraag naar voedsel rondom het meer halverwege de vorige eeuw toenam, werden er tijdens de jaren '50 verschillende vissoorten in het meer geïntroduceerd. Een van deze soorten was de roofzuchtige Nijlbaars die in de jaren '80 explosief in aantallen toe nam. Tegelijkertijd nam de eutrofiëring (de toename van anorganische nutriënten) van het meer ook toe wat resulteerde in lage zuurstofomstandigheden en troebel water. Deze veranderde omgevingsomstandigheden zorgden voor een veranderd voedselnetwerk met meer macro-invertebraten, maar zorgden er met name voor dat het aantal cichlidensoorten drastisch afnam in de jaren '80.

Naast de voorkeur van de Nijlbaars voor cichliden lijkt eutrofiëring en met de name de helderheid van het water een grote rol te hebben gespeeld in de biodiversiteitafname. De partnerkeuze van de Victoriameer cichliden is gebaseerd op lichaamskleur waarbij de vrouwtjes de mannetjes kiezen. Wanneer de helderheid van het water afneemt, beïnvloed dit het zicht van de vis en daarmee ook de partnerkeuze. Paait het vrouwtje met een mannetje van een andere soort, dan kunnen er hybriden gevormd worden. Hierdoor ontstaat er een nieuwe soort, maar als door competitie de twee oorspronkelijke soorten verdwijnen, betekent dat een afname van het aantal soorten.

In de jaren '90, nam de Nijlbaarspopulatie af door overbevissing wat uitmondde in de toename van populaties van een aantal cichlidensoorten, met name de zooplankti- en detritivoren. Deze soorten kwamen terug met een uitgebreider dieet dat voornamelijk bestond uit de in aantallen toegenomen macro-invertebraten zoals insecten, slakken en garnalen maar ook kleine vissen (dagaa). Naast deze ecologische aanpassingen vertoonde één van deze soorten, *Haplochromis* (*Yssichromis*) *pyrrhocephalus* ook morfologische aanpassingen. Hij bleek grotere kieuwen te hebben gevormd als een aanpassing aan de lage zuurstof omstandigheden. Daarnaast vertoonde dit soort ook een grotere omvang van de keelkaakspieren die nodig zijn om prooien te vermalen. Dit onderzoek legde de fundamenten waarop dit proefschrift is gebaseerd.

Het Haplochromis Ecology Survey Team (HEST) verzamelde al sinds de jaren '70 (nog voor de veranderde omgevingsomstandigheden) haplochrominen. Deze werden geconserveerd in formaline en in ethanol en opgeslagen in het Naturalis Biodiversity Center (waar nu ongeveer 125,000 cichliden exemplaren bewaard worden). Dit onderzoek concentreerde zich op een transect van ongeveer 5km lang in de Mwanza Golf in het zuiden van het Victoriameer (Figuur 1.1). Doordat er al vissen waren gevangen voordat de ecologische omstandigheden drastisch veranderden, ontstond een unieke kans om de effecten van de veranderde omgeving op de ecomorfologie van de haplochrominen te bestuderen. Doordat de vissen (en ook de omgevingsvariabelen) op bijna jaarlijkse basis werden verzameld, was het mogelijk om morfologische aanpassingen te ontdekken zodra ze in de haplochromine populatie voorkwamen. Voor dit onderzoek heb ik vier verschillende soorten gebruikt die na de veranderde omgevingsomstandigheden succesvol zijn teruggekeerd. Daarnaast heb ik ook gekeken of soorten die sinds de jaren '80 niet tot nauwelijks meer gevangen worden, en waarvan gedacht wordt dat ze uitgestorven zijn, geen morfologische aanpassingen of aanpassingen in de verkeerde richting vertoonden.

De belangrijkste doelstellingen van dit proefschrift waren: 1) Het aantonen van morfologische veranderingen. 2) Het bepalen van het tijdsbestek waarin de morfologische veranderingen zijn ontstaan. 3) Onderzoeken of de morfologische veranderingen een relatie hebben met de omgevingsveranderingen. 4) Vaststellen of de morfologische veranderingen adaptief zijn. 5) Aanwijzingen vinden voor het mechanisme achter de morfologische veranderingen (natuurlijke selectie, fenotypische plasticiteit, hybridisatie of een combinatie van die drie). Fenotypische plasticiteit wordt gedefinieerd als de door de omgeving geïnduceerde aanmaak van verschillende fenotypen met een gegeven genotype. Cichliden vertonen een hoge mate van plasticiteit in het lab en kunnen onder andere de lichaamsvorm, ogen en kaken aanpassen aan verschillende omgevingsomstandigheden tijdens hun leven.

Voor de bovenstaande onderzoeksdoelstellingen werden de volgende hypothesen opgesteld:

1) Als de haplochrominen morfologische veranderingen in dezelfde richting vertonen is het waarschijnlijk dat deze door de omgeving zijn geïnduceerd. Als de vissen geen morfologische veranderingen vertonen, zou dit een indicatie kunnen zijn dat de omgeving geen invloed heeft op de morfologie van de vissen of dat de vissen niet in staat zijn zich aan te passen.

2) Door het tijdbestek van de morfologische veranderingen te bepalen wordt er informatie verzameld die met name belangrijke is voor de bepaling van het mechanisme achter de morfologische veranderingen (doelstelling 5). Als morfologische veranderingen over de tijd geleidelijk worden waargenomen, is het waarschijnlijker dat deze worden veroorzaakt door genetisch gebaseerde veranderingen zoals natuurlijke selectie. Daarnaast kan een grotere variatie in de kenmerken van de oude populatie (van voor de veranderde omgeving) vergeleken met de kenmerken van moderne populaties (na de veranderde omgeving) ook een teken zijn van natuurlijke selectie. Door de kenmerken van verschillende soorten met elkaar te vergelijken op verschillende tijdstippen, kan er worden vastgesteld of deze kenmerken meer op elkaar gaan lijken en er eventueel hybridisatie is ontstaan tussen soorten.

3) Aangezien alle omgevingsveranderingen ongeveer tegelijkertijd plaats vonden (jaren '84-'87) kan het bepalen van het tijdstip van zowel morfologische, ecologische als omgevingsveranderingen inzicht bieden in de oorzaken en gevolgen. Daar komt bij dat het vergelijken van de morfologie van teruggekomen met uitgestorven soorten, informatie kan verschaffen waarom sommige soorten zijn teruggekomen terwijl andere dit niet deden.

4) Als de morfologische veranderingen adaptief zijn, zullen ze de fitness van de cichliden in de veranderde omgeving moeten verhogen. Aan de ander kant kunnen de morfologische veranderingen ook willekeurig en daarom neutraal of zelfs maladaptief zijn. Wanneer de morfologische kenmerken van de terug gekomen soorten in dezelfde richting veranderen is het waarschijnlijker dat deze relevant zijn om te overleven in de veranderde omgeving. Hierbij zal een vergelijking tussen teruggekomen en uitgestorven soorten ook meer duidelijkheid verschaffen.

5) Naast geleidelijke of directe aanpassingen van kenmerken, variatie van kenmerken en de inter-specifieke vergelijking van kenmerken, kan genetische informatie helderheid geven over welk mechanisme (natuurlijke selectie, fenotypische plasticiteit , hybridisatie of een combinatie van deze) verantwoordelijk is voor de morfologische veranderingen. Zo kan de afwezigheid van genetische veranderingen duiden op een belangrijke rol van fenotypische plasticiteit in de morfologische veranderingen.

Aanpassingen van het oog aan troebel water en prooigrootte (Hoofdstuk 2)

Twee van de doelstellingen van dit proefschrift waren het aantonen van morfologische veranderingen en het tijdsbestek ervan vaststellen. In **Hoofdstuk 2** wordt aangetoond dat de ogen van twee Victoriameer cichlidensoorten morfologische aanpassingen binnen twee decennia vertonen. Beide soorten kregen kleinere ogen, waarschijnlijk het resultaat van een soort morfologische "trade-off" (wisselwerking/uitruil). Deze trade-off vond vermoedelijk plaats met andere morfologische kenmerken die in grootte toegenomen waren zoals de wangdiepte (**Hoofdstuk 3**), de kieuwen (**Hoofdstuk 5**) en mogelijk ook andere kenmerken die gerelateerd zijn aan voedselopname zoals de omvang van de kauwspieren, terwijl het volume van het hoofd gelijk bleef of kleiner was geworden (**Hoofdstuk 3**). Hierdoor lijkt het dat sommige morfologische veranderingen niet direct aan de omgeving zijn gerelateerd, maar aan ruimtelijke beperkingen.

Het oog van *H. pyrrhocephalus* gevangen in de jaren '90 en '00 vertoonde een lagere dichtheid van de dubbele kegeltjes ten opzichte van *H. pyrrhocephalus* gevangen in de jaren '70, wat betekent dat de vis met een minder hoge resolutie ziet dan voorheen. Dubbele kegeltjes zijn gevoelig voor lange golflengtes en zijn daardoor meer gevoelig voor rood en groen licht. Ondanks dat het oog kleiner is geworden bleef de grootte van deze dubbele kegeltjes gelijk. Dit betekent dat de hoeveelheid licht wat opgevangen wordt door het oog hetzelfde blijft. Het oog van *H. tanaos* vertoonde zelfs grotere dubbele kegeltjes. De enkele kegeltjes waren bij beide soorten gereduceerd of helemaal verdwenen. Enkele kegeltjes zijn gevoelig voor korte golfengtes en vangen voornamelijk blauw licht op wat vrij zeldzaam is geworden in het troebele water van de Mwanza Golf. De afname van de helderheid van het water is waarschijnlijk de oorzaak van de morfologische veranderingen aan het oog.

Een ander doel van dit proefschrift was te onderzoeken of er een relatie was tussen de veranderde omgeving en de veranderde morfologie. In het geval van de cichliden ogen is het moeilijk om maar één omgevingsfactor aan te wijzen. Het behoud of het groter worden van de dubbele kegeltjes, en de degradatie van de enkele kegeltjes zijn waarschijnlijk een aanpassing op het troebeler geworden water. Het verlies van resolutie zou ook door een zogenaamde "trade-off" kunnen zijn veroorzaakt. Een kleiner oog met grotere kegeltjes kan ervoor gezorgd hebben dat er geen ruimte was voor een hoge dichtheid van de dubbele kegeltjes. Aan de andere kant zou dit gecompenseerd kunnen zijn door de afname of het verlies van de enkele kegeltjes.

Daarnaast zou de verschuiving naar grotere prooien in het dieet van de vissen (**Hoofdstuk 4**) een belangrijke rol kunnen hebben gespeeld. Het zou kunnen zijn dat, door deze verschuiving, een lagere resolutie "werd toegestaan" om de gevoeligheid van het oog te behouden. Deze gevoeligheid is onder andere belangrijk voor soortherkenning terwijl een hoge resolutie belangrijk is voor het zien van kleine prooien en misschien niet meer nodig was. Aan de andere kant zou de afname van de resolutie in het oog de vissen gedwongen kunnen hebben om op grotere prooien te foerageren. Alhoewel dit proefschrift geen duidelijk antwoord geeft op deze vraag, geeft dit onderzoek wel aan dat twee van deze haplochrominen al een verschuiving in hun dieet vertoonden in 1987 terwijl ze in 1984 nog voornamelijk zooplankton aten (**Hoofdstuk 4**; **Hoofdstuk 6**). De periode 1986-87 is kenmerkend voor een afname van zuurstof niveaus (**Hoofdstuk 5**), een afname van water helderheid (**Hoofdstuk 5**), maar ook een toename van grotere prooien, voornamelijk garnalen, en een afname van groot zooplankton (**Hoofdstuk 4**). Hoewel ze allemaal tot een trofische groep behoren, zijn de Victoriameer cichliden vrij opportunistisch met betrekking tot hun voedsel. Daarom is mijn opinie dat, de verschuiving naar grotere prooien het mogelijk heeft gemaakt dat het oog een lagere resolutie kon krijgen in plaats van dat de lage resolutie ervoor gezorgd heeft dat de vissen grotere prooien zijn gaan eten.

Veranderingen in lichaamsvorm als aanpassing aan predatie (Hoofdstuk 3)

Om te bepalen in welk tijdbestek de morfologische veranderingen plaats hebben gevonden, heb ik haplochrominen geselecteerd die in de afgelopen 30 jaar gevangen zijn met een tijdsinterval van ongeveer 3 jaar. Drie van de vier teruggekomen soorten vertoonden morfologische veranderingen binnen een decennium. Aangezien verschillende soorten vergelijkbare morfologische aanpassingen hadden, is het waarschijnlijk dat deze veranderingen adaptief zijn. Er lijkt een vrij duidelijk verband te zijn tussen het aantal Nijlbaarzen in de Mwanza Golf en de lichaamsvorm van de haplochrominen. Zodra de Nijlbaarspopulatie toeneemt in 1983-84 vertonen de cichliden een kleinere kop-staart ratio. Een kleinere kop en een grotere staart zijn essentieel voor het ontsnappen aan predatoren. De voordelen van een kleine kop-staart ratio bij het ontsnappen aan predatoren zijn al eerder aangetond bij andere vissoorten, zoals stekelbaarsjes en guppies. Een recent literatuur overzicht heeft aangetoond dat 16 verschillende vissoorten een kleinere kop-staart ratio hebben zodra ze in een omgeving zijn waar een predator is.

Opmerkelijk was dat, toen de Nijlbaarspopulatie in aantallen afnam, de lichaamsvorm van de cichliden terugkeerde naar de oorspronkelijke vorm van de jaren 70. Het terug veranderen van morfologische kenmerken na het terug veranderen van omgevingsomstandigheden is niet ongebruikelijk in vertebraten. Verschillende soorten waaronder Darwinvinken, driedoornige stekelbaarsjes en *Anolis* hagedissen vertoonden een terugverandering van morfologische kenmerken door middel van selectie nadat de verstoorde omgeving was hersteld. Dit zou ook het geval kunnen zijn bij de cichliden uit het Victoriameer. Hoewel de flexibiliteit die deze soorten vertonen ook een indicatie van fenotypische plasticiteit kan zijn, aangezien de lichaamsvorm van cichliden zeer plastisch is.

Een van de soorten die heel zeldzaam is of waarvan gedacht wordt dat deze uitgestorven is, *H. piceatus*, vertoonde in jaren '80 geen veranderingen in de kop-staart ratio, terwijl een ander "uitgestorven" soort *H. heusinkveldi*, lichaamsvorm veranderingen vertoonde in de tegenovergestelde richting vergeleken met de teruggekomen soorten. Dus het lijkt erop dat het ontbreken van, of de verkeerde richting van, aanpassingen aan predatie een rol gespeeld kan hebben in het verdwijnen van deze soorten. Aan de andere kant, deze soorten zijn voor het laatst gevangen in 1985-86 terwijl de succesvol teruggekomen soort *H. pyrrhocephalus* pas in 1987 een kleinere kop-staart ratio vertoonde. Daarom zou het zo kunnen zijn dat er andere er factoren een rol hebben gespeeld in het verdwijnen van deze soorten.

Veranderingen in de bovenkaak als aanpassing aan het verschoven dieet (Hoofdstuk 4)

Zodra de haplochrominen hun dieet verschuiven naar grotere en meer robuuste prooien, vertonen alle vier de teruggekomen soorten een bovenkaak (premaxilla) die meer aangepast lijkt aan bijten dan aan zuigen. Alle vier de soorten vertoonden een kortere opstijgende arm van de premaxilla, wat er voor zorgt dat de bijtkracht groter wordt, en een langere tandendragende arm die er voor zorgt dat bekgrootte toeneemt. In de zoöplanktivoren *H.* (*Y.*) *pyrrhocephalus* en *H.* (*Y.*) *laparogramma* was het oorspronkelijk tandeloze deel van de bovenkaak bijna helemaal gevuld met tanden. Dit is temeer opmerkelijk aangezien het tandenloze gedeelte van de premaxilla als diagnostisch kenmerk werd gebruikt bij de beschrijving van het genus *Yssichromis*. Vanwege de aangetoonde variatie is dit kenmerk niet meer te gebruiken om een geslacht te bepalen.

Alhoewel de daadwerkelijke methode om het nieuwe voedsel van deze haplochrominen op te nemen onbekend is, maakt het feit dat deze soorten vergelijkbare aanpassingen vertoonden het erg waarschijnlijk dat deze morfologische veranderingen adaptief zijn. Voor één soort, *H. laparogramma*, was er zelfs een duidelijke correlatie tussen de veranderingen in de premaxilla en de prooigrootte. Het tijdsbestek van de morfologische veranderingen was vrij kort. Drie van de vier soorten veranderden hun dieet en premaxilla in 1987 terwijl ze in 1984 nog hun oorspronkelijke dieet hadden met een nauwelijks veranderde premaxilla. Dit betekent dat de adaptieve aanpassingen hebben plaats gevonden binnen drie jaar. Bij dit soort snelle aanpassingen moet een snel adaptatie mechanisme betrokken zijn. De orale kaken van cichliden worden gereguleerd door relatief weinig genen wat kan resulteren in een extreem snelle reactie van de morfologie op selectie.

Net als bij de lichaamsvorm, was er een terugverandering van de premaxilla vorm. Tijdens de jaren '00, veranderde de premaxilla van de zoöplanktivoren *H. pyrrhocephalus* en *H. laparogramma* terug naar dezelfde vorm die ze hadden voor de dieet veranderingen. Deze morfologische terugverandering gebeurde tegelijkertijd met een gedeeltelijke terugverandering van het dieet naar zooplankton. De flexibiliteit van de terugverandering zou op fenotypische plasticiteit kunnen duiden alhoewel genetische veranderingen ook vrij waarschijnlijk een rol hebben gespeeld. Aanpassingen aan de hand van fenotypische plasticiteit in de orale kaken zijn al meerdere malen gevonden voor cichliden in het lab, en sommige van deze aanpassingen komen overeen met de aanpassingen die zijn gevonden in dit proefschrift onder natuurlijke omstandigheden.

De rol van klimaat variabiliteit in de veranderde omgeving (Hoofdstuk 5)

Naast predatie door de Nijlbaars wordt eutrofiëring genoemd als één van de hoofdoorzaken van de dramatische haplochromine cichliden soorten afname. Er wordt aangenomen dat de eutrofiëring en de gevolgen ervan vooral veroorzaakt zijn door menselijk toedoen. Verkeerde landbouw praktijken en ontbossing (met als gevolg bodemerosie) zijn in de afgelopen decennia toegenomen door de bevolkingsgroei en de toegenomen vraag naar voedsel. Aan de ander kant zijn er ook studies die suggereren dat klimaatverandering en met name verlaagde windsnelheden een rol zouden spelen bij de eutrofiëring en stratificatie van het Victoriameer.

In **Hoofdstuk 5**, heb ik meteorologische data en omgevingsvariabelen van de afgelopen 50 jaar verzameld en deze met elkaar en met het kieuwoppervlakte van de vier teruggekomen haplochromine cichliden gerelateerd. De omgevingsdata liet zien dat de lucht temperatuur was toegenomen en dat het waterniveau van het meer was afgenomen in de afgelopen 50 jaar. Interessant was dat gedurende de jaren '80 er een verandering van windrichting was waarbij de windsnelheid afnam. Tegelijkertijd namen de zuurstof niveaus, de waterhelderheid en de water temperatuur af, wat een indicatie van stratificatie is. Het kieuwoppervlak van drie van de vier teruggekomen soorten was toegenomen als een aanpassing op de veranderde omgeving.

Tijdens de jaren '00 veranderde de windrichting weer terug en nam de windsnelheid waarden aan die eerder nog niet zo hoog gemeten waren. Op hetzelfde moment namen de zuurstofniveaus, de waterhelderheid en de water temperatuur weer toe terwijl het kieuwoppervlakte van de haplochrominen weer afnam, waarschijnlijk ook een aanpassing op de terug veranderde omgeving. De toename van de windsnelheid lijkt ervoor gezorgd te hebben dat de stratificatie tijdens de jaren '00 gedeeltelijk werd opgeheven.

Aangezien de eutrofiëring van het meer inclusief de Mwanza Golf nog steeds gaande is, zijn deze terugveranderingen geen tekenen van verbetering van de staat van het meer, maar eerder een effect van klimaatverandering en met name een hogere windsnelheid. Dit is de reden waarom ik voorspel dat, wanneer er weer een afname van windsnelheid zal plaatsvinden in de komende jaren, de omgevingsomstandigheden nog zwaarder en ongunstiger zullen zijn voor de cichliden dan ze waren in de jaren '80. Wanneer de vissen niet in staat zijn om met deze nog zwaardere omstandigheden om te gaan, zal er een tweede biodiversiteitcrisis plaatsvinden.

Stabiele isotopen als indicators voor ecologische veranderingen (Hoofdstuk 6)

Stabiele isotopen worden over het algemeen gebruikt om de trofische positie en de koolstof doorgave te bepalen in aquatische ecosystemen. Aangezien stabiele isotopen de trofische groep en bron van het dieet weergeven in vissen, zijn deze een belangrijke toevoeging aan de tijdrovende maag en darminhoud analyses, die essentieel zijn om de diversiteit van de prooien te bepalen. Recentelijk is gebleken dat formaline en ethanol geconserveerde haplochromine cichliden gebruikt kunnen worden voor stabiele isotopen analyse.

Hoofdstuk 6 toont aan dat de stabiele isotopen van de vier teruggekomen cichliden zijn veranderd op een manier die overeenkomt met de dieet verandering. Alle vier de soorten vertoonden een toename van stikstof, $\delta^{15}N$ op het moment dat zij grotere prooien zoals insecten en vissen in het dieet opnamen. Naast dieet veranderingen lijken de verschuivingen in koolstof, $\delta^{13}C$, habitatverschillen weer te geven, waarbij zwaardere $\delta^{13}C$ waarden werden gevonden in vissen uit het litorale habitat en lichtere $\delta^{13}C$ waarden in vissen uit het sublitorale habitat.

Een opmerkelijk verschuiving naar zwaardere $\delta^{13}C$ waarden werd geobserveerd in 2011. Het zou kunnen zijn dat deze verschuiving een toename van de primaire productie van fytoplankton weergeeft en daarmee dus ook toegenomen eutrofiëring aanduidt, alhoewel er meer onderzoek nodig is om deze hypothese te bevestigen.

Implicaties voor toekomstig onderzoek (Hoofdstuk 7)

Behalve dat **Hoofdstuk 7** de onderzoeksresultaten van dit proefschrift samenvat geeft het ook aanwijzingen waar toekomstig onderzoek zich op zou kunnen en moeten richten. Dit proefschrift toont aan dat een aantal soorten Victoriameer cichliden zeer veerkrachtig zijn met betrekking tot de veranderde omgevingsomstandigheden en dat ze in staat zijn zich extreem snel hieraan aan te passen.

Aan de andere kant zouden toenemende eutrofiëring en soorten introducties verwoestende gevolgen kunnen hebben voor de biodiversiteit van het Victoriameer en de andere grote Afrikaanse meren. Wanneer hypereutrofiëring in het Victoriameer plaats zou vinden, zou dit resulteren in een extreem vijandige omgeving voor de cichliden en andere aquatische soorten waar ze waarschijnlijk niet mee om kunnen gaan. Daarbij komt nog dat een toename van eutrofiëring gepaard zal gaan met lagere zuurstof concentraties en waterhelderheid. Voor beide is aangetoond dat ze kunnen zorgen voor een afname van biodiversiteit door middel van hybridisatie. Daarom is het essentieel dat voortgaande eutrofiëring stop wordt gezet door middel van het beperken van de overmaat aan voedingsstoffen door menselijk toedoen. De resultaten van dit proefschrift tonen daarnaast aan dat gecontinueerde metingen van meteorologische en omgevingsdata uiterst belangrijk zijn voor ons begrip van de water dynamiek en de ichthyofauna van het meer.

Om te kunnen beantwoorden of de morfologische veranderingen worden veroorzaakt door fenotypische plasticiteit of genetische veranderingen door natuurlijke selectie of hybridisatie, zal er genetische informatie van deze vissen verzameld moeten worden.

Ongelukkigerwijs zijn al de vissen die gebruikt zijn in dit proefschrift gefixeerd en ook voor langere tijd geconserveerd in formaline, waarvan bekend is dat het zorgt voor het zogenaamde "cross linking" van DNA met eiwitten. Dit zorgt ervoor dat het extreem moeilijk is om met gebruikelijke methodes als Polymerase Chain Reaction (PCR) vermenigvuldigbaar DNA te verkrijgen dat groter is dan 200 basenparen. Desalniettemin hebben verschillende proeven in ons lab aangetoond dat, met behulp van microsatellieten, er af en toe vermenigvuldigbaar DNA verkregen kan worden van de vissen gebruikt in dit proefschrift. Deze uitkomst biedt veel mogelijkheden voor de toekomst, aangezien relatief nieuwe technieken als "Single Nucleotide Polymorphism (SNP) discovery" maar kleine DNA fragmenten nodig hebben, wat dus zal uitmonden in nog betere resultaten. Inmiddels is het genoom van de cichlide *Pundamilia nyererei* bekend. Door de genetica van soorten uit verschillende trofische groepen te vergelijken zouden er "kandidaat SNPs" voor morfologische kenmerken ontdekt kunnen worden. Deze kandidaat SNPs zouden weer kunnen leiden tot de ontdekking van genen die verantwoordelijk zijn voor (de verandering van) morfologische kenmerken.

Conclusies (Hoofdstuk 7)

Het tijdsbestek waarin de geobserveerde morfologische veranderingen plaats vinden, kan extreem snel zijn (drie jaar of zelfs minder, **Hoofdstuk 4**). Dit impliceert dat er een heel snel adaptatie mechanisme achter deze aanpassingen zit. Zowel genetisch gebaseerde veranderingen als fenotypische plasticiteit kunnen zo snel optreden.

De variatie van de morfologische kenmerken lijkt niet hoger te zijn in haplochrominen van voor de veranderde omgevingsomstandigheden vergeleken met de variatie tijdens en na deze veranderingen. Wanneer dit wel het geval zou zijn, zou dit een indicatie voor natuurlijke selectie kunnen zijn, alhoewel het gebrek aan verschil in variatie genetische veranderingen natuurlijk niet uitsluit. Nieuwe fenotypes zouden bijvoorbeeld ook kunnen zijn ontstaan door selectie op poligenetische kenmerken (**Hoofdstuk 4**).

Hybridisatie zou ook de morfologie van de vissen beïnvloed kunnen hebben. Convergentie van morfologische kenmerken is aangetoond in de ogen van *H. pyrrhocephalus* en *H. tanaos* (**Hoofdstuk 2**), in de kieuwen van alle vier de soorten (**Hoofdstuk 5**) en in de lichaamsvorm van *H. pyrrhocephalus* en *H. laparogramma*. Alhoewel de gebruikte vissen in dit proefschrift allemaal met grote zorg in het veld zijn gedetermineerd en daarna nogmaals in het lab zijn gecontroleerd om er zeker van te zijn dat de juiste soort was geselecteerd en geen hybriden, kan ik het effect van hybridisatie niet uitsluiten.

Alle geobserveerde veranderingen in de omgeving van de cichliden; toegenomen Nijlbaars predatie, afgenomen zuurstofniveaus, afgenomen waterhelderheid en de toename van macro-invertebraten en vis zowel in de omgeving als in het dieet van de cichliden, hebben de morfologie van de cichliden beïnvloed (**Hoofdstuk 2-6**). De vergelijking van teruggekomen cichliden met soorten waarvan wordt gedacht dat ze uitgestorven zijn suggereert dat een gebrek aan morfologische veranderingen of veranderingen in de verkeerde richting nadelig kunnen zijn voor de overleving en het voortbestaan van haplochromine cichlidensoorten (**Hoofdstuk 3**).

Bijna alle gevonden morfologische veranderingen hebben waarschijnlijk bijgedragen aan een toename van de fitness van de haplochrominen in de veranderde omgeving. Het feit dat de morfologische veranderingen in dezelfde richting en/of op hetzelfde tijdstip plaats vonden of wanneer de vissen geconfronteerd werden met dezelfde veranderingen in de omgeving, suggereert dat deze morfologische veranderingen daadwerkelijk adaptieve aanpassingen in de veranderde omgeving zijn. Het blijft onduidelijk of deze aanpassingen de oorzaak zijn van genetisch gebaseerde veranderingen, fenotypische plasticiteit of, meest waarschijnlijk, een combinatie van beide. Toekomstig onderzoek naar de genetica van de vissen gebruikt in dit proefschrift zal mogelijk kunnen ontrafelen welk(e) mechanisme(s) verantwoordelijk is/zijn voor de adaptaties. Wat de uitkomst hiervan ook moge zijn, het feit dat de cichliden in staat zijn om zich zo snel aan te passen, moet op de een of andere manier hebben bijgedragen aan hun extreem snelle adaptieve radiatie. Meer kennis van de adaptatie mechanismen zal ons begrip over speciatie (soortsvorming) en adaptieve radiatie van, niet alleen cichliden, maar ook van andere bekende radiaties zoals de honingzuigers van Hawaï en de Darwinvinken verbeteren.

Curriculum vitae

Jacobus Cornelis van Rijssel was born on the 28th of August 1985 in Lisserbroek, The Netherlands. He attended secondary school from 1997-2003 at the Herbert Vissers College in Nieuw-Vennep. In 2003, Jacco started his Bachelor in Biology at Leiden University. During this study he gained research experience by conducting an internship at the department of Integrative Zoology which involved the influence of swimming on testis development in silver eels. After receiving his Bachelor's degree, Jacco continued with a Master in Evolutionary and Ecological Sciences at Leiden University. During his Master education, Jacco conducted two research projects. In 2007, he studied the effect of ambient noise and blackbird density on the song of forest and city blackbirds at the Behavioural Biology department. In 2008, he continued his Master by conducting an internship at the Institute of Environmental Sciences (CML), Leiden. After he received grants and scholarships from various sources, he spent almost half a year in Waza National Park, Cameroon, studying the ecology and livestock conflicts of African lions. Later on that year, in October 2008, Jacco received his Master's degree in Evolutionary and Ecological Sciences.

In 2009, Jacco returned to the department of Integrative Zoology to start his PhD on the changing morphology of Lake Victoria cichlids under the supervision of Dr. Frans Witte and Prof. Dr. Michael K. Richardson. During his PhD, Jacco conducted fieldwork in Tanzania where he collected live Lake Victoria cichlids and transported them back to the Netherlands. Foreign lab experience was gained when Jacco visited the Great Lakes Observatory in the U.S.A to be trained in stable isotope analyses. Jacco gave several international conference talks, for one these, at the XIV European Congress of Ichthyology, he received a Best Oral communication Award. He published his research in several peer-reviewed scientific journals such as: *Biological Journal of the Linnean Society*, *Evolutionary Ecology* and *Evolution*. Jacco reviewed several papers on divergent topics in *Mammalian Biology*, *Biological Journal of the Linnean Society* and *Ecology*. After his PhD, Jacco will continue his research in the field of evolutionary ecology as a Postdoc at EAWAG, at the department of Fish Ecology and Evolution, led by Prof. Dr. Ole Seehausen, in Kastanienbaum, Switzerland.

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