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).

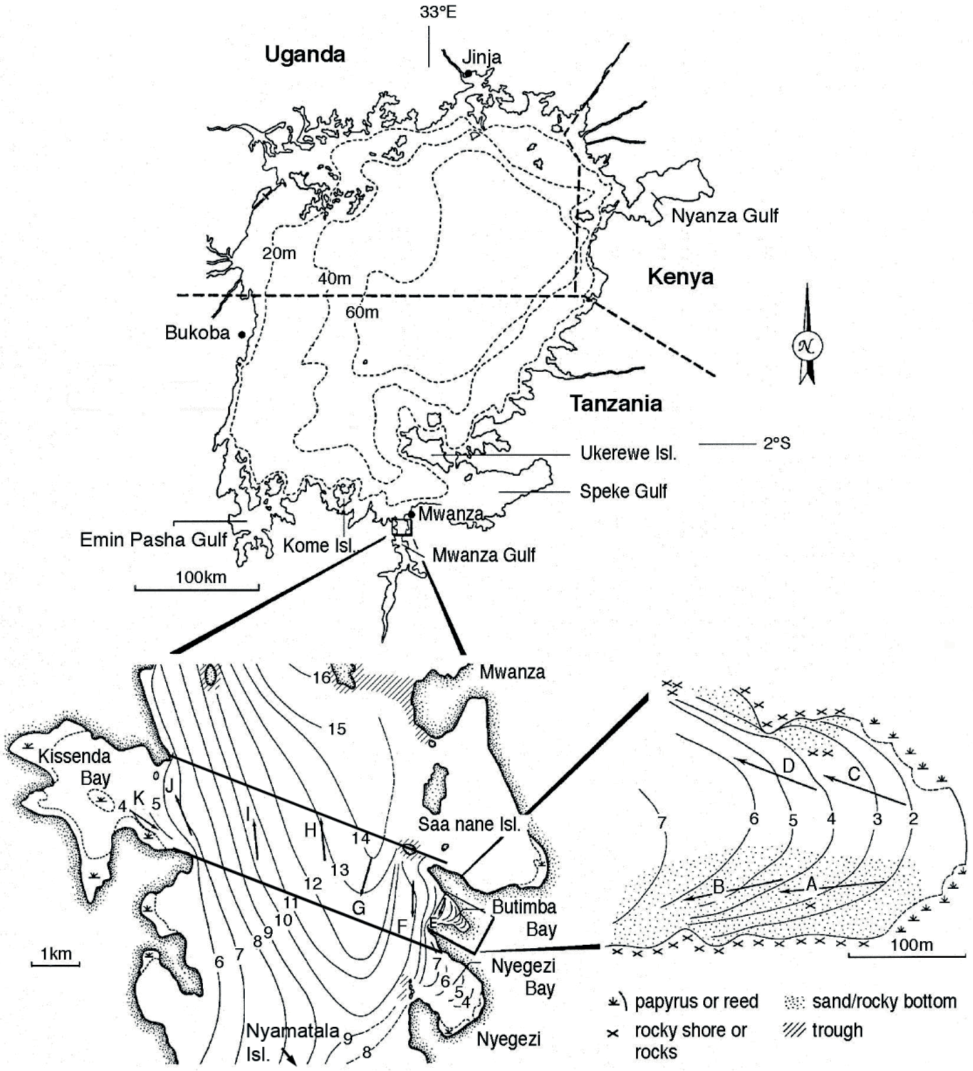

Figure 1.1 Map of Lake Victoria and the northern part of the Mwanza Gulf with the research transect (Station E-J) and the Butimba Bay with station A-D depicted. Numbers indicate depth in meters.

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.