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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 *Toxoplama gondi* 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; Hoole et al., 2010). This parasite has a complex life cycle involving two aquatic intermediate hosts and a fish-eating bird as a 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 x width x 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 x width x 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.

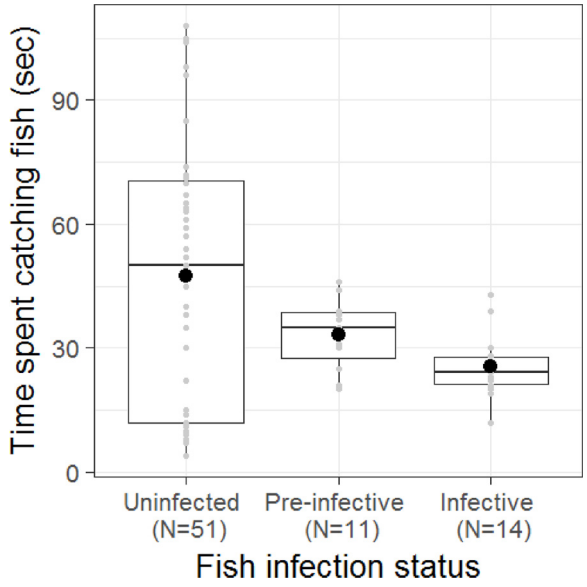

Fig. 1. Boxplot showing the influence of *L. intestinalis* on host ability to avoid simulated predation attack. The horizontal lines show the median, solid black circle show the mean and open grey circle show the raw data set.

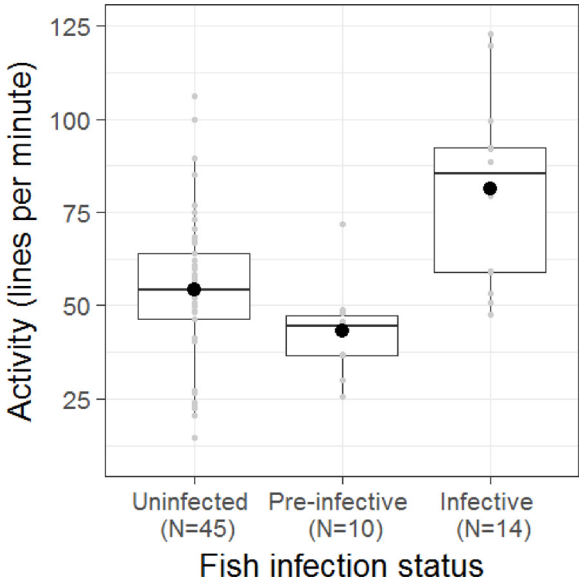

Fig. 2. Boxplot showing the influence of *L. intestinalis* on host swimming activity. The horizontal lines show the median, solid black circle show the mean and open grey circle show the raw data set.

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: Rs(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: Rs (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: Rs(22) $= 0.22$, $P = 0.34$)

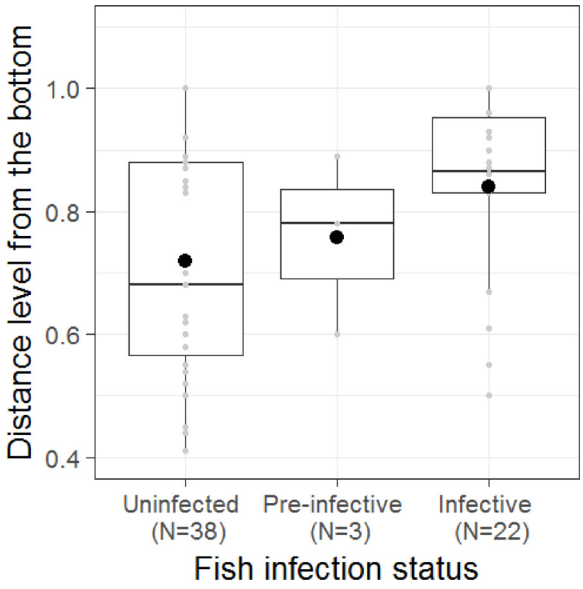

Fig. 3. Boxplot showing the influence of *L. intestinalis* on host depth preference. The horizontal lines show the median, solid black circle show the mean and open grey circle show the raw data set.

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 infective 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 (Arme 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 Skorping, 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 vigility 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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