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DISSERTATION

Titel der Dissertation

„PARASITES OF FISH AND TRACE ELEMENTS IN LAKES NAIVASHA AND TURKANA, KENYA“

Verfasser

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Abstract

Fish is an important protein source for many people living in the basins of Lakes Naivasha and Turkana and indeed for other people in Kenya. The fish in Lake Naivasha were all introduced while those of Lake Turkana are native. The dual aims of the study were to determine the occurrence of parasites of commercially important fish from the two lakes; and, to analyze trace elements concentrations in various lake matrices namely: water, sediments, fish organs and in selected fish parasites to establish any health risks for the fish consumers (humans and animals) and to evaluate bio-indicative potentials of some of the occurring parasite species. A total of 373 fish comprising of the common carp *Cyprinus carpio* (145), the blue spotted tilapia *Oreochromis leucostictus* (56), the red belly tilapia *Tilapia zillii* (18), the straightfin barb *Barbus paludinosus* *(*67) all from Lake Naivasha, and; the elongate tigerfish *Hydrocynus forskahlii* (43) and *T. zillii* (44) from Lake Turkana, were collected and examined for parasites during the periods February to September 2011 and in September 2012. From Lake Naivasha, 10 taxa of parasites were recovered from *C. carpio* dominated by the monogenean *Dactylogyrus minutus*, occurring with a prevalence (p) of 99.3%. 13 taxa of parasites were identified from *O. leucostictus* dominated by monogeneans *Cichlidogyrus* spp. (p = 91.1%). *Tilapia zillii* harbored 9 taxa of parasites with the digenean *Tylodelphys* sp. (p = 83.3%) being dominant and the *B. paludinosus* harbored 11 taxa of parasites dominated by an unidentified monogenean of the genus *Dactylogyrus* (p = 83.6%). *Cyprinus carpio* had the lowest helminth species diversity and richness while ectoparasites have been discovered in all the studied fish. These results have been summarized in a paper titled ``**Parasites of commercially important fish from Lake Naivasha, Rift Valley, Kenya**`` submitted to the Journal of Parasitology Research (Springer Verlag) on 28th of January 2013. In addition to this, the taxonomy, diversity and ecology of diplostomids infecting freshwater fishes in the African continent which are particularly poorly known, was addressed. Using diplostomid metacercariae collected from the eyes of 288 fish comprising two species in the Cyprinidae (*Cyprinus carpio*, n=145 and *Barbus paludinosus*, n=67), two Cichlidae (*Oreochromis leucostictus*, n=56 and *Tilapia zillii*, n=18) and one Centrarchidae (*Micropterus salmoides*, n=2) caught in Lake Naivasha, Kenya. Morphometric (14 characters and 8 indices in 111 specimens) and molecular (sequences from the barcode region of cytochrome c oxidase 1 in 11 specimens) data were used to discriminate species. All fish species except *B. paludinosus* were infected with *Tylodelphys* metacercariae that were initially separated into two types differing mainly in body

length. However, this morphological distinction received only intermediate support in quantitative morphological analysis and molecular data indicated both morphotypes were conspecific. All the specimens therefore are inferred to belong to a single unidentified species of *Tylodelphys*, which is not conspecific with any other diplostomid for which comparable molecular data are available, including four diplostomid species known from siluriforms in Nigeria and Tanzania. These findings have been discussed in a paper titled `` **Morphometric and molecular analysis of *Tylodelphys* sp. metacercariae (Digenea: Diplostomidae) from the vitreous humor of four fish species from Lake Naivasha, Kenya``** submitted to the Journal of Helminthology (Cambridge University Press) on 11th November 2013.

From Lake Turkana, 2 taxa of parasites infected *H. forskahlii*, the dominant one being an anisakid nematode *Contracaecum multipapillatum* (p=83.7%) while 12 taxa of parasites were recovered from *T. zillii*, with the gryporhynchid cestode *Amirthalingamia macracantha* (first report of a mix of merocercoids and plerocercoids) being dominant (p=79.5%). Therefore, *H. forskahlii* had a depauperate parasitic community but the infection levels with *C. multipapillatum* were high. *T. zillii* had a rich parasite fauna, although, most parasites occurred at low intensities except *A. macracantha*. These findings have been discussed in a paper titled `` **Parasite communities of the elongate tigerfish *Hydrocynus forskahlii* (Cuvier 1819) and redbelly tilapia *Tilapia zillii* (Gervais 1848) from Lake Turkana, Kenya``** to be submitted to the Journal of African Zoology (African Zoology Circulation Office).

In addition, the distribution of 15 major and trace elements was determined in sediments, fish (34 specimens of the *O. leucostictus*) and their pericardial parasites (pooled samples of the anisakid nematode, *C. multipapillatum*) from Lake Naivasha. Element concentrations were determined by inductively coupled plasma-optical emission spectroscopy (ICP-OES) and graphite-furnace-atomic-absorption spectrometry (GF-AAS). The concentrations of the elements in the sediments reflected the geology of the area and did not point to pollution, as all the investigated trace elements including Pb, Cd, Cu and Zn did not show elevated values. In contrast, concentrations in the fish muscle were elevated for Li, Sr, Cd and Zn with high target hazard quotient (THQ>0.1) indicating a potential health risk to the consumers of these fish. Fish liver showed significantly higher concentration of the trace elements Fe, Mn, Cd and Cu compared to the muscle and the *C. multipapillatum* ($p<0.05$). In the parasite, Zn had the highest concentration, but it showed a poor ability to accumulate trace elements in relation to its fish host. These findings have been summed in a paper titled `` **Trace elements in sediments, blue spotted tilapia *Oreochromis leucostictus* (Trewavas, 1933) and its parasite *Contracaecum multipapillatum* from Lake Naivasha, Kenya, including a comprehensive health risk analysis**`` submitted to the Environmental Science and Pollution Research (Springer Verlag) on 8th November 2013.

Zusammenfassung

Fische stellen sowohl für die Bevölkerung der Regionen um den Naivasha- und den Turkanasee als auch für die Gesamtbevölkerung Kenias eine wichtige Proteinquelle dar. Im Gegensatz zum Naivashasee, wo der Fischbestand aus Besatz stammt, ist der Fischbestand im Turkanasee autochthon. Die Ziele dieser Studie waren einerseits das Vorkommen von Parasiten in kommerziell bedeutsamen Fischen aus den beiden Seen zu untersuchen und andererseits die Analyse von Spurenelementen in mehreren Kompartimenten (Wasser, Sediment, Fischorgane und Fischparasiten) um eventuelle Gesundheitsrisiken für Konsumenten von Fischen aus den beiden Seen festzustellen. Außerdem wurde das Potential einiger Fischparasiten als Bioindikator untersucht. Insgesamt wurden 373 Fische zur Untersuchung herangezogen und zwar 145 Karpfen *Cyprinus carpio*, 56 Weissfleckenmaulbrüter *Oreochromis leucostictus*, 18 Zilles Buntbarsche *Tilapia zillii* und 67 Sumpfbarben *Barbus paludinosus* aus dem Naivashasee sowie 43 Exemplare von *Hydrocynus forskahlii* und 44 Zilles Buntbarsche aus dem Turkanasee. Die Fische wurden zwischen Februar und September 2011 sowie im Septemer 2012 gefangen und auf Parasiten untersucht.

In *C. carpio* wurden 10 unterschiedliche Parasitentaxa gefunden, wobei der monogenean *Dactylogyrus minutus* bei weitem am häufigsten identifiziert wurde (p=99,3%). In *O. leucostictus* war *Cichlidogyrus* spp. mit einer Prävalenz von 91,1% neben 12 anderen Taxa dominant. *Tilapia zillii* waren von neun Parasitentaxa befallen, allen voran die von *Tylodelphys* sp. (p=83,3%) während in *B. paludinosus* 11 verschiedene Taxa gefunden wurden, am häufigsten ein unidentifizierter Vertreter der Gattung *Dactylogyrus* (p=83,6%). Die wenigsten Helminthen, sowohl hinsichtlich der Anzahl an unterschiedlichen Arten als auch absolut, wurden im Karpfen gefunden. Ektoparasiten konnten an allen untersuchten Fischen festgestellt werden.

Die Ergebnisse dieser Studie wurden im Artikel "**Parasites of commercially important fish from Lake Naivasha, Rift Valley, Kenya**" zusammengefasst. Dieser wurde am 28. Jänner 2013 bei Springer Verlag zur Publikation in der Zeitschrift "Parasitology Research" eingereicht.

Zusätzlich wurde die Taxonomie, die Diversität und die Ökologie von, Süßwasserfische in Afrika befallenden, Diplostomidae, die bisher wenig erforscht wurden, untersucht. Dafür wurden Metacercarien von Diplostomidae aus den Augen von 288 Fischen aus dem Naivashasee untersucht. Von diesen stammten zwei Arten aus der Familie der Karpfenfische (Cyprinidae; *C. carpio*, n=145 und *B. paludinosus*, n=67), zwei aus der der Buntbarsche (Cichlidae; *O. leucostictus*, n=56 und *T. zillii*, n=18) sowie eine aus der Familie der Sonnenbarsche (Centrarchidae) nämlich der Forellenbarsch *Micropterus salmoides* (n=2). Alle Fische außer *B. paludinosus* waren von Metacercarien von *Tylodelphys* befallen. Für die Charakterisierung der Spezies dieser Cercarien wurden morphometrische (14 Eigenschaften und 8 Indizes von 111 Individuen) und genetische (Sequenzen der Barcode-Region von Cytochrom c-Oxidase 1 von 11 Individuen) Daten verwendet.

Anfangs wurden die Metacercarien aufgrund ihrer Körpergröße in zwei Klassen unterteilt. Eine quantitative morphologische und genetische Analyse ergab jedoch, dass beide Klassen derselben nicht identifizierten Spezies der *Tylodelphys* angehören. Diese gehört zu keiner anderen Art für die vergleichbare genetische Daten verfügbar sind, auch nicht zu vier aus Welsartigen in Nigeria und Tanzania bekannten Arten von Diplostomidae. Diese Erkenntnisse wurden unter dem Titel "**Morphometric and molecular analysis of *Tylodelphys* sp. metacercariae (Digenea: Diplostomidae) from vitreous humor of four fish species from Lake Naivasha, Kenya**" am 11. Novemer 2013 zur Publikation in der Zeitschrift "Journal of Helminthology" (Cambridge University Press) eingereicht.

Im Turkanasee wurden zwei Parasitentaxa in *H. forskahlii* gefunden, wobei der Nematode *Contracaecum multipapillatum* (L3) dominant war (p=83,7%). In *T. zillii* konnten 12 unterschiedliche Parasitentaxa identifiziert werden, am häufigsten Metacestoden des Bandwurmes *Amirthalingamia macracantha* mit einer Prävalenz von 79,5%. Hier konnte zum ersten Mal das gemeinsame Auftreten von Merocercoiden und Plerocercoiden in einem Fischwirt beschrieben werden.

Folglich wies *H. forskahlii* eine sehr artenarme Parasitengemeinschaft auf, allerdings war der Infektionsgrad mit *C. multipapillatum* hoch. Die Parasitenfauna von *T. zillii* ist vielfältig, wobei die meisten Parasiten, mit Ausnahme von *A. macracantha*, in niedrigen Intensitäten vorkommen. Diese Erkenntnisse wurden unter dem Titel "**Parasite communities of the elongate tigerfish *Hydrocynus forskahlii* (Curvier 1819) and the redbelly tilapia *Tilapia zillii* (Gervais 1848) from Lake Turkana, Kenya**" zusammengefasst. Diese Publikation wird bei der Zeitschrift "Journal of African Zoology" (African Zoology Circulation Office) eingereicht werden.

Weiter wurde die Verteilung von 15 Mengen- und Spurenelementen in Sediment, Fischen (34 Individuen von *O. leucostictus*) und deren perikardialen Parasiten *C. multipapillatum* im Naivashasee untersucht. Die Bestimmung der Elementkonzentrationen wurde mittels optischer Emissionsspektrometrie mit induktiv gekoppeltem Plasma (ICP-OES) und Graphitrohr-Atomabsorptionsspektrometrie (GF-AAS) durchgeführt. Die Konzentrationen der Spurenelemtente (einschließlich Pb, Cd, Cu und Zn) im Sediment wiesen keine erhöhten Konzentrationen auf die auf eine Verschmutzung hindeuten könnten, sondern entsprechen dem geologischen Hintergrund. Im Gegensatz dazu wurden im Fischmuskel erhöhte Konzentrationen von Li, Sr, Cd und Zn festgestellt. Der erhöhte Gefährdungsquotient (target hazard quotient, THQ>0,1) weist auf eine mögliche Gefährdung für die Konsumenten hin.

In der Fischleber konnten signifikant höhere Konzentrationen von Fe, Mn, Cd und Cu im Vergleich zum Muskel und zu *C. multipapillatum* ($p<0,05$) festgestellt werden. Zink wies im Parasiten die höchste Konzentration auf, jedoch zeigte dieser eine schlechte Anreicherungsfähigkeit für Spurenelemente in Relation zum Wirt. Die Ergebnisse dieser Untersuchung wurden unter dem Titel "**Trace elements in sediments, blue spotted tilapia**

Oreochromis leucostictus **(Trewavas, 1933) and its parasite *Contracaecum multipapillatum* from Lake Naivasha, Kenya, including a comprehensive health risk analysis**" am 8. November 2013 bei Springer Verlag zur Publikation in der Zeitschrift "Environmental Science and Pollution Research" eingereicht.

Dedication

To the Almighty Lord God, your grace is sufficient.

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CHAPTER ONE

INTRODUCTION

Fish parasite studies-perspectives on Kenya

In the Republic of Kenya, fisheries play a very important role in the economy, contributing approximately kshs. 4 Billion (40 Million Euros) as export earnings annually. Fish in itself, is an important source of animal proteins for human consumption and as raw materials for the manufacture of animal feeds (Aloo-Obudho & Owuor, 2010). It is not surprising therefore, that in recent years; efforts by the Government of Kenya for intensification of fish production have been in top gear. For example, the Government of Kenya through the economic stimulus program (ESP) in a supplementary budget of the year 2009/2010 allocated Kshs. 1.12 Billion for the establishment of 200 fish ponds in each of the 140 potential constituencies' in an effort aimed at enhancing food security by intensifying aquaculture (Otachi et al., 2011). With this expected growth in fish production, the quality and safety of the fish need to be guaranteed (State Department of Fisheries Kenya DOFK, 2013).

Parasites are important components of host biology, survival, population structure and indeed, ecosystem functioning (Marcogliese, 2004). Parasites can be found in any fish species and within any type of aquatic system. They range from Protozoans (Flagellates, Ciliates, Apicomplexans, etc.) to Metazoans (e.g. Myxozoans, Trematodes, Cestodes, Acanthocephalans, Nematodes, and Crustaceans) (Lewis, 1991).

Dobson et al. (2008) reported that over 40% of all known species on earth are parasitic with parasitism being ubiquitous in some taxa and either absent or rare in others. The rate of discovery of new parasite species has grown linearly or exponentially in some well-studied helminth taxa and in contrast, sampling of parasite diversity from the most diverse parts of the world is thin at best. The knowledge of the status of parasite diversity in the tropics is still inadequate (Dobson et al. 2008). For example, a literature search in scopus (www.scopus.com), with the combination of the key words: fish+parasites+Kenya, returns a paltry less than 30 research articles of which a few deal with parasites of fish (e.g. Aloo & Dezfuli 1997; Aloo 1999; 2002; Aloo et al. 2004; Amin & Dezfuli 1995; Cowx et al. 2008 amongst others). This indicates a slow progress in research in this field in Kenya considering that this combines marine and freshwater fish parasites. The continued discovery of new species from Kenyan lakes e.g. Lake Turkana (Moravec et al. 2009a, b; Prykrylova et al. 2012) is not only encouraging but also evidence of the understudied community of fish parasites. Additionally, there are gaps in knowledge of parasites from the two lakes: in Lake Naivasha, no reports of ectoparasites are available as the earlier study concluded that they were absent from the studied fish (Aloo 2002), while from Lake Turkana, data on parasite infection levels and dynamics is lacking.

The two sites chosen for this study have unique, interesting and contrasting features. For example, the Lake Naivasha is the only freshwater lake in the Rift valley without an outlet (Kamau et al. 2008) and was declared a Ramsar site in 1995 showing its recognition as a wetland of international importance. It is a relatively small lake ($160 km^2$) and shallow. All the fish, as well as several other biotas in the lake have been introduced either accidentally, or intentionally for stocking, re-stocking, sport fishing and biocontrol measures (Gherardi et al. 2011). On the other hand, Lake Turkana approximates a pristine and natural lake environment, with little anthropogenic influence (Odada et al. 2003; ILEC 2013). It is a large lake ($7560 km^2$), relatively deep lake and forms part of the Great Lakes Region of Africa. Its fish are native and diverse. The water of Lake Turkana is portable but not palatable, as it is slightly saline.

Trace element research (including heavy metals)-perspectives on Kenya

In Kenya, the need for baseline data on pollution studies to the aquatic biota of lakes and rivers was recognized three decades ago as a result of population expansion, burgeoning industries and increasing urbanization (Onyari, 1981). Several lakes have been investigated including the Lakes Victoria and its Winam gulf, Nakuru, Naivasha, Baringo, Turkana and Bogoria. For example, several studies on heavy metals in Lake Naivasha have been carried out (e.g. Bonzongo et al. 1996; Campbell et al. 2003; Kamau et al. 2007, 2008). However, there have been contradicting conclusions about the pollution status of the lake with regard to heavy metals. For example, Tarras-Wahlberg et al. (2002) found that the river and lake sediments of Lake Naivasha represent fairy undisturbed background conditions but observed a higher than expected concentrations of cadmium, iron, nickel and zinc found in both river and lake sediments and concluded that these were likely derivatives of volcanic rocks and/or lateritic soils found in the lake catchment. Ochieng et al. (2007) also concluded that there could be significant geological influence as input of heavy metals such as Co, Ni, Cu, Zn and Pb. Contrastingly; Njogu et al. (2011) and Mutia et al. (2012) showed that the lake is contaminated and that the most important sources of heavy metals pollution in the Lake Naivasha basin are River Malewa and the surrounding flower farms. As for Lake Turkana, little research has been done on heavy metals. To the best of our knowledge, only Campbell et al. (2003) presented the first data on one heavy metal: total mercury in fish from this lake.

Environmental pollution and Parasitism – the link

Many investigators have examined the effects of environmental stress on species of parasites in temperate aquatic systems (e.g. Khan & Thulin, 1991; Overstreet, 1993; MacKenzie et al., 1995; Marcogliese & Cone 1997; Williams & Mackenzie, 2003; Marcogliese, 2004; Sures, 2004; Blanar et al., 2009), despite the fact that it is difficult to predict the direction of effects of pollution impacts on parasite communities. Williams & Mackenzie (2003) provided a list of various pollutants and their effect on certain parasites. For example, if a site is organically polluted, bacteria and trichodinid populations in that area are more abundant and therefore *Trichodina* sp. presumably could be used as a pollution indicator (Palm & Dobberstein, 1999). Parasites with complex lifecyles also provide important information about the environmental conditions, because their presence and/or absence reflect a great deal about not only their host ecology but also food web interactions, biodiversity and environmental stress (Overstreet, 1997; Marcogliese, 2003, 2004; Ercument et al., 2008).

Interestingly, certain parasites, particularly intestinal helminthes, can accumulate heavy metals at concentrations that are orders of magnitude higher than those in the host tissues or the environment (e.g. Sures et al., 1994; 1997; 1999a, b; 2001; Jirsa et al. 2008). For example, some Acanthocephalans (Galli et al., 1998; Sures & Siddall, 1999; Sures et al., 2000; 2003a, b, 2005; Sures, 2004; Schludermann et al. 2003); Cestodes (Sures et al. 2003a; Retief et al. 2006; Jirsa et al. 2008; Oyoo-Okoth et al. 2010a, b; 2012); and Digeneans (Sures et al.1998) have been found to be efficient accumulators of heavy metals.

So far, from Kenya, Oyoo-Okoth et al. (2010a, b; 2012) were the first and the only ones to apply a host-parasite study of heavy metals bioaccumulation to our knowledge. They have investigated the accumulation of lead and copper in a cestode *Ligula intestinalis* and its fish host *Rastrineobola argentea* with promising results of a good host-parasite bio-indicator system.

Aims of the research

The dual aims of the study were to determine the occurrence of parasites of commercially important fish from two Rift Valley lakes: Lake Naivasha and Lake Turkana and evaluate the composition of the parasite community in connection with physico-chemical parameters. Additionally, to determine trace element concentrations in various fish organs and in selected fish parasites to establish if there was any health risk for fish consumers (humans and animals) in these areas and to evaluate bio-indicative potentials of the occurring parasite species.

Specific objectives

The specific objectives of the study were to:

1. Examine various commercially important fish for ecto and endo parasites

2. Identify the observed parasites to the lowest possible taxa

3. Determine levels of concentrations of various trace elements including heavy metals in water, sediment, fish organs in order to assess any health risks

4. Determine bioaccumulation of heavy metals in some helminth parasites in order to establish whether some parasites are potential biomarkers for heavy metal pollution

Scope of the dissertation

This is a cumulative dissertation and is structured such that the main research themes that were investigated are presented in chapters as standalone documents in the manner in which they have been accepted, prepared or submitted for publication in various journals as specified.

Chapter two discusses the findings on parasites of commercially important fish from Lake Naivasha, Kenya with a special emphasis on ectoparasites which were discovered for the very first time. Also, it presents results on parasites that have been reported for the first time from this lake.

Chapter three summarizes morphometric and molecular analyses of diplostomids occurring in the vitreous humor of the eyes of four fish species from Lake Naivasha. This is among the first attempts at identification of diplostomids infecting fish from Kenya and this study presents interesting data. Chapter four provides data on major and trace elements concentration including heavy metals in sediments, fish tissues and nematode parasites. It also presents a comprehensive health risk assessment using the Target Hazard Quotients (THQs) and a comparison of trace elements accumulation in an anisakid nematode *Contracaecum multipapillatum* and its host *Oreochromis leucostictus* tissues from the same lake. On the other hand, chapter five gives baseline data on the parasite communities of two commercially important fish species from the Lake Turkana including a detailed analysis of infection levels and host-parasite relationships. New parasites and new phenomenon observed are also discussed. Finally, chapter six provides the conclusions of the study.

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CHAPTER TWO

Parasites of commercially important fish from Lake Naivasha, Rift Valley, Kenya

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Abstract

In Lake Naivasha the common carp *Cyprinus carpio* L. 1758, was accidentally introduced from fish farms adjacent to River Malewa in 1999 and now forms the bulk of the total fish caught. Since its introduction, no study has been made on its parasitic community nor are there any reports on ectoparasites from other fish species in this lake to the best of our knowledge. Therefore, the aim of this study was to describe the parasitic community of *C. carpio* and two other commercially important fish species; *Oreochromis leucostictus* and *Tilapia zillii*. Additionally, the abundant *Barbus paludinosus* was included in the study. A total of 286 fish (145 *C. carpio*, 56 *O. leucostictus*, 18 *T. zillii* and 67 *B. paludinosus*) were collected during the year 2011 and examined. 10 taxa of parasites were recovered from *C. carpio* dominated by the monogenean *Dactylogyrus minutus*, occurring with a prevalence (p) of 99.3%. 13 taxa of parasites were identified from *O. leucostictus* dominated by monogeneans *Cichlidogyrus* spp. (p = 91.1%). *T. zillii* harbored 9 taxa of parasites with the digenean *Tylodelphys* sp. (p = 83.3%) being dominant and *B. paludinosus* harbored 11 taxa of parasites dominated by an unidentified monogenean of the genus *Dactylogyrus* (p = 83.6%). *C. carpio* had the lowest helminth species diversity and richness while monogenetic trematodes, which have never been reported from fish in Lake Naivasha, were the most prevalent parasites recovered.

Key words: Lake Naivasha; common carp; *Oreochromis leucostictus*; *Tilapia zillii*; ectoparasites; monogeneans.

Introduction

Fish is an important protein source for an estimated 650,000 people living in the Lake Naivasha basin and other local markets such as Nakuru and Nairobi (WWF 2011). Lake Naivasha fisheries depend on introduced species with variable catch composition, determined mainly by fishing intensity, water levels and changes in aquatic macrophyte densities (Muchiri et al. 1994; Hickley et al. 2004). For example, between the period 1987-2000 the commercial fish species composition caught was dominated by the blue spotted tilapia *Oreochromis leucostictus* (Trewavas, 1933) followed by largemouth bass *Micropterus salmoides* (Lacepède, 1802) and redbelly tilapia *Tilapia zillii* (Gervais, 1848) (Hickley et al. 2004). Muchiri and Hickley (1991) reported that commercial fishing in the lake began in 1959 being based on the largemouth bass and two tilapia species at that time. In recent years, the common carp *Cyprinus carpio* L. 1758, which was accidentally introduced into the lake from fish farms adjacent to its major water inflow River Malewa in the year 1999 (Hickley et al. 2004; Gherardi et al. 2011), now forms the bulk of the total fish caught (Ojuok et al. 2007). Apparently, *C. carpio* is one of the most widely distributed fresh water fish species worldwide (Hoole et al. 2001; Kir and Tekin-Özan 2007). Owing to its adaptation to a wide range of climatic and geographical conditions, *C. carpio* is one of the best examined fish species for parasites in different parts of the world such as Asia and Europe. As a consequence, a substantial number of almost all major taxa of parasites have been found in wild and farmed carp (Hoole et al. 2001; Kir and Tekin-Özan 2007). Most recent publications on the parasitic community of carp include integrative works by Hoole et al. (2001) on diseases of carp and other cyprinid fish and a checklist of carp parasites by Barus et al. (2002) and particular studies investigating diverse aspects of carp parasites (e.g. Bruňanska et al. 2011; Bazsalovicsová et al. 2011, 2012; Liu et al. 2011, 2013; Ondračková et al. 2012; Orosová and Oros 2012; Boerlage et al. 2012, 2013).

In Africa, Paperna and Thurston (1968) recognized parasites and particularly ectoparasites as important limiting factor in the development of intensified fish culture. These authors also acknowledged that carp had been introduced to some parts of the African continent and reported heavy losses in carp fry at Kajansi, in Uganda due to *Dactylogyrus* infection. In Kenya, and specifically in Lake Naivasha, several studies on fish parasites have been done (Malvestuto 1975; Malvestuto and Ogambo-Ongoma 1978; Amin and Dezfuli 1995; Aloo and Dezfuli 1997; Aloo 1999; 2002; Britton et al. 2009). They have described the occurrence of helminth parasites of four fish species namely; *T. zillii*, *M. salmoides*, *Barbus paludinosus* and *O. leucostictus.* Parasites such as the trematode *Clinostomum* sp., the cestodes *Armithalingamia macracantha*, *Ligula intestinalis* and *Cyclustera* sp., the nematode *Contracaecum* sp. and the acanthocephalan *Polyacanthorhynchus kenyensis* were reported in these studies. However, compared with the vast literature available from temperate regions, there is little information on the ecology of parasites of freshwater fish in tropical countries (Barson et al. 2008). For example, since the introduction of *C. carpio* into Lake Naivasha, more than a decade ago and its subsequent successful establishment leading to dominancy among fish species in the lake (Ojuok et al. 2007; Oyugi et al. 2011) there have been, to the best of our knowledge, no studies on its parasitic community. Furthermore, there are no reports of ectoparasites whatsoever of all the other fish in the Lake. Therefore, the aim of this study was to describe the parasitic community of *C. carpio* in the lake and to include two other commercially important fish, *O. leucostictus* and *T. zillii* and in addition the abundant *B. paludinosus* for the study of their ectoparasites in order to contribute to the existing body of knowledge.

Materials and methods

Study area

The study was conducted in Lake Naivasha, Kenya between February and August 2011. The lake is situated at 00º45'S and 36º20'E (Kamau et al. 2008) in a closed basin at an altitude of 1890m above sea level and covers approximately 160km^2 in the eastern Rift Valley of Kenya (Fig.1). It is the only freshwater lake in the Rift Valley without a surface outlet but with a substantial exchange with groundwater (Gaudet and Melack 1981; Clarke et al.1990). It is shallow (approximately 6m mean depth) with a volume of 4.6km^3 (Campbell et al. 2003). It is bordered by papyrus *Cyperus papyrus* in some sections and the overall composition of aquatic macrophytes are in a state of change (Tarras-Wahlberg et al. 2002) probably due to anthropogenic influence such as clearance of littoral vegetation, eutrophication and plant and animal introductions (Kitaka et al. 2002; Gherardi et al. 2011). Most of its fresh water inflow (approximately 80-90%) comes from River Malewa (Hickley et al. 2002; Kamau et al. 2008) with an estimated mean annual flow of 153 million m^3 and a catchment area of 1730km^2, followed by River Gilgil with an estimated average annual flow of 24 million m^3 and a catchment area of 420km^2 while the River Karati flows only intermittently (Ase et al. 1986; Ase 1987; Abila et al. 2008; Harper et al. 2011). The Lake Naivasha basin has three distinct components. The first component is the main lake with an area of 145km^2 and is the most important for fisheries (Harper et al. 1990). The second component is the Oloidien Lake with an area of 5.5km^2 (Harper et al. 1990). This lake had been separated from the main lake due to receding water levels a few decades ago and now has a considerably higher pH than the main lake (Harper et al. 1990). The third component is the Crescent Island Lake with an area of 2.1km^2 (Harper et al. 1990). It is the deepest lake (approximately 12-15m depth) but is constantly connected to the main lake (Abiya 1996; Harper et al. 2011). The basin area is generally semi-arid; receiving a mean annual rainfall of 620mm while the mean annual evaporation is estimated at 1735mm. Evaporation generally exceeds precipitation throughout the year except at peak rainfall with the rainfall trend being bimodal with a major peak in April-May and a minor peak in October-November (Abiya 1996). The water from Lake Naivasha is used extensively for agriculture (horticultural farms: approx. 77million m^3/year), geothermal power generation (approx. 1million m^3/year) (WWF 2011), domestic water supplies, commercial fishing, tourism and recreation as well as ranching and game farming (Abiya 1996). The key environmental problems facing the lake are water abstraction leading to changes in water level, eutrophication, pollution, invasive species, decline in fish stocks and biodiversity (Otiangà-Owiti and Oswe 2007; Abila et al. 2008).

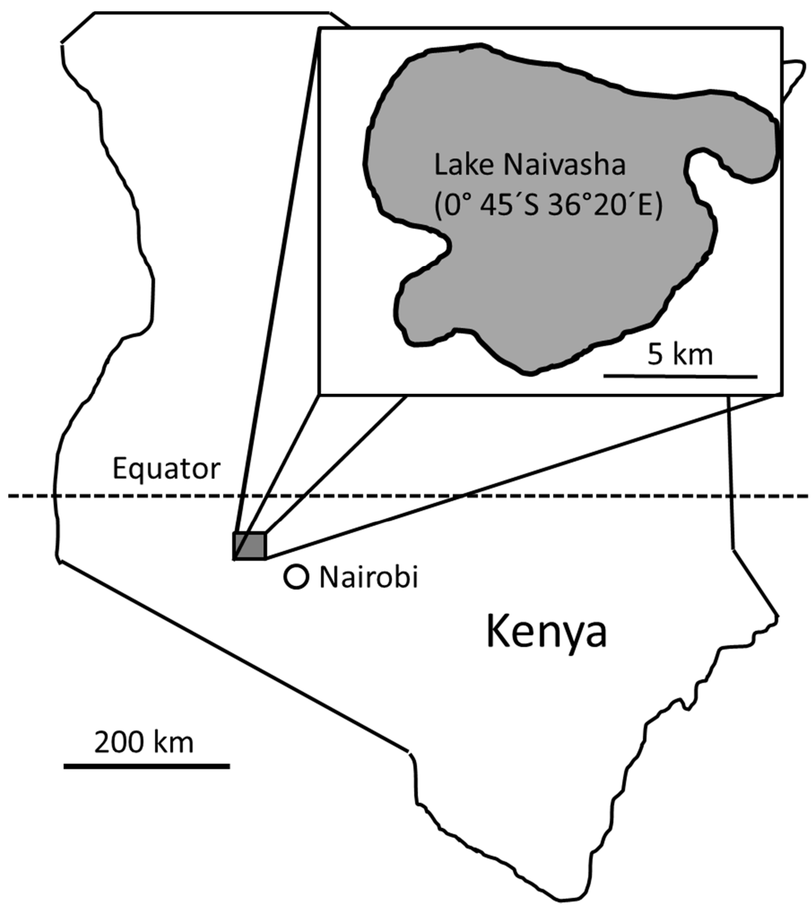

Fig.1. Map of Lake Naivasha, Rift Valley Kenya

Fish sampling and parasitological analyses

A total of 286 fish comprising of two cyprinids: 145 *C. carpio* and 67 *B. paludinosus* and two cichlids: 56 *O. leucostictus* and 18 *T. zillii* were collected from Lake Naivasha using a fleet of gill nets with mesh sizes 2, 2.5, 3, and 4 inches during the period February to August 2011. The fish were transported alive in a fish tank with lake water to the laboratory of the department of Biological sciences, Egerton University, Njoro. They were killed by cervical dislocation followed by a dissection as described by Schäperclaus (1990). Weight and total lengths (TL) were measured and the Fulton`s condition factor (Ricker 1975) calculated. For the estimation of intensity of infection of protozoans, gill and skin smears were prepared for examination under high magnification (X40-X100) as described by Lom and Dykovà (1992). The categories used to assess the intensity of infections were defined as follows: ``low´´<10 parasite individuals per field of view (at 100-fold magnification), ``medium´´ for 11-100 parasites per field of view and ``high´´ for more than 100 parasite individuals per field of view (Jirsa et al. 2011). Further, *C. carpio* gills were observed *in situ*, and each gill arch was removed sequentially and placed in numbered petri-dishes with tap water (1, 2, 3 and 4) with 1 representing outermost gill arch and the nearest to the operculum and 4 the innermost and nearest to the midline (transverse partitioning), and examined for monogeneans under a binocular microscope with no special attention given to the side of the fish since many studies have shown no significant differences in right and left sides (Chapman et al. 2000; Kadlec et al. 2003; Madanire-Moyo et al. 2010; Le Roux et al. 2011). The eyes, intestines, visceral organs and peri-cardial cavities were all examined for parasites. Parasites were identified directly using identification keys by: Bykhovskaya-Pavlovskaya et al. (1962), Khalil (1971), Lom and Dykovà (1992), Paperna (1980; 1996), Pariselle and Euzet (1995; 1998; 2009) and Le Roux and Avenant-Oldewage (2010).

Statistical analysis

The prevalence and mean intensities were determined according to Bush et al. (1997). The Fulton`s condition factor (Ricker 1975) was calculated using the formula: $k = (W/L^3)100$, where k, is the Fulton`s condition factor, W, is the weight of fish (g) and L, the total length of fish (cm). The measures of helminth community structure such as the Shannon-Wiener index, Simpson´s index, Margalef Richness index and Berger-Parker Dominance index as proposed by Magurran

(1988) were determined. To determine spatial distribution of monogeneans on the gill arches of *C. carpio*, the data was transformed (log x) to achieve homoscedasticity and an analysis of variance (Anova) and multiple comparisons (Tukey HSD) performed using R program version 2.15.1(R Foundation for Statistical Computing).

Results

An overview of the fish sample distribution, mean total lengths, mean weight and the Fulton's condition factors is presented in Table 1. *T. zillii* had the highest condition factor of 1.8 (±0.2) while *B. paludinosus* had the lowest at 1.4 (±3.2).

Table 1. Data of the fish species examined from Lake Naivasha

Fish species	Sample size (n)	Total length (cm) mean (min-max)	Weight (g) mean (min-max)	Condition factor (k) mean (±SD)
C. carpio	145	34.7 (19.0-55.0)	687.0 (158-2366)	1.5 (±0.2)
O. leucostictus	56	15.6 (6.7-28.4)	69.0 (5-418)	1.7 (±0.2)
T. zillii	18	15.4 (13.2-18.2)	66.5 (43-108)	1.8 (±0.2)
B. paludinosus	67	8.0 (3.2-11.0)	4.8 (0.5-8.6)	1.4 (±3.2)

99.3% (144 out of 145) of the *C. carpio* examined were infested with at least one taxon of parasites. A total of 10 taxa of parasites of *C. carpio* were recovered during the study (Table 2). The most dominant taxa were *Dactylogyrus minutus* and the digenean *Tylodelphys* sp. There was a low diversity of endoparasites infesting *C. carpio* with a Shannon-Wiener index of 0.14. Four species of protozoans namely *Trichodinella* sp., *Trichodina* sp., *Chilodonella* sp. and *Tetrahymena pyriformis* were recovered but all occurred at low intensities (Table 2). Three species of monogenetic trematodes with two belonging to the genus *Dactylogyrus* (*D. minutus* Kulwiec, 1927 and *D. extensus* Muller and Van Cleave, 1932) and the other of the genus *Acolpenteron* were found. There was a spatial distribution of the two monogenetic trematodes (*Dactylogyrus* spp.) across gill arches of *C. carpio* with the second gill arch being more preferred ($p<0.05$) (Fig. 2).

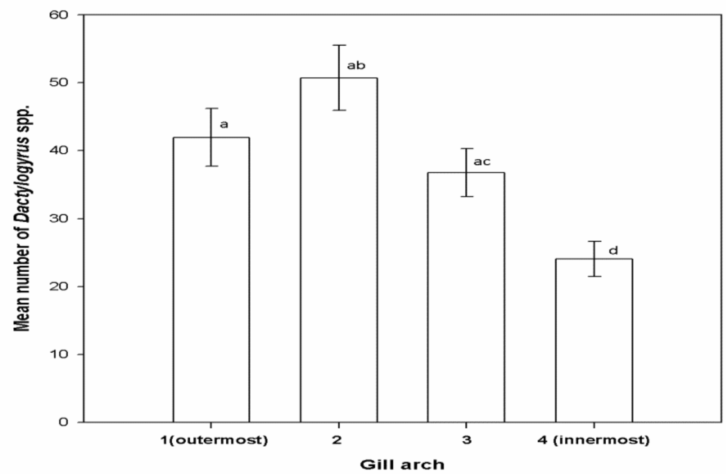

Fig.2. Distribution of *Dactylogyrus* spp. on gill arches of *C. carpio* from L. Naivasha, Mean±SE, means followed by the same letter are not significantly different from each other (Tukey HSD, $p<0.05$)

95.5% (64 out of 67) of the *B. paludinosus* examined were infested with at least one taxon of parasites. In total, 11 taxa of parasites were observed (Table 2). The most dominant parasite infesting *B. paludinosus* was a yet to be identified monogenean of the genus *Dactylogyrus* followed by a nematode *Contracaecum* sp. There were a number of protozoan parasites recorded but they occurred at low intensities as well. The genetic determination of the unidentified *Dactylogyrus* sp. and *Contracaecum* sp. found in *B. paludinosus* is on-going.

Table 2. Parasites of the two cyprinid fish from L. Naivasha: prevalence (P) and mean intensity (MI). n.d., not detected

Parasite species	Location on/in host	*C. carpio* n=145		*B. paludinosus* n=67	
		P	MI	P	MI
Trichodina sp.	gills+skin	39.3	low	11.9	medium
Trichodinella sp.	gills+skin	13.8	high	3.0	high
Chilodonella sp.	gills	0.01	low	n.d.	
Ichthyophthirius multifiliis	gills+skin	n.d.		10.4	low
Tetrahymena pyriformis	gills+skin	2.1	low	1.5	low
Cryptobia sp.	gills	n.d.		3.0	low
Epistylis sp.	gills	n.d.		3.0	low
Dactylogyrus minutus	gills	99.3	181.3	n.d.	
Dactylogyrus extensus	gills	25.5	2.1	n.d.	
Dactylogyrus sp.	gills	n.d.		83.6	11.2
Acolpenteron sp.	gills	0.01	3	n.d.	
Amirthalingamia macracantha	intestine	n.d.		3.0	9.5
Contracaecum sp.	intestine	n.d.		62.7	9.1
Nematoda (Larval)	intestine	0.01	1	4.5	1.7
Tylodelphys sp.	eye (vitreous humor)	54.5	4.7	n.d.	
Cestoda (Larval)	intestine	0.01	1	62.7	3.4
Digenean cysts	intestinal wall	0.01	>100	n.d.	

All (100%) of the *O. leucostictus* investigated were infested with at least one taxon of parasites and 13 taxa of parasites were recovered in total (Table 3). The dominant parasite taxa infesting *O. leucostictus* were monogenetic trematodes of the genus *Cichlidogyrus.* Protozoan and Myxozoan parasites were found occurring at low prevalence and intensities (Table 3).

Table 3. Parasites of two cichlid fish species from L. Naivasha: prevalence (P) and mean intensity (MI). n.d., not detected.

Parasite species	Location on/in host	*O. leucostictus* n=56		*T. zillii* n=18	
		P	MI	P	MI
Trichodina sp.	gills	21.4	low	3.2	medium
Trichodinella sp.	gills	10.7	low	3.2	low
Tetrahymena pyriformis	gills	3.6	low	3.2	low
Myxobolus sp.	gills	3.6	low	n.d.	
Epistylis sp.	gills	1.8	low	n.d.	
Cichlidogyrus spp.	gills	91.1	21.8	64.5	13.4
Amirthalingamia macracantha	intestine	21.4	8.1	3.2	27
Contracaecum multipapillatum	pericard	51.8	2.3	25.8	2
Heterophyes sp.	gills	19.6	1.6	22.5	2.2
Tylodelphys sp.	eye(vitreous humour)	66.1	6.8	80.6	8.7
Cestoda (Larval)	intestine	12.5	4.3	n.d	
Digenean cysts	intestinal wall	3.6	<100	6.5	>100

88.9% (16 out of 18) of the *T. zillii* examined were infested with at least one taxon of parasites. A total of 9 taxa of parasites were recorded (Table 3). The most dominant taxon of parasites of *T. zillii* was a digenean *Tylodelphys* sp. Several taxa of protozoans were also recorded but all occurred at low prevalence and intensities (Table 3). *T. zillii* had the most diverse helminth parasitic community of all the fish species examined (Shannon-Wiener index 1.62) (Table 4).

Table 4. Comparison of the diversity characteristics of the parasite communities of the four fish species from L. Naivasha.

Total component communities	*C. carpio*	*O. leucostictus*	*T. zillii*	*B. paludinosus*
Number of fish	145	56	18	67
Number of metazoan taxa	5	7	5	5
Shannon-Wiener index	0.14	1.43	1.62	1.51
Margalef Richness	0.39	0.81	0.63	0.57
Berger-Parker index	0.98	0.70	0.48	0.53
Simpson`s index	0.97	0.53	0.39	0.40
Dominant taxon	*D. minutus*	*Cichlidogyrus* spp.	*Tylodelphys* sp.	*Dactylogyrus* sp.

Discussion

Parasites of *C. carpio*

The results of this study indicate high prevalence and intensities of ectoparasites of *C. carpio* dominated by the monogenetic trematode *D. minutus* compared to other studies (Kir and Tekin-Özan 2007; Tekin-Özan et al. 2008; Theerawoot 2008). On the dominancy of *D. minutus* our results agree with findings by: Kir and Tekin-Özan (2007) who also found that *D. minutus* was the most dominant parasitic species infesting the native *C. carpio* from Kovada Lake (Turkey) (38.09%), Tekin-Özan et al. (2008) who found a 37.76% prevalence in Beysehir Lake, Turkey and Theerawoot (2008) who described a prevalence of 80% in Nakhonsithammarat, Southern Thailand although in the current study a higher prevalence and intensity were found (Table 2). Kàlmàn (2012) also reported that *D. minutus* has been among the species of *Dactylogyrus* infesting the European common carp subspecies for over 50years. In addition, our findings are supported by studies of Nematollahi et al. (2012) and Borji et al. (2012) in fish farms of Mashhad, northeast of Iran as well as the study by AL-Marjan and Abdullah (2009) in Ainkawa fish hatchery, Erbil province, Iraq, all of which reported that *Dactylogyrus* spp. are the most common parasites of *C. carpio*.

The high prevalence and intensities of *D. minutus* can be explained by the life cycle pattern. It is a monoxenous parasite and the risk factors favoring its spread such as warm tropical temperatures, low water exchange, poor bottom hygiene and short water column depth among others seems to be prevailing in Lake Naivasha (Florio et al. 2009). Lake Naivasha is a relatively shallow lake (Tarras-Wahlberg et al. 2002) and has a high year round temperature with a mean of 19-23°C (Campbell et al. 2003). In dactylogyrids, incubation time, life span of free-swimming larvae, development to maturity and the longevity of the adult worm are directly related to temperature (Paperna 1996). For example, with a temperature of 20-28 $^{\circ}$C eggs hatch within 2 to 6 days and development to maturity occurs within 5 days (Paperna 1996). This means that the dactylogyrids will have shorter life cycles with the resultant high reproduction capable of infesting many fish. Therefore, the high temperature and shallowness of the lake may present good conditions for the spread of monogeneans, as eggs laid at the bottom or on fish, miracidia hatch faster and within the small water column, swim and infest a suitable host more easily. Furthermore, currently *C. carpio* is the dominant species of fish in the lake in terms of biomass (Ojuok et al. 2007). This dominancy coupled with its benthopelagic habitat (Kottelat and Freyhof 2007) means host biomass and habitat play significant roles in the spread of these parasites.

Although there was a high intensity of *Dactylogyrus* spp. on gills of *C. carpio*, there were no obvious pathological effects such as haemorrhages and ulceration of host epithelium, development of epithelial outgrowths, and production of excessive amounts of mucus. Such effects could disturb the respiratory function of the gills and ionic exchange (Erasmus and Chapman 1972; Ukoli 1984).

Findings of this study on significant differences in distribution of *Dactylogyrus* spp. across gill arches are quite similar to other findings (Chapman et al. 2000; Kadlec et al. 2003; Madanire-Moyo et al. 2010; Le Roux et al. 2011; Ondràckova et al. 2011). In our study, the highest proportion (33%) of the two *Dactylogyrus* spp. recorded was located on the second gill arch. Similar findings have been reported (Chapman et al. 2000; Madanire-Moyo et al. 2010; Le Roux et al. 2011) thereby providing more evidence for strong site specificity. Microhabitat specificity is widespread among monogeneans and has been shown to occur in different ways (Chapman et al. 2000). Some monogeneans have shown a species-specific site selection (Wootten 1974; Koskivaara et al. 1991; Le Roux et al. 2011). For example, *Cichlidogyus philander* was found to predominantly occur on the second and third gill arches in *Pseudocrenilabrus philander philander* in South Africa (Le Roux et al. 2011). However, in this study the two *Dactylogyrus* spp. were not yet identified to species level at the time of enumeration and therefore, values presented here are for the two species together. Other monogeneans show a restriction to certain parts along the gill filaments and others to the external or internal filaments (Chapman et al. 2000). A number of factors have been recognized as determinants of microhabitat choice among monogeneans. They include intrinsic factors such as the need to increase chances of locating a mate by having a narrow microhabitat niche and environmental conditions. Among the environmental factors, water current has been identified as among the most important factor determining site specificity (Madanire-Moyo et al. 2010; Le Roux et al. 2011) with the suggestion that middle arches offer a hydro-dynamically protected site (Lo et al. 2001). It is an established fact that many parasites have free living stages (eggs or larvae) which have complimentary behavioral reactions to the ambient environment (Echi and Ezenwaji 2009) and therefore, the settling of oncomiracidia is likely to be influenced by water currents. Additionally, in oxygen-deficient waters, the difficulties of oxygen uptake in gill parasites and their fish hosts may influence host and parasite densities, site selection and indeed effects of the parasite on host condition (Chapman et al. 2000). Lake Naivasha being a eutrophic lake (Kitaka et al. 2002) in a tropical climate can experience declines in dissolved oxygen at certain depths and periods which could then influence the distribution of gill parasites.

Trichodina sp. and *Trichodinella* sp. occurred at a moderate prevalence in the *C. carpio* studied and at low intensities. These results compare quite well with studies by Mansoor and Al-Shaikh (2010) in *C. carpio* from Bab Al-Muatham fish markets in Iraq (*Trichodina domerguei* 21.6%) and that of Nematollahi et al. (2012) who described a 35% prevalence of *Trichodina* sp. in fish farms of Mashhad, northeast of Iran. Similar findings have also been reported by among others; Özer and Erdem (1998), Vulpe (2002), Daniela et al. (2008) and AL-Marjan and Abdullah (2009). There are data on the presence of trichodinids in a large number of aquatic systems in tropical Africa (Fryer and Iles 1972; El-Tantawy and Kazubsky 1986) but data on taxonomy are quite limited. All the ectoparasites found infesting *C. carpio* in this study have never been reported before in this lake.

Trichodinids are the most common ectoparasites of fish potentially capable of inflicting heavy damages and inducing chronic low-level mortalities in stressed or immune-suppressed fish, perhaps due to adverse environmental conditions (Lom and Dykovà 1992; Hoole et al. 2001; Florio et al. 2009). However, in the current study no mortalities were neither reported by the fishermen nor observed probably due to the low intensities observed. In otherwise healthy fish populations, *Trichodina* spp. could therefore be considered more as a nuisance than a serious parasite (Hoole et al. 2001) as they commonly use their host as a convenient feeding ground for waterborne particles, bacteria and debris from the fish surface (Woo 1995).

A low helminth parasite diversity and richness was found in *C. carpio*. Except for the endoparasitic metacercariae of the digenean *Tylodelphys* sp. which infested the vitreous humor of eyes virtually no other endohelminthes were recovered in this study, safe for a single nematode and cestode, thus agreeing with the study by Kennedy and Pojmanska (1996) who found that common carp helminth infra-communities were poor in a fish farm in Zabieniec, Poland. This was unexpected as this study had hypothesized a high diversity of endoparasites of *C. carpio* as has been reported from other parts of the world (Moravec 1984; Hoole et al. 2001; Barus et al. 2002; Vulpe 2002; Kir and Tekin-Özan 2007; Boane et al. 2008; Daniela et al. 2008). However, in agreement with our study, Kennedy and Pojmanska (1996) argued that studies indicating rich component communities of parasites of *C. carpio* may be somewhat misleading because many of the helminth species found occur very rarely and the component communities are often dominated by only a few species.

Perhaps the most important explanation for the low diversity of endo-parasitic fauna in *C. carpio* found in this study relates to the colonization time hypothesis, the invasion theory and the enemy release hypothesis. Invasion theory suggests that some of a species` initial colonization success may be due to temporary release from parasites and pathogens, with fewer in the new environment than in native habitats (Torchin et al. 2003). Release from parasites and pathogens is predicted to be greatest early in invasion, and forms part of the enemy release hypothesis (Keane and Crawley 2002). *C. carpio* was discovered in Lake Naivasha in the year 2002 barely a decade ago (Hickley et al. 2004; Ojuok et al. 2007) and thus the invasion theory and the enemy release hypothesis may explain the low diversity of endoparasites, since introduced species require considerable time to acquire a helminth fauna derived from the native species (Guegan and Kennedy 1993). Indeed some studies have reported the infestation of *C. carpio* with generalist parasites such as the nematode *Contracaecum* sp. (Boane et al. 2008; Karakisi and Demir 2012) which was also prevalent other fish species in our study (Table 2 and 3) and it was therefore surprising that none was found infesting *C. carpio*. However, exotic species may also serve as sources for new parasite species which may invade native host populations (Mack et al. 2000). Therefore, the finding of the strictly species-specific parasites such as *D. minutus* and *D. extensus* in this study reveals that they were introduced together with *C. carpio* thereby confirming studies by Ondràckova et al. (2011).

It was thus established that there was a high prevalence and intensity of ectoparasites dominated by the monogenean *D. minutus* newly introduced together with the *C. carpio*. Protozoan parasites were also present albeit at low intensities and a low diversity of helminth parasites was observed.

Parasites of *O. leucostictus, T. zillii,* and *B. paludinosus*

Ectoparasites particularly monogenetic trematodes; *Cichlidogyrus* spp. and *Dactylogyrus* sp. were found to be the dominant parasites infesting the two cichlid fish (*O. leucostictus* and *T. zillii*) and the cyprinid *B. paludinosus* with high prevalence and mean intensities (Table 2 and 3). Earliest studies on parasites of these fish in Lake Naivasha by Malvestuto (1975), Malvestuto and Ogambo-Ongoma (1978), Paperna (1980) and Aloo (1996; 2002) did not observe or report any ectoparasites on fishes from Lake Naivasha. For example, Aloo (2002) pointed out clearly that the lack of ectoparasites in *O. leucostictus* and *T. zillii* was a unique finding to Lake Naivasha and attributed it to a deteriorate water quality. Therefore, this is the first report of ectoparasites of fish in this lake.

This high prevalence of monogeneans is a sign that Lake Naivasha presents very good conditions for their diffusion. In farmed fish, stocking density has been recognized as another predisposing factor leading to heavy gill infestation in fish (Florio et al. 2009; Ibrahim 2012). Therefore, for Lake Naivasha the differences in prevalence and mean intensities of infestation with dactylogyrids *in O. leucostictus*, *T. zillii* and *B. paludinosus* might be partly attributed to their respective biomass in the lake and their preferred habitats. From the findings, it was deduced that the density or biomass of *T. zillii* was lowest compared to that of *O. leucostictus* and *B. paludinosus* hence the lowest prevalence of *Cichlidogyrus* spp. This corroborated well with evidence from other studies indicating declining stocks of *T. zillii* in the lake (Ojuok et al. 2007; Njiru et al. 2008; Oyugi et al. 2011). In a related study, Ibrahim (2012) found higher prevalences and mean abundances of monogeneans in *T. zillii* in Lake Manzalah, Egypt. However, in the study by Ibrahim (2012) cultured and wild *T. zillii* were compared and cultured fish had higher prevalence and mean abundance than wild fish. This was attributed to the high stocking density of cultured fish. Conversely, a low stocking density will imply a lower prevalence and intensity of monogeneans. Among monogenean dactylogyroids, the genus *Cichlidogyrus* Paperna, 1960, infesting the gills, and *Enterogyrus* Paperna, 1963, endoparasitic in the stomach, are host specific to diverse cichlid fish species from Africa (Paperna 1996). Monogeneans have been reported to cause severe mortalities in fish hatcheries in Nigeria (Obiakezie and Taege 1991) and South Africa in catfish, black bass and freshwater ornamental fish. Overcrowding of fish into culture ponds or tanks, together with different environmental and management factors, promote heavy infestations, which can lead to productive losses, tissue damages and in some cases mortality (Hecht and Endemann 1998). It was not possible to determine the species of *Dactylogyrus* from *B. paludinosus* and *Cichlidogyrus* recovered from cichlid fish in this study from a morpho-anatomical analysis of the opisthaptor and reproductive tubes. This was partly because most literature on records and the drawings in identification keys available were of species of the genus *Cichlidogyrus* from West Africa mainly (Khalil 1971; Pariselle and Euzet 1995; 1998; 2009; Paperna 1996; Le Roux and Avenant-Oldewage 2010; Mendlová et al. 2010; Řehulková et al. 2013), while the most detailed book by Bykhovskaya-Pavlovskaya et al. (1962) although sufficient could not permit such a species determination of the *Dactylogyrus* sp. Moreover, there were no records from Kenya of *Cichlidogyrus* spp. which could present the possibility of finding new species from these hosts as reported elsewhere (Bukinga et al. 2012; Gillardin et al. 2012; Řehulková et al. 2013). For these reasons, morpho-genetic species identification is currently on-going and the results will be published separately.

There was a good mix of monoxenous and heteroxenous parasites hence relatively high diversities were observed in *O. leucostictus*, *T. zillii* and *B. paludinosus* (Table 4). *Trichodina* sp. was also prevalent in the *O. leucostictus*, *T. zillii,* and *B. paludinosus*. These fish were also introduced into the lake (*O. leucostictus* in 1956, *T. zillii* in 1956 and *B. paludinosus* in 1920) (Gherardi et al. 2011) although they are within their native range unlike *C. carpio*. Therefore, it appeared that they have had enough time to acquire diverse parasite taxa as was observed in this study. These findings are similar to other studies that have reported a high diversity and richness of parasites in native fish (Kvach and Stepien 2008; Pèrez-Ponce de Leon et al. 2000; Kàlmàn 2012).

Although Aloo (2002) did not identify the species of *Contracaecum* found in *O. leucostictus* and *T. zillii*, the occurrence of a nematode identified genetically as *C. multipapillatum* in *O. leucostictus* in this study was similar to findings of *Contracaecum* sp. by Aloo (2002) with regard to prevalence (49.2% present study and 51.8% in Aloo 2002) and site of infestation. However, significantly higher prevalences of *C. multipapillatum* in *T. zillii* have been found in this study (25.8%) compared to the reported 2% by Aloo (2002). In this study *C. multipapillatum* in *T. zillii* were found un-encysted in the pericardial cavity as contrasted to the encysted state in the findings of Aloo (2002). Species of *Contracaecum* are generally known to occur either freely in the abdominal and pericardial cavities or encysting in musculature (Florio et al. 2009). The species of *Contracaecum* infesting *B. paludinosus* is yet to be identified.

Metacercariae of *Tylodelphys* sp. were the most frequent digeneans found in *O. leucostictus* and *T. zillii*, probably due to a huge diffusion of suitable snail hosts in the environment under study. *T. zillii* may have had a higher prevalence of *Tylodelphys* sp. due to its possible interaction and close proximity to snail intermediate hosts since they prefer shallow vegetated habitats/zones (Teugels and Thys van den Audenaerde 1991) as compared to *O. leucostictus* which dwell at inshore areas (Trewavas 1983). However, *B. paludinosus* was the only fish under this study found not to be infested with these metacercariae of diplostomatids. This was surprising as diplostomatid metacercariae have been reported in *B. paludinosus* in Transvaal dams in South Africa with 100% prevalence (Paperna 1996) and in other related fish such as *B. barbus* (Laimgruber et al. 2005; Nachev and Sures 2009). This could possibly be due to the particular ecological conditions of the microhabitat in which they were sampled, as they were caught in an abstraction channel draining from the lake. Additionally, the behavior of *B. paludinosus* to migrate to rivers (Skelton 1993) to breed may have reduced its chances of infestation with diplostomatids in Lake Naivasha because earlier studies on macro-invertebrates have shown that molluscan intermediate hosts of diplostomatids occur in the lake (Clarke et al. 1989) but not in the rivers draining it (Barnard and Biggs 1988).

Although the lens specific parasite *Diplostomum spathaceum* is unknown from water bodies in Africa, several infestations by other diplostomatid metacercariae have been reported, usually invading the anterior or vitreous humor (Florio et al. 2009; Otachi 2009). Eye infections by *Diplostomum* spp. metacercariae are considered of detrimental impact to fish activity and survival since feeding and therefore growth rates of the infested fish are greatly hampered. The greatest damage is caused when the lenses are infested by a non-encysted metacercariae of *D. spathaceum*, while eye flukes that live in vitreous humor as *Tylodelphys* spp. or other *Diplostomum* spp., are not yet correlated to pathological effect (Paperna 1996). Our findings reveal this kind of non-encysted metacercariae of *Tylodelphys* sp. with both a high prevalence and intensities comparable to earlier findings by Florio et al. (2009) and Otachi (2009) in cultured *Oreochromis niloticus* in Kenya.

Metacercariae of *Tylodelphys* sp. were found in almost all fish species examined in this study except for *B. paludinosus*, confirming the studies by Stables and Chappell (1986) on the ubiquitous presence of eye flukes in fresh and marine water fish. However, this is the first report of this parasite in Lake Naivasha. The current study has therefore partly confirmed the findings of earlier studies on fish parasites in Lake Naivasha by Amin and Dezfuli (1995), Aloo and Dezfuli (1997) and Aloo (1999; 2002). However, new parasite species such as ectoparasitic protozoans, monogenetic trematodes *Dactylogyrus* sp., *D. minutus*, *D. extensus*, *Cichlidogyrus* spp. and *Tylodelphys* sp. have been recovered in this study.

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CHAPTER THREE

Morphometric and molecular analysis of *Tylodelphys* sp. metacercariae (Digenea: Diplostomidae) from the vitreous humor of four fish species from Lake Naivasha, Kenya

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Short title: *Tylodelphys* sp. metacercariae from Lake Naivasha, Kenya.

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Abstract
Even in the relatively well characterized faunas of the developed world, it is difficult to discriminate species of metacercariae in the Diplostomidae using morphology, infection site, or host use. The taxonomy, diversity and ecology of diplostomids infecting freshwater fishes in the African continent are particularly poorly known, but recent morphometric and genetic studies have revealed four species of diplostomids in the eyes and brains of siluriform fishes. In the present study, diplostomid metacercariae were collected from the eyes of 288 fish comprising two species within the Cyprinidae (*Cyprinus carpio*, n=145 and *Barbus paludinosus*, n=67), two Cichlidae (*Oreochromis leucostictus*, n=56 and *Tilapia zillii*, n=18) and one Centrarchidae (*Micropterus salmoides*, n=2) caught in Lake Naivasha, Kenya. Morphometric (14 characters and 8 indices in 111 specimens) and molecular (sequences from the barcode region of cytochrome c oxidase 1 in 11 specimens) data were used to discriminate species. All fish species except *B. paludinosus* were infected with *Tylodelphys* metacercariae that were initially separated into two types differing mainly in body length. However, this morphological distinction received only intermediate support in quantitative morphological analysis and molecular data indicated both morphotypes were conspecific. All the specimens therefore are inferred to belong to a single unidentified species of *Tylodelphys*, which is not conspecific with any other diplostomid for which comparable molecular data are available, including four diplostomid species known from siluriform fish in Nigeria and Tanzania.

Introduction
The Diplostomidae (Digenea) comprise a large and cosmopolitan group of parasites in which adults infect a wide range of fish-eating birds (Chappell, 1995; McKeown & Irwin, 1995; Valtonen & Gibson, 1997). Fish serve as second intermediate hosts while gastropods are the first intermediate hosts (Hoffman & Hundley, 1957; Chappell, 1995; Niewiadomska, 1996; Morozińska-Gogol, 2007). In fish, diplostomid metacercariae occur in a variety of organs, particularly the eye and central nervous system, where they commonly occur free in tissues. In African freshwater fishes, diplostomid metacercariae have usually been reported in the vitreous humor of eyes or brain cavity (Musiba & Nkwengulila, 2006, Florio *et al.*, 2009; Chibwana & Nkwengulilia, 2010; Otachi *et al.*, 2011; Migiro *et al.*, 2012). Morphological identification of diplostomids has long been recognized to be problematic worldwide. Despite of attempts based

on infection site, chaetotaxy, multivariate analysis of morphometric data (Chappell, 1995; Niewiadomska & Niewiadomska-Bugaj, 1995; Gibson, 1996; Cavaleiro *et al*., 2012), considerable confusion remains. Taxonomic studies of diplostomids from the African continent are mainly limited to a few species described in the 1930s-1960s (King & Van As, 1997). In two recent surveys (Mwita & Nkwengulila, 2010; Chibwana *et al*., 2013) of African diplostomids in siluriform fishes, molecular data revealed three species of *Tylodelphys* in the brain of the North African catfish *Clarias gariepinus* in Tanzania and one species of *Diplostomum* in the lens of the catfish *Synodontis nigrita* from Nigeria, all of which were genetically distinct from the growing number of diplostomid species sequenced from Europe and North America (Galazzo *et al*., 2002; Niewiadomska & Laskowski, 2002; Locke *et al*., 2010a; Locke *et al*., 2010b; Rellstab *et al*., 2011; Cavaleiro *et al*., 2012; Behrmann-Godel, 2013; Georgieva *et al*., 2013).

Here we provide further morphological, molecular and ecological data on African diplostomids. We compare our material from the eyes of fishes in Lake Naivasha, Kenya with data from diplostomids from the brain of *C. gariepinus* collected approximately 700 km away in Tanzania (Chibwana *et al*., 2013). Secondly, we assess host specificity of the diplostomids encountered in five host species in three families from which no molecular data has been obtained from African diplostomids (Mwita & Nkwengulila, 2010; Chibwana *et al*., 2013), namely common carp *Cyprinus carpio* (L. 1758) and straightfin barb *Barbus paludinosus* (Peters 1852), both Cyprinidae; blue spotted tilapia *Oreochromis leucostictus* (Trewavas 1933), redbelly tilapia *Tilapia zillii* (Gervais 1848), both Cichlidae, and largemouth bass *Micropterus salmoides* (Lacepède 1802), Centrarchidae.

Molecular analyses have proved to be powerful tools in identification and discrimination of animal species. The mitochondrial cytochrome oxidase c subunit 1 (COI) gene is useful both for population genetic studies (Avise, 1994) and for discriminating species of animal congeners (Hebert *et al*., 2003). The utility of the ≈650 bp at the 5' end of COI in distinguishing closely related species has led to the designation of this fragment as the core target in DNA barcoding (Hebert *et al*., 2003), and it has provided useful information in diplostomids and other helminths (Locke *et al*., 2010b; Behrmann-Godel, 2013; Chibwana *et al*., 2013; Désilets *et al*., 2013; Georgieva *et al*., 2013; Sereno-Uribe *et al*., 2013).

Materials and Methods

Study area

Lake Naivasha (00º45'S and 36º20'E) covers approximately 160 km^2 in a closed basin 1890m above sea level in the eastern Rift Valley of Kenya (Fig. 1). It is the only freshwater lake in the Rift Valley without a surface outlet but with a substantial exchange with groundwater (Gaudet & Melack, 1981). It is shallow (approximately 6m mean depth) with a volume of 4.6 km^3 (Campbell *et al*., 2003). All the fish present in the lake have been introduced and as such none of the five fish species studied herein are native to the lake (Gherardi *et al*., 2011). The only native and endemic fish in the lake was black lampeye *Aplocheilichthys antinorii*, which was last recorded in 1962 and is believed to be extirpated from the lake (Harper *et al*. 2011). *Barbus paludinosus* was introduced in 1920s and restocked in 1982; *Oreochromis leucostictus* and *Tilapia zillii* were introduced in 1956 and thereafter restocked from populations in Lake Victoria, Kenya (Gherardi *et al*., 2011). *Micropterus salmoides* was introduced in 1929 and restocked in 1940 and 1951 as a sport fish, and *Cyprinus carpio* was accidentally introduced in 1999. Neither *M. salmoides* nor *C. carpio* are within their native ranges (Gherardi *et al*., 2011).

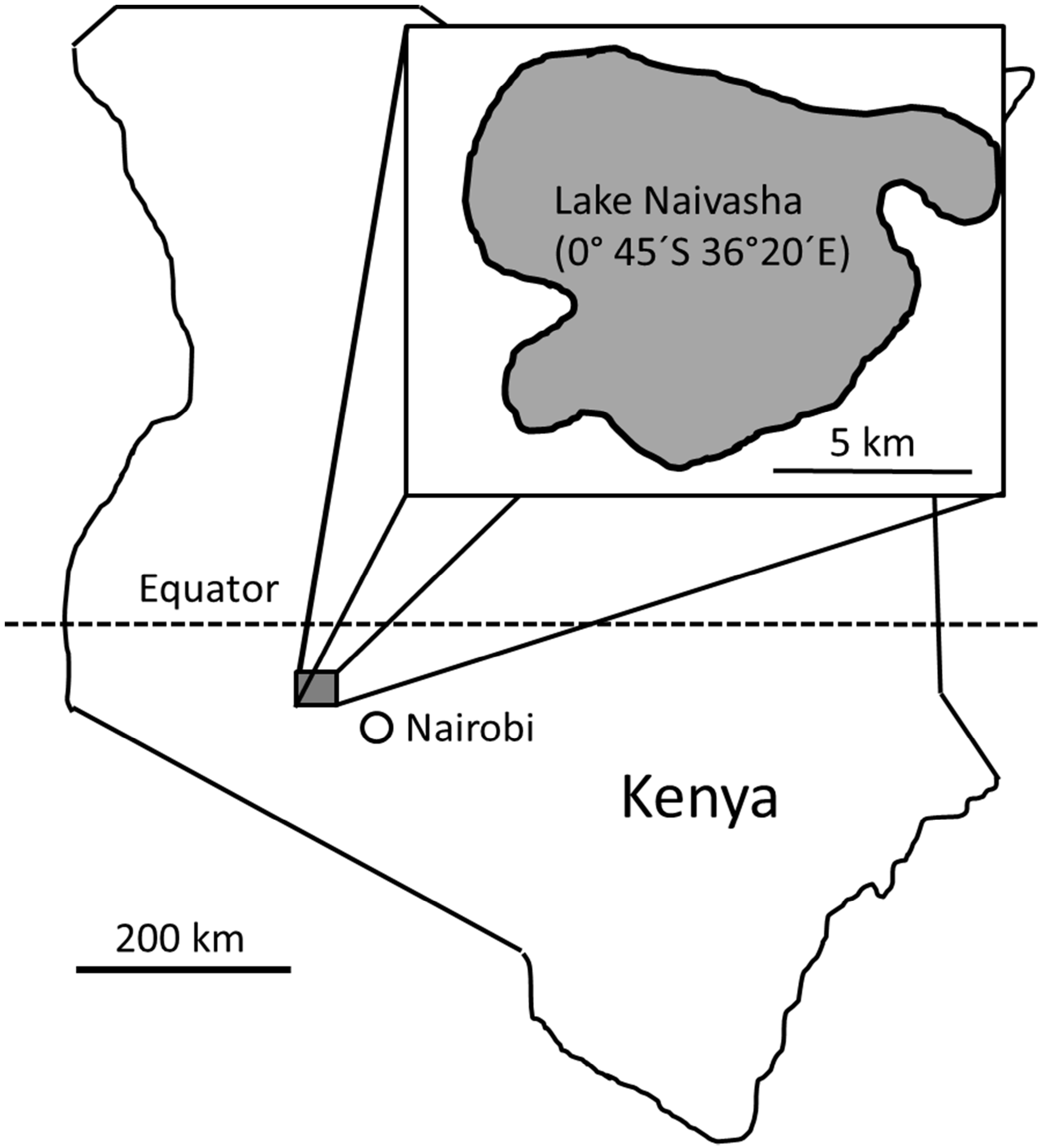

Fig. 1. Map of Kenya showing the location of Lake Naivasha

Fish sampling and ecological analyses

A total of 288 fish were collected from Lake Naivasha using a fleet of gill nets with mesh sizes 2, 2.5, 3, and 4 inches between February and August, 2011. These comprised two cyprinids, *C. carpio* (n=145) and *B. paludinosus* (n=67), two cichlids, *O. leucostictus* (n=56) and *T. zillii* (n=18), and the centrarchid *M. salmoides* (n=2). The fish were transported alive in a fish tank with lake water to the laboratory of the Department of Biological Sciences, Egerton University, Njoro, where they were killed by cervical dislocation (Schäperclaus, 1990), weighed and measured. All parasites in the eyes were counted. Parasites were preserved in either 4% formalin or absolute ethanol. Prevalence, mean intensity and mean abundance were determined as described (Bush *et al*., 1997).

Morphometric analysis

Using an approach previously recommended for distinguishing species in diplostomid metacercariae (Niewiadomska & Niewiadomska-Bugaj, 1995), 14 measurements were made on 111 metacercariae isolated from *C. carpio, M. salmoides, O. leucostictus* and *T. zillii*. An Olympus BX40 compound transmitted light microscope with phase contrast, fitted with a 35mm camera connected to a computer with digital software was used to measure body length (BL), body width (BW), oral sucker length (OL), oral sucker width (OW), length of pharynx (PHL), width of pharynx (PHW), ventral sucker length (AL), ventral sucker width (AW), Brandes' length (HL), Brandes' width (HW), length from centre of ventral sucker to end of oral sucker (AD), length of pseudosucker (LL), width of body at level of bifurcation of intestine (WaBI) and at the mid-length of oral sucker (WaO). In addition, the following indices were calculated: BW/BL (in %), BL×BW/HL×HW, BL×BW/AL×AW, OL×OW/AL×AW, HL×HW/AL×AW, OL×OW/PHL×PHW, AD/BL (in %) and WaO/WaBI. Multivariate analysis of these characters was performed using principal components analysis (PCA) extraction method (Flores & Baccala, 1998), while employing a varimax with Kaiser Normalization rotation technique. The appropriateness of PCA was ensured in several ways. More than 100 metacercariae were measured, which ensured the ratio of cases to variables exceeded 5:1 (Osborne & Costello, 2004). In addition, we iteratively eliminated variables with correlation coefficients $|r| < 0.3$ in the correlation matrix, examined the individual variables' Kaiser-Meyer-Olkin Measure of sampling adequacy (KMO)>0.5 in the anti-image correlation matrix, as well as the overall KMO>0.5 (Hutcheson & Sofroniou, 1999), Bartlett's Test of Sphericity (significant <0.05), and ensuring that communalities in initial extractions were >0.5. Outliers were identified on the basis of factor scores, i.e. metacercariae scoring >3.0 and excluded from the analysis. From the extracted components, the major characters were used as inputs in a two-step cluster analysis (CA) using Euclidean distances and the Schwarz's Bayesian Information Criterion (BIC) as the clustering criterion. Cluster quality was assessed with average Silhouette width, which is the ratio of each specimen's average morphometric distance to others in the same cluster to its smallest distance to specimens in other clusters (Rousseeuw, 1987). Statistical analysis was performed using predictive analytics software (PASW statistics 18, SPSS, Chicago, USA).

Molecular analysis

Specimens used for molecular analysis included representatives from *M. salmoides*, *T. zillii*, *O. leucostictus*, and *C. carpio*. Total DNA was extracted from 16 single specimens [see Moszczynska *et al*. (2009) for protocols], each of which was matched with a morphologically similar specimen collected from the same tissue of the same individual host. The latter specimens were stained with acetocarmine, mounted on slides in Canada balsam, and deposited as paragenophores (Pleijel *et al*., 2008) at the Canadian Museum of Nature (catalogue number CMNPA 2013-0001). The DNA barcode region of cytochrome c oxidase I (COI) was amplified and sequenced at the Canadian Centre for DNA Barcoding in Guelph, Canada, using previously described procedures, diplostomid-specific primers and cycling conditions (Moszczynska *et al*., 2009). Chromatograms were assembled using Geneious (Biomatters, New Zealand) and sequences were aligned and analyzed using MEGA 5.05 (Tamura *et al*., 2011). The resulting sequences were compared with data retrieved using BLAST searches (Altschul *et al*., 1990) and aligned with 215 published contiguous sequences from diplostomids in North America, Europe and Africa (Moszczynska *et al*., 2009; Locke *et al*., 2010a; Locke *et al*., 2010b; Behrmann-Godel, 2013; Chibwana *et al*., 2013; Georgieva *et al*., 2013). The mutation of COI nucleotides was best described by the General-Time-Reversible model with five categories of Gamma distributed rates and by assuming that a fraction of nucleotides are invariable. Published sequences of *Apatemon* (Digenea: Strigeidae) species (Moszczynska *et al*., 2009; Locke *et al*., 2010b) were used to root a phylogeny inferred using Maximum Likelihood, with 1000 bootstrap replicates, as strigeids are closely related and, in some cases, basal to diplostomids (Olson *et al*., 2003). For comparative purposes, outgroup sequences were the same as those used by (Chibwana *et al*., 2013). Molecular (chromatograms, sequences) and other (specimen images, morphological voucher accessions) data are published in project DIPLO at www.barcodinglife.com (see also Genbank accessions KF809484-KF809494).

Results

Among the four infected species, prevalence ranged from 54.5 to 80.6%, mean intensity from 4.7 to 8.7, and mean abundance from 2.5 to 7.1 (Table 1).

Table 1. Hosts and distribution of *Tylodelphys* sp. metacercariae in fish from Lake Naivasha, Kenya in 2011. TL: total length, P: prevalence, MI: mean intenstity, SE:standard error, for n=2 not calculated, MAI: maximum intensity, MA: mean abundance

Host (n)	Host TL (cm) mean (range)	P (%)	MI±SE	MAI	MA±SE
Cyprinus carpio (145)	35 (19-55)	54.5	4.7±0.6	28	2.6±0.4
Barbus paludinosus (67)	8 (3-11)	0			
Oreochromis leucostictus (56)	16 (7-28)	66.1	6.8±1.8	63	4.5±1.2
Tilapia zillii (18)	15 (13-18)	80.6	8.7±2.0	30	7.1±2.0
Micropterus salmoides (2)	29 (28-30)	50.0	5.0	5	2.5

Diplostomid metacercariae were the only parasites observed in fish eyes, and all were unencysted and found exclusively in the vitreous humor. No infection was detected in *B. paludinosus*. The metacercariae resembled *Tylodelphys* morphologically. The shapes varied from a short and ovate form (Fig. 2a) with offset pseudosuckers, which appeared to be distinct from an elongate and rounded form with recessed pseudosuckers (Fig. 2b).

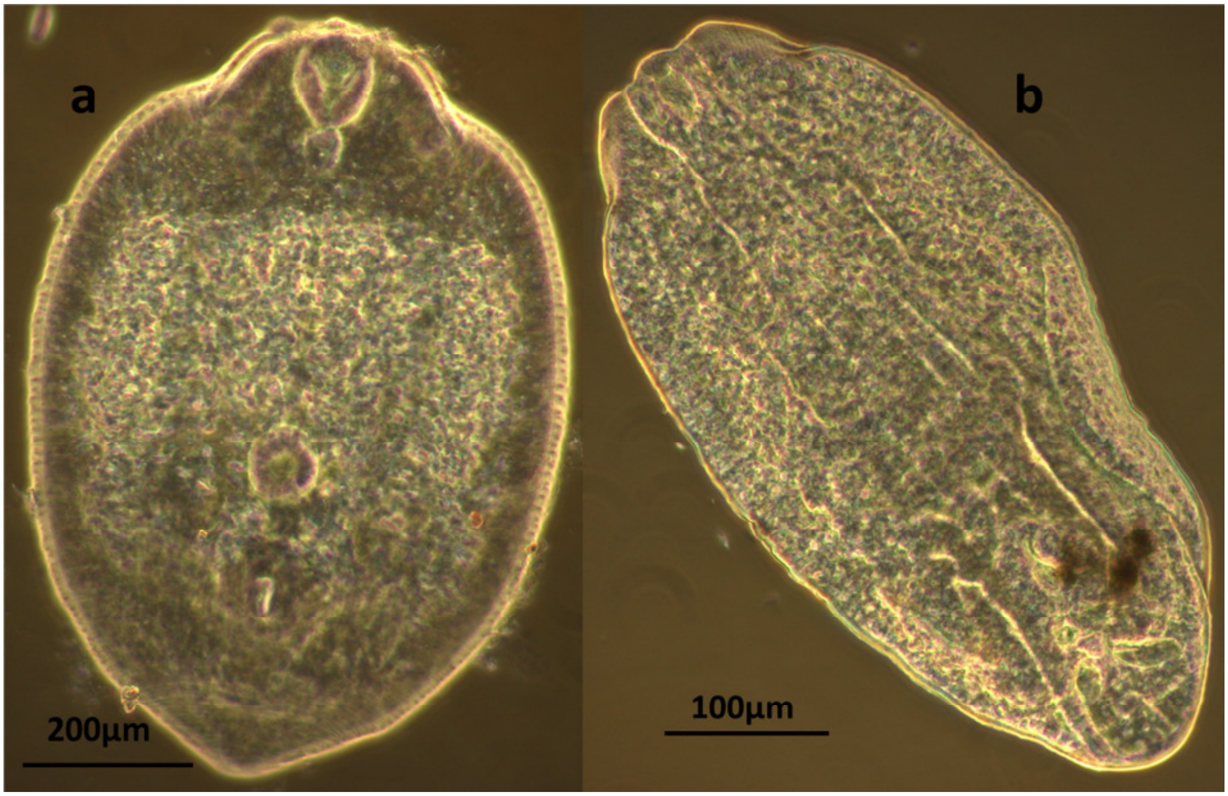

Fig. 2. Whole mounts of representatives of two morphological types of *Tylodelphys* sp. from the common carp *Cyprinus carpio* in Lake Naivasha: (a) short and ovate, (b) elongate and rounded

In all metacercariae, the oral sucker was circular and terminated in a conical pharynx. Posterior to the ventral sucker was a comparatively large holdfast organ. Although there were size and shape variations among the examined metacercariae, the dimensions of many features overlapped with morphometrics from diplostomids from *C. gariepinus* in Tanzania (Chibwana *et al.*, 2013), for example, in the BL, OL, OW, PHL, PHW, AL, AW, HL, HW and AD/BL (%) (Table 2).

Table 2. Morphometric dimensions (µm) of characters and indices (mean, SD, standard deviation, and range) in 111 *Tylodelphys* sp. metacercariae isolated from the vitreous humor of the eyes of fish from Lake Naivasha, Kenya as compared to the averages of two other species (Values in bold in a row indicate measurement overlaps) Data source: [1]Chibwana and Nkwengulila (2010)

	This study			*Tylodelphys* 1[1]	*Tylodelphys* 2[1]
Character	Mean	SD	Min-Max	Mean	Mean
body length (BL)	**490.88**	184.25	239.09-925.05	1022.13	**494.87**
body width (BW)	245.05	71.95	119.03-374.73	217.36	99.74
oral sucker length (OL)	**45.30**	14.24	23.51-73.48	**37.23**	28.45
oral sucker width (OW)	**35.21**	11.08	14.08-78.66	**37.04**	28.45
pharynx length (PHL)	**34.22**	10.23	16.15-65.63	**40.09**	22.96
pharynx width (PHW)	**17.74**	7.40	7.39-37.88	28.83	**22.96**
ventral sucker length (AL)	**32.09**	7.01	19.89-50.30	**36.3**	20.62
ventral sucker width (AW)	**35.41**	8.76	19.54-56.55	**36.32**	20.8
holdfast length (HL)	**58.08**	16.29	30.13-101.58	104.34	**61.19**
holdfast width (HW)	**41.00**	10.35	23.81-71.96	65.3	**36.61**
centre of ventral sucker to end of oral sucker (AD)	281.55	107.04	134.37-640.12	496.49	247.95
pseudosucker length (LL)	43.15	17.69	18.80-103.40		
width at intestinal bifurcation (WaBI)	187.71	69.42	81.90-328.04	908.8	396
mid-length OS (WaO)	88.34	38.73	40.15-213.21	113.33	98.88
BW/BL (%)	52.33	11.60	26.62-83.54	21	21
BLxBW/HLxHW	52.63	26.70	23.84-222.25		
BLxBW/ALxAW	106.36	37.53	38.69-275.27		
OLxOW/ALxAW	1.45	0.66	0.62-6.22		
HLxHW/ALxAW	2.17	0.67	0.53-4.19		
OLxOW/PHLxPHW	2.88	1.15	1.12-8.59		
AD/BL (%)	**57.45**	4.32	41.12-75.91	49	**51**
WaO/WaBI	0.47	0.11	0.16-0.76		

In PCA, three principal components with eigenvalues greater than one were extracted (Table 3), and they together accounted for 83.64% of the total morphometric variance in the specimens from Lake Naivasha (Fig. 3).

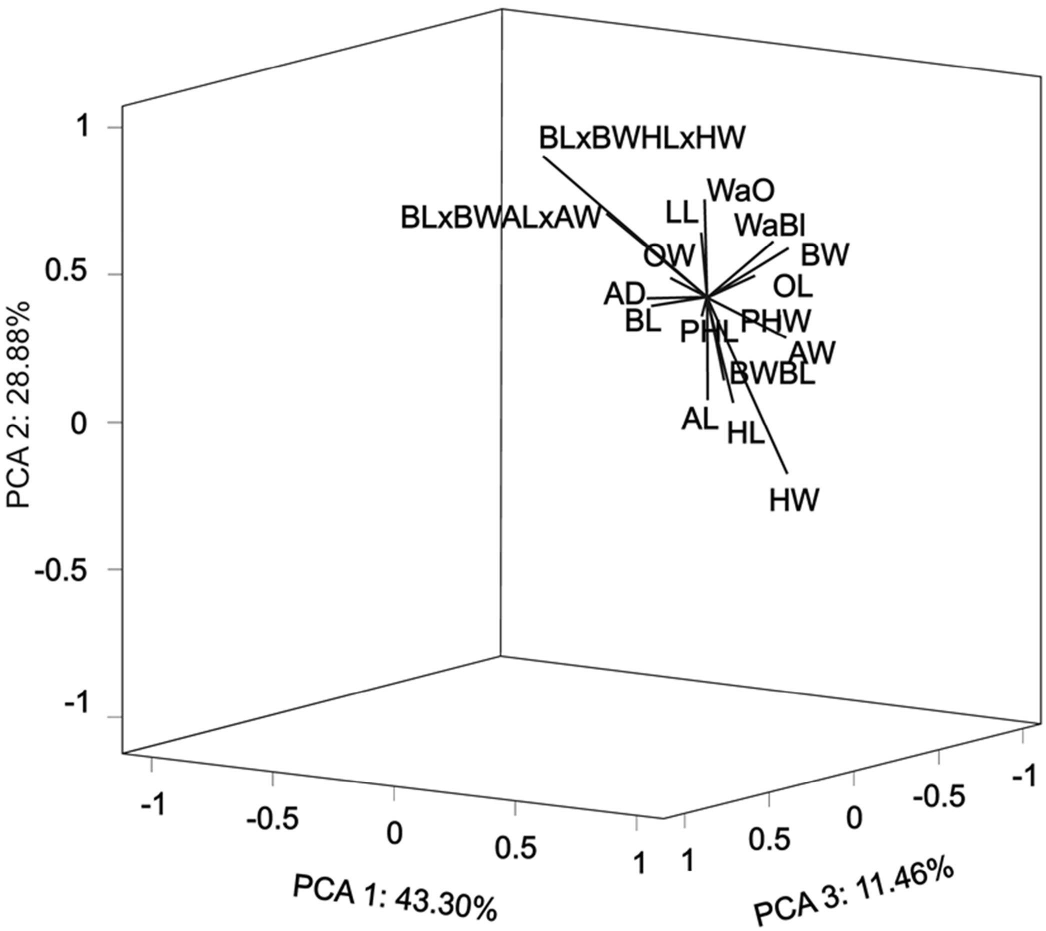

Fig. 3. Principal Components Analysis biplot of measurements of 17 morphological features which loaded strongly (component factor loadings) in 111 specimens of *Tylodelphys* sp. collected from four fish species in Lake Naivasha, Kenya. Highly and positively correlated morphological features point in the same direction, along the same axes. The proportion of total morphometric variance explained by the principal components (83.64 %) are shown on the three axes. BL=body length; BW=body width, OL=oral sucker length, OW=oral sucker width, PHL=length of pharynx, PHW=width of pharynx, AL=ventral sucker length, AW=ventral sucker width, HL=Brandes' length, HW=Brandes' width, AD=length from centre of ventral sucker to

end of oral sucker, LL=length of pseudosucker, WaBI=width of body at level of bifurcation of intestine and WaO=the mid-length of oral sucker.

Along the first PCA axis, which accounted for 43.30% of the total variance, BL, BW, PHW, PHL, AL, AW, OL, OW, HL, HW, AD and WaBI loaded strongly. The second PCA accounted for 28.88% of the total variance, and WaO, BL×BW/HL×HW, BL×BW/AL×AW and LL were associated with it, while the third PCA, which accounted for 11.46% of the total variance, had BW/BL (%) loading on it strongly (Table 4).

Table 3. Eigenvalues, number of components extracted, percent variance and the cumulative variance explained for the components from Principal Components Analyis of 14 morphometrics in 111 diplostomid metacercariae recovered from four fish species in Lake Naivasha, Kenya. Extraction Method: Principal Component Analysis. Cumul.: cumulative

Total Variance Explained

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumul. %	Total	% of Variance	Cumul. %	Total	% of Variance	Cumul. %
1	11.13	65.47	65.47	11.13	65.47	65.47	7.36	43.30	43.30
2	1.68	9.85	75.32	1.68	9.85	75.32	4.91	28.88	72.18
3	1.42	8.33	83.64	1.42	8.33	83.64	1.95	11.46	83.64
4	0.82	4.84	88.47						
5	0.43	2.53	91.01						
6	0.36	2.11	93.12						
7	0.32	1.87	94.99						
8	0.20	1.19	96.18						
9	0.15	0.88	97.06						
10	0.13	0.77	97.83						
11	0.11	0.63	98.46						
12	0.09	0.55	99.01						
13	0.06	0.38	99.39						
14	0.05	0.27	99.66						
15	0.03	0.18	99.84						
16	0.02	0.11	99.94						
17	0.01	0.06	100						

Extraction Method: Principal Component Analysis.

Table 4. Factor loadings of 14 morphometrics in 111 diplostomid metacercariae recovered from four fish species in Lake Naivasha, Kenya on the first three principal components extracted in Principal Components Analyis. Extraction Method: Principal Component Analysis. Rotation Method: Varimax with Kaiser Normalization. a. Rotation converged in 6 iterations.

	Rotated Component Matrix(a)		
	Component		
	1	2	3
ventral sucker length (AL)	0.76	0.18	0.33
ventral sucker width (AW)	0.81	0.34	-0.06
holdfast length (HL)	0.86	0.18	0.32
holdfast width (HW)	0.92	-0.09	0.10
BW/BL(%)	-0.08	-0.04	-0.97
BLxBW/HLxHW	-0.09	0.87	0.07
BLxBW/ALxAW	0.26	0.75	0.21
body length (BL)	0.67	0.51	0.51
body width (BW)	0.74	0.62	-0.17
oral sucker length (OL)	0.75	0.55	0.05
oral sucker width (OW)	0.59	0.56	0.31
pharynx length (PHL)	0.69	0.44	0.24
pharynx width (PHW)	0.74	0.47	0.17
centre of ventral sucker to end of oral sucker (AD)	0.64	0.53	0.50
pseudosucker length (LL)	0.58	0.68	0.11
WaBl	0.73	0.64	-0.10
WaO	0.50	0.76	-0.01

Extraction Method: Principal Component Analysis. Rotation Method: Varimax with Kaiser Normalization. a. Rotation converged in 6 iterations.

Two groups were apparent in CA indicating a possibility of two morphotypes with the larger cluster having 58 individuals (52.3%) and the smaller cluster having 53 individuals (47.7%). The most important variables in discriminating the two clusters were as follows: BL, AD, HL, PHW, AW, WaO, LL, OL, WaBI, BW; all other features contributed little to the discrimination of the two putative morphological types. The overall cohesion and separation of these clusters, according to the average Silhouette coefficient was 0.6, which is fair but not conclusive support for the presence of two morphotypes (Fig. 4).

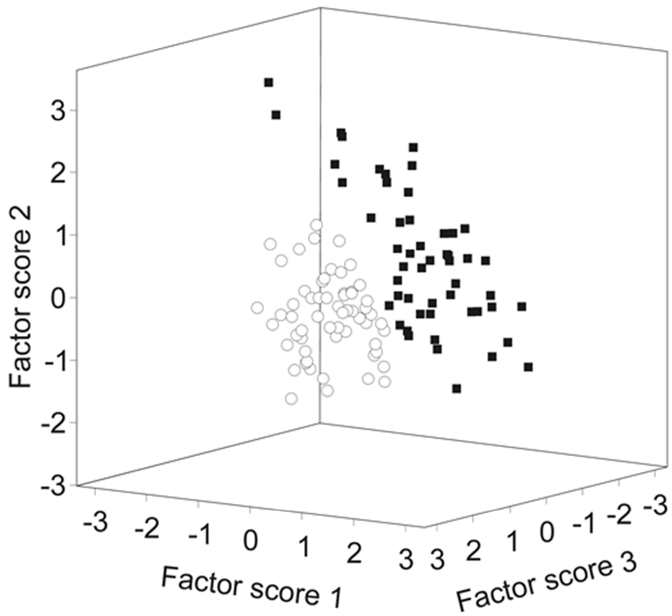

Fig. 4. Cluster analysis of morphometrics for 111 specimens of the *Tylodelphys* sp. with two initially distinguished morphotypes indicated by black squares (short and ovate morphotype) and white circles (elongate and rounded morphotype).

Sequences of COI 200-463-bp long were obtained from 11 of 16 specimens, including representatives from all four infected fish species. The 11 COI sequences differed from each other by mean 0.3% (range 0-1.0%, uncorrected p-distance), and there was no variation in amino acid translations. Among sequences published from 51 other specimens of *Tylodelphys* from Africa, Europe and North America, the most similar are from Tanzanian isolates of *T. mashonensis* (Chibwana *et al*., 2013), which differed from our material by an average of 9.3% (range 7.2 – 11.2%) and in three amino acids. In the phylogeny estimated using Maximum Likelihood, the two sequences 463-bp long were nested within other *Tylodelphys* species (Fig. 5), although statistical support for the *Tylodelphys* clade as whole was not strong. The variable length of the sequence precluded including all of them in phylogenetic analysis, however in separate analyses all fell within the same clade (not shown). Given the reciprocal monophyly and low divergence within our samples, and the high divergence from the most similar samples, lack of strong morphological distinctions, and phylogenetic placement within *Tylodelphys*, we consider the material collected here to represent a single species of *Tylodelphys* distinct from those previously sequenced. Under this hypothesis, the overall mean divergence in CO1 sequences among *Tylodelphys* species is 10.1% (range 7.2%-15.8%), and mean intraspecific divergence is 0.6% (range 0-2.4%).

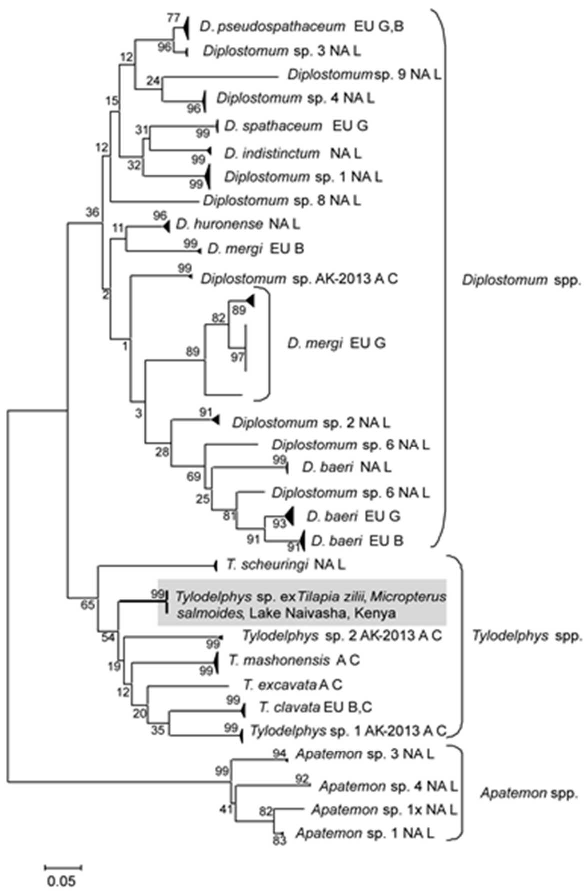

Fig. 5. Relationships among sequences of cytochrome oxidase I including representative sequences obtained from two specimens of *Tylodelphys* collected from two fish species in Lake Naivasha, Kenya in the present study (selected from 11 total similar sequences in order to maximize length and overlap with other data) and 215 sequences from diplostomids published in Genbank, inferred using Maximum Likelihood with 1000 bootstrap replicates, and pairwise deletion of gaps. The alignment was 463 bp long, and all sequences shared at least 297 sites. The vertical height of black triangles is proportional to the number of sequences in a clade. The width

of black triangles and horizontal branch lengths are both proportional to the scale = substitutions per site. Genbank accessions of data used are: KF809484-KF809494 (this study), FJ477183, FJ477185, FJ477186, FJ477194-6, FJ477198-200, FJ477223, GQ292475, GQ292476, GQ292478-502, HM064610, HM064636, HM064637, HM064644, HM064647, HM064660, HM064662-68, HM064670-80, HM064682-97, HM064699, HM064700, HM064702, HM064704-11, HM064909-15, JQ639170-204, JX986859-909, KC685327-60; NA= North America; EU=Europe; A=Africa; B=sequence from Behrmann-Godel 2013; C=sequence from Chibwana *et al*. 2013; G=sequence from Georgieva *et al*. 2013; L=sequence from Locke *et al*. 2010a, b.

Discussion

The diversity and ecology of diplostomids infecting fish from Kenya are poorly known. Our initial observation of two morphotypes led us to suspect the presence of two species in Lake Naivasha and, while this distinction received fair support in quantitative morphological analysis, the presence of two species was effectively ruled out by molecular data. Molecular data have also revealed other apparently morphologically distinct diplostomid metacercariae to be single species (Cavaleiro *et al*., 2012) and in other cases have confirmed separation of species in metacercarial morphotypes (Chibwana & Nkwengulila, 2010; Chibwana *et al*., 2013). Together with other published data (Chibwana & Nkwengulila, 2010), our measurements also confirmed interspecific morphometric overlap in *Tylodelphys*. Most characters and indices were within the range of those in other *Tylodelphys* spp. from the cranial cavity of *C. gariepinus* in Tanzania (Chibwana & Nkwengulila, 2010). Many factors cause phenotypic plasticity and morphological similarities between diplostomid metacercariae, including the species, size and age of hosts and the age, population size and density of metacercariae (Chappell *et al*., 1994; Chappell, 1995; Gibson *et al*., 1996; Niewiadomska, 1996; Chibwana & Nkwengulila, 2010). In retrospect, the morphological variation observed in our specimens was likely due to differences in contraction. Cavaleiro *et al.* (2012) hypothesized that similar phenotypic variation in two genetically identical but morphologically distinct metacercarial forms of *Diplostomum* arose from differences in specimen age. Age structure seems unlikely to be the cause of the morphotypes in diplostomids in our study, for the following reasons. Firstly, Rauch *et al.* (2005) found almost no clones in metacercariae of *Diplostomum pseudospathaceum* in the eyes of sticklebacks, which shows that cercariae are encountered singly, not in simultaneous exposures to batches of clonal cercariae emerging from single snails. Secondly, seasonality of recruitment (e.g., Marcogliese *et al.,* 2001a), which could also give rise to an age-structure in metacercariae, is unlikely to have played a role in our system because of both the local climate and the six-month interval over which we sampled fish. Thus, the two main sources of age-related parasite recruitment (acquisition of same-aged clones, and seasonal recruitment of same-aged parasites) are unlikely to apply in the present study, and fixation effects are a more plausible explanation for the two morphotypes.

The single species encountered here is genetically distinct from all those for which contiguous sequences have been published, including three *Tylodelphys* species from Tanzania. Whether this is a new species can only be determined through morphometric characterization of additional developmental stages, particularly the adult. Our results present an interesting contrast to a recent molecular survey in which three species of *Tylodelphys* were found in the brain of *Clarias gariepinus* in Tanzania (Chibwana *et al*., 2013). It may have been expected that we would recover some or all of the three species of *Tylodelphys* as these authors, and/or that we would observe similar or higher diversity, particularly because we sampled five host species in three families and two orders; instead, a single, oiöxenous *Tylodelphys* species was recovered. The small number (n=11) of specimens from which sequences were obtained may have been a factor in not detecting additional diversity. However, we note comparable sample sizes in other studies. For example, Chibwana *et al*. (2013) sequenced CO1 in 33 diplostomids, but only n=2, 3, 11, 17 were sequenced in any particular species. Facing a similar situation (two genetically identical morphotypes), Cavaleiro *et al*. (2012) sequenced ITS in 38 specimens.

The metacercaria of *Tylodelphys* sp. appears to be a true generalist (Poulin & Mouillot, 2005), as it occurred in similarly high intensities in four fish species in three families and two orders. Previous reports of diplostomid metacercariae in Kenya are from Nile tilapia *O. niloticus* and African catfish *C. gariepinus* (Florio *et al*., 2009; Otachi, 2009; Gichohi, 2010; Migiro *et al*., 2012). Indeed, diplostomids are frequently found in tilapias from all aquatic systems (Florio *et al*., 2009). Infection levels were higher than previously reported in Kenya from farmed tilapia

(Florio *et al*., 2009; Otachi, 2009; Gichohi, 2010; Migiro *et al*., 2012). As discussed by Otachi *et al*. (submitted), the factors influencing variation in infection levels among the fish species may include fish microhabitat and behavior, and subsequent variation in exposure, rather than variation in susceptibility (Valtonen & Gibson, 1997; Karvonen *et al*., 2004). For example, *B. paludinosus* is a riverine species (Welcomme, 1979; Mutia *et al*., 2010) that migrates into River Malewa for breeding, and spends considerable time there (Welcomme, 1979). Gastropods have not been observed in River Malewa (Barnard & Biggs, 1988), although they have been found in Lake Naivasha (Clarke *et al*., 1989), and this lack of contact with the first intermediate hosts of *Tylodelphys* could explain why *B. paludinosus* was uninfected. The other fishes sampled, such as *T. zillii*, prefer shallow vegetated zones within the lake where gastropod densities are higher (Styczynska-Jurewicz, 1959). This overlap in habitat use by the first and second intermediate hosts of *Tylodelphys* sp. is likely to increase the probability of infection (Styczynska-Jurewicz, 1959; Sweeting, 1974; Marcogliese *et al*., 2001b; Otachi *et al*. submitted). Additionally, innate immunity could also be important, as it has been suggested to play a major role in defence in sympatric hosts against the eye fluke *D. pseudospathaceum* (Kalbe & Kurtz, 2006).

This was the first study combining morphological, molecular and ecological analysis of *Tylodelphys* from Kenyan fishes. Quantitative morphometric analysis did not strongly support the distinct status of two initially distinguished types of metacercariae, and molecular data confirmed that both morphotypes represented a single species of *Tylodelphys*. The oiöxenous nature of this species was clear from its high and equitable distribution among four distantly related lacustrine fishes, and its absence in *B. paludinosus* is attributable to the riverine habits of this host. This may reflect different patterns of habitat use and/or interactions with host immunity in the different fish species. Clearly, much remains to be learned about the diversity and distribution of diplostomids in African fishes and molecular data, ideally in combination with appropriate morphological data from adult worms, provide an efficient way of moving forward, particularly in ecological studies.

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CHAPTER FOUR

Trace elements in sediments, blue spotted tilapia *Oreochromis leucostictus* (Trewavas, 1933) and its parasite *Contracaecum multipapillatum* from Lake Naivasha, Kenya, including a comprehensive health risk analysis

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Abstract

This study presents the distribution of 15 major and trace elements in sediments, fish and their pericardial parasites from Lake Naivasha, Kenya. The lake is one of the few freshwater lakes in the Great Rift Valley and is under strong anthropogenic pressure mainly due to agricultural activities. Its fish provide a valuable protein source for approximately 100 000 people in the area. Fish and their parasites have been acknowledged as indicators of environmental quality due to their accumulation potential for both essential and non-essential trace elements. A total of 34 specimens of the blue spotted tilapia *Oreochromis leucostictus* and pooled samples of their pericardial parasite, the anisakid nematode *Contracaecum multipapillatum* (Larvae 3), were examined. Element concentrations were determined by inductively coupled plasma-optical emission spectroscopy (ICP-OES) and graphite-furnace-atomic-absorption spectrometry (GF-AAS). The concentrations of elements in the sediments reflected the geology of the area and did not point to pollution: none of the investigated trace elements including Pb, Cd, Cu and Zn showed elevated values. In contrast, concentrations in the fish muscle were elevated for Li, Sr, Cd and Zn, with high target hazard quotients (THQ>0.1) indicating a potential health risk to the consumers of this fish. Fish liver showed significantly higher concentrations of the trace elements Fe, Mn, Cd and Cu compared to the muscle and *C. multipapillatum.* In the parasite, Zn had the highest concentration, but the worms only minimally accumulated trace elements in relation to their fish host.

Key words: Trace elements, *Contracaecum multipapillatum*, *Oreochromis leucostictus*, bioindicator, THQ, Lake Naivasha

Introduction

Trace elements are natural components of the aquatic environment (Ochieng et al. 2009), but their levels have significantly increased in some areas due to domestic, industrial, mining and agricultural activities, leading to pollution (Leland et al. 1978; Canli and Kalay 1998; Al-Weher 2008). In a recent report of the world's worst pollution problems, heavy metals are among the top threats; they jeopardize the health of tens of millions of people (McCartor and Becker 2010).

The report further states that more than 100 million people worldwide are estimated to be at risk from toxic pollution at levels above the international health standards. In Kenya, the need for studies on pollutants in aquatic ecosystems and their biota was recognized more than three decades ago as a result of rapid population expansion, growing industries and increasing urbanization (Onyari 1981). Since then, several studies have been conducted on trace elements including heavy metals in different lakes in Kenya, among them Lake Naivasha. Tarras-Wahlberg et al. (2003), Ochieng et al. (2007), Kamau et al. (2008) and others have already investigated the levels and sources of heavy metals such as Cu, Cd, Zn, Fe, Pb and Ni in Lake Naivasha. In addition, Njogu et al. (2011) and Mutia et al. (2012) showed that the most important sources of heavy metal pollution in Lake Naivasha basin are its major tributary, the Malewa River, and some flower farms surrounding the lake. Therefore, the studies of the latter and other authors on trace elements and heavy metals in the lake have focused on the anthropogenic sources of these metals and concentrations in the muscle of fish. No studies from Lake Naivasha have partitioned trace elements in different fish organ tissues or examined the accumulation of trace elements in fish parasites; trace elements such as Li, Mo, Sr, Rb and others have also been neglected.

Fish parasites have been recognized for their bioindicative potential regarding pollution in the last decades. Amongst other properties, certain parasites have shown a high potential for metal accumulation in relation to their fish hosts. Studies have mostly involved endo-helminthes, of which acanthocephalans and cestodes appear to be the best investigated taxa. Amongst others, Sures et al. (1994a, b, c), Sures et al. (1997a, c), Siddall and Sures (1998), Sures et al. (1999), Sures et al. (2000a, b), Scheef et al. (2000), Sures et al. (2003), Schludermann et al. (2003), Zimmermann et al. (2005), Jirsa et al. (2008), Oyoo-Okoth et al. (2010a, b) and Oyoo-Okoth et al. (2012) have shown the impressive ability of those intestinal worms to accumulate heavy metals to a high degree, showing biocontration factors (BCF) up to several thousand for parasites compared to fish muscle, but varying considerably from species to species.

Only a few studies are available on nematodes (Sures et al. 1998; Zimmermann et al. 1999; Barus et al. 2001; Azmat et al. 2008; Dural et al. 2011; Morsy et al. 2012; Nachev et al. 2013). From Africa, little research has been done linking parasitism and pollution. Regarding heavy metal accumulation, Retief et al. (2006), Oyoo-Okoth et al. (2010a, b) and Oyoo-Okoth et al. (2012) have examined cestodes and their fish hosts from South African river systems.

The blue spotted tilapia *Oreochromis leucostictus* is a very important commercial fish in Lake Naivasha. It is benthopelagic and occupies the inshore zone, being common in lagoons. It feeds on phytoplankton and detritus. The parasite chosen for this study was an anisakid nematode, *Contracaecum multipapillatum* (L3), from the pericard of the fish host because it was the only helminth parasite occurring in sufficient numbers and quantity. Anisakid nematodes are widespread and abundant in many regions around the world, including Africa, Europe, Asia, Australia, North and South America (Barson 2004; Barson and Marshall 2004). They therefore meet the basic criteria of sentinel organisms. They have been widely reported in cichlids and catfish from several African countries such as Egypt (Amin 1978), East Africa (Malvestuto and Ogambo-Ongoma 1978; Aloo 2001; Florio et al. 2009) and South Africa (Prudhoe and Hussey 1977; Mashego and Saayman 1981; Van AS and Basson 1984; Boomker 1994). Larval stages of *Contracaecum* spp. usually occur in the body cavity and mesenteries of fish, whereas the adults occur in the intestine of piscivorous birds, especially in pelicans, cormorants, herons and darters.

The current study was designed to determine a wide spectrum of trace element concentrations in sediments, in the muscle and liver of the blue spotted tilapia and in its pericardial parasite *C. multipapillatum*. The aim was to study bioaccumulation in both the fish host and its parasite and therefore gain new insights into this issue. In addition, a comprehensive health risk analyses with parameters adapted to Kenyan realities was undertaken to show possible risks for human fish consumers, including some elements that have been neglected so far in this area. This is a step forward in shedding new light on potential problems arising from fish consumption from Lake Naivasha.

Materials and Methods

2.1 Study site

The study was conducted in Lake Naivasha, Kenya, between February and August 2011. The lake is situated at 00º45'S and 36º20'E (Kamau et al. 2008) in a closed basin at an altitude of 1890 m above sea level and covers approximately 160 km^2 in the eastern Rift Valley of Kenya (Fig. 1). It is the only freshwater lake in the Rift Valley without a surface outlet but with a substantial exchange with groundwater (Gaudet and Melack 1981; Clarke et al. 1990). It is shallow (approximately 6 m mean depth) with a volume of 4.6 km^3 (Campbell et al. 2003). It is bordered by papyrus *Cyperus papyrus* in some sections, and the overall composition of aquatic macrophytes is in a state of change (Tarras-Wahlberg et al. 2002), probably due to anthropogenic influences such as destruction of littoral vegetation, eutrophication and plant and animal introductions (Kitaka et al. 2002; Gherardi et al. 2011). Most of its freshwater inflow (approximately 80-90%) comes from the Malewa River (Hickley et al. 2002; Kamau et al. 2008) with an estimated mean annual flow of 153 million m^3 and a catchment area of 1730 km^2, followed by the Gilgil River with an estimated average annual flow of 24 million m^3 and a catchment area of 420 km^2; an additional river, the Karati River, flows only intermittently (Ase 1987; Abila et al. 2008; Harper et al. 2011). The basin area is generally semi-arid, receiving a mean annual rainfall of 620 mm, while the mean annual evaporation is estimated at 1735 mm. Evaporation generally exceeds precipitation throughout the year except at peak rainfall, with the rainfall trend being bimodal with a major peak in April-May and a minor peak in October-November (Abiya 1996). The water from Lake Naivasha is used extensively for agriculture (horticultural farms: approx. 77 million m^3/year), geothermal power generation (approx. 1 million m^3/year) (WWF 2011), domestic water supply, commercial fishing, tourism and recreation as well as ranching and game farming (Abiya 1996). The key environmental problems facing the lake are water abstraction leading to changes in water level, eutrophication, pollution, invasive species as well as decline in fish stocks and biodiversity (Otiang'a-Owiti and Oswe 2007; Harper et al. 2011).

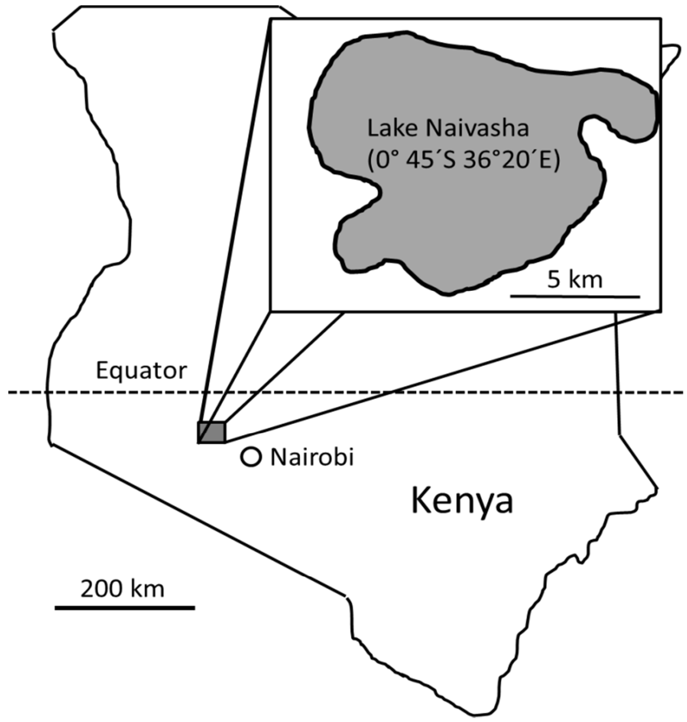

Fig. 1 showing the location of Lake Naivasha in Kenya

2.2 Water and sediment sampling

In situ measurements of the pH, dissolved oxygen, conductivity and temperature were performed 10 cm below the water surface at the point where the fishing nets were set using a portable Hach Field Case multi-parameter meter (Model Multi HQ40d, USA). A total of 10 water samples (about 20 ml each) were obtained biweekly from February to August 2011 from the same depth using a syringe and were immediately filtered with 0.2 µm nylon filters into pre-cleaned (acid washed) high density polyethylene (HDPE) bottles and acidified with 0.1 mL concentrated nitric acid (TraceSELECT®, Fluka). Similarly, 10 sediment samples were obtained biweekly from February to August 2011 using stainless Ekman grab samplers; caution was taken not to obtain the samples from sediment directly in contact with the surface of the Ekman grab sampler. The samples were placed into 50 mL polypropylene (PP) centrifuge tubes. In the laboratory, samples were first sieved through a 1 mm colander to remove pebbles, and the small fraction (<1 mm) was dried at 105ºC to a constant weight and used for further analyses.

2.3 Fish sampling and parasitological examination

In total, 34 fish were caught by fishermen using gill nets of 2.5 inch mesh size (2-4 fish obtained biweekly from February to August 2011). Fish were transported alive in aerated tanks to a laboratory at the Biological Sciences, Egerton University, where they were killed by cervical dislocation and dissected following standard procedures used in parasitological analyses. The fish were weighed and their total length (TL) determined. Where found, *C. multipapillatum* was collected from the pericardium of the fish; some were preserved in 4% formaline, others in absolute (95%) ethanol for further identification purposes, and still others were thoroughly rinsed with double distilled water and dried in the oven to weight constancy at 60ºC for trace element determinations. Fish tissues were carefully obtained using a ceramic knife and plastic tweezers. Approximately 1 g of dorso-ventral muscle and the identical mass of liver was washed with double distilled water and then dried in the same way as parasite samples.

2.4 Trace elements determination

The water, dried sediment, dried fish tissues and parasites were transported to the University of Vienna, Institute of Inorganic Chemistry. The sediment samples were first homogenized with mortar and pestle. Samples were then split, with about 1 g weighed into Teflon bombs for acid leaching, using 8 mL of HNO_3 34% (*Trace*SELECT® Fluka) in a microwave MARS XPRESS system (CEM Corporation), and another 1 g into crucibles for determination of organic matter (TOC) content using the loss on ignition (LOI) method determined as percent AFDW (Ash Free Dry Weight). After treatment in the microwave oven, samples were transferred quantitatively into 15 or 20 mL flasks and brought to volume with millipore water. Before measurement, samples were filtered through 0.2 µm PTFE syringe filters (VWR) and, where necessary, diluted.

Fish tissues and parasites (where possible 0.2 g dry weight) were digested in 8 ml of HNO_3 35% (*Trace*SELECT® Fluka). Reference samples comprising 0.2 g (dry weight) of fish protein DORM-3 and 0.2 g (dry weight) of marine sediment PACS-2 obtained from the National Research Council Canada (NRCC) were digested and diluted in the same manner as described above for fish tissues and sediments, respectively. To determine the detection limits, analytical blanks were prepared without insertion of a sample. Elements were determined using inductively coupled plasma-optical emission spectrometry (ICP-OES) using an Optima 5300DV (Perkin Elmer). When necessary, graphite furnace-atomic absorption spectrometry (GF-AAS) using a PinAAcle 900Z (Pelkin Elmer) was used. The results for the reference samples showed recovery rates between 95% and 104%, demonstrating the appropriateness of the sample preparation used.

2.5 Risk assessment

The target hazard quotients (THQ) were determined for the trace elements in order to assess the risk to the people who consume *O. leucostictus* in the area. THQ is the ratio between the potential exposure to a substance and the reference dose (level at which no adverse effects are expected) (USEPA 2012). If the resulting THQ is equal to or less than 1 there will be no significant risk regarding the health of individuals that consume these fish. If the THQ is greater than 1 it is indicative of a possible health risk associated with the consumption of the respective metals within the muscle of the fish. Furthermore, a THQ of 0.1 was later suggested for non-carcinogens to account for additive effects (USEPA 2012). Even though the standard fish equation inputs for fish consumption are given (USEPA 2012), we adapted some variables in order to reflect the local reality because of the wide discrepancies. For example, the body weight

(BWa) given by USEPA (2012) is 70kg whereas the average body weight in Africa is 60.7kg (Walpole et al. 2012) and the per capita fish consumption of Kenya is 5.2kg (Lokuruka 2009) which translates to a daily consumption of 14.2g/day while USEPA (2012) set the value at 54g/day. The equation for determining THQ according to USEPA (2012) is:

$$\mathrm{THQ} = \frac{\mathrm{EFr} \times \mathrm{EDr} \times \mathrm{IRFa} \times \mathrm{C}}{\mathrm{RfDo} \times \mathrm{BWa} \times \mathrm{AT}}$$

Where the variables are defined as follows: EF_r exposure frequency (350days/year), ED_r exposure duration, (30years), IRF_a fish consumption per day (0.0142kg/day) since the per capita 5.2kg/year, in Kenya (Lokuruka 2009), C is the metal concentration in edible portion of fish (mg/kg ww), RfDo is the reference dose, oral (mg/kg/day, according to the updated 2012 Regional Screening Level (RSL) in fish ingestion table (USEPA 2012), BW_a body weight, adult 60.7kg, for Kenya (Walpole et al. 2012), AT Averaging time for non-carcinogens (365days/year). The results for metal concentrations in mg/kg dry weight were multiplied by 0.2 to refer to wet weight.

Additionally, the mean concentrations of the trace elements were also compared with FAO/WHO recommended values. In doing all these comparisons, an average body weight of 60.7kg (adult) and a daily fish consumption of 14.2g (5.2kg per year) were adhered to as defined above.

2.6 Statistical analysis

Data was analyzed using Predictive Analytics Software (PASW statistics 18, SPSS, Chicago, USA). A non-parametric Friedman's test was used to analyze differences for Si, Al, Fe, Cu, Zn, Mn, Sr, Pb and Cd because data were not normally distributed. Multiple comparisons were performed using the Wilcoxon's signed-rank test with a Bonferroni correction of the level of significance (α=0.05). A Wilcoxon's signed-rank test was used in analysis for Rb and Mo differences.

Descriptive statistics for Li were also determined. All graphs were drawn using SigmaPlot 10.0 (Systat Software Inc., Chicago, IL, USA). The Bio concentration factors (BCF) were determined according to Sures et al. (1999) using the formula:

$$\text{BCF (x/y)} = \frac{\text{concentration of trace element in x}}{\text{concentration of the trace element in y}}$$

in which the variables x and y stand for biological compartments that are compared to each other, such as sediment, fish muscle, fish liver and parasites.

Results

Physico-chemical parameters

A summary of the physico-chemical parameters monitored during the study is presented in Table 1.

Table 1. Physico-chemical parameters of water (10cm below surface) measured *in situ* during the study period (February-August 2011) in Lake Naivasha and dissolved major cations (0.2µm filtered), n=10.

Parameter	Mean	Min-max
Temp. (°C)	21.4	19.6-24.4
pH	9.0	8.4-9.6
Diss.oxygen (mg/L)	6.6	5.5-8.8
Diss oxygen (% sat)	93.9	70.7-126.1
Conductivity (µS/cm)	355.0	238-426
Na (mg/L)	25.4	22.9-29.5
K (mg/L)	10.9	10.0-13.4
Ca (mg/L)	25.8	20.9-31.6
Mg (mg/L)	8.4	7.8-10.6

Characteristics of the fish samples

The average length (TL) of the *O. leucostictus* sampled was 15.6±0.3cm (6.7-28.4) and weight 69.0±6.9g (5.0-418.0) (mean±SE). The prevalence of *C. multipapillatum* was 51.8% with a mean intensity of 2.3. The parasite *C. multipapillatum* was located in the pericardium exclusively.

Trace elements concentration in the sediments

The order of concentration of trace elements in sediments was Al>Fe>Mn>Zn>Rb>Sr>Li>Pb>Cu>Cd. Mo was below detection limit. Most of the trace elements analysed in sediments were positively correlated ($p<0.05$) with the organic matter content of sediments with the exception of Sr (Table 2).

Table 2. Levels of elements in sediment samples from Lake Naivasha and organic matter content as compared to literature data of other Rift Valley lakes and the shale (mean±SE, mg/kg dw n=10 superscripts refer to data sources)

Element	Mean[a]	SE	r (TOC)	Lake Nakuru[1]	Lake Naivasha[2]	Lake Bogoria[1]	TEF[3]	Shale[4]
Li	17	6	0.85					66
Na	908	140		98100		79200		9600
Mg	3213	217						15000
Al	31677	2765	0.85					80000
K	6457	504		32800		28700		26600
Ca	8310	595						22100
Mn	633	306	0.76		1118			850
Fe	24803	6781	0.86	46300		53300		47200
Cu	11.5	6	0.90		10.33		35.7	45
Zn	138	51	0.89	236	229.6	222	123	95
Rb	82.9	32	0.89	120		108		140
Sr	63.3	10	0.17ns	48		39.4		300
Mo	<1.25							2.6
Cd	0.34	0.15	0.95		0.73		0.6	0.3
Pb	12.5	4	0.84		25.34		35	20
OM(%)	17.9	2.8						

[a] this study, r correlation coefficient for organic matter and the trace elements, ns correlation not significant $p<0.05$. TEF means the Threshold effect level as a sediment quality guideline. Shale represents the Earth's crust geochemical background value of sedimentary rocks. Data sources: [1]Jirsa et al. (2012), [2]Ochieng et al. (2007), [3]MacDonald et al. (2000) and [4]Turekian and Wedepohl (1961).

Bio concentration factors (BCF) for trace elements in *O. leucostictus*

Only a few trace elements showed BCF values >1. These included: Li, Cu, Cd and Zn (Table 3). *C. multipapillatum* had only Cd with a BCF greater >1. Pb had a BCF of 7.04 in *C. multipapillatum* compared to the host muscle.

Table 3. Bio concentration factors (BCF) of selected trace elements in *O. leucostictus* and *C. multipapillatum* from Lake Naivasha (mean)

Element	Muscle /sediment	Liver /sediment	*C. multipapillatum* /sediment	*C. multipapillatum* /muscle
Li	17.862			
Al	<0.001	<0.001	<0.001	0.746
Mn	0.004	0.032	0.002	0.500
Fe	0.001	0.075	0.002	2.937
Cu	0.047	40.859	0.092	1.963
Zn	4.370	0.528	0.326	0.075
Rb	0.217	0.183		
Sr	0.041	0.038	0.01	0.250
Cd	2.156	7.08	3.409	1.581
Pb	0.002	0.009	0.014	7.042

Trace element concentration in *O. leucostictus*

The liver had the highest concentration of the trace elements Fe, Mn, Cu and Cd compared to the muscle and *C. multipapillatum* ($p<0.05$) (Table 4). The concentration was, in descending order as follows liver>*C. multipapillatum*>muscle for Mn, Fe, Cu and Cd (Fig. 2). Although Al and Si concentrations were not statistically different between the muscle and *C. multipapillatum* ($Z=-0.744$, $p>0.05$ and $Z=-0.180$, $p>0.05$, respectively), they were both significantly lower than in the liver ($p<0.05$). Zinc (Zn) was highest in the muscle compared to the liver and *C. multipapillatum* ($p<0.05$) (Fig. 2). Strontium (Sr) concentration was not significantly different between the muscle and the liver ($Z=-1.222$, $p>0.05$) but was higher in both than in *C. multipapillatum* ($p<0.05$) (Table 4). Zn and Sr showed the pattern: muscle> liver>*C. multipapillatum* in a descending order of concentration. Mo was below the detection limit for the muscle but was higher in the liver compared to *C. multipapillatum* ($p<0.05$). Li was below detection limit in liver and in *C. multipapillatum*. Rb was below the detection limit in *C. multipapillatum* but was not significantly different between the muscle and the liver ($Z=-2.009$ $p>0.05$). The order of trace element concentration in the muscle was Zn>Li>Si>Rb>Fe>Al>Sr>Mn>Cd>Cu>Pb, while that of the liver was Fe>Cu>Si>Zn>Mo>Mn>Rb>Al>Cd>Sr>Pb; in *C. multipapillatum* the order was Zn>Fe>Si>Al>Mo>Cd>Mn>Cu>Sr>Pb.

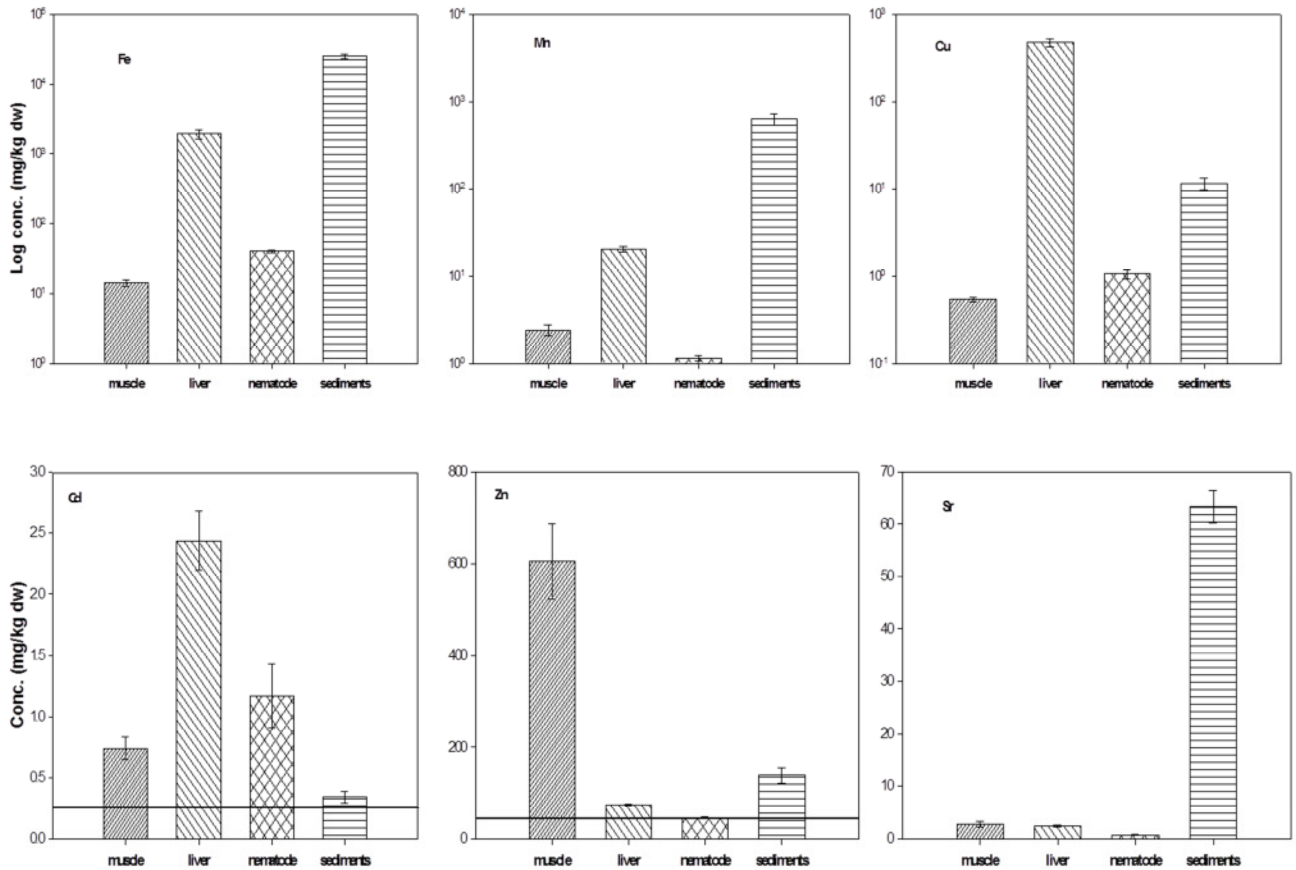

Fig. 2 pattern of concentration of some of the trace elements in various matrices from Lake Naivasha

Table 4. Trace elements concentration in muscle, liver and *C. multipapillatum* from *O. leucostictus* in Lake Naivasha: values given are means (mg/kg dw). SE: standard error. Values followed by* in the same line do not differ significantly (p<0.05).

Element	Muscle	SE	Liver	SE	*C. multipapillatum*	SE
Li	299	31	<0.5		<0.5	
Al	5.24*	0.74	11.11	1.75	3.91*	0.99
Si	34.43*	2.31	128.75	15.49	32.80*	6.15
Mn	2.43	0.3	20.46	1.55	1.14	0.09
Fe	14.26	1.48	1930	272	40.46	1.63
Cu	0.54	0.03	470.78	46.34	1.06	0.13
Zn	604	82	73.07	2.14	45.55	1.58
Rb	18.16*	0.76	15.55*	1.20	<2.9	
Sr	2.67*	0.52	2.38*	0.19	0.66	0.08
Mo	<0.375		48.49	3.09	2.06	0.41
Cd	0.74	0.09	2.44	0.24	1.17	0.26
Pb	0.024	0.015	0.108*	0.052	0.169*	0.117

Target Harzard Quotients

The THQ values are shown in Table 5. Lithium (Li) and Zn had the highest THQ values as compared to the other trace elements.

Table 5. Target Hazard Quotients (THQ) for various trace elements for *O. leucostictus* fish from Lake Naivasha (RfDo, Reference dose, oral according to USEPA [72])

	Al	Fe	Mn	Cd	Cu	Li	Sr	Zn
RfDo	1	0.7	0.14	0.001	0.04	0.002	0.006	0.3
THQ	0.007	0.027	0.023	0.980	0.018	197.971	0.590	2.674

Table 6. Trace element concentrations in *C. multipapillatum* from *O. leucostictus* in Lake Naivasha compared with published data on other nematode species (mg/kg ww, mean±SE, superscripts numbers indicate the data sources)

Nematode	Al	Si	Mn	Fe	Cu	Zn	Sr	Mo	Cd
C. multipapillatum (this study)	1.96	18.67	0.57	20.23	0.53	21.48	0.33	1.03	0.59
***Anisakis* sp.**[1]			25.86	233.77	21.04	43.16			4.28
***Echinocephalus* sp.**[2]				72		30			12
***Ascaris* sp.**[2]				80		32			15
Hysterothylacium aduncum[3]			3.60	102.26	28.01	62.55			2.05
Ascaris suum[4]									0.04
***Eustrongylides* sp.**[5]			1	250	80	50			0.05

[1]Dural et al. 2011, [2]Azmat et al. 2008, [3]Morsy et al. 2012, [4]Sures et al. 1998, [5]Nachev et al. 2013

Discussion
Physico-chemical parameters of water

The values of the physico-chemical parameters were agreed well with earlier studies from the lake and did not fluctuate highly during the study period (Campbell et al. 2003; Ochieng et al. 2007; Mutia et al. 2012). The concentration of major cations deviated from the typical ion balance of most freshwaters (Ca 63%, Mg 17%, Na 15%, K 4% and others 1%), with particularly a high K content, the latter implies effects in overall K metabolism and helps explain the relatively high intake of Rb as its biochemical analogue, as discussed under Rb.

Trace elements concentration in sediments

Of the analysed trace elements, the mean concentration of Al was highest in the sediment, followed by Fe, Mn, Zn and Rb among others. Table 2 shows a comparison of the mean concentration of trace elements in sediments from Lake Naivasha and other studies. Cu, Mn and Zn levels compared well with earlier studies from the same lake (Tarras-Wahlberg et al. 2002; Ochieng et al. 2007; Mutia et al. 2012). Cd and Pb levels were lower than those reported (Ochieng et al. 2007; Mutia et al. 2012). To our knowledge this is the first report of Li, Rb and Mo from the lake. Aluminium (Al), Li, Fe, Cu, Rb, Sr, Mo and Pb in sediment were below the average concentration for shale of sedimentary rocks, which is considered to be the normal background level in the Earth's crust (Turekian and Wedepohl 1961). These results reflected the geology of the lake. Manganese (Mn) and Cd were within the range of normal background levels, but Zn was elevated compared to the average shale (Turekian and Wedepohl 1961). An earlier study reported higher concentrations of Cd, Fe and Zn in the Lake Naivasha catchment and attributed this to the volcanic rocks or erosion of iron-enriched lateritic soils found in the catchment (Tarras-Wahlberg et al. 2002). Cu, Cd and Pb were below the threshold effect level (TEL), which is a sediment quality guideline below which no adverse effects are expected; Zn approximated the TEL.

Most trace elements, except for Sr, showed a positive significant correlation with the organic matter content of the sediment (Table 2). These findings are in agreement with other studies (Lin and Chen 1998; Ashworth and Alloway 2008; Yang et al. 2010; Deka and Sarma 2012). This is mainly because organic matter is known to form complexes with some metals by providing binding sites for their ions. Generally, organic matter contains different types of functional groups, such as the phenolic group (-OH), which are capable of complexing metals (Ashworth and Alloway 2008; Deka and Sarma 2012). Sr is known to form simple ionic complexes with other organic substances, but there are principal physico-chemical processes that transform Sr in soils and sediments. These processes are mediated by sorption and desorption reactions between the soil solution and matrix (precipitation, complexation and ion exchange) and controlled by the pH, ionic strength, mineral composition and temperature. A high concentration of ion-exchangeable Ca in soil enhances the complexation of Sr with organic matter and increases the removal of Sr from the solution, resulting in reduced mobility (Helal et al. 1998). Nitrate fertilizers, however, can inhibit the formation of Sr-organic matter complexes and increase Sr mobility. As Lake Naivasha is eutrophic (Kitaka et al. 2002), we expect nitrates to play a role in reducing he formation of Sr-organic matter complexes, which could partly explain the insignificant correlation obtained in this study.

Variation in trace elements concentrations in fish tissues

The liver of *O. leucostictus* had a significantly higher concentration of most trace elements than the muscle. These findings are similar to those elswhere (Jirsa et al. 2008; Mohamed and Gad 2008; Uysal et al. 2009; Wang et al. 2010; Gül et al. 2011; Poleksic et al. 2010, amongst others). These differences between the muscle and liver may reflect the different affinity of metals to fish tissues, different uptake, deposition and excretion rates (Jezierska and Witeska 2006). Different tissues have different accumulation capacities, which may also relate to the different metabolic roles of the metals and functions of organs. Accordingly, active metabolic organs such as the liver often accumulate more metals than the muscle (Wang et al. 2010). High accumulation by the liver could also be due to a greater tendency of some metals to react with the oxygen carboxylate, the amino group, the nitrogen and the sulphur of the mecarpto group in the metallothionein protein during its role in detoxification and storage (Al-Yousuf et al. 2000; Mohamed and Gad 2008). It has also been suggested that low levels of metals in the muscles of some fish such as Nile tilapia (*O. niloticus*) may be due to the minimal blood supply to the muscular tissue (Shenouda et al. 1992).

The above results show the general trend, but there were exceptions. For instance, whereas Zn and Sr concentration were highest in the muscle of *O. leucostictus*, Li and Rb were below the detection limit in the liver. This was in agreement with Mohamed and Gad (2008) on Zn but in contradiction with other findings by, amongst others: (Ussing 1960; Al-Yousuf et al. 2000; Stanek et al. 2005; Uysal et al. 2009; Poleksic et al. 2010; Gül et al. 2011), who found Zn highly concentrated in the liver. In this study, Zn had the highest BCF (against sediment) for the muscle (Table 3), which agrees with (Stanek et al. 2005), who reported a greater tendency of Zn to accumulate in the muscle of several fish: BCF (against water) of 22 for roach *Rutilis rutilus*, 47 for common bream *Abramis brama*, 60 for perch *Perca fluviatilis* and 61 for white bream *Blicca bjoerkna*. Zn is an essential element and fish actively regulate its concentration in the muscle. In contrast, Sr is a biochemical analogue of Ca, and its uptake and regulation are through the Ca pump system. Since Ca plays a role in the muscle function as a trigger for contraction, the muscle concentration of Sr may be related to that of Ca, hence higher in muscle than in the liver.

A brief discussion of each trace element concentration in the fish tissues is given below.

Li

We detected Li in high concentration only in the muscle of *O. leucostictus,* supporting the findings by Chassard-Bouchaud et al. (1984) but contrasting with the conclusion by Aral and Vecchio-Sadus (2008) that Li can occur in all organs and tissues. To our best knowledge, no comparable literature is available for Li concentration in freshwater fish, but the Li level observed in our study was much higher than that in several marine fish studies: Ashoka et al. (2011) reported concentrations between 0.08±0.03 and 0.18±0.03 mg/kg dw in various muscle tissues from a ling *Genypterus blacodes* off New Zealand, Tamari and Tsuchiya (2004) reported 2440±657 ng/g dw as the Li concentration in anchovy, Guèrin et al. (2011) reported low concentrations compared to our study (0.105 mg/kg in anchovy and 0.093 mg/kg in pilchard). In contrast, Hansen et al. (1978) reported 231.5 mg/kg fresh weight in tuna from the northern Pacific Ocean, which is higher than in the present study.

Li has no known biological use and does not appear to be an essential element for life (Aral and Vecchio-Sadus 2008). Metallic lithium has been classified as a health, physiological and ecotoxicological hazard. The primary target organ for lithium toxicity is the central nervous system (CNS) and, consequently, Li is used therapeutically on membrane transport proteins when treating bipolar disorder (manic depression).

Lithium in plants and animals interacts with sodium and potassium as well as with enzymes requiring magnesium. Its complexing properties are stronger than those of Na and K but weaker than those of Mg; this may explain why lithium preferentially affects the activity of Mg-containing enzymes (Birch 1976; 1988).

The THQ for Li was 197.971 (Table 5), which shows a very high risk for consumers of this fish in the area. Furthermore, a provisional recommended daily intake of 14.3 µg/kg body weight of Li for an adult has been suggested by Aral and Vecchio-Sadus (2008). On this basis too, our study found very high concentrations in the edible part of the fish, potentially posing a threat to fish consumers here.

Al

The Al concentration was higher in the liver than the muscle. This agrees with findings by Moiseenko and Kudryavtseva (2001) and by Bashir et al. (2012). Moreover, with regard to the muscle, the values we recorded compare very well with those reported by Bashir et al. (2012) in giant catfish *Arius thalassinus* (7.24 µg/g dw). With regard to the liver, our values are comparable to those of gelama *Pennahia anea* (13.72±1.12 µg/g dw) from Kapar and Mersing coastal waters. In contrast, in white fish *Coregonus lavaretus* from the Kola region of Russia, Moiseenko and Kudryavtseva (2001) recorded 4.8±0.6 µg/g dw in the muscle and 19±2.9 µg/g dw in liver; this area was considered polluted by metals due to mining and other activities. Higher Al values have been reported by Budambula and Mwachiro (2006) for redeye labeo *Labeo cylindricus* from the polluted Nairobi River, Kenya (70.0 µg/g ww for the muscle and 150µg/g ww for the liver), and Guèrin et al. (2011) recorded 9.68 mg/kg ww in gurnard.

For many years, Al was not considered a health threat because of its relatively low bioavailability. In 1965, however, animal experiments suggested a possible connection between

Al and Alzheimer's disease, and much research has been done on this link (e.g. Abreo 2001; Haese and De Broe 2001). The THQ for Al was 0.007, which means that the concentration in the fish poses no risk to consumers. Additionally, FAO/WHO (2011) reduced the provisional tolerable weekly intake value for Al from 7 mg/kg body weight/week to 1 mg/kg body weight/week: even on this basis, our results do not show a potential risk exposure here.

Mn

The Mn concentration was higher in the liver than the muscle. These results generally agree with findings by Al-Yousuf et al. (2000) and Budambula and Mwachiro (2006). The Mn levels in *O. leucostictus* muscle compare well with literature values of 1.75±0.05 µg/g dw in white fish *C. lavaretus* and 2.27±0.5 µg/g in brown trout *Salmo trutta* from the Kola region of Russia, considered polluted by metals due to mining and other activities (Moiseenko and Kudryavseva 2001). Lower values include: 1.0±0.1 µg/g dw in rainbow trout *Oncorhynchus mykiss* from the Yesilirmak River in Tokat, Turkey (Mendil et al. 2010), 0.74±0.04 mg/kg dw in belly flap (muscle) of a ling *G. blacodes* (Ashoka et al. 2011) and 1.11mg/kg in pilchard (Guèrin et al. 2011). Higher values than we found have been reported by Silva and Shimizu (2004) and Mendil et al. 2005). The former reported 6.62 µg/g dw in Nile tilapia *O. niloticus* from a hydropower reservoir in Sri Lanka.

Manganese is a key component of enzyme systems including oxygen-handling enzymes; it supports brain function and reproduction as well as blood sugar regulation. Mn has two roles, namely (i) as a co-factor for enzymes which form metal-enzyme complexes and; (ii) as an integral part of metalloenzymes (Watanabe et al. 1997). The THQ of Mn was 0.023, which means that Mn does not pose a risk to consumers of this fish in the area.

Cu

The Cu concentration was higher in the liver than the muscle, which generally agrees with many findings (e.g. Al-Yousuf et al. 2000; Avenant-Oldewage and Marx 2000; Budambula and Mwachiro 2006; Jirsa et al. 2008). This reflects the important role the liver plays in the storage and detoxification of Cu (Avenant-Oldewage and Marx 2000; Jezierska and Witeska 2006). The muscle levels we found compare well with the 0.78±0.2 mg/kg dw adjacent to belly flap in the marine *G. blacodes* (Ashoka et al. 2011) and in largemouth bass *Micropterus salmoides* from Lake Naivasha (0.31±0.05 µg/g dw) published by Njogu et al. (2011). Lower Cu values than in our study have been reported from Lake Naivasha: in blue spotted tilapia *O. leucostictus* (0.27±0.08 µg/g dw) and in common carp (*Cyprinus carpio*) 0.28±0.11 µg/g dw (Njogu et al. 2011). The Cu levels in this study are lower than those reported from many sites elsewhere (Cronin et al. 1998; Mendil et al. 2005; Jirsa et al. 2008; Ongeri et al. 2009; Ongeri et al. 2011; Mutia et al. 2012).

The THQ of Cu was 0.018. FAO/WHO (2011) has set a toxicological guidance value, PMTDI of 0.05-0.5 mg/kg body weight, while the WHO's (1989) permissible limit is 30 ppm. These values exceed the accumulation levels we found for the muscle, demonstrating no risk due to the consumption of this fish in the area.

Zn

In contrast to most trace elements discussed above, the Zn concentration was higher in the muscle than the liver. The reasons have been described above. The levels in the muscle were much higher than those reported by Ashoka et al. (2011) for ling fish filets (23±6 to 69±4 mg/kg dw) and by Mendil et al. (2010) for the chub *Squalius cephalus* (63.5±6.5 µg/g dw. Cronin et al. (1998) reported a range of 2.2-6.7 mg/kg ww in deep-sea fish from the North Atlantic. From Kenya the following authors have reported lower Zn concentrations than we found: Njogu et al. (2011) – 7.31±0.89µg/g ww in blue spotted tilapia *O. leucostictus* from Lake Naivasha; Budambula and Mwachiro (2006) – 70 µg/g ww in the redeye labeo *L. cylindricus* from Nairobi River; Mavura and Wangila (2003) – 238 µg/g dw in *Tilapia grahami* from Lake Nakuru. Similarly lower levels have been reported in Nile tilapia *O. niloticus* from Nakivuko wetland in Uganda (Birungi et al. 2007). Our findings are comparable to concentrations in the muscle of marine coney *Epinephelus fulvus* (410-630 mg/kg ash weight) and in six-lined trumpeter *Siphamia cephalotes* (480 mg/kg dw; Eisler 2010).

Zn is an essential element and fish actively regulate zinc concentration in their muscle tissue (Kumar et al. 2011). Although essential, at high concentrations Zn can be toxic to fish, causing mortality, growth retardation and reproductive impairment. Zinc is an essential part of more than

200 enzymes involved in digestion, metabolism, reproduction and wound healing, making it essential for human health. It plays a critical role in immune response and is an important antioxidant. Nonetheless, too much zinc can cause health problems such as stomach cramps, skin irritations, vomiting, nausea and anaemia. The THQ of Zn was 2.674. demonstrating a clear risk to fish consumers here. Additionally, the permissible limit for Zn set by WHO (1989) is 40 ppm – far below the concentration detected in *O. leucostictus* muscle.

Rb

Rb was higher in the muscle than the liver, but not significantly different ($p>0.05$). In *O. leucostictus* muscle, we found higher Rb concentrations than Campbell et al. (2005) did for several fish species from Lake Erie and two Arctic lakes (Lake Hazen and Resolute Lake), Ashoka et al. (2011) for *G. blacodes* fillet and Asante et al. (2009) for 22 fish species in the southwestern area of the Philippines. Thus the highest concentration reported by Campbell et al. (2005) was in freshwater drum *Aplodinotus grunniens* (4.5±0.4 µg/g ww), the highest by Asante et al. (2009) in Warming's lantern fish *Ceratoscopelus warmingii* from a composite (whole body) sample (4.0 µg/g dw). Conversely, the Rb concentrations we found are much lower than those reported by Guevara et al. (2006): mean value 57.6(±5.7) µg/g ww for brown trout *S. trutta* and 45.9(±6.2) µg/g ww for creole perch *Percichthys trucha* in lakes of Patagonia, Argentina. Similarly, Silva and Shimizu (2004) reported a range of 20.90-70.75 µg/g dw in the flesh of nine fish species in Sri Lanka. The latter authors also reported 30.10 µg/g dw in Mozambique tilapia *O. mossambicus*, 21.60 µg/g dw in Nile tilapia *O. niloticus*, and 70.75 µg/g dw in redbreast tilapia *Tilapia rendalli*. No literature is available on Rb in fish livers.

The Rb concentration Rb in the Earth's crust is 0.009% (Levinson 1974). Rubidium is an important requirement for bacterial growth, especially pigment biosynthesis in *Serratia marcescens*, where it can replace the need for potassium (Bruce and Duff 1968). Sander and Kirschner (1983) quantitatively determined that potassium and rubidium ions behaved similarly in exchanges within rainbow trout *O. mykiss* and sculpin *Leptocottus armatus*: more than 90% of an injected dose of both ions disappeared from the blood within one hour. This is because the physico-chemical behavior of Rb is similar to that of K. The main biological difference between the two is that they are not equally permeant in cell membranes and intracellular junctions

(Ussing 1960). Rb is a K analogue and can compete with K for enzymatic sites in fish and other organisms (Campbell et al. 2005).

Several factors determine the bioavailability and concentration of Rb in freshwater and marine ecosystems. They include the sources and ambient concentrations of Rb in water and sediment, the ambient K concentrations, ambient pH and temperature, and the trophic position (Campbell et al. 2005). Other variations might reflect the biological half-life of Rb, a parameter associated with Rb kinetics in fish and known to differ among species (Guevara et al. 2006).

We found Rb in both the muscle and liver, which agrees partly with Peters et al.´s (1999) study showing muscle to be the main location of Rb. The concentration in fish depends on diet and habitat (Chiasson 1980). Higher concentrations are associated with the littoral zone. *O. leucostictus* is a benthopelagic fish which prefers shallow vegetated zones and this could help explain the high concentration. Although there is some disagreement on the importance of Rb as an ultra-trace essential element to humans and other biota, even small concentrations of Rb in combination with other metals such as Pb, Mo, As can be toxic to fish and other organisms (Seixas and Pierce 2005; Yamaguchi et al. 2007). Neither safe concentrations nor the RfDo (reference dose) is known, preventing determining the THQ for Rb. Nonetheless, experimental toxic responses linked with elevated Rb have been observed in mammals fed low-K high-Rb diets, which are linked to physiological interference with K and Na (Kosla et al. 2002).

Adachi et al. (2012) classified Rb as a ``homeostasis type´´, meaning that its concentration varies within one order of magnitude throughout all trophic levels. Rb levels in organisms are maintained by the same mechanism that controls major electrolytes such as Na and K (Sakurai 2009).

Sr

More Sr was present in the muscle than the liver of *O. leucostictus,* in agreement with Moiseenko and Kudryavtseka (2001). Although those authors reported the highest concentrations in the skeleton and gills of white fish *C. lavaretus* and brown trout *S. trutta*, the values were notably higher in muscle versus liver, similar to this study. The values were generally comparable to those reported by Zumholz et al. (2006) in marine fish such as Baltic herring

Clupea harengus (3.1 mg/kg dw). In contrast, Levinson (1974), Ashoka et al. (2011) and Guèrin et al. (2011) have reported higher concentrations than we found. For example, Guèrin et al. (2011) reported 5.69 mg/kg ww in catshark *Chiloscyllium indicum*, while Levinson (1974) measured 371 µg/g dw in splendid seabass *Malakichthys elegans*, 661 µg/g dw in kamohara grenadier *Caelorinchus kamoharai*, and 690 µg/g dw in Japanese cusk *Glyptophidium japonicum* from the Sulu Sea, Philippines. Ashoka et al. (2011) measured 15±0.8 mg/kg dw in muscles adjacent to the belly flap *G. blacodes.*

Fish take up Sr and its analogue Ca from water, food and sediment. The uptake is through the Ca transport system located in the chloride cells of gills and enterocytes of the intestine. Sr is accumulated in bone and other calcareous tissues (Chowdhury and Blust 2001,2002). Furthermore, the higher concentration in muscle than in liver may reflect muscle function. Ca plays a crucial role in muscle function because it promotes the formation of action potentials which initiate muscle contractions. Sr is a non-essential element in fish. The THQ value for Sr was 0.590 and it could therefore pose a risk (additive effects for non-carcinogens) for the consumers of the fish here.

Mo

In this study, Mo was below the detection limit in *O. leucostictus* muscle but was detected in the liver. In general, lower Mo levels (0.063 mg/kg) have been reported by Guèrin et al. (2011) in tuna. Even lower levels are known in whole body composite samples from several fish species (Levinson 1974). Other values include 0.094µg/g dw in splendid seabass *M. elegans*, 0.060 µg/g dw in elongated bristlemouth fish *Gonostoma elongatum*, and 0.154 µg/g dw in ribbon sawtail fish *Idiacanthus fasciola*. Generally, the values are low in marine teleosts, never exceeding 0.5 mg/kg fresh weigh in edible muscle (Eisler 2010). Molybdenum contributes to normal growth and development and plays a key role in many enzyme systems, including those for detoxification. Since Mo was below the detection limit in the muscle, no THQ value was determined.

Cd

The concentration of Cd was higher in the liver than the muscle, which generally agrees with literature (Al-Yousuf et al. 2000; Budambula and Mwachiro 2006; Jirsa et al. 2008; Uysal et al. 2009; Vicente-Martorell et al. 2009; Wang et al. 2010; Poleksic et al. 2010; Gül et al. 2011). The levels we found compared quite well with those (0.75±0.03 µg/g dw) reported by Njogu et al. (2011) in largemouth bass *M. salmoides* from Lake Naivasha. Mendil et al. (2010) measured 0.75± 0.07 µg/g dw in Anatolian khramulya *Capoeta tinca* from a polluted area in the Yesilirmak River in Tokat, Turkey. Mendil et al. (2005) recorded 0.6±0.05 µg/g in seven khramulya *C. capoeta* from Uluöz Dam Lake in Tokat, Turkey, Mutia et al. (2012) 1.59± 0.002 mg/kg ww in common carp *C. carpio* from Hippo point in Lake Naivasha. Other literature values are lower than found in the present study (Cronin et al. 1998; Al-Yousuf et al. 2000; Ongeri et al. 2009; Mendil et al. 2010; Ashoka et al. 2011). Much higher values were reported by Budambula and Mwachiro (2006): 52.0 µg/g in muscle and 57.0 µg/g in liver of redeye labeo *L. cylindricus* from Nairobi River.

Cd has no known biological use in fish or humans. It is a relatively rare toxic metal that is released into air, land and water by human activities. Cd is also present in phosphate fertilizers (Tarras-Wahlberg et al. 2002). The intense agricultural activities in the Lake Naivasha catchment (Otiangà-Owiti and Oswe 2007) could represent a significant addition of Cd to the lake. The THQ value for Cd was close to 1 (0.980), which represents a risk for additive effects. The European Commission regulation set a maximum level of 0.05 mg/kg wet weight (EEC 2001). Therefore, based on the THQ value and EC regulation, Cd poses a risk to consumers of fish from the lake.

Pb

Our Pb results were unique and unexpected, differing greatly from earlier studies which had reported very high levels in various fish species from Lake Naivasha (Njogu et al. 2011; Mutia et al. 2012). The reasons for these differences remain unknown. For example, Njogu et al. (2011) reported a range of 5.12-58.11 mg/kg ww for *C. carpio* muscle, while Mutia et al. (2012)

reported 3.22±0.15, 1.49±0.1 and 1.56±0.19 mg/kg ww for *M. salmoides*, *O. leucostictus* and *C. carpio*, respectively. The levels reported in our study are safe and below the maximum permissible level according to the European Commission regulation (EEC 2001).

Accumulation of trace elements in *C. multipapillatum*

The trace elements with the highest concentration in *C. multipapillatum* were Zn, Fe and Si. Accordingly, *C. multipapillatum* strongly accumulates certain essential elements. This supports the findings by Nachev et al. (2013) on the nematode *Eustrongylides* sp.: it accumulates essential metals (Co, Cu, Fe, Se, Zn) better than its host fish, the barbel *Barbus barbus.* In our study, however, *C. multipapillatum* had significantly more Pb than *O. leucostictus* muscle. Also, *C. multipapillatum* seemed to accumulate more Pb than reported from another nematode, *Anguillicola crassus,* which infects the swim bladder of eels (*Anguilla anguilla*) (Sures et al. 1994b).The concentrations of Mn, Fe, Cu and Zn in *C. multipapillatum* were lower than those reported in literature from other anisakid nematodes (Table 6).

As compared to the host's muscle, the concentrations of Fe, Cd, Cu and Pb were 2.94, 1.58, 1.96 and 7.04 times higher, respectively, in *C. multipapillatum*. Mo was unique because it was below the detection limit in the muscle, but was detected in the parasite. Barus et al. (2001) found Pb bioconcentration factors between *C. rudolphii* tissue and the cormorant *Phalacrocorax carbo* muscle of 2.35 in male nematodes and 1.81 in females, whereas the values of Cd were 0.52 and 0.22 in males and females, respectively – lower than those found in the present study. Overall, these bioaccumulation capacities of *C. multipapillatum* are much lower compared to acanthocephalans and cestodes (Sures et al. 1994a; Jirsa et al. 2008). This points to an inefficient accumulation indicator, supporting the conclusion of Sures (2001) and Barus et al. (2001) that nematodes are not good accumulation indicators. Note that this differs with the conclusions of other studies on nematodes (Azmat et al. 2008; Dural et al. 2011; Morsy et al. 2012; Nachev et al. 2013). For example, Dural et al. (2011) found that *Hysterothylacium aduncum* accumulates more Cd, Cu and Pb than the tissues of its final host, sea bream *Sparus aurata*. Interestingly, their findings of no significant differences in the mean Cd and Cu concentrations in *H. aduncum* and the liver of sea bream corroborate our results.

There are three probable reasons for the observed concentration of trace elements in *C. multipapillatum*: (i) the parasite's development stage (ii) parasite location within the host (iii) feeding and excretion. Sures (2001) indicated that parasites that inhabit the intestine of their host fish tend to have a higher concentration of trace elements than those found in the body cavity; this is because of the uptake of bile salts and the entero-hepatic cycling of trace elements. In our study, *C. multipapillatum* inhabited the pericardial cavity of *O. leucostictus*. Secondly, larval (versus adult) parasites contain lower amounts of trace elements, a condition also closely connected with their location within the host: those in the intestines are better accumulators (Sures 2001). Sures (2001) clearly explained that the main uptake and accumulation of metals occur inside the gut of the final host and not in the larvae located elsewhere in the body cavity. The *C. multipapillatum* obtained from *O. leucostictus* in our study were larval-stage nematodes, and this could have contributed to the low trace element concentrations. The adult stage of *C. multipapillatum* occurs in piscivorous birds such as the pelican *Pelecanus onocrotalus*. Barus et al. (2001), however, who examined adult nematodes and their definitive host, found poor bioaccumulation factors. This suggests that other complex factors might play a role in influencing trace element uptake by nematodes. Thirdly, we hypothesize that, because *C. multipapillatum* inhabited the pericardial cavity where they primarily get nutrition from blood and other host body fluids, the trace element concentrations could be determined by the levels in the immediate muscle tissues. For example, Si, and Al levels were not significantly different between the muscle of *O. leucostictus* and *C. multipapillatum*. Accordingly, the regulation of these trace elements in *C. multipapillatum* is probably tightly linked to their concentration in the host fish muscle.

Finally, *C. multipapillatum* has a complete digestive system, setting it apart from cestodes and acanthocephalans, which absorb nutrients through their tegument. Therefore, the uptake, deposition and excretion of trace elements are different, although, like cestodes and acanthocephalans, nematodes cannot synthesize their own steroids and fatty acids and must ingest sterol precursors from their hosts.

Conclusions

The mean concentration of trace elements in the sediment reflected normal background levels due to the geology of the basin; Zn is the exception. The liver accumulated the highest concentration of most trace elements except Zn, Sr, Rb and Li. Our findings therefore support most studies that recommend considering the liver of fish when conducting studies on trace elements: it proved to be a good biomarker of most trace elements examined. Although *C. multipapillatum* showed potential to accumulate trace elements, its values were far lower than in the *O. leucostictus* liver and comparable to muscle levels. The parasite is therefore not an efficient accumulation bioindicator. Li, Sr, Cd and Zn had high THQ values and could represent a health risk to the local community that depends on fish for regular food.

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CHAPTER FIVE

Parasite communities of the elongate tigerfish *Hydrocynus forskahlii* (Cuvier 1819) and redbelly tilapia *Tilapia zillii* (Gervais 1848) from Lake Turkana, Kenya

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Abstract

Fish is an important alternative food source for an estimated 300,000 people inhabiting the shores of Lake Turkana, Kenya, and beyond. Despite its large size (7560 km^2) and importance for the people, the lake remains one of the least studied in the Great Lakes Region of Africa, and almost no literature is available on parasites of its inhabiting fish. This study describes the parasite community and the host-parasite relationships of the elongate tigerfish *Hydrocynus forskahlii* and the redbelly tilapia *Tilapia zillii*. A total of 87 individuals (43 *H. forskahlii* and 44 *T. zillii*) were dissected and examined for the presence of parasites. In both species, we recorded a very high prevalence (P) of 97.7% for at least one parasite taxon. Two taxa infected *H. forskahlii*, the dominant one being an anisakid nematode *Contracaecum multipapillatum* (P=83.7%, mean intensity (MI) =46.0, abundance (A) =38.5). Twelve parasite taxa were recovered from *T. zillii*, with the gryporhynchid cestode *Amirthalingamia macracantha* (co-occurrence of merocercoids and plerocercoids) being dominant (P=79.5, MI=10.3, A=8.2). We conclude that *H. forskahlii* had a depauperate parasitic community but that the infection levels with *C. multipapillatum* were high. *T. zillii* had a rich parasite fauna, although, except for *A. macracantha*, most parasites occurred at low intensities.

Keywords: Lake Turkana, *Hydrocynus forskahlii*, *Tilapia zillii*, *Contracaecum multipapillatum*, *Amirthalingamia macracantha*, parasites

Introduction

Fish is an important alternative food source for an estimated 300,000 people inhabiting the shores of Lake Turkana and beyond (Friends of Lake Turkana (FoLT), 2009; Avery, 2010; Hathaway and Annicucci, 2010). Located in an arid area, Lake Turkana is a lifeline for the impoverished dwellers surrounding the lake, firstly because of its water and secondly because of its rich and productive fishery (Kenya Marine Fisheries Research Institute (KMFRI), 2007; FoLT, 2009; Hathaway and Annicucci, 2010). The lake contains a high diversity of fish, with at least 48-50 species having been recorded (Kolding, 1989; Okeyo, 2003). This makes fish one of the most significant and most available food sources in the area. Amongst the challenges reported in the exploitation of Lake Turkana fish are: lack of appropriate offshore vessels and fishing gear, high post-harvest losses (approximately 90%), and a high infestation of fish with parasites (KMFRI, 2007; 2008). Several taxonomic studies have been done on parasites of fish from Lake Turkana. Moravec et al. (2009a; 2009b), for example, described new species such as *Mexiconema africanum* (Nematoda: Daniconematidae) from the Giraffe catfish *Auchenoglanis occidentalis* (Valenciennes, 1840), *Philometra lati* and *P. spiriformis* (Nematoda: Philometridae) from the Nile perch *Lates niloticus* (L. 1758), and *Afrophilometra hydrocyoni* (Fahmy, Mandour et El-Nafar 1976) from the elongate tigerfish *Hydrocynus forskahlii* (Cuvier 1819). Rushton-Mellor (1994) described a new crustacean parasite *Argulus fryeri* from Lake Turkana, Kenya, from an unknown host, while Přikrylová et al. (2012) described a new monogenean species *Gyrodactylus malalai* from Nile tilapia *Oreochromis niloticus* (L. 1758) and redbelly tilapia *Tilapia zillii* (Gervais 1848). Recently, Moravec et al. (2013) provided the morphology and systematics of *Rhabdochona paski* (Baylis, 1928) (Nematoda: Rhabdochonidae) from some specimens obtained from *H. forskahlii*, *Oreochromis niloticus* (L. 1758) and *T. zillii* among other fishes in Africa. Several taxa of parasites have been reported from the *H. forskahlii* and *T. zillii* in Africa (Khalil and Polling 1997; Paperna 1980; 1996 amongst others). Nonetheless, data on the diversity, prevalence, intensities and ecology of parasites from Lake Turkana is lacking.

This study addresses this critical gap by providing data on the diversity, prevalence, mean intensities, and abundances of parasites of *T. zillii* (local name – Loroto) and *H. forskahlii* (local name – Lokel) (KMFRI, 2007), which are commercially important but differ biologically (Froese and Pauly, 2013). *Hydrocynus forskahlii* is an open-water piscivorous predator often found near the water surface. *Tilapia zillii* is largely benthopelagic and potamodromous. It prefers shallow, vegetated areas. *Tilapia zillii* is mostly herbivorous, feeding on water plants and epiphyton and some invertebrates (Froese and Pauly, 2013).

Materials and methods

Study site description

Lake Turkana (formerly Lake Rudolf) is located at the north of the eastern Rift Valley, stretching from 35°50’ to 36°40’ E and 2°27’ to 4°40’N (KMFRI, 2007), with its northernmost tip stretching into Ethiopia (Fig 1). It is the world`s largest desert lake and the largest (7560 km^2) and deepest water mass in Kenya (mean depth 35 m, maximum 120 m). It is the fourth largest lake in Africa in terms of surface area (Avery, 2010; KMFRI, 2007). The water of the lake is alkaline (pH = 9.2) and has moderate salinity (total dissolved solids (TDS) = 2500 mg/L) (Yuretich and Cerling, 1983; Campbell et al., 2003). Volcanic activity was frequent during the creation of the Rift Valley, and lavas from the Quaternary and Tertiary ages cover much of the floor of the valley in Kenya. This lava is mainly of alkaline type, which has important implications for the chemical composition of the lake (International Lake Environment Committee (ILEC), 2013). The lake receives 90% of its water from Omo River from Ethiopia and seasonally from Turkwell and Kerio Rivers in Kenya. The lake loses water mainly by evaporation. The human population density in this lake basin is very low, 1-3 persons/km^2 (Odada et al., 2003; ILEC, 2013). The catchment area is approximately 130,860 km^2 and the land use is dominated by pasture (47.5%), herbaceous vegetation (45%), and woody plants (5%), whereas crop fields cover 2.4% (ILEC, 2013). Being in an arid area and with slightly saline water, there are no significant industrial activities in and around the lake. The only known pollution problem in Lake Turkana is that of suspended solids (Odada et al., 2003) coming from the drainage basin of Omo River in Ethiopia. An increased removal of vegetation cover for fuel and the conversion of forest land to agricultural fields may be leading to heavy sedimentation in the lake (Haack and Messina, 2001).

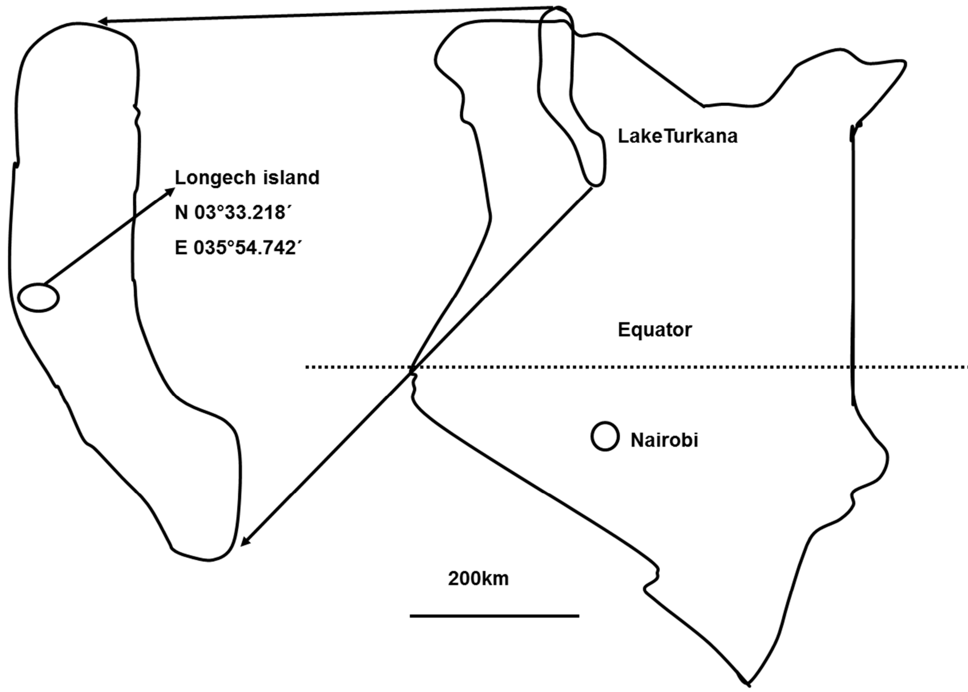

Fig.1. Map of Kenya showing the location of the study site in Lake Turkana

Sampling and parasitological examination

A total of forty-three (43) *H. forskahlii* and forty-four (44) *T. zillii* were caught using beach seines by fishermen in June 2011 and in September 2012 at Longech island (N 03º33.218' E 035º54.742') (Fig. 1). The fish were killed by cervical dislocation, weighed and their total lengths measured. This was followed by dissection (Schäperclaus, 1990). To estimate the intensity of infection by protozoans, gill and skin smears were prepared for examination under high magnification (×40-×100) as described previously (Lom and Dyková, 1992). The categories used to assess infection intensity were defined as follows: low- <10 parasite individuals per field of view (at 100-fold magnification); medium - 11-100 parasites per field of view; and high- >100 individuals per field of view. The gills, eyes, intestines, visceral organs and pericardial cavity were all examined for parasites. The gill filaments were examined under a dissecting microscope. Monogeneans were counted and removed carefully from the gills using fine forceps, fixed and preserved in either 4% formalin or absolute ethanol (95%). For identification, the monogeneans were mounted in either glycerine ammonium picrate (GAP) or glycerine jelly on slides and cover slips gently placed on top to flatten the worms and sealed with clear nail polish.

Most of the parasites were identified using identification keys and key literature at the University of Vienna and Museum of Natural History, Vienna (Paperna, 1980; Pariselle and Euzet, 1995; Paperna, 1996, Pariselle and Euzet, 1998; Scholz et al., 2004; Pariselle and Euzet, 2009, Le Roux and Avenant-Oldewage, 2010; Abowei and Ezekiel, 2011; Kuchta et al., 2012). The terms used to describe the metacestodes are according to Chervy (2002).

Genetic analysis of the nematode species from *T. zillii*

The targets of the molecular analysis were the internal transcribed spacers (ITS-1) of the ribosomal DNA (rDNA). The total DNA of the parasites was isolated using the Genomic Mini Kit (A&A Biotechnology, Gdynia, Poland) according to the manufacturer's recommendation (from *H. forskahlii* (2 specimens), from *T. zillii* (4 specimens) were sequenced). The ITS-1 was amplified using the primers NC5 (forward) and NC13 (reverse) (Zhu et al., 2000). The composition of the reaction mixtures and the PCR reaction conditions were identical to those described in Szostakowska and Fagerholm (2007). The PCR and the sequencing reaction products were purified using the Clean-Up Kit and the ExTerminator Kit (A&A Biotechnology), respectively. The analyses were performed using an automatic ABI PRISM 310 DNA sequencer (Applied Biosystems, Inc., California, USA), with the amplification primers and the standard procedure described by the manufacturer. The sequences obtained were then analyzed, aligned and blasted with the related sequences downloaded from GenBank using the GeneStudio Pro Software (GeneStudio, Inc., Suwanee, Georgia), and will be deposited in GenBank .

Statistical analyses

The prevalence, mean intensities and abundances were determined according to Bush et al. (1997). The Fulton's condition factor (Ricker, 1975) was calculated using the formula: $k = (W/L^3)100$, where k is the Fulton`s condition factor, W is the fish weight (g) and L is the total fish length (cm) for *T. zillii*. The parasite community structure was described using the Shannon-Wiener index, the Simpson´s index, the Margalef Richness index and the Berger-Parker Dominance index (Magurran, 1988).

Results

The mean length of *H. forskahlii* was 33.4±3.9cm, and that of *T. zillii* was 15.7±2.3cm. The average weight of *T. zillii* examined was 72.0±32.4g; and the Fulton's condition factor (k) was

1.79±0.22. The weight of *H. forskahlii* was not measured due to technical problems and consequently its condition factor was not evaluated.

Parasites were present in 97.7% of all the *H. forskahlii* examined. Two taxa of parasites were recovered from this host namely: *Annulotrema* sp. with a prevalence of 79.1%, mean intensity of 6.8 and abundance of 5.4 found in the gills and *Contracaecum multipapillatum* (Larvae 3) with a prevalence of 83.7%, a mean intensity of 46.0 and abundance of 38.5 found mainly aggregated in the body cavity and the intestines.

Almost all *T. zillii* examined (97.7%) were infected with at least one parasite taxon. The dominant species was *Amirthalingamia macracantha* (metacestodes), which occurred with a prevalence of 79.5% and a mean intensity of 10.3. Some of the metacestodes were encysted (in the intestinal wall) and others were unencysted (in the intestinal lumen). This co-occurrence of two developmental forms: encysted (merocercoids) and unencysted (plerocercoids) in the same host represented a new phenomenon (Fig. 2). The parasites included: *Trichodina* sp., *Cichlidogyrus* spp., *Gyrodactylus* sp., metacercariae cysts, *A. macracantha* (merocercoids and plerocercoids), *C. multipapillatum*, *C. bancrofti*, *Acanthogyrus* sp., *Paragorgorhynchus* sp., *Argulus fryeri*, *A. monodi* and *Lernaea cyprinacea* (Table 1). *Tilapia zillii* was infected by a relatively high diversity of parasite species (Shannon Wiener index 1.13 and Margalef Richness 1.12; Table 2).

Table 1. Site of infection, parasite prevalence, mean intensity and abundance in *Tilapia zillii* from Lake Turkana (n=44)

Parasite	Site of infection	Prevalence (%)	Mean intensity	Abundance
Trichodina sp.	gills	4.5	low	
Cichlidogyrus spp.	gills/skin	45.5	3.2	1.45
Gyrodactylus sp.	gills	4.5	6.0	0.27
Amirthalingamia macracantha	gut/intestine/liver	79.5	10.3	8.18
Contracaecum multipapillatum	intestine/body cavity/liver	18.2	2.6	0.48
Contracaecum bancrofti	gut/intestinal wall	15.9	12.7	2.02
Metacercariae cysts	intestinal wall	4.5	3.0	0.14
Acanthogyrus (Acanthosentis) sp.	intestinal wall	2.3	1.0	0.02
Paragorgorhynchus sp.	intestine wall	13.6	1.3	0.16
Argulus fryeri	body surface/skin	4.5	1.0	0.05
Argulus monodi	body surface/skin	2.3	1.0	0.02
Lernaea cyprinacea	gills	6.8	1.0	0.11

Table 2. Parasite component diversity characteristics of the fish studied from Lake Turkana, Kenya

Total component communities	*H. forskahlii*	*T. zillii*
Number of fish	43	44
Number of metazoan taxa	2	11
Shannon-Wiener index	0.37	1.22
Margalef Richness index	0.13	1.12
Berger-Parker Dominance index	0.88	0.63
Simpson`s index	0.78	0.88
Dominant taxon	*C. multipapillatum*	*A. macracantha*

For the identification of species of nematodes, *C. multipapillatum* and *C. bancrofti*, the ITS-1 sequences of the specimen were obtained. The comparison of the ITS-1 sequence with data from GenBank showed a 99% similarity with the ITS-1 sequences of *C. bancrofti* deposited under accession numbers EU839566-EU839569 (Shamsi, unpublished)

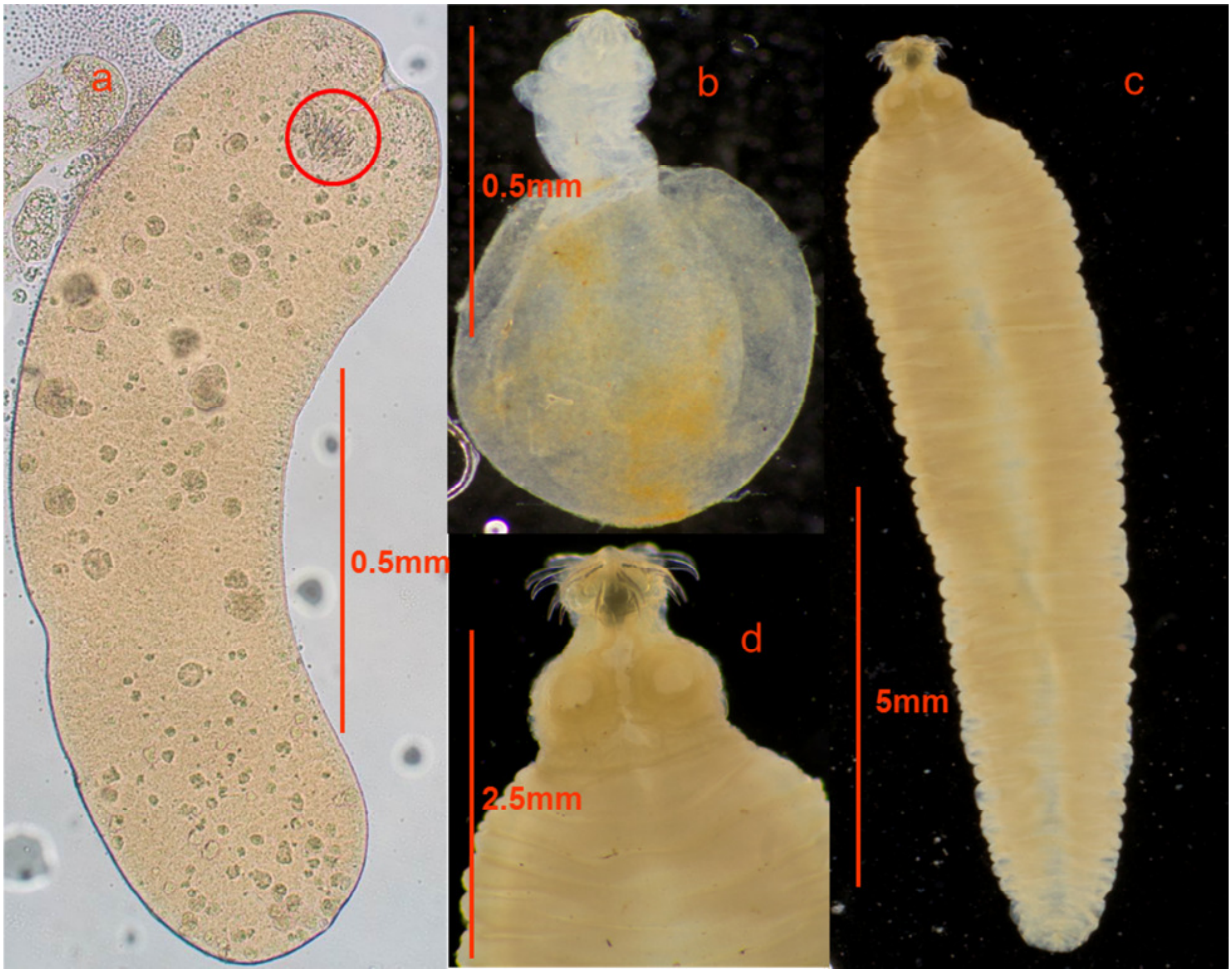

Fig. 2. Larval forms of *A. macracantha* recovered from *T. zillii*. (a) A merocercoid, usually encysted in the intestinal wall; primordial hooks are marked by a circle (b) A merocercoid emerging from a cyst (c) The typical plerocercoid found occurring freely in the intestine (d) details of the plerocercoid scolex

Discussion

Two taxa of parasites infected *H. forskahlii*. The anisakid nematode *C. multipapillatum* was dominant. Two parasites already described and reported from this host in the same lake i.e. *A. hydrocyoni* from *H. forskahlii* (Moravec et al., 2009a) and *R. paski* (Moravec et al. 2013), were not recovered during this study. This is probably because they are rare, for example, *A. hydrocyoni* was found in one of the 31 fish examined (3.2%) (Moravec et al. 2009a), while *R.*

paski had a prevalence of 11.6% (43 *H. forskahlii* examined) and one fish out of the72 *O. niloticus* (1.4%)and 3.7% of the 27 *T. zillii* examined (Moravec et al. 2013). The prevalence and the mean intensity of *C. multipapillatum* in *H. forskahlii* were significantly higher than in the following studies: Aloo (2002) reported a 49.2% prevalence of *Contracaecum* sp. in *Oreochromis leucostictus* (mean intensity 15.13) and a prevalence of 2% in *T. zillii* (mean intensity 1.42), both from Oloidien Lake in Naivasha basin, Kenya and Florio et al. (2009) reported a *C. multipapillatum* prevalence of 17.3% (mean intensity 4.1) from pooled wild tilapia from the Lakes Babogaya, Hora, Awassa and Chamo in Ethiopia. The high prevalence and mean intensity can be attributed, among other factors, to the feeding habits of *H. forskahlii*. This fish is a top piscivore in Lake Turkana. This diet could be a constant source of infection with *C. multipapillatum*. Piscivorous fish can act as parasite sinks (Paperna, 1980; Paterson et al., 2013) or as parasite concentrators, the latter by increasing the parasite's probability of finding a mate (Brown et al. 2001). However, as only larvae of the *C. multipapillatum* were present in the fish, finding a mate in this host was unlikely. Paperna (1980) suggested that fish which are too large a prey for piscivorous birds often have worms trapped in them and thus have no chance of reaching maturity in a definitive host. Such fish may play a significant role in maintaining balanced infection levels in the lakes (Paperna, 1980). *Hydrocynus forskahlii* could be such a fish because it is large (maximum recorded size 78cm; Froese and Pauly, 2013). Two different species of the genus *Contracaecum* infected *T. zillii*: *C. multipapillatum* in the intestine, liver and the body cavity, and *C. bancrofti*. This parasite was found almost exclusively aggregating in the gut and gut wall.

The overall prevalence of parasites in *T. zillii* was very high compared to other studies (Ramadan, 1991; Olofintoye, 2006; Abdel-Hady et al., 2008; Goselle et al., 2008; Bichi and Ibrahim, 2009; Morenikeji and Adepeju, 2009; Hassan et al., 2012; Olurin et al., 2012). We found several parasites infecting *T. zillii*, as did Ibrahim (2012) who reported 12 species, mainly monogeneans, from wild and cultured *T. zillii* from Lake Manzalah, Egypt. In contrast, several surveys have shown a less diverse parasite fauna in *T. zillii* elsewhere in Africa (Ramadan, 1991; Olofintoye, 2006; Abdel-Hady et al., 2008; Goselle et al., 2008; Bichi and Ibrahim, 2009; Morenikeji and Adepeju, 2009; Hassan et al., 2012; Olurin et al., 2012). The low infection intensity of most of the parasites that we found in *T. zillii* is similar to that reported by Aloo

(2002), who concluded that among the tilapiine species, *T. zillii* is probably more resistant to parasitic infections than the other species.

Cichlidogyrus spp. occurred at moderate prevalence and low intensities in *T. zillii*, lower than those reported in the literature (Ibrahim, 2012; Otachi et al. submitted). The identification of the species is currently ongoing. We had anticipated higher intensities of *Cichlidogyrus* spp. because this genus is known to have a shorter egg incubation time, faster hatching, longer lifespan and faster development to maturity in higher temperatures (Paperna, 1980; 1996). For example, at temperatures from 20-28℃, eggs hatch within two to six days. Furthermore, *T. zillii* is a substrate spawner, whereby both parents build and guard nests and protect their fry. This breeding behavior clearly predisposes them to oncomiracidia on the bottom where the eggs of *Cichlidogyrus* spp. hatch. Several factors could explain the observed low levels of infection, the most important being a possible low host density and a small fish population size. In farmed fish, high stocking densities result in high infections (Florio et al., 2009; Ibrahim, 2012). Additionally, Bagge et al. (2004) using a case of crucian carp *Carassius carassius* in ponds in Finland, demonstrated that fish population size *per se*, not density, determines monogenean infection levels. This is because the limited availability of hosts might constrain parasite population growth. Low infection levels could also indicate a successful host-parasite co-evolution.

In the present study, *Gyrodactylus* sp. occurred at lower prevalence and intensity than reported in the literature. Ibrahim (2012) reported a prevalence of 10.24% of *G. cichlidarum* in *T. zillii* in wild fish and 12.07% in cultured fish from Lake Manzalah, Egypt. Our study found *Gyrodactylus* sp. in the gills as opposed to the body surface (Ibrahim, 2012; Přikrylová et al., 2012). Similarly, *Gyrodactylus sturmbaueri* was reported from the gills of fish in Lake Tanganyika (Vanhove et al. 2011). *Gyrodactylus* spp. usually inhabit the body surface because this favors their transmission. Variations in microhabitat use are known, with some species changing microhabitats over time. For example, *G. cichlidarum* apparently migrate anteriorly from the caudal fin, ending up on the pectoral fin (Rubio-Godoy et al., 2012), whereas some gyrodactylids exhibit marked site specificity (topographical specialization) (Bakke et al., 2007). For instance, *G. turnbulli* prefers the caudal region of guppies, while *G. bullatarudis* has been found mostly on the head and mouth (Rubio-Godoy et al., 2012). *Gyrodactylus malalai* has been reported only from the fins of *O. niloticus* and *T. zillii* from Lake Turkana (Přikrylová et al., 2012). Therefore, our finding of *Gyrodactylus* sp. exclusively on the gills is unique. Several factors determine the microhabitat use in gyrodactylids. These include: access to resources, intra- and interspecific competition, parasite age, mating, transmission and host immunity (Rubio-Godoy et al., 2012). Although members of the family Gyrodactylidae are said to be ubiquitous ectoparasites of teleost fish (Florio et al., 2009), their reports have been rather uncommon, and the total number of *Gyrodactylus* species described from African fish is generally low. About 10 species of the genus *Gyrodactylus* are known to parasitize African cichlids, a small number compared with an estimated global diversity of 20,000 species (Přikrylová et al., 2012).

Amirthalingamia macracantha was the dominant parasite infecting *T. zillii* in Lake Turkana both in prevalence (79.5%) and mean intensity (10.3). The prevalence of *A. macracantha* reported in this study was higher than elsewhere. For example, Aloo (2002) found a prevalence of 10.7% of *A. macracantha* in *T. zillii* from Lake Naivasha; Florio et al. (2009) found a prevalence of 14.2% from pond- and cage-cultured *O. niloticus* in Kenya; Akoll et al. (2011) found a prevalence of 38% in *O. niloticus* in various water bodies in Uganda, and Otachi et al. (submitted) found a prevalence of 21.4% in *O. leucostictus* and a prevalence of 3.2% in *T. zillii* from Lake Naivasha, Kenya. Its high prevalence and intensity can be explained by the possible ingestion of abundant intermediate hosts (possibly the copepod, *Cyclops* spp.) infected with procercoids (Aloo, 2002; Akoll et al., 2011). Although the life cycle of *A. macracantha* has not been described (Bray, 1974; Aloo, 2002; Scholz et al., 2004), those of the related gryporhynchid taxa involve planktonic copepods as the first intermediate hosts, fish as the second intermediate hosts, and piscivorous birds as definitive hosts (Scholz et al., 2004). *Tilapia zillii* is thought to be herbivorous, but several studies reviewed by Hickley et al. (2002) indicate that it is an omnivorous browser, which could predispose it to *A. macracantha*. Furthermore, the co-occurrence of what we believe to be two distinct developmental forms – merocercoids and plerocercoids – in *T. zillii* has not been reported previously. This co-occurrence raises new questions, particularly regarding the parasite`s unknown life cycle. Several authors (Aloo, 2002; Florio et al., 2009; Akoll et al., 2011; Otachi et al. submitted) have reported *A. macracantha* plerocercoids encysted in the liver or intestinal wall of tilapias. To our knowledge, the only exception was in the original species description (Bray, 1974), which did not explicitly mention whether the specimens were found encysted or free in the liver of *T. nilotica* from Sudan. This raises the questions: (i) why would *A. macracantha* encyst and then excyst within a single host? (ii) is there a possibility of an unknown development within *T. zillii*, perhaps the results of some trigger mechanism? (iii) is the trigger mechanism specific for some study site? One potential factor might be the high mean annual water temperature of Lake Turkana, which is approximately 30ºC (Campbell et al., 2003). The excystment of the parasites when they transit from the fish hosts to birds is known to be triggered by, among other abiotic factors, the high body temperature of birds. Therefore, we hypothesize a scenario in which *A. macracantha* could develop into premature adult worms within *T. zillii* (considered to be intermediate host), as they are also known to mature in fish-eating birds (Scholz et al., 2004).

We found two different species of acanthocephalans. *Acanthogyrus* sp. (P=2.3%) and *Paragorgorhynchus* sp. (P=13.6%) infecting the intestinal lumen of *T. zillii*, albeit at low prevalence and intensities compared to the occurrence of other species in *T. zillii* elswhere. For example, Bayoumy et al. (2006) reported a higher prevalence of infection by *A. tilapiae* (24%) in *T. zillii* from the Nile River at Giza Governorate, Egypt. *Acanthosentis* sp. has been reported in the Nile tilapia *O. niloticus* in Kenya at a prevalence of 7.1% (Florio et al., 2009), while Ibrahim (2012) reported a prevalence of 25.90% in the wild and 24.14 % in cultured *T. zillii* from Lake Manzalah, Egypt. The low infection levels we observed may indicate a low abundance of suitable intermediate hosts. A study by Cohen (1986) of the benthic community of Lake Turkana found a higher abundance of ostracods belonging to the families Cyprididae, Ilyocyprididae, Darwinulidae and Cytheridae, but no amphipods were reported, indicating their low abundance in the lake. Also, *A. tilapiae* is specific to the Cichlidae, whereas *Paragorgorhynchus* spp. are indiscriminate in their host choice (Khalil, 1971). Although *P. albertianum* was reported in *H. forskahlii* (Khalil, 1971) from Congo, in the present study no acanthocephalan was found infecting this host, probably due to its piscivorous feeding habit. In contrast, other studies did not find acanthocephalans in *T. zillii*. For example, Goselle et al. (2008) found no acanthocephalans in the 80 *T. zillii* examined from Lamingo dam, Plateau State, Nigeria.

Three species of crustacean parasites were recovered during this study, represented by two argulids (*Argulus monodi* and *A. fryeri*) and the lernaeid *Lernaea cyprinacea*. All three crustaceans exhibited low infection levels compared to other studies (e.g., Ibrahim, 2012). One explanation is that argulids are more common in smooth-skinned fish such as the Clariidae and less so in scaly fish such as the Cichlidae (Paperna 1980). Ibrahim (2012), for example, determined that *L. cyprinacea* in *T. zillii* from Lake Manzalah, Egypt, occurred at a prevalence of 14.5% in the wild and 24.7% in cultured fish and classified it as a rare parasite in that population. Although Rushton-Mellor (1994) described *A. fryeri* from Lake Turkana, Kenya, the host was reported as unknown. Our study reveals *T. zillii* to be one of the hosts. In contrast to our findings, however, the *T. zillii* examined from Lake Naivasha, Kenya, were not infected with the crustacean parasites (Aloo, 2002; Otachi et al. submitted).

We can draw cautious conclusions even though our sampling episodes were short term and restricted to two sampling periods. *Hydrocynus forskahlii* had a depauperate parasitic community, with only two helminth parasites. The dominant parasite was the anisakid nematode *C. multipapillatum.* The infection levels of *C. multipapillatum* were high, which not only raises aesthetic issues in fish utilization, but also the need to control this parasite. In contrast, *T. zillii* had a rich parasite fauna, but occurred mostly at low intensities except for *A. macracantha.*

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CHAPTER SIX

Concluding discussions

Fish parasites from Lake Naivasha

It was thus established that there was a high prevalence and intensity of ectoparasites dominated by the monogenean *D. minutus* newly introduced together with the *C. carpio*. Protozoan parasites were also present albeit at low intensities and a low diversity of helminth parasites was observed. The current study has therefore partly confirmed the findings of earlier studies on fish parasites in Lake Naivasha by Amin and Dezfuli (1995), Aloo and Dezfuli (1997) and Aloo (1999; 2002). However, new parasite species such as ectoparasitic protozoans, monogenetic trematodes *Dactylogyrus* sp., *D. minutus*, *D. extensus*, *Cichlidogyrus* spp. and *Tylodelphys* sp. have been recovered in this study. Concerning diplostomids, this was the first study combining morphological, molecular and ecological analysis of *Tylodelphys* from Kenyan fishes. Quantitative morphometric analysis did not strongly support the distinct status of two initially distinguished types of metacercariae, and molecular data confirmed that both morphotypes represented a single species of *Tylodelphys*. The oiöxenous nature of this species was clear from its high and equitable distribution among four distantly related lacustrine fishes, and its absence in *B. paludinosus* is attributable to the riverine habits of this host. This may reflect different patterns of habitat use and/or interactions with host immunity in the different fish species. Clearly, much remains to be learned about the diversity and distribution of diplostomids in African fishes and molecular data, ideally in combination with appropriate morphological data from adult worms, provide an efficient way of moving forward, particularly in ecological studies.

Fish parasites from Lake Turkana

The sampling episodes were short term and restricted to two sampling periods, and cautious conclusions were drawn (Poulin, 1996). *H. forskahlii* had a depauperate parasitic community, with only two helminth parasites. The dominant parasite was the anisakid nematode *C. multipapillatum.* The infection levels of *C. multipapillatum* were high, which not only raises aesthetic issues in fish utilization, but also the need to control this parasite. In contrast, *T. zillii* had a rich parasite fauna, but most occurred at low intensities except for *A. macracantha*. Host sex did not influence parasite infection levels except for *C. multipapillatum* in *H. forskahlii*. In general, female fish seemed slightly more parasitized than males. Finally, host size proved to be a very important factor in determining infection levels, with positive correlations, the exceptions being the infection levels of *A. macracantha* in *T. zillii* and the prevalence of *C. multipapillatum* in *H. forskahlii*.

Trace elements concentration, bioaccumulation and bio-indicative aspects: Lake Naivasha

The mean concentration of trace elements in the sediments reflected normal background levels due to the geology of the basin with the exception of Zn. The liver accumulated the highest concentration of most trace elements except Zn, Sr, Rb and Li. Therefore, our findings support most studies that the liver of fish should be considered when carrying out studies on trace elements since it proved to be a good biomarker of most trace elements under this study. Although, *C. multipapillatum* showed potential to accumulate trace elements, it was far lower than in the liver of *O. leucostictus* and quite comparable to the levels in the muscle. Therefore, it is not an efficient accumulation bioindicator. Li, Sr, Cd and Zn had high THQ values and could present a health risk to the local community that depends on fish for regular food.

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Publications

1. Otachi E. O., Magana A. M., Simiyu L. N. and Omweri J. O. (2012) Fluctuating Asymmetry in Selected Fish Species in Lake Naivasha: Biomarkers for Environmental Stress. *Journal of Environmental Natural Resources Management and Society* (JENRS): 29-44
2. Otachi, E., Wathuta, E., Magana, A., Fioravanti, M. L., Florio, D., and Konecny, R. (2011) Comparative Ecological Analysis of Fish Parasitofauna in a Hub-Plot Aquaculture System: Implications for Aquaculture in Kenya. *Egerton Journal of Science and Technology,* 11: 87-103
3. Andrea Gustinelli * Monica Caffara * Daniela Florio * Elick O. Otachi * Euty M. Wathuta * Maria L. Fioravanti.*(2010). First description of the adult stage of *Clinostomum cutaneum* Paperna, 1964 (Digenea: Clinostomidae) from grey herons *Ardea cinerea* L. and a redescription of the metacercaria from the Nile tilapia *Oreochromis niloticus niloticus* (L.) in Kenya. *Syst Parasitol* 76:39-51.
4. Daniela Florio, Andrea Gustinelli, Monica Caffara, Francesco Turci, Francesco Quaglio, Robert Konecny, Tanja Nikowitz, Euty M. Wathuta, Adiel Magana, Elick O. Otachi, Geraldine K. Matolla, Hellen W. Warugu, David Liti, Raphael Mbaluka, Benson Thiga, Jonathan Munguti, Peter Akoll, Wilson Mwanja, Kassahun Asaminew, Zenebe Tadesse, (2009). Veterinary and public health aspects in tilapia (*Oreochromis niloticus niloticus*) aquaculture in Kenya, Uganda and Ethiopia (*Aspetti veterinari e di sanità pubblica nell'allevamento della tilapia (*Oreochromis niloticus niloticus*) in Kenya, Uganda ed Etiopia). Ittiopatologia*. 6: 51-93

Papers submitted

1. Otachi, E.O., Magana, A. M., Jirsa, F. & Frank-Fellner, C. Parasites of commercially important fish from Lake Naivasha, Rift Valley, Kenya (*Submitted: Parasitology Research*)

2. Otachi, E.O., Körner W., Avenant-Oldewage A., Frank-Fellner, C., Jirsa, F. Trace elements in sediments, blue spotted tilapia *Oreochromis leucostictus* (Trewavas, 1933) and its parasite *Contracaecum multipapillatum* from Lake Naivasha, Kenya, including a comprehensive health risk analysis (*Submitted: Environmental Science and Pollution*)

3. Otachi, E.O., Locke, S.A., Franz Jirsa, F., Frank-Fellner, C. Distribution, morphology, morphometrics and genetics of *Tylodelphys* sp. metacercariae (Digenea: Diplostomidae) from the vitreous humor of four fish species from Lake Naivasha, Kenya (*Submitted: Journal of Helminthology*)

In preparation:

1. Otachi, E.O., Jirsa, F. & Frank-Fellner, C. Trace metal concentrations in water, sediments, *Contracaecum multipapillatum* and its fish host *Hydrocynus forskahlii* from Lake Turkana, Rift Valley, Kenya: bioindicative aspects (*Prepared for submission: Environmental Monitoring and Assessment*)

2. Otachi, E.O., Szostakowska B., Jirsa, F. & Frank-Fellner, C. Parasite communities of the elongate tiger fish Hydrocynus forskahlii (Cuvier 1819) and red belly tilapia Tilapia zillii (Gervais 1848) from Lake Turkana, Kenya: influence of host size and sex (*Prepared for submission: African Journal of Zoology*)

SELECTION OF CONFERENCE/ SEMINARS

1. June 2012: EMOP XI in Cluj Napoca, Romania

Paper presented: Parasites of fish from Lakes Naivasha and Turkana, Rift Valley, Kenya

2. November 2011: ÖGTP, Austrian Society of Tropical Medicine and Parasitology, Vienna, Austria

Poster presented: Parasites of fish from Lakes Naivasha and Turkana, Kenya: first results

3. June 2013: ÖGTP Parasitological Day in Klagenfurt

Paper presented: Parasites of commercially important fish from Lake Naivasha, Rift Valley, Kenya.