Response of an African tilapia (*Oreochromis hunteri*) to long-term environmental fluctuations: an integrated approach combining the fossil record and modern-day system

–

Respons van een Afrikaanse tilapia (*Oreochromis hunteri*) op langetermijn omgevingsschommelingen: een geïntegreerde aanpak die het fossiel archief en huidig systeem combineert

Jorunn Dieleman

2018

Ghent University, Faculty of Sciences, Department of Biology, Limnology Unit

Dissertation submitted in partial fulfillment of the requirements for the degree of Doctor (Ph.D.) in Science: Biology

Supervisors:
Prof. Dr.Dirk Verschuren
Dr. Bert Van Bocxlaer

Members of the examination committee:
Prof. Dr. Luc Lens (chairman)
Prof. Dr. Ann Huysseune (secretary)
Prof. Dr. Dominique Adriaens
Prof. Dr. Walter Salzburger
Prof. Dr. Jos Snoeks

Please refer to this work as: Dieleman, J. (2018). *Response of an African cichlid fish to long-term environmental fluctuations: an integrated approach combining the fossil record and modern-day system.* Ph.D. thesis, Ghent University, Ghent, Belgium.

Table of Contents

Summary

Studying the processes that underlie organismal diversity is of major importance to understand the origin of new species. Lineages that have speciated rapidly within a geographically confined area, for example in lakes or on oceanic islands, provide valuable model systems to study these processes. Cichlid fishes inhabiting the Great East African Lakes have thus been studied extensively as a prime example of an adaptive radiation, as they have adapted rapidly to a variety of ecological niches and speciated accordingly. However, the lack of a good fossil record has hampered long-term eco-morphological studies in cichlids. In this respect, abundant fossil cichlid teeth, scales and bones preserved in lake sediments may provide a continuous fossil archive for cichlid species inhabiting a lake, but these remain relatively unexplored.

In this PhD thesis, I studied a 25,000-year old fossil cichlid record preserved in the sediments of isolated crater lake Chala (Kenya/Tanzania). This lake has been prone to strong lake-level changes during this period, which presumably impacted habitat availability for its only endemic cichlid, the tilapiine *Oreochromis hunteri*. As oral tooth shape can function as a proxy for past ecology and may thus reflect past changes in habitat exploitation, I hypothesised that these lake-level changes may be visible in the shape of *O. hunteri*'s fossil oral teeth. Our aim was thus to trace long-term morphological changes in oral teeth in response to past lake-level fluctuations.

As this study was the first of its kind, I first had to develop a quantitative method that captures the morphology of individual oral teeth. I therefore compared the performance of two geometric-morphometric techniques, elliptic Fourier analysis (EFA) and semi-landmark analysis (SLM), and found that

SLM performed slightly better. Combined with multivariate analysis, this method was capable of distinguishing between oral teeth from two congeneric species (*O. hunteri* and *Oreochromis* cf. *korogwe*), and was successfully used to assign a small set of fossil oral teeth to *O. hunteri*.

Moreover, thorough understanding of a fossil record requires adequate knowledge of its modern-day representatives, yet very little was known about the ichthyofauna currently inhabiting Lake Chala. Part of this thesis therefore focussed on resolving phylogenetic relationships and assess feeding ecology of extant Lake Chala cichlids. Although *O. hunteri* is the only indigenous cichlid species inhabiting Lake Chala, two other tilapiine species, *O.* cf. *korogwe* and *Coptodon rendalli*, were introduced in the second half of the 20^{th} century. Hybridisation with *O.* cf. *korogwe* may have compromised *O. hunteri*'s genetic and morphological integrity and could undermine its potential as a modern-day reference frame. I therefore analysed the species integrity of *O. hunteri* using a combination of overall body morphology and mitochondrial DNA (mtDNA) analysis. I found no signs of past or ongoing hybridisation between *O. hunteri* and the introduced *O.* cf. *korogwe*, and concluded that the morphological integrity of *O. hunteri* is currently intact. Moreover, using mtDNA from native *Oreochromis* species from nearby Lake Jipe and man-made Nyumba ya Mungu reservoir, *Oreochromis jipe* was established as the closest relative of *O. hunteri*.

In order to interpret the ecological significance of past oral tooth shape changes in *O. hunteri*, an assessment of their current use was crucial. Cichlid oral teeth are mostly of importance in obtaining food, hence I analysed the diet of *O. hunteri* using a combination of gut content and stable isotope analysis. I found that this species retains an herbivorous/detritivorous diet throughout the year, but shows seasonal variation in gut content. By comparing gut content composition to monthly fluctuations in the composition of limnetic phytoplankton and to the composition of littoral food source samples, I determined that *O. hunteri* feeds in the littoral zone of Lake Chala for most of the year, but migrates to the limnetic zone in response to an extensive phytoplankton bloom between July and September. I found similar trends in the guts of the introduced *O.* cf. *korogwe* and *C. rendalli*, which may thus be competing for food with the endemic *O. hunteri*. However, stable isotope composition revealed significant interspecific differences, likely reflecting a discrepancy in the exploitation of the phytoplankton bloom. I thus concluded that *O. hunteri* currently retains a competitive advantage over the introduced newcomers.

As *Oreochromis hunteri* spends most of the year feeding close to the shore of Lake Chala, I also aimed to establish how individual teeth are eventually buried at the lake's depositional centre, which is approximately one kilometre away from the nearest shoreline. I collected a short sediment core (27 cm) near shore and described fossil tooth distribution throughout this core in relation to sedimentological properties and the distribution of other biological fossils. I found very high abundances of fossil teeth in the large-grained lower sections of this core, indicating that the majority of fossil teeth is permanently buried in the lake periphery. Moreover, the strong correlation with grain size and other dense fossil remains suggests that the hydrostatic properties of fossil teeth do not allow their transport over very long distances, even during occasional extreme events induced by exceptionally intense mixing of the water column. I therefore surmised that teeth retrieved from cores collected at the centre of the lake were deposited *in situ*.

Finally, I analysed both extant and fossil oral tooth shape of *Oreochromis hunteri*. I quantified oral tooth shape in twelve *O. hunteri* specimens and observed a gradual shift in oral tooth shape linked to body size. Small- and medium sized specimens had a bicuspid outer series, followed by multiple series of tricuspids, and these were gradually replaced by unicuspid teeth in very large specimens, possibly reflecting changes in feeding behaviour or territoriality. Moreover, I found a strong linear relationship between tooth size and body size, indicating that fossil tooth size can function as a proxy for past body size. I analysed six fossil assemblages from alternating high- and low lake-level stands in the last 25,000 years and found that the morphospace of fossil and extant oral teeth overlapped completely. I thus concluded that *O. hunteri* is the only species that has inhabited Lake Chala in the last 25,000 years, and that it has retained its overall ecology throughout this period. However, significant shifts in the proportion of unicuspid teeth were observed between different fossil assemblages. Specifically, a 10% increase in the proportion of unicuspid teeth during low lake-level stands was not linked to an increase in median body size. I interpreted this as a shift in the ontogenetic trajectory of oral tooth development in *O. hunteri*, in which the species obtains 'adult-like tooth shapes earlier in life when lake levels are low, likely in response to a more extensive soft-bottom habitat. I concluded that *O. hunteri* has been coping with climate-driven environmental change through rapid morphological responses in oral dentition. Moreover, it has responded repeatedly in the same way to similar stimuli over the past 25,000 years, thus providing evidence of the morphological versatility of *O. hunteri* over extended periods of time.

This study demonstrates the potential of fossil cichlid remains preserved in lake sediments. I successfully used fossils to trace long-term morphological trends in response to severe environmental change in the form of lake-level fluctuations, which have been considered an important driver of cichlid evolution. Studies of continuous fossil records in lakes with more complex species flocks may elucidate long-term patterns of eco-morphological change on much larger scales, and may further enhance our understanding of the processes that underlie cichlid species diversity. This thesis provides a potential approach for these studies.

Samenvatting

Het bestuderen van processen die aan de basis van de huidige biodiversiteit liggen is van belang om te begrijpen hoe nieuwe soorten ontstaan. Goede modelsystemen voor deze studies zijn taxa die zeer snel nieuwe soorten hebben gevormd binnen een geografisch afgebakend gebied, zoals in meren of op oceanische eilanden. De cichliden die de Afrikaanse Grote Meren bevolken zijn om die reden uitgebreid bestudeerd. Deze vissen zijn een typevoorbeeld van een adaptieve radiatie, omdat ze zich zeer snel hebben aangepast aan een waaier van ecologische niches, en al doende nieuwe soorten hebben gevormd. Maar hun gebrekkige fossiele record maakt het moeilijk om eco-morfologische patronen te bestuderen over zeer lange periodes. Fossiele cichlidentanden, -botten en -schubben die werden bewaard in meersedimenten kunnen een ononderbroken archief bieden van de soorten in dat meer, maar werden tot op heden slechts beperkt onderzocht.

In deze doctoraatsthesis werd een 25000 jaar oud fossiel record van een cichlide bestudeerd in de meersedimenten van het kratermeer Lake Chala (Kenia/Tanzania). Het meerniveau van Lake Chala heeft in die periode sterk geschommeld, en die schommelingen hebben hoogst waarschijnlijk een invloed gehad op de beschikbaarheid van habitats voor de enige endemische cichlidensoort in het meer, de tilapia *Oreochromis hunteri*. De vorm van de orale tanden kan iets zeggen over de ecologie van deze soort, en hoe die in het verleden is gewijzigd na veranderingen in beschikbaar habitat. De hypothese was daarom dat meerniveauschommelingen in Lake Chala zichtbaar kunnen zijn in de vorm van fossiele orale tanden van *O. hunteri*. Om die reden werd getracht om langetermijn morfologische veranderingen in fossiele orale tanden op te sporen

die in verband stonden met meerniveauschommelingen in het verleden.

Vergelijkbare studies waren niet beschikbaar, dus er werd eerst een kwantitatieve methode ontwikkeld die de vorm beschrijft van individuele orale tanden. Twee geometrisch-morfometrische technieken werden daartoe vergeleken: elliptische Fourier analyse (EFA) en semi-landmark analyse (SLM). SLM bleek beter in staat om vormvariatie accuraat te beschrijven en werd gecombineerd met multivariate data-analyse. De methode kon een onderscheid maken tussen de orale tanden van twee nauw gerelateerde soorten (*Oreochromis hunteri* en *Oreochromis* cf. *korogwe*), en kon een beperkte dataset van fossiele orale tanden toewijzen aan *O. hunteri*.

Een goed begrip van een fossiel record van een soort steunt op een goede kennis van de huidige populatie van die soort. Bij aanvang van het onderzoek was er echter zeer weinig gekend over de visfauna die op dit moment in Lake Chala voorkomt. Een deel van deze thesis behandelde daarom de fylogenetische relaties van de vissoorten in het meer, en bestudeerde hun huidige voedselvoorkeuren. *O. hunteri* is de enige vissoort die van nature voorkomt in Lake Chala, maar twee andere tilapia-soorten werden ergens in de tweede helft van de 20^{e} eeuw ingevoerd: *O.* cf. *korogwe* en *Coptodon rendalli*. Hybridisatie met *O.* cf. *korogwe* heeft mogelijks zowel de genetische als de morfologische integriteit van *O. hunteri* beïnvloed, en zou de bruikbaarheid van deze soort als huidige vertegenwoordiger van het fossiel record in het gedrang kunnen brengen. Daarom werden anlyses van lichaamsvorm en mitochondriaal DNA (mtDNA) gecombineerd om de integriteit van *O. hunteri* als soort na te gaan. Er werden geen tekenen gevonden van vroegere of huidige hybridisatie tussen *O. hunteri* en de geïntroduceerde *O.* cf. *korogwe*, en de morfologische integriteit van *O. hunteri* is dus intact. Bovendien werd ook het mtDNA van andere *Oreochromis* soorten uit de nabijgelegen meren Lake Jipe en Nyumba ya Mungu geanalyseerd, en bleek *Oreochromis jipe* de dichtst gerelateerde soort aan *O. hunteri*.

Voor een correcte interpretatie van vroegere veranderingen in de vorm van orale tanden is kennis van hun huidige functie van belang. Over het algemeen worden orale tanden in cichliden gebruikt voor het vergaren van voedsel, dus werd het dieet van *O. hunteri* bestudeerd met een combinatie van tellingen van maaginhouden en stabiele isotopenanalyse. Deze soort blijkt een herbivoor/detritivoor dieet te hebben doorheen het hele jaar, maar vertoont seizoenale variatie in de maaginhoud. Door deze maaginhouden te vergelijken met de samenstelling van limnetisch fytoplankton en van littorale voedselbronnen kon ik vaststellen dat *O. hunteri* het grootste deel van het jaar in de littorale delen van het meer voedsel vergaart, maar naar de limnetische zone migreert wanneer er daar een fytoplanktonbloei plaatsvindt, jaarlijks in de maanden juli, augustus en september. Ik vond gelijkaardige seizoenale trends in de gentroduceerde *O.* cf. *korogwe* en *C. rendalli*, die dus mogelijks in competitie treden voor voedsel met *O. hunteri*. De stabiele isotopenanalyse leverde echter significante verschillen op tussen de soorten, die waarschijnlijk het resultaat zijn van de verschillende manieren waarop deze soorten gebruik maken van de jaarlijkse fytoplanktonbloei. Op dit moment heeft *O. hunteri* waarschijnlijk een competitief voordeel ten opzichte van de ingevoerde soorten.

Dit betekent echter wel dat *O. hunteri* zich het merendeel van de tijd dicht bij de kust bevindt in Lake Chala, en dus werd ook getracht om te achterhalen hoe individuele tanden uiteindelijk in het midden van het meer terecht komen, waar de bestudeerde boorkernen werden ingezameld. Een korte boorkern (27 cm) werd daartoe genomen nabij de kust, en er werd een vergelijking gemaakt tussen de distributie van fossiele tandjes en andere biologische fossielen enerzijds, en de textuur van het sediment anderzijds. Er werden zeer hoge abundanties van fossiele tanden aangetroffen in grofkorrelige secties onderaan de boorkern, die erop wijzen dat het merendeel van de tanden die in het meersediment terecht komen permanent worden begraven aan de rand van het meer. Bovendien werd er een sterke positieve correlatie waargenomen tussen korrelgrootte en de abundanties van fossielen met een hoge densiteit, die er op wijst dat de hydrostatische eigenschappen van fossiele tanden geen transport over grote afstanden toelaten. De fossiele tanden die werden aangetroffen in de lange boorkernen die ver uit de kust werden ingezameld werden dus waarschijnlijk ter plaatse afgezet.

Uiteindelijk werd zowel de huidige als de fossiele orale tandvorm van *O. hunteri* in detail bestudeerd. De orale tanden van twaalf *O. hunteri* specimens werden geanalyseerd, en er werd een graduele verschuiving waargenomen in tandvorm die is gelinkt aan de grootte van het individu. Kleinere specimens hebben een bicuspide eerste rij orale tanden, gevolgd door meerdere rijen tricuspide tanden. Die worden echter geleidelijk aan vervangen door unicuspide tanden in zeer grote individuen, wat mogelijks een verschuiving in hun dieet of in territoriaal gedrag betekent. Er was ook een sterke lineaire correlatie tussen tandgrootte en lichaamsgroote, zodat de grootte van fossiele tandjes kon worden gebruikt als proxy voor de grootte van de vis waaruit ze afkomstig zijn. Vervolgens werden zes fossiele assemblages bestudeerd die afwisselende hoog- en laagstanden vertegenwoordigen in de afgelopen 25000 jaar. De vorm van de orale tanden uit deze assemblages overlapte volledig met tandvorm in de huidige *O. hunteri*, zodat ik kon concluderen dat deze soort de enige aanwezige vissoort is geweest in Lake Chala in deze periode, tenminste vóór de recente introducties. Deze overlap wees er ook op dat de algemene ecologie van *O. hunteri* in Lake Chala zeer gelijkaardig is gebleven. Er werden echter wel significante verschuivingen aangetroffen in de proportie unicuspide tanden tussen de verschillende assemblages. De proportie unicuspen steeg met ongeveer 10% in laagstand-assemblages, zonder gepaard te gaan met een stijging in gemiddelde lichaamsgrootte. Deze waarneming werd genterpreteerd als een verschuiving in het ontogenetische traject van tandontwikkeling in *O. hunteri.* De soort kreeg op die manier reeds ‘adulte’ tandtypes bij een kleinere lichaamsgrootte wanneer het meerniveau laag was, mogelijks als respons op een meer uitgestrekt zanderig habitat. Dit toont aan dat *O. hunteri* het hoofd heeft geboden aan klimaatgedreven omgevingsveranderingen door een snelle morfologische respons in orale tandvorm. Bovendien heeft de soort zich herhaaldelijk op dezelfde manier aangepast in de afgelopen 25000 jaar, wat de morfologische veranderlijkheid van *O. hunteri* over zeer lange periodes bevestigt.

Deze studie toont het potentieel aan van fossiele cichlidenresten die worden bewaard in meersedimenten. Fossiele orale tanden werden met succes gebruikt om langetermijn verschuivingen in morfologie op te sporen die het gevolg zijn van klimaatgedreven omgevingsveranderingen. Meerniveauschommelingen worden bovendien beschouwd als een belangrijke drijfveer van evolutie in cichliden in het algemeen, en onze observaties suggereren een potentieel mechanisme dat snelle adaptatie aan een nieuwe omgeving mogelijk maakt. Het bestuderen van gelijkaardige ononderbroken fossiele records in meren met een complexere cichlidengemeenschap kan langetermijn patronen in eco-morfologie blootleggen op veel grotere schaal, en kan op die manier bijdragen aan een beter begrip van de processen aan de basis van de huidige soortendiversiteit. Deze thesis beschrijft hoe zo’n studie kan worden aangepakt.

CHAPTER 1

General introduction

1.1 Cichlid adaptive radiation

The study of diverse yet relatively young species assemblages that have radiated in a geographically confined area has been essential to understand the causes and consequences of speciation (Coyne & Orr, 2004). Especially adaptive radiations, in which the exploitation of and adaptation to a variety of different environments results in species differentiation (Schluter, 2000; Losos, 2011), have been used to document the origin and proliferation of species. Well-known examples of terrestrial species radiations include *Anolis* lizards in the Caribbean islands (e.g., Williams, 1972; Jackman *et al.*, 1997; Losos, 2011; Mahler *et al.*, 2013), Darwin's finches on the Galápagos (e.g., Lack, 1947; Boag & Grant, 1981; Grant & Grant, 1989; Lamichhaney *et al.*, 2015) and the Hawaiian silversword alliance (e.g., Carlquist *et al.*, 1965; Carlquist, 1980; Carr, 1985; Baldwin & Robichaux, 1995). Undoubtedly, the most dramatic case of aquatic species radiation is provided by the cichlid fishes (e.g., Fryer & Iles, 1972; Stiassny & Meyer, 1999). These tropical freshwater fishes occur in Africa, Madagascar, India and southern and central America, and, with an estimated total of 2300 species, they comprise a considerable part of worldwide vertebrate diversity (Van Steenberge, 2014). Especially in the East African lakes Malawi, Tanganyika and Victoria, cichlids have radiated independently into exceptionally diverse species flocks (Fryer

& Iles, 1972), which nevertheless share many phenotypes due to convergence (e.g., Kocher *et al.*, 1993; Muschick *et al.*, 2012). The evolutionary mechanisms underlying cichlid diversity are slowly being unveiled by a combination of information that is embedded in species' genetic codes (e.g., Meyer, 1993; Brawand *et al.*, 2014), detailed morphological analysis (e.g., Elmer *et al.*, 2010a; Muschick *et al.*, 2012), ecological assessment (e.g., Fryer, 1959a; Greenwood & Gee, 1969; Reinthal, 1990) and behavioural studies (e.g., Goodwin *et al.*, 1998; Verzijden *et al.*, 2010). Based on these studies, a complex interplay of natural selection, hybridisation and sexual selection is now suggested to be responsible for the observed organismal diversity (Salzburger, 2009; Salzburger *et al.*, 2014).

Most studies on cichlid fishes have focused on evolutionary mechanisms within historical times, because the lack of a continuous fossil record hampers examination of long-term patterns in morphology and their underlying processes (Murray, 2001). In this respect, cichlid fossils such as teeth, scales and bones preserved in lake sediments may provide a continuous fossil record representing a lake's past ichthyofauna and have been tentatively explored in Lake Malawi (Reinthal *et al.*, 2011) and more recently Lake Victoria (Muschick *et al.*, 2018). Especially fossil teeth could function as a proxy for niche exploitation, as they reflect past feeding habits. The study of cichlid fossils in lake sediments could thus greatly enhance our understanding of cichlid evolution on much longer timescales, but the great complexity of many cichlid communities and complicated geological history of the lakes they inhabit obstruct easy interpretation of these fossil records. Ideally, a small system with limited species diversity and a continuous fossil record is studied first. In this dissertation, I study the potential of such a fossil record in isolated crater lake Chala. Moreover, the well-resolved climatic past of Lake Chala enables tracing the morphological response of cichlid fossils to past environmental change, in particular strong lake-level fluctuations.

1.2 Ecological opportunity

Ecological opportunity provided by the availability of complex and discordant habitats is one component that has been considered crucial for the diversification of cichlid species (Huber *et al.*, 1997; Danley & Kocher, 2001). The dynamic geological history and paleoclimate of the East African region has greatly influenced the availability of such habitats in the past (e.g., Rossiter, 1995; Danley *et al.*, 2012). During the Quaternary period, this region has witnessed alternating wet and dry cycles linked to fluctuations in monsoon rainfall due to variations in solar insolation forcing (e.g., Verschuren *et al.*, 2009; Lyons *et al.*, 2015), with obvious effects on African palaeohydrology and lake levels (De Cort *et al.*, 2017). In the African Great Lakes (Fig. 1.1), lake-level fluctuations caused extreme contraction (Lake Malawi; Delvaux, 1995; Scholz *et al.*, 2007; Lyons *et al.*, 2011), fragmentation (Lake Tanganyika; Scholz & Rosendahl, 1988; Rossiter, 1995) or even desiccation (Lake Victoria, Johnson *et al.*, 1996), and have had a profound impact on the lakes' biota (Cohen *et al.*, 2007; Danley *et al.*, 2012). Indeed, rapid population expansion of inshore demersal haplochromine cichlids in Lake Malawi has been linked to the lake rising to current levels (Genner & Turner, 2014), and fragmentation of Lake Tanganyika is reflected in the phylogeographic patterns of its rock-dwelling cichlids (Sturmbauer & Meyer, 1992; Verheyen *et al.*, 1996; Rüber *et al.*, 1999; Nevado *et al.*, 2013). The relatively recent desiccation of Lake Victoria (∼14,000 yr BP; Johnson *et al.*, 1996), which is nevertheless populated with an extremely diverse cichlid species flock (∼500 species; Snoeks, 2000), is perhaps the most striking example of the ecological opportunity created by these lake-level changes.

At least in the case of Lake Tanganyika, lake-level fluctuations have been considered to have functioned as a 'species pump'. Fragmentation of this lake into several sub-basins due to strong lake-level drops facilitated allopatric speciation among cichlids, greatly enhancing species diversity even when lake levels rose again and reunited communities from different sub-basins (Rossiter, 1995). More complex effects of lake-level fluctuations on speciation have been suggested for Lake Malawi. Based on phylogenetic patterns in Malawi cichlids, Ivory *et al.* (2016) hypothesised that an expansion of rocky habitats allowed their diversification during past periods of high lake level. In addition, lake lowstands were accompanied by more turbid conditions, leading to episodes of extinction but also to interspecific hybridisation, which is known to be an important source of evolutionary novelty for selection to act upon (Verheyen *et al.*, 2003; Seehausen, 2004; Meier *et al.*, 2017a). Thus, both lake-level states are suggested to have contributed to the phenomenal species diversity observed in African cichlid fishes today.

1.3 The trophic apparatus as key innovation

Ecological opportunity alone does however not predict whether cichlid lineages radiate or not. Several lineage-specific traits have been proposed to be of importance for diversification and speciation in cichlid fishes. The evolutionary potential of cichlids is likely contingent on unique genomic properties (Seehausen, 2006; Brawand *et al.*, 2014), and the evolution of egg spots (Hert, 1989; Salzburger *et al.*, 2005), maternal mouthbrooding (Greenwood, 1979; Sefc, 2011) and a sophisticated trophic apparatus (Liem, 1973; Hulsey *et al.*, 2006) have all been proposed to have promoted their rapid speciation. The trophic apparatus has even been advanced as a 'key innovation' (Liem, 1973; Hulsey *et al.*, 2006), as it provides a clear tool for ecological adaptation and niche diversification, eventually contributing to adaptive speciation (Schluter, 2000).

The evolution of jaws has been a major driver of vertebrate evolution in general (Mallatt, 1996; Liem *et al.*, 2001), and the diversification of trophic morphology in cichlids is a great example of the evolutionary potential of this trait (Fryer & Iles, 1972). Cichlid fishes are not unique in their possession of a

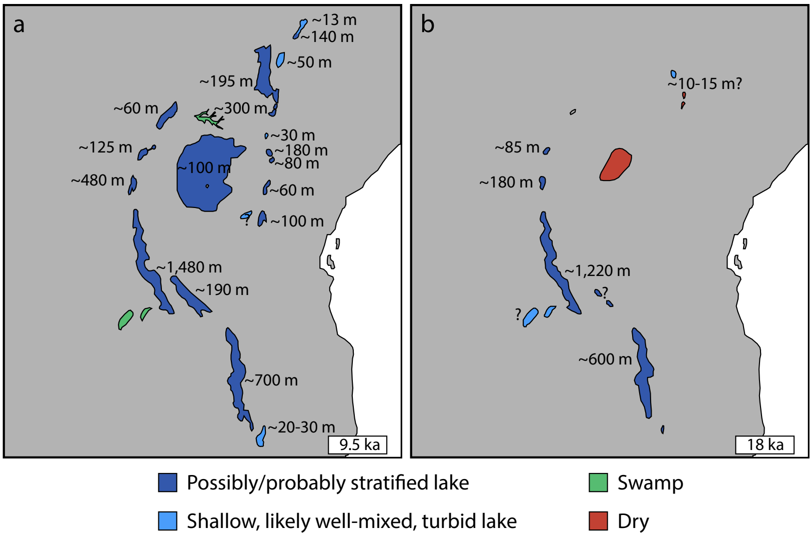


Figure 1.1: Palaeogeographic maps of East Africa showing lake-level stands 9,500 (**a**) and 18,000 (**b**) year BP. Numbers represent estimated water depths. Modified from Salzburger *et al.* (2014)

second set of jaws (i.e. pharyngeal jaws; Schaeffer & Rosen, 1961; Liem, 1986; Wainwright, 1989), but the fusion of the pharyngeal plates and the modified musculature are unique innovations that have enhanced this jaw's mobility and led to a functional decoupling from the oral jaws (Liem, 1973; Meyer *et al.*, 1990; Hulsey *et al.*, 2006; Fig. 1.2a). This way, the oral jaws were released to specialise on prey collection while the pharyngeal jaws do the prey processing (Liem, 1973; Lauder *et al.*, 1989). The resulting independent divergence of jaws, combined with diverging tooth shapes, has produced remarkable levels of morphological specialisation (Fryer & Iles, 1972; Hulsey *et al.*, 2006) and facilitated extraordinary cases of fine-scaled resource partitioning (Fryer, 1959a; Reinthal, 1990). Nevertheless, convergence of trophic structures is common in cichlids, both within (Rüber *et al.*, 1999; Rüber & Adams, 2001) and between water bodies (Kocher *et al.*, 1993; Muschick *et al.*, 2012), even across continents (Winemiller *et al.*, 1995). That similar ecological problems generate similar ecomorphological solutions indicates that certain morphological constraints may influence the direction of evolution (Albertson & Kocher, 2006), and suggests that natural selection is shaping phenotypic diversity.

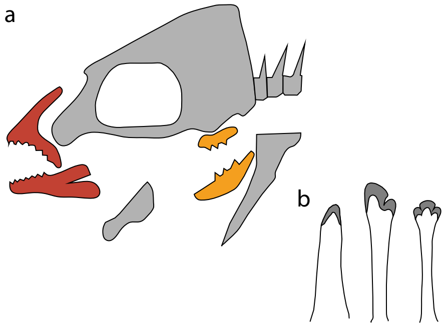


Figure 1.2: **a** Schematic representation of oral (red) and pharyngeal (yellow) jaws in cichlid fishes, modified from Machubi *et al.* (2007). **b** Schematic drawing of oral jaw teeth of *Oreochromis hunteri*, representing the three general tooth types present in cichlid fishes. From left to right: unicuspid, bicuspid and tricuspid tooth

This unique combination of trophic divergence within clades and morphological convergence between clades has therefore stimulated investigations of the functional morphology of cichlid jaws. In this context, the development of a quantitative method to analyse shape (i.e. geometric morphometrics; Bookstein, 1991; Zelditch *et al.*, 2004) has proven extremely valuable in cichlid research (reviewed in Kerschbaumer & Sturmbauer, 2011). Such studies have highlighted for example the importance of physical constraints on jaw morphology (Hulsey, 2006), the presence of structural trade-offs (Tkint *et al.*, 2012), and the existence of intraspecific polymorphisms in the lower pharyngeal element (e.g., Trapani, 2004; Muschick *et al.*, 2011). Although the resulting variation in jaw shape is astounding, the diversity in cichlid tooth shapes is equally impressive (Greenwood & Gee, 1969; Fryer & Iles, 1972). Tooth shape variation can be summarised in three major tooth types, based on the number of cusps they bear: unicuspid, bicuspid and tricuspid teeth (Fig. 1.2b). Teeth are often implanted in the jaws in multiple series, in which each separate series bears similarly-shaped teeth (homodonty; Streelman *et al.*, 2003), but shape may differ substantially between series. Subtle differences in cusp shape aid species to specialise in a certain niche, and these have enabled the fine-scaled resource partitioning observed in the Great Lakes' cichlid communities (Fryer, 1959a; Greenwood & Gee, 1969; Reinthal, 1990).

The developmental pathways and genetic architecture controlling cichlid jaw and tooth morphology are now relatively well-understood (e.g., Huysseune & Sire, 1992; Streelman *et al.*, 2003; Albertson & Kocher, 2006; Hulsey *et al.*, 2017; Singh *et al.*, 2017). Increasing numbers of genes underlying the observed craniofacial diversity are being discovered (Albertson & Kocher, 2006; Gunter *et al.*, 2013) and substantial genetic covariance exists between the oral and pharyngeal jaws (Hulsey *et al.*, 2017). Cichlids typically replace their teeth continuously via *de novo* formation of tooth germs (polyphyodonty), and tooth shape can vary considerably throughout a fish's life due to temporal changes in gene expression (Streelman *et al.*, 2003). Such ontogenetic changes are possibly associated with alterations in feeding habits when juveniles reach adulthood, or with sexual maturation. Differences in the timing and rate of ontogenetic trajectories are an important source of phenotypic diversity, and may provide cichlids with the means to rapidly adapt to a changing environment (Meyer, 1987; Streelman *et al.*, 2003), as has for example been documented in the visual system of neotropical cichlids (Härer *et al.*, 2017).

1.4 Small systems and non-radiating lineages

Many evolutionary mechanisms and processes have been elucidated by studying the species flocks that populate the Great Lakes of Africa, and their extraordinary outcome is also best observed in these lakes. Yet the enormous habitat diversity and difficulties in determining the relationships between species have complicated untangling certain principles (e.g., Verheyen *et al.*, 2003; Joyce *et al.*, 2011; Ford *et al.*, 2015; Kavembe *et al.*, 2016; Meyer *et al.*, 2017), and have compelled scientists to investigate the inhabitants of smaller lake systems. Such systems have thus been used to characterise the genomic properties of the early stages of adaptive radiation (Ford *et al.*, 2015; Malinsky *et al.*, 2015; Kavembe *et al.*, 2016; Ronco & Salzburger, 2016), but they have also functioned as 'natural laboratories' to study adaptive responses to, for example, changes in light environments (Härer *et al.*, 2017; Torres-Dowdall *et al.*, 2017). Famously, the most compelling evidence for the enigmatic process of sympatric speciation – divergence in the presence of gene flow – has been found in small, monophyletic species flocks inhabiting isolated crater lakes (Schliewen *et al.*, 1994; Barluenga *et al.*, 2006; Elmer *et al.*, 2010b), although recently new debate has emerged about some of the interpretations made (Martin *et al.*, 2015; Foote, 2018).

Small systems harbouring non-radiating cichlid lineages have also been pivotal in explaining which factors influence diversification. Through a comparative analysis of radiating and non-radiating cichlid lineages in both large and small lakes across tropical Africa, Wagner *et al.* (2012) found that a combination of lake depth, solar radiation and sexual dichromatism predicted whether cichlids radiated or not. Not all cichlid lineages thus have the same propensity for radiation. One group of cichlids whose members rarely radiate is the paraphyletic species group formerly referred to as 'Tilapia' (Ribbink, 1990; Nagl *et al.*, 2001; Klett & Meyer, 2002; Dunz & Schliewen, 2013), although small incipient radiations of tilapiine cichlids have been found in some systems (Schliewen & Klee, 2004), often driven by extreme environmental conditions (Ford *et al.*, 2015; Kavembe *et al.*, 2016). Despite sharing many characteristics with radiating cichlid lineages, tilapiines differ fundamentally in certain inherent traits, for example in their genomic properties (Brawand *et al.*, 2014). Moreover, whereas radiations often produce highly stenotopic communities in which each species is perfectly adapted to its relatively narrow ecological niche, tilapia species display exceptionally high levels of versatility in behaviour, ecology and morphology (Fryer & Iles, 1972; Trewavas, 1983; Ribbink, 1990).

Tilapiines are indeed remarkably tolerant to wide ranges of temperature, dissolved oxygen, salinity, alkalinity and pH (reviewed in Philippart & Ruwet, 1982), and several species occupy a range of different habitats during their lifespan (Trewavas, 1983; Ribbink, 1990). Their diet is generally herbivorous or detritivorous, but changes in feeding habits are commonly reported and have been linked to ageing (Bruton & Boltt, 1975; Whyte, 1975; Gophen, 1980; Njiru *et al.*, 2004), to seasonal differences in food availability (Spataru, 1976; Man & Hodgkiss, 1977; Spataru, 1978a), to breeding status (Spataru, 1978b) or simply to opportunity (Bruton & Boltt, 1975). Moreover, there is a large amount of intraspecific variability in growth and onset of sexual maturation between populations from different localities (Brummett, 1995; Duponchelle & Panfili, 1998; Bwanika *et al.*, 2007), strongly depending on environmental variables.

As a result, requirements for tilapia aquaculture are not very demanding, and a considerable part of the global freshwater fish production is currently tilapia-based (FAO, 2014). Especially large *Oreochromis* species are used for this purpose, such as *O. niloticus*, *O. mossambicus*, *O. aureus*, and their different hybrids. Although part of the fish production is confined to artificial fish ponds, natural systems worldwide have been stocked with alien *Oreochromis* species to boost fisheries (Eknath & Hulata, 2009), where their versatile nature has allowed them to readily adapt to new circumstances. In many places, these anthropogenic introductions have resulted in ecological disasters. The effects of invasive *Oreochromis* species are typically most apparent in the native ichthyofauna (reviewed in Canonico *et al.*, 2005), but in Mexico for example the introduction of *O. niloticus* has also been linked to population decline in the iconic yet critically endangered axolotl (Zambrano *et al.*, 2010; Alcaraz *et al.*, 2015). The negative impacts of invasive aliens on a natural system are often the result of a complex interplay of factors (Gozlan *et al.*, 2010), and in *Oreochromis* include direct predation (De Moor *et al.*, 1986) and competition for food (Ogutu-Ohwayo, 1990; Daga *et al.*, 2016) or prime breeding grounds (Canonico *et al.*, 2005). Additional concern is raised by the propensity of introduced *Oreochromis* species to hybridise with indigenous tilapiine species (Agnèse *et al.*, 1998; D'Amato *et al.*, 2007; Nyingi & Agnèse, 2007; Angienda *et al.*, 2011; Deines *et al.*, 2014; Ndiwa *et al.*, 2014). The introgression of alien genes into local species may lead to homogenisation of the gene pool and thus induce a loss of genetic diversity (Nyingi & Agnèse, 2007; Crispo *et al.*, 2011; Firmat *et al.*, 2013), but it also potentially impacts important morphological features (Parnell *et al.*, 2012; Holzman & Hulsey, 2017). Interspecific hybridisation is considered as an important mechanism in generating natural cichlid diversity (Salzburger *et al.*, 2002; Seehausen, 2004; Meier *et al.*, 2017a), but from a conservational point of view it creates a worrying prospect (Pullin, 2002).

1.5 The fossil record of cichlids

Molecular phylogenetic techniques have proven invaluable in elucidating many of the evolutionary processes driving cichlid speciation (e.g., Wagner *et al.*, 2012; Brawand *et al.*, 2014), but also in reconstructing the origin of certain biogeographic patterns (e.g., Joyce *et al.*, 2005; Meier *et al.*, 2017a). Moreover, they can be used to estimate divergence times between species based on the principle of the 'molecular clock', which assumes a correlation between the age of species divergence and the number of genetic differences (Zuckerkandl & Pauling, 1962). But large differences exist in the rate of genetic change between lineages (Drummond *et al.*, 2006), and a good calibration of the molecular clock is necessary to accurately estimate the timing of past divergence events. For an estimation of absolute minimum age, calibration is commonly done using fossils, and clocks calibrated in this manner have for example been used to estimate the divergence times of large cichlid species flocks (Genner *et al.*, 2007), or to link phylogeographic patterns to past lake-level fluctuations (Genner & Turner, 2014). However, the cichlid fossil record has thus far proven inadequate to answer such important questions as the timing of divergence between African and neotropical cichlids, so that the long-standing debate between their Gondwanan vicariance or transatlantic dispersal remains unresolved (e.g., Genner *et al.*, 2007; Azuma *et al.*, 2008; Smith *et al.*, 2008; Friedman *et al.*, 2013; Matschiner *et al.*, 2017).

Proper fossil records are useful in many other ways, notably in extrapolating evolutionary and ecological observations over longer time scales. In this respect, the study of morphology and wear of fossil teeth has proven effective in diverse vertebrate taxa, such as hominids (reviewed in Ungar, 2017), bovids (e.g., Solounias & Moelleken, 1992; Fortelius & Solounias, 2000; Blondel *et al.*, 2010) and fish (e.g., Purnell *et al.*, 2007; Bellwood *et al.*, 2014; Hundt & Simons, 2018). But for cichlids, the scarcity of continuous fossil deposits and the disarticulated nature of remains has hampered a detailed study of long-term trends in their ecology and evolution (Murray, 2001). The cichlid teeth and scales preserved in lake sediments hold the best hope for a continuous record, and are hence beginning to be explored in Africa's Great Lakes, e.g. in Lake Malawi (Reinthal *et al.*, 2011) and Lake Victoria (Muschick *et al.*, 2018). However, the species richness in these large lakes makes it difficult to assign fossils to specific lineages. Also, the past lake-level fluctuations that presumably influenced cichlid speciation (cf. above) potentially impacted important taphonomic processes and the depositional environment in which the fish are preserved, impeding straightforward interpretation of these records. In this context, studying the fossil record of cichlid fishes in a small and simple lake system prone to similar environmental dynamics becomes appealing to assess the true potential of this approach. In this thesis, I present the results of such a study on the sediment archive of crater Lake Chala.

1.6 Lake Chala

1.6.1 Local climate & sediment archive

Lake Chala (03°19'S, 37°42'E) is a 4.2 km^2 crater lake at the border between Kenya and Tanzania, located on the lower east slope of Mount Kilimanjaro (Fig. 1.3a). It is contained within a steep-sided caldera, and the narrow shoreline quickly drops down to a depth of nearly 55 m (Opitz, 2008), from where soft sediments slope more gently towards a maximum depth of around 92 m in the middle of the lake (Fig. 1.3b; Moernaut *et al.*, 2010). The local climate is tropical semi-arid, with mean temperatures varying between 26 and 30°C. Lake-surface evaporation supersedes total annual rainfall (~565 mm), but sub-surface in- and outflow strongly influence the lake's water budget and keep it fresh (Moernaut *et al.*, 2010). The latitudinal zone of peak insolation and the intertropical convergence zone (ITCZ) pass across this equatorial region twice per year, resulting in a characteristic bimodal pattern of rainfall seasonality:

southeasterly monsoon winds bring ‘long rains’ from March to May, whereas the northeasterly monsoon winds bring ‘short rains’ from late October to December. During most of the year, wind-driven turbulence and night-time convection in this sheltered crater basin are relatively modest, limiting frequent oxygenation to the upper 15-30 m of the water column. The surface water is cooled by a combination of strong southerly winds and reduced seasonal insolation during the southern hemisphere winter from late June to September, which promotes convection and expands the mixed layer to between 35 m and 45 m (Buckles *et al.*, 2014). This deep mixing brings up dissolved nutrients that have been accumulating in the lower water column and induces an extensive phytoplankton bloom at the end of the long dry season (Barker *et al.*, 2011; Wolff *et al.*, 2011; Buckles *et al.*, 2014).

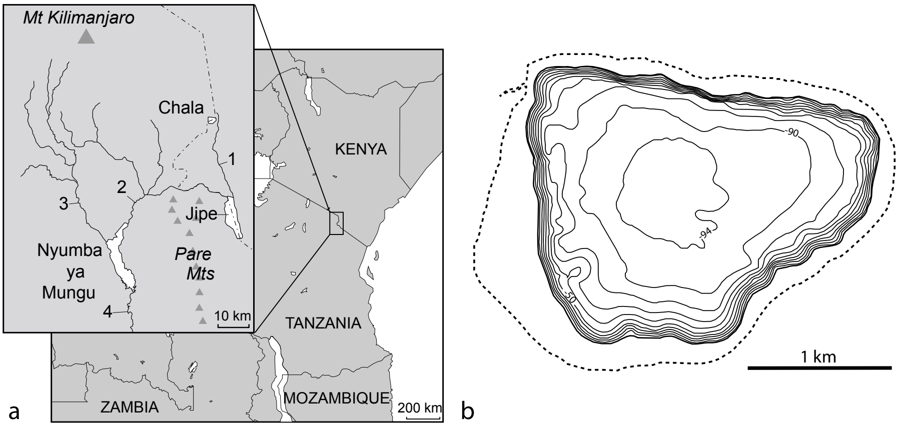


Figure 1.3: Skeleton maps of East Africa and the Upper Pangani River basin (inset) in northern Tanzania, with indication of the sampled surface waters Chala, Jipe and Nyumba ya Mungu (**a**). River systems are indicated by numbers. 1: Lumi, 2: Ruvu, 3: Kikuletwa, 4: Pangani. Bathymetry of Lake Chala (**b**) with dotted line representing its catchment. Modified from Moernaut *et al.* (2010)

The sediment record of Lake Chala demonstrates long-term continuity of lacustrine conditions (Verschuren *et al.*, 2009; Moernaut *et al.*, 2010), and is currently the subject of a multifaceted research programme aiming to reconstruct the long-term climate history and ecosystem dynamics of equatorial East Africa (Verschuren *et al.*, 2013). Notably, seismic-reflection data revealed that the lake has experienced pronounced lake-level fluctuations since its origin approximately 250,000 years ago. These fluctuations probably ranged from

~30-40 m below to ~10 m above current lake levels during low- and highstands respectively (Moernaut *et al.*, 2010). This change in total lake depth likely had an impact on the availability and distribution of different types of aquatic habitat and food resources, and can thus be expected to have influenced the ecology and evolution of indigenous ichthyofauna in Lake Chala.

1.6.2 *Oreochromis hunteri*, the mountain Chromis

Lake Chala currently harbours the only known population of the Chala Tilapia, *Oreochromis hunteri* Günther, 1889 (Fig. 1.4a). This was the only fish species present in the lake when A. Günther described it in 1889 as the type species for a new genus: *Oreochromis*, 'the mountain Chromis', in reference to its home at the foot of Mount Kilimanjaro (Dadzie *et al.*, 1988). Primarily because of its restricted distribution in a single small lake, this endemic species has been listed as 'critically endangered' on the IUCN red list (Bayona *et al.*, 2006), yet very little is known about its ecology. Based on morphological similarities Trewavas (1983) proposed a close relationship to *Oreochromis jipe* from nearby Lake Jipe, but this hypothesis has never been tested phylogenetically. Similarly, Trewavas (1983) could not elaborate on its feeding habits and breeding behaviour, except that Lowe (1955) reported having observed the young fish feeding on algae and debris from the bottom between the rocks. Indeed, in the modern-day lake system the steep rocky crater walls and open-water (limnetic) zone provide the only oxygenated habitats available to *O. hunteri.* The rocks are covered by epilithic algae and interspersed with small sandy patches trapping plant litter and other organic detritus, thus providing a dependable food source throughout the year. In contrast, primary production in the limnetic zone is very low (secchi-disk transparency ranging between 6 and 9 m; van Bree *et al.*, unpublished data) except during the phytoplankton bloom between July and September. However, a detailed quantitative assessment of *O. hunteri*'s diet is still lacking.

Investigations into the origin and ecology of *Oreochromis hunteri* are especially relevant because it now shares Lake Chala with three anthropogenically introduced cichlid species. These introductions probably took place sometime in the second half of the 20^{th} century (Caxton Oluseno, pers. comm.), most likely after 1980 as surveys carried out on Lake Chala in 1889, 1902, 1946, 1952

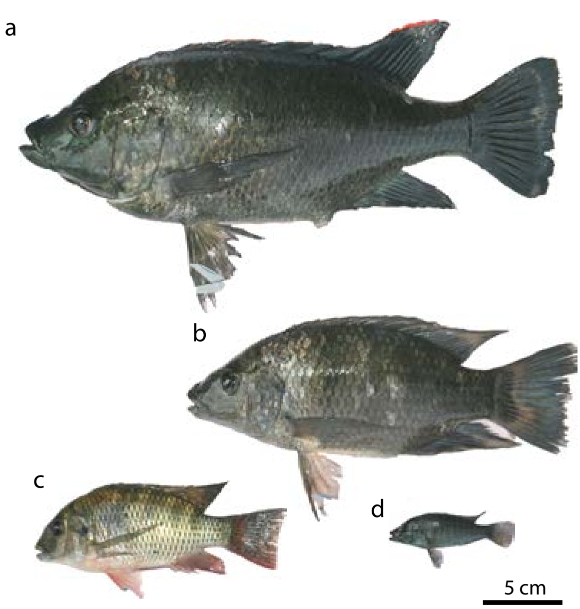


Figure 1.4: The extant ichthyofauna of Lake Chala consists of the endemic *Oreochromis hunteri* (**a**), introduced tilapiines *Oreochromis* cf. *korogwe* (**b**) and *Coptodon rendalli* (**c**), and the small haplochromine *Haplochromis* sp. 'Chala' (**d**)

and 1980 only found *O. hunteri* present (Günther, 1889; Dadzie *et al.*, 1988). Two tilapiine species have been found in catches since then, being *Oreochromis korogwe* Lowe, 1955 (Fig. 1.4b) and *Coptodon rendalli* Boulenger, 1896 (Fig. 1.4c; Dadzie *et al.*, 1988; Seegers *et al.*, 2003), although the former of the two was identified with some doubt. Because of this uncertainty, this taxon is identified as *O.* cf. *korogwe* in this thesis (see Chapter 3). A small cichlid species identified as *Haplochromis* sp. "Chala" was also introduced in that period (Seegers *et al.*, 2003). These species may interfere with the endemic *O. hunteri* in multiple ways. First, the congeneric nature of *O.* cf. *korogwe* and *O. hunteri* increases the risk of hybridisation and loss of genetic diversity (Nyingi & Agnèse, 2007; Crispo *et al.*, 2011; Firmat *et al.*, 2013). Second, similar habitat requirements of the introduced tilapiines may lead to the indigenous species being outcompeted, as has happened for example with the indigenous oreochromines of Lake Victoria after the introduction of *O. niloticus* (Twongo, 1995; Goudswaard *et al.*, 2002).

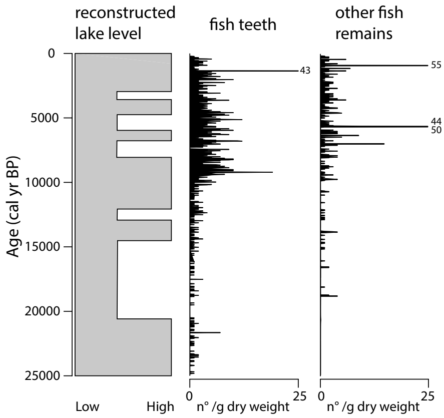


Figure 1.5: Counts of cichlid fossils with respect to reconstructed lake level (after Moernaut *et al.*, 2010) in a sediment core of Lake Chala spanning the last 25,000 years

1.6.3 The fossil record of *Oreochromis hunteri*

Fish remains in the form of fossil teeth, scales and bones are abundantly present in the sediment archive of Lake Chala throughout at least the last 25,000 years (Fig. 1.5). These most probably belong to *O. hunteri*, or to another species of cichlid that has since gone extinct; the introduced species are not represented in the fossil record except possibly in the uppermost centimetres of the sediment column. However, the seismic-reflection data reveal that considerable lake-level fluctuations have occurred also during the last 25,000 years (Fig. 1.5), with prolonged lowstands primarily during the early late-Glacial period (~19,500-14,500 years ago), the Younger Dryas (~13,000-11,500 years ago) and much of the mid-Holocene (~8500-3000 years ago). These fluctuations potentially influenced the ecology of *O. hunteri* by changing the availability of different aquatic niches. Specifically, the lowstands were probably severe enough (~30-40 m, Moernaut *et al.*, 2010) to oxygenate soft-bottom areas in the lake periphery at the foot of the rocks, which currently experience permanent anoxia due to their great depth (at least 55 m; Opitz, 2008). Hence, a soft-bottom benthic habitat and associated food source may have become available during such lowstands (Fig. 1.6). Presumably, such a shift would be reflected in ecologically important morphological traits, for example tooth shape. The excellent preservation of fish teeth in the sediment record of Lake Chala creates a unique opportunity to trace such morphological shifts over extended periods of time, as a proxy for the fishes' changes in niche exploitation in response to climate-driven lake-level fluctuations.

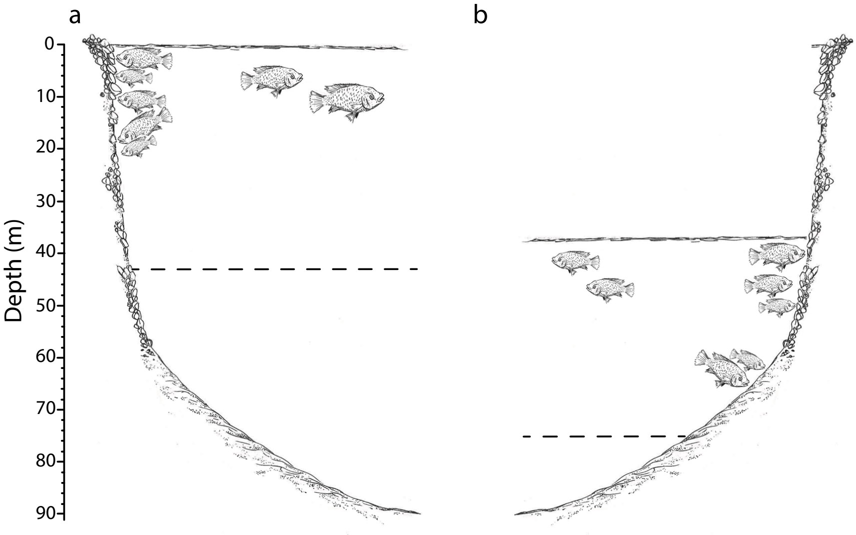


Figure 1.6: Schematic representation of the hypothesised effect of strong lake-level fluctuations on the available habitat for *O. hunteri*. During high lake-level stands, oxygenated habitats are limited to rocks and small sandy patches in the littoral zone, and the open-water (limnetic) zone (**a**). During lake lowstands, oxygenation of soft sediments near the lake periphery may bring a soft-bottom benthic habitat and associated food sources within reach (**b**). Dashed line represents the maximum depth of the oxycline during the mixing season. Drawing by Tim Goethals

1.7 Outline of this thesis

The principal aim of this PhD thesis is a detailed study of the fossil cichlid teeth preserved in the sediment record of Lake Chala. The isolated nature of the lake, its well-studied environmental past, and the known timing of recent fish introductions provide a uniquely controlled setting for morphological analysis of its fossil record. However, correct interpretation of this fossil record requires thorough understanding of the ecology and genetic relationships of modern-day *Oreochromis hunteri*, and of the taphonomic processes that might influence the fossil record. Consequently, the research programme of this PhD project has also given due attention to these aspects. The results of this study can thus be structured in six chapters, as outlined below.

1.7.1 Method development for geometric-morphometrics of fossil fish teeth

Chapter 2 of this thesis describes the development of several methods used in subsequent chapters. Important morphological traits are examined using geometric-morphometrics. Based on existing literature, a set of landmarks is defined to analyse the general body shape of the fishes. These landmarks are then applied to personal collections of the two *Oreochromis* species now inhabiting Lake Chala (*O. hunteri* and *O.* cf. *korogwe*) to test the potential of this method to discriminate between species. A quantitative method capturing cichlid tooth shape had been developed earlier by Wautier *et al.* (2002), but required undamaged teeth, which is not always possible in fossil teeth. I therefore develop a method combining length-width measurements of complete teeth with quantitative shape analysis of the tooth top alone (i.e. the enameloid-covered crown). I further compare the outcome of two alternative geometric-morphometric techniques in describing the shape of oral tooth crowns: elliptic Fourier analysis (EFA) and semi-landmark analysis (SLM). The optimal technique is then used to compare and describe the tooth shapes of *O. hunteri* and *O.* cf. *korogwe*, and a first set of fossil teeth is added to the dataset to assess the feasibility of this approach.

1.7.2 Genetics and ecology of the extant ichthyofauna of Lake Chala

Morphological integrity of the trophic apparatus of modern-day *O. hunteri* is fundamental to its function as modern-day reference for the fossil record. However, hybridisation with recent newcomers may compromise this integrity (Parnell *et al.*, 2012; Holzman & Hulsey, 2017). In **Chapter 3**, I therefore search for traces of interbreeding by combining whole-body geometric morphometry with molecular phylogenetic analysis of mitochondrial loci from *O. hunteri* and *O.* cf. *korogwe*. Comparing the obtained sequences to relevant GenBank entries, I aim to establish the true identity of the *O.* cf. *korogwe* population in Lake Chala. Using fishes from Lake Jipe and Nyumba ya Mungu reservoir, I expand the analysis to all four *Oreochromis* species currently inhabiting the Upper Pangani River system to determine the closest living relative of *O. hunteri*, and hence the possible source population of the ancestral species which colonised Lake Chala. Based on the observed haplotype diversity, I also intend to provide an estimated timing of arrival for *O. hunteri* in Lake Chala.

Oral tooth shape is strongly linked to the feeding habits of a species, yet very little is known about the diet of *O. hunteri*. The feeding ecology of *O. hunteri*, *O.* cf. *korogwe* and *C. rendalli* in Lake Chala is therefore studied in **Chapter 4** using a combined analysis of gut content composition and stable isotopes. Monthly collecting of fish over the course of 20 months enables tracing of seasonal variation in gut content, which is expected to occur in relation to the annual phytoplankton bloom. I combine these observations with monthly counts of the limnetic phytoplankton, and with the composition of potential food sources collected from rocks and sandy patches at the lake periphery. This way, I aim to assess the food sources these species are exploiting at different times of the year. The isotopic composition of fish tissue is then compared between species to trace any general interspecific differences in diet, and interpreted in conjunction with observations on gut content. This chapter concludes with a discussion of the consequences of anthropogenic fish introductions on the ecology and conservation status of *O. hunteri*.

1.7.3 Sedimentation processes influencing fossil distributions

The results of Chapter 4 suggest that *O. hunteri* spends most of its life close to shore. Correct interpretation of the fossil record therefore requires a proper understanding of how *O. hunteri*'s teeth end up at the depositional centre of the lake, where the 25,000-year sediment sequence has been extracted. The distribution of fossil teeth throughout a short core collected near the lake periphery is analysed in **Chapter 5**. I link the distribution and size variation of the fish teeth to important sedimentological variables such as the grain size of the clastic-mineral fraction, as well as to the distribution of other types of biological fossils. This may aid in understanding processes of tooth burial and transport, and how these may influence our observations from sediment cores collected offshore.

1.7.4 Long-term trends in oral tooth morphology of *Oreochromis hunteri*

In **Chapter 6**, I assess the effects of past lake-level fluctuations (Verschuren *et al.*, 2009; Moernaut *et al.*, 2010) on the local *O. hunteri* population through analysis of fossil tooth shape. The Chapter starts with implementation of the methods developed in Chapter 2 to achieve a thorough description of oral tooth morphology of extant *O. hunteri.* I then collected oral teeth from six time windows in the last 25,000 years representing alternating high and low lake-level stands. These six fossil assemblages are interpreted using the generated modern-day framework. I explore whether *O. hunteri* has indeed been the only species present in Lake Chala in this period, and compare morphospace occupation of fossil lowstand and highstand assemblages. This way, I aim to track long-term changes in the oral tooth morphology in relation to climate-driven environmental change.

1.7.5 General discussion

In final **Chapter 7**, the findings of all previous chapters (Chapter 2-6) are integrated into a general discussion. I make an assessment of the methods used and discuss their potential application in other systems. Genetics of the oreochromines of Lake Chala are briefly discussed, and tilapiine oral tooth shape is linked to feeding habits. Taphonomic processes influencing offshore fossil tooth distribution in Lake Chala sediments are discussed with respect to observations in the fossil record, and I elaborate on the origin of *O. hunteri* in Lake Chala and its subsequent eco-morphological response to past environmental fluctuations.

CHAPTER 2

Exploring the methods

Jorunn Dieleman[1]
Bert Van Bocxlaer[1,2,3]
Claudia Manntschke[1,4]
Wanja Dorothy Nyingi[5]
Dominique Adriaens[6]
Dirk Verschuren[1]

Modified* from: Dieleman, J., Van Bocxlaer, B., Manntschke, C., Nyingi, W.D., Adriaens, D. & Verschuren, D. (2015). Tracing functional adaptation in African cichlid fishes through morphometric analysis of fossil teeth: exploring the methods. *Hydrobiologia* 755: 73-88

[1] Limnology Unit, Department of Biology, Ghent University, K. L. Ledeganckstraat 35, B-9000 Gent, Belgium
[2] Departments of Paleobiology and Invertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington, DC 20013, USA
[3] Department of Animal Ecology and Systematics, Justus Liebig Universität, Heinrich-Buff-Ring 26-32 IFZ, 35392 Giessen, Germany
[4] Institute of Earth and Environmental Science, Universität Potsdam, Karl-Liebknecht-Str. 24-25, 14476 Potsdam, Germany
[5] Ichthyology Section, Zoology Department, National Museums of Kenya, P.O. Box 40658-00100, Nairobi, Kenya
[6] Evolutionary Morphology of Vertebrates, Department of Biology, Ghent University, K. L. Ledeganckstraat 35, 9000 Ghent, Belgium

* See Section 2.7 for a list of modifications.

2.1 Abstract

The sedimentary archive of Crater Lake Chala in East Africa contains abundant fossil teeth of cichlid fishes throughout at least the last 25,000 years. Here, we use morphometric analyses of oral teeth from the two extant *Oreochromis* species inhabiting Lake Chala to explore the feasibility of tracing adaptive modification of the cichlid trophic apparatus in the lake's fossil record. We compared the performance of semi-landmark analysis (SLM) and elliptic Fourier analysis in capturing morphological variation in oral tooth crowns, and found that SLM, supplemented with tooth neck measurements, ensured consistency with whole-body landmark analyses. Whole-body and tooth morphology data both allowed to discern between the indigenous *Oreochromis hunteri* and the recently introduced *Oreochromis* cf. *korogwe*. Both species have an oral jaw arrangement of outer-series bicuspid and inner-series tricuspid teeth, but *O. hunteri* has generally more slender teeth with a proportionally taller major cusp than *O.* cf.*korogwe*, suggesting fine-scaled resource partitioning. Exploratory analysis of three fossil samples showed that the major tooth types of modern-day *Oreochromis* are also represented in the fossil record. Their total morphological variation is largely restricted to the morphospace occupied by contemporary teeth, suggesting a close functional relationship between ancient populations and their likely descendant, *O. hunteri*.

2.2 Introduction

The study of relatively young evolutionary radiations in geographically confined areas has been the key to understanding mechanisms of speciation and adaptive divergence. In this respect, cichlid fishes have provided one of the best 'natural experiments' to study radiations and their prevalence in nature; hence, they function as a model system to examine animal diversification and its underlying processes (reviewed in Salzburger, 2009). The natural history of African cichlid radiations is profoundly influenced by the tectonic and climate history of the continent, and the East African Rift in particular (Koblmüller *et al.*, 2008; Salzburger *et al.*, 2014). Lake-level fluctuations during climatic dry-wet cycles and the development of the rift have alternatingly created and eliminated freshwater habitats, and they changed the hydrographic connectivity of rivers and lakes, thereby affecting gene flow between populations. Extrinsic environmental factors, such as these tectonic and climatic events, are believed to interact in complex ways with intrinsic biological characteristics to drive speciation and evolutionary radiation (Koblmüller *et al.*, 2008; Salzburger *et al.*, 2014).

One trait responsible for the evolutionary success of cichlids is their complex and extraordinarily adaptable trophic apparatus (e.g., Fryer & Iles, 1972). The oral and pharyngeal jaws and their associated dentition can change over the course of a few generations (van Rijssel *et al.*, 2015) or even within the lifetime of a single specimen (Meyer, 1987; Gunter *et al.*, 2013). Because of this flexibility, cichlid fishes can exploit an extensive array of food resources, and do so with impressively fine-scaled resource partitioning, especially in rock-dwelling species (e.g., Fryer, 1957; Reinthal, 1990). As a result, disruptive natural selection may lead to rapid speciation and the maintenance of distinct species, even if differences in feeding ecology and occupied niches appear to be modest (Schluter, 2000).

Current knowledge about the processes of diversification and speciation in cichlids is often derived from evolutionary patterns revealed in the phylogenetic trees of extant taxa (e.g., Danley & Kocher, 2001; Sato *et al.*, 2003; Barluenga *et al.*, 2006; Wagner *et al.*, 2012). With little concrete information on extinct species, however, a large knowledge gap remains. In this regard, fossil data extracted from African lake sediment records possess great potential. They may increase our understanding of both biological evolution and long-term ecological processes such as habitat specialisation and niche partitioning, provided that these data are continuous across environmental transitions (Park & Cohen, 2011) and allow analysis at sufficiently high temporal resolution (Van Bocxlaer & Hunt, 2013). Cichlid fish fossils preserved in sediments of Africa's large tectonic lakes remain relatively unexplored in this respect (Reinthal *et al.*, 2011), partly because a combination of great species richness, enormous habitat diversity and complex biotic interactions (Fryer & Iles, 1972) hampers the constraint of fossil data and the correct interpretation of fossil assemblages.

The sediment record of crater Lake Chala in East Africa (Kenya/Tanzania) contains abundant fossil teeth, bones and scales of cichlid fishes (Fig. 1.4). These remains accumulated throughout at least the last 25,000 years and have, hence, produced a unique system to study long-term ecological and evolutionary dynamics. The lake's extant fish community consists of four cichlid species, of which only one is indigenous and endemic: the Lake Chala Tilapia, *Oreochromis hunteri* Günther, 1889 (Trewavas, 1983; Seegers *et al.*, 2003). This is the fish known locally as 'Chala', and here abbreviated as 'OH'. Three other species have been introduced during the second half of the 20^{th} century: the Korogwe tilapia, *O.* cf.*korogwe* Lowe, 1955 ('Bandia', here abbreviated as 'OK'); the redbreast tilapia, *Coptodon rendalli* Boulenger, 1896 ('Kijiji') and a small haplochromine cichlid referred to as *Haplochromis* spec. "Chala" by Seegers *et al.* (2003). Therefore, the only cichlid remains we expect to find in the pre-20^{th} century sediment record are those of the endemic *O. hunteri.* Other fish remains may belong to other indigenous fishes that have become (locally) extinct since.

Lake Chala is the focus of multidisciplinary investigations into the long-term climate and environmental history of equatorial East-Africa, and these provide a highly suitable context for detailed study of the lake's fossil record. A wide array of paleo-environmental proxies extracted from its sediments have allowed reconstruction of lake-level fluctuations (Moernaut *et al.*, 2010), temperature change (Sinninghe Damsté *et al.*, 2012) and diverse other aspects of the lake's environmental history, which facilitate linking long-term aquatic ecosystem dynamics with pacers of environmental change.

Although the continuous sediment record of Lake Chala creates interesting opportunities to study evolutionary and ecological changes through time, preserved fossil fish remains mainly consist of individual teeth. Notwithstanding the vast amount of descriptive literature on trophic morphology and its ecological significance (e.g. Greenwood & Gee, 1969; Witte & Van Oijen, 1990), contemporary ecomorphological studies focus mainly on morphometric analyses of complete oral and pharyngeal jaws (see e.g. Kerschbaumer & Sturmbauer, 2011). A knowledge gap thus remains, concerning the application of morphometric methods on individual teeth with the objective to infer feeding ecology in cichlids.

In this Chapter, we develop a methodology to quantify variation in the morphology of oral teeth using geometric morphometrics. These efforts are a first step towards the ecological or evolutionary interpretation of changes in the morphology of fossil teeth through time. To build a reference framework for the interpretation of fossil data, we assessed different components of the overall variability in tooth shape present in the two *Oreochromis* species inhabiting Lake Chala today. First we used landmark analyses to document variation in overall body morphology of the endemic *O. hunteri* and introduced *O.* cf.*korogwe*, with the aim to unambiguously differentiate individuals of both taxa. Using representative specimens of each species, we then performed traditional length measurements on complete oral teeth and geometric-morphometric analyses of tooth crowns. We also compared the performance of semi-landmark analysis (SLM) and Elliptic Fourier Analysis (EFA) in the quantitative characterisation of tooth crown morphology. Our results allowed us to draw tentative inferences about the feeding ecology of both species. Finally, we compared the combined morphospace occupied by oral teeth of the extant Lake Chala fishes with those found in three fossil assemblages of up to 24,000 years old, as a first exploration of morphological variation through time.

2.3 Materials and methods

2.3.1 Specimen collection and preparation

Modern material

A total of 75 specimens of *O. hunteri* (OH) and 20 of *O.* cf. *korogwe* (OK) were used to examine overall body morphology (Fig. 2.1). Freshly caught specimens were identified by local fishermen and positioned on graph paper with their left side up and fins spread out. They were photographed from a standard angle (i.e. perpendicular to the dorsoventral and anteroposterior axis) using a Canon EOS400D SLR camera with an 18-55 mm lens. The photographs were pre-processed in Photoshop CS5 to increase contrast, and subsequently stacked in a .tps file using tpsUtil version 1.58 (Rohlf, 2013c) for morphometric analyses.

Qualitative microscopic examination of the trophic apparatus of both *Oreochromis* species revealed great variation in the morphology of oral teeth within a single specimen, compared to their overall variation between specimens of the same species or even between the two species. Therefore, we performed an exhaustive study of all teeth in the left half of the oral jaws, of one representative specimen of each species. Visual inspection of the pharyngeal apparatus showed a large number (ca. 1000) of slender (‘papilliform’) unicuspid teeth in both

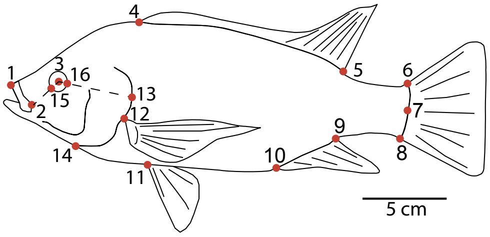


Figure 2.1: Outline drawing of a female *Oreochromis hunteri* from Lake Chala with indication of the digitised landmarks. *1* rostral tip of the upper jaw, *2* caudo-dorsal tip of maxillary bone, *3* centre of the eye, *4* rostral insertion of the dorsal fin, *5* caudal insertion of the dorsal fin, *6* base of the dorsal caudal fin ray, *7* intersection between lateral line and insertion of the caudal fin, *8* base of the ventral caudal fin ray, *9* caudal insertion of the anal fin, *10* rostral insertion of the anal fin, *11* rostral insertion of the pelvic fin, *12* base of the dorsal pectoral fin ray, *13* most caudal point of the operculum, *14* ventral intersection between the branchiostegal membrane and body outline, *15* intersection between the line connecting landmarks 2 and 3 and the eye outline, *16* intersection between the line connecting landmarks 3 and 13 and the eye outline

species. Some preliminary analyses were undertaken on these pharyngeal teeth, but are not discussed here.

After fixation of fish heads in formalin 35%, dentaries and premaxillae were dissected and stained using an Alizarin red S solution, and soft tissues were removed by maceration in 0.5% KOH following Wautier *et al.* (2002). Because each oral jawbone contains ca. 100-160 teeth and because of bilateral symmetry, all data presented are from teeth in the left dentary (DTL) and premaxilla (PML). Overview photographs of each jawbone were taken to keep track of the exact location of individual teeth (Fig. 2.2), which were divided into five groups. The first group (DTL1/ PML1) comprises the outer series of oral teeth. Group DTL2/PML2 constitutes the outer row of the inner series of teeth, from here on referred to as the second series, which is similarly well defined. The other teeth are more chaotically positioned and not evidently arranged in series. Consequently, we split them in an anterior (DTLA/PMLA), middle (DTLM/PMLM) and posterior (DTLP/PMLP) group. Soft tissue maceration for 20-25 h allowed easy extraction of all teeth from the jawbones, under a Nikon SMZ-U binocular microscope (zoom 1:10).

Each tooth was mounted in glycerin on a microscope slide and oriented with its convex side up. Photographs were taken at 60x magnification, using an

Olympus Camedia C5060 Wide Zoom, which is a 5.1 megapixel camera with HQ lens and 4x optical zoom, attached to the optic mount of the binocular microscope. Because the strong curvature of large teeth hampered standardised orientation, their base was clipped with a scalpel in such a way that the crown could be mounted in a flat plane. After imaging, all teeth were stored individually in 100% ethanol.

The contrast of tooth images was enhanced in Photoshop CS5. Photographs of PML teeth were mirrored to allow direct comparison with DTL teeth, because teeth in the premaxilla develop as counterparts to those in the dentary. For consistency, also tricuspid teeth were mirrored, although they are bilaterally symmetrical and mirroring does not generally affect their morphology. Photographs of individual teeth were combined in a .tps file using tpsUtil and served as input files for length measurements and geometric-morphometric digitisation.

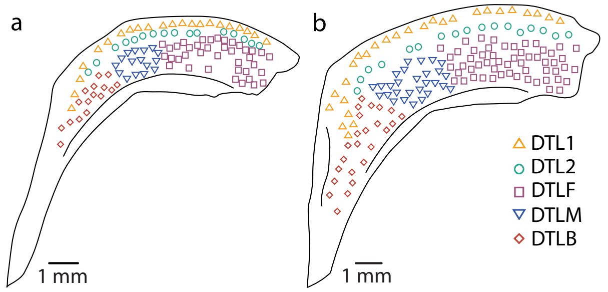


Figure 2.2: Left dentaries (dorsal view) of *Oreochromis hunteri* (**a**) and *O.* cf.*korogwe* (**b**) showing the position of individual oral teeth, subdivided into five groups: outer series (DTL1), second (outer row of inner) series (DTL2), and the anterior (DTLA), middle (DTLM) and posterior (DTLP) portions of the other inner series

Fossil material

In 2003 and 2005, the CHALLACEA project recovered a 21.65-m-long sequence of mostly finely laminated organic muds from the middle of Lake Chala (Verschuren *et al.*, 2009). Excision of five turbidite sections yielded a 20.60-m-long sequence of continuous offshore lacustrine sedimentation spanning the last 25,000 years (Blaauw *et al.*, 2011). Four large-volume sediment samples (Table 2.1), each representing a ca.100-year time interval, had been extracted partly from the master core sequence and partly from overlapping core sections, originally for the purpose of compound-specific radiocarbon dating. For this pilot study, these bulk samples were subsequently processed by adding 10% NaOH and heating to 75℃ for ca. 30 min, while stirring continuously. Subsequently, the samples were sieved on stacked 250- and 150-μm mesh sieves to recover all particles larger than 150 μm in two size fractions. Both fractions were searched using a Nikon SMZ-U Zoom 1:10 binocular microscope. All fossil teeth, oral and pharyngeal, were picked with a brush, photographed and stored in Eppendorf vials. The sample dated to 19,100 year BP did not contain any fossil fish teeth. Measurement and morphological analysis of fossil teeth was identical to that of the modern teeth. Fossil bicuspid teeth derived from right dentaries and left premaxillae were mirrored, for direct comparison with the left dentary and right premaxilla. Given the bilaterally symmetric nature of tricuspid teeth, it was impossible to determine which lateral side they derived from, and hence no mirroring of fossil tricuspid teeth was performed. Teeth from pharyngeal and oral jaws have strongly different morphologies, so that they can be assigned unambiguously to the correct jaw.

Table 2.1: Sediment samples from Lake Chala screened for recovery of fossil fish teeth. Samples are indicated with their depth in the sequence, calibrated age range, sample size (estimated dry sediment weight) and the number of oral teeth recovered; any pharyngeal teeth found are not included. The oldest sample originates from a turbidite section later excised from the composite sediment sequence, and hence its approximate age (ca. 500 years older than adjacent muds; Blaauw *et al.*, 2011) and identical top and basal depths

Depth top (cm)	Depth base (cm)	Age range (cal yr BP)	Estimated dry weight (g)	# fossil oral teeth
572	581	7,000–7,100	17.83	25
666	674	8,200–8,300	21.02	15
1520	1527	19,100–19,200	91.22	0
1951	1951	ca. 24,000	60.62	11

2.3.2 Measurements and morphometric analyses

Overall body morphology

Overall body morphology was documented largely following Kerschbaumer & Sturmbauer (2011) with sixteen traditional landmarks (Fig. 2.1) in tpsDig2 v.2.17 (Rohlf, 2013a). Size was calibrated using the graph paper on which specimens were placed (see above). The digitised landmark dataset was aligned via Procrustes superimposition in the program CoordGen6h of the Integrated Morphometrics Package software (IMP; Sheets, 2008). After all variation in size, position and orientation was removed, Procrustes coordinates and rescaled centroid size were saved as a data matrix file in IMP format. Centroid size is a reliable size measure (e.g., Bookstein, 1997), and other fish studies report strong correlations between centroid size and traditional length measurements (Langerhans & DeWitt, 2004; Leinonen *et al.*, 2006). The overall variation in body shape was then analysed via principal component analysis (PCA) on the Procrustes superimposed coordinates. We performed model-based clustering on the first two principal components (PC) without *a priori* species designation, to test whether OH and OK can be reliably differentiated based on body shape. These analyses were performed in 'Mclust' v.4.3 (Fraley *et al.*, 2012) in R (R Development Core Team, 2016), which was used for all statistical analyses. The performance of different clustering algorithms was evaluated using a Bayesian Information Criterion (BIC), which documents the optimal number of clusters that can be assigned to the data without over-fitting. We also determined the axes of maximal group separation using discriminant function analysis (DFA). A partial warp matrix was generated from the Procrustes superimposed coordinates using tpsRelw v.1.53 (Rohlf, 2013b), and we performed a DFA on these partial warp scores in PAST v.2.17 (Hammer *et al.*, 2001), in order to reveal more subtle morphological differences between the species. Finally, because the analysed OH specimens were generally larger than OK specimens, we studied the extent to which variation is caused by growth allometry. First, we performed a general least-squares regression of PC1 versus log centroid size (LCS), and subsequently we executed a MANCOVA on PC1 and LCS using tpsRegr version 1.40 (Rohlf, 2011) to compare slopes and intercepts between both species. If both nominal species would display similar slopes and intercepts, they would share the same relationship between shape and size (LCS), which would suggest that all variation is caused by allometry. This scenario would then imply that we had collected a biased sample of one species, rather than of the two morphologically distinct species identified by Trewavas (1983) and Seegers *et al.* (2003).

Tooth measurements

Traditional length measurements on complete teeth were used to evaluate possible differences in tooth shape between the two nominal *Oreochromis* species, parallelling overall differences in body morphology. Although these measurements are less time-consuming than geometric-morphometric analysis of tooth crown morphology, they cannot readily be used for measuring fossil teeth, because these are often broken in ways that do not allow reconstructing general tooth morphology. Moreover, the measurements may reveal information that is complementary to that obtained via geometric morphometrics. Length and width of complete, unclipped teeth were measured in number of pixels, using the measurement tool in tpsDig2. Length was measured along the median axis of the tooth neck as the distance from the top of the major cusp to the tooth base (Fig. 2.3); width was measured at the narrowest point of the neck, and subsequently the length/width ratio was calculated. We performed a non-parametric KruskalWallis Rank test with these ratios to discern differences between the two species.

Tooth digitisation for geometric morphometrics

We examined crown morphology in more detail with geometric-morphometric methods. To ascertain the most suitable method for our data, we compared two common methods, semi-landmark (SLM) and elliptic Fourier analysis (EFA). For SLM, a number of homologous points must be established, which can be supplemented with open curves. We positioned landmarks one and three (LM1 and LM3) at the base of the crown, on both sides where the enameloid-dentin junction reaches the tooth outline (Fig. 2.3). Landmark two (LM2) was positioned at the top of the major/middle cusp, by shifting the baseline defined by LM1 and 3 until it lies tangential to the most apical point of the major cusp. If, due to wear or abrasion, the location of these landmarks was not clear, the tooth was excluded from analysis. Eventually, we analysed 468 teeth, 51 of which were fossils. Although the homology criterion is somewhat relaxed for LM2, inclusion of this landmark significantly increased alignment accuracy compared to analyses that only used LM1 and LM3 (C. Manntschke, unpublished data). Two open curves, each with 20 equidistant semi-landmark points digitised in clockwise direction, were anchored between

LM1 and LM2, and between LM2 and LM3. Digitisations for SLM were performed in tpsDig2. The generated semi-landmarks were initially specified as landmarks using tpsUtil, so that partial Procrustes super-impositioning could be performed in CoordGen6h. Generated Procrustes coordinates and centroid size were saved as a data matrix file in IMP format. Centroid sizes are comparable between teeth without additional calibration, because all teeth were imaged at the same magnification (60x). These data were subsequently loaded in SemiLand6 to align the semi-landmarks by sliding them along the curves using the perpendicular projection criterion (Perez *et al.*, 2006).

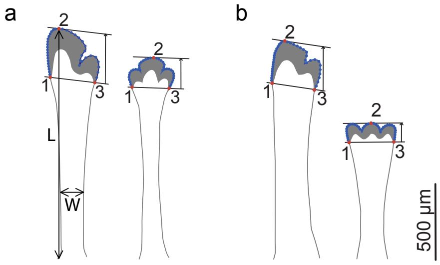


Figure 2.3: Outline drawings of representative bicuspid and tricuspid oral teeth from *Oreochromis hunteri* (**a**) and *O.* cf. *korogwe* (**b**), with enameloid-covered area shaded grey. Semi-landmark analysis (SLM) involved three landmarks (1-2-3), supplemented with two open curves consisting of twenty equally spaced semilandmarks

EFA was performed using a closed curve tracing the outline of the tooth crown and connecting the LM1 and LM3 positions on the enameloid-dentin boundary. Before outlines were generated, tooth images were converted into black and white bitmap files with Photoshop CS5 to maximise contrast. Further analyses were carried out using the SHAPE software package (Iwata & Ukai, 2002). All generated bitmap files were loaded into SHAPE ChainCoder v.1.3, where outlines were traced in counter-clockwise direction, and a chain code file was generated. Contour data were aligned automatically, and the digitised chain codes were imported in SHAPE CHC2NEF v.1.3a to obtain Fourier coefficients. The maximum number of harmonics was set to 50, and standardisation for size, rotation and starting point was based on the first harmonic.

Comparison of SLM and EFA

For comparison of SLM and EFA, data were ordinated using nonmetric multidimensional scaling (NMDS) based on Procrustes superimposed coordinates (SLM) and Fourier coefficients (EFA). We used NMDS because of its limited assumptions, because the number of components can be chosen and because the analysis generates a stress value (i.e. goodness of fit), which makes comparison between the methods more straightforward. We constructed Euclidean distance matrices for both datasets and performed ordination in two dimensions. Preliminary runs reached convergence before 20 iterations, and hence we set the maximum number of iterations to 50 and performed the analyses with 1000 random starting configurations. Stress values were obtained, multiplied by 100 and evaluated following Kruskal & Clarke's rules of thumb (Kruskal, 1964; Clarke, 1993), indicating ordination to be good if stress values are ≤ 10. Analyses were performed in R, using the packages 'vegan' (v.2.0.10; Oksanen *et al.*, 2013) and 'MASS' (v.7.3.34; Venables & Ripley, 2002). Furthermore, we analysed the correlation of pairwise distances in order to quantify the similarity in how both methods handle tooth shape variation. We performed Mantel tests on Euclidean distance matrices constructed on the basis of superimposed coordinates (SLM) and Fourier coefficients (EFA); these permutate rows and columns randomly and yield distribution matrix correlations for unrelated matrices (Dietz, 1983). We used the package 'ade4' (v.1.6.2; Chessel *et al.*, 2004) with 1,000 random permutations.

Comparison of tooth morphology in modern and fossil fishes

Morphospace occupation of fossil and modern fish teeth was assessed with PCA. To check whether our *a priori* classification of bi- and tricuspid teeth was consistent with their location in morphospace, we performed model-based clustering with 2 models on the first 2 PCs and examined the error rate. Subsequently, the first principal component score was analysed and compared between groups to assess the significance of any differences in average tooth morphology between the fossil and the modern-day *Oreochromis* populations in Lake Chala. We therefore performed a Kruskal-Wallis test in R, for bicuspid and tricuspid teeth separately, followed by *post-hoc* comparisons using Tukey HSD tests (included in the standard package of R).

2.4 Results

2.4.1 Overall body morphology

Ordination (PCA) of the landmark coordinates of 75 *O. hunteri* (OH) and 20 *O.* cf. *korogwe* (OK) specimens from Lake Chala shows a strongly bimodal pattern in overall body shape along the first principal component (PC1; Fig. 2.4a). Main sources of variation on PC1 reflect the length of the tail (long for OH, short for OK) and body depth (slender, shallow habitus for OH; more robust, deeper body for OK). Variation on PC2 mainly reflects differences in the shape of the head, as represented by the relative distance between the rostral tip of the upper jaw and the most caudal point of the operculum (Fig. 2.1). Applying model-based clustering to the first two principal components yielded greatest support for solutions with only two clusters (ΔBIC ≥ 5.5 over models with fewer or more clusters). These two clusters coincide entirely with the two species as identified in the field, apart from one aberrant, slender OK specimen that clusters with OH. These results are further corroborated by the DFA, which separates groups along a highly similar morphological axis. Regression of PC1 versus centroid size indicates a strong and significant relationship between specimen size and shape, and hence allometric variation (t-value = 11.45, $p < 0.001$). MANCOVA results indicated that OH and OK do not differ significantly in the slope of allometric growth (Fig. 2.4b: Wilks' K = 0.64, p = 0.99), but they do in intercepts (Wilks' K = 0.071, $p < 0.001$), which suggests that the relationship between size and shape differs between the two species, supporting the taxonomic division between OH and OK. Hence, the shape differences between both species do not solely result from allometry.

2.4.2 Shape of oral teeth

Qualitative visual inspection of tooth shape and arrangement in the oral jaw bones (dentary and premaxilla) hints towards a strong similarity between the two studied *Oreochromis* species (Fig. A.1). In both species, the outer series (DTL1/PML1; Fig. 2.2) consists of bicuspid teeth, whereas the second series (DTL2/ PML2) and more inward placed teeth are mostly tricuspid, except for some of the posterior teeth (DTLP/ PMLP) which are more unicuspid in shape (but distinct from unicuspid pharyngeal teeth), mostly in the dentaries. No obvious differences were observed between teeth from the upper (PML) and

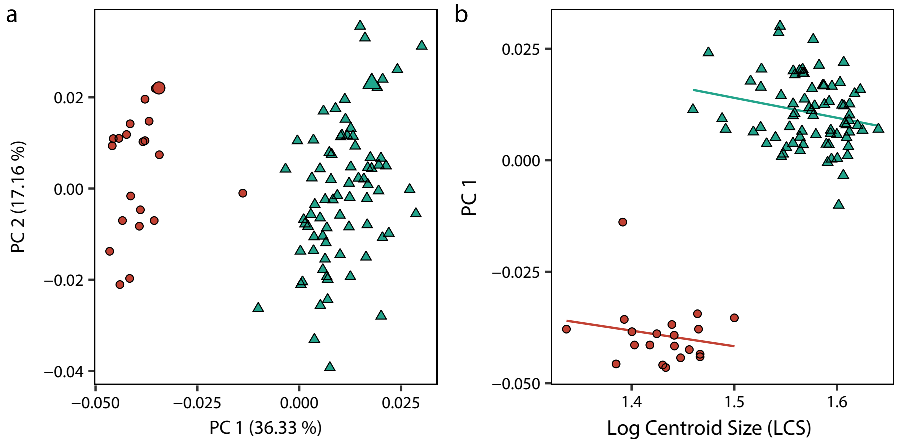


Figure 2.4: **a** PCA ordination plot showing the variation of overall body morphology in modern-day Lake Chala *Oreochromis*, based on 16 landmarks (cf. Fig. 2.1) measured on 75 *O. hunteri* (green △) and 20 *O.* cf.*korogwe* (red ○) specimens. The two enlarged symbols represent the specimens used for analysis of tooth morphology. **b** MANCOVA test on overall body morphology of both species corrected for size, showing similar regression slopes of growth allometry but significantly different intercepts

lower (DTL) jaws for either species. The total number of oral teeth differed considerably between OH (n = 268) and OK (n = 307), but examination of more specimens is required to examine whether this difference is consistent. However, the number of oral teeth that could be included in the geometric morphometric dataset is similar (Supplementary Table A.1).

Traditional length measurements revealed that the length/width ratio of oral teeth of both species is significantly different (Kruskal-Wallis $\chi^2 = 116.3$, $p < 0.001$; Fig. 2.5). The range in tooth length is roughly equal for both species, but OK teeth are wider at their narrowest point. The difference is most pronounced in the longest teeth. Shorter teeth show a clear overlap between the species, but nevertheless the teeth of OK can be considered more stout than those of OH. Teeth with overlapping morphology mostly derive from the inner series (DTL/ PML A-M-P).

Model-based clustering on the first two principal components classified six out of 468 teeth differently (1% error rate), and hence both methods of clustering are considered useful. For further analyses, we classified specimens following the generated model.

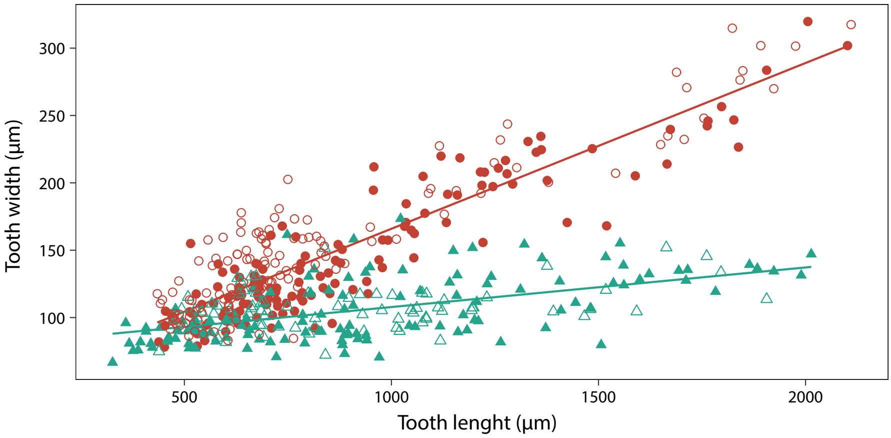


Figure 2.5: Length/width ratio of oral teeth from modern-day *Oreochromis hunteri* (green △; n = 212) and *O.* cf.*korogwe* (red ○; n = 259), distinguishing between teeth from dentaries (hollow symbols) and premaxillae (filled symbols). Best-fit linear regression lines are shown per species. Length is the distance from the top of the major cusp to the base of implantation, and width is measured at the narrowest point of the neck (cf. Fig 2.3)

2.4.3 Comparative analysis of SLM and EFA

The stress values associated with NMDS were fairly low for both SLM (8.17) and EFA (10.13), indicating that the data can be fit well in two dimensions, and that NMDS ordination plots (Fig. 2.6) can be considered a reliable representation of the variation in the data. Both methods reveal a marked separation between bicuspid and tricuspid teeth along NMDS1. The few unicuspids present in the DTLP/PMLP group form an elongated 'tail' of NMDS scores on the negative end of NMDS2 in both graphs. Most importantly, the position of the five groups is similar for both methods, suggesting that they capture the overall variability in tooth crown morphology in a very comparable manner. This conclusion is supported by a Mantel test between the Euclidean distance matrices of the two datasets, which reveals a strong and highly significant positive correlation ($z = 0.81$, $p < 0.001$).

2.4.4 Tooth crown morphology of extant *Oreochromis*

Ordination based on the SLM coordinates of all modern tooth crowns resulted in a major separation between outer-series (mainly bicuspid) teeth (DTL1/

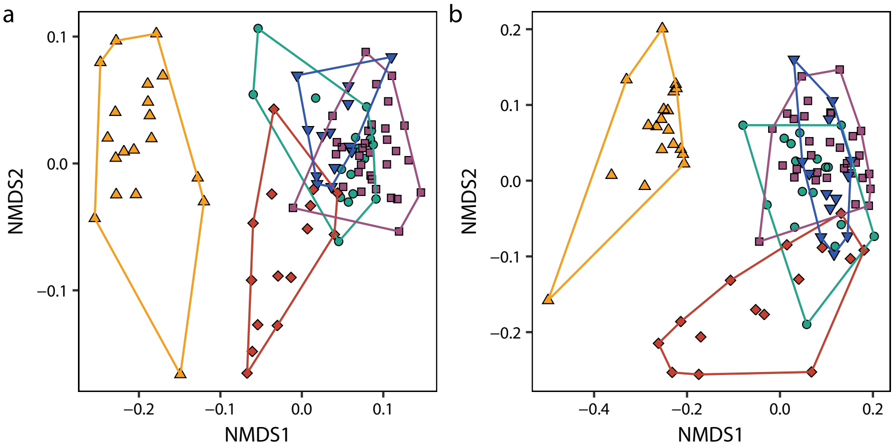


Figure 2.6: Nonmetric multidimensional scaling (NMDS) plots for the crown morphology of mandibular oral teeth in *O.* cf.*korogwe*, assessed using semi-landmark analysis (SLM; **a**) and elliptic Fourier analysis (EFA; **b**). Symbols represent the five pre-defined groups displayed in Fig. 2.2: First (yellow △) and second series teeth (green ○), and anterior (purple □), middle (blue ▽) and posterior (red ◇) inner teeth.

PML1: PC1-values < -0.1) and inner-series (mainly tricuspid) teeth (other groups: PC1-values > -0.1; Fig. 2.7a). Crowns with a somewhat aberrant unicuspid shape plotted mostly on the negative side of PC2 and display some overlap with tricuspid teeth. No obvious differences were observed between DTL and PML teeth belonging to the same group, suggesting that, within an individual specimen, the upper and lower jaw bones bear highly similar teeth. Notably however, both species have several unicuspid teeth in the posterior part of the dentary, but not in the premaxilla (Supplementary Figure A.3). For each tooth type, teeth of the two species occupy largely distinct regions in morphospace, separated along PC2 (Fig. 2.7a).

Because large differences between the major tooth types may obscure more subtle differences of tooth crown variation, outer-series and inner-series teeth were subjected to separate ordinations. After omitting two highly aberrant specimens to enhance spread of datapoints, the PCA of outer-series teeth (Fig. 2.7b) shows that the separation between OH and OK is mainly driven by differences in the relative size of the major crown cusp: bicuspid teeth of OH tend to have a large major cusp, and hence more positive PC1 values, whereas bicuspid teeth of OK, with a relatively smaller major cusp, have negative

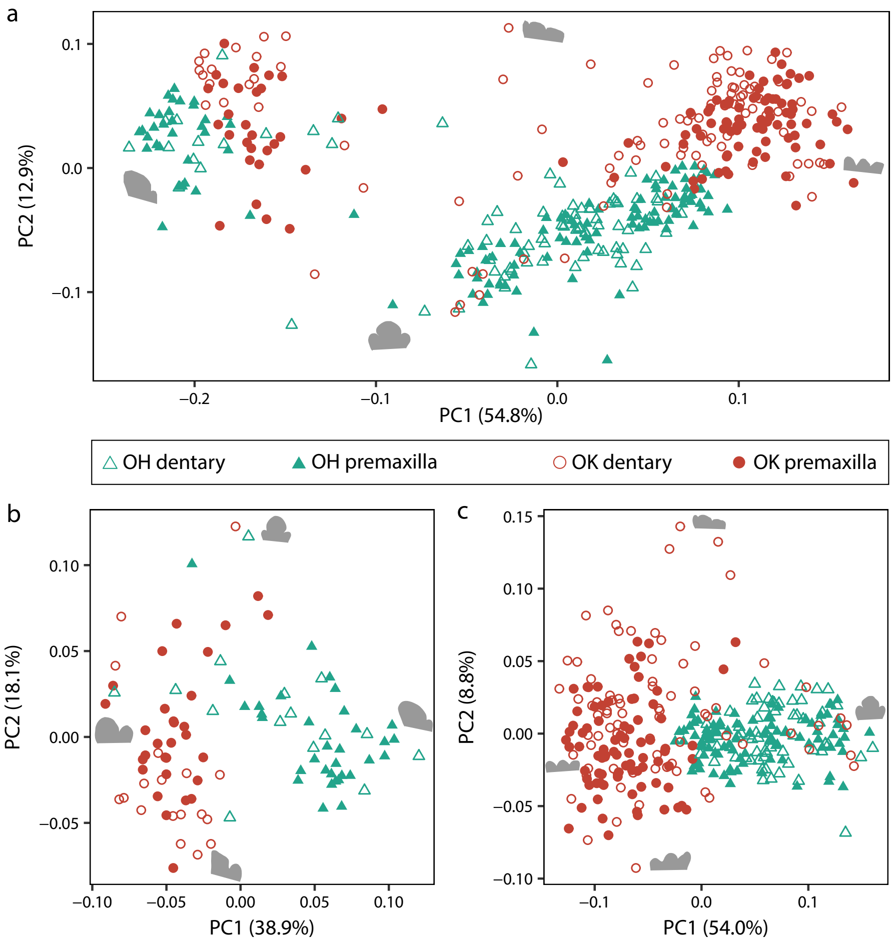


Figure 2.7: PCA ordination plots on SLM coordinates documenting crown morphology in *Oreochromis hunteri* (green △) and *O.* cf. *korogwe* (red ○), distinguishing between teeth from dentaries (hollow symbols) and premaxillae (filled symbols). **a** All modern-day teeth analysed; **b** outer-series (mostly bicuspid) teeth only; **c** inner-series (mostly tricuspid) teeth only. Silhouettes of some representative crown shapes facilitate interpretation of the morphological variation along PC1 and PC2

PC1 values. Variation on PC2 seems to primarily reflect differences in the occlusion surface. OK bicuspids tend to have a sharp major cusp (positive PC2 values), whereas those of OH have a convex, more broadly rounded major cusp (negative PC2 values). Aberrant teeth are also singled out in morphospace, for example, the small number of unicuspid DTLP/PMLP teeth (located between the clusters, but slightly closer to the bicuspids cluster). In addition, one outer-series tooth of the analysed OH specimen had a tricuspid, rather than the usual bicuspid, appearance.

Ordination of inner-series teeth separately (Fig. 2.7c) shows that both taxa are well-differentiated on PC1, which mainly captures variation in the relative size of the central cusp: negative values indicate a small central cusp, as typically found in OK, whereas positive values indicate a large central cusp, as found in OH. PC2 captures information related to asymmetry, a biological signal that is largely related to tooth wear and, hence, rather to function than to original morphology. Original morphology and tooth wear are easily distinguished from one another based on the enameloid-dentin boundary: a sloping boundary suggests an originally asymmetric morphology, whereas a level boundary points towards asymmetry after wear. Highly negative PC2-values indicate that the right side of the crown is worn down, whereas crowns with the left side worn down have highly positive PC2-values. Most crowns, however, have PC2-values close to zero, and are more or less symmetrical, attesting to rather limited wear. The range of PC1-scores is larger for OH than for OK, indicating greater variation in the relative size of the middle cusp in OH, whereas OK showed greater variation along PC2, indicating a greater degree of asymmetry caused by wear. Teeth from the inner series (DTL/PML A-M-P) of the two species overlap partly in crown shape.

2.4.5 Teeth from the fossil archive of Lake Chala

The crowns of 51 fossil teeth from Lake Chala were analysed, and these teeth occupy a combined morphospace that is very similar to that of modern-day teeth (Fig. 2.8), demonstrating that the morphology of fossil teeth is similar to those of the extant *Oreochromis* inhabiting Lake Chala. Hence, cichlid fishes with bicuspid and tricuspid teeth have been present in Lake Chala over at least the past 24,000 years. Nevertheless, some fossil teeth plot outside the morphospace bounds of the major modern-day teeth types, indicating that their morphology is slightly unusual.

The elongated cluster with largely positive PC1-values represents tricuspid teeth. The 10 fossil tricuspid teeth dated to 7,000 years ago (year BP) are spread throughout this cluster, but do not exceed the morphospace boundary of modern inner-series teeth. Average crown morphology was thus similar to that observed within the two examined extant specimens, and no notably different oral tooth shapes were encountered in the fossil record. Roughly, three clusters

Table 2.2: Mean PC1 of bicuspid and tricuspid teeth in extant *Oreochromis hunteri* (OH), extant *O.* cf. *korogwe* (OK) and the three samples of fossil teeth, and adjusted p-values of *post-hoc* comparisons using the Tukey HSD test. P-values indicated with an * highlight significant results. The table illustrates that fossil tricuspid teeth resemble those of the native OH more closely in morphology than those of the introduced OK

	Mean PC1 ± SD	Adjusted P values of Tukey HSD test			
		OH	OK	7,000 yr BP	8,200 yr BP
Bicuspid teeth					
OH	-0.19 ± 0.04				
OK	-0.15 ± 0.04	<0.001*			
7,000 yr BP	-0.18 ± 0.02	0.96	0.04*		
8,200 yr BP	-0.15 ± 0.04	0.52	1	0.72	
24,000 yr BP	-0.18 ± 0.08	0.99	0.52	1	0.83
Tricuspid teeth					
OH	0.03 ± 0.04				
OK	0.10 ± 0.04	<0.001*			
7,000 yr BP	0.05 ± 0.06	0.57	0.02*		
8,200 yr BP	0.00 ± 0.08	0.32	<0.001*	0.10	
24,000 yr BP	-0.02 ± 0.10	0.10	<0.001*	0.03*	0.90

can be distinguished among tricuspids from this time window. One cluster has a crown shape similar to OK-tricuspids, one cluster resembles the OH tricuspids most similar to those of OK, and a third cluster, with negative PC1-values, has typical OH-crowns with a pronounced middle cusp. Almost all tricuspids from this time window are located close to the longitudinal axis of the point cluster, indicating great similarity and limited wear (compare with Fig. 2.7c). The 13 fossil tricuspid teeth dated to 8,200 year BP are distributed in a similar way.The same three clusters (of roughly equal size) can be distinguished, but overall they are displaced slightly towards more negative PC1-values compared to those dated to 7,000 year BP, indicating that they possess a highly pronounced middle cusp, similar to that of some outlier tricuspids in extant OH (cf. Fig. 2.7C). The 7 tricuspid teeth dated to 24,000 year BP show only two distinct crown morphologies: one that is intermediate between the tricuspids of OH and OK, and one with an extremely pronounced middle cusp, sometimes even more pronounced than in outlier modern-day OH tricuspids. In general, the average crown morphology of the tricuspid teeth migrates over time (from 24,000, 8,200 and 7,000 year BP to the modern fauna) towards more positive values on PC1.

This shift is partly corroborated by the significance of a Kruskal-Wallis test on the differences between mean PC1-scores (Kruskal-Wallis $\chi^2 = 156.3$, p $< 2.2 \times 10^{-16}$). *Post-hoc* comparisons using the Tukey HSD test (Table 2.2) indicate that modern-day OH and OK tricuspids differ significantly in the average pronunciation of their middle cusp. All fossil tricuspid groups differ significantly from OK, and hence more closely resemble the native OH than the introduced OK. Between fossil groups, tricuspids from 24,000 year BP have a significantly different morphology than the ones from 7,000 year BP.

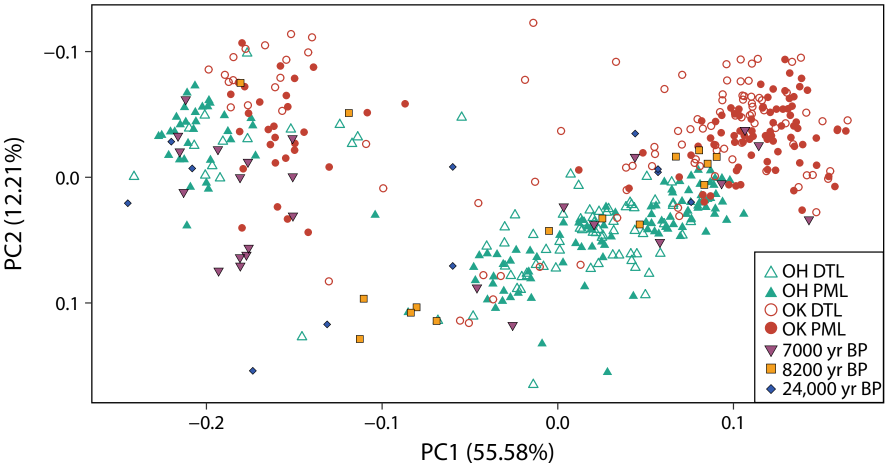


Figure 2.8: PCA ordination of SLM-coordinates documenting crown morphology of all modern-day teeth, and fossil teeth of the three age intervals (7,000, 8,200 and 24,000 years ago) analysed

Fossil bicuspid teeth all plot within or near the morphospace occupied by modern bicuspids, with negative PC1-values. The 15 fossil bicuspid teeth dated to 7,000 year BP span the variation seen in modern-day OH and OK, and some specimens with negative PC2-scores plot outside the modern morphospace occupation (Fig. 8). Bicuspids from sediments dated to 8,200 year BP (n = 2) fall within the crown variability found in OK, whereas those dated to 24,000 year BP (n = 4) more closely resemble bicuspids of modern-day OH. *Post-hoc* comparisons, however, indicate that only bicuspids from 7,000 year BP are significantly different from modern-day OK bicuspids; none of the three groups of fossil bicuspid teeth differ significantly from one another (Table 2.2).

2.5 Discussion

2.5.1 Comparative analysis of SLM and EFA

The EFA and SLM methods both have merits that make them useful tools to document tooth crown morphology. The main advantage of EFA is that this method is more robust against violations of the homology assumption (Van Bocxlaer & Schultheiß, 2010). At the downside, closing the crown outline with a straight line along the (poorly-defined) crown-neck boundary results in propagation of error along this line, because points along it are not independent. This creates artificial shape variation which potentially blurs other, more biologically relevant shape information. However, difficulty to accurately define the crown-neck boundary implies that locating LM1 and LM3 for SLM was not always unambiguous either. Furthermore, our method to locate LM2 directly depends on the positions of LM1 and LM3, so that some uncertainty may have been propagated towards LM2 as well. Because both methods produced very similar and fully consistent results, and because landmark-based approaches are conceptually more straightforward, we adopted SLM as the principal method in this study. Moreover, SLM data are more directly comparable to those of the landmark analysis used to characterise overall body morphology.

2.5.2 The modern-day *Oreochromis* of Lake Chala: morphology and feeding ecology

The clearly bimodal distribution of overall body morphology, based on 16 landmark characters, of modern-day *Oreochromis* in Lake Chala supports the presence of two species, *O. hunteri* and *O.* cf. *korogwe* (Trewavas, 1983), which are also recognised by local fishermen as morphologically distinct. Body depth accounts for most of the shape variation between the two species. The lack of overlap and limited number of intermediate forms supports the taxonomic status of these species and argues against the occurrence of significant interbreeding between both species in Lake Chala. One slender OK specimen clusters with OH and potentially represents a rare hybrid specimen. However, the mitochondrial DNA of this specimen (BANDIA cha022) is identical to other *O.* cf. *korogwe* specimens (Chapter 3), arguing against its hybrid status. The natural distribution of *O. korogwe* is limited to the lower parts of the Pangani River basin in northeastern Tanzania (Trewavas, 1983). It was introduced in

Lake Chala (Seegers *et al.*, 2003), most likely in the 1950s or early 1960s, by a colonial-era owner (Caxton Oluseno, pers. comm. 2014). Hence, we do not expect to find remains of this species in the lake's (pre-20^{th} century) fossil record.

The ecology and feeding habits of *O. hunteri* and *O.* cf. *korogwe* are poorly known. A concise description in Trewavas (1983) suggests that the young of *O. hunteri* feed on algae and organic debris, but no information on adults is given. The diet of *O. korogwe* is unknown, except that in fish-culture ponds the species feeds on algae and insects. This information clearly does not suffice to draw conclusions on their respective diets in Lake Chala, whereas our biometric and geometric-morphometric data on the oral jaw teeth of both species do allow tentative inferences on feeding ecology. We revealed substantial morphological differences between OH and OK in overall body shape as well as in the morphology of their oral teeth, both general and crown-specific. Given these recurrent and consistent differences, it is likely that the two *Oreochromis* species inhabiting Lake Chala exploit different ecological niches.

The large number of oral teeth in wide, U-shaped jaws, and the combination of an outer series of bicuspid teeth with multiple inner series of tricuspid teeth (as well as a large number of papilliform pharyngeal teeth, not studied in detail here) suggest that both *O. hunteri* and *O.* cf. *korogwe* graze on epilithic algae (e.g., Witte & Van Oijen, 1990; Rüber & Adams, 2001). In the large African rift lakes, such epilithic grazers can form ecologically stable cichlid communities with very high species diversity (Reinthal, 1990). Notwithstanding that multiple species may all appear to feed on highly similar resources, namely algae growing on rocks, fine-scaled resource partitioning within such communities has been described extensively (e.g., Fryer, 1957; Genner *et al.*, 1999), and this partitioning has been linked to an equally great diversity of trophic morphologies (Greenwood & Gee, 1969). The consistent and marked differences in tooth morphology of OH and OK, as revealed by this study, suggests that co-occurrence of the native and introduced *Oreochromis* in Lake Chala is facilitated by diet-related ecological differentiation.

The widely separated, mobile and slender oral teeth of *O. hunteri* (Fig. 2.3a; Supplementary Fig. A.1) seem most appropriate for a diet of the so-called 'Aufwuchs', i.e. the organisms that live loosely attached within epilithic algae (Fryer, 1957). This Aufwuchs may include diatoms, other microscopic autotrophic organisms, microcrustacea and occasionally the nymphs and larvae of aquatic insects. The slender shape of the teeth and their widespaced arrangement allows them to function both as a comb and as a sieve. Moreover, the bicuspid and second-series tricuspid teeth of OH have a relatively larger and more concave central cusp than those of OK, which gives these teeth a shovel-like appearance that is adapted to scooping loose material from between filamentous algae attached to rocks.

The oral teeth of *O.* cf. *korogwe* are much more robust than those of *O. hunteri* (Fig. 2.3b; Supplementary Fig. A.2), and they are more closely spaced and more firmly attached to the jaw bones. All of these observations indicate that OK is well equipped to scrape filamentous algae from rocks (Yamaoka, 1983). The crowns of its outer-series (bicuspid) teeth are typically obliquely truncated, with a minor cusp that is taller in comparison to the bicuspids of OH. This type of tooth crown, and close tooth spacing, gives the outer series a continuous but notched appearance, like the blade of a jagged knife. The second series of teeth forms a flattened and even surface of densely spaced tricuspid teeth, allowing the fish to scrape algae thoroughly from the rocks. The flattened appearance of second-series tricuspids is due to their specific crown morphology, with minor cusps that almost equal the major cusp in size.

Both species have a number of unicuspid teeth in the posterior part of the dentary, which probably are new first-generation teeth that were added to the jaw as a result of growth (Huysseune & Witten, 2018). These unicuspid teeth are largely absent from the premaxilla, suggesting that new teeth are first added to the dentary in these *Oreochromis* species.

The functional interpretation of tooth morphology corresponds to the observed patterns of tooth wear. Greater tooth wear is expected in OK than in OH, because of the functional implications of scraping and brushing feeding styles, respectively. Our observation of greater differences in the degree of cusp asymmetry in oral teeth of OK compared to OH is consistent with more substantial wear. The greater bite forces needed for scraping may moreover explain the more robust dentaries of OK (Fig. 2.2).

2.5.3 Tooth morphology of fossil cichlids from Lake Chala

The high amount of intraspecific morphological variation in the modern-day *Oreochromis* species of Lake Chala ideally requires large numbers of fossil teeth to trace morphological change through time (Chapter 6). The limited number of fossil teeth examined in this Chapter therefore urges caution in the interpretation of our results. Nevertheless, the morphospace occupied by the fossil tooth crowns shows a number of aspects that are worth discussing in more detail.

First, fossil teeth recovered from lake sediments of any past time interval are very likely derived from multiple specimens. Consequently, the broadly similar morphospace occupation of fossil and modern tooth crowns indicates that, as anticipated, variation in tooth morphology between individuals of a species is significantly more limited than the morphological disparity of teeth within the jaws of a single specimen. This result suggests that teeth from a limited number of specimens provide a reasonable estimate of the morphological variation within a population. However, this methodological convenience comes with a potential disadvantage: if great variation is observed within populations (groups of interbreeding individuals), but limited variation exists between populations or species, the opportunities to reconstruct ecological or evolutionary trends in trophic morphology through time may be limited. Given that this issue may compromise our primary research objectives, we explore potential trends in the fossil data further.

Although the morphospace occupied by our collection of fossil teeth does not fully overlap with those of modern-day *O. hunteri* in Lake Chala (Fig. 2.8), the recorded differences between fossil and modern teeth are rather limited and suggest that the fossil teeth also derive from a large cichlid feeding on epilithic algae. Our fossil data further revealed a modest but consistent gradual shift of mean tooth morphology through time, expressed mainly in tricuspid teeth and towards the morphology of modern-day *O. hunteri*. In consideration of the small size and disjunct time line of our current fossil collection, we refrain from drawing inferences about a possible long-term shift in the feeding ecology of the *Oreochromis* from Lake Chala. Nevertheless, these results suggest that the presumed disadvantageous combination of limited variation between populations with large variation within specimens does not seem to hamper detection of a long-term trend in tooth morphology, even if the magnitude of these changes through time is limited in comparison with variation within assemblages.

The available paleo-environmental (e.g. Verschuren *et al.*, 2009; Barker *et al.*, 2011) and geochronological (Moernaut *et al.*, 2010; Blaauw *et al.*, 2011) data indicate that a deep, freshwater aquatic ecosystem with rocky shoreline existed continuously in the Chala Basin throughout the last 25,000 years; there is no indication for large ecological disturbances that could have eradicated the local fish community. Hence, the lack of fossils in the sample from 19,200 year BP is notable, and more detailed sampling around that period is required to figure out what may have cause this absence. Although natural colonisation of an isolated crater lake by fish is considered largely a rare chance event (Elmer *et al.*, 2012), the presence of cichlids in even very young isolated lakes suggests that on longtime scales, colonisation of vacant habitat may occur relatively frequently (Elmer *et al.*, 2010b). In this regard, re-colonisation of Lake Chala from closely-related parent populations could explain similarity in shape of fossils before and after a temporary absence.

2.6 Conclusion

This exploratory methodological study demonstrates the potential of fossil cichlid teeth extracted from lake-sediment cores to trace morphological change in their trophic apparatus through time. At least in a well-controlled setting such as Lake Chala, morphological analysis of fossil cichlid teeth may thus provide a means to elucidate the natural history of local fish communities including their ecological and evolutionary dynamics. Contingent upon the availability of greater fossil collections, more intensive and finer-spaced sampling of fossil teeth will be required to confirm whether the apparent trend in the morphology of Lake Chala *Oreochromis* is real. Moreover, comprehending the evolutionary and ecological changes behind the observed morphological trends will require better understanding of the feeding ecology of modern-day *O. hunteri* and related species, and its relationship with their trophic morphology.

2.7 List of modifications

- The species name *Oreochromis korogwe* is replaced by *O.* cf. *korogwe*, in accordance with findings in Chapter 3.
- *Tilapia rendalli* is now *Coptodon rendalli* (Dunz & Schliewen, 2013).
- In Material and methods, the rationale behind the selection of the four fossil assemblages is now addressed (Section 2.3.1).
- The presence of several unicuspid teeth in the posterior part of the dentaries is now addressed in the Results (Section 2.4.4) and in the Discussion (Section 2.5.2).
- An intermediate *O.* cf. *korogwe* specimen is discussed in more detail in the Discussion (Section 2.5.2).
- The high number of oral teeth required to overcome intraspecific shape variation, with reference to Chapter 6, is addressed in the Discussion, Section 2.5.3.
- Photographs of cleared-and-stained jaws of *O. hunteri* and *O.* cf. *korogwe* have been added to Appendix A.
- Appendix A now contains a table listing the number of teeth per jaw region included in the morphometric dataset.
- A new figure indicating the location of the different tooth types (unicuspid – bicuspid – tricuspid) in morphospace is added in Appendix A.

CHAPTER 3

Species integrity and origin of *Oreochromis hunteri*

Jorunn Dieleman[1,*]
Moritz Muschick[2,3,4,*]
Wanja Dorothy Nyingi[5]
Dirk Verschuren[1]

Modified from**: Dieleman, J., Muschick, M., Nyingi, W.D., & Verschuren, D. (2018). Species integrity and origin of *Oreochromis hunteri* (Pisces: Cichlidae), endemic to crater Lake Chala (Kenya-Tanzania). *Hydrobiologia* Online first. doi.org/10.1007/s10750-018-3570-7.

[1] Limnology Unit, Department of Biology, Ghent University, K. L. Ledeganckstraat 35, B-9000 Ghent, Belgium
[2] Department of Animal and Plant Sciences, University of Sheffield, Sheffield, S10 2TN, UK
[3] Aquatic Ecology & Evolution, Institute of Ecology and Evolution, University of Bern, CH-3012, Switzerland
[4] Department of Fish Ecology & Evolution, EAWAG, Swiss Federal Institute for Aquatic Science and Technology, CH-6047, Kastanienbaum, Switzerland
[5] Ichthyology Section, Zoology Department, National Museums of Kenya, P.O. Box 40658-00100, Nairobi, Kenya

[*] Equal contribution

[**] See Section 3.6 for a list of modifications.

3.1 Abstract

Extensive transfer of tilapia between lakes throughout East Africa has often led to hybridisation with indigenous fish populations. The endemic *Oreochromis hunteri* of Lake Chala, an isolated crater lake near Mount Kilimanjaro, is potentially susceptible to such introgression from a species formerly identified as *Oreochromis korogwe*, introduced 30 years ago. We combined whole-body geometric morphometry on 104 Lake Chala specimens of both taxa with molecular phylogenetic analysis of mitochondrial loci from 15 *O. hunteri* and 9 *O.* cf. *korogwe* specimens to assess whether hybridisation has occurred. Using fishes from Lake Jipe and Nyumba ya Mungu reservoir, we expanded our analysis to all four *Oreochromis* species currently inhabiting the Upper Pangani River system to determine the closest living relative of *O. hunteri*, and hence the possible source population of the ancestral species which colonised Lake Chala. Our results indicate that no interbreeding occurs between *O. hunteri* and *O.* cf. *korogwe*, and infer *O. jipe* to be the closest living relative of *O. hunteri*. The introduced *O.* cf. *korogwe* is a phenotypically uniform but genetically variable population, the identity of which remains unknown. The high haplotype diversity of *O. hunteri* is consistent with available fossil evidence indicating that its ancestor colonised Lake Chala at least 25,000 years ago.

3.2 Introduction

Tilapia is one of the most productive food fishes in Africa. Tilapia-based fisheries provide an often indispensable protein source for local food security, and especially *Oreochromis* species have been utilised for this purpose in global aquaculture (Eknath & Hulata, 2009). This has led to extensive transfer from their natural ranges into other regions and countries (Eknath & Hulata, 2009). The invasive nature of some *Oreochromis* species and their propensity for hybridisation rightfully has raised concern when they are being introduced to regions with indigenous tilapiine communities (e.g. Agnèse *et al.*, 1998, D'Amato *et al.*, 2007, Nyingi & Agnèse, 2007, Angienda *et al.*, 2011, Deines *et al.*, 2014, Ndiwa *et al.*, 2014). It has been suggested that the introgression of alien genes into local species may also contribute to rapid speciation in cichlids

(Salzburger *et al.*, 2002, Meier *et al.*, 2017b). However, most often it simply induces a loss of genetic diversity, through the homogenisation of gene pools (Nyingi & Agnèse, 2007, Crispo *et al.*, 2011, Firmat *et al.*, 2013).

Lake Chala (locally 'Challa', after a nearby village) is a crater lake bridging the border between Kenya and Tanzania, immediately to the southeast of Mount Kilimanjaro in East Africa. It harbours the only natural population of *Oreochromis hunteri* (Günther, 1889, Seegers *et al.*, 2003), the type species of the genus *Oreochromis* Günther, 1889 (Trewavas, 1983). Until the early 1980s it also seems to have been the only fish species inhabiting Lake Chala, as surveys carried out in 1889, 1902, 1946, 1952 and 1980 did not reveal other species (Günther, 1889, Dadzie *et al.*, 1988). However, sometime in the late 20^{th} century two other tilapiine species were introduced, namely *Coptodon rendalli* Boulenger, 1896 and *Oreochromis korogwe* Lowe, 1955 (Dadzie *et al.*, 1988, Seegers *et al.*, 2003), as well as a small cichlid identified as *Haplochromis* spec. "Chala" (Seegers *et al.*, 2003). The two *Oreochromis* species have the potential to hybridise, as the deep open-water environment of the lake encircled by near-vertical rocky crater walls appears to offer limited potential for reproductive or niche segregation.

Recent research on the long-term evolutionary and ecological dynamics of *O. hunteri* in Lake Chala is based on analysis of its fossil teeth recovered from the sediment record (Chapter 2, Chapter 6). This study uses the extant *O. hunteri* population as principal reference for variation in fossil tooth morphology, and assumes that it is genetically pure. However, introgression of genetic material due to interbreeding with an introduced species could potentially impact important morphological features (Parnell *et al.*, 2012, Holzman & Hulsey, 2017). Such a recently compromised species integrity would complicate the comparison of modern phenotypes with the fossil record. Quantitative examination of general body morphology and the shape of oral teeth (Chapter 2) found the two local *Oreochromis* species to be clearly distinct, arguing against recent and/or ongoing hybridisation. However, demonstration of the presence or absence of shared haplotypes with molecular-genetic methods would provide a more sensitive test of hybridisation. Such genetic assessment is particularly relevant for the endemic *O. hunteri* population in Lake Chala, where detection of recent hybridisation would have implications for both taxonomy and conservation. Also, comparing genetic data of *O. hunteri* with that of *Oreochromis* populations from nearby waters might help reveal its phylogenetic associations, by providing information on the possible source population(s) from where the isolated crater lake was colonised, and on the approximate timing of this colonisation (Barluenga *et al.*, 2006, Elmer *et al.*, 2012, Genner & Turner, 2014).

In this Chapter, we aim to (i) validate with genetic evidence the quantitative morphological differences and apparent lack of interbreeding between the endemic and introduced *Oreochromis* in Lake Chala; (ii) identify the closest relative of *Oreochromis hunteri* by molecular phylogenetic analysis of the *Oreochromis* fauna inhabiting Lake Chala and the only two nearby lakes, namely Lake Jipe and the Nyumba ya Mungu reservoir; and (iii) provide an estimate of the timing of *O. hunteri*'s arrival in Lake Chala.

3.2.1 Study area

Lake Chala (3°19'S, 37°42'E) is a freshwater lake with a surface area of 4.5 km^2 and a maximum depth that has fluctuated between 92 m and 98 m since 1999 (Wolff *et al.*, 2014). The lake fills a volcanic caldera basin at ~880 m above sea level immediately east of Mount Kilimanjaro, and is hydrologically mainly influenced by subsurface in- and outflow (Moernaut *et al.*, 2010). Despite its isolated location, biogeographically it is considered part of the Upper Pangani River basin (Seegers *et al.*, 2003), which also contains Lake Jipe (Kenya/Tanzania) and the man-made reservoir Nyumba ya Mungu (Tanzania; Fig. 3.1). The former is a medium-sized (30 km^2) but very shallow (<3 m) muddy lake located immediately east of the Pare Mountains. It is fed by the Lumi River and drains into the Ruvu River, both at the northern end of the lake, and its shoreline is fringed with swamps. Nyumba ya Mungu was created in 1965, has a surface area of 110 - 180 km^2 depending on rainfall and drawdown, and gradually increases in depth from 4 m in the north to 41 m at the dam (Denny, 1978). It lies in the north-south trending valley between the Lelatema and Pare Mountains, at the former confluence of the Ruvu and Kikuletwa rivers. The reservoir drains into the Pangani River, which flows into the Indian Ocean 500 km to the southeast.

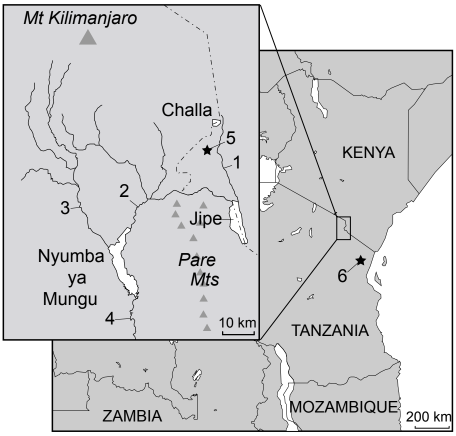


Figure 3.1: Skeleton maps of East Africa and the Upper Pangani River basin (inset) in northern Tanzania, with indication of the sampled surface waters Chala, Jipe and Nyumba ya Mungu. River systems (1-4) and towns (5-6) mentioned in the text are indicated by numbers. 1: Lumi, 2: Ruvu, 3: Kikuletwa, 4: Pangani, 5: Taveta, 6: Korogwe

3.2.2 Regional ichthyofauna of the Upper Pangani system

The indigenous *Oreochromis* fauna of the Upper Pangani region is rather modest. Apart from *O. hunteri*, endemic to Lake Chala, *O. jipe* is considered indigenous to both Lake Jipe and the Pangani River itself (Lowe, 1955, Trewavas, 1983, Seegers *et al.*, 2003). Trewavas (1983) also reports the species *O. pangani*, with subspecies *O. pangani pangani* in the Pangani River and *O. pangani girigan* in Lake Jipe. However, several recent studies regard *O. pangani* as conspecific to *O. jipe* (Seegers *et al.*, 2003; Seegers, 2008; Froese & Pauly, 2017; Shechonge *et al.*, 2018), and it is treated here as such. Morphologically, *O. jipe* is considered the closest relative of *O. hunteri*, sharing high numbers of vertebrae, scales and dorsal fin rays (Trewavas, 1983), but to our knowledge this has never been confirmed by genetic data. Transfer of tilapiine fishes to improve local fisheries started influencing the ichthyofauna in this region from the 1970s onwards. For most of these transfers, no written records are available, and nearly all dates mentioned below are based on observations made during field surveys, rather than actual accounts.

A survey in 1980 found only the endemic *O. hunteri* in Lake Chala, but in 1985 catches also included *Coptodon rendalli* and one specimen that the collectors identified as *O. pangani* (Dadzie *et al.*, 1988). Since the latter species does not occur in any recent catches, it either rapidly disappeared after its introduction, or the specimen was in fact *O. hunteri* misidentified as *O. pangani*. More recently a second *Oreochromis* species has been found in significant numbers, and it has been thought to be *O. korogwe* (Seegers *et al.*, 2003). Morphologically, however, Lake Chala specimens do not fully correspond to the *O. korogwe* holotype, which naturally inhabits the Lower Pangani River. Seegers *et al.* (2003) thus recommended to confirm this association, but no such study has been carried out so far.

The first written account of catches of *O. esculentus* in Lake Jipe (which naturally occurs only in Lake Victoria) dates from 1983, followed by *C. rendalli* (naturally distributed throughout the Congo River basin, lakes Tanganyika and Malawi, and southern Africa) in 1985 (Dadzie *et al.*, 1988). However, Trewavas (1983) suggests that at least *O. esculentus* must have been introduced there already in the 1950s, but that it went unnoticed in previous surveys. In Nyumba ya Mungu, *O. esculentus* and *C. rendalli* were caught for the first time in respectively 1973 and 1974 (Bailey *et al.*, 1978). It is unclear whether *O. jipe* colonised this reservoir in a natural way, or was introduced (Trewavas, 1983).

This general lack of written records complicates determining the exact source populations of introduced species, but some inferences can be made. Probably, most of the region's lakes were stocked with fish from nearby fish ponds, from which transporting living fry is more probable and logistically more feasible. The fish ponds still present today around the town of Taveta and near Lake Jipe are good candidates, as they date back to the late 1940s when the British colonel Ewart Scott Grogan settled in the region and established a sisal farm that included such ponds (Dadzie *et al.*, 1988). Tilapiines were also reared in governmental fish ponds at the town of Korogwe, located downstream along the Pangani River, for stocking reservoirs and ponds throughout the Tanganyika region (Lowe, 1955, Bailey *et al.*, 1978). Among those species were *O. jipe* and *O. korogwe*, but also *O. esculentus* and *O. variabilis* from Lake Victoria, and *C. rendalli* and *O. macrochir* that had previously been raised in ponds in the D.R. Congo province of Katanga (Lowe, 1955, Dadzie *et al.*, 1988). It seems thus most plausible that species introduced in the Upper Pangani

Region, including Lake Chala, derive from populations that were reared in the Korogwe or Taveta ponds.

3.3 Methods

3.3.1 Specimen collection

For this study, we obtained 104 specimens from local fishermen servicing nets on the Kenya (southeast) side of Lake Chala, in September 2012, January 2014 and September 2015. Similarly, 15 specimens were obtained from fishermen along the southeast shore of Lake Jipe and 10 from the north shore of Nyumba ya Mungu (hereafter, NyM) during the same periods. Initially the local names given to the diverse species were recorded for each specimen; the corresponding nominal species names were assigned afterwards. From Lake Chala, two *Oreochromis* species were distinguished, being *O. hunteri* ('Chala') and *O.* cf. *korogwe* ('Bandia'). From Lake Jipe, we collected *O. jipe* ('Asilia') and *O. esculentus* ('Polana'). At NyM we collected *O. jipe* ('Asilia') and *O. esculentus* ('Polana') (Supplementary Table B.1).

3.3.2 Geometric-morphometric analysis

Whole-body photographs of fresh specimens were taken in the field prior to preservation, from a perpendicular angle. This was carried out by positioning specimens on graph paper with their left side facing up and fins spread out.

Overall body morphology was analysed as in Chapter 2, using 16 traditional landmarks (Fig. 2.1) digitised in tpsDig2 version 2.17 (Rohlf, 2013a). The present chapter includes data on 95 Lake Chala specimens previously presented in Chapter 2. Size was calibrated using the graph paper visible on the photograph. The digitised landmark dataset was aligned via Procrustes superimposition in the program CoordGen6h of the Integrated Morphometrics Package software (Sheets, 2008). Procrustes coordinates and rescaled centroid size were saved as data matrix file in IMP format. All further analyses were performed on these Procrustes coordinates.

Overall variation in body shape was analysed using principal component analysis (PCA) in R (R Development Core Team, 2016), and the number of principal components to consider was assessed using scree-plot analysis and a broken-stick model. Multivariate Analysis of Variance (MANOVA) with four constraints was used to determine whether the fish taxa as distinguished by their local names indeed reflect significantly distinct shape morphs, and Canonical Variate Analysis (CVA) was used to determine the axes of maximal group separation. Both analyses were conducted on minimal Mahalanobis distances in PAST v.2.17 (Hammer *et al.*, 2001), and results were cross-validated by comparing leave-one-out (jack-knifed) classifier tables to the original confusion matrix. Substantial differences between these two classifier tables would reflect the important influence of one or few specimens on the observed outcome, and hence reveal any unreliable results.

3.3.3 MtDNA genotyping & analysis

We isolated DNA from tissue sample collected and stored in the field in 99.5% absolute ethanol using the Blood and Tissue DNA isolation kit (Qiagen) following the manufacturer's specifications. Two widely-used mitochondrial loci were chosen for this study, NADH dehydrogenase subunit 2 (ND2) and a fast-evolving part of the control region (CR), allowing straightforward comparison with GenBank sequences available for other *Oreochromis* species. These loci were amplified via polymerase chain reaction (PCR), using the published primers MET and TRP for ND2 (Kocher *et al.*, 1995), and L-Pro-F (Meyer *et al.*, 1994) and TDK-D (Lee *et al.*, 1995) for CR. PCR was performed in 25 μL reaction volumes, containing 1 μL DNA extract, 2.5 μL PCR buffer II (Applied Biosystems), 0.5 μL of either primer [10 μM], 1 μL $MgCl_2$ solution [25 mM], 0.4 μL dNTP solution [10 mM], 0.1 μL AmpliTaq Gold DNA polymerase [5U/μL] (Applied Biosystems), and 19 μl water. Thermocycling was performed with an initial denaturation for 3 minutes at 95°C, then 30 cycles with 30 seconds at 95°C, 30 seconds at 55°C, and 1 minute at 72°C, followed by final elongation for 7 minutes at 72°C. Reaction products were cleaned up using ExoSAP-IT PCR Product Cleanup Reagent (Affymetrix), following the manufacturer's instructions. Purified PCR products were used for cycle sequencing reactions using the BigDye Terminator Mix v3.1 (Applied Biosystems). Cycle sequencing was performed for each sample and primer combination using 4 μL of BigDye Terminator 3.1 Ready Reaction Mix, 1.5 μL of primer [10 μM], 2 μL cleaned PCR product, and 2.5 μL water. Thermocycling was performed with 1 minute at 96°C, then 25 cycles with 10 seconds at 94°C, 5 seconds at 50°C, and 4 minutes at 60°C. Cycle sequencing reactions were cleaned using ethanol precipitation. Sanger sequencing was performed on an ABI 3730 48-well capillary DNA Analyser (Applied Biosystems, Foster City, CA, USA). Electropherograms and their automatic translation were checked by eye and trimmed. Overlapping sequence reads from either direction were merged for each sample and locus. For each locus, 38 sequences were generated for this study (Supplementary Table B.1).

In total, four sequence alignments were generated. Lengths differed between alignments due to the occurrence of gaps with more distantly related species, and as a result of trimming positions with excess missing data from the alignment ends. *O. hunteri* CR sequences (N=15) were aligned (430 positions) and used for demographic analyses. All new *Oreochromis* sp. sequences (N=38) were aligned for each locus separately (1051 positions for ND2, 435 for CR), and concatenated to generate a haplotype network. Additional, published sequences of each locus were downloaded from Genbank (https://www.ncbi.nlm.nih.gov, Supplementary Tables B.2 and B.3) and analysed jointly with those new to this study. The datasets were pruned and trimmed, resulting in two alignments with 1040 and 437 positions, and 71 and 90 sequences, for ND2 and CR, respectively. These alignments were used for locus-wise phylogenetic analysis in RAxML (v.8.2.4; Stamatakis, 2014). For each locus, a separate maximum-likelihood tree was generated to make best use of available GenBank sequences. To find the best-scoring tree in each case, we performed rapid bootstrap analyses using the GTRGAMMAI model of sequence evolution and 100 alternative runs from distinct starting trees. The resulting trees were visualised in, and figures created with, FigTree v.1.4.3 (Rambaut, 2009) and fitchi (Matschiner, 2016), for the locus-wise molecular phylogenies and the haplotype network, respectively.

To test for a genetic signature of past population expansion in *O. hunteri*'s CR sequences, we performed a haplotype mismatch distribution analysis in Arlequin v.3.5 (Excoffier *et al.*, 2005) and a coalescent Bayesian skyline plot analysis (BSP; Drummond *et al.*, 2005) in BEAST2, v.2.4.5 (Bouckaert *et al.*, 2014). Populations that have undergone a period of sudden or exponential growth in the past exhibit a characteristically unimodal, wave-like pattern in the distribution of haplotype mismatches. If present, the mode of this distribution together with estimates of generation time and mutation rate can be used to infer the approximate timing of population expansion (e.g., Barluenga *et al.*, 2006). We performed the BSP analysis using a strict molecular-clock model with the base-substitution-rate estimate of 0.0324 changes per site per million years (SE 0.0139) of Genner *et al.* (2010). Our analysis employed a non-coding site model, and the Hasegawa-Kishino-Yano (HKY) substitution model with empirical base frequencies (Hasegawa *et al.*, 1985), identified as best choice for these data, using jModelTest v.2.1.10 (Darriba *et al.*, 2012). Chain length was 25,000,000 steps, and the first 10% were discarded as burn-in. This analysis also estimated the timing of the deepest coalescence event of the *O. hunteri* CR sequences.

3.4 Results

3.4.1 Morphological characterisation of regional tilapiine taxa

Although the broken-stick model indicates that 6 principal components (PCs) are significant, the scree plot (Supplementary Figure B.1) shows that only PC1-3 explain at least 10% of the morphological variation. Therefore, we only retained principal components explaining at least 10% of the morphological variatio (PC1-3). The first two axes of the PCA (Fig. 3.2a) together explain 47.14% of the observed variation in general body morphology. Principal component 1 (PC1) mainly reflects variation in body elongation and depth, with long and slender bodies on the positive side and shorter, deeper bodies on the negative side of the axis (Fig. 3.2c). PC1 distinguishes *O. hunteri* from the other *Oreochromis* species. PC2 reflects differences within body depth: specimens with positive PC2-values are ventrally flattened, whereas negative PC2-values correspond to ventrally-extended specimens (Fig. 3.2d). PC3, which explains an additional 12% of the observed variation, captures differences in the length of the head: specimens with positive PC3-values have shorter heads (i.e., relatively smaller distance between mouth and operculum), whereas specimens with negative PC3-values have more elongated heads.

Although assumptions for CVA and MANOVA were not met, jack-knifed confusion matrices did not differ substantially from the original classification table. Therefore, we consider these results as reliable, except for *O. esculentus*, where the small sample sizes (2 specimens from Jipe, 4 from NyM) prevented comparison with the other species except for *O. hunteri*. Uncorrected and

Bonferroni-corrected pairwise comparison results indicate that *O. hunteri*, *O.* cf. *korogwe* and *O. jipe* specimens as identified by their local names differ significantly in overall body morphology at the (overall) 5% confidence level (Table 3.1; Wilks'$\lambda = 0.003$, $p < 0.001$); the CVA scatter plot (Fig. 3.2b) shows all four taxa to be clearly distinct from one another. *O. hunteri* is separated from the other species along the first axis (CV1), whereas the other species are separated along the second axis (CV2).

Table 3.1: Uncorrected (above the diagonal) and Bonferroni-corrected (below the diagonal) *post-hoc* results, showing that all taxa as identified by their local names differ significantly in overall body morphology. The limited sample size of Polana caused pairwise comparisons to be non-applicable (N.A.) in some cases

		'Chala'	'Asilia'	'Bandia'	'Polana'
O. hunteri	'Chala'		<0.001	<0.001	<0.001
O. jipe	'Asilia'	<0.001		<0.001	N.A.
O. cf. *korogwe*	'Bandia'	<0.001	0.003		N.A.
O. esculentus	'Polana'	<0.001	N.A.	N.A.	

3.4.2 Genetic characterisation of regional *Oreochromis* taxa

Phylogenetic trees

Figures 3.3 and 3.4 depict the phylogenetic trees of a selection of relevant African *Oreochromis* taxa based on sequence data from the mitochondrial control region (CR, Fig. 3.3) and NADH dehydrogenase subunit 2 (ND2, Fig. 3.4) loci, respectively. Specimens of the four *Oreochromis* taxa as identified by local fishermen, and as validated by geometric-morphometric analysis, largely cluster into distinct mitochondrial clades. Further, CR and ND2 sequences suggest very similar phylogenetic affiliations, as could be expected from a non-recombining pair of markers. Only few of the studied specimens appear genetically distinct from these clusters. Specimens representing 'Chala' (*O. hunteri*) from Lake Chala and 'Asilia' (*O. jipe*) from both Lake Jipe and NyM cluster together in one clade, but with a distinct, albeit nested, split between the two species. Comparison with relevant GenBank sequences confirms high sequence similarity of 'Asilia' to gene fragments of *O. jipe* and suggests a close relationship with *O. amphimelas* specimens from an unknown locality. The latter species occurs naturally in lakes of the Eastern Rift Valley in Tanzania

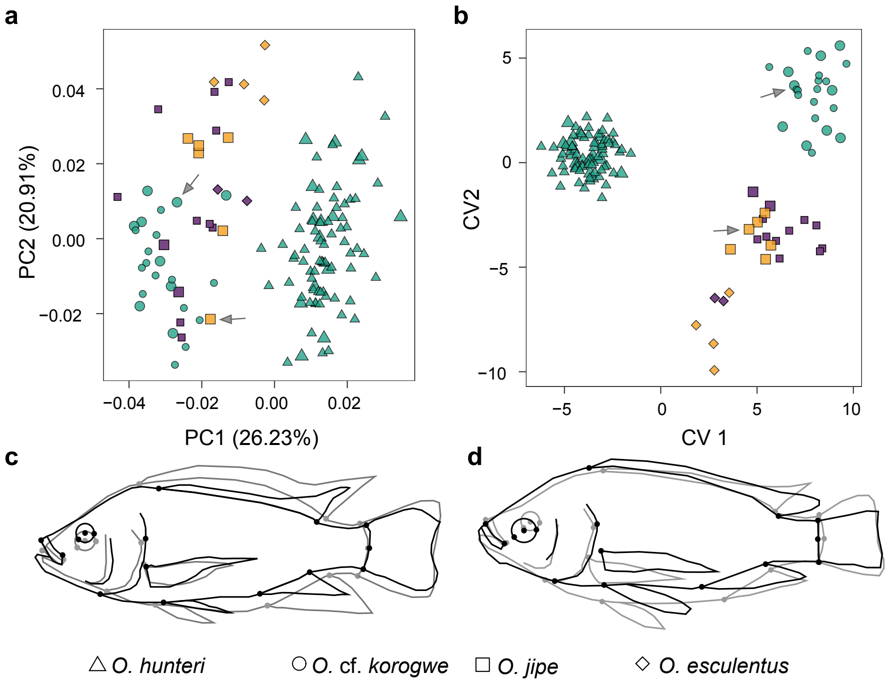


Figure 3.2: **a** PCA ordination plot synthesising overall variation in body morphology among the four *Oreochromis* species currently inhabiting the Upper Pangani River basin. Green, purple and yellow symbols represent specimens from respectively Lake Chala, Lake Jipe and NyM reservoir, with large symbols of each type and colour indicating sequenced specimens. The two arrows point to the specimens of *O. jipe* and *O.* cf. *korogwe* with genetically distinct positions in the phylogenetic trees. **b** CVA scatterplot showing maximal phenotypic separation of the four groups of specimens attributed to each of the four taxa. The outline drawings represent the specimens at the **c** positive (black) and negative (grey) extremes of PC1, and **d** the positive (black) and negative (grey) extremes of PC2.

(Manyara, Eyasi, Kitangiri, Singida), situated to the west of the Upper Pangani region. However, CR-sequences of *O. amphimelas* from Lake Manyara (clade containing *O. amphimelas* AF296490) occur in very different parts of the tree. Nevertheless, the latter clade is separated from *O. hunteri* and *O. jipe* by nodes with very low support values, so that their close relationship with *O. amphimelas* cannot be ruled out.

One ‘Asilia’ specimen from NyM (ASILIA nym011) clusters with *O. niloticus* genotypes, although morphologically it groups with the other ‘Asilia’ examined in this study (Fig. 3.2a). The Lake Chala fishes identified by local

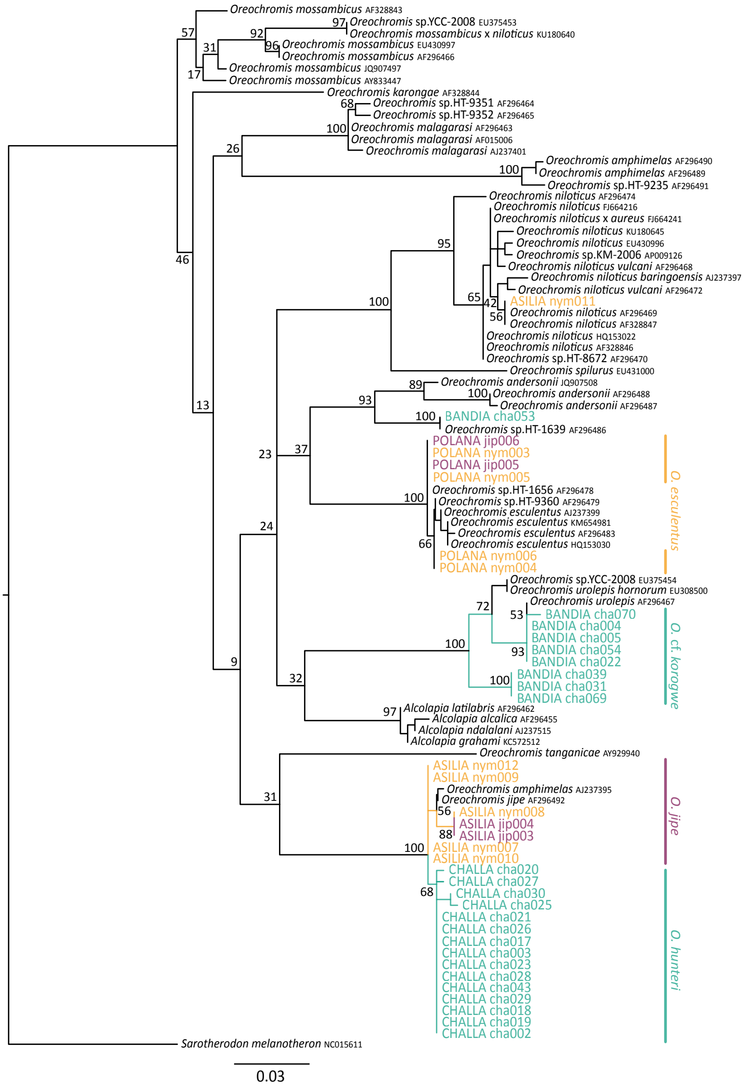


Figure 3.3: Maximum-likelihood phylogenetic tree of relevant African *Oreochromis* taxa based on sequences of the control region (CR) locus in mtDNA. *Sarotherodon melanotheron* was used as outgroup. The specimens sequenced in this study are colour-coded per site, as in Fig. 3.2: Chala (green), Jipe (purple) and NyM (yellow). Node support values are given in percent and are based on 100 bootstrap replicates. Note the low support for deeper nodes, but high support for those nodes on which identification of the closest relatives of the target species relies

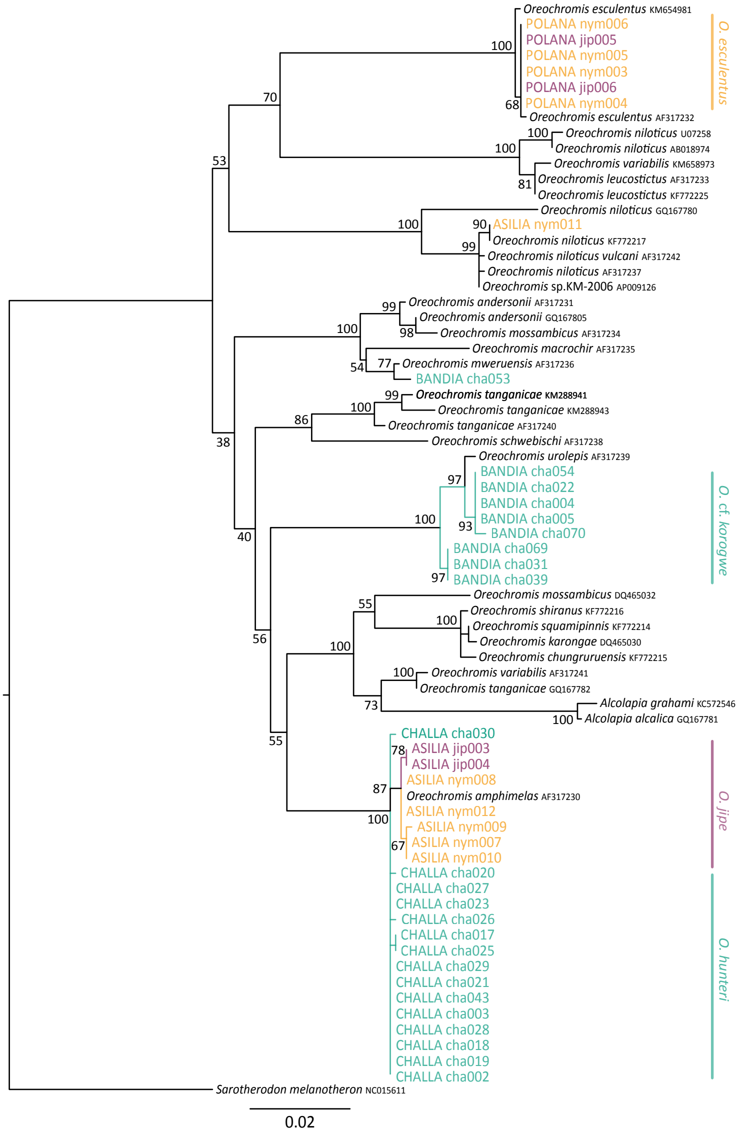


Figure 3.4: Maximum-likelihood phylogenetic tree based on sequences of the NADH dehydrogenase subunit 2 (ND2) locus in mtDNA. *Sarotherodon melanotheron* was used as outgroup. The specimens sequenced in this study are colour-coded per site, as in Fig. 3.2: Chala (green), Jipe (purple) and NyM (yellow). Node support values are given in percent and are based on 100 bootstrap replicates. Note the low support for deeper nodes, but high support for those nodes on which identification of the closest relatives of the target species relies

fishermen as ‘Bandia’ (*O.* cf. *korogwe*), although morphologically uniform (Fig. 3.2a), display a distinct split within their main clade, with CR and ND2 sequences separating the same two sub-groups of specimens. This differentiation appears greater than the variation within *O. hunteri*, or even the difference between *O. hunteri* and *O. jipe* (Fig. 3.3 & 3.4). Most of our ‘Bandia’ specimens cluster phylogenetically with *O. urolepis*. One available *O. urolepis* CR sequence, representing a specimen from the Wami river, was even identical to a subset of ‘Bandia’ sequences. This river belongs to the natural range of *O. urolepis*, and is located just south of the Pangani River basin. One particular Lake Chala specimen (BANDIA cha053) is phylogenetically even further removed from the main ‘Bandia’ clade. Our phylogenetic reconstruction based on the CR gene suggests close affinity with an unidentified *Oreochromis* specimen (HT-1639) collected from Pangani River, and dubbed *Oreochromis* ‘Korogwe’ in the original publication (Nagl *et al.*, 2001). Its ND2-sequence reveals that a specimen of *O. mweruensis*, collected in Lake Mweru-Wantipa (Zambia), may be a close relative (Klett & Meyer, 2002).

Haplotype network

An unrooted haplotype network of all genotyped *Oreochromis* specimens based on concatenated CR and ND2 sequences reveals 20 different haplotypes (Fig. 3.5). Again, the four taxa locally recognised as such (and distinguished by morphology, Fig. 3.2a) also group into distinct mitochondrial clusters. Among our 15 *O. hunteri* specimens, haplotype 20 is the most abundant (9 specimens), with six less common haplotypes (one specimen each) differing from haplotype 20 by up to five mutation steps. These seven *O. hunteri* haplotypes share a hypothetical common ancestor (haplotype 22) with *O. jipe*. Our seven *O. jipe* specimens comprise five haplotypes, of which one is found in Lake Jipe (2 specimens) and four in NyM, three of which are more closely related to *O. hunteri* (Fig. 3.5).

The deep split in the main group of Lake Chala ‘Bandia’ (*O.* cf. *korogwe*) specimens separates haplotype 16 (3 specimens) from haplotypes 14, 15 and 17 (together 5 specimens), and reiterates the observations in the phylogenetic trees. Haplotypes of the aberrant ‘Bandia’ specimen from Lake Chala (12; near *O. mweruensis*) and ‘Asilia’ specimen from NyM (10; near *O. niloticus*) are also here strongly isolated from their respective, morphology-based, clusters.

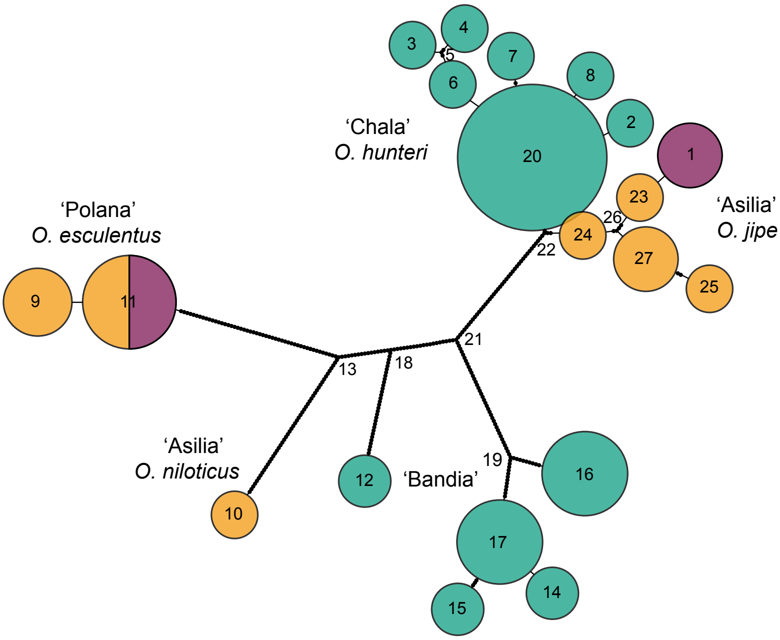


Figure 3.5: Unrooted haplotype network of all 38 *Oreochromis* specimens genotyped in this study, based on mitochondrial CR and ND2 sequences. Each circle represents one haplotype, numbered 1 to 27 including the seven hypothetical haplotypes at network nodes. The diameter of the circles is proportional to the number of individuals with a certain haplotype (1 to 9); the black dots represent undocumented mutation steps between the haplotypes. Colour codes are by water body, as in Figs. 3.2, 3.3 and 3.4: Chala (green), Jipe (purple) and NyM (yellow)

Mismatch distribution and skyline plot

The observed haplotype mismatch distribution of the 15 genotyped *O. hunteri* specimens is not unimodal (Fig. 3.6a), hence no distinct event of past population expansion can be defined. Likewise, the Bayesian skyline plot (Fig. 3.6b) does not provide evidence of a sudden large change in population size that might indicate a post-colonisation expansion. Instead, both results suggest that the size of the Lake Chala population has been relatively stable, or only slightly and continuously increasing, over an extended period of time. We estimate the oldest coalescence event for the CR sequences to have occurred approximately 100,000 years ago (100 ka), with a median of 92.4 ka and a 95% highest posterior density (HPD) interval ranging from 230 ka to 19 ka.

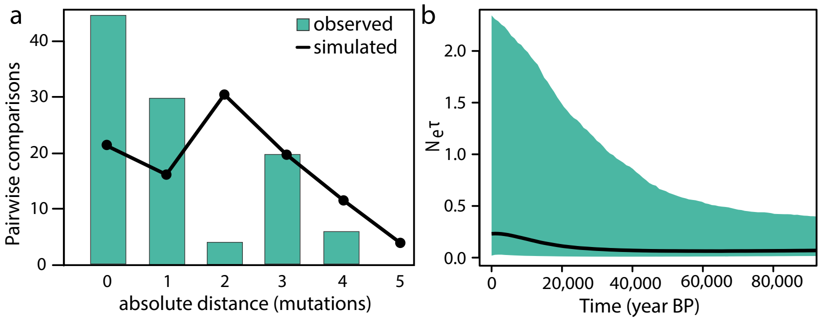


Figure 3.6: **a** Empirical distribution of haplotype mismatches among the CR sequences of 15 *O. hunteri* individuals (green bars) and their expected distribution from simulations (black line). **b** Bayesian Skyline Plot visualising the modelled median effective female population size (NeT, scaled by generation time τ) versus time in years before present (BP)

3.5 Discussion

3.5.1 *Oreochromis* in Lake Chala

To allow treatment of the extant *O. hunteri* as a modern-day reference for the fossil record of Lake Chala, hybridisation with species recently introduced to Lake Chala should ideally be ruled out. Although clear morphological distinction between *O. hunteri* and *O.* cf. *korogwe* was demonstrated in Chapter 2 and is confirmed by this study (Fig. 3.2), a complementary molecular-genetic approach enabled direct assessment of the likelihood of past hybridisation events. The results of our phylogenetic analyses (Figs. 3.3, 3.4 & 3.5) show that Lake Chala specimens assigned to these two *Oreochromis* species form distinct, well-supported genetic clades and do not share mitochondrial haplotypes, suggesting that hybridisation in Lake Chala is absent or at least very rare. Hybridisation can be detected more reliably with a combination of mitochondrial and nuclear markers, as introgression does not necessarily always affect both genomes at the same time (Nyingi & Agnèse, 2007, Angienda *et al.*, 2011). However, mitochondrial introgression appears to happen more readily than introgression of nuclear loci, due to, for example, interspecific matings being more likely to occur between females of the (initially) rare invading species and males of the normally more abundant native species (Wirtz, 1999). Mitochondrial alleles might also be comparatively neutral in a new genetic background, as opposed to alleles of nuclear genes, or of loci those genes are linked to (Martinsen *et al.*, 2001). In *Oreochromis*, cases of mitochondrial introgression have been demonstrated to occur without apparent nuclear introgression (Rognon & Guyomard, 2003, Nyingi & Agnèse, 2007), or with parallel introgression of only a few nuclear loci (Ndiwa *et al.*, 2014). While this discrepancy might in part reflect a bias towards traditionally-studied mitochondrial loci, these studies at least demonstrate that mitochondrial introgression in *Oreochromis* appears to occur readily and frequently. As *O. hunteri* and *O.* cf. *korogwe* from Lake Chala can easily be distinguished phenotypically and are not found to share mitochondrial haplotypes despite three decades of syntopy, we consider the species integrity of *O. hunteri* to be currently intact, and regard its morphology as a reliable modern-day reference frame for interpretation of its fossil record.

The uniform and spatially contiguous habitat of Lake Chala does not provide much opportunity for species segregation, but the observed lack of mitochondrial exchange between the native and the introduced *Oreochromis* does suggest some form of reproductive isolation. Postzygotic barriers are not very prominent in closely-related cichlid species (Stelkens *et al.*, 2010), but various forms of premating isolation may prevent the two species from interbreeding. Visual identification of species-specific coloration patterns, olfactory cues and sound recognition are important segregation mechanisms in cichlid species (Fryer & Iles, 1972). In fact, Stelkens & Seehausen (2009) found that phenotypic divergence predicts assortative mating better than does genetic distance between species. Visual cues may be less important in tilapias, but other mechanisms of isolation have been suggested in this group, such as separation in spawning time and distinctions in microhabitat preference (Pullin & Lowe-McConnell, 1982, Lowe-McConnell, 1987, Beveridge & McAndrew, 2000).

Although distinction between species within tilapiine genera is often notoriously difficult (Nagl *et al.*, 2001), the phenotypic/genotypic clusters of the four species analysed in this study coincide largely with usage of their local names, indicating that fishermen in each lake most often differentiate accurately and consistently between these fish taxa. However, one ‘Asilia’ specimen (in principle *O. jipe*) has a mitochondrial genotype clustering with *O. niloticus*, despite its phenotypical similarity to *O. jipe.* Our morphometric dataset does not contain *O. niloticus* specimens, and although this species has never been encountered in past Lake Jipe surveys (Dadzie *et al.*, 1988, Seegers *et al.*, 2003), the possibility exists that it has been introduced in recent years, and that this specimen is an actual *O. niloticus* or an individual carrying an introgressed mitochondrial haplotype. Importantly, the phenotypically uniform 'Bandia' (*O.* cf. *korogwe*) in Lake Chala also contains one individual with a very distinct genotype and a pronounced subdivision of the other specimens into two clades. A possible explanation for the occurrence of phenotype-genotype mismatches is that the aberrant mitochondrial genotype introgressed via an interbreeding event, either ancient or recent (Rognon & Guyomard, 2003, Ndiwa *et al.*, 2014). This process does not explain the genotypic division within the main 'Bandia' cluster, however. Historical collections suggest that *O.* cf. *korogwe* has been introduced to Lake Chala only during the 1980s (Dadzie *et al.*, 1988). Therefore, it is unlikely that this deep split, more pronounced than even the split between *O. hunteri* and *O. jipe*, has arisen locally in such a short time, while the morphological uniformity of 'Bandia' implies that the population indeed consists of one species. We propose that multiple stocking events, or one single event containing a mix of genotypes, are a likely cause for these distinct genotypes currently coexisting in Lake Chala.

There also remains doubt about the true identity of 'Bandia', which Seegers *et al.* (2003) attributed to *O. korogwe* with some reticence. Whereas all 9 specimens genotyped in this study are morphologically similar (Fig. 3.2a), most of their CR- and ND2-sequences cluster with *O. urolepis*. The one exception has a CR-sequence most similar to that of a specimen identified by Nagl *et al.* (2001) as *O.* 'Korogwe', but this only refers to the eastern Tanzanian village where it was collected (in Genbank this specimen is listed as *Oreochromis* sp.). The ND2-sequence of the same specimen suggests close relationship with a completely different species, *O. mweruensis*. No other *O. korogwe* sequences are currently available, and at this point we cannot rule out that our sequences would align with other *O. korogwe* specimens. Our data nevertheless suggest that the Lake Chala 'Bandia' (*O.* cf. *korogwe* in this paper) were stocked from at least two fish ponds, each containing a distinct *O. urolepis* or *O. korogwe* population which itself may already have undergone prior interbreeding with other *Oreochromis* taxa. Future genetic studies including nuclear markers could elucidate the exact identity of 'Bandia'.

3.5.2 The likely ancestor of *O. hunteri*

Trewavas (1983) first suggested that *O. jipe* and *O. hunteri* might be closely related, on the basis of their similar number of vertebrae, which is generally higher (31-34) than in other *Oreochromis* species such as *O. esculentus* (30-31). One would therefore expect the Upper Pangani tilapiines to have relatively elongated bodies, and to cluster together in a PCA where body elongation is an important character separating phenotypes along the principal axis of variation (PC1). Our geometric-morphometric data do not support this suggestion. Based on morphological data alone (Fig. 3.2), *O. hunteri* is distinct from the other three species, whereas *O. jipe* clusters with *O.* cf. *korogwe* and *O. esculentus.*

Our molecular-phylogenetic analyses, in contrast, do reveal *O. jipe* and *O. hunteri* to be each other's closest relative (Figs. 3.3 & 3.4). The two species form a polytomy in both phylogenetic trees, but based on CR-sequences *O. hunteri* is nested within *O. jipe*, whereas the opposite is true for ND2. This indicates that the employed markers may not be optimal to resolve the exact relationship of the two species, and we wish to be prudent with further interpretations. Nevertheless, genetic diversity of the *O. jipe* indigenous to Lake Jipe seems to be nested in the greater genetic diversity of modern-day *O. jipe* from NyM for both markers, despite the fact that the lacustrine habitat of NyM reservoir is only 50 years old. We cautiously suggest that this high diversity reflects standing variation retained through time in riverine populations of *O. jipe*, which seeded the newly formed NyM as well as the natural but climate-sensitive Lake Jipe, after a (relatively recent) environmental perturbation had eradicated its lacustrine population. In this context, we follow Seegers *et al.* (2003) in considering the riverine *O. pangani* as conspecific with the lacustrine *O. jipe*, notwithstanding some morphological differences in oral and pharyngeal teeth that had led Bailey *et al.* (1978) and Trewavas (1983) to describe them as two distinct species. Although no *O. pangani* specimens were available for us to address this issue with genetic analyses, we surmise that *O. pangani* may well be the riverine representative of *O. jipe*, which ensured the species' survival in the Upper Pangani River basin through past episodes of climatic drought when the regions shallow lakes fell dry. Although *O. jipe* clusters with an *O. amphimelas* specimen in both trees, most well-described specimens of the latter occur in other parts of the tree, and that particular specimen may be misidentified or may have been subject of mitochondrial introgression. Therefore, also the apparent relationship between *O. amphimelas* and *O. jipe* should be treated with caution.

3.5.3 Timing and mode of the colonisation of Lake Chala

The colonisation of isolated crater lakes by fish is still an enigmatic process (Barluenga & Meyer, 2010, Elmer *et al.*, 2012). The main mechanisms considered are human introduction, a hypothetical former aquatic connection and natural introduction by air (Elmer *et al.*, 2012). Although undocumented, late-20^{th} century human introduction is almost certainly how *O.* cf. *korogwe* and *C. rendalli* arrived in Lake Chala. Yet fossil fish teeth and bones occurring throughout the presently recovered part of the sediment record reveal presence of *O. hunteri* in Lake Chala since at least 25,000 years ago (Chapter 6). Ancient, or at least pre-colonial, stocking of fishless lakes in this region of East Africa, if it did occur, was most likely restricted to the period after ca. 1000 AD, when Bantu farmers first settled in the Mt. Kilimanjaro region (Håkansson, 2008). The high-rimmed volcanic caldera basin holding Lake Chala was formed after roof collapse of a magma chamber of Mt. Kilimanjaro, and hence it has always been hydrographically isolated from Upper Pangani tributary streams. Therefore, the introduction of fish by air, such as the transfer of fertilised eggs by birds, arguably remains the only plausible explanation of how the ancestor of *O. hunteri* arrived in Lake Chala.

Assuming that the niche space available to the colonising *O. hunteri* ancestor was not filled by other (now locally extinct) fish species, the ancestral *O. hunteri* population probably expanded rapidly after this initial colonisation. If so, the genetic signature of this ancient population expansion in today's *O. hunteri* population should provide an estimate of the time passed since then. However, as neither the haplotype mismatch distribution nor the Bayesian skyline plot of the 15 *O. hunteri* specimens we sequenced (Fig. 3.6) reveal an unambiguous signal of rapid population expansion, we cannot conclude with certainty that such rapid population expansion has actually occurred. Although sample sizes similar to ours have allowed the detection of past population expansions in some studies (e.g., Genner & Turner, 2014), some authors suggest that sample sizes must be on the order of 20-40 (Drummond & Bouckaert, 2015) or even 50 (Grant, 2015) for this purpose. Estimating the approximate timing of the putative population expansion which followed the colonisation of Lake Chala by the ancestor of *O. hunteri*, using a coalescence approach, must hence await the sequencing of additional specimens.

The structure of the modern-day haplotype network of *O. hunteri*, in which 15 specimens yield seven haplotypes with up to five mutations between them, does suggest that this endemic population is relatively ancient, i.e., in line with the fossil evidence. The age of Lake Chala itself is estimated at approximately 250,000 years, based on the total depth of its sedimentary record as revealed by seismic-reflection stratigraphy (Moernaut *et al.*, 2010) relative to the radiocarbon-dated upper portion of this record (Verschuren *et al.*, 2009, Blaauw *et al.*, 2011). The HPD interval derived from our genetic data suggests the age of the oldest coalescence event within *O. hunteri* to range between 230,000 and 19,000 years. Although this is a rather wide interval of time, its upper (older) end is consistent with the current best estimate of the age of Lake Chala, whereas its lower (younger) end is only a slight underestimation of the minimum age of the population based on fossil evidence. Given the modest number of sequences currently available, and therefore the potential for more distant haplotypes to remain undocumented at this time, we consider an early rather than late colonisation most plausible.

3.6 List of modifications

- The conspecific nature of *O. jipe* and *O. pangani* is discussed in more detail in the Introduction, Section 3.2.2.
- An objective method to determine the number of principal components (PCs) to consider is added to Material and methods, Section 3.3.2. The results are discussed in Section 3.4.1, and visualised in Appendix B.
- An explanation for the choice of mitochondrial loci is added to Material and methods, Section 3.3.3.
- The reasoning behind the generation of separate trees for the two loci is added to Material and methods, Section 3.3.3.
- The caption of Fig. 3.2 is changed to explain how the outline drawings were generated.
- A more detailed explanation of the origin of Lake Chala has been added to the Discussion, Section 3.5.3.

CHAPTER 4

Feeding habits of endemic *Oreochromis hunteri* and introduced tilapiines in Lake Chala

---

Jorunn Dieleman[1]
Christine Cocquyt[1,2]
Wanja Dorothy Nyingi[3]
Dirk Verschuren[1]

After: Dieleman, J., Cocquyt, B., Nyingi, W.D. & Verschuren, D. Feeding habits of endemic *Oreochromis hunteri* and introduced tilapiine cichlids in crater lake Chala. *In preparation for Journal of Fish Biology*

[1] Limnology Unit, Department of Biology, Ghent University, K. L. Ledeganckstraat 35, B-9000 Ghent, Belgium
[2] Meise Botanic Garden, Nieuwelaan 38, 1860 Meise
[3] Ichthyology Section, Zoology Department, National Museums of Kenya, P.O. Box 40658-00100, Nairobi, Kenya

4.1 Abstract

Two tilapiine cichlid species that were recently introduced into isolated crater lake Chala (Kenya/Tanzania) may interfere with the endemic and critically endangered Chala tilapia (*Oreochromis hunteri* Gnther, 1889) by increased competition for food. However, nothing is known about the feeding habits of these fish species in Lake Chala, hampering a comprehensive assessment of the threat these introduced species may pose. Here, we describe the feeding habits of both the endemic *O. hunteri* and introduced *Oreochromis* cf. *korogwe* and Redbreast tilapia (*Coptodon rendalli* (Boulenger, 1897)), with special focus on seasonal changes in food availability and interspecific differences. We used a combination of gut content analysis (n=36) and stable-isotope composition (n=17) to asses feeding behaviour over 20 consecutive months. We compared these samples to the limnetic phytoplankton composition in these months, and to the composition of littoral food sources. We found that all species feed in the littoral zone for most of the year, but migrate to open water in response to an annual phytoplankton bloom. However, interspecific differences were apparent in fish stable-isotope composition, and potentially reflect differences in food preference during the phytoplankton bloom. We suggest that *O. hunteri* is making better use of feeding opportunities provided by the lake, and may thus retain its competitive advantage over recent newcomers.

4.2 Introduction

Anthropogenic introductions of non-native species into natural systems are a major concern in conservation biology, as they often bring considerable hazards for the system's indigenous populations (Vitousek *et al.*, 1997; Strayer, 2010). For example, the extensive transfer of teleost fishes in aquaculture (Gozlan *et al.*, 2010; FAO, 2014) has caused widespread decline and even extinctions of native ichthyofauna, notably in Africa (e.g., Barel *et al.*, 1985; Witte *et al.*, 1992; Canonico *et al.*, 2005). Negative impacts of invasive aliens on a natural system are often the result of a complex interplay of factors, which may include direct predation (e.g. Barel *et al.*, 1985; Witte *et al.*, 1992), hybridisation (e.g. D'Amato *et al.*, 2007; Angienda *et al.*, 2011), and competition for food (e.g. Ogutu-Ohwayo, 1990) or breeding grounds (Canonico *et al.*, 2005).

The cichlid species *Oreochromis hunteri* (Günther, 1889) is endemic to Lake Chala (locally 'Challa' after a nearby village; Kenya/Tanzania, Fig. 1.3) and is currently listed on the IUCN red list as 'critically endangered', primarily because of its exclusive occurrence in this isolated crater lake (Bayona *et al.*, 2006). Fish fossils in the lake's sediments indicate that this species has been present for at least 25,000 years (Fig. 1.5), but it may already have colonised the lake much earlier, not long after the crater basin was formed >250,000 years ago (Chapter 3). Despite being the type species of the genus *Oreochromis* (Günther, 1889), the general ecology of *O. hunteri* has never been studied in detail. Very little information is available on *O. hunteri*'s breeding and feeding behaviour (Trewavas, 1983): although Lowe (1955) observed juveniles feeding on algae and detritus at the shore, nothing is known about the adults, and an in-depth study of its feeding habits is currently lacking.

Three other cichlid species were introduced to Lake Chala sometime in the second half of the 20^{th} century, possibly as late as the early 1980s (Dadzie *et al.*, 1988; Seegers *et al.*, 2003). These include a haplochromine from the *Astatotilapia bloyeti* (Sauvage, 1883) complex and two tilapiines, the Redbreast tilapia (*Coptodon rendalli* (Boulenger, 1897)) and an unknown *Oreochromis* species that we here refer to as *Oreochromis* cf. *korogwe*, after Chapter 3. These alien species are surviving well, and especially *O.* cf. *korogwe* and *C. rendalli* may interfere with the endemic *O. hunteri*, because they are phylogenetically closely related and have similar ecological preferences. Moreover, the deep open waters and steep rocky crater walls of Lake Chala provide limited opportunity for niche segregation. Tilapiine cichlids have in many places outcompeted native species (Canonico *et al.*, 2005) or hybridised with indigenous tilapia species (D'Amato *et al.*, 2007; Angienda *et al.*, 2011) due to their tolerance to a wide range of environmental conditions. Although a combined morphometric and genetic analysis did not find traces of hybridisation between the introduced and endemic *Oreochromis* in Lake Chala (Chapter 3), the direct competition for resources has not yet been assessed.

The trophic versatility of tilapiine cichlids is not only advantageous when coping with new competitors, it is also highly useful in (seasonally) changing environments. Particularly in tropical lakes, wind-induced seasonal upwelling can be an important driving factor of temporary increases in food availability (Lowe-McConnell, 1987), and tilapia species have been described to adapt their feeding behaviour to such events (Spataru, 1976; Man & Hodgkiss, 1977; Spataru, 1978a). In the case of Lake Chala, the short-lived phytoplankton bloom which often develops during the windy mixing season (Barker *et al.*, 2011; Wolff *et al.*, 2011; Buckles *et al.*, 2014) may therefore create seasonal changes in the diet composition of the Lake Chala cichlids, and potentially to a different extent in the indigenous and the introduced species.

In this context, this Chapter aims to document the feeding habits of *Oreochromis hunteri* and the two introduced tilapiine cichlids in Lake Chala, with special emphasis on the link with seasonal fluctuations in resource availability. In this way, we aim to assess the current and potential future impact of the introduced species on the endemic *O. hunteri* population.

4.3 Material and methods

4.3.1 Study system

Lake Chala (03°19'S, 37°42'E; Fig. 1.3) is a deep and permanently stratified crater lake, located at an elevation of ~880 m on the border between Kenya and Tanzania immediately east of Mount Kilimanjaro. Contained within a steep-sided caldera basin, its rocky shoreline drops down near-vertically to a depth of ca. 55 m (Opitz, 2008), from where soft fine-grained sediments slope more gently towards a maximum depth of around 92 m in the middle of the lake (Moernaut *et al.*, 2010; Fig. 4.1). For most of the year oxygenated water reaches down to between 15 and 30 m only (Wolff *et al.*, 2011; Wolff *et al.*, 2014), effectively restricting viable fish habitat to the upper half of the rocky crater walls and the open-water (limnetic) zone. Submerged rocks are covered with an algal biofilm and are interspersed with small sandy patches filling up crevices between them. These patches probably increase in areal extent as they fill up the rock crevices at water depths beyond wave-induced turbulence. Trapping the sinking litter of near-shore trees and shrubs, they are likely inhabited by a diverse zoobenthos community down to where dissolved-oxygen levels become too low. For most of the year, feeding opportunity for the Lake Chala fishes is limited to this near-shore habitat, as very low productivity of the limnetic zone (secchi-disk transparency ranging between 6 and 9 m; van Bree *et al.*, unpublished data) implies poor feeding conditions. However, combination of the seasonal minimum in solar insolation with strong monsoon winds causes intermittent deep water-column mixing down to between 35 and 45 m depth between late June and September. These short-lived mixing events induce upwelling of the dissolved nutrients that had been accumulating in deeper water layers, often (but not always) leading to an extensive phytoplankton bloom towards the end of the dry season (Barker *et al.*, 2011; Wolff *et al.*, 2011; Buckles *et al.*, 2014). This bloom dramatically enhances limnetic feeding opportunity for the local fishes.

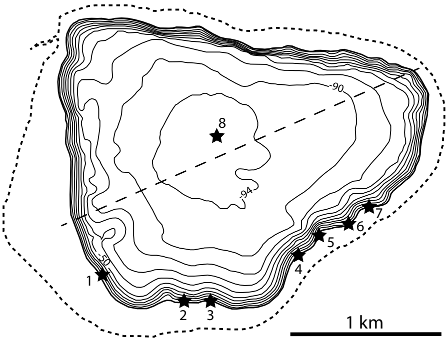


Figure 4.1: Bathymetric map of Lake Chala with indication of food source sampling locations. Location 1-7 represent samples collected from rocks and sandy patches, location 8 is the position where limnetic samples were collected. Dashed line represents the country border between Kenya and Tanzania

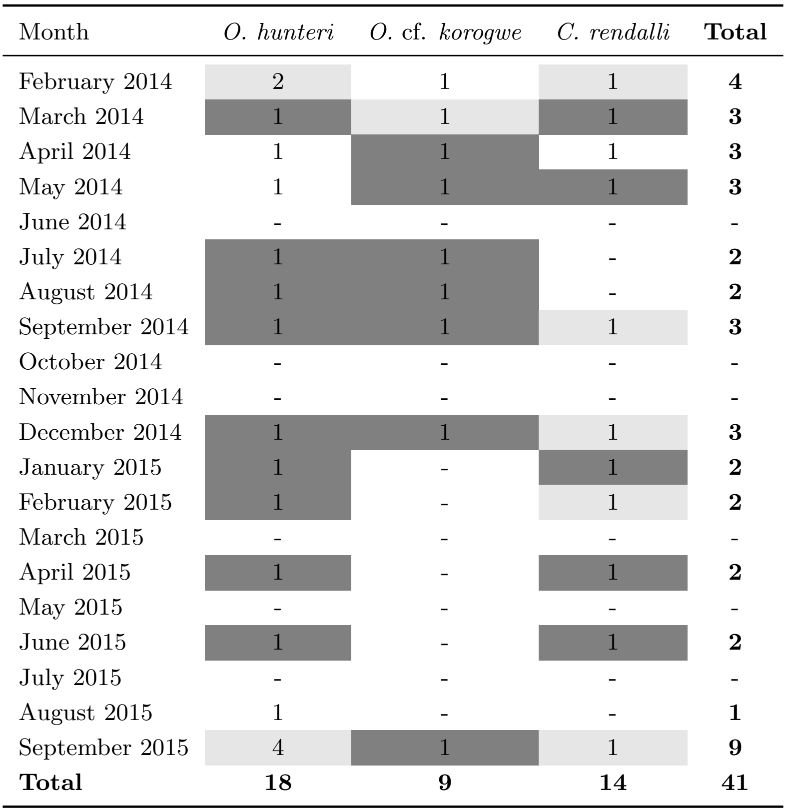
4.3.2 Fish and food source collection

Fish samples were collected at monthly intervals over a 20-month period between February 2014 and October 2015, via purchase from local fishermen using gill nets suspended from floats and set along the shoreline. Fishermen adapt the location of their nets to the presence of fish, which fluctuates from the surface (July – August) to >6m (May – June; November – December). Fishing in Lake Chala has strongly dwindled over the last decade mainly due to interference from illegal alcohol distillers, and most fishermen go home to tend their farms during the seasons when the large tilapiines dwell at depths beyond the reach of their nets (May – June and November – December). We nevertheless succeeded to collect fish specimens during most months, yielding a total of 41 specimens of which 36 contained sufficient gut content for analysis (Table 4.1). We used a combination of gut content and stable isotope analyses to document both short and long-term feeding habits of Lake Chala fish. The guts were immediately dissected to halt further digestion, divided into their rostral, middle and caudal part, and stored in absolute ethanol (99.8%). For stable-isotope analysis, dorsal muscle tissue was collected from 17 specimens using a sterile scalpel, and stored frozen up to the moment of sample processing. Potential food source samples were obtained in September 2015 by scraping off the epilithic algal mats from shoreline rocks and by rinsing sand from submerged sandy patches at 14 different localities on the Kenyan side of the lake (Fig. 4.1). These samples were frozen for later analyses. Limnetic phytoplankton samples were collected by filling 100 mL bottles with surface water, monthly between February 2014 and October 2015, and at two additional depth intervals (5 and 10 m) between January 2014 and January 2015 using a UWITEC water sampler. Immediately after collection these water samples were fixed *in situ* with an alkaline Lugol's solution and formalin.

4.3.3 Laboratory methods

The abundance of gut content was determined qualitatively upon return to Ghent University, Belgium. Guts that were filled over the entire length of the rostral part were considered full, whereas guts with large empty gaps but still sufficient material for counting were considered half-full. All others were considered empty for the purpose of this study. The middle and caudal parts of the guts contained strongly digested material only and were not further analysed. The content of the first cm of each gut was transferred to a 2-mL Eppendorf vial and diluted with distilled water. If the first cm did not contain material, which sometimes occurred in specimens with half-full guts, the first food bolus encountered within the anterior 10 cm of the guts was used. Littoral food-source samples were similarly diluted in distilled water. One mL of the resulting suspension was pipetted into a sedimentation chamber of 10 mL and left to settle down. The limnetic phytoplankton samples were analysed following the Ütermöhl method (Utermöhl, 1931, 1958) and contained Chlorophyta (green algae), Bacillariophyta (diatoms), Dinophyta, Euglenophyta, Chrysophyta, Cryptophyta and Cyanobacteria (blue-green algae) in variable quantities and proportions. In each gut and littoral sample 200 algal/cyanobacterial cells,

Table 4.1: Overview of fish specimens available for gut analysis, collected in Lake Chala between February 2014 and September 2015. Values indicate the number of collected specimens per month and per species, whereas cell colour represents the abundance of gut content: dark grey cells indicate full guts, light grey cells indicate half-full guts and white cells indicate empty guts



Month	*O. hunteri*	*O.* cf. *korogwe*	*C. rendalli*	**Total**
February 2014	2	1	1	**4**
March 2014	1	1	1	**3**
April 2014	1	1	1	**3**
May 2014	1	1	1	**3**
June 2014	-	-	-	-
July 2014	1	1	-	**2**
August 2014	1	1	-	**2**
September 2014	1	1	1	**3**
October 2014	-	-	-	-
November 2014	-	-	-	-
December 2014	1	1	1	**3**
January 2015	1	-	1	**2**
February 2015	1	-	1	**2**
March 2015	-	-	-	-
April 2015	1	-	1	**2**
May 2015	-	-	-	-
June 2015	1	-	1	**2**
July 2015	-	-	-	-
August 2015	1	-	-	**1**
September 2015	4	1	1	**9**
Total	**18**	**9**	**14**	**41**

colonies or detritus items were counted; in the phytoplankton samples at least 500 solitary cells or colonies. Enumeration and taxonomic identification was done using a Kyowa inverted microscope for gut and littoral samples (Ghent University), and an Olympus CKX 41 inverted microscope for the phytoplankton (Meise Botanic Garden). All analyses were done with a 100x immersion oil objective. Different algal/cyanobacterial taxa made up the bulk of most studied samples, and were identified down to genus level for the dominant taxa and down to order level for other taxa (31 in total, cf. below). Colonies were counted as one item, but the number of individual cells in each colony was also recorded. The average number of cells per colony was compared between guts and food sources for each colonial taxon. If this average number of cells differed, for example due to rapid disintegration of colonial genera in the fish gut, colony counts were rescaled accordingly. Plant remains were classified as detritus; most of these must originate from terrestrial plant litter because submerged or emergent aquatic macrophytes are almost absent in Lake Chala. The loose sponge spicules that were occasionally found were also regarded as detritus, rather than evidence for direct consumption of sponges.

For stable-isotopic analysis of fish tissue, samples were dried overnight at 60°C and pulverised using a mortar. Subsamples were weighed to the nearest 0.01 mg before being put into tin capsules (8x5 mm). Replicates of the same sample were analysed for specimens of *O. hunteri* and *C. rendalli*. Stable isotope analyses were carried out at the Isotype Bioscience Laboratory (ISOFYS) of Ghent University. The samples were run for nitrogen (N) and carbon (C) isotope ratios, which are expressed in delta notation:

$$\delta^{15}\text{N or } \delta^{13}\text{C} = [(\text{Rsample / Rstandard}) \quad 1\ ] \text{ x } 1000$$

with R = $^{15}\text{N}/^{14}\text{N}$ or $^{13}\text{C}/^{12}\text{C}$ relative to a standard, which is atmospheric nitrogen for N and Pee Dee Belemnite limestone for C.

4.3.4 Data analyses

Further data analyses were performed in R version 3.3.2 (R Development Core Team, 2016). Species accumulation curves (package ‘vegan’ v.2.4-3; Oksanen *et al.*, 2017) were used to establish that 200 algal/cyanobacterial cells, colonies or detritus items were sufficient to capture the composition of ingested food, and that enough fish specimens were analysed for comprehensive assessment of fish diets in Lake Chala. To assess seasonal differences in gut content, fishes of each of the three cichlid species considered were initially analysed collectively per season. Guts of fishes caught during the mixing season (July to September) were assembled into one group, and those collected during the predominantly stratified season (October to June) into another group, per consecutive year. Our gut collections during 2014 and 2015 hence resulted in observations from four seasons, for which diet composition was compared with multivariate techniques using the packages ‘vegan’ and ‘MASS’ v.7.3.45 (Venables & Ripley, 2002). A Bray-Curtis similarity matrix was generated based on square-root transformed counts of the gut contents. We compared the multivariate spread between samples, as homogeneity of spread is an important assumption for permutational multivariate analysis of variance (PERMANOVA). We tested for differences in seasonal diet using PERMANOVA (999 permutations), followed by pairwise comparison *post-hoc* tests. Results were visualised using non-metric multidimensional scaling (NMDS).

To compare observed seasonal differences in diet composition with seasonal variation in food sources, we related the gut content of the three fish species combined per consecutive month to the limnetic and littoral food-source samples. Counts were summed per month and divided by the total number of food items in that month to calculate a percentage by number (%N; after Hyslop, 1980). A similar method was applied to estimate the relative importance of different algal/cyanobacterial taxa in the phytoplankton samples, by combining samples from 0, 5 and 10 m depth when applicable, into the percentage by number (%N) per month. Littoral samples were only available for September 2015, hence %N for rock scrapes and sandy patches was calculated from the overall average of the 14 sampled localities. All the above-mentioned metrics were calculated both at the phylum level (including the categories of detritus and sand grains), and at the highest taxonomic resolution feasible. The results were visualised and compared using bar plots.

Generalised (i.e., long-term average) interspecific diet differences were assessed using δ^{15}N and δ^{13}C values. A possible influence of high lipid values on δ^{13}C results can be controlled for by simultaneous lipid extraction or mathematical normalisation. Animal lipid content has a strong relationship with the C:N ratio, and a C:N ratio <3.5 (corresponding to a 5% lipid content) is preferred (Post *et al.*, 2007). None of our fish specimens crossed that threshold, hence normalisation of the δ^{13}C values was deemed unnecessary. ANOVA followed by *post-hoc* Tukey-tests was used to test the significance of differences in δ^{15}N and δ^{13}C values between the fish species. We also assessed diet disparity (i.e. isotopic ranges) for each species by calculating the mean Euclidean distance of each point in the δ^{13}C: δ^{15}N plot to its group centroid, and using the sum of variances on the two axes.

Gut composition between species was compared using PERMANOVA after the assumption of homogeneity was checked. We used the same similarity matrix as for seasonal comparisons and visualised the results using NMDS. We then determined the dietary importance of food items per fish species and per season using percentage by number (%N) and visualised the results in bar plots to assess the exact diet differences between the respective fish species. Although ontogenetic diet shifts are widely reported in tilapiine species (Ribbink, 1990; Piet, 1998; Njiru *et al.*, 2004), we found no influence of body size on the diet of the studied species (Supplementary Information C).

4.4 Results

4.4.1 Seasonal differences in gut contents and food sources

The guts of 18 *Oreochromis hunteri*, 9 *O.* cf. *korogwe* and 14 *Coptodon rendalli* from Lake Chala together contained 28 different prey types identified at the lowest-feasible taxonomic level (Supplementary Table C.1), including two types of cyanobacteria, 24 types of algae, detritus and sand grains. Unicellular algae and cyanobacteria make up between 45 and 95% of the analysed gut contents. The 29 samples of limnetic phytoplankton of Lake Chala contained a total of 27 different prey types, namely three types of cyanobacteria and 24 types of algae (Supplementary Table C.2). The 12 littoral rock samples and 2 littoral sand samples contained 24 and 18 different prey types, respectively (Supplementary Table C.3). .

Table 4.2: Bonferroni-corrected p-values of i) *post-hoc* homogeneity Tukey-tests per season (above diagonal) and ii) of *post-hoc* PERMANOVA pairwise comparisons of Bray-Curtis similarity matrices per season (below diagonal). The asterisks (*) signify significance at the adjusted 5% level.

	Feb ’14 - Jun ’14	Jul ’14 - Sep ’14	Dec ’14 - Jun ’15	Sep ’15
Feb ’14 - Jun ’14		0.91	0.16	0.24
Jul ’14 - Sep ’14	0.01^*		0.54	0.07
Dec ’14 - Jun ’15	0.47	$< 0.01^*$		$< 0.01^*$
Sep ’15	0.09	0.02^*	$< 0.01^*$	

A two-dimensional NMDS plot was generated with acceptable stress values (0.16) and hence represents the data well. This plot (Fig. 4.2) shows that gut content composition varies greatly between seasons, whereas interspecific differences are less pronounced. We found sample homogeneity to differ significantly between seasons ($F = 6.36$, $p < 0.01$), but *post-hoc* Tukey tests revealed only one significant pairwise distinction (between the two seasons of 2015; Table 4.2). Therefore, assumptions are met for the majority of inter-season comparisons and PERMANOVA can be safely executed, although the results of one comparison should be treated with caution. Overall, we found a highly significant impact of seasonality on diet composition (F = 4.63, $p < 0.01$), and *post-hoc* testing revealed that all pairwise differences between mixing and predominantly stratified periods were borderline to highly significant on the Bonferroni-corrected 5% confidence interval (Table 4.2).

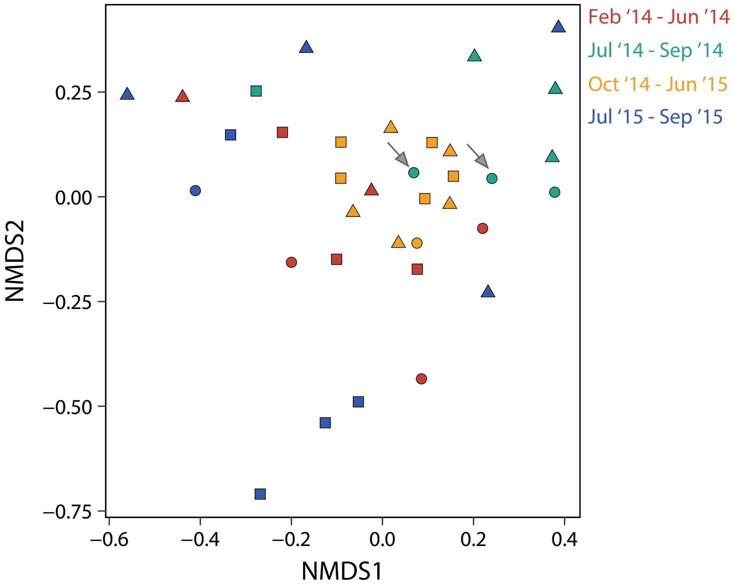


Figure 4.2: Non-metric multidimensional scaling ordination (NMDS; stress = 0.16) visualising similarity in gut content. Shapes represent interspecific differences between the endemic *O. hunteri* (△) and introduced *O.* cf. *korogwe* (○) and *C. rendalli* (□). Colour describes seasonality, in which a distinction is made between predominantly stratified seasons (2014, red; 2015, yellow) and mixing seasons (2014, green; 2015, blue). Arrows indicate specimens of *O.* cf. *korogwe* collected in July and August 2014.

To comprehend the sources of seasonal variation in gut contents, we compared the relative abundances of different food types in the fishes' guts with those of the limnetic and littoral food sources, both at the phylum level (Fig. 4.3) and with a higher taxonomic resolution (Fig. 4.4). Apart from February 2014, the proportion of detritus and sand grains in the guts is much lower during the during the mixing season (6-13%) than during the rest of the year (27-53%; Fig. 4.3b). Whereas all phyla of algae/cyanobacteria, except Chrysophyta, were found to occur in both the limnetic and littoral zone, the detritus and sand in the fish guts have a littoral origin, as these non-algal food types are (predictably) absent in the open surface water but represent almost half of the littoral food sources (Fig. 4.3c). Chlorophyta are abundant in the gut contents at the start of the 2014 mixing season (from 10% to 30-50%), which coincides with high abundances of the chlorophyte *Tetraedron* Kützing (1845) in the open water column (Fig. 4.4). Indeed, *Tetraedron* is during that period abundant in the fish guts. It also occurs in large proportions in the guts in certain months of the predominantly stratified season, but is in those months less abundant in the limnetic zone. The proportion of Bacillariophyta (diatoms) in the guts remains comparatively stable throughout the year, but composition of diatom genera is highly variable. *Nitzschia* Hassall (1845) and *Staurosirella* Williams & Round (1987) represent a significant part of the gut contents during the predominantly stratified season (10-45% and 5-18%, respectively). *Nitzschia* makes up a considerable part of littoral food sources (15-30%), and although *Staurosirella* represents only a small fraction of the littoral samples (∼0.5%), it requires a substrate for growing, arguing for a littoral habitat. *Staurosirella* also occurs in the limnetic zone during the mixing season, when zooplankton abundance peaks in response to the phytoplankton bloom and their faecal pellets provide abundant substrates for this diatom. Simultaneously, however, *Staurosirella* abundance in fish guts strongly decreases (0-1%), and it is replaced by *Fragilaria* s.l. Lyngby (1819) in July and August (12-28%) and *Afrocymbella* Krammer (2003), more specifically the endemic species *A. barkeri* Cocquyt & Ryken (2016), in September (11%). These genera also successively peak in the limnetic phytoplankton at that time. Notably, the Chlorophyte *Treubaria* Bernard (1908) is highly abundant in the water column in July 2014, but is rare in the guts of fish collected in that month.

The expected similar trend during the mixing season of 2015 was not realised. Instead, gut contents from September 2015 largely consist of Cyanobacteria, although the gut of one specimen of *O. hunteri* was filled with green algae. All guts from specimens collected at that time have at least some content, but in most guts food boluses are interspersed with large stretches of empty gut (Table 4.1). Absolute abundances of the major algal/cyanobacterial taxa present in the water column (Fig. 4.5) show that a seasonal diatom and green algae bloom was mostly absent that year. Other important seasonal abundance shifts that take place in the limnetic phytoplankton are not reflected in the fish

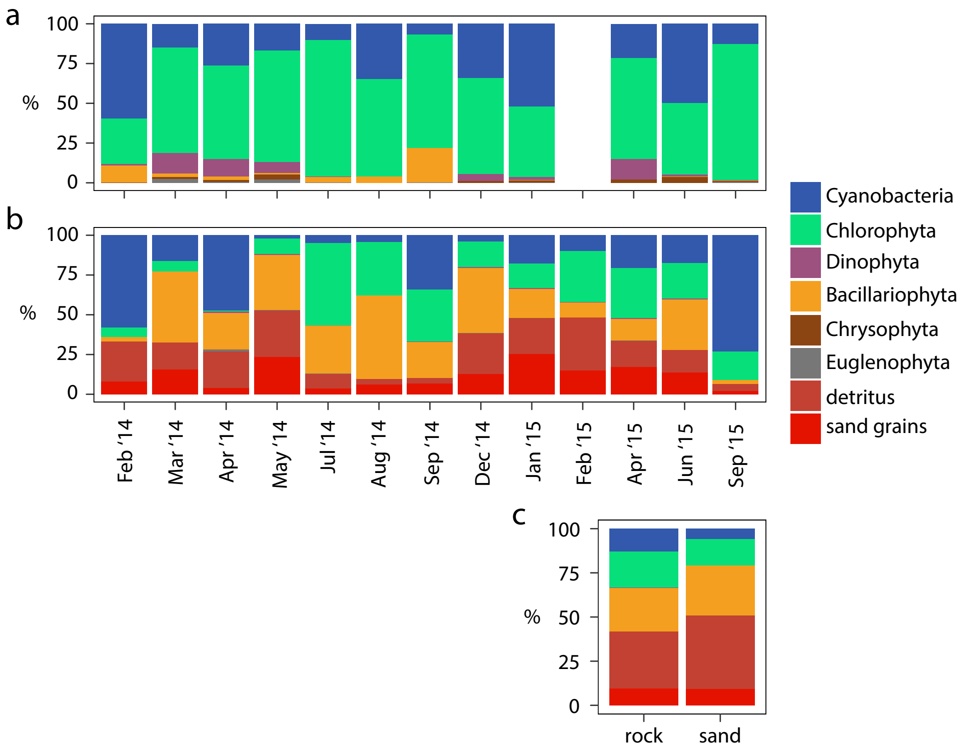


Figure 4.3: Proportion (%N) per month of algal/bacterial phyla in limnetic phytoplankton (**a**), and algal/bacterial phyla, detritus and sand in fish guts (**b**) and littoral food sources (**c**). 'Rock' refers to samples scraped from rocks; 'sand' refers to patches of sand.

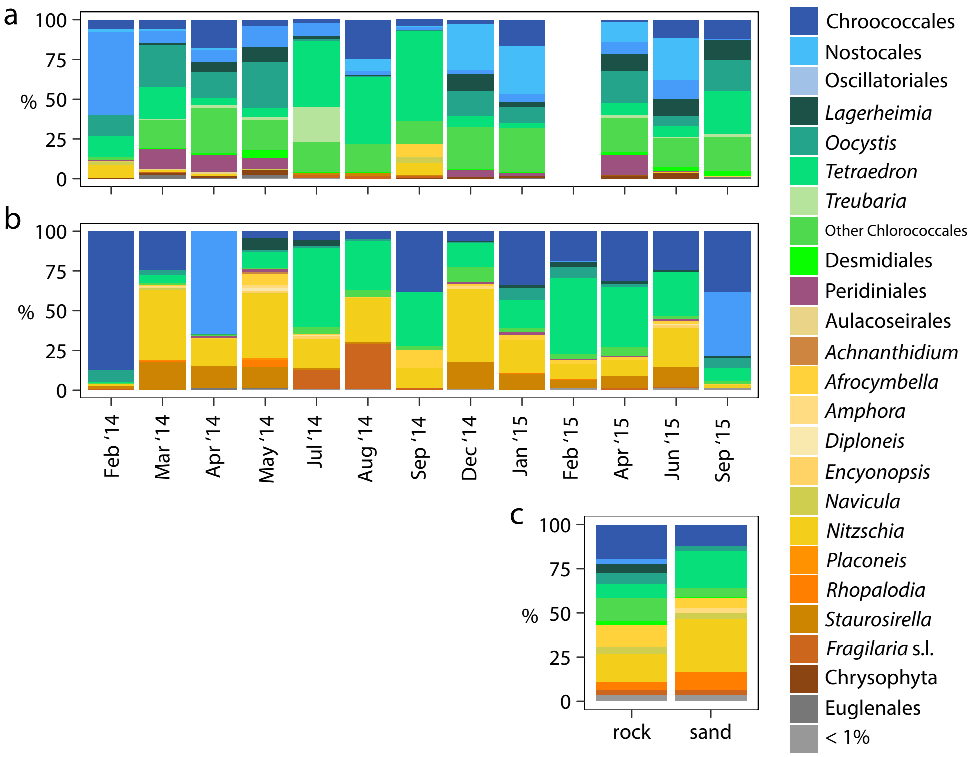


Figure 4.4: Proportion (%N) per month of algal/bacterial genera and orders in the limnetic phytoplankton (**a**), fish guts (**b**) and littoral food sources (**c**).

gut contents, for example high abundances of Dinophyta and the chlorophyte genus *Oocystis* Braun (1909) from March to May, and increased numbers of Nostocales Borzi (1914) (Cyanobacteria), mainly *Cylindrospermopsis* Seenayya & Subbarayu (1972), in December and January. Overall, gut samples taken during the mixing season resemble the limnetic phytoplankton composition at that time, whereas gut samples taken at different times of the year contain higher littoral contents.

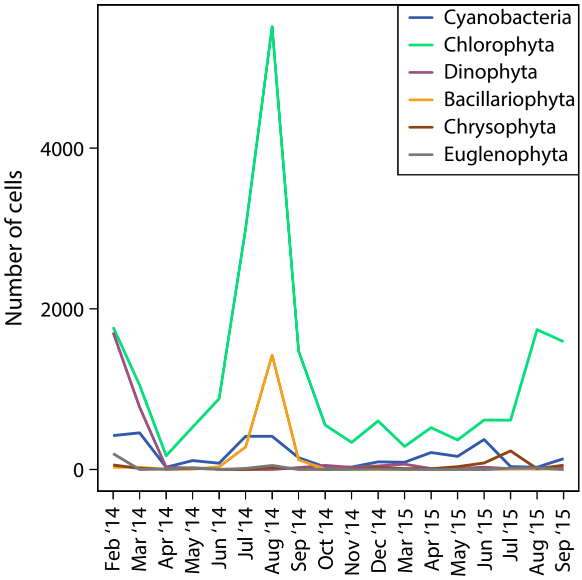


Figure 4.5: Total abundance (counts in 100 mL surface water) of cells or colonies of the six most common algal/bacterial phyla in Lake Chala, during 20 consecutive months from February 2014 to September 2015.

4.4.2 Interspecific and size-related differences

There was no significant difference in the homogeneity of gut contents grouped per fish species (F = 0.31, p = 0.73), hence assumptions for PERMANOVA are fulfilled. Although PERMANOVA indicated a borderline significant difference in gut content composition between fish species (F = 1.90, p = 0.05), *post-hoc* tests revealed that none of the pairwise differences were significant at the Bonferroni-adjusted 5% significance level (Table 4.3). The available sample sizes did not allow us to simultaneously test for the influence of both species and season, but bar plots taking into account both factors (Fig. 4.6) show some interesting interspecific differences. Although all species show the seasonal shift between littoral and limnetic food sources (Fig. 4.6a), during the mixing season of 2014 the diet of *O. hunteri* has a much higher contribution of the blooming limnetic algae *Tetraedron* and *Fragilaria* s.l. (Fig. 4.6b). In contrast, *O.* cf. *korogwe* specimens caught during that time (particularly those from July and August 2014) show diet compositions similar to those observed in all species during the predominantly stratified season. This is also reflected in the NMDS plot (Fig. 4.2), where *O.* cf. *korogwe* specimens from July and August cluster with specimens collected in the stratified season. Only the individual caught in September 2014 contains high abundances of the limnetic algae blooming at that time, such as *Afrocymbella* and *Tetraedron.* The gut content of the single *C. rendalli* specimen obtained during the mixing season of 2014 consisted mostly of Cyanobacteria. Some minor interspecific differences can also be observed during the predominantly stratified season, for example that *O.* cf. *korogwe* has higher abundances of the diatom *Staurosirella* in its gut than the other two cichlids.

Table 4.3: Bonferroni-adjusted p-levels of pairwise comparison on Bray-Curtis similarity matrices of gut composition per species (*post-hoc* PERMANOVA).

	O. hunteri	*O.* cf. *korogwe*
O. cf. *korogwe*	0.22	
C. rendalli	0.18	1.00

The total isotopic range in all Lake Chala tilapiines combined is higher for δ^{13}C (6.18) than for δ^{15}N (2.88). Muscle tissue of the three fish species differed significantly in stable-isotopic composition (Fig. 4.7), both for δ^{15}N ($F = 19.12$, $p < 0.01$) and somewhat less for δ^{13}C ($F = 4.5$, $p = 0.03$). Average δ^{15}N was higher for *O. hunteri* ($\overline{x} = 10.11$) and *C. rendalli* ($\overline{x} = 10.22$) than for *O.* cf. *korogwe* ($\overline{x} = 8.02$), whereas *O. hunteri* ($\overline{x} = -24.60$) was on average more depleted in δ^{13}C than both *O.* cf. *korogwe* ($\overline{x} = -21.84$) and *C. rendalli* ($\overline{x} = -22.76$). Disparity metrics indicate that isotopic disparity is higher in *O. hunteri* than in the two introduced species (Table 4.4), particularly in δ^{13}C where its range approaches the total observed range (Fig. 4.7).

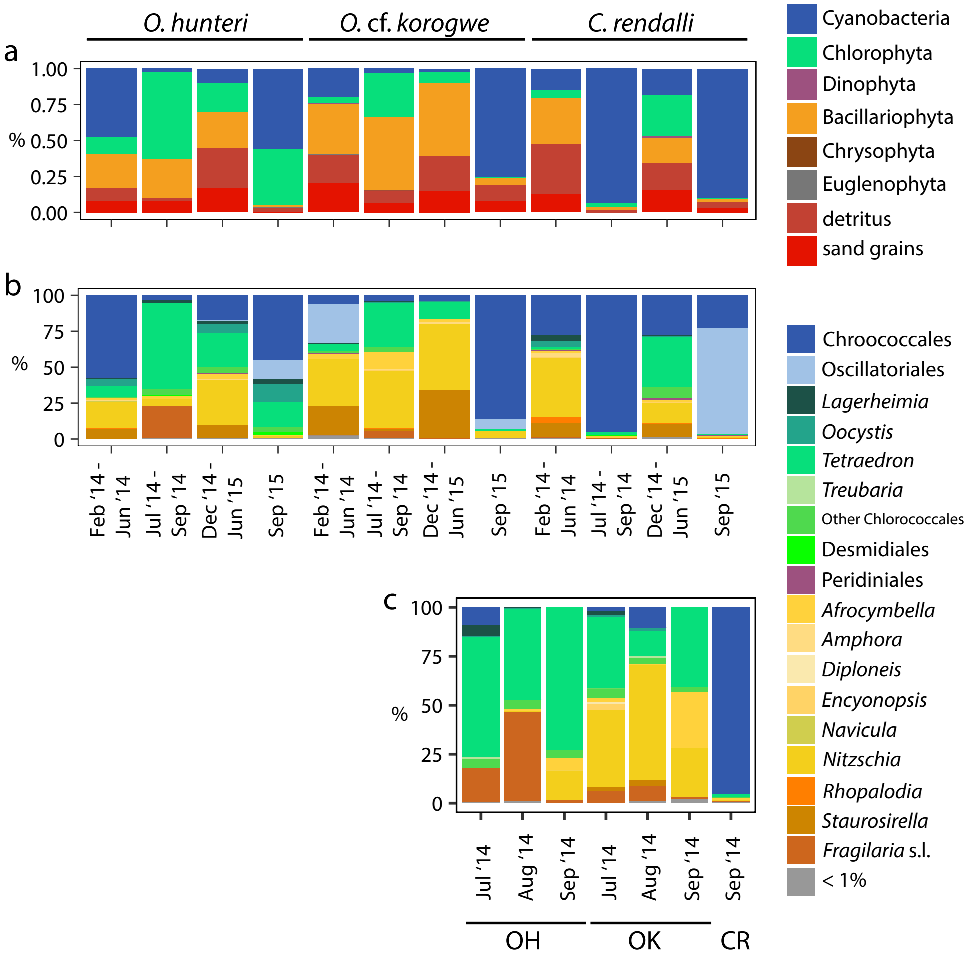


Figure 4.6: Proportion (%N) of algal phyla, detritus and sand (**a**); and algal genera and orders (**b**) in fish gut content per season and per fish species. Panel **c** displays the gut content per month and per species during the annual period of phytoplankton bloom in 2014

Table 4.4: Disparity measures of C:N-ratios in the three tilapiine species of Lake Chala.

	O. hunteri	*O.* cf. *korogwe*	*C. rendalli*
Average Euclidean distance	1.28	1.02	1.14
Sum of Variance	2.71	2.07	1.85

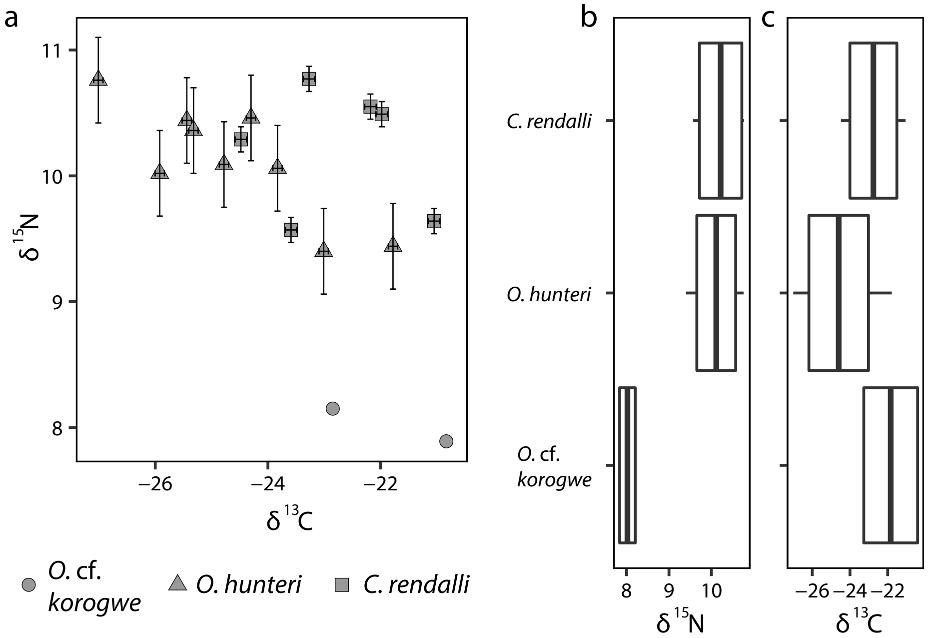


Figure 4.7: **a** Carbon and nitrogen stable-isotope bi-plot with symbols representing the endemic *O. hunteri* (yellow △) and introduced *O.* cf. *korogwe* (green ○) and *C. rendalli* (red □). Error bars represent the standard deviation in stable-isotopic composition of two replicate samples of the same specimen, which were only available for *O. hunteri* and *C. rendalli*. Box plots display mean, standard deviation (SD) and minimum and maximum values per species, for δ^{15}N (**b**) and δ^{13}C (**c**).

4.5 Discussion

Apart from some periods during the predominantly stratified season, when fish reside somewhat deeper and stay very close to shore, and are hence more difficult to catch, specimens were collected in 14 out of 20 months (Table 4.1). Sample sizes are relatively modest; nevertheless, our study is the first to provide insights into the feeding habits of both the endemic *O. hunteri* and introduced *O.* cf. *korogwe* and *C. rendalli* in Lake Chala, although we wish to be prudent with certain interpretations. In the examined guts we did not find evidence of live consumption of animals, not from the rocks nor benthic fauna nor pelagic zooplankton. Similarly, the isotopes do not give indication of the high mean trophic level occupied by omnivores. The results of our gut analyses therefore indicate that all three species currently inhabiting Lake Chala retain a herbivorous diet supplemented with detritus throughout the year, like most other tilapias (Trewavas, 1983). However, we found highly significant seasonal shifts in the feeding behaviour of all three species, that were much larger than the observed interspecific differences. These shifts are strongly linked to seasonal fluctuations in phytoplankton abundance, highlighting the role of resource availability in foraging behaviour.

The three cichlids appear to be restricted to exclusively littoral food sources when the water column is predominantly stratified (February – June 2014 and October – June 2015), but the extensive bloom of green algae (Chlorophyta) and diatoms (Bacillariophyta) during the mixing season (July – September) provides plenty of food to temporarily draw fish towards the open water, where they feed on the successive peaks of different algal taxa (Fig. 4.4). Remarkably, the short-lived yet considerable limnetic Dinophyta bloom, generally taking place from February to March (at least in 2014; Fig. 4.5), is not exploited, indicating that Lake Chala cichlids prefer to retain their littoral feeding habits over abundant ingestion of Dinophyta. Also, the Chlorophyte *Treubaria* is largely absent from the guts in July 2014, although it is at that time highly abundant in the water column (Fig. 4.4). Other planktivorous *Oreochromis* species use their pharyngeal jaws to mechanically process phytoplankton (Greenwood, 1965; Trewavas, 1983), and potentially, the rather large *Treubaria* ($\sim 40\ \mu$m, including the spines) is broken beyond recognition by these jaws before it enters the gut. In contrast, the strong siliceous valves of diatoms may enable them to pass these jaws relatively unscathed.

We detected crucial differences in gut content composition between mixing seasons of subsequent years. The lack of an extensive phytoplankton bloom in 2015 (Fig. 4.5) is reflected in the guts by high proportions of Cyanobacteria (Fig. 4.4) yet low food abundance (Table 4.1), indicating a decrease in food consumption. Although some littoral material (detritus, sand grains) is present in the guts from these months, it is not abundant, suggesting that fish venture out into open waters despite sub-optimal feeding conditions. The presence of some individuals with guts filled with green algae demonstrates that abundant phytoplankton can be locally available, and may still trigger fish to migrate to the limnetic zone.

Importantly, similar seasonal shifts in feeding habits were observed for all species (Fig. 4.6a), indicating that the introduced species have rapidly learned to exploit the seasonal productivity patterns characteristic for Lake Chala. Although this observation is not sufficient to directly demonstrate competition between species (Zengeya *et al.*, 2011), it provides strong circumstantial evidence that *O. hunteri* is presented with serious competition for food. Indeed, we could not find significant interspecific differences in year-round gut content. However, substantial distinctions between species are present in the isotopic signal (Fig. 4.7), with lower δ^{13}C, higher δ^{15}N and higher isotopic disparity in *O. hunteri* than in its non-native counterparts, suggesting at least some form of resource partitioning (Zengeya *et al.*, 2011). The distinction is mostly reflected in the guts of specimens collected during the mixing season, potentially indicating interspecific segregation during the phytoplankton bloom only. Indeed, whereas *O. hunteri* guts contain limnetic phytoplankton (*Fragilaria* s.l., *Tetraedron*) from July onwards, *O.* cf. *korogwe* appears to linger in the littoral until *Afrocymbella* peaks in September (Fig. 4.6b). The one specimen of *C. rendalli* collected during this period had only Cyanobacteria in its gut, hence this species potentially does not feed on the green algae/diatom bloom at all. Phytoplankton is on average more depleted in δ^{13}C than epilithic algae and detritus (Finlay & Kendall, 2007). As regards Lake Chala, Hurrell et al. (2011) found very low δ^{13}C values for the blooming diatom genera *Nitzschia* and *Afrocymbella*. The longer exposure of the endemic *O. hunteri* to phytoplankton could explain the more depleted δ^{13}C values, and its enhanced isotopic disparity.

Gut content composition and stable isotopes thus indicate that the indigenous *O. hunteri* is taking more advantage of the opportunities provided by the annual phytoplankton bloom in Lake Chala than introduced cichlids. The abundance of easily accessible food present in the limnetic zone at that time is likely preferable over scarcer littoral food sources that require active exploration and competition with other individuals. The introduced tilapiines seemingly have not (yet) synchronised their behaviour to the seasonal patterns in food availability in Lake Chala, whereas the presence of *O. hunteri* dates back at least 25,000 years (Chapter 6), which has resulted in a perfect synchrony between the endemic species and lake dynamics. Therefore, *O. hunteri* potentially has a competitive advantage over the recent newcomers. Nevertheless, this advantage strongly depends on the annual recurrence of a strong bloom of diatoms and green algae in the limnetic zone. The strong effect of low phytoplankton abundances on gut content during the mixing season of 2015 therefore raises concerns about the effects of predicted climate warming in East Africa. Wolff et al. (2011) highlighted the consequences of climate variability on the strength of the annual phytoplankton bloom in Lake Chala, as wetter, less windy years can strongly reduce its intensity. The prognoses for a warmer climate in East Africa include higher rainfall and higher rainfall variability (Held *et al.*, 2006) and could thus severely impact the intensity of the phytoplankton bloom. Although *O. hunteri* has coped with severe climate-driven habitat changes in its past (Moernaut *et al.*, 2010), in all likelihood the species has never had to deal with competition from other species during such events (Chapter6). Climate change could therefore affect the faith of endemic and introduced fish species in Lake Chala.

CHAPTER 5

Distribution and burial of fish remains in near-shore sediments of Lake Chala

Jorunn Dieleman[1]
Els Ryken[1]
Wanja Dorothy Nyingi[2]
Dirk Verschuren[1]

[1] Limnology Unit, Department of Biology, Ghent University, K. L. Ledeganckstraat 35, B-9000 Gent, Belgium
[2] Ichthyology Section, Zoology Department, National Museums of Kenya, P.O. Box 40658-00100, Nairobi, Kenya

5.1 Abstract

The isolated crater lake Chala (Kenya/Tanzania) has a continuous offshore sediment record containing abundant fossil fish remains, yet its only endemic cichlid, *Oreochromis hunteri*, spends most of its life close to the shore. This raises questions about how fish fossils are transported to and eventually buried in the depositional centre of the lake. Here, we analyse the distribution of fish fossils (teeth, bones, scales) as well as the remains of diverse other lacustrine biota throughout a short core spanning the last ∼180 years, collected near the shore of Lake Chala, in order to gain insights into sedimentation processes influencing their burial and preservation. This core displays a strong top-to-bottom gradient of sediment characteristics, from soft, fine-grained and finely laminated sediments to consolidated basal muds containing sand and gravel-sized rock particles. We found that the median depth of occurrence of each type of fossil is strongly related to its hydrostatic properties: fossils with low specific density are more abundant in the fine-grained upper core section, whereas fossils with high specific density and/or compact shape are concentrated in the coarse-grained lower section. This suggests that the lower core section is equivalent to a lag deposit, predominantly containing those types of fossils that are least susceptible to the winnowing effect of repeated re-suspension and transport towards greater water depths offshore. In the calm bottom waters of meromictic Lake Chala, not many fish bones are likely to be transported over long distances before final burial. We therefore argue that fish teeth preserved in the offshore sediment record have predominantly been deposited *in situ*. Although we observed a significant correlation between fossil teeth abundance and median grain size, sediment properties did not have a strong influence on tooth size or fractional abundance of teeth from different jaws. Considering further that the offshore sediments of Lake Chala are uniformly fine-grained and laminated, at least throughout the last 25,000 years, we suggest that any changes in sedimentation dynamics that may have occurred during that time must have had a negligible influence on the fish fossil assemblages buried at Lake Chala's depositional centre.

5.2 Introduction

For several decades now, cichlid fishes have been under study as a model system for evolution. Rapid adaptation of the trophic apparatus to a wide variety of available niches, coupled to sexual selection and subsequent reproductive isolation, has been considered pivotal in the origin of hundreds of species in what is a prime example of adaptive radiation (e.g., Stiassny & Meyer, 1999; Danley & Kocher, 2001; Salzburger *et al.*, 2014). Morphological change in the cichlid's trophic apparatus in response to environmental pressures has been described to occur on decadal timescales (Witte *et al.*, 2008; van Rijssel *et al.*, 2015), and the plasticity of this apparatus is often suggested to have influenced speciation (e.g., Meyer, 1990; Muschick *et al.*, 2011; Gunter *et al.*, 2013). Yet the absence of a good fossil record for this group has hampered the study of morphological change through time beyond historical records (Murray, 2001). In this context, fossil fish remains preserved in continuous lake-sediment records could fill an important knowledge gap. Especially fossil fish teeth can potentially be used to trace trophic adaptation to local habitat changes over very long timescales. The cichlid fossil archives available in sediment sequences recovered through lake drilling are thus starting to be explored (Reinthal *et al.*, 2011). These cores are typically collected in deepwater areas offshore to guarantee continuity in sediment deposition (Wilke *et al.*, 2016). However, in many lakes the majority of cichlid species live close to shore (e.g., Fryer, 1959b; Ribbink *et al.*, 1983), raising the question of how their remains reach their final burial place offshore.

This issue is also relevant to the fish fossil record of Lake Chala (Kenya/-Tanzania). Abundant fossil cichlid teeth have been recovered from a 25,000-year sediment sequence collected at Lake Chala's depositional centre in the middle of the lake (Chapter 2 & 6), yet gut contents of the endemic cichlid *Oreochromis hunteri* (Günther, 1889) indicate that this species feeds on littoral food sources for most of the year, and thus must typically reside close to shore (Chapter 4). Understanding how the fossil remains of this species end up in the middle of the lake is an important prerequisite for correct interpretation of the recovered fossil assemblages.

Lake Chala is a 92-meter deep, steep-sided crater lake at the foot of Mount Kilimanjaro with a uniform basin morphometry (Fig. 5.1). Its near-vertical rocky crater walls drop down to a depth of 50-55 m, from where soft sediments slope down more gently to approximately 92 m at the depositional centre (Opitz, 2008; Moernaut *et al.*, 2010). For most of the year, oxygenation of the water column is limited to a depth of 15-20 m, but cooling of the water surface from late June to September promotes convective mixing down to between 35 and 45 m (Buckles *et al.*, 2014; De Crop *et al.*, unpublished data). However, considering the strong control of inter-annual climate variability on water-column mixing in Lake Chala (Wolff *et al.*, 2011), extreme climate anomalies occurring once every several decades (e.g., Buckles *et al.*, 2016) may induce mixing to even greater depths, reaching down to soft-sediment bottom areas near the lake periphery. Seismic-reflection data revealed that pronounced lake-level fluctuations have occurred since Lake Chala's origin approximately 250,000 years ago (Moernaut *et al.*, 2010; Verschuren *et al.*, 2017), but although these were accompanied by slightly altered patterns of sedimentation, at least in the past 25,000 years they were never severe enough to expose near-shore soft sediments. Also, the depositional centre of Lake Chala has likely always been anoxic and devoid of a zoobenthos community (Meyer *et al.*, 2018), reflected in the absence of bioturbation implied by the prevalence of seasonal-scale fine lamination throughout the sediment sequence (Verschuren *et al.*, 2009; Wolff *et al.*, 2011). Therefore, overall sedimentation dynamics in these profundal bottom areas must have remained relatively stable through time.

There are three possible ways in which cichlid teeth may end up at the centre of Lake Chala. *Oreochromis hunteri* displays seasonal migration into the limnetic zone in response to an annual phytoplankton bloom (Chapter 4). Although fish would not have to venture far into open waters to feed off this bloom, they may occasionally do so, and fossils could during this period be deposited *in situ*. Such *in situ* deposition may, firstly, be the result of cichlids continuously replacing their teeth throughout their lives (polyphyodonty; e.g., Streelman *et al.*, 2003), and individual teeth in offshore sediments may thus result from shedding events at that location. Secondly, they could represent remains after death in various stages of disintegration (Elder & Smith, 1988). Thirdly, fossil teeth are largely deposited close to the lake periphery, and may subsequently be transported from the shore to the centre through repeated resuspension and sediment-focussing processes. Occasional extreme deep-mixing events (on decadal and longer time scales) may distort the upper layers of soft sediment and induce focussing towards the depositional centre. Cichlid fossils that are present in these upper layers may thus be transported further and further from shore until they reach their final burial place in the middle of the lake. Importantly, the likelihood of such extreme events increases during lake lowstands, potentially increasing the proportion of teeth (relative to the total amount of teeth produced by the entire lake population in a given period of time) that is eventually deposited in the middle of the lake.

To shed light on some of the processes involved, in this Chapter we study the distribution of fish fossils (teeth, bones and scales) as well as the remains of diverse other lacustrine biota throughout a short surface core collected near-shore. This way, we aim to determine which of the abovementioned processes is more likely to influence fish fossil distributions in sediments from the centre of Lake Chala. Whereas the monotonous sediment composition at the depositional centre hampers correlation of fossils with sediment properties, the variable nature of these near-shore sediments facilitates comparison between fossil distributions and sedimentology. We thus intend to gain insight into potential influences of altered sedimentation patterns on the composition of fossil assemblages during lake lowstands.

5.3 Material and methods

5.3.1 Core collection

A 27 cm-long gravity core (CH16-34G) was collected near the shore of Lake Chala (Fig. 5.1; 53 m depth) in November 2016 using a large-diameter UWITEC gravity corer (ϕ 9cm). To preserve the original gradient of water content and degree of sediment compaction the core was extruded upright in the field and stored in Whirlpack bags for transport to the lab in Ghent, Belgium. The upper 16 cm was extruded in 2-cm intervals, whereas the lower 11 cm was extruded in 1-cm intervals, resulting in a total of 19 sediment intervals.

Finely laminated sediments occur in approximately the top 9 cm of the core (Fig. 5.2a). A detailed count of the number of years represented by these laminated layers cannot be obtained from our extruded core. However, correlation of photographs from the freshly-collected core (Fig. 5.2a) with split-core photographs of partly dewatered surface cores also collected in November 2016 (Fig. 5.2b) in the same near-shore area and at comparable water depths

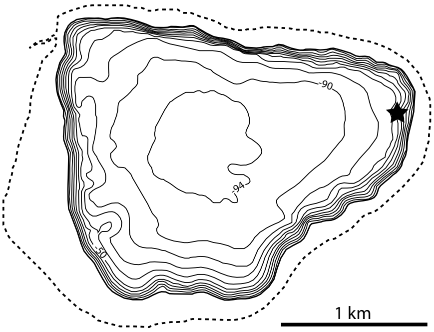


Figure 5.1: Bathymetry of Lake Chala, with indication of near-shore coring location at 53-m water depth

(60 and 65 m; Fig. 5.2b & c), indicates that the 9 cm of laminated sediment were deposited over a time span of approximately 25 years. Extrapolation of the obtained sediment accumulation rate, corrected for the down-core decrease in water content and thus compaction (Fig. 5.3c), indicates that the age of the basal interval of core CH16-34G may be on the order of 180 years (but see below).

5.3.2 Assessment of sediment properties

We used sequential Loss-On-Ignition (LOI) to estimate the organic and carbonate content for each interval (Heiri *et al.*, 2001). We transferred 1 mL of wet sediment from each interval into a crucible and assessed weight loss after 14 h at 105℃ to determine its H_2O content and porosity (H_2O content by volume). Organic matter (OM) content was determined in a similar fashion after burning for 4 h at 550℃, and carbonate content ($CaCO_3$) was determined after ashing for 2 h at 1000℃. The rest fraction (calculated by subtracting the percentages of OM and $CaCO_3$ from 100) is here considered to exclusively represent siliciclastic material, but in reality it also includes an unknown fraction of biogenic silica derived from diatoms and sponge spicules.

Determining the grain-size distribution of the siliciclastic sediment component requires removal of all carbonate and biogenic material from the sediment,

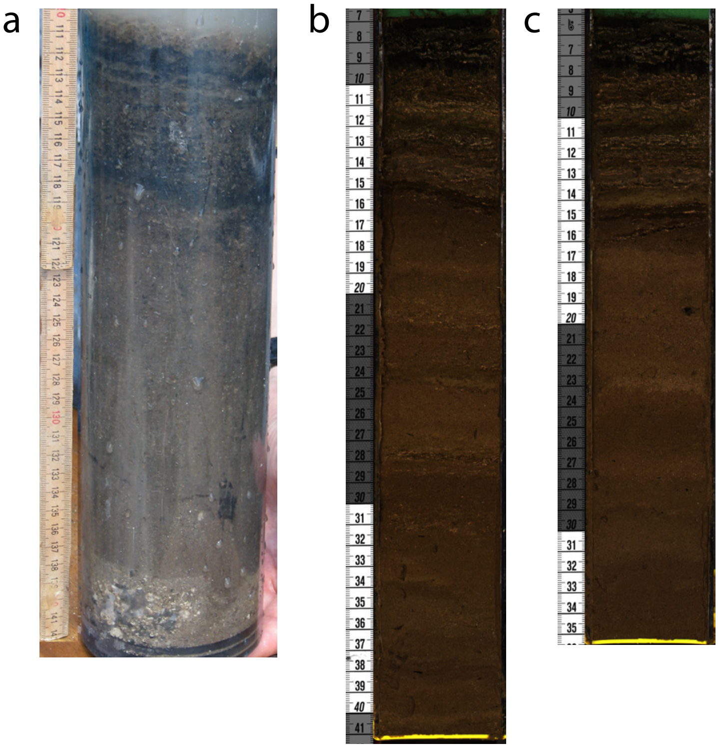


Figure 5.2: **a** Sediment-surface core CH16-34G, collected in November 2016 at 53-m water depth from near the base of the near-vertical rock face. Sediment-surface cores CH16-14G (**b**) and CH16-15G (**c**) were collected in the same month at nearby locations, at 65-m and 60-m water depth, respectively

hence pre-treatment of sediment samples was done following Vaasma (2008). Four gram of wet sediment was treated for 3 h with 10 mL of 10% HCl at 80℃ in order to remove $CaCO_3$. Samples were subsequently washed by centrifuging three times at 3500 rpm, after which 10 mL of H_2O_2 was added to the sediment. Samples were kept at 80℃ and more H_2O_2 was added if necessary until all organic material was removed (after ~2 weeks), and then the suspension was centrifuged three times at 3500 rpm. In a final step, treatment with 10 mL of 10% KOH for 6 h at 80℃ removed (most of the) biogenic silica. After centrifugation, samples were stored in Graham's salt ($NaPO_3$) to avoid coagulation until execution of grain-size analysis by laser diffraction.

Grain-size measurements were performed using a Malvern Mastersizer 3000. Samples were sonicated for 10 min prior to measurement, after which the suspension was pipetted onto a sieve with 1-mm mesh size and placed into the Mastersizer, until obscuration level reached 7-15%. Three consecutive measurements were performed on the same sample for 12 seconds under continuous stirring at 2500 rpm, and a grain size distribution was generated for each measurement. Additionally, 3-4 g of wet sediment was sieved over a 1-mm mesh-size sieve, and the number of inorganic (either siliciclastic or carbonate) particles >1 mm was counted under a binocular microscope.

5.3.3 Collection of fossil material

The distribution of fossil remains from fish and other lacustrine biota with depth in the core was assessed by sampling ~2 cc of wet sediment from each of the 19 intervals, stirring it in H_2O for 30 min and sieving the resulting solution into two size fractions (> 250 μm and 150-250 μm). These samples were then stored in Graham's salt ($NaPO_3$) to prevent coagulation, and counted under a binocular microscope. Counts included fish fossils (scales, bones), gastropod molluscs (snails and limpets), ostracods (seed shrimps), cladocerans (water fleas) and their resting eggs (ephippia), chironomid larvae, sponge spicules, and non-diagnostic insect and plant remains.

To obtain sufficient fossil fish teeth for numerical analysis, an additional ~20 g of wet sediment from each interval was stirred in H_2O for 30 min. This solution was then sieved into two size fractions (> 250 μm and 150-250 μm) that were both searched for fossil teeth under a binocular microscope. Teeth were counted and allocated to their respective jaws (oral or pharyngeal). Oral teeth were classified into the three principal tooth types (unicuspid, bicuspid or tricuspid). All recovered teeth were subsequently photographed at 60x magnification for later measurement.

5.3.4 Data analysis

Tooth length was measured using ImageJ v.1.49m (Rasband, 1997) after the method used in Chapter 2. We used the Malvern Mastersizer 3000 software v.3.62 to calculate the average grain-size distribution in each depth interval (i.e. average of three consecutive measurements performed on the same sample). This average grain-size distribution was then used to calculate a median grain size for each sediment interval. All further analyses were performed in R

(R Development Core Team, 2016). After assumption testing we examined correlations between fossil abundances and median grain size, and between the median tooth size and median grain size, using Spearman rank tests for non-parametric correlations. Moreover, the proportions of oral and pharyngeal teeth, and of the three oral tooth types, were calculated for each interval to assess changes in tooth assemblage composition between depth intervals, again for comparison with sedimentological variables.

5.4 Results

The upper section of core CH16-34G (0-9 cm depth) consists of very soft, finely laminated and fine-grained sediments with high water content (>90%; Fig. 5.3b & 5.3f top panel). Based on the overall thickness of the sequence of alternating dark and light layers (Fig. 5.2a), this upper section represents approximately the last 25 years of lake history (i.e. its lower boundary can be tentatively dated to the early 1990s). The middle section of the core (9-16 cm depth) consists of similarly soft and fine-grained but unlaminated (massive) sediments. The organic matter (OM) content of the upper and middle sections is also similar, and ranges between 15 and 21% (mean 19%; Fig. 5.3a). From 17 cm depth onwards, water content starts to decrease (Fig. 5.3b) but OM content and median grain size remain stable until 19 cm depth (Fig. 5.3d & 5.3f middle panel). Median grain size starts to increase below 19 cm, with silt-sized particles becoming the dominant fraction (Fig. 5.3f bottom panel). Median grain-size is again slightly lower between 22 and 25 cm (but still silt-sized) and then rises more dramatically at 25 cm depth with sand-sized particles becoming predominant at the base of the core (Fig. 5.3d & 5.3f), and OM content drops to 5-7% (Fig. 5.3a). Clastic mineral particles exceeding 1 mm in size are absent in the upper 22 cm, but become abundant in the bottom intervals (Fig. 5.3e) so that the base of the core is distinctly gravelly in appearance. These large particles consist mostly of either pyroclastic material (basalt, lava, tuff) or carbonate.

The depth distribution of fossils of lacustrine biota other than fish throughout core CH16-34G is highly taxon-specific (Fig. 5.4). Arranging the taxa by increasing median depth of occurrence indicates that chitinous remains (Fig. 5.4a-d: ephippia , 0 cm; chironomid larvae, 14-16 cm; other insect remains,

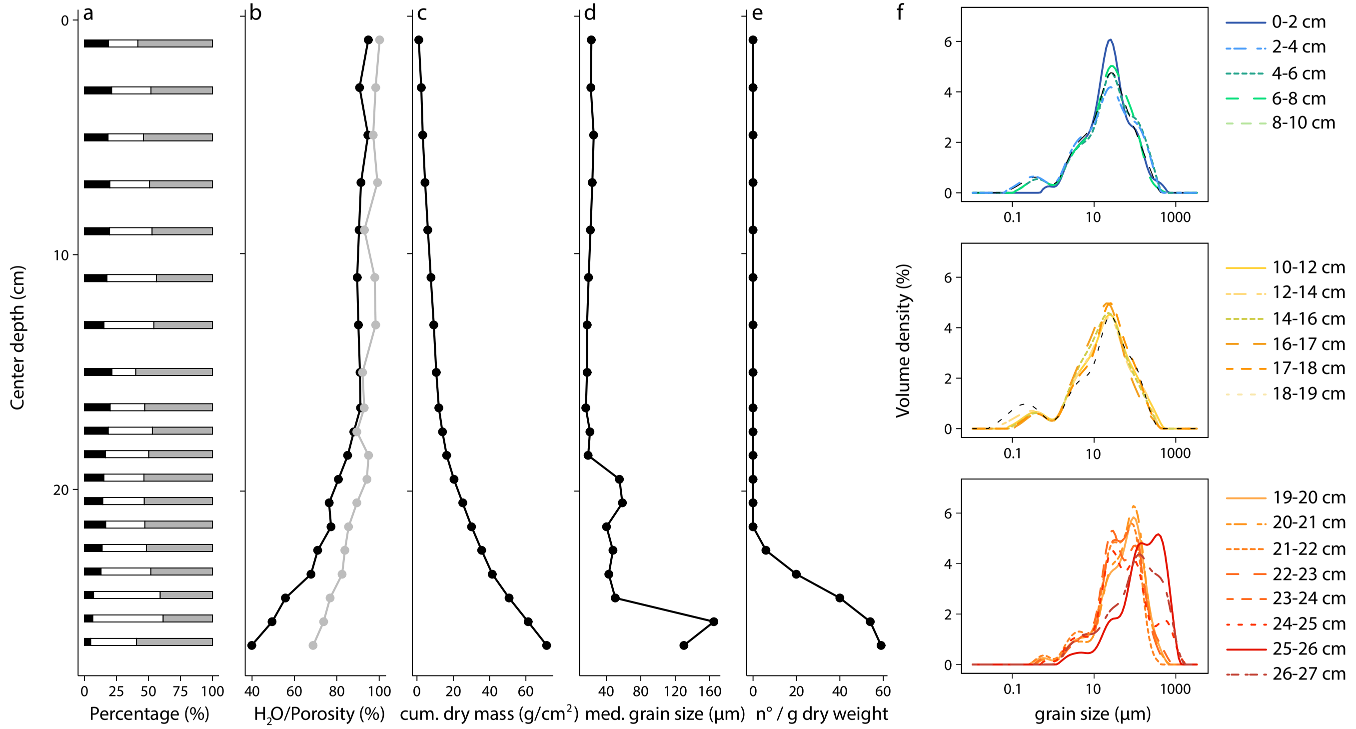


Figure 5.3: Physical properties and bulk composition of near-shore surface core CH16-34G. **a** Proportion of sediment consisting of organic matter (black), carbonate (white) and siliciclastic material including diatoms (grey). **b** H_2O content (black) and porosity (grey) **c** Cumulative dry mass downcore, showing the transition from soft to more consolidated sediments around 16 cm depth **d** median grain size, showing the transition from a predominance of clay-sized particles in the upper 18 cm to silty sediments between 18 and 24 cm, and fine sand at the base of the core. **e** number of inorganic particles >1 mm per gram dry weight, showing the increasing abundance of coarse sand and gravel in the lower 5 cm of the core. **f** Grain-size distribution per interval for the laminated fine-grained sediments (0-10 cm; top panel), the unlaminated fine-grained sediments (10-19 cm; middle panel) and the more coarse-grained sediments in the bottom section of the core (19-27 cm; bottom panel).

16-17 cm; cladocerans, 17-18 cm) occur on average higher up in the core than do fossils consisting of silica (Fig. 5.4e: sponge spicules (*Spongilla* macroscleres), 19-20 cm) and fossils consisting of carbonate (Fig. 5.4g-h: ostracods, 22-23 cm; gastropod molluscs, 25-26 cm). However, the relative size of the fossil remains also matters, with all chitinous fossils being relatively small (<1 mm), and the carbonate fossils typically larger (2-10 mm). *Spongilla* macroscleres are small (250-350 μm) but consist of solid silica, and assume an intermediate position with significant presence both in the upper laminated section and in the silty basal section (Fig. 5.3e). Also, the relatively light-weight but mostly large terrestrial plant remains are retrieved in high abundance from all sections of the core, but with a distinct peak in the upper part of the silty basal section (Fig. 5.4f; median depth 21-22 cm).

Fish fossils are relatively scarce in the fine-grained upper and middle sections of the core (Fig. 5.5a-c), although a short-lived increase is evident in the 8-10 cm interval, more prominently so in the abundance of scales and bones than of teeth. The abundances of all three types of fish fossils increase more dramatically from 19 cm depth onwards, with a general dip in abundance between 23 and 25 cm depth. Overall these abundances are significantly correlated with median grain size (teeth: $r = 0.69$, $p = 0.01$; scales: $r = 0.56$, $p = 0.01$; bones: $r = 0.68$, $p = 0.01$). The median depth of occurrence of fish scales (Fig. 5.5a; 19-20 cm) is rather low, being heavily influenced by the peak in the 8-10 cm interval. The median depths of fish bones (Fig. 5.5b; 23-24 cm) and fish teeth (Fig. 5.5c; 24-25 cm) are similar to those of other fossils with high carbonate contents (ostracods and molluscs), indicating similar hydrostatic properties. There is no significant correlation between median tooth size (Fig. 5.5f) and median grain size ($r = -0.10$, $p = 0.68$). Further, the absence of a correlation between the proportion of pharyngeal versus oral teeth (Fig. 5.5d) and median grain size ($r = -0.36$, $p = 0.14$) suggests that the representation of teeth from the two jaws in the fossil record is not strongly dependent on sediment texture. However, a positive correlation can be observed between the proportion of tricuspids ($r = 0.57$, $p = 0.01$) and unicuspids ($r = 0.66$, $p < 0.01$) and median grain size (Fig. 5.5e).

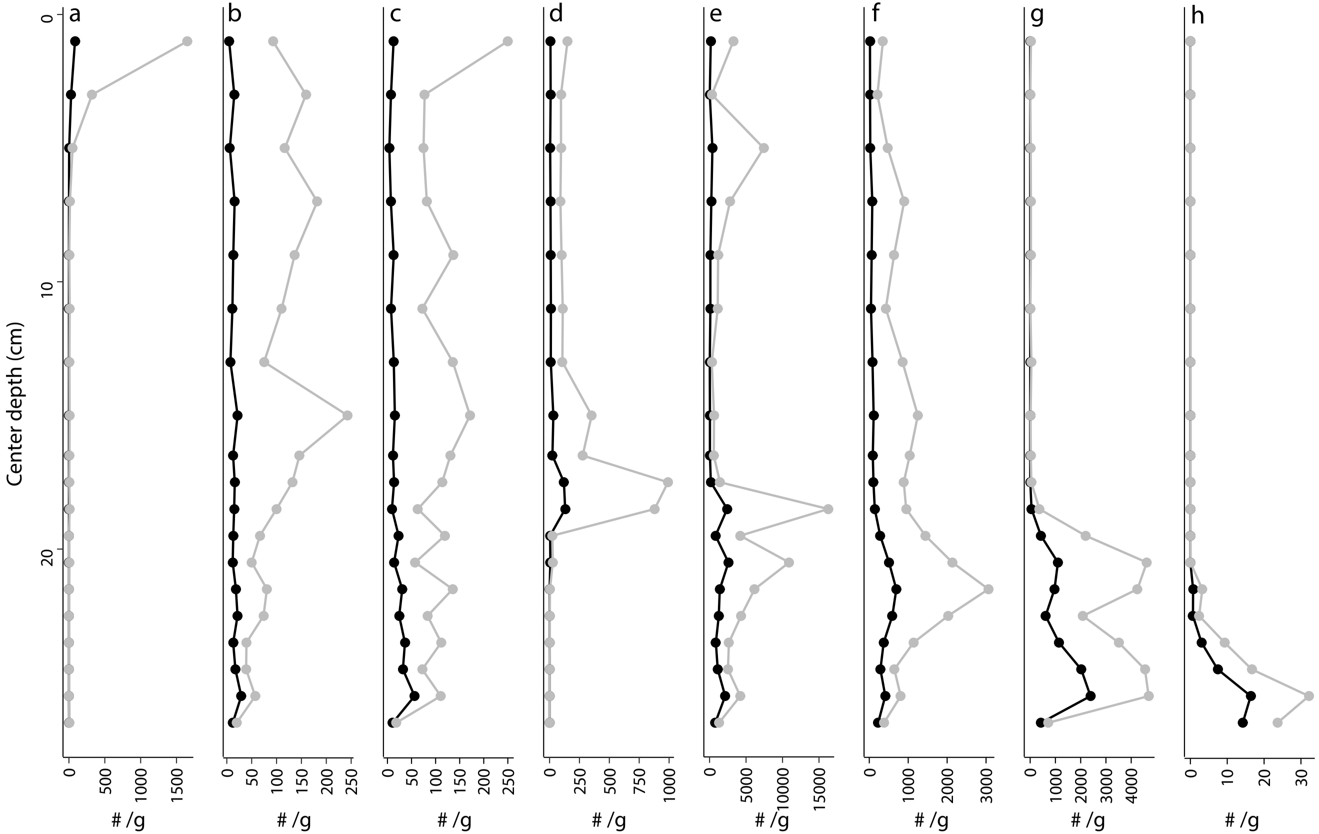


Figure 5.4: Distribution of the fossil remains of aquatic biota other than fishes throughout near-shore surface core CH16-34G. The panels are arranged from left to right by increasing median depth of occurrence, and show the number of fossils per gram wet weight (black) and dry weight (grey). From left to right: cladoceran resting eggs (**a**), chironomid larvae (**b**), other insect remains (**c**), cladocerans (**d**), sponge spicules (**e**), plant remains (**f**), ostracods (**g**) and gastropod molluscs (**h**).

5.5 Discussion

The large inorganic particles found in the bottom intervals of core CH16-34G suggest that the gravity corer touched down on rock bottom, and that the recovered section represents the entire sedimentary deposit present on top of these rocks at 53 m depth. Alternatively, the nearby 1950s construction of the pylon for a mechanical lake-level gauge may have caused small pieces of concrete, likely manufactured with locally available sand and thus difficult to distinguish from natural rock, to be deposited on top of the soft sediments near the lake periphery. The high water content of these sediments may have allowed such concrete fragments to sink through the core, but no evidence of drop stones traveling through the sediment was found during the extrusion process. Moreover, several intervals with low water content lie on top of the intervals with high abundances of large particles, arguing against easy post-depositional protrusion.

The total amount of dry sediment deposited at this location in the last ~25 years equals the value of cumulative dry weight at 9 cm depth (0.78 g/cm^2). Assuming that sediment has accumulated at a similar rate before that time, we can assign an approximate age of 55 years (ca. 1960 AD) to the base of the fine-grained unit at 19 cm depth. Since there is no evidence that lake level was significantly lower than today during most of the 20th century prior to the 1990s, we propose that the lack of fine sediment lamination beyond 9 cm depth is presumably the result of multiple events of unusually deep seasonal-mixing, the last of which occurred ~25 years ago. These events either created enough bottom turbulence to reshuffle the surficial sediment and destroy its lamination, or it injected sufficient oxygen to allow temporary development of a zoobenthos community which would have destroyed the lamination through burrowing (i.e. bioturbation). Considering that this part of the lake bottom is located >10 m lower than the usual depth of seasonal oxygen injection today, and that the oxygen values required for development of a zoobenthos community (~2 mg/l) typically persist for only about two months (De Crop *et al.*, unpublished data), the former mechanism of sediment disturbance may be the most likely.

During such an event, the very soft surface sediment (containing >90% water) is first re-suspended into the lower water column, then resettles with a rate proportional to the size of the particles it is composed of. Large-

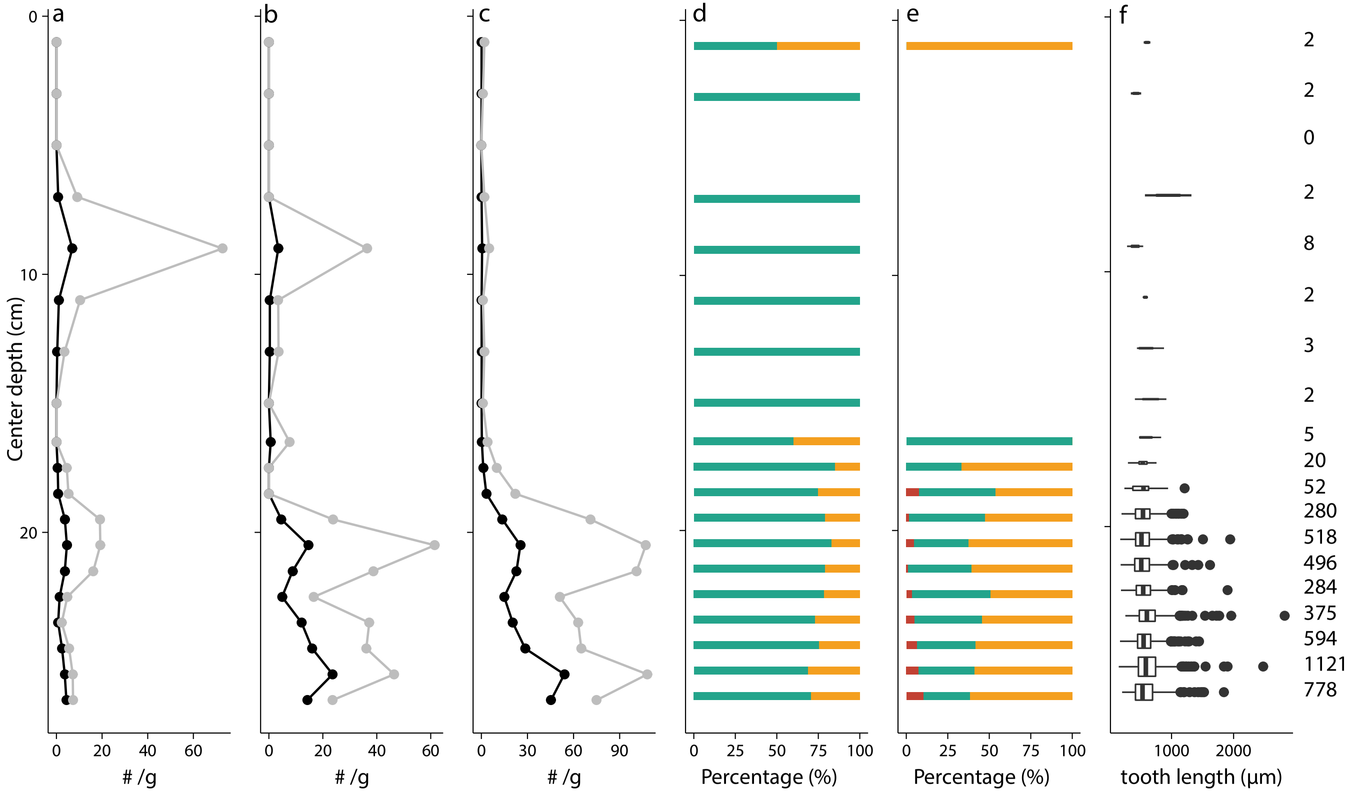


Figure 5.5: Distribution of fish fossils throughout near-shore surface core CH16-34G. The number of fish scales, bones and teeth per gram wet weight (black) and per gram dry weight (grey) are visualised in panels **a**, **b** and **c**, respectively. The proportion of pharyngeal (green) and oral (yellow) teeth is displayed in panel **d**, whereas the proportions of the three principal types of oral teeth is displayed in panel **e**, distinguishing between bicuspid (green), tricuspid (yellow) and unicuspid (red) teeth. Boxplots in **f** represent the size distribution of all recovered teeth per sediment interval, with interquartile ranges, medians and outliers. Numbers on the right indicate the number of fish teeth retrieved from each interval. Note that the median size value remains stable throughout the core, except that low yields handicap its estimation in the upper section of the core. Also, the apparent concentration of large-sized outliers in the lower section is a result of the much higher number of observations in those intervals.

sized sediment grains and biological fossils with high specific density settle down rapidly after disturbance, whereas re-deposition of smaller-sized mineral particles (clay and fine silt) and light-weight fossils can take hours to days (Ferrara & Hildick-Smith, 1982). While in suspension, these small and/or light particles have a higher probability than the larger and/or heavy particles (and fossils) to be transported offshore and to eventually settle in deeper water. Therefore, due to this winnowing process the proportion of large/heavy particles can be expected to increase with depth in the sediment. This seems to be the case here, with the relatively abrupt increase in median grain size between 19 and 21 cm potentially indicating the maximum depth of sediment disturbance which has occurred during these extreme mixing events since the 1960s. Two important caveats must be mentioned, however. First, since a substantial fraction of fine-grained sediment may have been winnowed away from this location during each of these disturbance events, the base of the middle core section at 19 cm depth may well be significantly older than the second half of the 20^{th} century. Second, for most of the time which has elapsed since then, the package of sediment deposited at this location was substantially thinner than it is today. Thus, even the most severe sediment-mixing event will not have mixed much more than 10 cm of soft surface muds. Finally, the decline in median grain size between 22 and 25 cm depth, followed by a steep increase towards the gravelly base of the core, may be a relic from a previous phase in lake history with slightly different bottom dynamics in this near-shore area.

A sequence of short-lived disturbance events is also reflected in the ordered fashion in which biological fossils are distributed throughout the core (Fig. 4.4). The hydrostatic properties of large, carbonate-rich fossils such as gastropod molluscs imply that they are not easily relocated in the first place, and immediately sink after disturbance. Other calcified and heavy fossils like ostracods and fish fossils would likewise sink rapidly, followed by sponge spicules consisting of dense biogenic silica, and only then chitinous fossils such as cladocerans, chironomid larvae and insect remains. The lightest of all fossils are the cladoceran ephippia, which consist of a porous chitinous structure. Their complete disappearance below 3 cm core depth does not necessarily imply poor preservation, since these resting eggs are commonly preserved in lake sediments for multiple millennia (e.g., Iglesias *et al.*, 2016). Rather, we surmise that a large fraction of freshly deposited ephippia is being winnowed away from this near-shore site by bottom currents that are too weak to even disturb the seasonal sediment lamination. The distribution of the densest fossil remains mirrors the double peak observed in median grain size, corroborating our suggestion of the possibility of a second, more ancient series of disturbance events occurring under different conditions of near-shore sedimentation.

The peak in fish fossils observed at 8-10 cm depth likely reflects the localised disintegration and partial removal of a fish carcass. Overall, however, the numbers of fish fossils, especially teeth, in the top 18 cm of the core are very low, suggesting that the slope at this near-shore coring location may expose also fish fossils to at least some gravity-driven focussing offshore. The majority of the fossil teeth encountered in this top section are pharyngeal teeth, yet relative abundances of oral and pharyngeal teeth resemble those encountered at the lake's depositional centre (Chapter 6). Our results indicate that fish fossils and the densest fossils of other lacustrine biota possess highly similar hydrostatic properties, hence the likelihood that fish teeth and bones are being transported over very long distances, even during extreme deep-mixing events, must be rather low. This argues against frequent burial at the lake centre of teeth that initially accumulated elsewhere. We therefore surmise that most teeth retrieved from offshore core locations, including our 25,000-year sediment record (Chapter 6) were deposited *in situ* when fish occasionally ventured offshore, likely through the process of tooth replacement. Fish carcasses appear to become permanently buried in articulated form (Verschuren *et al.*, 2017), as can be expected in the quiet environment of permanently anoxic bottom waters. In core intervals they are easily distinguished by the strongly increased local abundance of fish fossils. *Post mortem* transportation of fish carcasses has likely occurred (Elder & Smith, 1988), but only during a relatively short amount of time. Therefore, both individual teeth and fish carcasses preserved in the sediment represent the lakes' ichthyofauna at the time of their burial.

Our observations in this study thus indicate that sediment focussing currently has very little influence on fish fossil distributions at the lake's depositional centre. This result supports the argumentation that the modest changes in sediment focussing patterns induced by lake-level fluctuations in the last 25,000 years (Moernaut *et al.*, 2010) probably had no influence on fossil distributions offshore. The fractional abundance of teeth derived from different jaws (i.e. oral and pharyngeal) is not correlated to grain size, but oral tooth type proportions are to some extent, indicating that severe changes in sedimentation patterns potentially influence fossil assemblage composition. The extreme downcore gradient in the texture (grain size) of sediments deposited near the lake periphery is however never observed in cores from the depositional centre of Lake Chala, arguing against a strong influence of sedimentology and taphonomy on its fish fossil assemblages. Overall, fossil assemblages retrieved from offshore cores likely represent the fish population inhabiting Lake Chala at that time well.

CHAPTER 6

Long-term trends in oral tooth morphology of *Oreochromis hunteri* linked to past lake-level fluctuations

Jorunn Dieleman[1]
Bert Van Bocxlaer[1,2]
Wanja Dorothy Nyingi[3]
Anna Lyaruu[4]
Dirk Verschuren[1]

After: Dieleman, J., Van Bocxlaer, B., Nyingi, W.D., Lyaruu, A. & Verschuren, D. Long-term trends in oral tooth morphology of *Oreochromis hunteri* (Pisces: Cichlidae) linked to past lake- level fluctuations. *In preparation*

[1] Limnology Unit, Department of Biology, Ghent University, K. L. Ledeganckstraat 35, B-9000 Ghent, Belgium
[2] CNRS, Univ. Lille, UMR 8198 Evolution, Ecology, Paleontology, F-59000 Lille, France
[3] Ichthyology Section, Zoology Department, National Museums of Kenya, P.O. Box 40658-00100, Nairobi, Kenya
[4] Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, 1090 GE Amsterdam, The Netherlands

6.1 Abstract

Climate-induced habitat changes have often been invoked as important pacers of evolution in African cichlid fishes, yet studies directly linking morphological changes to environmental fluctuations are scarce. Here, we track changes through time in the oral tooth morphology of a cichlid fish in relation to past lake-level fluctuations. We analysed the variability in oral tooth morphology of modern-day *Oreochromis hunteri*, the only indigenous fish species inhabiting crater lake Chala (Kenya/Tanzania) as a reference for studies of fossil teeth. Fossils were recovered from six time windows representing alternating high- and low lake-level stands during the last 25,000 years. We find that, as in other cichlids, modern-day *O. hunteri* gradually replace their bicuspid/tricuspid oral dentition with more unicuspid teeth as they grow. Also, *O. hunteri* has likely been the only fish species inhabiting Lake Chala throughout the last 25,000 years. However, we observe a recurrent trend linked to lake level in the relative abundance of oral tooth types, with an increasing proportion of unicuspids during lowstand periods. This increase is not associated with a larger median body size, as teeth from lowstands are not significantly larger. Thus, fishes from lowstand populations developed a unicuspid dentition at smaller size than today. These shifts are systematic and can be linked directly to climate-driven habitat change.

6.2 Introduction

The ability of species to adapt their ecology and behaviour to a changing environment is considered a major driving force of speciation. Especially when environmental changes give rise to new, unexploited habitats (i.e. ecological opportunity), rapid adaptation to these habitats provides ample opportunity for populations to diverge and speciate (Schluter, 2000). Such ecological speciation is famously prominent in African cichlid fishes (Cichlidae), where it has rapidly produced highly diverse species flocks (Fryer & Iles, 1972; Johnson *et al.*, 1996; Salzburger *et al.*, 2014). Both major and minor lake-level fluctuations induced by Quaternary climate change have been invoked as pacers of cichlid radiation, alternatingly creating and eliminating particular types of freshwater habitats (Rossiter, 1995; Danley *et al.*, 2012).

Intrinsic biological factors also influence whether cichlids radiate, and the colonisation of new lakes and habitats does not automatically result in diversification and radiation (Gante & Salzburger, 2012; Wagner *et al.*, 2012). One group of cichlids whose members rarely radiate is the paraphyletic species group formerly referred to as 'tilapia' (Klett & Meyer, 2002; Dunz & Schliewen, 2013; Brawand *et al.*, 2014). In contrast to the more stenotopic haplochromine cichlids, tilapiines, including the genus *Oreochromis*, display a less specialised but highly versatile morphology, making them more tolerant to habitat change and less prone to extinction (Ribbink, 1990; Klett & Meyer, 2002). One trait that has enabled cichlids in general to cope with new environments is great flexibility of their trophic apparatus, which consists of oral and pharyngeal jaws (Muschick *et al.*, 2011; van Rijssel *et al.*, 2015). Oreochromines are generally herbivorous mouthbrooders and hence have similar overall trophic morphologies to accommodate multiple functions (Trewavas, 1983), yet subtle differences in jaw and tooth morphology permit them to specialise in certain ecological niches, especially during periods of food scarcity.

Morphological variation among *Oreochromis* populations is often directly linked to differences in local environment (Ndiwa *et al.*, 2016), but studies of naturally-induced morphological shifts in response to changing surroundings are rare, especially over extended periods. In this respect, the study of fossils has proven effective for some other groups of teleost fish (Purnell *et al.*, 2007; Bellwood *et al.*, 2014). But for cichlids, the scarcity of continuous fossil deposits, the typically disarticulated nature of remains and the limited ability to assign individual fossils to specific lineages has hampered detailed study of long-term trends in their ecology and evolution (Murray, 2001). Cichlid teeth and scales preserved in lake sediments hold the best hope for a continuous record and are increasingly being explored (Reinthal *et al.*, 2011; Muschick *et al.*, 2018), but because of confounding factors (Chapter 2) detailed studies of environment-phenotype relations in cichlid species lineages are still lacking.

The isolated crater lake Chala (Kenya/Tanzania) has a sediment record demonstrating long-term continuity of lacustrine conditions (Verschuren *et al.*, 2009; Moernaut *et al.*, 2010), which contain abundant fossil teeth, bones and scales of cichlid fishes throughout at least the last 25,000 years (Fig. 6.1). The lake currently harbours the endemic Chala tilapia (*Oreochromis hunteri* Günther 1889), which is also the only indigenous fish species. Due to a series

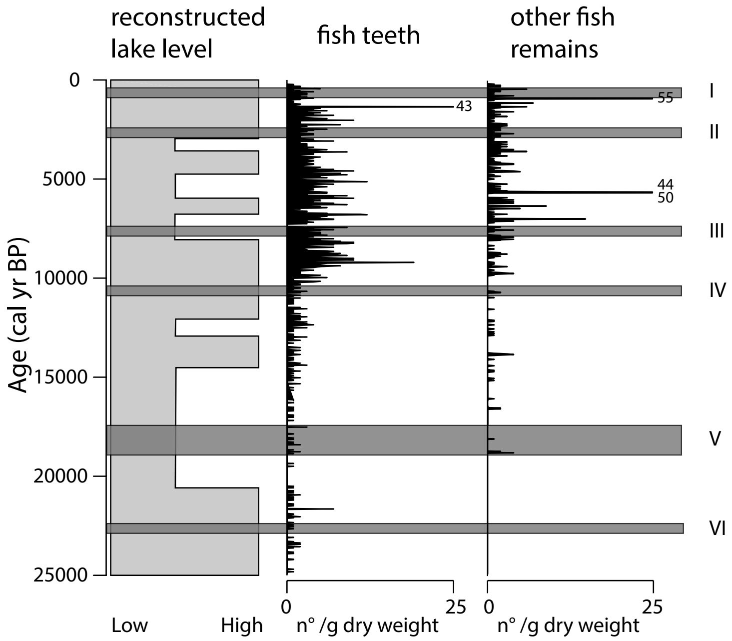


Figure 6.1: Counts of cichlid fossils with respect to reconstructed lake level (after Moernaut *et al.*, 2010). The green bars indicate the six time zones of alternating high- and lowstands that were sampled intensively for fossil teeth.

of anthropogenic introductions in the 1980s, it now shares its habitat with two other tilapiines, *Oreochromis* cf. *korogwe* and Redbreast tilapia (*Coptodon rendalli* (Boulenger, 1897)), and one haplochromine cichlid (Dadzie *et al.*, 1988; Seegers *et al.*, 2003).

Lake Chala is the focus of a multifaceted research programme aiming to reconstruct the long-term environmental and climate history of equatorial East Africa. These studies revealed that the lake has experienced a sequence of pronounced lake-level fluctuations during the last 25,000 years (Moernaut *et al.*, 2010; Fig. 6.1). These lake level drops likely had an impact on the nature of aquatic habitats available to *O. hunteri*. In Chapter 2, we hypothesised that morphological change allowing *O. hunteri* to exploit habitat changes caused by lake-level fluctuations are reflected in the fossil record, in particular in the shape of its oral teeth, and that these teeth can be used as a proxy for changes in trophic ecology. The isolated nature of Lake Chala, its well-studied environmental past, and the known timing of the arrival of other species provide a uniquely controlled setting for a detailed morphological analysis of its fossil record. However, despite being the type species of its genus, only a general morphological and ecological description of *Oreochromis hunteri* is currently available (Trewavas, 1983). Therefore, a quantitative modern-day reference framework of oral jaw morphology must be constructed before the fossil record can be optimally explored. In this context, we first provide a detailed quantification of the intraspecific variability in oral tooth morphology of living *O. hunteri*. This is followed by an analysis of the diversity in oral tooth morphology during six time windows in the last 25,000 years, and assessment whether any changes in tooth shape observed through time can be linked to the independently documented environmental change.

6.3 Material and methods

6.3.1 Study system

The modern-day water level of Lake Chala is relatively high, with a maximum depth of ∼90 m (Moernaut *et al.*, 2010). The narrow, rocky shoreline quickly drops down to a depth of nearly 55 m, from where soft sediments slope more gently towards the middle of the lake (Fig. 5.1; Opitz, 2008). Monthly monitoring of dissolved oxygen throughout the water column indicates that the well-oxygenated zone extends to a maximum of 45 m during mixing events at the end of the long dry season of July to September. For most of the year oxygenated water is limited to the uppermost 15 to 30 m (Wolff *et al.*, 2011), thereby restricting viable fish habitats to the steep rocky crater walls and the open-water (pelagic) zone. The rocks are covered by epilithic algae and interspersed with small sandy patches, providing a relatively continuous food source throughout the year. By contrast, pelagic productivity is largely restricted to a short-lived phytoplankton bloom in August-September (Buckles *et al.*, 2014); low phytoplankton productivity outside of this period implies poor open-water feeding conditions during the rest of the year. The depositional centre of Lake Chala has most probably been continuously anoxic throughout documented lake history (Verschuren *et al.*, 2009), allowing for excellent preservation of fossils (Meyer *et al.*, 2018). Nevertheless, several prolonged lake-level drops in the past 25,000 years were probably severe enough (∼30-40 m, Moernaut *et al.*, 2010) to bring oxygenated conditions to soft-bottom sediments at the base of the rocks around the lake periphery, which currently occur at depths too great (at least 55 m; Opitz, 2008) to be supplied with adequate oxygen. Therefore, soft-bottom benthic habitats may have become available or even predominant during such lowstands (Fig. 1.6).

6.3.2 Modern-day study material

To study the extant population of *O. hunteri* in Lake Chala, we obtained 88 fresh-caught specimens purchased from fishermen between January 2014 and September 2015. Fishermen also determined the sex of these specimens, but later comparison with photographs of these specimens showed that this sex information was unreliable (although fishermen proved very adept at species-level identification, Chapter 3). Fish were photographed in lateral view, and standard length (SL; distance between the tip of the snout and the posterior end of the last vertebra) was measured to generate a body-size frequency distribution of the population. The heads of 12 specimens with SL values covering the full body-size gradient were stored frozen until transport to Belgium. Upon arrival in the lab they were preserved in 10% formaldehyde for later tooth extraction. For this purpose, the oral jaws (left dentary, DTL; left premaxilla, PML) were dissected, cleared and stained using a protocol adapted from Taylor & van Dyke (1985; for details see Chapter 2). *Oreochromis* species typically possess very high numbers of oral teeth implanted in the jaws in multiple rows (Trewavas, 1983; between ∼350 and 950 in *O. hunteri*; Supplementary Figs. D.3, D.4 & D.5). We therefore extracted a random subset of 60 teeth from each specimen, which was determined by systematically lowering the number of teeth included in the morphometric analysis of two test specimens (Chapter 2), without loss of information in the generated morphospace (no decrease in convex hull area). *O. hunteri* generally has bicuspid outer-series teeth (FR) and tricuspid second- (SR) and inner-series teeth (IR). For this study, we extracted 10 FR, 5 SR and 15 IR teeth, from both the DTL and PML of each analysed specimen, all randomly within the respective row classes.

Teeth were photographed at 60x magnification under a binocular microscope. The curved nature of longer teeth hampered standardised orientation of the tooth crown. Therefore, the crowns were clipped off and photographed again in a standardised manner for geometric morphometrics. To optimise morphometrics, the photographs were enhanced in contrast using Photoshop CS6, and PML teeth were mirrored to homologise them with DTL teeth for direct comparison. The edited photographs were bundled per jaw location and per specimen into .tps files using tspUtil version 1.58 (Rohlf, 2013c).

6.3.3 Fossil study material

In 2003 and 2005, the CHALLACEA project recovered a 21.65 m-long sequence of mostly finely laminated muds from the centre of Lake Chala (Verschuren *et al.*, 2009). After excision of five turbidites, a 20.82 m-long sequence of continuous lacustrine sediments represents the last 25,000 years of lake history (Blaauw *et al.*, 2011). The distribution of fish fossils (teeth, scales, bones) in Lake Chala sediments was first assessed by recording their presence in each contiguous 2 cm interval throughout the master composite core sequence below 30 cm depth (a total of 1026 depth intervals, after turbidite excision). This was done by rinsing 2 g of untreated wet mud through a 250 μm mesh sieve, and scanning the retained residue under a binocular microscope at 25-40x magnification. These counts were visualised using package rioja v.0.9.15 in R v.3.3.2 (R Development Core Team, 2016). Next, we used a lake-level reconstruction based on seismic stratigraphy (Moernaut *et al.*, 2010) to select six time windows of between ∼500 and 1500-year duration from alternating lake highstands and lowstands over the last 25,000 years that were certain to contain sufficient fossil teeth for comprehensive morphometric analyses (Fig. 6.1). Given the considerable sample volumes required to extract substantial numbers of fossil teeth (383-1858 g of wet mud), the exact length and position of the selected time windows also depends on the availability of sufficient core material. The fossil teeth were therefore not sampled from the (already partly depleted) master core sequence, but from overlapping duplicate core sections. The visually distinct lamination of Lake Chala sediments permitted unambiguous cross-correlation of these overlapping sections to the master sequence.

Extraction of teeth was done by stirring sediments for 30 min in H_2O, after which the solution was sieved into two size fractions (150-250 μm and >250 μm) that were both searched for fossils under a binocular microscope. Only oral teeth were retrieved, representing 27% of all fossil teeth encountered. Pharyngeal teeth were counted but not analysed further. The collected teeth were photographed at 60x magnification, clipped re-photographed and treated following the same procedure as adopted on modern-day teeth. Photos of bicuspid teeth that had their major cusp on the left were flipped to ascertain homology in direct comparisons, but the quasi-symmetrical tricuspid and unicuspid teeth were left unchanged. Fossil teeth were grouped in .tps files per time window.

6.3.4 Analysis of tooth size and shape

Analysis of tooth size and shape followed the method described in Chapter 2. Tooth length and narrowest width were measured in ImageJ v. 1.49m (Rasband, 1997) and further analysed in R. The necks of fossil teeth were often broken, preventing measurements of their total length, however, the crowns of these teeth could usually still be included in the morphometric dataset. Therefore, we used the width of the tooth crown (more specifically, the enameloid width as represented by the Euclidean distance between landmarks 1 and 3, see below) as a proxy for tooth length. For this purpose, we first analysed the correlations between enameloid width (EW), tooth length (TL) and body size (SL) in modern-day specimens using nonparametric Spearman rank tests, for each of the three tooth types separately. We then compared the median EW of fossil teeth from highstands and lowstand episodes using a nonparametric two-sample Wilcoxon tests, also grouped per tooth type. Nonparametric tests were used because the assumption of normality for parametric tests was not fulfilled, a common issue in fish studies because their indeterminate growth tends to produce skewed size distributions (Nash *et al.*, 2014). Finally, we also compared median EW of teeth between modern-day and fossil assemblages.

Tooth crown shape was analysed using semi-landmark analysis, in which two curves containing 20 semilandmarks each were attached to three fixed landmarks (LM): the left and right lower margin of the enameloid-covered area of the tooth (LM1 and 3) and the most distal location on the tip (highest cusp) of the crown relative to the baseline connecting LM 1 and 3 (LM2). The landmarks were digitised in tspDig2 v.2.17 (Rohlf, 2013a), after which IMP software was used for Procrustes alignment of landmarks and semilandmarks (Sheets, 2008). Teeth in which unambiguous assignment of one or more landmarks was problematic (e.g., due to severe abrasion) were excluded, resulting in a dataset of 671 modern-day teeth and 886 fossil teeth available for further morphometric analyses (Supplementary Table D.1). All further analyses were performed in R. To generate a shape space, we ordinated the aligned landmark data of both modern-day and fossil teeth using non-metric multidimensional scaling in two dimensions (NMDS; packages vegan v.2.4.3 (Oksanen *et al.*, 2017) and MASS v.7.3.45 (Venables & Ripley, 2002)). Ordination of all teeth combined produced an acceptable stress value (8.9) according to the rule of thumb proposed by Kruskal (1964) and Clarke (1993), which indicates that ordination is good if stress values are ≤ 10. Additional statistical analyses were performed on the NMDS coordinates of each tooth.

The diversity in tooth crown shape present within the modern-day *O. hunteri* population was analysed using the NMDS plots of modern-day teeth only. Although a cichlid's oral teeth are generally described as bicuspid, tricuspid or unicuspid (e.g., Trewavas, 1983), the assignment of teeth to these tooth types is not often clear-cut. Hence, to avoid subjectivity, we examined the robustness of our results under a classification method based on quantitative morphometry, using angles between cusps. Cusps were considered distinct if separated by an incision with an angle of less than 135°. Thus, tooth crowns were classified as tricuspid if they possess two incisions with angles $<135°$, bicuspids one and unicuspids none. The performance of this classification method was assessed based on its ability to assign teeth from the modern-day samples to their known jaw location, and its capacity to reflect changes in dentition linked to body size. We also compared angle-based classification with a second quantitative method based on modelled clustering using Gaussian mixture models (Appendix D).

After a general comparison of fossil tooth shape with modern-day teeth, all fossil teeth from the two lowstand and four highstand episodes were aggregated to compare the morphospace occupation of teeth deposited during these two contrasting habitat phases. We used a permutated Hotelling T^2-test packages ICSNP v.1.1.0 (Nordhausen *et al.*, 2015) and Hotelling v.1.0.4 (Curran, 2017) in two dimensions (NMDS1 and NMDS2) to examine differences, as assumptions of multivariate normality and common variance-covariance matrices for parametric tests were not met. This was followed by pairwise comparison of tooth assemblages from each of the six time windows, likewise using permutated Hotelling T^2-tests. Angle-based classification was then applied to the ordination data to determine and compare the relative abundances of the three tooth types. Differences in abundance were validated by randomly resampling the complete dataset and comparing observed abundances with resampled abundances of tooth types.

6.4 Results

6.4.1 Modern-day specimens

The standard length (SL) of the 88 *O. hunteri* specimens collected in Lake Chala ranged from 16 to 30 cm, with peak distributions (81% of all specimens) between 23 and 29 cm (Supplementary Fig. 6.2). Tooth length measurements of modern-day teeth show a clear relation with body size: larger specimens have longer and wider teeth, and they are more robust (Fig. 6.3). Enameloid width (EW) correlates strongly with tooth length for all three tooth types (unicuspids: $r = 0.85$, $p < 0.001$; bicuspids: $r = 0.82$, $p < 0.001$; tricuspids: $r = 0.90$, $p < 0.001$; Fig. 6.4a) and also with body size (unicuspids: $r = 0.55$, $p < 0.001$; bicuspids: $r = 0.71$, $p < 0.001$; tricuspids: $r = 0.74$, $p < 0.001$; Fig. 6.4b). Therefore, we can use EW as a measure of tooth length for all tooth types, and fossil EW distributions as an estimate of the median body size of fossil fish assemblages.

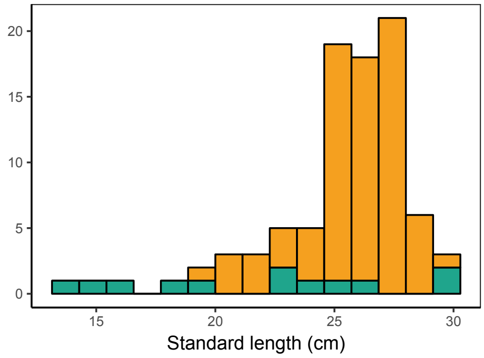


Figure 6.2: Body-size frequency distribution based on standard length of 88 specimens of *O. hunteri*. Colour codes indicate whether teeth were collected (green) or not (yellow).

The shape space containing all 671 teeth from the modern populations is plotted in Figure 6.5a, with the gradient of symbol colour reflecting body size. NMDS-axis 1 discriminates between bicuspids (left, negative side) and tricuspids (right, positive side; Fig. 6.5b), whereas NMDS-axis 2 reflects the

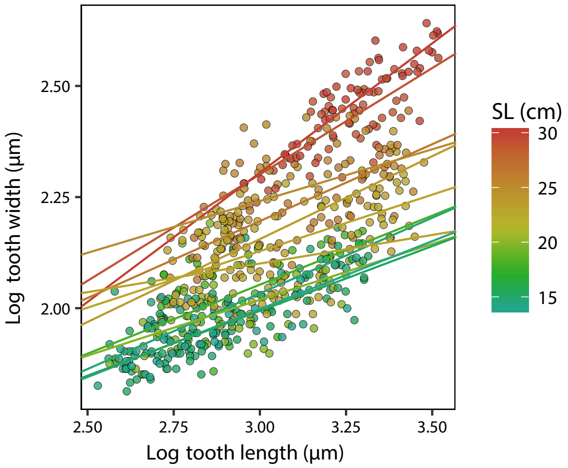


Figure 6.3: Log length-width measurements of modern-day teeth, colour-coded by body size (standard length, SL). Linear regression lines are displayed for each specimen and colour-coded accordingly.

distinction between unicuspids (bottom, negative side) and teeth with multiple cusps (top, positive side; Fig 6.6c). Tooth shape trends related to body size are evident along both NMDS-axes, with teeth from small specimens (more positive values) gradually shifting towards more negative values along both axes, representing more unicuspid teeth in larger specimens.

Shape space occupation of the different tooth types as classified by intercusp angle (unicuspid, bicuspid and tricuspid) is visualised in Figure 6.6a. Despite some overlap, teeth assigned to each of the three tooth types mostly occupy distinct locations in NMDS shape space. Front-series teeth are predominantly bicuspid, whereas the second and inner series consist mostly of tricuspid teeth (Fig. 6.6b). Unicuspid teeth occur in all jaw regions. The increasing trend of unicuspid teeth in larger specimens is clearly reflected in the relative abundances of each tooth type (Fig. 6.6c), mirroring the patterns observed in NMDS shape space. In contrast to the model-based approach (Supplementary information), this classification method thus captures biologically relevant information and is retained for further examination of tooth-type distribution in the fossil record.

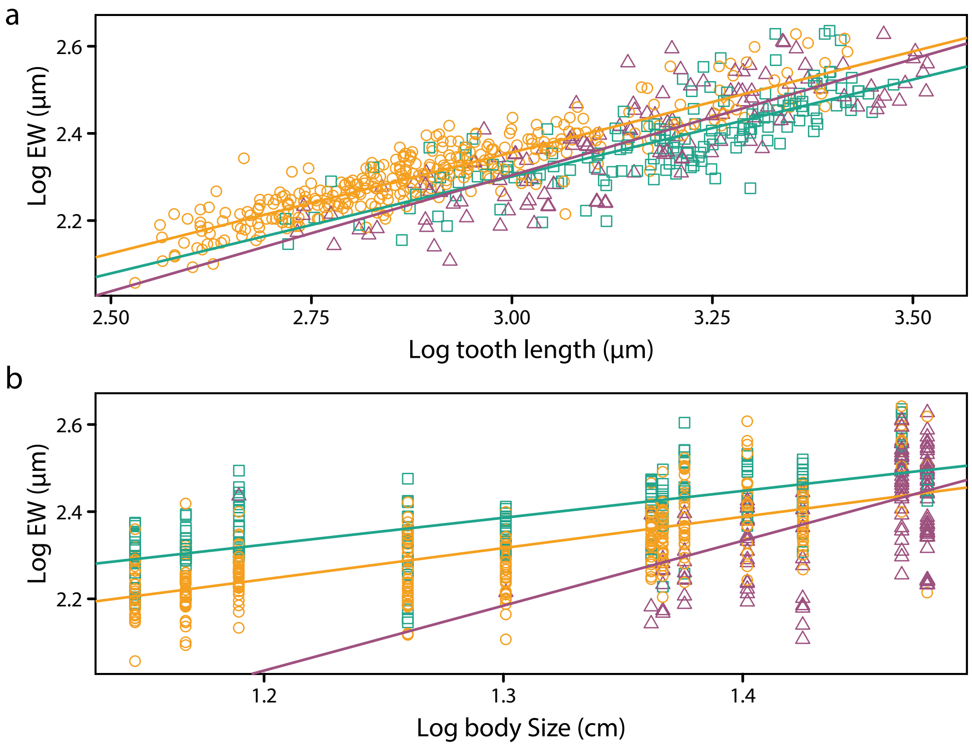


Figure 6.4: Correlations between log enameloid width (EW) and log tooth length (**a**), and between log EW and log body size (**b**). Colour codes and symbols represent the different tooth types: unicuspids (purple △), bicuspids (green □) and tricuspids (yellow ○). Linear regression lines are displayed for each tooth type and colour-coded accordingly.

6.4.2 Fossil teeth

Fossil fish remains are (almost) continuously present throughout 25,000-year sediment record of Lake Chala (Fig. 6.1), but their abundances vary substantially through time. The most notable pattern is a marked increase around 10,500 years ago, i.e. at the onset of the Holocene.

Enameloid width (EW) distributions show that the recovered fossil oral teeth (n = 886) are on average significantly smaller than our reference collection of modern-day teeth of *O. hunteri* (Fig. 6.7a; W=114,790; $p < 0.001$). Aggregating fossil teeth assemblages according to lake level reveals that teeth deposited during two lowstand episodes (subtotal n = 526) are significantly larger, on average, than those from four highstand episodes (subtotal n =

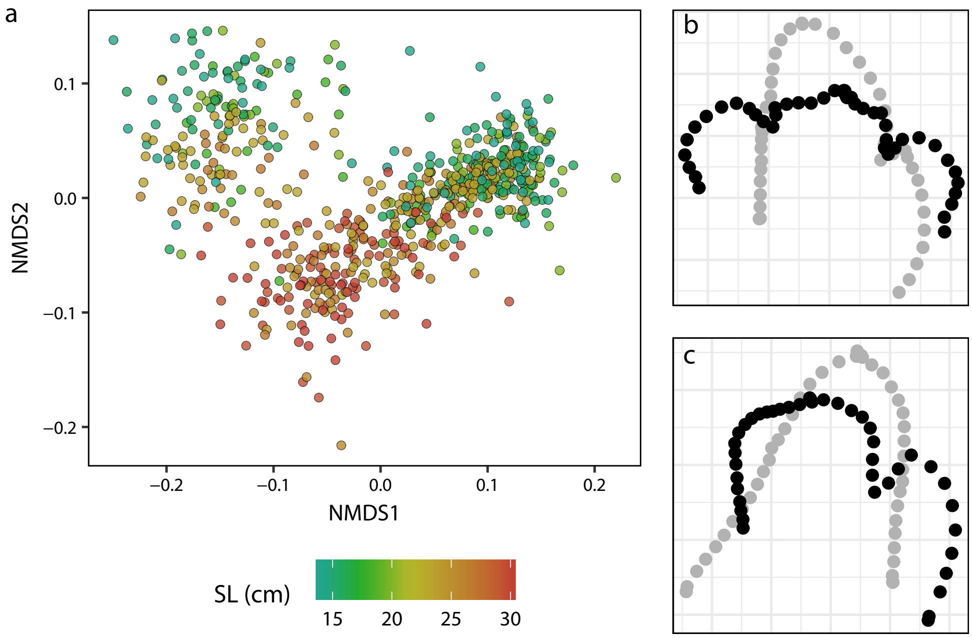


Figure 6.5: NMDS shape space of modern-day teeth with colour reflecting body size (**a**), with outline drawings of specimens representing minimum (grey) and maximum (black) values along NMDS-axis 1 (**b**) and along NMDS-axis 2 (**c**).

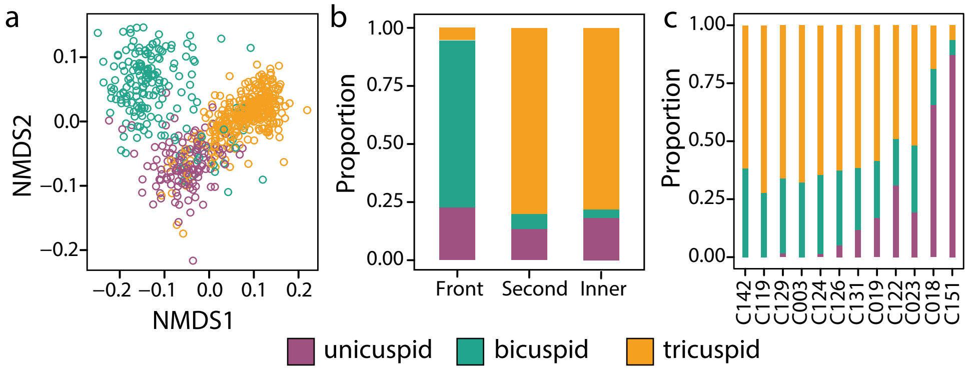


Figure 6.6: **a** Modern-day NMDS shape space colour-coded by tooth type as classified by intercusp angle, and **b** distribution of tooth types in the jaw. **c** displays proportions of tooth types in each specimen, ordered by increasing body size.

360; W = 77,434; $p < 0.001$). However, non-aggregated assemblages display a marked decline in median tooth size from older to younger time windows in all three tooth types (Fig. 6.8), indicating that the previous result may have been biased by the large sample from lowstand episode III (Supplementary Table D.1), and that the trend in median tooth size is potentially influenced by time passed since deposition, rather than by lake level at the time of deposition. Although most samples are skewed towards larger teeth, this skewness is enhanced in older samples (Fig. 6.8), suggesting that post-depositional corrosion results in a disproportionate loss of smaller teeth over long time periods.

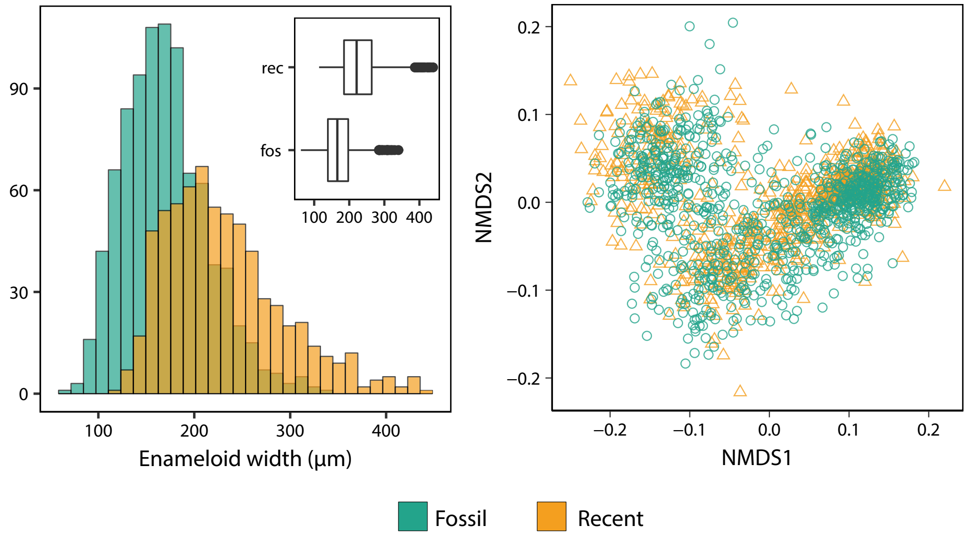


Figure 6.7: **a** Enameloid width (EW) distributions of fossil (green) and modern-day teeth (yellow), with boxplots (inset) capturing median EW and interquartile ranges. **b** NMDS shape space including both fossil (green) and modern-day (yellow) teeth.

Fossil teeth are uni-, bi- and tricuspid, similar to those in modern-day *O. hunteri*. Viewing morphological variation in the oral tooth crowns as a continuum through time, the variability in tooth shapes represented by our six fossil assemblages overlaps almost completely with that in the modern-day population of *O. hunteri* (Fig. 6.7b), strongly suggesting that all recovered fossil teeth belong to ancestral *O. hunteri*. When fossil assemblages are aggregated according to lake level, mean oral tooth shape of *O. hunteri* living during past lowstand phases is significantly different from that of *O. hunteri* during highstand episodes ($T^2 = 85.25$, $p < 0.001$; Fig. 6.9a). The difference is mainly situated along NMDS2, with on average more negative values for 'lowstand' teeth. The variation in tooth shape explained by the two NMDS-axes

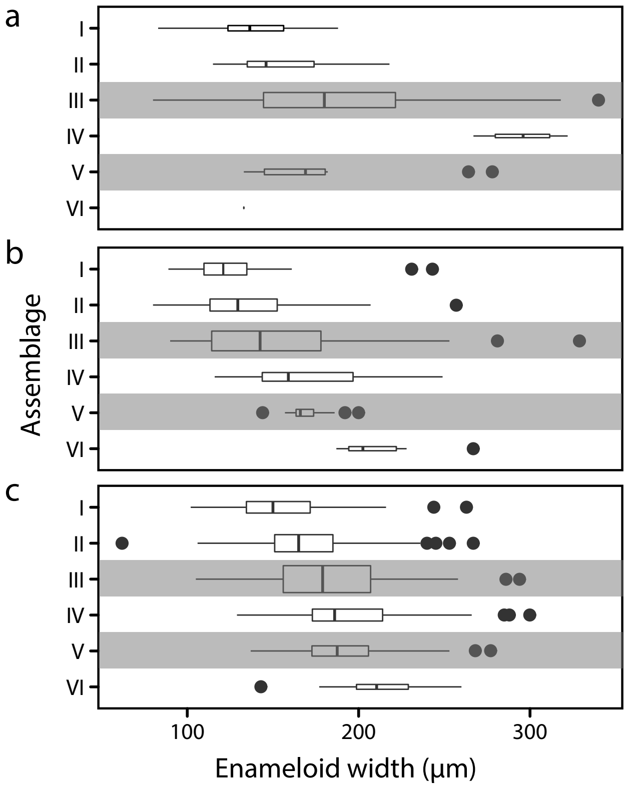


Figure 6.8: Boxplots representing median EW values and interquartile ranges per fossil assemblage, and per tooth type: **a** unicuspids, **b** bicuspids and **c** tricuspids. Grey bars indicate fossil assemblages derived from lowstand periods

is identical to that in modern-day teeth (Fig. 6.5b-c), with more negative values along NMDS-axis 2 similarly indicating a greater proportion of unicuspid teeth. Moreover, when comparing the two lowstand and four highstand assemblages separately, this shift in tooth morphology is shown to be recurrent through time (Fig. 6.9b). More specifically, permutated Hotelling T^2-tests yield significant differences in five out of eight Bonferroni-corrected pairwise comparisons between NMDS-axes values of individual high- and lowstand assemblages (Table 6.1).

Plotting fossil oral teeth according to tooth type as classified by intercusp angle reveals a higher amount of overlap between types than in modern-day shape space (Fig. 6.10a). Nevertheless, consistent with our observations in NMDS shape space, the proportion of unicuspid teeth as classified by intercusp

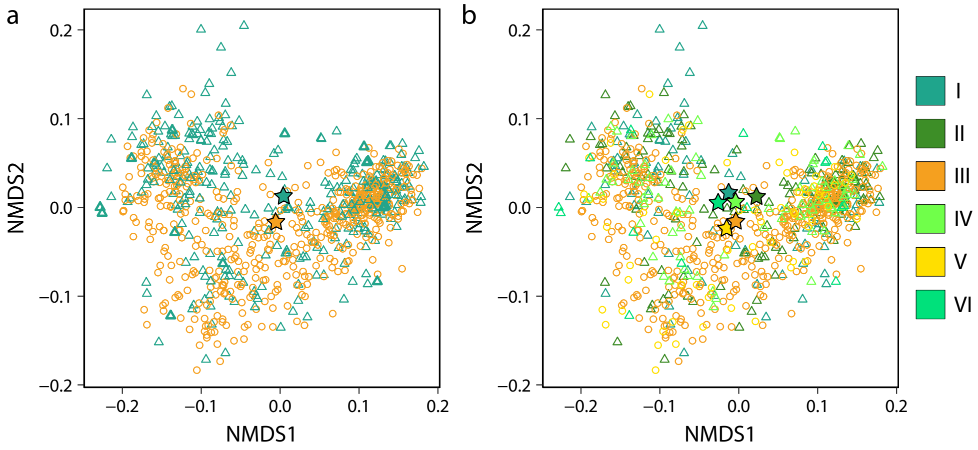


Figure 6.9: NMDS shape space of fossil teeth displaying the distinction between fossil crown shape during lowstands (yellow circles) and highstands (green triangles; **a**), and between fossil crown shape of six assemblages from alternating high- and low lake level phases (**b**). Stars indicate centroid location.

Table 6.1: Bonferroni-corrected p-values of permutated Hotelling T^2-tests for each pairwise comparison between assemblages. Comparisons between assemblages from contrasting lake-level stands have grey cell colours. P-values significant on the 5% signifance level are indicated with an asterisk (*). .

		H	H	L	H	L
		I	II	III	IV	V
H	II	0.74				
L	III	$<0.01^*$	$<0.01^*$			
H	IV	1.00	1.00	0.03^*		
L	V	0.02^*	$<0.01^*$	1.00	0.17	
H	VI	1.00	1.00	1.00	1.00	1.00

angle is markedly higher during lowstand episodes than during the highstand episodes before and after (Fig. 6.10b). The average increase (∼10%) is significantly ($p < 0.01$) higher than what can be expected by chance.

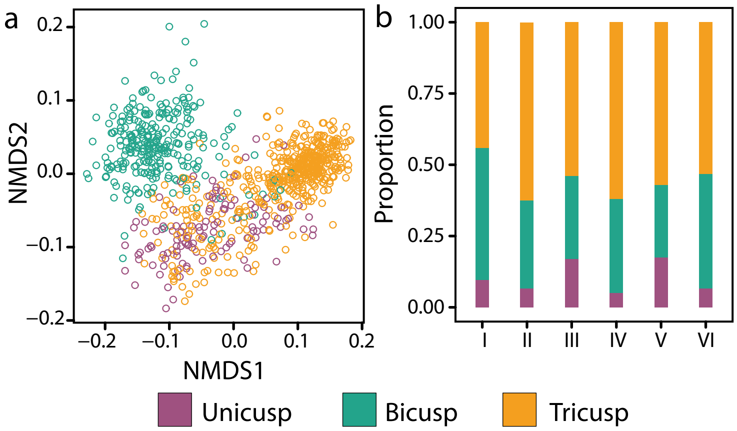


Figure 6.10: NMDS shape space of tooth types assigned using the intercusp angle (**a**), and their relative proportions in the six sampled time intervals (**b**).

6.5 Discussion

6.5.1 The oral dentition of modern-day *O. hunteri*

Our data show that during their lifetime, modern-day *Oreochromis hunteri* in Lake Chala undergo significant shifts in oral dentition. First, the teeth become gradually wider and more robust with increasing body size (Fig. 6.3). Secondly, larger fishes develop a greater proportion of unicuspid teeth (Figs. 6.5 & 6.6). Specifically, small fish have bicuspid FR teeth followed by tricuspid SR and IR teeth, whereas very large fishes possess an almost entirely unicuspid oral dentition. Individuals of intermediate body size, which make up the majority of the specimens available for this study, have an intermediate dentition: they largely retain the bicuspid/tricuspid arrangement, but the major/middle tooth cusp becomes more dominant relative to its side cusp(s). The shift towards a predominantly unicuspid dentition in very large specimens is thus gradual rather than abrupt (Fig. 6.6).

The shift to unicuspid FR teeth or an entirely unicuspid oral dentition is known to occur in large individuals of several other *Oreochomis* species, mainly in sexually mature males (Trewavas, 1983). Most cichlids display dietary shifts as they grow (Njiru *et al.*, 2008), and these are regularly accompanied by changes in different aspects of their trophic morphology (Streelman *et al.*, 2007). To our knowledge, this is the first study documenting such ontogenetic change in oral dentition with geometric morphometrics, and it reveals that, at least in *O. hunter*i, the changes ensue gradually until completely transforming the dentition. *Oreochromis* species are generally herbivorous (Trewavas, 1983), including modern-day *O. hunteri* in Lake Chala (Chapter 4). Given the lack of submerged macrophytes and a reliable pelagic food source, it feeds mostly on epilithic algae growing on the steep rocky shores (Chapter 4). In other cichlid taxa a unicuspid oral dentition is often linked to feeding at a higher trophic level (Fryer & Iles, 1972; Witte & Van Oijen, 1990), but oreochromines are rarely entirely piscivorous or insectivorous. We therefore surmise that the shift to unicuspid dentition in sexually mature *O. hunteri* is associated with their enhanced territorial behaviour linked to breeding (Trewavas, 1983).

6.5.2 Trends in fossil teeth abundance and preservation

The presence of fossils throughout the core demonstrates that fish have been (almost) continuously present in Lake Chala over at least the past 25,000 years (Fig. 6.1). Lack of a systematic contrast in the abundance of fish fossils between highstand and lowstand episodes argues for a negligible influence of changes in *post-mortem* transport on the abundance of fish fossils recovered from the mid-lake sediment record (Chapter 5). Nevertheless, fossil abundances vary considerably during this period, showing a distinct rise at the Pleistocene-Holocene transition approximately 10,500 years BP. The organic-carbon content in Lake Chala sediments shows a marked increase in aquatic primary production during this transition (Blaauw *et al.*, 2011; Barker *et al.*, 2013; Meyer *et al.*, 2018), which is presumably associated with regional climate warming around the same time (Loomis *et al.*, 2017) and may have enhanced the lake's carrying capacity to sustain a larger fish population. Based on genetic analyses, we found no evidence of a pronounced demographic expansion within the last 25,000 years (Chapter 3), although the sample size was relatively small for the used approach. Taphonomic effects cannot be excluded at this stage. Chemical corrosion makes older teeth more brittle and possibly reduces their preservation potential, which not only decreases the overall abundance of fossils in older samples, but also the proportion of small teeth. However, the relatively modest trend in mean tooth size with age (Fig. 6.8a) compared to the abrupt, order-of-magnitude increase in fossil abundance at the start of the Holocene (Fig. 6.1) suggests that the latter mostly reflects a true increase of the Lake Chala

O. hunteri population at that time.

Our data on enameloid width (EW) indicates that fossil teeth are on average smaller than those of modern-day fishes (Fig. 6.7a). No specimens smaller than 16 cm were collected in the modern-day survey, presumably due to large fish-net mesh. Fry and juvenile fishes are abundantly present in natural populations, and typically experience high mortality (Cushing, 1974; Peterson & Wroblewski, 1984). Teeth of these small fishes should be well-represented in the fossil record, except that fry teeth may suffer more chemical corrosion affecting their preservation (and in any case are not retained by the used sieve if smaller than 150 μm). Moreover, cichlids continuously replace their teeth as they grow (polyphyodonthy; Fryer & Iles, 1972; Hulsey *et al.*, 2016), and a tooth has an average lifespan of around 100 days (Tuisku & Hildebrand, 1994). If *O. hunteri* has a life span similar to other *Oreochromis* species (up to ~10 years; Froese & Pauly, 2017), each large specimen has contributed up to three dozen of generations of teeth to the sediment record, increasing the proportion of small teeth further. The smaller median size of fossil teeth thus conforms to expectation. In effect, the minimum size of teeth in our modern-day reference collection is controlled by the mesh size of the fish nets, whereas the minimum size of teeth in the fossil assemblage is controlled by the sieve mesh used for sediment processing. Recent and fossil assemblages are hence not directly comparable in terms of tooth size, but their tooth shapes show great similarities (Fig. 6.7b). This implies that fossil *O. hunteri* tooth morphology can be interpreted within the modern-day framework.

6.5.3 Temporal trends in oral tooth shape

The large overlap between fossil and modern-day oral teeth in shape spaces (Fig. 6.7b) confirms that *O. hunteri* has been the only fish species inhabiting Lake Chala before the recent introductions, and this for at least the last 25,000 years. The overall variability in tooth shape existing in the current population has thus been remarkably stable over time. However, we observe significant and systematic trends in occupied morphospace that can be related to the documented changes in lake level (Figs. 6.9 & 6.10). During periods of low lake level, the dentition shifts towards more unicuspid, and we found this trend to occur repeatedly over time. The observed trends are clearly reflected in the relative abundances of different tooth types, in which an average increase of

10% in the abundance of unicuspid teeth is observed during lowstand periods (Fig. 6.10).

The morphological changes from highstands to lowstands are overall similar to the changes in oral dentition occurring in the modern-day population as specimens grow larger. However, fossil teeth from lowstand assemblages are not systematically larger than those from highstand assemblages (Fig. 6.8), hence a larger median body size during low lake levels is not a plausible explanation for the observed shifts in tooth shape. Rather, our data suggest that, during low lake level phases, *O. hunteri* shifted towards a unicuspid dentition earlier in life, likely triggered by a change in available habitat. In cichlids, unicuspids are often related to feeding at a higher trophic level (Fryer & Iles, 1972), and in Lake Chala may have been advantageous to exploit a new food source that is present in the soft sediments made accessible during lowstands, such as insect larvae, annelid worms and other macrobenthos. However, insectivorous oreochromines are rare, although juveniles may opportunistically feed on insects or even fish fry (Njiru *et al.*, 2004). Alternatively, an earlier shift towards unicuspid teeth might indicate that sexual maturity is reached at smaller body sizes (i.e. at an earlier age) during low lake level phases than during highstands. Intraspecific population variability in body size and age at maturity is common in *Oreochromis* species (Duponchelle & Panfili, 1998) and is strongly linked to environmental factors, such as temperature and population density (Brummett, 1995).

Both phenotypic plasticity (Bouton *et al.*, 2002; Muschick *et al.*, 2011; van Rijssel *et al.*, 2015) and genetic differences (Albertson & Kocher, 2006; Hulsey *et al.*, 2017) have been invoked to explain rapid morphological change in the trophic ecology of cichlid fishes. We can currently only hypothesise on the exact ecological and evolutionary mechanisms underlying the observed trends in oral tooth shape, yet the observation in itself is remarkable. Recurrent shifts in oral tooth shape directly linked to lake-level fluctuations provide long-term evidence of the ability of *O. hunteri* to respond to climate-driven environmental variation, and confirm the versatility of this species over extended periods of time. Rapid morphological responses in the trophic apparatus of *O. hunteri* may thus have enabled this species to cope with past environmental change.

CHAPTER 7

General discussion

7.1 Producing a long-term record of evolutionary change in cichlid fishes

For almost a century, cichlid fishes populating freshwater bodies in both the Old and New World tropics have been under study as a model system for ecological speciation and radiation (Fryer & Iles, 1972; Stiassny & Meyer, 1999). Although this research has led to a better understanding of the processes governing evolution, many questions as to how various factors contributed to differentiation and reproductive isolation remain unanswered. A major drawback is the lack of a continuous fossil record of this group (Murray, 2001), hampering long-term inquiries of morphological patterns and their underlying processes. Indeed, rapid morphological responses to environmental change, especially of ecologically important traits, have been documented to occur on very short timescales (e.g., Witte *et al.*, 2008; van Rijssel *et al.*, 2015) and they have been proposed to function as a driver of diversification and speciation in cichlids (e.g., Meyer, 1990; Muschick *et al.*, 2011; Gunter *et al.*, 2013). Extending morphological observations beyond the historical time scale could provide long-term evidence of such morphological responses and corroborate their importance in cichlid evolution, yet these studies are currently lacking.

Fossil remains of cichlid fishes, like teeth, scales and bones are, however, often well-preserved in lake sediments. These sediments may in some lakes provide a continuous palaeontological record, stretching back to the lake's early origins. Cichlid fossils have been tentatively explored in the sediment record of the great African rift lake Malawi (Reinthal *et al.*, 2011), but the enormous species diversity and major past lake-level fluctuations in this lakes (Chapter 1) complicate the analysis and interpretation of its fossil records. Appointing fossils to a certain species is difficult in these lakes, and changes in the position of the shoreline likely influenced the composition of fossil assemblages at any fixed coring location. Ideally, a small and isolated system with limited species diversity yet a continuous fossil record is examined first to explore the feasibility of paleontological studies of cichlids as a baseline for later investigations in more complex systems. Moreover, if this small system has a well-resolved climatic past and has been prone to environmental fluctuations over extended periods of time, it could be used to document morphological change in response to such fluctuations and it may provide insight into long-term ecological and evolutionary processes underlying cichlid diversity.

This thesis had the general aim of tracing long-term morphological change in a single cichlid species in response to past lake-level fluctuations. The isolated crater lake Chala (Kenya/Tanzania) harbours only one indigenous fish species, the cichlid *Oreochromis hunteri* Günther, 1889. The lake has been prone to major lake-level changes throughout at least the last 25,000 years (Moernaut *et al.*, 2010), which presumably impacted habitat diversity and niche availability for *O. hunteri* (Chapter 1). Lake Chala's finely-laminated and continuous sediment record also contains abundant cichlid fossils (Fig. 1.5), of which especially the morphology of fossil teeth may reflect the species' past ecology and could therefore be used to trace how feeding habits changed in function of environmental changes. Lake Chala thus provides a controlled setting that is ideal to develop studies of long-term morphological change in cichlids in response to climatically-driven hydrological fluctuations in the past.

Chapter 6 presents the findings as to the overall aim to document long-term patterns in oral tooth shape of *Oreochromis hunteri* in response to strong lake-level fluctuations. As this study is the first of its kind in cichlid fishes, it required a feasibility analysis and methodological exploration, which I developed in Chapter 2 and further improved in Chapter 6. Moreover, to fully understand morphological trends observed in the fossil record, an integrated approach that included the extant ichthyofauna as a modern-day reference framework was required. As Lake Chala fishes had thus far been relatively unexplored, I investigated species integrity, colonisation history and feeding habits of the enigmatic *O. hunteri* in Chapter 3 and 4, respectively, with special attention to the impact of anthropogenically introduced cichlid species on *O. hunteri*. Chapter 6 also includes a detailed description of oral tooth shape in extant *O. hunteri*. Beyond these data on the extant fishes, insight into the taphonomic processes that affect how fossil teeth accumulate in the fossil record is required. Therefore, the impact of sedimentation dynamics and taphonomic processes on the distribution of fossil teeth in Lake Chala sediments was assessed in Chapter 5. In the following discussion, I aim to integrate the findings of these respective Chapters. I start with a thorough assessment of the methods used, and elaborate on their potential application in future studies of other systems. I then discuss the results of my examination of the extant ichthyofauna of Lake Chala, touching on species integrity and conservation of *O. hunteri* in light of recent anthropogenic introductions and integrating the obtained insights in its diet and oral tooth shape. Important taphonomic influences on the distribution of tooth fossils in the sediment are discussed before I elaborate on the origin of *Oreochromis hunteri* in Lake Chala and its eco-morphological response to past environmental change.

7.2 Quantitative analysis of tooth shape

7.2.1 Geometric morphometrics for cichlids

The use of geometric-morphometrics to quantify organismal shape is now well-established in many fields of biology (Bookstein, 1991; Zelditch *et al.*, 2004), and a large body of literature exists of its application to the study of cichlid fishes (reviewed in Kerschbaumer & Sturmbauer, 2011). In this thesis, geometric-morphometrics were used in two aspects of the research, namely to first describe the whole-body morphology of Lake Chala *Oreochromis*, and then to describe the shape of their extant and fossil oral teeth. Whole-body morphology is described in Chapter 2 using a landmark-based approach. This method was capable to consistently distinguish the two *Oreochromis* species currently inhabiting Lake Chala: the endemic *O. hunteri* and the introduced

Oreochromis cf. *korogwe*. In Chapter 3, the same method was found to be effective to distinguish all *Oreochromis* species currently present in the Upper Pangani River region, even between the phylogenetically close species *O.hunteri* and *O. jipe*.

Fossil fish remains in Lake Chala sediments (and in other lakes; Reinthal *et al.*, 2011) consist largely of individual teeth, yet contemporary ecomorphological studies on cichlids mostly analyse complete oral (Albertson & Kocher, 2001; van Rijssel *et al.*, 2015) and pharyngeal jaws (Hellig *et al.*, 2010; Muschick *et al.*, 2011). Geometric morphometrics have been applied successfully to individual fossil teeth of other biota, for example hominids (Martinón-Torres *et al.*, 2006; Gómez-Robles *et al.*, 2007), sharks (Nyberg *et al.*, 2006; Whitenack & Gottfried, 2010) and teleost fish (Bellwood *et al.*, 2014; Gauchey *et al.*, 2014), but studying individual teeth has rarely been attempted for cichlids (but see Wautier *et al.*, 2002). I therefore developed a geometric-morphometric approach tailored specifically to the analysis of oral tooth shape in the *Oreochromis* species of Lake Chala, *O. hunteri* and *O.* cf. *korogwe*. A working method required solutions to several obstacles, first and foremost the problem that the diversity in tooth shape within a single specimen is generally much larger than interspecific differences. Indeed, the oral jaws of both species contain an outer series of bicuspid teeth and several inner series of tricuspid teeth (Fig. 2.2). Because of this intraspecific disparity, a sensitive method was required to distinguish between the oral teeth from different species, and to trace subtle changes in those oral teeth through time. Moreover, fossil teeth are often broken in ways that do not allow reconstruction of general tooth morphology but nevertheless have their tops (crowns) intact, potentially because the strongly mineralised enameloid layer (Sasagawa, 1997) protects crowns from breaking. I therefore focused on documenting shape variation in crowns because it allows maximal data retrieval from the fossil record. I found that semi-landmark analysis (SLM) followed by principal component analysis (PCA) on the tooth tops best suited the needs.

Semi-landmark analysis was put to the test in Chapter 6, where extension of the analysis to 12 extant *O. hunteri* specimens of different body sizes revealed considerable ontogenetic change in oral tooth shape. The gradual nature of these changes was clearly reflected in the generated morphospace (Fig. 6.5), indicating that SLM could not only separate ontogenetic extremes but also more subtle shape differences. However, the gradual transition from bicuspid and tricuspid oral teeth to unicuspid teeth in large individuals resulted sometimes in uncertainty as to assigning teeth to tooth types. I therefore also compared the results of two classification mechanisms, one based on angles between cusps and the other based on all the variation in the morphometric dataset and Gaussian mixture models. Moreover, I found a strong linear relationship between tooth size and body size, which was then used for inferences about body size in the past.

The very high numbers of oral teeth in the premaxillae and dentaries of *Oreochromis* species required selection of a randomised yet morphologically informative subset of teeth from each analysed modern-day specimen. This subsampling strategy was developed in Chapter 6, comprised 60 teeth per individual and, after exclusion of teeth that could not be digitised, resulted in a modern-day reference frame consisting of a total of 671 teeth. Yet these high numbers of oral teeth had to be matched by a comparable amount of fossil teeth being analysed if subtle shape shifts through time were to be detected. Eventually, 886 fossil teeth were included in the morphometric dataset of Chapter 6, but sample sizes varied greatly between different time windows, so that non-parametric statistical tests had to be applied to analyse the digitised datasets. Non-metric multidimensional scaling (NMDS) returned highly similar results as PCA, but it has less assumptions and, if stress values are acceptable, it allows representing all shape variation on a predefined number of axes (here two). Overall, the methods presented in Chapter 6, largely based on earlier findings in Chapter 2, proved ideal to trace oral tooth shape change within a species through time in relation to past environmental change.

7.2.2 Potential of the developed method for future studies

The method of tooth shape analysis used in this thesis can be applied on both extant and fossil cichlid species in other systems. Combining geometric-morphometric analysis of the tooth crown with length-width measurements of the neck allows distinguishing between species (Chapter 2) and tracing subtle shape changes associated with ontogeny (Chapter 6). It could therefore potentially be implemented in morphological studies of tooth development, for example enabling more detailed analyses of tooth replacement patterns. Currently, such studies use qualitative tooth shape descriptions (e.g., Huysseune, 1995; Streelman *et al.*, 2003), yet a quantitative approach of oral tooth shape analysis could facilitate comparison between studies. It could also be used to study convergence in oral tooth morphology between more distantly related taxa, as has for example been done for the shape of the lower pharyngeal element (Muschick *et al.*, 2012). Although this method is very sensitive for subtle changes in oral tooth crown shape, such studies may nevertheless require extra biometric measures for thorough description of complete oral tooth shape and interspecific differences. For example, tooth curvature is an important source of shape variation in oral cichlid teeth (Fryer & Iles, 1972) that has been standardised in this analysis.

The main aim was however the development of a tool that enables a quantitative analysis of individual fossil cichlid teeth in lake sediments. I successfully implemented this approach on the fossil record of Lake Chala, yet some important caveats should be made for other systems. First and foremost, very high numbers of fossil teeth are required for complete examination of shape diversity in the fossil record. In total, 5 kg of wet sediment extracted from cores was sieved and analysed for the results presented in Chapter 6 alone. Many large scientific drilling programs become increasingly multi- and interdisciplinary (Wilke *et al.*, 2016), restricting the amount of sediment that can be dedicated to the analysis of a single palaeoenvironmental proxy. A sampling strategy generating large amounts of sediment is thus required to adequately study fossil cichlid teeth, and is ideally delineated before coring is initiated.

The workload associated with a comprehensive analysis of modern-day tooth shape is also considerable. I found that incorporation of oral teeth from several individuals of different body sizes is a prerequisite in morphologically versatile genera like *Oreochromis* (Chapter 6), which occupy a wide range of habitats throughout their lives and functionally adapt their trophic traits accordingly (Trewavas, 1983; Ribbink, 1990). Stenotopic lineages, such as the haplochromines, have an ecomorphology that is more closely linked to the exploitation of very narrow niches (Fryer, 1959b; Fryer & Iles, 1972; Ribbink *et al.*, 1983; Reinthal, 1990), and for such taxa smaller sample sizes may suffice to obtain a complete image of extant intraspecific diversity in tooth shape. Changes in oral tooth shape associated with ontogeny have nevertheless also been documented in haplochromine species (Streelman *et al.*, 2003, 2007). Moreover, the enormous species diversity combined with considerable convergence of trophic structures among haplochromines (Chapter 1) may impede assignment of individual fossil teeth to a certain species or even genus. Especially in the cichlid species flocks of the African Great Lakes, studying general ecotypes rather than species-specific ecomorphology may provide a viable alternative.

7.3 The extant ichthyofauna of Lake Chala

The presence of only one indigenous cichlid species (*Oreochromis hunteri*) in Lake Chala made interpretation of shape changes in fossil oral tooth assemblages relatively straightforward, as all teeth could be assigned to this species (Chapter 6). However, since very little was known about the extant *O. hunteri* at the start of this project, development of a comprehensive modern-day framework was an important prerequisite for thorough understanding of its fossil record. In this respect, the recent introductions of three other cichlid species by humans (*Oreochromis* cf. *korogwe*, *Coptodon rendalli* and *Haplochromis* sp. "Chala") potentially complicated this study, as interspecific hybridisation with one of these species could compromise the extant trophic morphology of *Oreochromis hunteri* (Holzman & Hulsey, 2017). This is especially relevant in Lake Chala, where a deep open-water environment surrounded by near-vertical crater walls offers limited potential for reproductive or niche segregation. Moreover, a correct interpretation of changes in oral tooth shape requires understanding of the feeding habits of modern-day *O. hunteri*.

7.3.1 Species integrity of *Oreochromis hunteri*

To examine the influence of hybridisation, I first studied the genetic and morphological integrity of *Oreochromis hunteri*. Hybridisation is most likely to occur between congeneric species, hence I focussed on potential interbreeding of *O. hunteri* with *O.* cf. *korogwe*. In Chapter 2, I first performed geometric morphometrics to assess body and oral tooth morphology of both taxa and found their overall morphology to be clearly distinct, already arguing against recent and/or ongoing hybridisation. In Chapter 3, I extended this study with a molecular-genetic assessment of two loci in the mitochondrial DNA (mtDNA) of *O. hunteri* and *O.* cf. *korogwe*, and found no signs of introgression between the two. I therefore concluded that both the genetic and morphological integrity of *Oreochromis hunteri* is currently intact, and that it can be used as a modern-day representative for *O. hunteri* remains in the fossil record of Lake Chala.

Potential mechanisms of reproductive isolation acting between *O. hunteri* and *O.* cf. *korogwe* are briefly touched upon in Chapter 3, and include separation in spawning time and distinctions in microhabitat preference (for example described in Pullin & Lowe-McConnell, 1982; Lowe-McConnell, 1987; Beveridge & McAndrew, 2000). Although tilapia in near-equatorial regions generally spawn year-round, many species display breeding peaks at a certain time of the year (e.g., Lowe-McConnell, 1987; Brummett, 1995). It is not known whether the two Lake Chala *Oreochromis* species have a delineated spawning time. If they do, then fishes probably stick to small sandy patches for spawning and substrate brooding (in *Coptodon rendalli*), whereas mouthbrooding *Oreochromis* females often reside in sheltered areas (Lowe-McConnell, 1987) and thus, in Lake Chala, likely hide out in crevices between rocks. Obtaining fish is generally difficult for all tilapiine species at the end of May and in June, and in November and December, when nets are lowered and fishermen resort in part to line fishing to supplement the net catches (Caxton Oluseno, pers. comm.; Table 4.1). I hypothesise that these may well represent periods of peak spawning in Lake Chala, although assessment of ovary conditions throughout the year would be required to corroborate this suggestion. The synchronised timing of disappearance of both *O. hunteri* and *O.* cf. *korogwe* then argues against a separation in spawning time as a mechanism of reproductive isolation. However, differential preferences for certain spawning locations could also induce a premating reproductive barrier between *O. hunteri* and *O.* cf. *korogwe*. Different depths of spawning, reinforced by male breeding colour differences, keep for example *O. squamipinnis* and *O. saka* from interbreeding in Lake Malawi, despite similar overall ecologies (Lowe-McConnell, 1987).

7.3.2 Relationship between tooth shape and feeding habits of Lake Chala tilapiines

Gut content analysis of *O. hunteri*, *O.* cf. *korogwe* and *C. rendalli* over the course of 20 months combined with stable-isotope analysis (Chapter 4) revealed that all three species have a mixed herbivorous-detritivorous diet in Lake Chala, but display significant seasonal shifts in feeding habits in response to an annual phytoplankton bloom developing towards the end of the long dry season (July – September). Nevertheless, evidence for interspecific diet differences was found, most notably in the stable-isotopic data. In Chapter 2 I hypothesised that subtle differences in oral tooth shape between *O. hunteri* and *O.* cf. *korogwe* might facilitate fine-scaled resource partitioning of certain food sources at the shore (cf. below). However, gut content composition suggested interspecific divergence in feeding habits to occur mainly during the annual phytoplankton bloom, when *O. hunteri* appeared to venture out into the limnetic zone for three full months whereas *O.* cf. *korogwe* (and potentially also *C. rendalli*) lingers close to shore until September (Chapter 4).

This seeming mismatch between different oral tooth shapes yet similar feeding habits during the least favourable times of the year is notable, given that tooth shape often reflects exploitation of less accessible food sources when preferred food is unavailable (so-called 'fallback foods'). Perhaps oral tooth shape of the recently introduced *O.* cf. *korogwe* and *C. rendalli* still reflects the feeding habits of their respective source populations. Indeed, *C. rendalli* is generally a voracious feeder on aquatic and semi-aquatic macrophytes (Ruwet, 1963; Fryer & Iles, 1972), and they are often anthropogenically introduced as a means of weed control (Dadzie *et al.*, 1988). Considering the almost complete absence of submerged macrophytes in Lake Chala, it is quite remarkable that this species has been able to survive and reproduce in the lake for almost half a century, although the trophic versatility of *C. rendalli* is well-known. Very little is known about the feeding habits of *O. korogwe* within its natural range in the lower Pangani basin (Trewavas, 1983). Insight in the diet of the source population of *O.* cf. *korogwe* is moreover complicated by the uncertain identity of this species in Lake Chala (Chapter 3). The strong (asymmetry linked to) dental wear which I observed in oral teeth of both *O.* cf. *korogwe* (Chapter 2) and *C. rendalli* (data not shown) suggests that their exploitation of Lake Chala resources comes at a cost.

In this respect, only the oral dentition of *Oreochromis hunteri* probably truly reflects its feeding habits for most of the year, when feeding conditions in Lake Chala are rather harsh in comparison to the short-lived annual phytoplankton bloom. In Chapter 2, I suggested that the slender, shovel-like appearance of oral *O. hunteri* teeth may allow them to function as a comb and a sieve, ideal for scooping up loose material from between filamentous epilithic algae covering the rocks (‘Aufwuchs’, Fryer, 1959a). I did not find high abundances of macroscopic filamentous algae in the analysed littoral rock samples (Chapter 4), arguing against frequent combing as a mechanism of obtaining loose Aufwuchs. Closely-spaced, long and moveable teeth, as found in small *O. hunteri* specimens, have also been suggested to enable the removal of organic detritus from hard substrates in some coral reef fishes (Bellwood *et al.*, 2014; Hundt & Simons, 2018). Likewise, the detritus, green algae and diatoms found abundantly in the guts of *O. hunter*i outside of the annual period of phytoplankton bloom may have been scooped up from the rocks by comb-like oral teeth. Nevertheless, the gradual tooth shape shift of *O. hunteri* associated with growth (Chapter 6) suggests that there is at least some degree of change in feeding behaviour linked to ageing (see Section 7.3.3).

The annual phytoplankton bloom in Lake Chala provides highly favourable feeding conditions for a short period of time (July – September), and the ability of fish to feed off this bloom probably does not rely on the shape of their oral teeth. In fact, the capacity of suspension-feeding has in some *Oreochromis* species been attributed to a combination of mucus entrapment and pharyngeal jaw motion, rather than mechanical sieving by gill rakers (Sanderson *et al.*, 1996). Greenwood (1953) found that a raking motion of the upper pharyngeal element over the lower pharyngeal element may explain why large phytoplankton elements occur broken in the gut of *O. esculentus*. This is reminiscent of my observations in Chapter 4, where I suggested that the apparent absence of the large chlorophyte *Treubaria* in fish stomachs, despite its common occurrence in the phytoplankton, could be due to it being shattered beyond recognition by the pharyngeal jaws. The interspecific divergence in fish feeding habits observed during the annual bloom is then potentially linked to differences in pharyngeal rather than oral tooth shape. An in-depth morphological study of pharyngeal teeth is outside the scope of this thesis, but qualitative inspection reveals that pharyngeal teeth of *C. rendalli* are shaped very different, and that pharyngeal teeth of *O.* cf. *korogwe* resemble, but are more robust than, those of *O. hunteri* (Fig. 7.1). I hypothesise that these pharyngeal tooth shape differences may compromise consumption of certain Lake Chala phytoplankton taxa by the introduced tilapiines, and may explain why *O.* cf. *korogwe* and possibly also *C. rendalli* feed in the littoral area longer than *O. hunteri*, where their oral dentition guarantees at least some food intake.

Figure 7.1: Pharyngeal tooth extracted from the lower pharyngeal element of *O. hunteri* (**a**), *O.* cf. *korogwe* (**b**) and *C. rendalli* (**c**).

7.3.3 Ontogenetic shifts in the oral dentition of *O. hunteri*

When developing a modern-day morphological framework of the oral dentition of *O. hunteri* (Chapter 6), I found a replacement of bicuspid and tricuspid teeth by unicuspid teeth in very large individuals, comparable to observations in other *Oreochromis* species (Trewavas, 1983). This replacement happened gradually, with both bicuspids and tricuspids displaying a progressively more dominant major cusp as specimens grow larger, until nearly all teeth are unicuspid. This gradual ontogenetic change in oral tooth shape may well reflect changes in microhabitat preference as *O. hunteri* grow older. Limits on available sample sizes did not allow us to assess differences in the gut content of *O. hunteri* of different body sizes that could possibly be related to a changing microhabitat preference (Chapter 4), yet such changes have been documented in other *Oreochromis* species (Njiru *et al.*, 2004). Fryer & Iles (1972) moreover indicate that *Oreochromis* species move progressively into deeper water as they grow. By doing so in Lake Chala, they may encounter altered habitat conditions that require changes in the shape of the oral dentition for optimal exploitation. For example, the areal extent of sandy patches between the rocks may increase at depths with less wave action, and collecting detritus from this sand likely exerts different forces on oral teeth than scooping up detritus from rocks surfaces.

The final replacement with unicuspid teeth in the largest adults, however, probably no longer reflects a change in feeding habits. In many cichlid taxa, an entirely unicuspid oral dentition is often associated with piscivory or insectivory

(Fryer & Iles, 1972; Witte & Van Oijen, 1990; Streelman *et al.*, 2003; Burress, 2015) but optimising such feeding habits would require modification of the entire jaw apparatus, as piscivorous and insectivorous species typically possess long snouts and gracile jaws (Streelman *et al.*, 2003). *Oreochromis* species are rarely entirely piscivorous or insectivorous (Trewavas, 1983), although juveniles have been observed to opportunistically feed on insects and smaller fish (De Moor *et al.*, 1986; Njiru *et al.*, 2004). Moreover, none of the *O. hunteri* specimens with a unicuspid dentition in my study material contained fragments of fish, insects or even zooplankton in their gut contents, nor do their isotopic compositions show evidence of an omnivorous diet. This is despite the presence of a diverse zoobenthos community inhabiting the mentioned sand patches, as indicated by fossils preserved in a sediment core collected near-shore (Chapter 5). Thus, the unicuspid dentition of large *O. hunteri* is not linked to feeding at a higher trophic level. More likely, the unicuspid teeth are useful in agonistic behaviour and territorial defence. *Oreochromis* males usually build bowers where they display for females and subsequently reproduce (Turner, 1986; Turner *et al.*, 1991), and a unicuspid dentition enhances the ability to defend such territories. Indeed, the shift to unicuspid teeth has been documented to occur in very large, sexually mature *Oreochromis* individuals of several other species (e.g., *O. aureus*, *O. mossambicus*, *O. spilurus*), especially in males (Trewavas, 1983). In Lake Chala, this shift probably comes at a considerable cost in feeding efficiency, as truly unicuspid teeth are far from ideal for feeding at the rocks or in the sand. However, they may increase breeding success by enhancing dominance over other males.

7.4 Taphonomy on fossil cichlid teeth in Lake Chala

7.4.1 Burial and transport to a core site in the lake's depositional centre

The diet composition of *O. hunteri* and the two introduced tilapiine species indicated that the three larger cichlids in Lake Chala spend most of their time at the lake periphery (Chapter 4). The majority of cichlid teeth thus probably end up in the sediment in close proximity of the fishes' principal habitat at the lakeshore. These fossils mostly represent teeth shed during the process of tooth replacement (approximately every 100 days for tilapiines; Tuisku & Hildebrand, 1994), but may also be the result of the occasional burial of an entire fish carcass (Elder & Smith, 1988). Nevertheless, cores collected at the depositional centre also contain abundant cichlid fossils (Fig. 1.5), which may either have been deposited *in situ* or were transported to the centre by sedimentation processes. In order to assess which scenario is most likely, the distribution of cichlid teeth (both oral and pharyngeal) in sediments deposited near Lake Chala's periphery is discussed in Chapter 5. I found a strong correlation between the depth distribution of cichlid teeth or bones and sediment texture: large-grained (coarse) sediments contain very high numbers of fossil fish remains. The remains of various groups of zoobenthos, such as ostracods (seed shrimp) and gastropod molluscs (snails and limpets) were in the same way correlated with larger grain sizes. I therefore suggested that the high specific density of all these fossils limits long-distance transport over the flat offshore lake floor. Fish fossils are thus buried near the lake periphery in relatively coarse sediments from which the fine and light materials have gradually been removed by resuspension and winnowing. Likely only an exceedingly small part of the fish fossils is transported to the depositional centre by repeated winnowing, except that occasional landslides from a failing underwater slope may transport higher abundances of fossils to the centre in turbidite flows (Van Daele *et al.*, 2017). I therefore concluded that most fossil teeth in the offshore sediment record have been deposited *in situ*. Moreover, although corrosion linearly altered the size distribution of fossil teeth (Chapter 6), there was no link between median tooth size and lake level, indicating limited changes in taphonomic effects linked to lake-level fluctuations. I therefore assume that fossil oral teeth from the six fossil assemblages studied in Chapter 6 represent the oral dentition of the Lake Chala fish population at that time, and that they represent it well.

Also, the characteristic basin morphometry of Lake Chala with near-vertical crater walls surrounding a flat profundal lake bottom (Fig. 1.4) ensures that the distance between the principal location of fish occurrence (littoral) and the location of final burial in the long fossil record (offshore depositional centre) has remained relatively stable over time. Only extremely severe lake-level drops (> 55 m) could have exposed soft sediments at the lake periphery and forced *O. hunteri* to move away from the rocky crater walls, closer to the depositional centre. No indication of such an event was found in the sediment record of the last 25,000 years, yet it may have occurred briefly during the penultimate glacial maximum (~140-128 kyr BP; Moernaut *et al.*, 2010). Lake-level fluctuations therefore probably did not significantly influence fossil tooth distributions throughout the sediment record of Lake Chala until at least 160,000 years ago, when the central ash cones became exposed and provided a second near-shore environment (Moernaut *et al.*, 2010). This is in stark contrast to what is expected in more gently-sloping lake basins, where past lake-level fluctuations not only greatly affect the distance between the locations of fossil deposition and final burial when past shorelines were displaced (e.g., Danley *et al.*, 2012), but also local sedimentation dynamics at the burial site.

7.4.2 Tooth preservation in the offshore sediment record

A considerable proportion of fossil teeth retrieved from the 25,000-yr sediment record of Lake Chala had broken necks (Chapter 2). If most teeth are indeed deposited *in situ* and the influence of tooth abrasion during transport is thus neglegible, these broken teeth suggest the occurrence of other taphonomic influences after teeth have been buried. The dentin part of fossil teeth becomes more brittle with age, whereas the enameloid-covered tooth crown is more resistant, but may eventually also break as its dentin base is being corroded (pers. obs.). It is known that the mineralisation process differs between enameloid and dentin (Sasagawa, 1997), which may induce differences in their preservation potential. The near-perfect state of preservation of ~170,000 year-old fossil teeth retrieved from the DeepCHALLA core sequence (discussed in section 7.5.1), as compared to the near-complete absence of intact tooth necks older than 8000 yr BP and progressively larger teeth with increasing age in the last 25,000 years (Chapter 6), indicates that the state of preservation is not necessarily correlated with the age of the assemblages. These very old intact teeth (~170,000 year BP) were collected from the coarse-grained base of a turbidite interval, which in Lake Chala have a higher proportion of clastic material (Van Daele *et al.*, 2017). The relatively lower proportion of organic material present in these layers may result in lower concentrations of the organic acids that are produced during decomposition of organic material, and thus create better conditions for the preservation of fossil teeth, as more acid conditions could enhance corrosion of calcified fossils such as teeth. The lack of fossils in one interval studied in Chapter 2 (~19,200 yr BP) could thus also be the result of increased corrosion in this interval. Moreover, the rapid burial of fossil remains at the base of a turbidite may further enhance fossil preservation.

7.5 Long-term history of *Oreochromis hunteri* in Lake Chala

7.5.1 Origin of *O. hunteri* in Lake Chala

Phylogenetic analysis of the mitochondrial DNA (mtDNA) of all *Oreochromis* species currently inhabiting the Upper Pangani River system enabled us to assign *O. jipe* as the closest living relative of *O. hunteri* in Lake Chala (Chapter 3). As the isolated nature of this lake does not allow direct colonisation through hydrographic connections, introduction of the first fishes by air is the only plausible explanation for the colonisation of Lake Chala by *O. hunteri*. Transportation of live fishes by birds of prey (potentially carrying mouthbrooding females), or natural events such as storms causing 'fish rains' have been suggested as means of aerial introduction (Elmer *et al.*, 2012), yet very little empirical evidence for either of these mechanisms exists (Hirsch *et al.*, 2018). In Chapter 3, I suggested that a riverine ancestral population of *O. jipe* may have seeded both Lake Jipe and Lake Chala, and more recently also the reservoir Nyumba ya Mungu (constructed in 1965; Denny, 1978). This riverine seeding population likely occurred close to Lake Chala, as smaller distances increase the probability of a transfer by air.

Notwithstanding its elusive mode of arrival, the ancestral *O. hunteri* colonised Lake Chala at least 25,000 years ago, based on its continuous fossil record (Chapter 6). Referring to observations in neotropical cichlids in Nicaraguan crater lakes (Elmer *et al.*, 2010b; Elmer *et al.*, 2012), *O. hunteri* could either have arrived soon after the lake's formation approximately 250,000 years ago (Moernaut *et al.*, 2010; Verschuren *et al.*, 2017), as happened in Lake Xiloá; or Lake Chala may have been uninhabited for long periods of time or have been inhabited by earlier fish populations that later went extinct, as has for example been suggested for Lake Apoyeque. I hypothesised in Chapter 3 that the ancestral *O. hunteri* population, provided with ecological opportunity, expanded quickly after colonisation, and that the genetic signature of this expansion could provide us with an estimated time of arrival, as has been done on cichlids in other lakes (Barluenga *et al.*, 2006; Elmer *et al.*, 2012; Genner & Turner, 2014). I did not find an unambiguous sign of past population expansion, but the structure of the haplotype network suggested that the *O. hunteri* population of Lake Chala is relatively ancient.

Discovery of abundant fish fossils in a turbidite ('landslide') layer at a sub-bottom depth of ∼133 m during the recent DeepCHALLA drilling appears to corroborate an ancient origin of the *O. hunteri* population in Lake Chala. Based on an age model for the Lake Chala sedimentary sequence proposed by Moernaut *et al.* (2010), the landslide occurred at least 170,000 years ago, which is thus also the minimum age of the fish fossils it dislodged. The discovery of well-preserved fish fossils at such great depths raises hopes for the opportunity to study oral tooth shape change over very long time scales. The DeepCHALLA drilling project eventually acquired a sediment core representing ∼260,000 years, thus covering most of the lake's history. Although the fossil cichlid record may be interrupted in some sediment intervals (Chapter 2, and see also Section 7.4.2), geometric morphometrics would quickly reveal whether teeth belonged to *O. hunteri*, and thus provide a very good estimate of the time of arrival of this species in Lake Chala. Moreover the lake has witnessed several more (severe) lowstands during this period (Moernaut *et al.*, 2010), and additional samples of alternating high and low lake-level stands may expand the observations of changes in tooth type abundance linked to lake-level change (Chapter 6) on even longer time scales.

7.5.2 Long-term trends in the oral tooth shape of *O. hunteri*

Overall the variation in the shape of oral teeth is highly similar between all analysed fossil assemblages and the extant fauna (Chapter 2, Chapter 6). Therefore, it is most parsimonious to consider *O. hunteri* as the only species to have inhabited Lake Chala throughout the last 25,000 years, at least before recent anthropogenic introduction of *O.* cf. *korogwe*, *C. rendalli* and *Haplochromis* sp. "Chala". Moreover, the close resemblance of fossil and modern-day oral teeth indicates that *O. hunteri* unlikely experienced major diet shifts, and that besides periods of phytoplankton blooms it has probably always been feeding in the littoral zone (Chapter 4), with only subtle ontogenetic changes in feeding behaviour (Section 7.3.3). Qualitative inspection of fossil pharyngeal teeth (pers. obs.) indicated that these teeth have similarly retained their slender, unicuspid morphology throughout Lake Chala's fossil record. If the pharyngeal teeth indeed assist in feeding off phytoplankton (as proposed in Section 7.3.2), seasonal migration to the limnetic zone in response to an annual phytoplankton bloom may have been occurring for at least the last 25,000 years, similar to what is observed in the lake today. The magnitude of the seasonal phytoplankton bloom is strongly related to interannual climate variability, and tends to be less developed during El Niño years (Wolff *et al.*, 2011). Nevertheless, certain blooming taxa, such as the endemic diatom *Afrocymbella barkeri* (Cocquyt & Ryken, 2016), have continuously been present in Lake Chala during the last 25,000 years (Barker *et al.*, 2011), indicating that this temporary food source has at least to some extent always been seasonally available to *O. hunteri*.

Importantly, although all recovered fossil oral teeth can be assigned to *O. hunteri*, I documented recurrent shifts in the proportions of oral tooth types linked to past lake-level fluctuations (Chapter 6). Apart from an approximately 10% increase in the proportion of unicuspid teeth during low lake-level stands, there is an overall higher abundance of teeth occupying the section of morphospace that is currently taken up by larger fish. However, the enameloid width of fossil teeth suggested that the median body sizes of fish assemblages did not increase during lowstands. I therefore surmised that fishes living during lowstands developed a predominantly unicuspid dentition at smaller body sizes (and thus probably at a younger age). Below, I elaborate somewhat on the possible ecological interpretations of my findings, and discuss potential mechanisms that may be responsible.

Potential mechanisms underlying long-term trends in oral tooth shape

When the lake level of Lake Chala is high, soft-bottom habitats are restricted to small patches of sand in between rocks, likely slightly increasing in areal extent at water depths beyond wave-induced turbulence (Chapter 4). In Chapter 1, I hypothesised that low lake levels in Lake Chala may have brought an extensive soft-bottom benthic habitat (and associated food sources) within reach at the lake periphery (Fig. 1.6), and that this might be reflected in oral tooth shape. In this respect, I propose two alternative ecological explanations for the apparent shift in tooth shapes during past lowstand episodes.

Earlier in this discussion (Section 7.3.3), I suggested that modern-day *O. hunteri* may move into progressively deeper water with age, as has been observed in other *Oreochromis* species (Fryer & Iles, 1972; Ribbink, 1990), and that such migration may explain subtle differences in the shape of the oral dentition linked to age. During low lake level phases, *O. hunteri* may thus have encountered soft-bottom habitats earlier in life, in particular a large expanse of soft sediment. Optimal exploitation of food sources would then have required changes in oral dentition earlier in life. Alternatively, a shift in oral tooth shape during low lake-level stands may be an indication of earlier sexual maturation. Changes in the onset of sexual maturation in *Oreochromis* species are well-described, mostly in the context of aquaculture (Pullin & Lowe-McConnell, 1982; El-Sayed, 2006), and are strongly linked to environmental influences (reviewed in Brummett, 1995). Changes in the timing of first sexual maturity have for example been linked to temperature, food availability and quality, and light regimes, but also to lake-level fluctuations. Specifically in Lake Sibaya (South Africa), *Oreochromis mossambicus* starts reproducing at smaller sizes during episodes of higher lake level, as young males attempt to exploit the extension of available habitat by breeding in the marginal, recently inundated regions of the flooded lake (Noakes & Balon, 1982). Although I observed the opposite signal in Lake Chala (potential reproduction at smaller sizes during low lake levels), similar underlying mechanisms may be acting. In Lake Chala, reduced lake levels could increase suitable nesting habitat (i.e. by extension of sandy substrate), which could allow subordinate (often smaller) males to start reproducing, thus inducing sexual maturity at earlier ages. Both cues are not mutually exclusive, and the observed trends in oral tooth morphology in response to changing lake levels may well be the result of an interplay between the two.

Based on observations of changing oral tooth shape in the fossil record of Lake Chala, I hypothesise that a modified developmental trajectory of the oral teeth enabled *O. hunteri* to respond to past environmental change (in the form of major lake-level fluctuations). During low lake-level stands, I found an increase in the relative proportion of unicuspid oral teeth that was decoupled from a larger median body size. The developmental trajectory of *O. hunteri* teeth may either be altered by increasing the rate of oral tooth replacement, or by increasing the morphological differences between subsequent tooth generations, resulting in 'adult-like' dentition occurring in smaller fishes when lake levels are low.

Modification of developmental trajectories is a straightforward way to alter the phenotype, and may provide a valuable mechanism for organisms to rapidly respond to environmental cues (West-Eberhard, 2003; Shapiro *et al.*, 2004). Such a mechanism has been suggested to lie at the basis of the distinction between substrate spawning and mouthbrooding (Noakes & Balon, 1982), and more recently it has been invoked in the rapid adaptation of cichlids to novel photic environments upon colonisation of Nicaraguan crater lakes (Härer *et al.*, 2017). Continuous tooth replacement (polyphyodonty) combined with the potential to rapidly adjust gene expression regulating tooth morphology in subsequent developmental stages has been suggested to provide cichlids with a mechanism to quickly adapt to new ecological circumstances (Streelman *et al.*, 2003). I propose that a similar mechanism has assisted *O. hunteri* in its adaptation to major past lake-level fluctuations in Lake Chala, either as a direct response to climate-driven change in suitable feeding habitat, or indirectly as the result of climate-driven changes in suitable breeding grounds through a shift in the onset of sexual maturation.

Long-term trends in oral tooth morphology in response to lake-level fluctuations

I can currently only hypothesise on the exact ecological and evolutionary mechanisms underlying trends in oral tooth shape of *O. hunteri*, but the recurrent shifts in oral tooth shape directly linked to long-term environmental pacers such as lake-level change are a remarkable observation. They provide evidence of the ability of *O. hunteri* to rapidly adapt to a changing environment, and to systematically do so over very long timescales, highlighting the versatility that typifies the genus *Oreochromis*. Moreover, rapid phenotypic responses of the trophic apparatus to environmental change have been observed in other cichlid lineages (Witte *et al.*, 2008; Muschick *et al.*, 2011; Huysseune, 1995), and are increasingly accepted as a promotor of diversification (Schneider & Meyer, 2017). In this thesis, I provided evidence of such responses, and of their recurrent nature, on very long timescales (∼25,000 years), indicating that they may well have enabled cichlids to cope with past environmental change, and promoted their subsequent diversification and radiation when presented with ecological opportunity.

7.6 Future prospects

Many ecological aspects of modern-day and fossil *O. hunteri* populations have been elucidated in this thesis, yet some important questions remain unresolved. Certain hypotheses that are presented in this Chapter could however be tested in future research.

Elucidating the exact timing of peak spawning of tilapiines in Lake Chala was considered outside the scope of this thesis, but may to some extent influence the migratory behaviour observed in the lake. Breeding season in tilapia species is generally studied by monthly analysis of gonad development in a sufficiently high number of specimens (Babiker & Ibrahim, 1979; Admassu, 1996). Higher frequencies of breeding fish in certain months then indicate periods of peak spawning.

The function of unicuspid teeth in large specimens of *O. hunteri* is an important knowledge gap for the conclusions of this thesis. I hypothesised that the shift towards a more unicuspid dentition is either the result of changes in feeding behaviour, or linked to sexual maturation and increased territoriality (Section 7.5.2). To test the former hypothesis, a first step towards a better understanding of the ecological function of different tooth types would be to quantify jaw morphology associated with a certain dentition. Indeed, differences in jaw shape have been linked to tooth shape and body size in other cichlid species (Streelman *et al.*, 2007), and could give an indication of the predominant feeding strategy (Kassam *et al.*, 2004). Moreover, morphological comparison of *O. hunteri* to other *Oreochromis* species associated with a soft-bottom environment could reveal whether the observed shifts in oral dentition indeed reflect an adaptation to a soft-bottom feeding mode. Truly benthic *Oreochromis* species are rare (Trewavas, 1983), but the *Nyasalapia* species complex (an *Oreochromis* subgenus) inhabiting the ecologically diverse Rungwe crater lakes in Tanzania (Pillay, 2016) provides an ideal control system. These crater lakes represent a host of different habitats, ranging from entirely soft-bottom to rocky crater walls interspersed with sandy patches. Quantifying the trophic morphology of *Nyasalapia* species associated with each of these habitats may provide an eco-morphological framework in which the morphology of *O. hunteri* could be interpreted.

Testing whether an earlier shift to a unicuspid dentition is linked to earlier sexual maturation is ideally done in a controlled setting. However, differences in the timing of onset of sexual maturation are widely documented in *Oreochromis* species grown in ponds (Pullin & Lowe-McConnell, 1982; El-Sayed, 2006). A straightforward approach could be to quantify oral tooth shape in a representative subset of *Oreochromis* specimens from ponds with known differences in the timing of onset of sexual maturation, and test whether unicuspid teeth occur at smaller body sizes in ponds where earlier sexual maturation occurs.

7.7 General conclusions

This general discussion integrated the results of five chapters describing the extant and past life history of the endemic cichlid *Oreochromis hunteri* in Lake Chala based on its oral dentition, and elaborated on several of my findings. The main conclusions which can be drawn from this doctoral thesis research are summarised below.

1. I developed a geometric-morphometric approach for quantitative analysis of oral tooth crown shape with landmarks and semi-landmarks. This method was highly suitable to distinguish oral tooth shape at the species level, yet also for the detection of subtle ontogenetic changes in the extant *O. hunteri*, and of significant shifts in oral tooth shape between different fossil assemblages preserved in Lake Chala sediments.

2. I found no signs of ongoing hybridisation between the indigenous *O. hunteri* and the recently introduced *O.* cf. *korogwe*, neither in whole-body morphology, nor in two mitochondrial loci. I thus confirmed both the genetic and morphological integrity of the modern-day *O. hunteri* population inhabiting Lake Chala, and its suitability as extant reference framework for fossil tooth assemblages preserved in the lake's sediment record.

3. Both *O. hunteri* and the recently arrived tilapiines *O.* cf. *korogwe* and *C. rendalli* feed most of the year in the littoral zone, but they migrate seasonally to the limnetic zone to exploit the phytoplankton bloom that

develops at the end of the long dry season. Although oral tooth shape was clearly distinctive between species, a significant interspecific difference in feeding habits appears to occur only during the phytoplankton bloom, when oral dentition is presumably of less importance. I hypothesise that the pharyngeal rather than oral dentition may play a role in optimal exploitation of phytoplankton by Lake Chala tilapiines. As yet undocumented shape differences in pharyngeal dentition may thus explain why the two introduced tilapiines have not yet completely adapted to the seasonally variable food availability in Lake Chala.

4. The distribution of cichlid fossils throughout a core collected near-shore indicated that the hydrostatic properties of fossil teeth are not favourable for transportation over very long distances, and suggested that such transportation is rare. Sedimentological evidence is indicative for *in situ* deposition of fossil teeth in offshore locations.

5. Oral tooth shape studied in six fossil assemblages from the last 25,000 years indicate that *O. hunteri* has been the only species inhabiting Lake Chala during this period. However, I found clear trends in the shape of oral dentition linked to major lake-level fluctuations driven by long-term climate variability. During low lake-level stands, a higher proportion of unicuspid teeth, typical for large specimens in the modern-day population, is found. However, this change in dentition was not coupled to increases in median body size, and thus appears to occur earlier in life. I interpret this as a change in the ontogenetic trajectory of oral tooth development in response to changes in feeding or breeding habitats, or a combination of both.

To my knowledge, I have provided the first evidence of long-term recurrent changes in the oral dentition of a cichlid species in relation to climate-driven environmental fluctuations using the fossil record. More generally, I conclude that building the required control to accurately study individual lineages is difficult, but the specific setting and well-resolved environmental history of Lake Chala made it possible for *O. hunteri*. I anticipate that it will be more difficult in larger, less isolated lakes which harbour many cichlid lineages, but a different approach based on the overall tooth diversity at the community level may be possible there. Moreover, great numbers of teeth are required to reliable reconstruct patterns through time, and these numbers would need to be further increased for more complex fish communities. Nevertheless, studying a continuous fossil record in lakes with a more complex history and more diverse species flocks may elucidate patterns of long-term eco-morphological change on a larger scale, and further enhance our understanding of processes that underlie cichlid species diversity.

Bibliography

Admassu, D. (1996). The breeding season of tilapia, *Oreochromis niloticus* l. in lake awassa (ethiopian rift valley). *Hydrobiologia*, 337(1-3):77–83.

Agnèse, J. F., Adépo-Gourène, B. & Pouyaud, L. (1998). Genetics and Aquaculture in Africa. In A. . Actes du collogue Genetique et aquaculture en Afrique, Abidijan, editor, *Genetics and Aquaculture in Africa*, pp. 95–103. Paris: Orstom Editions.

Albertson, R. C. & Kocher, T. D. (2001). Assessing morphological differences in an adaptive trait: A landmark-based morphometric approach. *Journal of Experimental Zoology*, 289(6):385–403.

Albertson, R. C. & Kocher, T. D. (2006). Genetic and developmental basis of cichlid trophic diversity. *Heredity*, 97(3):211–221.

Alcaraz, G., López-Portela, X. & Robles-Mendoza, C. (2015). Response of a native endangered axolotl, *Ambystoma mexicanum* (Amphibia), to exotic fish predator. *Hydrobiologia*, 753(1):73–80.

Angienda, P. O., Lee, H. J., Elmer, K. R., Abila, R., Waindi, E. N. & Meyer, A. (2011). Genetic structure and gene flow in an endangered native tilapia fish (*Oreochromis esculentus*) compared to invasive Nile tilapia (*Oreochromis niloticus*) in Yala swamp, East Africa. *Conservation Genetics*, 12(1):243–255.

Azuma, Y., Kumazawa, Y., Miya, M., Mabuchi, K. & Nishida, M. (2008). Mitogenomic evaluation of the historical biogeography of cichlids toward reliable dating of teleostean divergences. *BMC Evolutionary Biology*, 8(1):215.

Babiker, M. & Ibrahim, H. (1979). Studies on the biology of reproduction in the cichlid *Tilapia nilotica* (l.): gonadal maturation and fecundity. *Journal of Fish Biology*, 14(5):437–448.

Bailey, R. G., Churchfield, S., Petr, T. & Pimm, R. (1978). The ecology of the fishes in Nyumba ya Mungu reservoir, Tanzania. *Biological Journal of the Linnean Society*, 10:109–137.

Baldwin, B. & Robichaux, R. (1995). Historical biogeography and ecology of the Hawaian silversword alliance (Asteraceae). In W. Wagner & F. Funk, editors, *Hawaiian biogeography*, pp. 259–287. Smithsonian Institution Press, Washington D.C.

Barel, C. D., Dorit, R., Greenwood, P. H., Fryer, G., Hughes, N., Jackson, P. B., Kawanabe, H., Lowe-McConnell, R. H., Nagoshi, M., Ribbink, A. J., Trewavas, E., Witte, F. & Yamaoka, K. (1985). Destruction of fisheries in Africa's lakes. *Nature*, 315(6014):19–20.

Barker, P. A., Hurrell, E. R., Leng, M. J., Plessen, B., Wolff, C., Conley, D. J., Keppens, E., Milne, I., Cumming, B. F., Laird, K. R., Kendrick, C. P., Wynn, P. M. & Verschuren, D. (2013). Carbon cycling within an East African lake revealed by the carbon isotope composition of diatom silica: A 25-ka record from Lake Challa, Mt. Kilimanjaro. *Quaternary Science Reviews*, 66:55–63.

Barker, P. a., Hurrell, E. R., Leng, M. J., Wolff, C., Cocquyt, C., Sloane, H. J. & Verschuren, D. (2011). Seasonality in equatorial climate over the past 25 k.y. Revealed by oxygen isotope records from Kilimanjaro. *Geology*, 39(12):1111–1114.

Barluenga, M. & Meyer, A. (2010). Phylogeography, colonization and population history of the Midas cichlid species complex (*Amphilophus* spp.) in the Nicaraguan crater lakes. *BMC Evolutionary Biology*, 10:326.

Barluenga, M., Stölting, K. N., Salzburger, W., Muschick, M. & Meyer, A. (2006). Sympatric speciation in Nicaraguan crater lake cichlid fish. *Nature*, 439:719–723.

Bayona, J., Odhiambo, E. & Hanssens, M. (2006). *Oreochromis hunteri*. *The IUCN Red List of Threatened Species.*

Bellwood, D. R., Hoey, A. S., Bellwood, O. & Goatley, C. H. (2014). Evolution of long-toothed fishes and the changing nature of fish–benthos interactions on coral reefs. *Nature Communications*, 5:3144.

Beveridge, M. & McAndrew, B. (2000). *Tilapias: biology and exploitation.* Springer Science & Business Media.

Blaauw, M., van Geel, B., Kristen, I., Plessen, B., Lyaruu, A., Engstrom, D. R., van der Plicht, J. & Verschuren, D. (2011). High-resolution ^{14}C dating of a 25,000-year lake-sediment record from equatorial East Africa. *Quaternary Science Reviews*, 30(21-22):3043–3059.

Blondel, C., Merceron, G., Andossa, L., Taisso, M., Vignaud, P. & Brunet, M. (2010). Dental mesowear analysis of the late Miocene Bovidae from Toros-Menalla (Chad) and early hominid habitats in Central Africa. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 292:184–191.

Boag, P. T. & Grant, P. (1981). Intense natural selection in a population of Darwin's finches (Geospizinae) in the Galapagos. *Science*, 214(4516):82–85.

Bookstein, F. (1991). *Morphometric tools for landmark data: Geometry and biology.* Cambridge University Press, Cambridge.

Bookstein, F. L. (1997). Landmark methods for forms without landmarks: Morphometrics of group differences in outline shape. *Medical Image Analysis*, 1(3):225–243.

Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C. H., Xie, D., Suchard, M. A., Rambaut, A. & Drummond, A. J. (2014). BEAST 2: A software platform for Bayesian evolutionary analysis. *PLoS Computational Biology*, 10(4):e1003537.

Boulenger, G. A. (1896). Descriptions of new fishes from the upper shiré river, british central africa, collected by dr percy rendall, and presented to the british museum by sir harry h. johnston, k.c.b. *Proceedings of the Zoological Society of London*, 1896:915–920.

Bouton, N., Witte, F. & Van Alphen, J. J. M. (2002). Experimental evidence for adaptive phenotypic plasticity in a rock-dwelling cichlid fish from Lake Victoria. *Biological Journal of the Linnean Society*, 77(2):185–192.

Brawand, D., Wagner, C., Li, Y., Malinsky, M., Keller, I., Fan, S., Simakov, O., a.Y. Ng, Lim, Z., Bezault, E., Turner-Maier, J. Johnson, J., Alcazar, R., Noh, H., Russell, P., Aken, B., Alföldi, J., Amemiya, C., Azzouzi, N., Baroiller, J.-F., Barloy-Hubler, F., Berlin, a., Bloomquist, R., Carleton, K., Conte, M., D'Cotta, H., Eshel, O., Gaffney, L., Galibert, F., Gante, H., Gnerre, S., Greuter, L., Guyon, R., Haddad, N., Haerty, W., Harris, R., Hofmann, H., Hourlier, T., Hulata, G., Jaffe, D., Lara, M., A.P., L., MacCallum, I., Mwaiko, S., Nikaido, M., Nishihara, H., Ozouf-Costaz, C., Penman, D., Przybylski, D., Rakotomanga, M., Renn, S., Ribeiro, F., Ron, M., Salzburger, W., Sanchez-Pulido, L., Santos, M., Searle, S., Sharpe, T., Swofford, R., Tan, F., Williams, L., Young, S., Yin, S., Okada, N., Kocher, T., Miska, E., Lander, E., Venkatesh, B., Fernald, R., Meyer, A., Ponting, C., Streelman, J., Lindblad-Toh, K., Seehausen, O. & Di Palma, F. (2014). The genomic substrate for adaptive radiation in African cichlid fish. *Nature*, 513:375–381.

Brummett, R. E. (1995). Environmental regulation of sexual maturation and reproduction in tilapia. *Reviews in Fisheries Science*, 3(3):231–248.

Bruton, M. N. & Boltt, R. E. (1975). Aspects of the biology of *Tilapia mossambica* Peters (Pisces: Cichlidae) in a natural freshwater lake (Lake Sibaya, South Africa). *Journal of Fish Biology*, 7(4):423–445.

Buckles, L. K., Verschuren, D., Weijers, J. W. H., Cocquyt, C., Blaauw, M. & Damsté, J. S. S. (2016). Interannual and (multi-)decadal variability in the sedimentary BIT index of Lake Challa, East Africa, over the past 2200 years: Assessment of the precipitation proxy. *Climate of the Past*, 12:1243–1262.

Buckles, L. K., Weijers, J. W. H., Verschuren, D. & Sinninghe Damsté, J. S. (2014). Sources of core and intact branched tetraether membrane lipids in the lacustrine environment: Anatomy of Lake Challa and its catchment, equatorial East Africa. *Geochimica et Cosmochimica Acta*, 140:106–126.

Burress, E. D. (2015). Cichlid fishes as models of ecological diversification: patterns, mechanisms, and consequences. *Hydrobiologia*, 748(1):7–27.

Bwanika, G. N., Murie, D. J. & Chapman, L. J. (2007). Comparative age and growth of Nile tilapia (*Oreochromis niloticus* L.) in lakes Nabugabo and Wamala, Uganda. *Hydrobiologia*, 589(1):287–301.

Canonico, G. C., Arthington, A., Mccrary, J. K. & Thieme, M. L. (2005). The effects of introduced tilapias on native biodiversity. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 15(5):463–483.

Carlquist, S. (1980). *Hawaii: a natural history.* Pacific Tropical Botanical Garden, Lawai, Hawaii, 2^{nd} edition.

Carlquist, S., Janish, J. & Papp, C. (1965). *Island life.*

Carr, G. (1985). Monography of the Hawaiian Madiinae (Asteraceae): Argyroxiphium, Dubautia and Wilkesia. *Allertonia*, 4(1):1–123.

Chessel, D., Dufour, A. B. & Thioulouse, J. (2004). The ade4 package - I : One-table methods. *R News*, 4(1):5–10.

Clarke, K. R. (1993). Nonparametric multivariate analyses of changes in community structure. *Australian Journal of Ecology*, 18(1):117–143.

Cocquyt, C. & Ryken, E. (2016). *Afrocymbella barkeri* sp. nov. (Bacillariophyta), a common phytoplankton component of Lake Challa, a deep crater lake in East Africa. *European Journal of Phycology*, 51(2):217–225.

Cohen, A. S., Stone, J. R., Beuning, K. R. M., Park, L. E., Reinthal, P. N., Dettman, D., Scholz, C. A., Johnson, T. C., King, J. W., Talbot, M. R., Brown, E. T. & Ivory, S. J. (2007). Ecological consequences of early Late Pleistocene megadroughts in tropical Africa. *Proceedings of the National Academy of Sciences*, 104(42):16422–7.

Coyne, J. A. & Orr, H. (2004). *Speciation.* Sinauer Associates.

Crispo, E., Moore, J. S., Lee-Yaw, J. a., Gray, S. M. & Haller, B. C. (2011). Broken barriers: Human-induced changes to gene flow and introgression in animals. *BioEssays*, 33(7):508–518.

Curran, J. (2017). Hotelling: Hotelling's T^2 test and variants. R package version 1.0-4.

Cushing, D. (1974). The possible density-dependence of larval mortality and adult mortality in fishes. In *The early life history of fish*, pp. 103–111. Springer.

Dadzie, S., Haller, R. & Trewavas, E. (1988). A note on the fishes of Lake Jipe and Lake Chale on the Kenya-Tanzania border. *Journal of East African Natural History*, 192:46–51.

Daga, V. S., Debona, T., Abilhoa, V., Gubiani, É. A. & Vitule, J. R. S. (2016). Non-native fish invasions of a Neotropical ecoregion with high endemism: A review of the Iguaçu River. *Aquatic Invasions*, 11(2):209–223.

D'Amato, M. E., Esterhuyse, M. M., Van Der Waal, B. C. W., Brink, D. & Volckaert, F. A. M. (2007). Hybridization and phylogeography of the Mozambique tilapia *Oreochromis mossambicus* in southern Africa evidenced by mitochondrial and microsatellite DNA genotyping. *Conservation Genetics*, 8:475–488.

Danley, P. D., Husemann, M., Ding, B., Dipietro, L. M., Beverly, E. J. & Peppe, D. J. (2012). The impact of the geologic history and paleoclimate on the diversification of East african cichlids. *International Journal of Evolutionary Biology*, 2012:574851.

Danley, P. D. & Kocher, T. D. (2001). Speciation in rapidly diverging systems: Lessons from Lake Malawi. *Molecular Ecology*, 10(5):1075–1086.

Darriba, D., Taboada, G. L., Doallo, R. & Posada, D. (2012). JModelTest 2: More models, new heuristics and parallel computing. *Nature Methods*, 9(8):772.

De Cort, G., Chen, C., McGee, D., Ge, Y., Zhang, F., Shuman, B., Metcalfe, S., Primmer, N. & Harrison, S. (2017). Updating the Global Lake Status Data Base. In *First Paleoclimate Modelling Intercomparison Project Phase 4 (PMIP4), Stockholm, Sweden.*

De Moor, F. C., Wilkinson, R. C. & Herbst, H. M. (1986). Food and feeding habits of *Oreochromis mossambicus* (Peters) in hypertrophic Hartbeespoort Dam, South Africa. *South African Journal of Zoology*, 21(2):170–176.

Deines, A. M., Bbole, I., Katongo, C., Feder, J. L. & Lodge, D. M. (2014). Hybridisation between native *Oreochromis* species and introduced Nile tilapia *O. niloticus* in the Kafue River, Zambia. *African Journal of Aquatic Science*, 39(1):23–34.

Delvaux, D. (1995). Age of Lake Malawi (Nyasa) and water level fluctuations. *Musée royale d' Afrique centrale, Tervuren (Belgique), Deptartement Gédynamique et Ressources Minérales, Rapport annuel 1993 & 1994*, 108:99–108.

Denny, P. (1978). Nyumba ya Mungu reservoir, Tanzania, the general features. *Biological Journal of the Linnean Society*, 10:5–28.

Dietz, E. J. (1983). Permutation tests for association between two distance matrices. *Systematic Zoology*, 32(1):21–26.

Drummond, A. J. & Bouckaert, R. R. (2015). *Bayesian evolutionary analysis with BEAST 2.* Cambridge University Press, Cambridge.

Drummond, A. J., Ho, S. Y., Phillips, M. J. & Rambaut, A. (2006). Relaxed phylogenetics and dating with confidence. *PLoS Biology*, 4(5):699–710.

Drummond, A. J., Rambaut, A., Shapiro, B. & Pybus, O. G. (2005). Bayesian coalescent inference of past population dynamics from molecular sequences. *Molecular Biology and Evolution*, 22(5):1185–1192.

Dunz, A. R. & Schliewen, U. K. (2013). Molecular phylogeny and revised classification of the haplotilapiine cichlid fishes formerly referred to as Tilapia. *Molecular Phylogenetics and Evolution*, 68(1):64–80.

Duponchelle, F. & Panfili, J. (1998). Variations in age and size at maturity of female Nile Tilapia, *Oreochromis niloticus*, populations from man-made lakes of Côte d'Ivoire. *Environmental Biology of Fishes*, 52:453–465.

Eknath, A. E. & Hulata, G. (2009). Use and exchange of genetic resources of Nile tilapia (*Oreochromis niloticus*). *Reviews in Aquaculture*, 1(3-4):197–213.

El-Sayed, A.-F. M. (2006). *Tilapia Culture.* CABI Publishing.

Elder, R. L. & Smith, G. R. (1988). Fish taphonomy and environmental inference in paleolimnology. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 62(1-4):577–592.

Elmer, K. R., Kusche, H., Lehtonen, T. K. & Meyer, A. (2010a). Local variation and parallel evolution: morphological and genetic diversity across a species complex of neotropical crater lake cichlid fishes. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 365(1547):1763–82.

Elmer, K. R., Lehtonen, T. K., Fan, S. & Meyer, A. (2012). Crater lake colonization by neotropical cichlid fishes. *Evolution*, 67:281–288.

Elmer, K. R., Lehtonen, T. K., Kautt, A. F., Harrod, C. & Meyer, A. (2010b). Rapid sympatric ecological differentiation of crater lake cichlid fishes within historic times. *BMC biology*, 8:60.

Excoffier, L., Laval, G. & Schneider, S. (2005). Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, 1(1):47–50.

FAO (2014). *The state of world fisheries and aquaculture*, volume 2014.

Ferrara, R. A. & Hildick-Smith, A. (1982). A modeling approach for stormwater quantity and quality control via detention basins. *Water Resources Bulletin AWRA*, 18(2):975–981.

Finlay, J. C. & Kendall, C. (2007). Stable isotope tracing of temporal and spatial variability in organic matter sources to freshwater ecosystems. In L. K. Michener R, editor, *Stable isotopes in ecology and environmental science.*, pp. 283–333. Blackwell, Oxford.

Firmat, C., Alibert, P., Losseau, M., Baroiller, J. F. & Schliewen, U. K. (2013). Successive invasion-mediated interspecific hybridizations and population structure in the endangered cichlid *Oreochromis mossambicus*. *PLoS ONE*, 8(5):e63880.

Foote, A. D. (2018). Sympatric speciation in the genomic era. *Trends in Ecology & Evolution*, 33(2):85–95.

Ford, A. G. P., Dasmahapatra, K. K., Rüber, L., Gharbi, K., Cezard, T. & Day, J. J. (2015). High levels of interspecific gene flow in an endemic cichlid fish adaptive radiation from an extreme lake environment. *Molecular Ecology*, 24(13).

Fortelius, M. & Solounias, N. (2000). Functional characterization of ungulate molars using the abrasion-attrition wear gradient: A new method for reconstructing paleodiets. *American Museum Novitates*, 3301:1–36.

Fraley, C., Raftery, A. E., Murphy, T. B. & Scrucca, L. (2012). Mclust version 4 for R: Normal mixture modeling for model-based clustering, classification, and density estimation. *Technical Report 597, University of Washington*, pp. 1–50.

Friedman, M., Keck, B. P., Dornburg, A., Eytan, R. I., Martin, C. H., Hulsey, C. D., Wainwright, P. C. & Near, T. J. (2013). Molecular and fossil evidence place the origin of cichlid fishes long after Gondwanan rifting. *Proceedings of the Royal Society B: Biological Sciences*, 280(1770):20131733.

Froese, R. & Pauly, D. (2017). FishBase. URL `www.fishbase.org`.

Fryer, G. (1957). The trophic interrelationships and ecology of some littoral communities of Lake Nyasa with especial reference to the fishes, and a discussion of the evolution of a group of rock-frequenting cichlidae. *Journal of Zoology*, 132(2):153–281.

Fryer, G. (1959a). Some aspects of evolution in Lake Nyasa. *Evolution*, 13:440–451.

Fryer, G. (1959b). The trophic interrelationships and ecology of some littoral communities of Lake Nyasa with especial reference to the fishes, and a discussion of the evolution of a group of rock-frequenting cichlidae. *Northern Rhodesia-Nyasaland Joint Fisheries Research Organization*, pp. 153–281.

Fryer, G. & Iles, T. (1972). *The Cichlid Fishes of the Great Lakes of Africa, their Biology and Distribution.* Oliver and Boyd, Edinburgh.

Gante, H. F. & Salzburger, W. (2012). Evolution: Cichlid models on the runaway to speciation. *Current Biology*, 22(22).

Gauchey, S., Girard, C., Adnet, S. & Renaud, S. (2014). Unsuspected functional disparity in Devonian fishes revealed by tooth morphometrics? *Naturwissenschaften*, 101(9):735–743.

Genner, M. J., Knight, M. E., Haesler, M. P. & Turner, G. F. (2010). Establishment and expansion of Lake Malawi rock fish populations after a dramatic Late Pleistocene lake level rise. *Molecular Ecology*, 19:170–182.

Genner, M. J., Seehausen, O., Lunt, D. H., Joyce, D. A., Shaw, P. W., Carvalho, G. R. & Turner, G. F. (2007). Age of cichlids: New dates for ancient lake fish radiations. *Molecular Biology and Evolution*, 24(5):1269–1282.

Genner, M. J. & Turner, G. F. (2014). Timing of population expansions within the Lake Malawi haplochromine cichlid fish radiation. *Hydrobiologia*, 748:121–132.

Genner, M. J., Turner, G. F. & Hawkins, S. J. (1999). Foraging of rocky habitat cichlid fishes in Lake Malawi: coexistence through niche partitioning? *Oecologia*, 121(2):283–292.

Gómez-Robles, A., Martinón-Torres, M., Bermúdez de Castro, J. M., Margvelashvili, A., Bastir, M., Arsuaga, J. L., Pérez-Pérez, A., Estebaranz, F. & Martínez, L. M. (2007). A geometric morphometric analysis of hominin upper first molar shape. *Journal of Human Evolution*, 53(3):272–285.

Goodwin, N. B., Balshine-Earn, S. & Reynolds, J. D. (1998). Evolutionary transitions in parental care in cichlid fish. *Proceedings of the Royal Society of London Series B-Biological Sciences*, 265(1412):2265–2272.

Gophen, M. (1980). Food sources, feeding behaviour and growth rates of *Sarotherodon galilaeum* (Linnaeus) fingerlings. *Aquaculture*, 20(2):101–115.

Goudswaard, P., Witte, F. & Katunzi, E. (2002). The tilapiine fish stock of Lake Victoria before and after the Nile perch upsurge. *Journal of Fish Biology*, 60:838–856.

Gozlan, R. E., Britton, J. R., Cowx, I. & Copp, G. H. (2010). Current knowledge on non-native freshwater fish introductions. *Journal of Fish Biology*, 76(4):751–786.

Grant, B. & Grant, P. (1989). *Evolutionary dynamics of a natural population.* Chicago University Press, Chicago.

Grant, W. S. (2015). Problems and cautions with sequence mismatch analysis and Bayesian skyline plots to infer historical demography. *Journal of Heredity*, 106:333–346.

Greenwood, P. H. (1953). Feeding mechanism of the cichlid fish, *Tilapia esculenta* Graham. *Nature*, 172(4370):207.

Greenwood, P. H. (1965). Environmental effects on the pharyngeal mill of a cichlid fish, *Astatoreochromis alluaudi*, and their taxonomic implications. *Proceedings of the Linnean Society of London*, 176(1):1–10.

Greenwood, P. H. (1979). Towards a phyletic classification of the 'genus' *Haplochromis* (Pisces, Cichlidae) and related taxa. Part 1. *Bulletin of the British Museum (Natural History) Zoology Series*, 35(4):265–322.

Greenwood, P. H. & Gee, J. M. (1969). A revision of the Lake Victoria *Haplochromis* species (Pisces, Cichlidae). *Bulletin of the British Museum (Natural History)*, 18(2):1–65.

Gunter, H. M., Fan, S., Xiong, F., Franchini, P., Fruciano, C. & Meyer, A. (2013). Shaping development through mechanical strain: The transcriptional basis of diet-induced phenotypic plasticity in a cichlid fish. *Molecular Ecology*, 22(17):4516–4531.

Günther, A. (1889). On some fishes from the Kilima-Njaro district. In *Proceedings of the Scientific Meetings of the Zoological Society of London for the Year 1889*, pp. 70–72.

Håkansson, N. (2008). The decentralized landscape: regional wealth and the expansion of production in northern Tanzania before the eve of colonialism. In *Economies and the transformation of landscape*, pp. 239–266. AltaMira Press.

Hammer, Ø., Harper, D. & Ryan, P. D. (2001). PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica*, 4(1):1–9.

Härer, A., Torres-Dowdall, J. & Meyer, A. (2017). Rapid adaptation to a novel light environment: The importance of ontogeny and phenotypic plasticity in shaping the visual system of Nicaraguan Midas cichlid fish (*Amphilophus citrinellus* spp.). *Molecular Ecology*, 26(20):5582–5593.

Hasegawa, M., Kishino, H. & aki Yano, T. (1985). Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution*, 22(2):160–174.

Heiri, O., Lotter, A. & Lemcke, G. (2001). Loss on Ignition as a method for estimating organic and carbonate content in sediments : Reproducibility and comparability of results. *Journal of Paleolimnology*, 25:101–110.

Held, I. M., Soden, B. J., Held, I. M. & Soden, B. J. (2006). Robust responses of the hydrological cycle to global warming. *Journal of Climate*, 19(21):5686–5699.

Hellig, C. J., Kerschbaumer, M., Sefc, K. M. & Koblmüller, S. (2010). Allometric shape change of the lower pharyngeal jaw correlates with a dietary shift to piscivory in a cichlid fish. *Naturwissenschaften*, 97(7):663–672.

Hert, E. (1989). The function of egg-spots in an African mouth-brooding cichlid fish. *Animal Behaviour*, 37(5):726–732.

Hirsch, P., N'Guyen, A., Muller, R., Adrian-Kalchhauser, I. & Burkhardt-Holm, P. (2018). Colonizing islands of water on dry land on the passive dispersal of fish eggs by birds. *Fish and Fisheries*, Early view.

Holzman, R. & Hulsey, C. D. (2017). Mechanical transgressive segregation and the rapid origin of trophic novelty. *Scientific Reports*, 7:40306.

Huber, R., Van Staaden, M. J., Kaufman, L. S. & Liem, K. F. (1997). Microhabitat use, trophic patterns, and the evolution of brain structure in african cichlids. *Brain, Behavior and Evolution*, 50(3):167–182.

Hulsey, C. D. (2006). Function of a key morphological innovation: Fusion of the cichlid pharyngeal jaw. *Proceedings of the Royal Society of London B: Biological Sciences*, 273(1587):669–675.

Hulsey, C. D., Fraser, G. J. & Meyer, A. (2016). Biting into the genome to phenome map: Developmental genetic modularity of cichlid fish dentitions. *Integrative and comparative biology*, 56(3):373–388.

Hulsey, C. D., García de León, F. J. & Rodiles-Hernández, R. (2006). Micro- and macroevolutionary decoupling of cichlid jaws: A test of Liem's key innovation hypothesis. *Evolution; international journal of organic evolution*, 60(10):2096–2109.

Hulsey, C. D., Machado-Schiaffino, G., Keicher, L., Ellis-Soto, D., Henning, F. & Meyer, A. (2017). The integrated genomic architecture and evolution of dental divergence in east african cichlid fishes (*Haplochromis chilotes* x *H. nyererei*). *G3: Genes, Genomes, Genetics*, 7(9):3195–3202.

Hundt, P. & Simons, A. (2018). Extreme dentition does not prevent diet and tooth diversification within combtooth blennies (Ovalentaria: Blenniidae). *Evolution*, Early view.

Hurrell, E. R., Barker, P. A., Leng, M. J., Vane, C. H., Wynn, P., Kendrick, C. P., Verschuren, D. & Alayne Street-Perrott, F. (2011). Developing a methodology for carbon isotope analysis of lacustrine diatoms. *Rapid Communications in Mass Spectrometry*, 25(11):1567–1574.

Huysseune, A. (1995). Phenotypic plasticity in the lower pharyngeal jaw dentition of *Astatoreochromis alluaudi* (Teleostei: Cichlidae). *Archives of Oral Biology*, 40(11):1005–1014.

Huysseune, A. & Sire, J. Y. (1992). Development of cartilage and bone tissues of the anterior part of the mandible in cichlid fish: A light and TEM study. *The Anatomical Record*, 233(3):357–375.

Huysseune, A. & Witten, P. E. (2018). Tooth shape in teleosts and its link to tooth spacing and replacement. *Cybium*, 42(1):19–27.

Hyslop, E. J. (1980). Stomach contents analysis-a review of methods and their application. *Journal of Fish Biology*, 17:411–429.

Iglesias, C., Bonecker, C., Brandão, L., Crispim, M. C., Eskinazi-Sant'Anna, E. M., Gerhard, M., Laço Portinho, J., Maia-Barbosa, P., Panarelli, E. & Santangelo, J. M. (2016). Current knowledge of south american cladoceran diapause: A brief review. *International Review of Hydrobiology*, 101(3-4):91–104.

Ivory, S. J., Blome, M. W., King, J. W., McGlue, M. M., Cole, J. E. & Cohen, A. S. (2016). Environmental change explains cichlid adaptive radiation at Lake Malawi over the past 1.2 million years. *Proceedings of the National Academy of Sciences of the United States of America*, 113(42).

Iwata, H. & Ukai, Y. (2002). SHAPE: a computer program package for quantitative evaluation of biological shapes based on elliptic Fourier descriptors. *The Journal of heredity*, 93(5):384–385.

Jackman, T., Losos, J., Larson, A. & de Queiroz, K. (1997). Phylogenetic studies of convergent adaptive radiations in Caribbean *Anolis* lizards. In *Molecular evolution and adaptive radiation*. Cambridge University Press, Cambridge.

Johnson, T. C., Scholz, C. & Talbot, M. R. (1996). Late Pleistocene dessication of Lake Victoria and rapid evolution of cichlid fishes. *Science*, 273:1091–1093.

Joyce, D., Lunt, D., Genner, M., Turner, G., Bills, R. & Seehausen, O. (2011). Repeated colonization and hybridization in Lake Malawi cichlids. *Current Biology*, 21(3):R108–R109.

Joyce, D. A., Lunt, D. H., Bills, R., Turner, G. F., Katongo, C., Duftner, N., Sturmbauer, C. & Seehausen, O. (2005). An extant cichlid fish radiation emerged in an extinct Pleistocene lake. *Nature*, 435(7038):90–95.

Kassam, D., Adams, D. C. & Yamaoka, K. (2004). Functional significance of variation in trophic morphology within feeding microhabitat-differentiated cichlid species in lake malawi. *Animal Biology*, 54(1):77–90.

Kavembe, G. D., Kautt, A. F., Machado-Schiaffino, G. & Meyer, A. (2016). Eco-morphological differentiation in Lake Magadi tilapia, an extremophile cichlid fish living in hot, alkaline and hypersaline lakes in East Africa. *Molecular Ecology*, 25(7):1610–1625.

Kerschbaumer, M. & Sturmbauer, C. (2011). The utility of geometric morphometrics to elucidate pathways of cichlid fish evolution. *International Journal of Evolutionary Biology*, 2011:290245.

Klett, V. & Meyer, A. (2002). What, if anything, is a Tilapia? - Mitochondrial ND2 phylogeny of tilapiines and the evolution of parental care systems in the African cichlid fishes. *Molecular Biology and Evolution*, 19(6):865–883.

Koblmüller, S., Sefc, K. M. & Sturmbauer, C. (2008). The Lake Tanganyika cichlid species assemblage: Recent advances in molecular phylogenetics. *Hydrobiologia*, 615(1):5–20.

Kocher, T. D., Conroy, J. A., McKaye, K. R. & Stauffer, J. R. (1993). Similar morphologies of cichlid fish in lakes Tanganyika and Malawi are due to convergence. *Molecular Phylogenetics and Evolution*, 2(2):158–165.

Kocher, T. D., Conroy, J. A., McKaye, K. R., Stauffer, J. R. & Lockwood, S. F. (1995). Evolution of nadh dehydrogenase subunit 2 in east african cichlid fish. *Molecular phylogenetics and evolution*, 4(4):420–432.

Kruskal, J. B. (1964). Multidimensional scaling by optimizing goodness of fit to a nonmetric hypothesis. *Psychometrika*, 29(1):1–27.

Lack, D. (1947). *Darwin's finches.* Cambridge University Press, Cambridge.

Lamichhaney, S., Berglund, J., Almén, M. S., Maqbool, K., Grabherr, M., Martinez-Barrio, A., Promerová, M., Rubin, C.-J., Wang, C., Zamani, N., Grant, B. R., Grant, P. R., Webster, M. T. & Andersson, L. (2015). Evolution of Darwin's finches and their beaks revealed by genome sequencing. *Nature*, 518(7539):371–375.

Langerhans, R. B. & DeWitt, T. J. (2004). Shared and unique features of evolutionary diversification. *The American Naturalist*, 164(3):335–349.

Lauder, G., Crompton, A., Gans, C., Hanken, J., Liem, K., Maier, W., Meyer, A., Presley, R., Rieppel, O., Roth, G., Schluter, D. & Zweers, G. (1989). How are feeding systems integrated and how have evolutionary innovations been introduced? In D. Wake & G. Roth, editors, *Complex Organismal Functions: Integration and Evolution in Vertebrates*, pp. 97–115. John Wiley & Sons, Ltd.

Lee, W. J., Conroy, J., Howell, W. H. & Kocher, T. D. (1995). Structure and evolution of teleost mitochondrial control regions. *Journal of Molecular Evolution*, 41(1):54–66.

Leinonen, T., Cano, J. M., Mäkinen, H. & Merilä, J. (2006). Contrasting patterns of body shape and neutral genetic divergence in marine and lake populations of threespine sticklebacks. *Journal of Evolutionary Biology*, 19(6):1803–1812.

Liem, K. (1986). The pharyngeal jaw apparatus of the Embiotocidae (Teleostei): A functional and evolutionary perspective. *American Society of Ichthyologists and Herpetologists*, 2:311–323.

Liem, K., Bemis, W., Walker, W. & Grande, L. (2001). *Functional anatomy of the Vertebrates, an evolutionary perspective.* Brooks/Cole - Thomson Learning, 3^{rd} edition.

Liem, K. F. (1973). Evolutionary strategies and morphological innovations: Cichlid pharyngeal jaws. *Systematic Zoology*, 22(4):425.

Loomis, S. E., Russell, J. M., Verschuren, D., Morrill, C., Cort, G. D., Olago, D., Eggermont, H., Street-perrott, F. A. & Kelly, M. A. (2017). The tropical lapse rate steepened during the Last Glacial Maximum. *Science Advances*, 3:e1600815.

Losos, J. B. (2011). Convergence, adaptation, and constraint. *Evolution*, 65(7):1827–1840.

Lowe, R. H. (1955). New species of Tilapia (Pisces, Cichlidae) from Lake Jipe and the Pangani River, East Africa. *Bulletin of the British Museum (Natural History) Zoology.*

Lowe-McConnell, R. H. (1987). *Ecological studies in tropical fish communities.* University Press Cambridge.

Lyons, R. P., Scholz, C. A., Buoniconti, M. R. & Martin, M. R. (2011). Late Quaternary stratigraphic analysis of the Lake Malawi Rift, East Africa: An integration of drill-core and seismic-reflection data. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 303(1-4):20–37.

Lyons, R. P., Scholz, C. A., Cohen, A. S., King, J. W., Brown, E. T., Ivory, S. J., Johnson, T. C., Deino, A. L., Reinthal, P. N., McGlue, M. M. & Blome, M. W. (2015). Continuous 1.3-million-year record of East African hydroclimate, and implications for patterns of evolution and biodiversity. *Proceedings of the National Academy of Sciences*, 112:15568–15573.

Mabuchi, K., Miya, M., Azuma, Y. & Nishida, M. (2007). Independent evolution of the specialized pharyngeal jaw apparatus in cichlid and labrid fishes. *BMC Evolutionary Biology*, 7:10.

Mahler, D. L., Ingram, T., Revell, L. J. & Losos, J. B. (2013). Exceptional convergence on the macroevolutionary landscape in island lizard radiations. *Science*, 341(6143):292–295.

Malinsky, M., Challis, R. J., Tyers, A. M., Schiffels, S., Terai, Y., Ngatunga, B. P., Miska, E. A., Durbin, R., Genner, M. J. & Turner, G. F. (2015). Genomic islands of speciation separate cichlid ecomorphs in an East African crater lake. *Science*, 350(6267):1493–1498.

Mallatt, J. (1996). Ventilation and the origin of jawed vertebrates: A new mouth. *Zoological Journal of the Linnean Society*, 117(4):329–404.

Man, H. S. & Hodgkiss, I. J. (1977). Studies on the ichthyofauna in Plover Cove Reservoir, Hong Kong: I. Sequence of fish population changes. *Journal of Fish Biology*, 10(5):493–503.

Martin, C. H., Cutler, J. S., Friel, J. P., Dening Touokong, C., Coop, G. & Wainwright, P. C. (2015). Complex histories of repeated gene flow in Cameroon crater lake cichlids cast doubt on one of the clearest examples of sympatric speciation. *Evolution*, 69(6):1406–1422.

Martinón-Torres, M., Bastir, M., Bermúdez de Castro, J. M., Gómez, A., Sarmiento, S., Muela, A. & Arsuaga, J. L. (2006). Hominin lower second premolar morphology: evolutionary inferences through geometric morphometric analysis. *Journal of Human Evolution*, 50(5):523–533.

Martinsen, G. D., Whitham, T. G., Turek, R. J. & Keim, P. (2001). Hybrid populations selectively filter gene introgression between species. *Evolution*, 55(7):1325–1335.

Matschiner, M. (2016). Fitchi: Haplotype genealogy graphs based on the Fitch algorithm. *Bioinformatics*, 32(8):1250–1252.

Matschiner, M., Musilová, Z., Barth, J. M., Starostová, Z., Salzburger, W., Steel, M. & Bouckaert, R. (2017). Bayesian phylogenetic estimation of clade ages supports trans-atlantic dispersal of cichlid fishes. *Systematic Biology*, 66(1):3–22.

Meier, J. I., Marques, D. A., Mwaiko, S., Wagner, C. E., Excoffier, L. & Seehausen, O. (2017a). Ancient hybridization fuels rapid cichlid fish adaptive radiations. *Nature Communications*, 8:14363.

Meier, J. I., Sousa, V. C., Marques, D. A., Selz, O. M., Wagner, C. E., Excoffier, L. & Seehausen, O. (2017b). Demographic modelling with whole-genome data reveals parallel origin of similar *Pundamilia* cichlid species after hybridization. *Molecular Ecology*, 26(1):123–141.

Meyer, A. (1987). Phenotypic plasticity and heterochrony in *Cichlasoma managuense* (Pisces, Cichlidae) and their implications for speciation in cichlid fishes. *Evolution*, 41(6):1357–1369.

Meyer, A. (1990). Ecological and evolutionary consequences of the trophic polymorphism in *Cichlasoma citrinellum* (Pisces: Cichlidae). *Biological journal of the Linnean Society*, 39:279–299.

Meyer, A. (1993). Phylogenetic relationships and evolutionary processes in East African cichlid fishes. *Trends in Ecology & Evolution*, 8(8):279–284.

Meyer, A., Kocher, T. D., Basasibwaki, P. & Wilson, A. C. (1990). Monophyletic origin of Lake Victoria cichlid fishes suggested by mitochondrial DNA sequences. *Nature*, 347(6293):550–553.

Meyer, a., Morrissey, J. M. & Schartl, M. (1994). Recurrent origin of a sexually selected trait in *Xiphophorus* fishes inferred from a molecular phylogeny. *Nature*, 368(6471):539–542.

Meyer, B. S., Matschiner, M. & Salzburger, W. (2017). Disentangling incomplete lineage sorting and introgression to refine species-tree estimates for Lake Tanganyika cichlid fishes. *Systematic Biology*, 66(4):531–550.

Meyer, I., Van Daele, M., Fiers, G., Verleyen, E., De Batist, M. & Verschuren, D. (2018). Sediment reflectance spectroscopy as a paleo-hydrological proxy in East Africa. *Limnology and Oceanography Methods*, 16:92–105.

Moernaut, J., Verschuren, D., Charlet, F., Kristen, I., Fagot, M. & De Batist, M. (2010). The seismic-stratigraphic record of lake-level fluctuations in Lake Challa: Hydrological stability and change in equatorial East Africa over the last 140 kyr. *Earth and Planetary Science Letters*, 290(1-2):214–223.

Murray, A. M. (2001). The fossil record and biogeography of the Cichlidae (Actinopterygii: Labroidei). *Biological Journal of the Linnean Society*, 74:517–532.

Muschick, M., Barluenga, M., Salzburger, W. & Meyer, A. (2011). Adaptive phenotypic plasticity in the Midas cichlid fish pharyngeal jaw and its relevance in adaptive radiation. *BMC evolutionary biology*, 11(1):116.

Muschick, M., Indermaur, A. & Salzburger, W. (2012). Convergent evolution within an adaptive radiation of cichlid fishes. *Current Biology*, 22(24):2362–2368.

Muschick, M., Russell, J. M., Jemmi, E., Walker, J., Stewart, K. M., Murray, A. M., Dubois, N., Stager, J. C., Johnson, T. C. & Seehausen, O. (2018). Arrival order and release from competition does not explain why haplochromine cichlids radiated in lake victoria. *Proceedings of the Royal Society B*, 285(1878):20180462.

Nagl, S., Tichy, H., Mayer, W. E., Samonte, I. E., McAndrew, B. J. & Klein, J. (2001). Classification and phylogenetic relationships of African tilapiine fishes inferred from mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution*, 20(3):361–374.

Nash, K. L., Allen, C. R., Barichievy, C., Nyström, M., Sundstrom, S. & Graham, N. A. (2014). Habitat structure and body size distributions: Cross-ecosystem comparison for taxa with determinate and indeterminate growth. *Oikos*, 123(8):971–983.

Ndiwa, T. C., Nyingi, D. W. & Agnese, J. F. (2014). An important natural genetic resource of *Oreochromis niloticus* (Linnaeus, 1758) threatened by aquaculture activities in Loboi Drainage, Kenya. *PLoS ONE*, 9(9):e106972.

Ndiwa, T. C., Nyingi, D. W., Claude, J. & Agnèse, J. F. (2016). Morphological variations of wild populations of Nile tilapia (*Oreochromis niloticus*) living in extreme environmental conditions in the Kenyan Rift-Valley. *Environmental Biology of Fishes*, 99(5):473–485.

Nevado, B., Mautner, S., Sturmbauer, C. & Verheyen, E. (2013). Water-level fluctuations and metapopulation dynamics as drivers of genetic diversity in populations of three Tanganyikan cichlid fish species. *Molecular Ecology*, 22(15):3933–3948.

Njiru, M., Getabu, A., Jembe, T., Ngugi, C., Owili, M. & Van Der Knaap, M. (2008). Management of the Nile tilapia (*Oreochromis niloticus* (L.)) fishery in the Kenyan portion of Lake Victoria, in light of changes in its life history and ecology. *Lakes and Reservoirs: Research and Management*, 13(2):117–124.

Njiru, M., Okeyo-Owuor, J. B., Muchiri, M. & Cowx, I. G. (2004). Shifts in the food of Nile tilapia, *Oreochromis niloticus* (L.) in Lake Victoria, Kenya. *African Journal of Ecology*, 42(3):163–170.

Noakes, D. & Balon, E. (1982). Life histories of tilapias: an evolutionary perspective. In R. Pullin & R. Lowe-McConnell, editors, *The biology and culture of tilapias.*, pp. 61–87. WorldFish.

Nordhausen, K., Sirkia, S., Oja, H. & Tyler, D. (2015). ICSNP: Tools for Multivariate Nonparametrics. R package version 1.1-0.

Nyberg, K. G., Ciampaglio, C. N. & Wray, G. A. (2006). Tracing the ancestry of the great white shark, *Carcharodon carcharias*, using morphometric analyses of fossil teeth. *Journal of Vertebrate Paleontology*, 26(4):806–814.

Nyingi, D. W. & Agnèse, J. F. (2007). Recent introgressive hybridization revealed by exclusive mtDNA transfer from *Oreochromis leucostictus* (Trewavas, 1933) to *Oreochromis niloticus* (Linnaeus, 1758) in Lake Baringo, Kenya. *Journal of Fish Biology*, 70:148–154.

Ogutu-Ohwayo, R. (1990). The decline of the native fishes of lakes Victoria and Kyoga (East Africa) and the impact of introduced species, especially the Nile perch, *Lates niloticus*, and the Nile tilapia, *Oreochromis niloticus*. *Environmental Biology of Fishes*, 27(2):81–96.

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E. & Wagner, H. H. (2017). Vegan: Community Ecology Package. R Package Version. 2.4-3. *http://CRAN.R-project.org/package=vegan.*

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H. & Wagner, H. (2013). Package 'vegan'. *R package ver. 2.08*, p. 254.

Opitz, S. (2008). *Mikrofazielle untersuchungen an einem transekt von kurzkernen aus dem Lake Challa- kratersee in Kenia.* Master's thesis, Universität Potsdam.

Park, L. E. & Cohen, A. S. (2011). Paleoecological response of ostracods to early Late Pleistocene lake-level changes in Lake Malawi, East Africa. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 303(1-4):71–80.

Parnell, N. F., Hulsey, C. D. & Streelman, J. T. (2012). The genetic basis of a complex functional system. *Evolution*, 66(11):3352–3366.

Perez, S. I., Bernal, V. & Gonzalez, P. N. (2006). Differences between sliding semi-landmark methods in geometric morphometrics, with an application to human craniofacial and dental variation. *Journal of Anatomy*, 208(6):769–784.

Peterson, I. & Wroblewski, J. (1984). Mortality rate of fishes in the pelagic ecosystem. *Canadian Journal of Fisheries and Aquatic Sciences*, 41(7):1117–1120.

Philippart, J. & Ruwet, J. (1982). Ecology and distribution of tilapias. In *The biology and culture of tilapias.*, p. 432. WorldFish.

Piet, G. J. (1998). Ecomorphology of a size-structured tropical freshwater fish community. *Environmental Biology of Fishes*, 51(1):67–86.

Pillay, G. K. (2016). *Discriminating between Oreochromis populations using morphological and molecular analysis.* Master's thesis, Bangor Unversity.

Post, D. M., Layman, C. A., Arrington, D. A., Takimoto, G., Quattrochi, J. & Montaña, C. G. (2007). Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. *Oecologia*, 152(1):179–189.

Pullin, A. (2002). *Conservation biology.* Cambridge University Press, Cambridge.

Pullin, R. & Lowe-McConnell, R. (1982). The Biology and Culture of Tilapias. In *Proceedings of the International Conference on the Biology and Culture of Tilapias, 2-5 September 1980 at the Study and Conference Center of the Rockefeller Foundation, Bellagio, Italy. (Vol. 7). WorldFish.*, p. 432.

Purnell, M. A., Bell, M. A., Baines, D. C., Hart, P. J. B. & Travis, M. P. (2007). Correlated evolution and dietary change in fossil stickleback. *Science*, 317(5846):1887–1887.

R Development Core Team (2016). R: a language and environment for statistical computing.

Rambaut, A. (2009). FigTree v1.3.1: Tree figure drawing tool. *http://tree. bio. ed. ac. uk/software/figtree.*

Rasband, W. S. (1997). ImageJ. *http://imagej.nih.gov/ij/.*

Reinthal, P. N. (1990). The feeding habits of a group of rock-dwelling cichlid fishes (Cichlidae: Perciformes) from Lake Malawi, Africa. *Environmental Biology of Fish*, 27:215–233.

Reinthal, P. N., Cohen, A. S. & Dettman, D. L. (2011). Fish fossils as paleo-indicators of ichthyofauna composition and climatic change in Lake Malawi, Africa. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 303(1-4):126–132.

Ribbink, A. J. (1990). Alternative life-history styles of some African cichlid fishes. *Environmental Biology of Fishes*, 28(1-4):87–100.

Ribbink, A. J., Marsh, B. A., Marsh, A. C., Ribbink, A. C. & Sharp, B. J. (1983). A preliminary survey of the cichlid fishes of rocky habitats in Lake Malawi. *South African Journal of Zoology*, 18(3):149–310.

Rognon, X. & Guyomard, R. (2003). Large extent of mitochondrial DNA transfer from *Oreochromis aureus* to *O. niloticus* in West Africa. *Molecular Ecology*, 12(2):435–445.

Rohlf, F. J. (2011). TpsRegr: Thin Plate Spline Regress (v. 1.40). *Stony Brook University, New York.*

Rohlf, F. J. (2013a). TpsDig2: Thin Plate Spline Digitise (v. 2.17). *Stony Brook University, New York.*

Rohlf, F. J. (2013b). TpsRelw: Thin Plate Spline Relative Warp (v.1.53). *Stony Brook University, New York.*

Rohlf, F. J. (2013c). TpsUtil: Thin Plate Spline Utility (v.1.58). *Stony Brook University, New York.*

Ronco, F. & Salzburger, W. (2016). Speciation: Genomic archipelagos in a crater lake. *Current Biology*, 26(5).

Rossiter, A. (1995). The cichlid fish assemblages of Lake Tanganyika: Ecology, behaviour and evolution of its species flocks. *Advances in Ecological Research*, 26:187–252.

Rüber, L. & Adams, D. C. (2001). Evolutionary convergence of body shape and trophic morphology in cichlids from Lake Tanganyika. *Journal of Evolutionary Biology*, 14(2):325–332.

Rüber, L., Verheyen, E. & Meyer, A. (1999). Replicated evolution of trophic specializations in an endemic cichlid fish lineage from Lake Tanganyika. *Proceedings of the National Academy of Sciences*, 96(18):10230–10235.

Ruwet, R.-C. (1963). *Tilapia melanopleura* (Dum.), poissons cichlides et la lutte contre la vegetation semi-aquatique au lac banage de la Lufira (Haut-Katanga). *Bulletin de la Société Royale des Sciences de Liège*, 32:516–528.

Salzburger, W. (2009). The interaction of sexually and naturally selected traits in the adaptive radiations of cichlid fishes. *Molecular Ecology*, 18(2):169–185.

Salzburger, W., Baric, S. & Sturmbauer, C. (2002). Speciation via introgressive hybridization in East African cichlids? *Molecular Ecology*, 11(3):619–625.

Salzburger, W., Bocxlaer, B. V. & Cohen, A. S. (2014). Ecology and evolution of the African Great Lakes and their faunas. *Annual Review of Ecology, Evolution and Systematics*, 45:519–545.

Salzburger, W., Mack, T., Verheyen, E. & Meyer, A. (2005). Out of Tanganyika: Genesis, explosive speciation, key-innovations and phylogeography of the haplochromine cichlid fishes. *BMC Evolutionary Biology*, 5(1):17.

Sanderson, S., Stebar, M., Ackermann, K., Jones, S., Batjakas, I. & Kaufman, L. (1996). Mucus entrapment of particles by a suspension-feeding tilapia (Pisces: Cichlidae). *The Journal of Experimental Biology*, 199:1743–56.

Sasagawa, I. (1997). Fine structure of the cap enameloid and of the dental epithelial cells during enameloid mineralisation and early maturation stages in the tilapia, a teleost. *Journal of Anatomy*, 190(4):589–600.

Sato, A., Takezaki, N., Tichy, H., Figueroa, F., Mayer, W. E. & Klein, J. (2003). Origin and speciation of haplochromine fishes in East African crater lakes investigated by the analysis of their mtDNA, Mhc genes, and SINEs. *Molecular Biology and Evolution*, 20(9):1448–1462.

Schaeffer, B. & Rosen, D. E. (1961). Major adaptive levels in the evolution of the actinopterygian feeding mechanism. *Integrative and Comparative Biology*, 1(2):187–204.

Schliewen, U., Tautz, D. & Pääbo, S. (1994). Sympatric speciation suggested by monophyly of crater lake cichlids. *Nature*, 368:629–632.

Schliewen, U. K. & Klee, B. (2004). Reticulate sympatric speciation in Cameroonian crater lake cichlids. *Frontiers in Zoology*, 1(1):5.

Schluter, D. (2000). *The ecology of adaptive radiation.* Oxford University Press, Oxford.

Schneider, R. F. & Meyer, A. (2017). How plasticity, genetic assimilation and cryptic genetic variation may contribute to adaptive radiations. *Molecular Ecology*, 26(1):330–350.

Scholz, C. A., Johnson, T. C., Cohen, A. S., King, J. W., Peck, J. A., Overpeck, J. T., Talbot, M. R., Brown, E. T., Kalindekafe, L., Amoako, P. Y. O., Lyons, R. P., Shanahan, T. M., Castaneda, I. S., Heil, C. W., Forman, S. L., McHargue, L. R., Beuning, K. R., Gomez, J. & Pierson, J. (2007). East African megadroughts between 135 and 75 thousand years ago and bearing on early-modern human origins. *Proceedings of the National Academy of Sciences*, 104(42):16416–16421.

Scholz, C. A. & Rosendahl, B. R. (1988). Low lake stands in lakes Malawi and Tanganyika, East Africa, delineated with multifold seismic data. *Science*, 240:1645–1648.

Seegers, L. (2008). The fishes collected by ga fischer in east africa in 1883 and 1885/86. *Zoosystematics and Evolution*, 84(2):149–195.

Seegers, L., De Vos, L. & Okeyo, D. O. (2003). Annotated Checklist of the Freshwater Fishes of Kenya (excluding the lacustrine haplochromines from Lake Victoria). *Journal of East African Natural History*, 92(1):11–47.

Seehausen, O. (2004). Hybridization and adaptive radiation. *Trends in Ecology and Evolution*, 19(4):198–207.

Seehausen, O. (2006). African cichlid fish: a model system in adaptive radiation research. *Proceedings. Biological sciences / The Royal Society*, 273(1597):1987–1998.

Sefc, K. M. (2011). Mating and parental care in Lake Tanganyika's Cichlids. *International Journal of Evolutionary Biology*, 2011:1–20.

Shapiro, M. D., Marks, M. E., Peichel, C. L., Blackman, B. K., Nereng, K. S., Jónsson, B., Schluter, D. & Kingsley, D. M. (2004). Genetic and developmental basis of evolutionary pelvic reduction in threespine sticklebacks. *Nature*, 428(6984):717–723.

Shechonge, A., Ngatunga, B. P., Bradbeer, S. J., Day, J. J., Freer, J. J., Ford, A. G., Kihedu, J., Richmond, T., Mzighani, S., Smith, A. M., Sweke, E. A., Tamatamah, R., Tyers, A. M., Turner, G. F. & Genner, M. J. (2018). Widespread colonisation of tanzanian catchments by introduced *Oreochromis* tilapia fishes: the legacy from decades of deliberate introduction. *Hydrobiologia*, p. Online first.

Sheets, H. D. (2008). IMP: integrated morphometrics package. *Department of Physics, Canisius College, Buffalo, NY.*

Singh, P., Börger, C., More, H. & Sturmbauer, C. (2017). The role of alternative splicing and differential gene expression in cichlid adaptive radiation. *Genome Biology and Evolution*, 9(10):2764–2781.

Sinninghe Damsté, J. S., Ossebaar, J., Schouten, S. & Verschuren, D. (2012). Distribution of tetraether lipids in the 25-ka sedimentary record of Lake Challa: Extracting reliable TEX_{86} and MBT/CBT palaeotemperatures from an equatorial African lake. *Quaternary Science Reviews*, 50:43–54.

Smith, L., Chakrabarty, P., Sparks, J. S. *et al.* (2008). Phylogeny, taxonomy, and evolution of neotropical cichlids (teleostei: Cichlidae: Cichlinae). *Cladistics*, 24(5):625–641.

Snoeks, J. (2000). How well known is the ichthyodiversity of the large East African lakes? *Advances in Ecological Research*, 31:17–38.

Solounias, N. & Moelleken, S. M. (1992). Dietary adaptations of two goat ancestors and evolutionary considerations. *Geobios*, 25(6):797–809.

Spataru, P. (1976). The feeding habits of *Tilapia galilaea* (Artedi) in Lake Kinneret (Israel). *Aquaculture*, 9:47–59.

Spataru, P. (1978a). Food and feeding habits of *Tilapia aurea* (Steindachner) (Cichlidae) in Lake Kinneret (Israel). *Aquaculture*, 13(1):67–79.

Spataru, P. (1978b). Food and feeding habits of *Tilapia zillii* (Gervais) (Cichlidae) in Lake Kinneret (Israel). *Aquaculture*, 14(4):327–338.

Stamatakis, A. (2014). RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, 30(9):1312–1313.

Stelkens, R. B. & Seehausen, O. (2009). Phenotypic divergence but not genetic distance predicts assortative mating among species of a cichlid fish radiation. *Journal of Evolutionary Biology*, 22(8):1679–1694.

Stelkens, R. B., Young, K. A. & Seehausen, O. (2010). The accumulation of reproductive incompatibilities in African cichlid fish. *Evolution*, 64(3):617–633.

Stiassny, M. L. J. & Meyer, A. (1999). Cichlids of the Rift Lakes. *Scientific American*, 280(2):64–69.

Strayer, D. L. (2010). Alien species in fresh waters: ecological effects, interactions with other stressors, and prospects for the future. *Freshwater Biology*, 55(s1):152–174.

Streelman, J. T., Albertson, R. C. & Kocher, T. D. (2007). Variation in body size and trophic morphology within and among genetically differentiated populations of the cichlid fish, *Metriaclima zebra*, from Lake Malawi. *Freshwater Biology*, 52(3):525–538.

Streelman, J. T., Webb, J. F., Albertson, R. C. & Kocher, T. D. (2003). The cusp of evolution and development: A model of cichlid tooth shape diversity. *Evolution and Development*, 5(6):600–608.

Sturmbauer, C. & Meyer, A. (1992). Genetic divergence, speciation and morphological stasis in a lineage of African cichlid fishes. *Nature*, 358(6387):578–581.

Taylor, W. & Van Dyke, G. C. (1985). Revised procedures for staining and clearing small fishes and other vertebrates for bone and cartilage study. *Cybium*, 9(2):107–119.

Tkint, T., Verheyen, E., de Kegel, B., Helsen, P. & Adriaens, D. (2012). Dealing with food and eggs in mouthbrooding cichlids: Structural and functional trade-offs in fitness related traits. *PLoS ONE*, 7(2):e31117.

Torres-Dowdall, J., Pierotti, M. E., Härer, A., Karagic, N., Woltering, J. M., Henning, F., Elmer, K. R. & Meyer, A. (2017). Rapid and parallel adaptive evolution of the visual system of neotropical Midas cichlid fishes. *Molecular Biology and Evolution*, 34(10):2469–2485.

Trapani, J. (2004). A morphometric analysis of polymorphism in the pharyngeal dentition of *Cichlasoma minckleyi* (Teleostei: Cichlidae). *Archives of Oral Biology*, 49(10):825–835.

Trewavas, E. (1983). *Tilapiine fishes of the genera Sarotherodon, Oreochromis and Danakilia*. British Museum (Natural History).

Tuisku, F. & Hildebrand, C. (1994). Evidence for a neural influence on tooth germ generation in a polyphyodont species. *Developmental biology*, 165:1–9.

Turner, G. (1986). Territory dynamics and cost of reproduction in a captive population of the colonial nesting mouthbrooder *Oreochromis mossambicus* (Peters). *Journal of Fish Biology*, 29(5):573–587.

Turner, G. F., Witimani, J., Robinson, R. L., Grimm, A. S. & Pitcher, T. J. (1991). Reproductive isolation and the nest sites of Lake Malawi chambo, *Oreochromis (Nyasalapia)* spp. *Journal of Fish Biology*, 39(6):775–782.

Twongo, T. (1995). Impact of fish species introductions on the tilapias of Lakes Victoria and Kyoga. In T. Pitcher & P. Hart, editors, *The Impact of Species Changes in African Lakes*, pp. 45–57. Chapman & Hall, London.

Ungar, P. (2017). *Evolution's bite: A story of teeth, diet and human origins.* Princeton University Press.

Utermöhl, H. (1958). *On the perfecting of quantitative phytoplankton methods.*

Utermöhl, v. H. (1931). Neue wege in der quantitativen erfassung des plankton.(mit besonderer berücksichtigung des ultraplanktons.). *Internationale Vereinigung für theoretische und angewandte Limnologie: Verhandlungen*, 5(2):567–596.

Vaasma, T. (2008). Grain-size analysis of lacustrine sediments: A comparison of pre-treatment methods. *Estonian Journal of Ecology*, 57(4):231–243.

Van Bocxlaer, B. & Hunt, G. (2013). Morphological stasis in an ongoing gastropod radiation from Lake Malawi. *Proceedings of the National Academy of Sciences*, 110(34):13892–13897.

Van Bocxlaer, B. & Schultheiß, R. (2010). Comparison of morphometric techniques for shapes with few homologous landmarks based on machine-learning approaches to biological discrimination. *Paleobiology*, 36(3):497–515.

Van Daele, M., Meyer, I., Moernaut, J., De Decker, S., Verschuren, D. & De Batist, M. (2017). A revised classification and terminology for stacked and amalgamated turbidites in environments dominated by (hemi)pelagic sedimentation. *Sedimentary Geology*, 357:72–82.

van Rijssel, J. C., Hoogwater, E. S., Kishe-Machumu, M. a., Reenen, E. V., Spits, K. V., van der Stelt, R. C., Wanink, J. H. & Witte, F. (2015). Fast adaptive responses in the oral jaw of Lake Victoria cichlids. *Evolution*, 69(1):179–189.

Van Steenberge, M. (2014). *Species and speciation in Tropheus, Simochromis and Pseudosimochromis: A multidisciplinary approach on a cichlid radiation from Lake Tanganyika.* Ph.D. thesis, KU Leuven/Royal Museum for Central Africa.

Venables, W. N. & Ripley, B. D. (2002). *MASS: modern applied statistics with S.* March.

Verheyen, E., Ruber, L., Snoeks, J. & Meyer, A. (1996). Mitochondrial phylogeography of rock-dwelling cichlid fishes reveals evolutionary influence of historical lake level fluctuations of Lake Tanganyika, Africa. *Philosophical Transactions of the Royal Society London B, Biological Sciences*, 351(1341):797–805.

Verheyen, E., Salzburger, W., Snoeks, J. & Meyer, A. (2003). Origin of the superflock of cichlid fishes from Lake Victoria, East Africa. *Science*, 300:325–328.

Verschuren, D., Olago, D., Rucina, S. & Odhengo, P. (2013). DeepCHALLA: Two Glacial Cycles of Climate and Ecosystem Dynamics from Equatorial East Africa. *Scientific drilling*, 15(15):72–76.

Verschuren, D., Sinninghe Damsté, J. S., Moernaut, J., Kristen, I., Blaauw, M., Fagot, M. & Haug, G. H. (2009). Half-precessional dynamics of monsoon rainfall near the East African Equator. *Nature*, 462(7273):637–641.

Verschuren, D., Van Daele, M. E., Wolff, C., Waldmann, N., Meyer, I., Lane, C. S., Van der Meeren, T., Ombori, T., Kasanzu, C. & Olago, D. (2017). Icdp project deepchalla: Reconstructing ∼250,000 years of climate change and environmental history on the east african equator. In *American Geophysical Union Fall Meeting*.

Verzijden, M. N., Van Heusden, J., Bouton, N., Witte, F., Ten Cate, C. & Slabbekoorn, H. (2010). Sounds of male Lake Victoria cichlids vary within and between species and affect female mate preferences. *Behavioral Ecology*, 21(3):548–555.

Vitousek, P. M., Mooney, H. A., Lubchenco, J. & Melillo, J. M. (1997). Human Domination of Earth's Ecosystems. *Science*, 277(5325):494–499.

Wagner, C. E., Harmon, L. J. & Seehausen, O. (2012). Ecological opportunity and sexual selection together predict adaptive radiation. *Nature*, 487(7407):366–369.

Wainwright, P. (1989). Functional morphology of the pharyngeal jaw apparatus in Perciform fishes: An experimental analysis of the Haemulidae. *Journal of Morphology*, 200:200–231.

Wautier, K., Huysseune, a. & Verheyen, E. (2002). Tooth shape differences analyzed by biometric and morphometric approaches: a case study on two morphologically very similar lacustrine cichlid species. *Connective Tissue Research*, 43(2-3):103–108.

West-Eberhard, M. J. (2003). *Developmental plasticity and evolution*. Oxford University Press.

Whitenack, L. B. & Gottfried, M. D. (2010). A morphometric approach for addressing tooth-based species delimitation in fossil mako sharks, *Isurus* (Elasmobranchii: Lamniformes). *Journal of Vertebrate Paleontology*, 30(1):17–25.

Whyte, S. (1975). Distribution, trophic relationships and breeding habits of the fish populations in a tropical lake basin (Lake Bosumtwi, Ghana). *Journal of Zoology*, 177:25–56.

Wilke, T., Wagner, B., Van Bocxlaer, B., Albrecht, C., Ariztegui, D., Delicado, D., Francke, A., Harzhauser, M., Hauffe, T., Holtvoeth, J., Just, J., Leng, M. J., Levkov, Z., Penkman, K., Sadori, L., Skinner, A., Stelbrink, B., Vogel, H., Wesselingh, F. & Wonik, T. (2016). Scientific drilling projects in ancient lakes: Integrating geological and biological histories. *Global and Planetary Change*, 143:118–151.

Williams, E. E. (1972). The origin of faunas. evolution of lizard congeners in a complex island fauna: A trial analysis. In *Evolutionary Biology*, pp. 47–89. Springer.

Winemiller, K. O., Kelso-Winemiller, L. C. & Brenkert, A. L. (1995). Ecomorphological diversification and convergence in fluvial cichlid fishes. *Environmental Biology of Fishes*, 44(1-3):235–261.

Wirtz, P. (1999). Mother species-father species: Unidirectional hybridization in animals with female choice. *Animal Behaviour*, 58(1):1–12.

Witte, F., Goldschmidt, T., Wanink, J., van Oijen, M., Goudswaard, K., Witte-Maas, E. & Bouton, N. (1992). The destruction of an endemic species flock: quantitative data on the decline of the haplochromine cichlids of Lake Victoria. *Environmental Biology of Fishes*, 34(1):1–28.

Witte, F. & Van Oijen, M. J. P. (1990). Taxonomy, ecology and fishery of Lake Victoria haplochromine trophic groups. *Zoologische Verhandelingen*, 262(1):1–47.

Witte, F., Welten, M., Heemskerk, M., Van Der Stap, I., Ham, L., Rutjes, H. & Wanink, J. (2008). Major morphological changes in a Lake Victoria cichlid fish within two decades. *Biological Journal of the Linnean Society*, 94(1):41–52.

Wolff, C., Haug, G. H., Timmermann, A., Damste, J. S. S., Brauer, A., Sigman, D. M., Cane, M. A. & Verschuren, D. (2011). Reduced interannual rainfall variability in East Africa during the last ice age. *Science*, 333(6043):743–747.

Wolff, C., Kristen-Jenny, I., Schettler, G., Plessen, B., Meyer, H., Dulski, P., Naumann, R., Brauer, A., Verschuren, D. & Haug, G. H. (2014). Modern seasonality in Lake Challa (Kenya/Tanzania) and its sedimentary documentation in recent lake sediments. *Limnology and Oceanography*, 59(5):1621–1636.

Yamaoka, K. (1983). Feeding behaviour and dental morphology of algae scraping cichlids (Pisces: Teleostei) in Lake Tanganyika. *African Study Monographs*, 4:77–89.

Zambrano, L., Valiente, E. & vander Zanden, M. J. (2010). Food web overlap among native axolotl (*Ambystoma mexicanum*) and two exotic fishes: Carp (*Cyprinus carpio*) and tilapia (*Oreochromis niloticus*) in Xochimilco, Mexico City. *Biological Invasions*, 12(9):3061–3069.

Zelditch, M. L., Swiderski, D. L., Sheets, H. D. & Fink, W. L. (2004). *Geometric morphometrics for biologists.* 3. Academic Press.

Zengeya, T. A., Booth, A. J., Bastos, A. D. S. & Chimimba, C. T. (2011). Trophic interrelationships between the exotic Nile tilapia, *Oreochromis niloticus* and indigenous tilapiine cichlids in a subtropical African river system (Limpopo River, South Africa). *Environmental Biology of Fishes*, 92(4):479–489.

Zuckerkandl, E. & Pauling, L. (1962). *Molecular Disease, Evolution, and Genetic Heterogeneity.* Academic Press.

A

Supplementary information for Chapter 2

Table A.1: Number of oral teeth included in geometric morphometric analyses. OH: *Oreochromis hunteri*; OK: *Oreochromis* cf. *korogwe*; DTL1/PML1: outer series; DTL2/PML2: outer row of inner series; DTLA/PMLA: anterior group of the inner series; DTLM/PMLM: middle group of the inner series; DTLP/PMLP: posterior group of the inner series.

	left dentary					left premaxilla					**total**
	DTL1	DTL2	DTLA	DTLM	DTLP	PML1	PML2	PMLA	PMLM	PMLP	
OH	16	11	33	13	10	34	36	23	18	14	**208**
OK	19	17	34	14	15	29	20	36	9	16	**209**

Figure A.1: Overview pictures of *in toto* cleared-and-stained jaws of *Oreochromis hunteri*. **a** Ventral and **b** labial view of the right premaxilla, and **c** dorsal and **d** labial view of the right dentary.

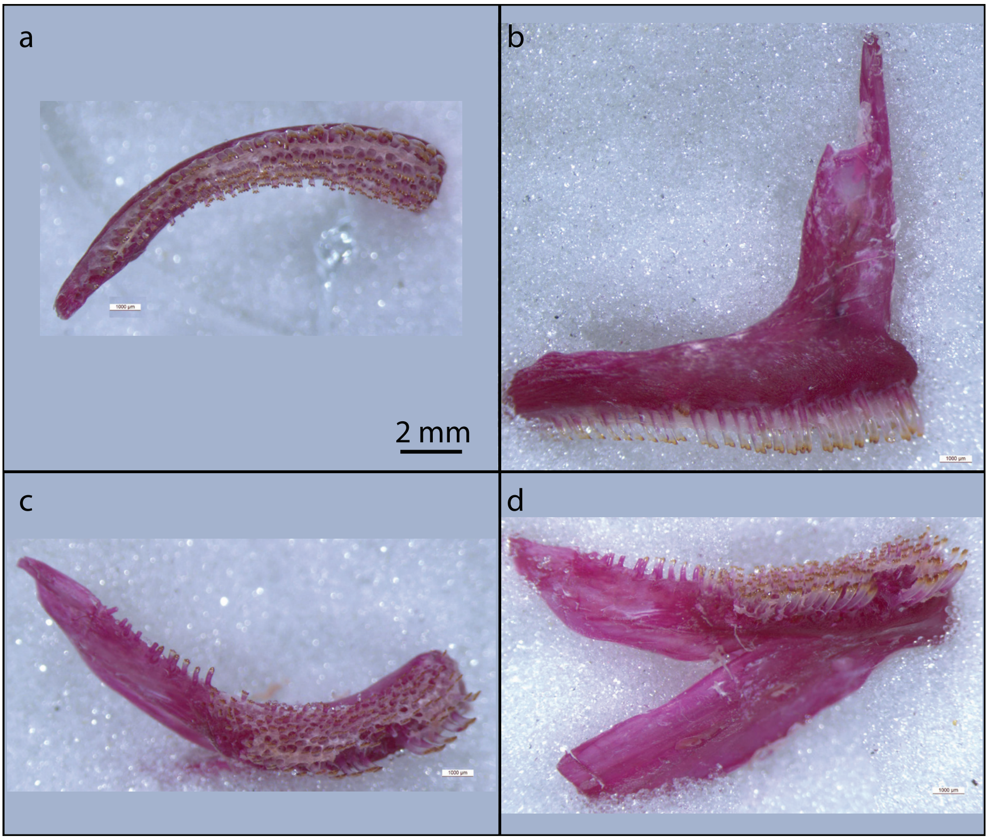


Figure A.2: Overview pictures of *in toto* cleared-and-stained jaws of *Oreochromis* cf. *korogwe*. **a** Ventral and **b** labial view of the right premaxilla, and **c** dorsal and **d** labial view of the right dentary.

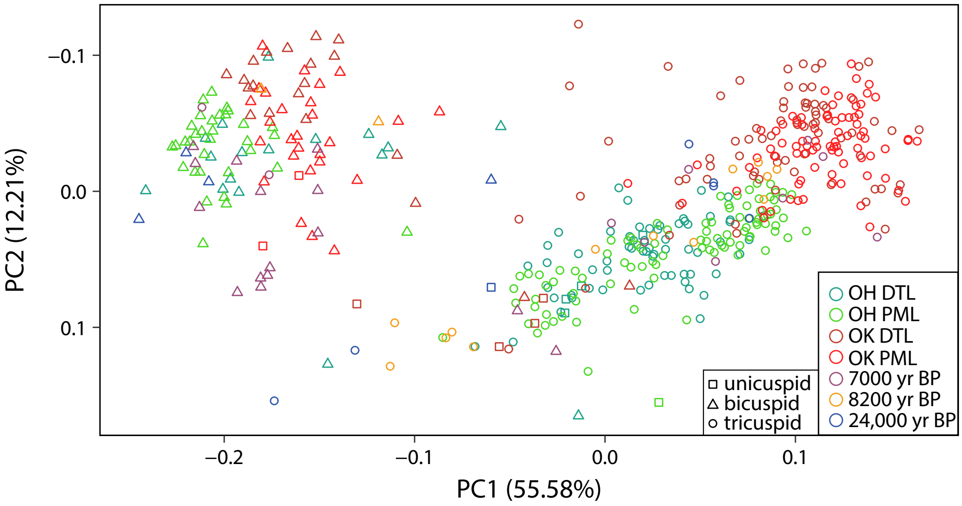


Figure A.3: PCA ordination of SLM-coordinates documenting crown morphology of all modern-day teeth, and fossil teeth of the three age intervals (7,000, 8,200 and 24,000 years ago) analysed. Symbols represent the three different tooth types present in both *Oreochromis* species: unicuspid, bicuspid and tricuspid teeth.

B

Supplementary information for Chapter 3

Table B.1: List of specimens.

species	local name	n	n (genotype)	specimen ID	GenBank accession n° CR	GenBank accession n° ND2	locality
Oreochromis hunteri	Chala	81	15	CHALA_cha002	MG922031	MG922069	Lake Chala
				CHALA_cha003	MG922032	MG922070	Lake Chala
				CHALA_cha017	MG922035	MG922073	Lake Chala
				CHALA_cha018	MG922036	MG922074	Lake Chala
				CHALA_cha019	MG922037	MG922075	Lake Chala
				CHALA_cha020	MG922038	MG922076	Lake Chala
				CHALA_cha021	MG922039	MG922077	Lake Chala
				CHALA_cha023	MG922041	MG922079	Lake Chala
				CHALA_cha025	MG922042	MG922080	Lake Chala
				CHALA_cha026	MG922043	MG922081	Lake Chala
				CHALA_cha027	MG922044	MG922082	Lake Chala
				CHALA_cha028	MG922045	MG922083	Lake Chala
				CHALA_cha029	MG922046	MG922084	Lake Chala
				CHALA_cha030	MG922047	MG922085	Lake Chala
				CHALA_cha043	MG922050	MG922088	Lake Chala
Oreochromis cf. korogwe	Bandia	23	9	BANDIA_cha004	MG922033	MG922071	Lake Chala
				BANDIA_cha005	MG922034	MG922072	Lake Chala
				BANDIA_cha022	MG922040	MG922078	Lake Chala
				BANDIA_cha031	MG922048	MG922086	Lake Chala
				BANDIA_cha039	MG922049	MG922087	Lake Chala
				BANDIA_cha053	MG922051	MG922089	Lake Chala
				BANDIA_cha054	MG922052	MG922090	Lake Chala
				BANDIA_cha069	MG922053	MG922091	Lake Chala
				BANDIA_cha070	MG922054	MG922092	Lake Chala
Oreochromis jipe	Asilia	18	8	ASILIA_jip003	MG922055	MG922093	Lake Jipe
				ASILIA_jip004	MG922056	MG922094	Lake Jipe
				ASILIA_nym007	MG922063	MG922101	Nyumba ya Mungu
				ASILIA_nym008	MG922064	MG922102	Nyumba ya Mungu
				ASILIA_nym009	MG922065	MG922103	Nyumba ya Mungu
				ASILIA_nym010	MG922066	MG922104	Nyumba ya Mungu
				ASILIA_nym011	MG922067	MG922105	Nyumba ya Mungu
				ASILIA_nym012	MG922068	MG922106	Nyumba ya Mungu
Oreochromis esculentus	Polana	6	6	POLANA_jip005	MG922057	MG922095	Lake Jipe
				POLANA_jip006	MG922058	MG922096	Lake Jipe
				POLANA_nym003	MG922059	MG922097	Nyumba ya Mungu
				POLANA_nym004	MG922060	MG922098	Nyumba ya Mungu
				POLANA_nym005	MG922061	MG922099	Nyumba ya Mungu
				POLANA_nym006	MG922062	MG922100	Nyumba ya Mungu

Table B.2: Genbank accession numbers for Control Region (CR) sequences.

species or hybrid	Genbank accession n°
Alcolapia alcalica	AF296455
Alcolapia grahami	KC572512
Alcolapia latilabris	AF296462
Alcolapia ndalalani	AJ237515
Oreochromis amphimelas	AJ237395
Oreochromis amphimelas	AF296490
Oreochromis amphimelas	AF296489
Oreochromis andersonii	JQ907508
Oreochromis andersonii	AF296488
Oreochromis andersonii	AF296487
Oreochromis esculentus	KM654981
Oreochromis esculentus	AJ237399
Oreochromis esculentus	AF296483
Oreochromis esculentus	HQ153030
Oreochromis jipe	AF296492
Oreochromis karongae	AF328844
Oreochromis malagarasi	AF015006
Oreochromis malagarasi	AF296463
Oreochromis malagarasi	AJ237401
Oreochromis mossambicus	AY833447
Oreochromis mossambicus	AF328843
Oreochromis mossambicus	JQ907497
Oreochromis mossambicus	EU430997
Oreochromis mossambicus	AF296466
Oreochromis mossambicus x *niloticus*	KU180640
Oreochromis niloticus	KU180645
Oreochromis niloticus	HQ153022
Oreochromis niloticus	FJ664216
Oreochromis niloticus	AF296474
Oreochromis niloticus	EU430996
Oreochromis niloticus	AF328847
Oreochromis niloticus	AF296469
Oreochromis niloticus	AF328846
Oreochromis niloticus baringoensis	AJ237397
Oreochromis niloticus vulcani	AF296468
Oreochromis niloticus vulcani	AF296472
Oreochromis niloticus x *Oreochromis aureus*	FJ664241
Oreochromis sp. HT-1639	AF296486
Oreochromis sp. HT-1656	AF296478
Oreochromis sp. HT-8672	AF296470
Oreochromis sp. HT-9235	AF296491
Oreochromis sp. HT-9351	AF296464
Oreochromis sp. HT-9352	AF296465
Oreochromis sp. HT-9360	AF296479
Oreochromis sp. KM-2006	AP009126
Oreochromis sp. YCC-2008	EU375454
Oreochromis sp. YCC-2008	EU375453
Oreochromis spilurus	EU431000
Oreochromis tanganicae	AY929940
Oreochromis urolepis	AF296467
Oreochromis urolepis hornorum	EU308500
Sarotherodon melanotheron	NC015611

Table B.3: Genbank accession numbers for ND2 sequences.

species or hybrid	Genbank accession n°
Alcolapia alcalica	GQ167781
Alcolapia grahami	KC572546
Oreochromis amphimelas	AF317230
Oreochromis andersonii	AF317231
Oreochromis andersonii	GQ167805
Oreochromis chungruruensis	KF772215
Oreochromis esculentus	KM654981
Oreochromis esculentus	AF317232
Oreochromis karongae	DQ465030
Oreochromis leucostictus	AF317233
Oreochromis leucostictus	KF772225
Oreochromis macrochir	AF317235
Oreochromis mossambicus	DQ465032
Oreochromis mossambicus	AF317234
Oreochromis mweruensis	AF317236
Oreochromis niloticus	AF317237
Oreochromis niloticus	AB018974
Oreochromis niloticus	U07258
Oreochromis niloticus	KF772217
Oreochromis niloticus	GQ167780
Oreochromis niloticus vulcani	AF317242
Oreochromis schwebischi	AF317238
Oreochromis shiranus	KF772216
Oreochromis sp. KM-2006	AP009126
Oreochromis squamipinnis	KF772214
Oreochromis tanganicae	AF317240
Oreochromis tanganicae	KM288941
Oreochromis tanganicae	KM288943
Oreochromis tanganicae	GQ167782
Oreochromis urolepis	AF317239
Oreochromis variabilis	AF317241
Oreochromis variabilis	KM658973
Sarotherodon melanotheron	NC015611

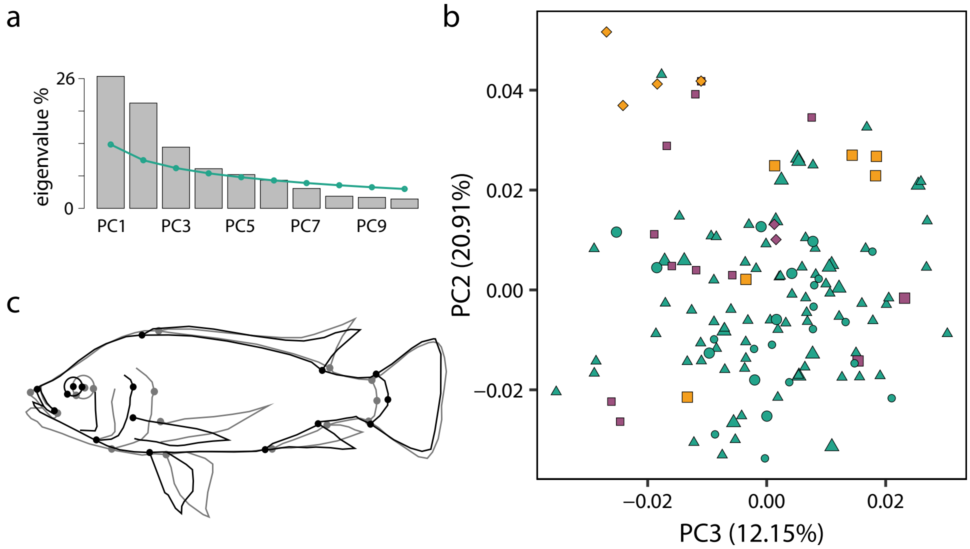


Figure B.1: **a** Scree plot (grey bars) and broken-stick model (green line) for the first 10 principal components (PCs) capturing body morphology. **b** PCA ordination plot synthesising variation in body morphology as captured by PC2 and PC3, among the four *Oreochromis* species currently inhabiting the Upper Pangani River basin. Green, purple and yellow symbols represent specimens from respectively Lake Chala, Lake Jipe and NyM reservoir, with large symbols of each type and colour indicating sequenced specimens. **c** The outline drawings represent the specimens at the positive (black) and negative (grey) extremes of PC3.

C

Supplementary information for Chapter 4

C.1 Supplementary analyses

We assessed whether gut content composition of each of the three species is influenced by body size. Correlations were tested between NMDS-scores and standard length (SL; distance between the tip of the snout and the posterior end of the last vertebra) for each species separately, using non-parametric Spearman-rank tests. Similarly, we tested for correlations between stable-isotopic values (δ^{15}N and δ^{13}C) and SL. None of these correlations yielded a significant result, and we conclude that body size does not influence diet.

Table C.1: Numerical abundance (%N) per month for each food item in the guts of *Oreochromis hunteri*, *O.* cf. *korogwe*, and *Coptodon rendalli* combined. Bold values represent the relative abundance of phyla but take into account detritus and sand. Normal fonts are used for relative abundances of algal genera and orders, excluding detritus and sand.

	Feb '14 n = 2	Mar '14 n = 3	Apr '14 n = 1	May '14 n = 2	Jul '14 n = 2	Aug '14 n = 2	Sep '14 n = 3	Dec '14 n = 3	Jan '15 n = 2	Feb '15 n = 2	Apr '15 n = 2	Jun '15 n = 2	Sep '15 n = 9
Cyanobacteria	**58.1**	**16.2**	**47.3**	**2**	**4.8**	**4.3**	**34.2**	**3.9**	**17.7**	**10**	**20.7**	**17.5**	**73.2**
Chroococcales	87.2	24.1		4.2	5.5	4.8	38.1	6.3	34	18.4	31.1	24.3	38.1
Oscillatoriales			64.9							0.8			40.1
Chlorophyta	**5.8**	**6.3**	**0.5**	**9.6**	**52**	**33.4**	**32.6**	**16.1**	**15.4**	**31.8**	**31.3**	**22.2**	**17.8**
Lagerheimia		0.5		7.3	4.1	0.3		0.3	1.5	2.9	2.1	1.1	1.6
Oocystis	7.6	2.8		1.6	0.9	1.1		0.8	7.5	7.1	2.1	0.4	5.9
Tetraedron	0.6	5.1		9.9	49.6	30.7	34.3	14.9	18	47.7	37.4	27.6	8.7
Treubaria				0.5	0.6	0.3							0.2
other Chlorococcales	0.6	1	0.7	1	4.6	4.2	2	9.9	2.5	3.3	5.5	1.8	1.6
Desmidiales						0.5	0.2	0.3		0.4			1
Dinophyta			**1**	**0.7**			**0.1**	**0.5**	**0.8**	**0.4**	**0.6**	**0.8**	**0.1**
Peridiniales			1.4	1.6			0.2	0.8	1.5	0.8	0.8	1.1	0.1
Bacillariophyta	**2.7**	**44.7**	**23.2**	**34.6**	**30.1**	**52.5**	**22.8**	**41.1**	**18.2**	**9.5**	**13.7**	**31.5**	**2.4**
Achnanthidium				1									
Afrocymbella		0.8	0.7	7.3	0.9	0.8	11.5	1.8	3.5	2.1	2.1	2.2	0.4
Amphora		1.5		2.1		0.3	0.2	1.6		0.4			0.1
Cocconeis		0.3										0.4	
Cymatopleura								0.3	0.5	0.4			
Diploneis				1.6	0.6							0.7	
Encyonema													0.1
Encyonopsis				1.6	1.4							1.5	0.3
Navicula		1.3										0.4	
Nitzschia	1.2	44.2	16.9	40.6	18.6	27.5	12.2	45.7	20.5	9.2	9.7	24.3	1.3
Placoneis				1									
Rhopalodia		0.8		4.7			0.3	0.3	1	0.4			0.3
Sellaphora				0.5		0.3						1.1	0
Staurosirella	2.3	17.8	14.2	13	0.9	1.3		16.7	9.5	5	7.6	12.9	0.1
Surirella										0.4			
Fragilaria s.l.	0.6				12.2	28	1.2	0.3		0.4	1.3	0.4	0.1
Euglenophyta			**1**	**0.2**	**0.3**			**0.2**			**0.3**		**0.2**
Euglenales			1.4	0.5	0.3			0.3			0.4		0.2
detritus	**25.2**	**17.2**	**23.2**	**29.2**	**9.3**	**3.6**	**3.4**	**25.4**	**22.4**	**33.3**	**16.2**	**14.3**	**4.1**
sand grains	**8.1**	**15.5**	**3.9**	**23.6**	**3.5**	**6.2**	**6.9**	**12.9**	**25.5**	**14.9**	**17.3**	**13.8**	**2.4**

Table C.2: Numerical abundance (%N) for each algal individual in the combined limnetic samples, collected per month. Bold values represent the relative abundance of phyla, whereas normal fonts are used for relative abundances of algal genera and orders. Supplement to Chapter 4.

	Feb ’14 n = 2	**Mar ’14** n = 3	**Apr ’14** n = 3	**May’14** n = 3	**Jul ’14** n = 3	**Aug’14** n = 2	**Sep ’14** n = 3	**Dec ’14** n = 3	**Jan ’15** n = 3	**Apr ’15** n = 1	**Jun ’15** n = 1	**Sep ’15** n = 1
Cyanobacteria	**59.6**	**14.8**	**26.3**	**16.7**	**10**	**34.6**	**6.7**	**34.1**	**52**	**21.4**	**49.9**	**12.7**
Chroococcales	5.8	5.5	17.8	3.7	1.9	24.4	3.8	2.3	16.6	1.2	11.1	11.9
Nostocales	1.8	1.4	1	0.1	0.3	7.9	0.5	29	29.8	12.9	26.4	0
Oscillatoriales	52	7.8	7.6	12.9	7.8	2.3	2.4	2.8	5.6	7.2	12.4	0.8
Chlorophyta	**28.3**	**66.5**	**58.6**	**70**	**86.1**	**61.4**	**71.3**	**60.1**	**44.3**	**63.6**	**44.9**	**85.5**
Lagerheimia	0.1	0.7	6.3	9.9	2	0.6	0	10.8	2.8	11.1	10.8	12.4
Oocystis	13.2	26.9	16.5	28.7	1	0.4	0.3	15.9	10.3	19.6	6.2	19.8
Tetraedron	13	20.4	4.3	5.8	42.1	42.7	56.5	6.4	2.9	8	6.5	26.6
Treubaria		0.6	1.8	1.8	21.7			0.1	0.2	2	0.8	1.9
other Chlorococcales	1.9	17.3	28.6	19.3	18.8	17.6	14.5	26.7	27.8	21.1	18.3	21.4
Desmidiales		0.6	1	4.6	0.5	0.1		0.2	0.3	2	2.3	3.3
Zygnematales			0.1									
Dinophyta	**1.1**	**12.9**	**11.1**	**7.2**	**0.2**	**0**	**0.1**	**4.4**	**1.9**	**12.8**	**1**	**0.3**
Peridiniales	1.1	12.9	11.1	7.2	0.2		0.1	4.4	1.9	12.8	1	0.3
Bacillariophyta	**10.6**	**2.1**	**2.2**	**0.9**	**3.3**	**3.9**	**21.5**	**0.2**	**0**	**0.2**	**0.3**	**0.4**
Aulacoseirales		0.3	1	0.1								
Thalasiosirales				0.2								
Achnanthidium					0.3							
Amphora			0.1		0.1							
Cymbopleura			0.1									
Encyonema		0.1										0.1
Afrocymbella		0.1				0.2	8.2					0.3
Navicula	2.2		0.2	0.1		0.3	3.6					
Nitzschia	8.4	1.1	0.6		0.1		7.5			0.2	0.2	
Rhopalodia								0.1				
Staurosirella		0.2		0.3	1.3	1.3	0.5					
Fragilaria s.l.		0.2	0.1	0.1	1.7	2	1.7	0.1			0.1	
Chrysophyta	**0.3**	**1.3**	**1.3**	**2.9**	**0.3**	**0.1**	**0.1**	**1**	**1.4**	**2.1**	**3.8**	**0.4**
Euglenophyta	**0**	**2.5**	**0.5**	**2.4**	**0.1**	**0.1**	**0.2**	**0.1**	**0.3**			**0.8**
Euglenales		2.5	0.5	2.4	0.1	0.1	0.2	0.1	0.3			0.8

Table C.3: Numerical abundance (%N) for each item in the combined littoral samples, collected in September 2015. Bold values represent the relative abundance of phyla but take into account detritus and sand. Normal fonts are used for relative abundances of algal genera and orders, excluding detritus and sand. Supplement to Chapter 4.

	rock n = 12	**sand n = 2**
Cyanobacteria	**12.9**	**5.8**
Chroococcales	19.4	11.8
Nostocales	0.1	
Oscillatoriales	2.7	
Chlorophyta	**20.3**	**14.9**
Lagerheimia	5.1	
Oocystis	6	3.3
Tetraedron	8.3	20.9
Treubaria	0.5	0.5
other Chlorococcales	13.3	4.7
Desmidiales	1.8	0.9
Dinophyta	**0.3**	**0**
Peridiniales	0.6	0
Bacillariophyta	**24.5**	**28.4**
Achnanthidium	0.5	0
Afrocymbella	12.7	5.2
Amphora	0.3	3.3
Cymatopleura		0.5
Diploneis		0.5
Encyonopsis	1	
Geissleria		1.4
Gomphonema	0.4	
Navicula	3.9	3.3
Nitzschia	15.3	29.9
Placoneis	0.1	
Rhopalodia	4.7	10
Staurosirella	0.2	0.5
Fragilaria s.l.	3.1	3.3
Euglenophyta	**0.1**	
Euglenales	0.1	
detritus	**32.1**	**41.5**
sand grains	**9.7**	**9.3**

D

Supplementary information for Chapter 6

D.1 Supplementary analyses

Classification of oral teeth – We examined the robustness of the angle-based classification method by comparing it to a classification using model-based clustering applying Gaussian mixture models. We used the package Mclust v.5.3 (Fraley *et al.*, 2012) to fit mixture models with different underlying assumptions as to the variance-covariance of the data, and we evaluated different clustering solutions based on a Bayesian information criterion (BIC). This method had a tendency to overfit the data with multiple clusters that represent biologically unrealistic scenarios, however, if constrained to fewer clusters (i.e. <5) a three-cluster model was preferred and the best-fitting three-cluster model was adopted as a grouping variable.

Results of the two tooth-type classification methods are shown in Figure D.1a-c (based on intercusp angle) and D.1d-f (based on Mclust modelling). Teeth assigned to each of the three types occupy highly comparable distributions in NMDS shape space with either method, although type assignments show slightly greater overlap when based on the intercusp angle (Fig. D.1a & D.1d). However, the way in which the three tooth types are distributed in the jaw differs between the two methods (Fig. D.1b & D.1e). Especially many second-row (SR) tricuspid teeth are classified as unicuspid by the Mclust method. We described these particular tricuspids in Chapter 2 as possessing a dominant middle cusp, causing their general shape to resemble a unicuspid tooth despite having two clear incisions in the crown's outline. Both methods capture a clear increasing trend of unicuspid-like teeth in larger specimens (Fig. D.1c & D.1f). However, tooth type classification based on angles reflects biologically relevant information better, and this method was therefore retained.

As in the modern-day collection, either classification method produces highly comparable distributions in NMDS shape space when fossil teeth are assigned to each of the three main types of oral teeth (Fig. D.2a & D.2c). Moreover, both classification methods capture the increase in the proportion of unicuspid-like teeth during lowstand episodes (Fig. D.2b & D.2d).

Table D.1: Overview of fossil oral teeth retrieved from six time windows, representing alternating high- and low lake-level stands

Assemblage	Corrected composite depth (cm)		Approximate age range (yrs BP)	Reconstructed lake level	Wet weight (g)	# oral teeth retrieved	# oral teeth in morphometric dataset
	Top	Bottom					
I	66.5	123	500 - 1000	High	645	148	104
II	239	275	2500 - 3000	High	383	199	162
III	600.5	640	7500 - 8000	Low	491	544	463
IV	787	830.5	10,500 - 11,000	High	399	86	79
V	1328.5	1501	17,500 - 19,000	Low	1890	77	63
VI	1861.5	1959	22,000 - 23,500	High	1224	26	15
Total					**5002**	**1080**	**886**

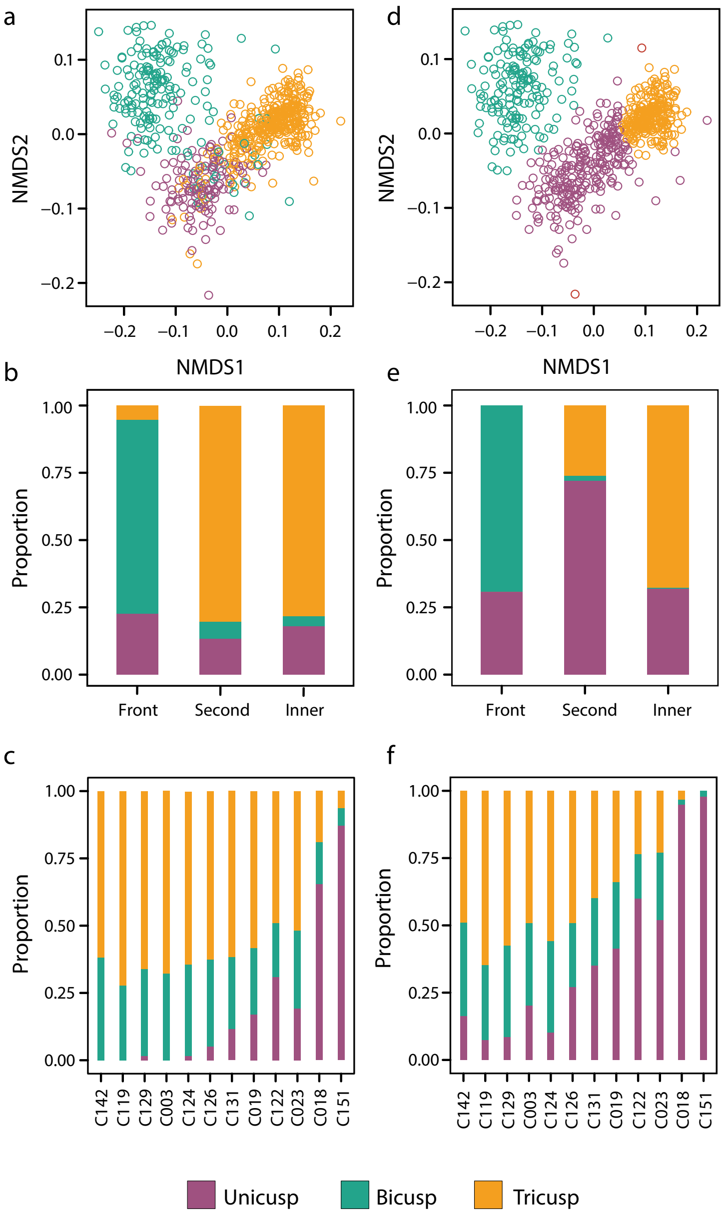


Figure D.1: Results of different tooth-type classification methods in modern-day teeth, with colour codes reflecting unicuspids (purple), bicuspids (green) and tricuspids (yellow). NMDS shape space of tooth types assigned using the intercusp angle (**a**), and distribution of tooth types in the jaw (**b**). Proportions of 'traditional' tooth types in each specimen, ordered by increasing body size (**c**). Tooth type occupation of NMDS shape space based on modelled clustering (**d**), and their location in the jaw (**e**). Model-based tooth types per individual (**f**).

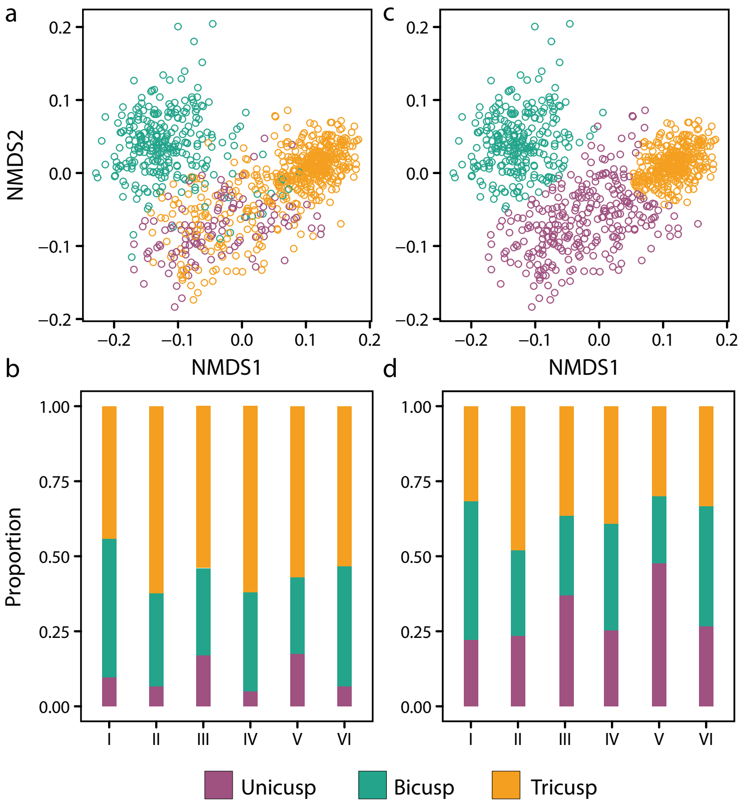


Figure D.2: Results of different tooth type classification methods in fossil teeth, with colour codes reflecting unicuspids (purple), bicuspids (green) and tricuspids (yellow). NMDS shape space of tooth types assigned using the intercusp angle (**a**), and their relative proportions in the six sampled time intervals (**b**). Tooth type occupation of NMDS shape space based on modelled clustering (**c**), and their relative proportions in the six sampled time intervals (**d**).

Figure D.3: Overview pictures of *in toto* cleared-and-stained jaws of a small specimen (SL = 18 cm) of *Oreochromis hunteri*. **a** Ventral and **b** labial view of the right premaxilla, and **c** dorsal and **d** labial view of the right dentary.

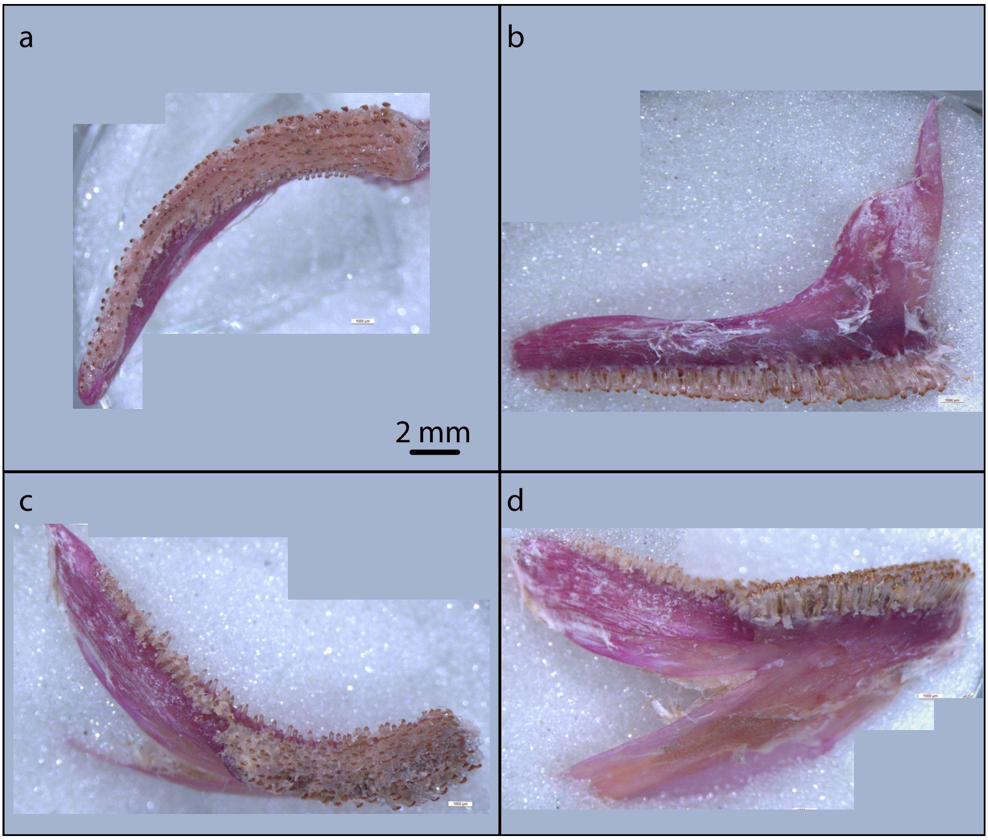


Figure D.4: Overview pictures of *in toto* cleared-and-stained jaws of a medium-sized specimen (SL = 25 cm) of *Oreochromis hunteri*. **a** Ventral and **b** labial view of the right premaxilla, and **c** dorsal and **d** labial view of the right dentary.

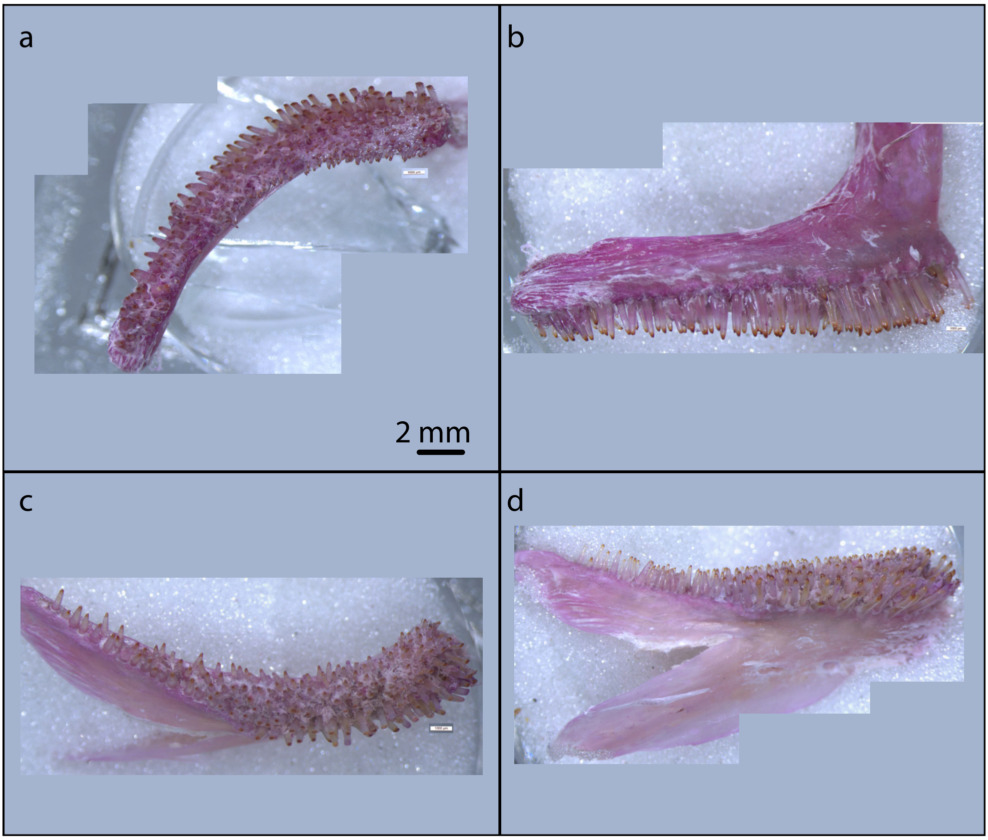


Figure D.5: Overview pictures of *in toto* cleared-and-stained jaws of a large specimen (SL = 30 cm) of *Oreochromis hunteri*. **a** Ventral and **b** labial view of the right premaxilla, and **c** dorsal and **d** labial view of the right dentary. Note the entirely unicuspid dentition of this specimen.