

Biological and ecological effects of
Ligula intestinalis (Linnaeus, 1758)
in a fish host *Engraulicypris sardella*
(Gütter, 1868) from Tanzanian side
of Lake Nyasa

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Thesis for the degree of Doctor Philosophiae (dr. philos.)
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Dedication

To my parents: Peter Martin Gabagambi and Victoria Protaz Mikimba. I also dedicate this work to my wife Nelusigwe Simon Mwakigonja and my children Alice Nyakato and Collins Kyaruzi.

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First and foremost, I would like to thank my supervisor Arne Skorping for supporting my PhD research project for funding through the Norwegian High Education Loan Board (Lånekassen) under the quota scheme scholarships. Not only that but also for his constant help, encouragements, support and always being available for supervision and guidance. Likewise, I would like to thank my co-authors, Anne-Gro Vea Salvanes for her contribution to Paper II. Adele Mennerat, for data analysis and useful comments on manuscript III. I also recognize the support of Jonathan Kihedu and Mwita Chacha for shaping my thinking in manuscript III. In the same tune, I thank Frank Midtøy for travelling to Tanzania to make sure that all experiments were conducted as planned.

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Bergen, June 2020

Nestory

Abstract

The current thesis investigates the epidemiological, behavioral and evolutionary effects of the tapeworm *Ligula intestinalis* in the ‘Nyasa sardine’ (*Engraulicypris sardella*), an intermediate fish host, endemic to Lake Nyasa, Tanzania.

Most of the available knowledge on this parasite, and its host interactions, comes from small lakes and water reservoirs in Northern Europe, where the parasite tends to show epidemic cycles, where prevalence increases very rapidly, and causing population crashes in its host. This is followed by a rapid decline in infection levels as transmission rates are reduced, and ultimately leads to the local extinction of the parasite. My research investigated the dynamics between the parasite and its intermediate host in Lake Nyasa, a vast tropical lake which differs markedly in its ecological conditions compared to the small and relatively homogenous lakes that have been studied previously.

Given the size of Lake Nyasa, we predicted that transmission rates and levels of infection of this parasite would vary markedly, both in time and space within Lake Nyasa. In accordance with this, we found that the population dynamics of the parasite appeared to change into an endemic state with small variations in prevalence from year to year. Therefore, we suggested that it is unlikely that *L. intestinalis* infestation would have a devastating effect on the *E. sardella* population within Lake Nyasa, as has been reported for smaller water bodies in the European freshwater systems (**Paper I**).

In this thesis, we also investigated the influence of the cestode *L. intestinalis* on the behavior of its intermediate host *E. sardella* and explored if this parasite could cause adaptive modifications of its intermediate host (depending on the parasite infectivity stage) that could lead to increased rates of parasite transmission. We found that fish hosts infected with infective stage parasite larvae significantly altered antipredator defense behavior compared to

uninfected individuals, and that hosts infected with pre-infective stage larvae behaved more or less the same as uninfected hosts (**Paper II**).

Finally, based on our findings that we did not observe any indications of local extinctions of the cestode *L. intestinalis* in Lake Nyasa (**Paper I**), this suggests the parasite may act as a continuous selective force on the fish host *E. sardella* and may drive shifts in life history traits of *E. sardella* towards a higher reproductive investment.

Consistent with our predictions, we found that the cestode *L. intestinalis* had a strong negative effect on the fecundity of *E. sardella* which may have driven the fish host to increase investment in reproduction at the expense of growth. Our findings therefore suggest that the cestode *L. intestinalis* might have selected for increased investment in current reproduction in its fish host *E. sardella* in Lake Nyasa. These results are discussed with respect to other selective factors that could have also influenced the life history traits of *E. sardella* in Lake Nyasa (**Paper III**).

List of papers

The intellectual content of this thesis is built on the following papers.

Paper 1: Gabagambi, N., & Skorping, A. (2018). **Spatial and temporal distribution of *Ligula intestinalis* (Cestoda: Diphylobothriidea) in usipa (*Engraulicypris sardella*) (Pisces: Cyprinidae) in Lake Nyasa.** *Journal of Helminthology* **92**: 410-416.

Paper II: Nestory P. Gabagambi, Anne-Gro V. Salvanes, Frank Midtøy, Arne Skorping (2019). **The tapeworm *Ligula intestinalis* alters the behavior of the fish intermediate host *Engraulicypris sardella*, but only after it has become infective to the final host.** *Behavioural processes* **158**: 47-52.

Paper III: Nestory Peter Gabagambi., Arne Skorping., Mwita Chacha., Kwendwa Jonathan Kihedu., Adele Mennerat (2020). **Increased reproductive investment in an exploited African cyprinid fish following invasion by a castrating parasite in Lake Nyasa, Tanzania.** (Under review *Journal of Ecology and Evolution*).

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1.0 Introduction

It is well known that the transmission success and epidemiology of parasites depend on both environmental conditions (Marcogliese, 2001; Wolinska and King, 2009; Karvonen *et al.*, 2013; Scharsack *et al.*, 2016) and on their interaction with their hosts (Lafferty, 1997; Lafferty and Kuris, 1999; Lazzaro and Little, 2009; Schmid-Hempel, 2011; Kołodziej-Sobocińska, 2019). This is the main reason we find different parasites in different environments, as well as different parasites in different hosts.

When a parasite finds itself in a new environment, it must adapt to new environmental signals, such as a change in temperature, humidity and salinity, as well as to different biotic conditions including unknown predators, new intermediate hosts and different final hosts which might deviate in behavior and biology from the ones they are adapted to.

Clearly, many species of parasites will not be able to cope with such circumstances and will therefore go extinct when they are transferred to a completely new set of environmental conditions. But then there are some groups of parasites that can be found almost everywhere on the globe. The protozoan *Toxoplasma gondii* is a prime example. This parasite is distributed all over the world and is capable of infecting a wide range of intermediate hosts (Dubey and Beatty, 2010). For the final host it is restricted to species within the family Felidae, but can infect all members of this taxon. Another example is the tapeworm *Echinococcus granulosus*, which can also use many different intermediate hosts but is again restricted in its final host, to the family Canidae (Long *et al.*, 2012).

What we typically observe for such parasites is that they have relatively low host specificity (i.e. ability to infect a broad range of host species) and that their developmental stages can live and grow under a range of different environmental conditions.

However, such parasites are the exception rather than the rule; at least among eukaryotic organisms. Most of the parasitic species we know have a highly restricted range of suitable host species (Poulin *et al.*, 2006a; Poulin and Keeney, 2008) and both free-living transmission stages and parasitic developmental stages can only survive and develop under specific environmental conditions. One example is the digenean *Schistosoma mansoni* which can only develop in a few snail species, particularly of the genus *Biomphalaria*, and therefore has a restricted geographic distribution (Basch, 1976). Another is the tapeworm *Schistocephalus solidus* which has a high specificity at the second intermediate host level and consequently can only occur where the suitable fish host is found (Henrich, 2014).

Parasites with a wide geographical distribution allow us to ask questions that cannot be answered by studying parasites with a high host specificity (i.e. parasites with a highly restricted range of suitable host species) and a more narrow geographic range. Parasites that occur over a large geographical area usually have to switch to new host species (Vanacker *et al.*, 2012). We have then the opportunity to study how these parasites adapt to a new host and the effect of this new adaptation on the physiology, ecology, behavior and evolution of the host. Moreover, such parasites are also subjected to different environmental conditions which allow us to explore how a change in environment will affect parasite transmission and epidemiology.

In this thesis we investigated the dynamics of the cestode *Ligula intestinalis* in a new intermediate host species *Engraulicypris sardella* in Lake Nyasa, which represents very different ecological conditions compared to the lakes that have been previously studied.

We therefore expected that the ecological environment of Lake Nyasa should considerably affect the epidemiological patterns of the cestode *L. intestinalis* when infecting its new intermediate host *E. sardella*.

1.1 The life cycle, growth and development of the tapeworm *Ligula intestinalis*

The tapeworm *Ligula intestinalis* is one of the most widespread and economically important parasites infecting freshwater fish as the second intermediate host (Kennedy, 1974; Dubinina, 1980; Hoole *et al.*, 2010). The parasite has been reported infecting fish hosts in many parts of the world, including Africa, Europe, the former USSR, North America, Middle East, China and Australasia (Hoole *et al.*, 2010). The cyprinid fish species that have had reported infections with this parasite include dagaa (*Rastrineobola argentea*) (Marshall and Cowx, 2003), barbus (*Barbus paludinosus*) (Barson and Marshall, 2003), carp (*Chanodichthys erythropterus*) (Sohn *et al.*, 2016), roach (*Rutilus rutilus*), common bream (*Abramis brama*), silver bream (*Blicca bjoerkna*), barbel (*Barbus barbus*), gudgeon (*Gobio gobio*), tench (*Tinca tinca*), common carp (*Cyprinus carpio*), spottail (*Notropis hudsonius*), bleak (*Alburnus alburnus*), minnow (*Phoxinus phoxinus*), rudd (*Scardinius erythrophthalmus*), crucian (*Carassius carassius*), nase (*Chondrostoma nasus*) and dace (*Leuciscus leuciscus*) (Bouزيد Lamine, 2008). The parasite therefore has very low host specificity although most of its intermediate fish hosts are reported to come from the family Cyprinidae. Moreover, this shows that the parasite can be found both from cold temperate regions to the hot and humid climate of Africa and Australasia.

This parasite has a complex life cycle involving two aquatic intermediate hosts and a fish-eating bird as the final host (Dubinina, 1980; Loot *et al.*, 2001b). The life cycle begins in the intestinal tract of the final host, where the parasite reaches sexual maturity and produces eggs. Eggs are introduced to the aquatic ecosystem through bird's faeces, and hatch into free swimming larvae which are eaten by the first intermediate host, a planktonic copepod. After being consumed by the copepod it develops into a small larva, called a proceroid. The cycle proceeds when the second intermediate host, usually a cyprinid fish, ingests infected copepods. Within the body cavity of the fish the proceroid larva develops into a bigger and

more developed larva, called a plerocercoid. The life cycle is completed when fish with developed plerocercoids are eaten by birds.

1.2 Research gaps

The transmission dynamics of the cestode *L. intestinalis* in some host-parasite systems in Northern Europe is well documented (Burrough *et al.*, 1979; Burrough and Kennedy, 1979; Wyatt and Kennedy, 1988; Kennedy *et al.*, 2001; Loot *et al.*, 2001b; Museth, 2001; Vanacker *et al.*, 2012).

In this region, *L. intestinalis* have been reported to show epidemic cycles over periods of 4-5 years, with prevalence increasing very rapidly to reach almost 100%, followed by a rapid decline in infection levels as transmission rates are reduced (Sweeting, 1976; 1977; Kennedy *et al.*, 2001).

This parasite, therefore, has a significant effect on the population dynamics of its intermediate fish hosts in these ecosystems by suppressing the development of the fish host reproductive organs, and thereby significantly reducing reproduction, sometimes towards more or less complete castration, rendering the fish host sterile even when they harbour only a single worm (Kennedy *et al.*, 2001; Hoole *et al.*, 2010).

This has been evidenced by the works of Sweeting (1976), Burrough *et al.* (1979), Kennedy and Burrough (1981), Kennedy *et al.* (2001) and Wilson (1971) who reported reductions in natural populations of roach in Lakes Gravel Pit, Slapton Ley and Chew Valley in England, respectively, due to *L. intestinalis* infections.

Furthermore, it has been previously shown that this tapeworm can affect their intermediate fish host in a way that modifies fish behavior to make them more vulnerable to predation to the avian final host, a mechanism which has been suggested to increase the fitness of the parasite (Brown *et al.*, 2001; Loot *et al.*, 2001c; Museth, 2001; Carter *et al.*, 2005).

This has been evidenced by the works of Loot and colleagues (Loot *et al.*, 2001a; Loot *et al.*, 2002a) who observed clear behavioral alterations in parasitized roach. Loot *et al.* (2001a) showed that infected fish preferred to stay close to the lake shore in the field, and also preferred to swim higher in the water column, compared to uninfected conspecifics, under experimental conditions (Loot *et al.*, 2002a). These behavioral modifications were suggested to favour the predation of infected roach by avian final hosts (Loot *et al.*, 2001a; Loot *et al.*, 2002a). Further evidence of host manipulation by *L. intestinalis* in other cyprinids hosts has been well documented in the works of Brown *et al.* (2001), Museth (2001) and Carter *et al.* (2005),

Taken together, these combined effects (i.e. effects of the parasite on the reproduction capabilities and behavioral alteration) have shown to have a direct major impact on the recruitment dynamics of the host fish stocks. Consequently, these effects have been shown to cause a complete crash in the fish population in these systems (Burrough and Kennedy, 1979; Wyatt and Kennedy, 1988).

However, at present, most of the information available on *L. intestinalis* host-parasite interactions comes from small lakes, dams and reservoirs located in the Northern hemisphere, and from common cyprinids hosts such as roach (Wyatt and Kennedy, 1988; Kennedy *et al.*, 2001), minnow (Museth, 2001) and silver bream (Vanacker *et al.*, 2012).

Less is known about the host-parasite dynamics of *L. intestinalis* from larger lakes, and more specifically deeper lakes such as Lake Nyasa. Studies investigating the effect of this parasite on other cyprinid species in tropical lakes of Africa have not been previously well studied, with the exception of studies conducted by Cowx *et al.* (2008); Msafiri *et al.* (2014) and Rusuwa *et al.* (2014). We must assume that tropical cyprinids differ markedly in physiology,

behavior and ecology from species living in temperate regions, and we would therefore expect that there would be marked differences in the effect of the parasite on such species.

In addition, studying host-parasite interactions in tropical lakes gives us the possibility to explore behavioral effects of this parasite with higher resolution compared to European lakes. Tropical lakes are often huge in area compared to the small lakes, dams and reservoirs studied in Northern Europe. They are thermally and permanently stratified (Darwall *et al.*, 2010; Weyl *et al.*, 2010; Mziray *et al.*, 2018), in contrast to small shallow lakes, where the layers of water are often regularly mixed throughout the year (Vanacker *et al.*, 2012). Thermal stratification provides marked changes in biotic and abiotic factors with water depth (Mziray *et al.*, 2018).

Furthermore, large and deep lakes such as Lake Nyasa provide more environmental heterogeneity compared to most lakes in the temperate region. Given the high level of environmental heterogeneity within Lake Nyasa, we assumed that some sites within the lake may experience local extinction of the parasite while other sites may simultaneously experience increasing parasite abundance. This might prevent population crashes of the intermediate host and the subsequent loss of the parasite, as has been frequently observed in smaller lake ecosystems in the Northern hemisphere (Sweeting, 1976; 1977; Bean and Winfield, 1992; Kennedy *et al.*, 2001).

The novelty of this thesis therefore lies in the fact that we have investigated the epidemiological, behavioral and evolutionary effects of the tapeworm *L. intestinalis* in a hitherto poorly studied intermediate host species *E. sardella*, which lives under highly different ecological conditions compared to the studies that already exist.

The overall aim of this thesis was to increase our understanding of the host-parasite dynamics between the cestode *L. intestinalis* and its intermediate host *E. sardella* in Lake Nyasa.

In this thesis, I present three papers that each advance our knowledge of host-parasite interactions between *L. intestinalis* and *E. sardella*, and provide an initial platform to assess the biological and ecological impacts this parasite might inflict in Lake Nyasa. Here, I outline the broad motivation behind each paper.

In **Paper I**, I investigated the spatial and temporal changes in prevalence rates of *L. intestinalis* in *E. sardella* in Lake Nyasa. Although there have been a few previous studies investigating the prevalence rate of *L. intestinalis* on the *E. sardella* in the Tanzania part of Lake Nyasa (Msafiri *et al.*, 2014) and (TAFIRI;2006 unpublished data), our understanding of the biological and ecological effects of this cestode on its intermediate host *E. sardella* is not well known. In this paper, I examined whether, and how, these host-parasite interactions differ in a large, deep and tropical lake as compared to earlier studies by exploring the variation in the prevalence rate of *L. intestinalis* in *E. sardella* over different spatial and temporal gradients within the lake.

In **Paper II**, I examined whether *L. intestinalis* induces behavioral alterations in *E. sardella* in Lake Nyasa. Previous studies investigating the effects of *L. intestinalis* infection on its host have reported behavioral alterations that were suggested to be adaptive to increase the transmission rate of the parasite to the final host (Brown *et al.*, 2001; Loot *et al.*, 2001a; Loot *et al.*, 2001c; Museth, 2001; Carter *et al.*, 2005). However, these studies were all conducted in small and shallow lakes in Northern Europe, and in different fish species. Before these studies, we did not know if this parasite induced similar behavioral alterations in *E. sardella*. Moreover, I discuss the more general question if such behavioral changes in infected hosts are adaptive to the parasite.

Leading on from this, in **Paper III**, I investigated to what degree parasite infections have led to changes in reproductive investment in the fish host *E. sardella*. Earlier studies reported that

the cestode *L. intestinalis* has the ability to induce castration in several intermediate hosts (Wyatt and Kennedy, 1988; Kennedy *et al.*, 2001; Loot *et al.*, 2002b; Cowx *et al.*, 2008; Hoole *et al.*, 2010) including our study fish model *E. sardella* (Msafiri *et al.*, 2014; Rusuwa *et al.*, 2014). Therefore, in this paper, I investigated whether a strong reduction in the reproduction capacity in *E. sardella* by *L. intestinalis* could have selected for increased investment into current reproduction in this intermediate fish host.

1.3 Hypotheses addressed in this thesis

To this end, the current study explored the following hypotheses:

I: We hypothesized that in Lake Nyasa we would find much higher variation in levels of infection in both time and space, and that this would change the epidemiology of the infection from an epidemic to endemic state.

II: We hypothesized that fish infected with *L. intestinalis* would affect the behavior of its intermediate host in a way that should make it less able to avoid predation, be more active, and show differing preferences in water depth compared to uninfected individuals, depending on the parasite developmental stage.

III: We explored if the cestode *L. intestinalis* selected for changes in life history traits in its intermediate fish host *E. sardella*. Data used for this analysis were collected for a period of ten years in order to elucidate whether *L. intestinalis* caused evolutionary changes in the fish population of *E. sardella*. We hypothesized a shift in reproductive schedule towards a higher investment in current reproduction.

2.0 Study system

2.1 The study site

Lake Nyasa (also known as Lake Malawi in Malawi and Lake Niassa in Mozambique) is situated at the southernmost part of the African rift valley, between Malawi, Mozambique and Tanzania (Fig.1). This is the third largest lake in Africa based on surface area after lakes Victoria and Tanganyika, and is the second largest lake by volume after Lake Tanganyika (Darwall *et al.*, 2010; Macuiane *et al.*, 2015). The lake has a mean surface area of 29,000 Km², a maximum depth of 785 m, and it is 550 Km long and around 48-60 Km wide. It has an estimated volume of 8,400 Km³, and is located at an altitude of 472 m above the sea level (Gonfiantini *et al.*, 1979; Bootsma and Hecky, 1993; Patterson and Kachinjika, 1995; Darwall *et al.*, 2010). The total catchment area of the lake is 126,500 Km² (Kumambala and Ervine, 2010) with land occupying 97,750 Km² of the total catchment area (Menz, 1995). The age of the lake is estimated to be around 2 million years (Scholz and Rosendahl, 1988).

Lake Nyasa is meromictic, although it may experience mixing during the dry season in the southern tip of the lake where the depth is relatively shallow (Vollmer *et al.*, 2005; Darwall *et al.*, 2010). The mean surface temperature of the lake ranges between 24 and 28 °C (Vollmer *et al.*, 2005) with annual rainfall ranging between 1,000 and 2,800 mm (LNBWB, 2013). The lake experiences two main seasons, the dry season (May-August) and wet season (November-April) which are governed by the regional climate (Vollmer *et al.*, 2005; Lyons *et al.*, 2011).

Due to the stratification, together with the great depth of the lake, the nutrients available to the plankton community are generally very low, and thus, the lake is considered 'oligotrophic' (Irvine *et al.*, 2001; Mwambungu and Ngatunga, 2001). The phytoplankton community in the lake is mainly dominated by diatoms and cyanobacteria while the crustacean zooplankton community is dominated mainly by cladocerans and copepods (Irvine *et al.*, 2001). The shore

area of this lake is mainly composed of three ecological zones, namely the rocky shores, the sandy beaches and the rivers estuaries which is associated with swampy areas covered with reeds.

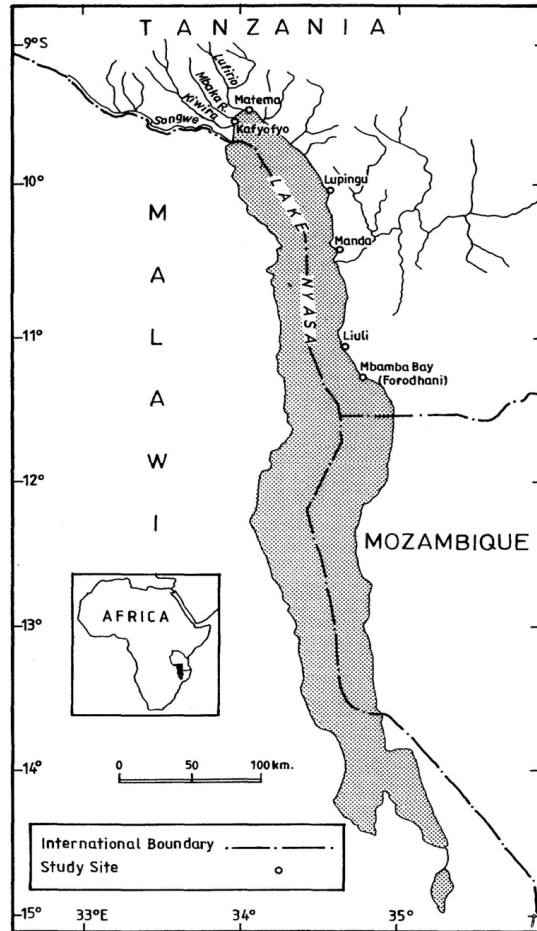


Figure 1. Map of Lake Nyasa showing the administrative riparian states of Malawi, Mozambique and Tanzania. Source; Modified from Msafiri *et al.* (2014).

Lake Nyasa is home to more than 1,000 species of fish, many of which are endemic (Salzburger *et al.*, 2014) including a cyprinid pelagic fish *E. sardella* (Rufli and Van Lissa, 1982), which is currently the only known intermediate fish host of the parasite *L. intestinalis* in Lake Nyasa (Fig. 2).



Fig 2. Laboratory picture of the tapeworm *Ligula intestinalis* and its host fish *Engraulicypris sardella*. Source: Courtesy of Nestory Peter Gabagambi (2015).

E. sardella is a small, slender, silvery, zooplanktivorous fish that occurs in shoals, which is widely distributed within the lake and found in both near shore areas and offshore pelagic water, down to a depth of approximately 200 m (Maguza-Tembo *et al.*, 2009). *E. sardella* is a typically pelagic shoaling species (Lowe-McConnell, 1993) and therefore does not display diurnal vertical migration. *E. sardella* is an annual species, where hatchlings grow and age to reproduce and die in a yearly cycle (Iles, 1960), although some studies have reported that they can live longer (Thompson and Bulirani, 1993; Rusuwa *et al.*, 2014). During early developmental stages, *E. sardella* feeds exclusively on phytoplankton, then switches to feeding on zooplankton once reaching adulthood (Degnbol, 1982; Allison *et al.*, 1996).

E. sardella demonstrate rapid growth rates and can attain a maximum total length of about 130 mm in a year (Tweddle and Lewis, 1990; Thompson, 1996). This species matures at a size around of 70 to 75 mm and have been reported to breed throughout the year but with bi-annual recruitment peaks; which notably occurs during both the wet season and dry season (Morioka and Kaunda, 2005; Rusuwa *et al.*, 2014). We continuously observe small individuals of *E. sardella* in the lake suggesting a continuous spawning behavior in this species (pers. obs.).

E. sardella forms an important part of the food web of Lake Nyasa. The species is a primary consumer of zooplankton (Degnbol, 1982; Konings, 1990), but they are also an important prey of pelagic piscivorous fishes within the lake, particularly *Diplotaxodon* spp. and *Rhamphochromis* spp. (Allison *et al.*, 1996), as well as piscivorous birds (Linn and Campbell, 1992). *E. sardella* is of high commercial value and, for many decades, it has been the main animal protein source for most of the local human population (Manyungwa-Pasani *et al.*, 2017).

Recently however, the species was reported to be infected with the tapeworm *L. intestinalis* (Msafiri *et al.*, 2014; Rusuwa *et al.*, 2014), with the infections in Lake Nyasa first observed by Mwambungu *et al.* (1996). This parasite is believed to have been introduced into the lake by migrating infected fish-eating birds such as White-breasted Cormorant (*Phalacrocorax carbo*) which are among the most abundant fish-eating birds in the Lake Nyasa basin (Linn and Campbell, 1992) and one of the final hosts of *L. intestinalis*. The Lake Nyasa basin also supports (in low numbers) other birds of prey such as, Long-tailed Cormorant (*P. africanus*), many species of heron such as Grey heron (*Ardea cinerea*), and Great Crested Grebe (*Podiceps cristatus*), all of which are also known to be potential final hosts of *L. intestinalis* (Rosen, 1920).

Another group of intermediate hosts necessary for *L. intestinalis* development are aquatic copepods (the first intermediate host). In Lake Nyasa, the crustacean copepod is mainly composed of five copepods communities; the cyclopoids *Mesocyclops aequatorialis aequatorialis* (Keifer) and *Thermocyclops neglectus* (Sars), the calanoid *Tropodiatomus cunnington* (Sars) and the cladocerans *Diaphanosoma excisum* (Jenkin) and *Bosmina longirostris* (Müller) (Thompson and Irvine, 1997; Darwall *et al.*, 2010). The *Mesocyclops* spp. and *Cyclops* spp. are known to be potential agents of *L. intestinalis* transmission (Meyer and Olsen, 1975; Piasecki *et al.*, 2004).

The parasite infects the fish host through ingestion of the infected copepod and then penetrates the intestine to establish in the body cavity. Within the fish host the parasite grows very rapidly, ultimately reducing the space available in the visceral cavity (Dence, 1958). Previous studies have shown that successful infection depends on fish size (Msafiri *et al.*, 2014; Rusuwa *et al.*, 2014). Below the threshold of 50 mm length the infection rate has been found to be zero (Msafiri *et al.*, 2014). This is linked to the change in feeding behavior since juveniles *E. sardella* feed mainly on algae particularly chlorophytes (Allison *et al.*, 1995) and switch to zooplankton diet when they are at the size of 40 mm onwards (Rusuwa *et al.*, 2014).

2.2 Methodological limitations

Our study fish model *E. sardella* were sampled from Lake Nyasa using traditional fishing methods by local fishermen, as well as by our research team using the same fishing procedures. To avoid sampling bias, the fish were sampled lake-wide (i.e. in the Tanzanian waters) across all year seasons at various water depths up to 200 m habitat where *E. sardella* were not found in the catch.

However, there are few potential research limitations in this thesis that could be addressed in future research. For instance, in **Paper I**, although samples of *E. sardella* were collected

across different months of the year 2015, during the analysis all samples were analysed together per each sampling station (i.e. in Matema, Lupingu and Mbamba Bay) to investigate the parasite prevalence in Lake Nyasa. Given that *E. sardella* spawn throughout the year with two peak spawning periods, it would have been interesting to look at differences in size and ontogenic stage of individuals sampled at different times throughout the year (e.g. dry versus wet season), and how this influenced infection rates.

Another limitation was in our behavioral experiments (**Paper II**), where we used wild caught fish that were naturally infected or not infected with the parasite, rather than experimentally infected fish. Since we used wild-caught fish we could not know at the point of collection whether a fish was infected or not, or at what developmental stage any harboured parasites were at (i.e. pre-infective or infective). This could only be confirmed after the experimental tests were carried out and we had dissected the fish. The major limitation of this method is that you cannot attain with any certainty that you will collect even sample sizes for each group (i.e. not infected, pre-infective and infective) to generate a robust statistical analysis. For example, the pre-infective stage test group in this study had a small sample size of three, compared to sample sizes of 38 for not infected and 22 for infective stages. This is one of the main limitations of using naturally infected samples in the experiment. Therefore, it would be informative for future studies to address the same research question using experimental infection studies to determine whether the patterns we observe in the current research remain.

Furthermore, another potential limitation was in **Paper III**. The study is correlative and therefore, it does not allow to firmly demonstrating the causative link between parasitism and life history changes. However, being aware that our study remains correlative, we discussed our results with respect to other potential selective factors that could have also influenced the life history traits of *E. sardella* in Lake Nyasa. In addition, in this paper, samples were not

collected equally each year and not at the same season. With a more balanced sampling procedure, we could have a more powerful statistical analysis.

3.0 Results

This thesis reports the biological and ecological effects of the parasitic tapeworm *L. intestinalis* on its intermediate fish host, *E. sardella*, from the Tanzanian side of Lake Nyasa. Overall, we found that the infection prevalence of *L. intestinalis* in *E. sardella* in Lake Nyasa was generally low (i.e. close to 15%) during the year 2015. However, we did find that infection prevalence differed spatially within the lake. We found higher prevalence in the littoral zone compared to the pelagic zone, as well as a marked effect of water depth on parasite prevalence, with a consistent decrease in prevalence with increasing depth. We also observed clear patterns of seasonal variation in infection, with parasite prevalence significantly higher during the rainy season compared to during the dry season.

Furthermore, we also found that fish hosts infected with the tapeworm *L. intestinalis* differed in their behavior, compared to uninfected individuals. Infected individuals were more easily caught, demonstrated more active swimming, and stayed higher in the water column. The impact of the parasite on host behavior was significantly higher when hosts were infected with infective stage parasites compared to the smaller, pre-infective stage parasite larvae, which had considerably lower effects on fish host behavior. Additionally, we did not observe any correlation between the size of the infective stage parasite larvae and the size of the fish host, or the magnitude of the behavioral modifications (i.e. vigilance, activity and depth preference) observed in the infected fish.

Finally, we found that the tapeworm *L. intestinalis* had a strong negative effect on the fecundity of its intermediate host. Infected *E. sardella* had an overall lower fecundity than uninfected conspecifics. In uninfected fish we observed an increase in relative gonadal weight

over the full 10 year study period, while somatic growth at maturity decreased during the study period

4.0 Discussion

In this thesis, we found that the patterns of epidemic cycles previously observed in small, shallow temperate lakes in Northern Europe (Wilson, 1971; Burrough and Kennedy, 1979; Wyatt and Kennedy, 1988; Kennedy *et al.*, 2001; Loot *et al.*, 2001a; Loot *et al.*, 2001c; Vanacker *et al.*, 2012) were not present in Lake Nyasa. Instead, we observed that the prevalence of the parasite appeared to show large variations, both in time and space. These conditions may have facilitated this parasite to change into an endemic state (in epidemiological terms) rather than an epidemic state as has been observed in the previously studied small, shallow temperate lakes in Northern Europe (**Paper I**).

The observed differences in the population dynamics of this parasite in our study system did not seem to be caused by the fact that *L. intestinalis* uses a different fish host species within Lake Nyasa other than the endemic sardine species *E. sardella*. Instead, we suggested that the observed differences in parasite transmission and epidemiology pattern were associated with the new environmental conditions within Lake Nyasa where this parasite has been introduced over the last two decades.

Moreover, we found that the effects of the parasite on the behavior and physiology of *E. sardella* appeared to be remarkably similar to what has been described previously in fish host species from Northern Europe. For instance, we found that *L. intestinalis* tended to affect the behavior of *E. sardella* in the similar way as reported in roach (Loot *et al.*, 2002a), gudgeon (Bean and Winfield, 1992) and carp (Sohn *et al.*, 2016) despite the fact that these intermediate host species show quite different patterns of natural behavior (**Paper II**). For instance, roach shows a batch spawning behavior (i.e. ability to spawn more than once within

the spawning period) (Wedekind, 1996), whereas *E. sardella* shows a continuous spawning behavior (i.e. ability to spawn throughout the year) (Thompson, 1996).

Furthermore, we found that *L. intestinalis* induced partial castration (i.e. reduction of the host fecundity) in *E. sardella* in a similar way as was reported in roach (Kennedy *et al.*, 2001; Hoole *et al.*, 2010), rudd (Orr, 1966), gudgeon (Fuhrmann, 1934), dace (Arme, 1968) and carp (Sohn *et al.*, 2016) despite the fact that these host species must be quite different in physiology and immune responses (**Paper III**). We also found that the reduction in host fecundity by *L. intestinalis*, in conjunction with the effect from fishing pressure, may have acted as selective agents driving evolutionary changes in the life history of *E. sardella* by increasing reproductive investment into current reproduction (**Paper III**).

The results of this thesis, therefore, suggest that the cestode *L. intestinalis* uses a very general mechanism to modify the behavior and physiology of its intermediate fish host species so that it can be successfully applied in many other different alternative host species across latitudes (**Papers II & III**). However, these findings were observed in a new host species living in a new ecological environment where the prevalence of this parasite was found to be changing significantly from year to year (**Paper I**).

From an evolutionary perspective, a fundamental question is whether this observation is the general rule - do parasite species that are able to use different alternative intermediate hosts tend to affect these host species in the same way?

According to Poulin and Keeney (2008), different parasite groups show different levels of host specificity. Some of the parasites are able to establish in a broad range of alternative hosts (i.e. generalist parasites). A typical example of a generalist parasite that has been reported to exploit a broad range of intermediate mammal hosts, and affects these intermediate host species in a similar way to reach to its definitive host, is *T. gondii*, an

intracellular apicomplexan protozoan which is globally distributed. Extensive studies carried out under different experimental conditions showed that *T. gondii* altered the behavior of different rodents species (its intermediate host prey) in a similar way by either increasing the host activity (Piekarski *et al.*, 1978; Witting, 1979; Webster, 1994) or by decreasing the fear of novelty (i.e. neophobia) (Webster *et al.*, 1994; Berdoy *et al.*, 1995); mechanisms that make the intermediate host more likely to be predated on by cats, the parasite's definitive host.

Another example of a host-generalist parasite comes from the lancet liver fluke model, where the parasitic fluke *Dicrocoelium dendriticum* infects several snail and ant species (as its intermediate hosts) across the world. This parasite has been observed to consistently apply the same strategy to ensure its arrival at the definitive host (the sheep) by modifying the ant's behavior to remain attached to the upper region of grass blades and stay there patiently waiting for a grazing sheep (Carney, 1969; Wickler, 1976; Moore, 2002). Other patterns and processes of alternative host use have been described for many other generalist parasites in the work of Lootvoet *et al.* (2013).

Taken together, the aforementioned findings suggest that it seems to be a general pattern that parasite species which use different alternative intermediate hosts tend to interact with their alternative hosts in the same way to ensure their transmission to the final host. Although the transmission success may differ between alternative hosts; the mechanism used by the parasite to infect the host remains similar across a broad range of hosts. This also appears to apply for *L. intestinalis*.

Following this, why are some parasites selected towards lower host specificity and thereby adopt a more generalist strategy?

A host-generalist strategy, whereby the parasites are less selective and always use the same mechanism to infect many taxonomically diverse hosts, has been shown to be beneficial in

some cases. For instance, Streicker *et al.* (2013) suggested that parasites that infect a broad range of host species are more resilient to changing environmental conditions, and Viana *et al.* (2014) showed that reservoir hosts are often critical for the continuation of the transmission. Another key factor that might explain the benefit of evolving host-generalist strategies is that this approach reduces the risk of parasite extinction in the event that principle host species becomes rare or disappears (Bush *et al.*, 2001). Parasites employing host-generalist mechanisms can more readily switch hosts when needed (Vanacker *et al.*, 2012), and therefore are more likely to survive events such as host population crashes, over parasites that employ host-specific mechanisms. Additionally, according to the ecological similarity hypothesis by Timms and Read (1999) and Cooper *et al.* (2012), a generalist approach offers a decreased risk of mortality when entering new host species that are ecologically related to the principle host species; i.e. host species that share resources, such as diet or habitat, with the principle host (Combes, 2001). A further benefit of exploiting a broad range of alternative hosts includes exposure to multi-immune systems that cannot evolve to eliminate the parasite since that generalist parasite can co-evolve to survive in their new alternative hosts (this follows the Red Queen hypothesis) (Schmid-Hempel, 2011).

However, despite of the evolutionary advantages of employing a generalist strategy, several studies has suggested that across taxa, many parasite species are not extreme generalists (Sasal *et al.*, 1999; Poulin *et al.*, 2006b; Poulin and Keeney, 2008; Agosta *et al.*, 2010).

This indicates that there are fitness costs of being a generalist parasite. For instance, Poulin (2005) and Leggett *et al.* (2013) suggested that, the ability of generalist parasites to exploit many host species has made this group of parasites less efficient in exploiting the host because they are not specifically adapted to that host. Other fitness costs of being a generalist parasite includes the higher probability of finding themselves in a multiple infections scenario

within their alternative intermediate hosts, which consequently might require the parasite to invest more in outcompeting co-infecting parasites (Leggett *et al.*, 2013).

Our finding that epidemiology of *L. intestinalis* in Lake Nyasa differs from that reported in earlier studies of small, temperate lakes (**Paper I**), but that behavioral and physiological effects the parasite has on its intermediate host *E. sardella* are comparable across systems (**Papers II and III**), provides strong evidence that the cestode *L. intestinalis* consistently induces behavioral modifications that increase transmission, and significantly reduces fish fecundity, independent of the host species or geographic location. Therefore, this thesis expands our understanding of how the tapeworm *L. intestinalis* can affect novel host populations in varying freshwater systems.

The observed relatively small changes in prevalence between sampling years in Lake Nyasa means that, in this locality, the parasite acts as a continuous selective force on the fish host, as opposed to in Northern European lakes where the parasite frequently tends to go locally extinct. Consistent with our predictions, we found an increase in the reproductive investment (at the expense of growth) in the fish populations of *E. sardella* in Lake Nyasa. Our findings are in agreement with the life-history theory which predicts a higher reproductive investment as an adaptive response to reduction in future reproductive value, such as the presence of a castrating parasite (Blair and Webster, 2007), increased predation pressure (Gordon *et al.*, 2009; Walsh and Reznick, 2009; Sharpe *et al.*, 2012) or increased fishing mortality (Jørgensen *et al.*, 2007; Jørgensen *et al.*, 2009). Facultative adjustment of reproductive investment observed in this study provides further evidence that selection frequently favours plasticity in investment that can compensate for environmental change (Harris and Uller, 2009; Ebner *et al.*, 2016). In the light of life-history theory, the fish population of *E. sardella* in Lake Nyasa seems to be responding to such change, a strategy which may compensate or reduce the costs of parasitism. Fecundity compensation as an adaptive

response to infection has also been observed in other host-parasite systems, for instance in *Drosophila nigrospiracula* infected with a mite (*Macrocheles subbadius*) (Polak and Starmer, 1998) and in crickets (*Acheta domesticus*) and *Daphnia magna* following infection with the bacterium *Serratia marcescens* and microsporidian *Glugoides intestinalis* respectively (Adamo, 1999; Chadwick and Little, 2005). Together with our study this suggests that parasites have the potential to affect the future reproductive success in several host-parasite systems and thus drive changes in host life history traits.

5.0 Conclusions and future research

This thesis shows that the ecological conditions of a large and deep tropical lake significantly affects the transmission dynamics of the parasite *L. intestinalis* compared to smaller and shallow lakes found in European freshwater systems. However, the current findings were limited to three locations (i.e. Matema, Lupingu and Mbamba-Bay) in the Northern part of the lake, while the fish host *E. sardella* is widely distributed throughout the lake. It would therefore be worthwhile to extend the research to other parts of the lake to confirm the current findings are consistent across the whole of the lake, which would aid in formulating a common management approach in all three countries sharing the lake (i.e. Malawi, Mozambique and Tanzania) and its resources. Long term data monitoring of observed trends in parasite-host dynamics in Lake Nyasa would be extremely informative and would help to elucidate the potential impacts of this parasite on the productivity of this important fishery in the lake.

The apparent evolutionary change that was observed in the fish host *E. sardella* was suggested to be associated with the cestode *L. intestinalis* in conjunction with fishing pressure. However, our data are only correlative, and therefore, it would be interesting to provide more data in the future that could also help to explain how other potential selective pressures, such as changes in natural predation by native piscivorous predators, changes in

water temperature and fluctuations in plankton abundance could affect the life history of *E. sardella*. In addition, there is also a need to assess the level of genetic variation between local populations of *E. sardella* within the Lake Nyasa. This study will help us to determine whether some locations within the lake experience stronger selection pressure than others.

Finally, in our experiment on parasite-induced changes in host behavior, we used a model which is an endemic pelagic schooling species (Lowe-McConnell, 1993) that is widely distributed within the lake and can be found to depths of approximately 200 m (Maguza-Tembo *et al.*, 2009). Based on the nature of the distribution ranges exhibited by this fish species in this lake, and considering its good swimming abilities, I suggest further behavioral studies which build on the questions dealt with in this thesis. I would also suggest enhancing the experimental set-up by using larger research vessels and advanced acoustic equipment with opening and closing devices that could sample this fish species at the specific depth ranges.

6.0 References

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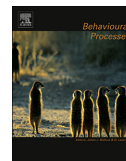
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The tapeworm *Ligula intestinalis* alters the behavior of the fish intermediate host *Engraulicypris sardella*, but only after it has become infective to the final host



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ABSTRACT

Ligula intestinalis is a tapeworm using copepods and cyprinid fish as intermediate hosts and fish-eating birds as final hosts. Since some parasites can increase their own fitness by manipulating the behavior of the intermediate host, we explored if this parasite affected predator avoidance, swimming activity and depth preference of the fish intermediate host, *Engraulicypris sardella*. We found that when *L. intestinalis* had reached a developmental stage that is able to establish in the bird host, it had a significant impact on *E. sardella* behavior, while the tapeworm that was not fully developed had little effect and fish hosts showed a behavior more similar to uninfected fish. These results are discussed with respect to two different processes: the manipulation hypothesis and the energy drain hypothesis.

1. Introduction

Parasites sometimes induce morphological or behavioral changes in their intermediate host (*i.e.*, a host that is used for developmental stages of the parasite before reaching the final host) in order to increase their own fitness through enhanced transmission success (Lafferty, 1999). If parasites are transmitted through the food chain, the most obvious way of increasing transmission is by making the host more vulnerable to predators. Parasites may achieve this by weakening the host (Temple, 1987; Joly and Messier, 2004; Hafer and Milinski, 2016), or actively manipulating its appearance (Yanoviak *et al.*, 2008) or behavior (Barber *et al.*, 2000; Berdoy *et al.*, 2000; Holmstad *et al.*, 2006; Poirotte *et al.*, 2016). It is therefore not surprising that final hosts (*i.e.*, a host where parasite reproduction takes place) tend to catch a disproportionate number of infected intermediate host prey (Brown *et al.*, 2001; Loot *et al.*, 2001a; Moore, 2013).

Parasites, by definition, use the host as a resource by exploiting energy and nutrients that could otherwise have been used to sustain the growth, activity and reproduction of the host. Since energy and proteins are usually limited, a parasitized host tends to be harmed or weakened by the parasite. One reason that parasitized individuals tend to be captured by predators more often than non-parasitized ones could simply be because they are easier to catch. This is usually referred to as the “energy drain hypothesis” (Hafer and Milinski, 2016)

However, some infected hosts show behavioral changes that are difficult to explain simply by the harm done by the parasite. For example, why would a rat infected with *Toxoplasma gondii* tend to be less afraid of the presence of a cat, which serves as the final host for this parasite (Berdoy *et al.*, 2000), or why would some digeneans change the antennae of their snail hosts to look like insect caterpillars, which is the preferred food for the bird final hosts (Combes, 2001)? Observations such as these have led to an alternative hypothesis where it is assumed that the parasite has been selected for characters that specifically enhance their transmission. This hypothesis is referred to as “the parasite manipulation hypothesis”, although, as pointed out by Thomas *et al.* (2005), the energy drain hypothesis and the manipulation hypothesis are not necessarily mutually exclusive. Several workers have stressed that we need more examples and a better understanding of how parasites affect trophic transmission (Lafferty, 1999; Kuris, 2005; Poulin, 2010), since it has the potential to influence fundamental processes and patterns within an ecosystem, such as predator-prey interactions and the abundance and distribution of predators (Joly and Messier, 2004).

Manipulation of the intermediate host would only be adaptive after the parasite is ready for establishment in its definitive host (Parker *et al.*, 2009; Poulin, 2010). A parasite which is ready to be transmitted to the final host can increase its fitness by manipulating its intermediate host to be more easily taken by the predator, but for parasites that are not yet fully developed (*i.e.*, pre-infective stages), an early transfer to

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the next host could be fatal (Bethel and Holmes, 1974; Dianne et al., 2011).

The parasite that is at a pre-infective stage should therefore manipulate the intermediate host in the opposite direction to reduce predation and avoid a premature transfer to the final host (Parker et al., 2009; Hafer and Milinski, 2015). This strategy is known as predation suppression hypothesis (Parker et al., 2009; Dianne et al., 2011). In a modelling study, Parker et al. (2009) concluded that predation suppression should be easier to evolve than predation enhancement, because enhancement needs to be selective towards the final host, while parasite stages not yet ready to establish in a host will gain fitness by suppressing predation in general. Despite the logic behind this idea, most published evidence for parasite manipulation is related to enhancement. However, since intermediate hosts are often infected with parasites at different stages of infectivity, any effects of parasite manipulation can be difficult to detect without careful experimental studies.

The tapeworm *Ligula intestinalis* (L. 1758) is a common and widespread parasite mainly infecting cyprinid fish as the second intermediate host (Dubinina, 1980; Hooile et al., 2010). This parasite has a complex life cycle involving two aquatic intermediate hosts and a fish-eating bird as the final host (Dubinina, 1980). The life cycle began in the intestinal tract of the final host, where the parasite reaches sexual maturity and produces eggs. Eggs are introduced to the aquatic ecosystem through bird's faeces, and hatch into free swimming larvae which are eaten by the first intermediate host, a planktonic copepod. After being consumed by the copepod it develops into a small larva. The cycle proceeds when the second intermediate host, usually a cyprinid fish, ingests infected copepods. Within the body cavity of the fish host the parasite develops into a bigger larva. The life cycle is completed when infected fish are eaten by birds.

Several studies show that avian final hosts tend to consume a higher proportion of intermediate hosts infected with *L. intestinalis* than the proportion present in a lake (Dobben, 1952; Brown et al., 2001; Loot et al., 2001a). Furthermore, cyprinid fish infected with this parasite tend to have a different behavior compared to uninfected individuals, for example by swimming closer to the surface (Loot et al., 2002). These observations suggest that *L. intestinalis* in the intermediate hosts could be a parasite that is selected to increase its transmission through host manipulation. In addition, to date there is a lack of empirical studies on how different developmental stages of this parasite affect host behavior.

In this study we investigated the effects of *L. intestinalis* on its intermediate fish host *Engraulicypris sardella* by examining three different behavioral traits namely; predation avoidance, swimming activity and depth preference. We also studied how different stages of infectivity of the parasite, that is the infective and non-infective stages, affected host behavior.

We predicted that fish infected with *L. intestinalis* would be less able to avoid predation, be more active due to higher energetic needs, and show a different preference for water depth as compared to uninfected fish, depending on the parasite infectivity stage. For intermediate hosts infected with parasite larvae that are in the infective stages and ready to be transmitted to the final host, we expected the parasite to induce behavioral changes that would make the intermediate host more vulnerable to predation by avian predators. However, for intermediate hosts infected with parasite larvae that are in the pre-infective stages, we predicted a reversed manipulation and expected to observe behavioral changes that are consistent with a smaller risk of being predated upon. We also examined whether our results are best explained by active manipulation by the parasite or by the energy drain hypothesis.

2. Methods

2.1. Fish used

Experimental fish *E. sardella* were caught in Lake Nyasa, also known

as Lake Malawi or Lake Niassa, which is situated in southernmost of the large African rift valley lakes between S 9°30' and 14°30' S (Twombly, 1983). *E. sardella* is a pelagic shoaling cyprinid species (Lowe-McConnell, 1993). The fish were caught between December 2016 and January 2017 at Matema fishing station that is situated northern part of the lake (S 9°29'; E34°01').

The fishing procedure was to first attract the fish to artificial light and then encircle them using a seine net with 10 mm mesh size. We used seven fluorescent light bulbs each with 12 W (total effects 84 W) operated by one dry cell battery with 12.6 V for 30 min. The fish came voluntarily near the light zone and were caught. About 60 live fish were transferred to three holding tanks in the boat using a bucket containing water from the lake (about 20 individuals per tank) each with 120 L water capacity filled with ca 80 L fresh lake water and transported 1–2 km to the field station.

On arrival to the field station, the three holding tanks were aerated using air stones fitted to air pumps (Karlie aquarium air pump high tech 1500) to keep oxygen level > 90%. Oxygen concentration was monitored by using Oximeter (WTW oxi 3310 fitted with WTW cell Ox 325 sensor). During the experiment, the water temperature in the aquaria was ≤ 25 °C and the oxygen saturation level was > 90%.

The experimental protocol of Gopko et al. (2015) was modified and used to test if infected and uninfected individuals; i) responded differently to simulated predator attacks; ii) differed in swimming activity; and iii) had different depth preferences. Immediately after completing each experiment trial, the test fish was euthanized using an overdose of Tricaine methanesulfonate (MS-222; 100 mg/l). The fish was measured for length to the nearest millimeter and weighed to the nearest 0.01 g. The fish were later examined for parasite and the cestodes were identified according to the protocol of Dobben (1952). The parasite was also measured to nearest millimeter and weighed to nearest 0.01 g. Maturation stage of *L. intestinalis* was categorized based on its weight. Previous work by Wyatt and Kennedy (1988) has shown that *Ligula* larva weighting > 0.57 g can be regarded as a fully developed larva. They have shown that larva above this weight are capable of maturation in vitro, and therefore supposedly, of maturation in the bird final host. In the present study, the status of infected fish with *Ligula* larva weighing < 0.57 g were categorized to be infected with the parasite which is at in the pre-infective stage and those weighing > 0.57 g as infected by parasite which is at the infective stage. Detailed information about the weight distribution of the parasite larva is provided in Supplementary data (Table S1).

The experimenter was blind to whether a fish was infected or uninfected since the parasite lives in the host's abdominal cavity (Loot et al., 2001b). Following Gopko et al. (2015) we conducted behavioral tests until a sufficient number of 25 infected individuals was reached. Therefore, the sample size of infected and uninfected fish differed. After we had completed the experiments on 25 infected and on 51, 45 and 38 uninfected individuals for Experiments 1, 2 and 3 respectively (Table 1), experiments stopped. Each test fish was used only one time, such that different fish were used in the 3 experiments trials.

2.2. Experimental protocol

On arrival to the Matema field station, fish were allowed to acclimate for 60 min in the holding tanks before the trials began. Each fish was caught from the holding tanks using a 4 L bucket containing ca 0.4 L water and transferred to the test aquaria where it acclimated for 10 min before the behavioral trial started.

The water in the aquarium was changed between each test fish and the aquarium was thoroughly cleaned using fresh lake water. Video footages were recorded using GoPro Hero 3+ edition and Canon Legria HF R506 cameras which were fixed above and positioned at the centre of the aquariums for Experiment 1 and Experiment 2 and on the side of the aquarium for Experiment 3. All tests were done in shelter and all aquaria and test fish had similar light conditions during the tests, and

Table 1

Sample size of infected and uninfected test fish on each of the experimental days in Matema station, December 2016 and January 2017.

Experiment	Day	Number of Experimental Aquarium	Infected		Uninfected	Total
			Pre-infective	Infective		
Predation test	1	3	2	7	21	30
	2	2	2	4	17	23
	3	2	7	3	13	23
Activity test	1	1	11	14	45	70
Depth test	1	1	3	22	38	63

no direct sun light.

2.2.1. Experiment 1: simulated predation

The aim of the experiment was to test if infected *E. sardella* were more easily caught than the uninfected fish. Rectangular glass aquariums (length × width × height: 30.0 cm × 20.2 cm × 17.0 cm) were filled with 4.8 L fresh lake water (ca 8 cm high). The length of the aquaria was thus equivalent to 3–4 body lengths of the test fish allowing approximately normal swimming activity, and having ability to possess burst escape from the simulated attack. All four sides of the aquariums including the bottom part were covered with black plastic sheets to reduce mirror image effect and to prevent visual contact and minimize disturbance to the test fish from the outside. Two investigators were collaborating during the test. One investigator simulated predator attack using a rectangular dip-net (8 cm × 7 cm). To avoid that the test fish swim over the dip-net when simulating predator attacks, the water height in the test aquarium was chosen to be same as the dip-net height. The dip-net was slowly and blindly moved from one end of the aquarium to the other with relative constant speed, and as the investigator that was moving the net had blinds covering the eyes, the dip-net movement along the aquarium length was approximately random as to the where along the aquarium length the dip-net was moved. This continued until the second investigator confirmed that the fish was caught. The time from start of the simulated predator attack and until the test fish was captured by the dip-net was recorded.

2.2.2. Experiment 2: swimming activity

The aim of the experiment was to test whether swimming activity differed between infected and uninfected *E. sardella*. Behavioral tests were conducted in a rectangular glass aquarium (length × width × height: 30.0 cm × 40.0 cm × 50.0 cm) filled with 50 L fresh lake water (height ca 42 cm). All sides including the bottom of the aquarium were covered using black plastic sheets to reduce disturbance of the fish. The bottom of the aquarium was gridded using silver duck tape (10.0 cm × 10.0 cm grids).

The grid on the bottom allowed quantifying swimming activity (*i.e.*, horizontal moving) by counting the number of grid lines crossed by the test fish during a standardized 4 min observation period. During footage analysis, we found one test fish swimming abnormally, thus we removed this fish from the analysis. All other test fish showed approximately normal swimming activity and had the ability to swim 3–4 body lengths and showing both horizontal and vertical turns within the test aquarium. Therefore, final sample size for analysis in this experiment consisted of 24 infected fish and 45 uninfected fish.

2.2.3. Experiment 3: depth preference

The aim of the experiment was to test whether the depth preference differed between infected and uninfected *E. sardella*. The experiment was conducted in a rectangular glass aquarium (length × width × height: 30.0 cm × 40.0 cm × 50.0 cm) filled with 50 L fresh lake water (height ca 42 cm). Three sides of the aquarium including the bottom part were covered using black plastic sheets to reduce disturbance of the fish, but one side of the aquarium was left uncovered to be able to observe the vertical position (depth preference) of the test fish.

Six horizontal lines were drawn on the non-covered side of the aquarium and depths were categorized into 1, 2, 3, 4, 5, and 6. For statistical analysis these categories were transformed into 0, 0.2, 0.3, 0.5, 0.7, 0.8 and 1 with 0 being the lowest line at the bottom of the aquarium (minimum value) and 1 being the line located at the surface (maximum value). The videos were analyzed and for each test fish the vertical position in the water column was noted for every 30 s over a 10 min period. For each test fish, mean transformed score for the 20 recording events was calculated and used in the statistical analysis.

2.3. Statistical analysis

Statistical analysis and graphics were carried out using R, versions 3.2.5 (<http://r-project.org>). The fish size differences in Experiments 1, 2 and 3 were tested using a one-way ANOVA test.

Analyses of time used by a fish to avoid the dip-net catch were performed using Linear Mixed Effect Model (LME). Time was response variable in the model and infection (Levels: uninfected, pre-infective and infective) were the explanatory variables. Because the test experiment was conducted for three different days at three different aquariums, day of experiment and aquarium were included as random factors in the models.

Analyses of overall swimming activity of a fish were carried out using Generalized Linear Model (GLM) fitted with a quasi-poisson distribution. In the model, number of lines crossed by the fish in the aquarium grids was fitted as a response variable and predictors are the same as described in the previous model.

Analyses of depth preference of a fish were carried out using Generalized Linear Model (GLM) fitted with a quasi-binomial distribution. In the model vertical position (mean score) preferred by the fish in the aquarium was fitted as a response variable and predictors are the same as described in the previous model. Mean scores were scaled from 0 being the lowest line at the bottom of the aquarium (minimum value) to 1 being the line located at the surface of the aquarium (maximum value) to meet the assumption of the GLM model fitted with quasi-binomial error term.

Since change in fish behavior may be positively correlated with how much energy the parasite takes from the host, we estimated the size of the infective parasites in relation to the host by dividing the fish weight by the parasite weight. This index was compared with the fish host behavioral traits to check if they were positively correlated using Spearman's rank correlation test.

3. Results

The total lengths (mean ± SD) of fish for Experiments 1, 2 and 3 were 101.10 ± 8.10 mm; 101.81 ± 4.92 mm and 103.25 ± 6.62 mm respectively. Fish size did not differ among groups (one-way ANOVA; $F_{(2, 205)} = 1.86, P = 0.16$). The total lengths (mean ± SD) of the infected fish host, uninfected fish and the parasite were 101.62 ± 6.63; 101.19 ± 8.14 and 111.10 ± 25.43 mm respectively. The mean intensity (*i.e.*, number of parasite in a single host) of *L. intestinalis* in infected fish was 1.0.

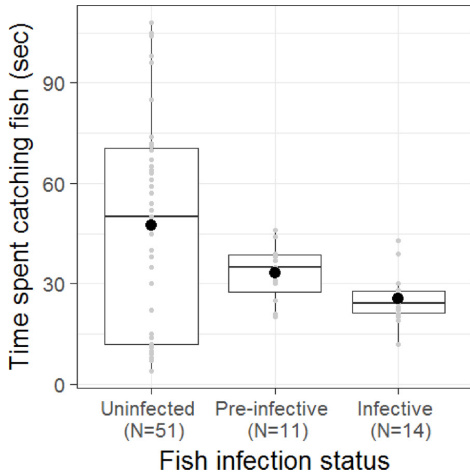


Fig. 1. Boxplot showing the influence of *L. intestinalis* on host ability to avoid simulated predation attack. The horizontal lines show the median, solid black circle show the mean and open grey circle show the raw data set.

3.1. Experiment 1: simulated predation

Uninfected *E. sardella* took longer to be caught by a dip net than infected fish (LME: $F_{(1, 68)} = 18.18, P < 0.0001$). When we split the group of infected fish into those carrying infective and pre-infective parasite larvae, both these groups were caught quicker than uninfected fish: pre-infective vs uninfected: (LME: $F_{(1, 54)} = 14.55, P < 0.0001$), infective vs uninfected: (LME: $F_{(1, 57)} = 9.21, P < 0.004$; Fig. 1). However, we also observed a difference in the time needed to catch a fish within the infected group; fish carrying pre-infective larvae took significantly longer to be caught than fish with infective larvae (LME: $F_{(1, 18)} = 5.07, P < 0.03$; Fig. 1). Additionally, there was no correlation between the size of the infective larvae in relation to fish size and the time used by a fish to avoid the dip-net catch (Spearman's Rho: $R_s(14) = -0.31, P = 0.28$).

3.2. Experiment 2: swimming activity

There was no difference in the swimming activity between fish infected with *L. intestinalis* and uninfected fish (GLM: $F_{(1, 67)} = 3.77, P = 0.06$). Fish infected with infective parasite larvae were more active than uninfected fish (GLM: $F_{(1, 57)} = 16.07, P < 0.001$; Fig. 2), while fish infected with pre-infective larvae did not differ in activity from uninfected fish (GLM: $F_{(1, 53)} = 2.77, P = 0.10$; Fig. 2). Fish infected with infective larvae were more active than fish infected with pre-infective larvae (GLM: $F_{(1, 22)} = 23.05, P < 0.001$; Fig. 2). We found no correlation between the size of the infective larvae in relation to fish size and the swimming activity of the fish host (Spearman's Rho: $R_s(14) = 0.24, P = 0.93$).

3.3. Experiment 3: depth preference

Infected *E. sardella* tended to position themselves higher up in the water column as compared to uninfected fish (GLM: $F_{(1, 61)} = 17.59, P < 0.001$). Fish infected with infective parasite larvae stayed shallower than uninfected fish (GLM: $F_{(1, 58)} = 17.50, P < 0.001$; Fig. 3).

There was no correlation between the size of the infective larvae in relation to fish size and the depth preference of a fish host (Spearman's Rho: $R_s(22) = 0.22, P = 0.34$).

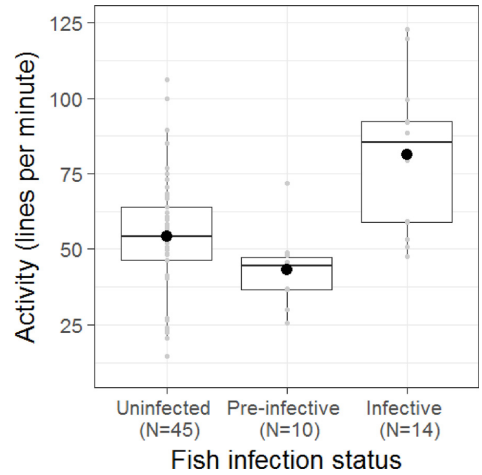


Fig. 2. Boxplot showing the influence of *L. intestinalis* on host swimming activity. The horizontal lines show the median, solid black circle show the mean and open grey circle show the raw data set.

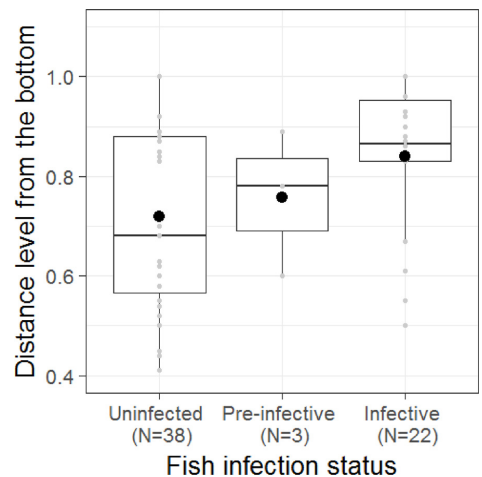


Fig. 3. Boxplot showing the influence of *L. intestinalis* on host depth preference. The horizontal lines show the median, solid black circle show the mean and open grey circle show the raw data set.

4. Discussion

We found that *E. sardella* infected with *L. intestinalis* were more easily caught and positioned themselves higher up in the water column than fish that were not infected, suggesting that this parasite may affect the behavior of its fish intermediate host as earlier suggested by Loot et al. (2002). As for swimming activity we observed no difference between infected and non-infected individuals.

When we split the infected group into two categories, depending on infection stage of the parasite, the results became clearer. Fish with parasite larvae ready to be transmitted to the final host, differed in their behavior from uninfected fish in all our tests. They were more easily caught, showed higher swimming activity, and tended to stay higher up in the water column. The overall picture of these three tests is that when the tapeworm has reached the stage of development when it can

establish in the final host, it changes the behavior of the intermediate host in a way that makes it more vulnerable to predation. Our simulated predation test suggests that fish infected with parasites in the infective stage were less vigilant and therefore less able to avoid being caught by the dip net than uninfected fish, despite showing a higher swimming activity. A fish that tends to move around more is also more likely to be caught by a bird predator, since birds are visual predators. It is also reasonable to expect that fish closer to the surface are more vulnerable to predation by birds than fish in deeper waters. Our first prediction is therefore confirmed.

Our next question was whether fish infected with pre-infective larvae are manipulated in the opposite direction? Since it is not in the interest for these parasites to be transmitted to a bird host quite yet, do they induce behavioral changes that makes the intermediate host less vulnerable to predation? We could not see any evidence for this in our results. As for swimming activity, fish carrying pre-infective larvae showed no difference in their behavior compared to uninfected fish, and in the simulated predation test we found that these fish also were more easily caught than uninfected ones. However, when we compared the behavior of the two categories of infected fish, those carrying pre-infective larvae differed significantly from fish with infective larvae. Fish infected with pre-infective larvae took a longer time to be caught and showed lower swimming activity as compared to the group harboring infective *L. intestinalis* larvae. The picture that emerges from these three experiments is therefore that when *L. intestinalis* has developed to a stage that is able to establish in the bird host, it has a significant impact on fish behavior, while when the tapeworm is pre-infective this effect is significantly smaller and fish with parasites in this maturational stage are more similar in their behavior to uninfected fish.

One concern in the interpretation of our results is that we used naturally infected fish. In theory there could have been genetic differences between individuals that affected both susceptibility and behavior patterns, without any causal link between the two. However, since *L. intestinalis* is regarded as a highly virulent parasite (Arne and Owen, 1968), there should be strong counter selection for such genes.

Another complication using naturally infected fish is that we had not complete control of other parasite species that might also affect host behavior. Apart from *L. intestinalis*, the only other parasite that has been reported from *E. sardella* is the nematode *Camallanus* sp. (Mgwede and Msiska, 2018). In our sample we did not observe any infections with *Camallanus* sp. Besides, this nematode uses *E. sardella* as the final host and we would therefore not expect this parasite to induce similar behavioral changes as we observed.

Since our results came from fish studied in small aquariums, we cannot conclude that *L. intestinalis* will affect the fish host in a similar way under natural conditions. Experimental studies are sometimes likely to induce more stress to a fish than it experiences in the wild, exacerbating the negative effects of parasitism. However, in a field study, (Gabagambi and Skorpung, 2018) observed that *E. sardella* sampled from the upper water levels (at 50 m depth) of the lake had significantly higher prevalence than fish captured at 100 m depth. Together with earlier studies showing that avian final hosts tend to take a higher proportion of fish infected with *L. intestinalis* compared to what is observed in lakes (Brown et al., 2001; Loot et al., 2001a), this suggests that the behavioral effects of this parasite are not just experimental artefacts, but are also affecting predation rates under natural conditions.

If the state of development of the parasite affects the predation rate of *E. sardella*, as our results indicate, we would expect to see a higher prevalence of immature larvae as compared to mature larvae in the fish population. We did not find this in our data (see Supplementary data, Table S1). There could be several reasons for this, for example that bird predation, as a contribution to total fish mortality, is too low to detect such an effect. Another factor that could affect these results is that small immature larvae are more easily overlooked as compared to the big mature larvae (plerocercoids) ready for transmission.

Although our study suggests that, under experimental conditions, the tapeworm *L. intestinalis* alters the behavior of the host, it is more problematic to conclude that this is the result of a specific parasite adaptation, i.e., that individual parasites have been selected for traits that increase predation rate. The observed changes in host behavior could be due to pathological side effects, for example that an increased level of activity results from a higher demand for nutrients (Milinski, 1990), that infected fish prefer a microhabitat closer to the water surface due to a higher oxygen requirement (Lester, 1971), or that the behavioral changes are caused by an energy drain from the parasite (Hafer and Milinski, 2016). If changes in host behavior were just a side-effect of the amount of energy taken from the host, we would expect to see a positive correlation between the relative size of the parasite in relation to the host and the observed changes in behavior. We did not find any sign of this in any of our tests. This observation therefore suggests that the manipulation hypothesis is most consistent with our results. However, this conclusion is based on the assumption that infected fish do not compensate for energy loss by higher food intake, as has been observed for *Schistocephalus solidus* in sticklebacks (Milinski, 1990). This mechanism has also been suggested for *L. intestinalis* (Britton et al., 2009). Without a detailed energy-budget for infected and uninfected fish, we therefore cannot reject the energy-drain hypothesis.

Poulin (1995), listed four criteria; complexity, purposiveness of design, convergence and fitness effects as tools for exploring if an apparent change in host phenotype or behavior is a parasite adaptation. In our study, we should regard the changes in behavior in hosts infected with *L. intestinalis* to be rather complex, both because several different components of behavior are changed and because the effect of the parasite depends on its state of maturation. Moreover, higher activity combined with reduced vigilance and a change in the preferred microhabitat to the upper water layers all seem to serve the purpose of increasing the predation success of infected intermediate hosts by fish-eating birds. The first two of Poulin's criteria therefore seem to be fulfilled.

We also notice that there is some convergence in the behavior of fish infected with different species of parasites having fish-eating birds as final hosts. For example, both the tapeworm *Schistocephalus solidus* (Tierney et al., 1993) as well as the digenean *Diplostomum spathaceum* (Seppälä et al., 2005) have been reported to change the behavior of their intermediate hosts in a similar way as reported in our study. When similar adaptations have evolved independently in several different lineages, this provides an argument for a specific adaptation as opposed to an accidental side-effect.

The most obvious criterion in demonstrating that a trait is adaptive is that it leads to an increase in fitness in individuals that possess it. This is also the most difficult criterion to measure, because it requires the demonstration of a correlation between the phenotypic variability in the trait and fitness. Since fitness is difficult to measure, Poulin (1995) has suggested that transmission success can be used as a proxy. We have no such measures of transmission in this study, but the observations of earlier studies that bird hosts tend to take a higher proportion of fish infected with *L. intestinalis* than the proportion present in a lake (Dobben, 1952; Brown et al., 2001; Loot et al., 2001a), suggest that the behavioral manipulations of this parasite are positively associated with transmission. Therefore, all of the criteria proposed by Poulin (1995) are to some extent fulfilled, which lead us to conclude that the observed behavioral changes induced by the parasite appear to be an adaptive trait evolved in the parasite to increase its fitness. Some of these parasite-induced behavioural effects have been earlier reported from small lakes in northern Europe (Wyatt and Kennedy, 1988; Museth, 2001; Vanacker et al., 2012). The fact that we observe the same behavioural effects of this parasite in an endemic fish species from an African lake, suggests that behavioral manipulation by *L. intestinalis* is widespread, both geographically and in terms of host range.

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Conflict of interest

None

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.beproc.2018.11.002>.

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