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head size and/or volume of these fishes, however, remained the same or even decreased (**Chapter 3**; J. C. van Rijssel unpublished data).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.