Worm in the eye makes for easy prey: evidence of behavioural alterations induced by *Diplostomum* metacercariae in *Synodontis zambezensis* in South Africa

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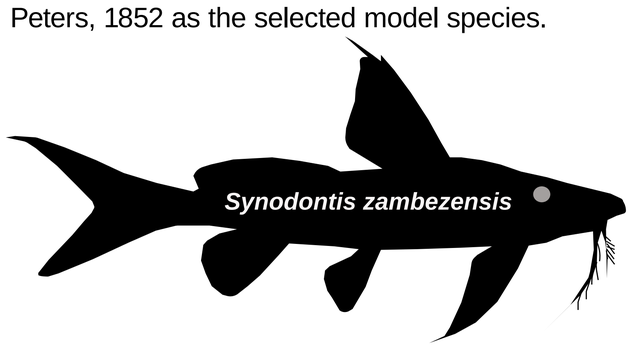
Introduction

Behavioural changes induced by metacercariae of *Diplostomum* von Nordmann, 1832 in fish hosts have become an increasingly investigated field of interest in the field of parasitology. Metacercariae of *Diplostomum* are known fish pathogens that infect the eyes and brain of their host and may even at high densitites cause mortality. Numerous studies, predominantly done in North America and Europe, found that in cases of extreme infections, the metacercariae significantly changes host behaviour.

Although there are a number of studies focusing on the influence of metacercariae of *Diplostomum* on the behaviour of various fishes inhabiting different ecoregions within an aquatic system, in Africa none have focused on parasite induced behavioural changes on bottom-dwelling fish inhabiting the profundal zone of an aquatic ecosystem. Moreover, data on the effect of metacercariae of *Diplostomum* on fish behaviour in South Africa are virtually lacking.

Therefore this study aimed to determine the influence of metacercaie of *Diplostomum* on the behaviour of their naturally infected fish hosts, with the Plain squeaker *Synodontis zambezensis* Peters, 1852 as the selected model species.



***Diplostomum* sp.**

***Diplostomum* sp. 14**

Materials & Methods

Fish sampling were done on the Phongolo River in the Ndumo Game Reserve (NGR), KwaZulu-Natal. A total of 10 *S. zambezensis* (NGR, 2017) were used to establish baseline bahaviour prior to field experiments. Fish were transported to a behaviour analysis tank (90 cm x 40 cm) where individual (n=1) as well as group (n=3) behaviour were evaluated. All behaviour were recorded with a Basler GigE camera for 4 hours. After analysis, an accclimation time of 60 minutes were determined.

The field-based experiments consisted of 22 *S. zambezensis* (NGR, 2018) that were transferred to a behaviour analysis tank and left for the required acclimatisation period. Fish were exposed to an attack stimuli (Heron/ Fly-by) and behaviour were recorded with a Panasonic HD camera. All analysis were performed at the National Aquatic Bioassay Facility (NABF), North-West University using EthoVision XT software.

Parameters measured:

- Distance moved
- Swimming speed
- Acceleration (Min & Max)
- In Zone (Top & Bottom)
- Zone alternations
- Mobility state (M, HM & IM)

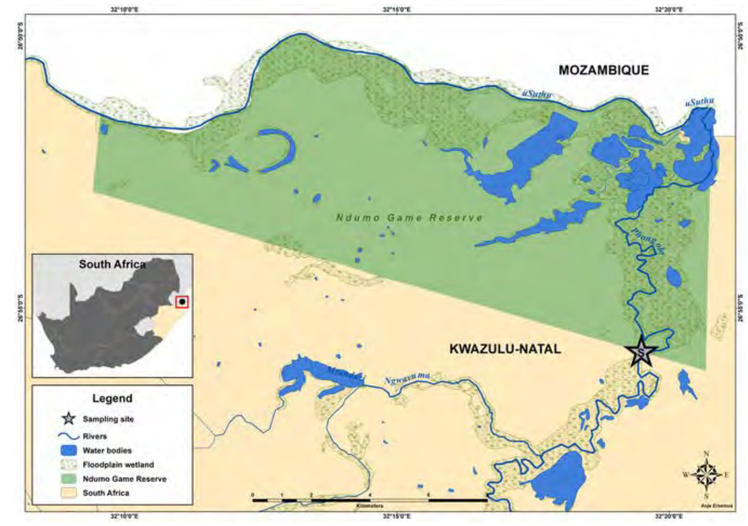
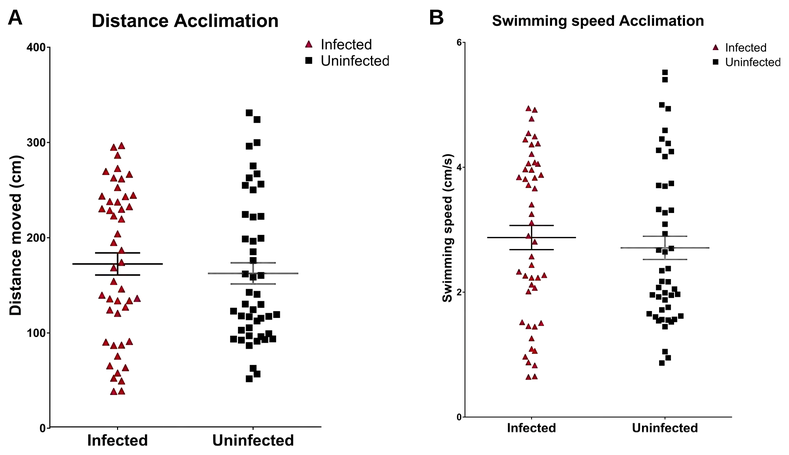


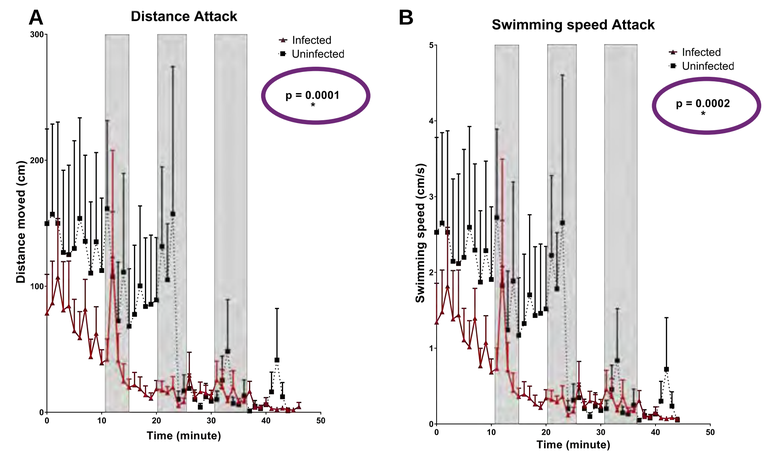
Fig. 1: Map of sampling sites in the KwaZulu-Natal, South Africa \(sampling sites marked with a star).

Results

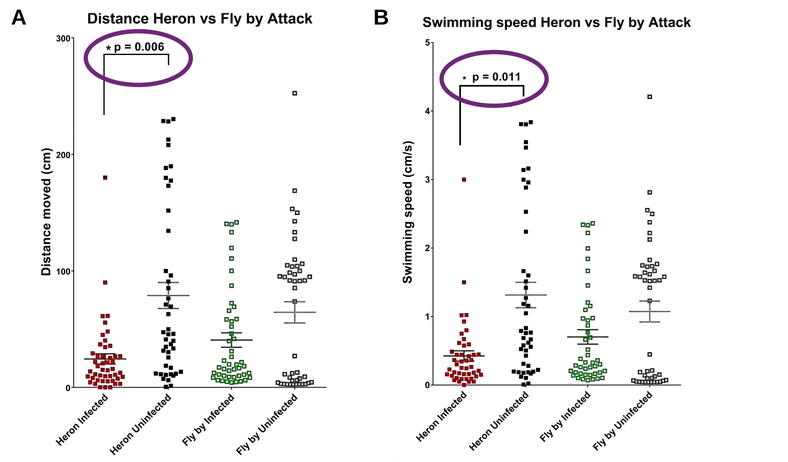
Field lab Acclimation



Field lab Attack



Attacks combined



Significant differences observed during acclimation between infected and uninfected fish in the top and bottom zones as well as zone alternation.

This shows that infected fish spent more time in the top zones as well as spending more time alternating between top and bottom zones compared to uninfected fish. Previous behaviour studies supports that this will make infected fish more susceptible to predation.

*Fig. 2: Mean ± SEM activity during field acclimation of infected vs uninfected fish of Synodontis zambezensis. (A) Distance (cm). (B) Swimming speed (cm/s). (C) In Zone (Top) (s). (D) In Zone (Bottom) (s). (E) Zone alternation. *Asterisks indicate significant differences between the infected and uninfected S. zambezensis (Welch's t-tests; p < 0.05).*

Significant differences observed during 3x attacks (10 minute intervals) between infected and uninfected fish in the distance moved, swimming speed as well as zone alternation.

This shows that infected fish overall moved less, had a slower swimming speed and moved fewer between top and bottom zones compared to uninfected fish. As a result, this makes infected fish easier targets for predators to strike due to their slow movement and their time spent in the top zones of the tank.

*Fig. 3: Mean ± SEM activity for all attacks on infected vs uninfected fish of Synodontis zambezensis during field exposures. (A) Distance (cm). (B) Swimming speed (cm/s). (C) In Zone (Top) (s). (D) In Zone (Bottom) (s). (E) Zone alternation. *Asterisks indicate significant differences between the infected and uninfected S. zambezensis (Welch's t-tests; p < 0.05).*

Significant differences observed between infected and uninfected fish during all the heron attacks (distance, swiming speed and mobility state). In contrast, no significant differences observed for none of the variables tested during the fly-by attacks.

This indicates that the type of attack method used to test behavioural changes should be a consideration when doing comparison studies of naturally infected and unifected fish in future research.

*Fig. 4: Mean ± SEM activity between heron vs fly-by attacks of infected vs uninfected fish of Synodontis zambezensis. (A) Distance (cm). (B) Swimming speed (cm/s). (C) Mobility state - highly mobile(s). (D) Mobility state - Mobile (s). (E) Mobility state - Immobile. *Asterisks indicate significant differences between the infected and uninfected S. zambezensis (Welch's t-tests; p < 0.05). Hash indicates no significant differences between variables.*

Discussion

Statistical comparisons were made between acclimation and attacks based on the distance moved, swimming speed, time spent in top and bottom zones, frequency of zone alternations, minimum and maximum acceleration and mobility state. Graphs highlighted with purple circles indicate significant differences in behaviour between infected and uninfected fish for acceleration and time spent in the top zone but not for distance moved or swimming speed.

Until recently, It was generally accepted that only high intensity of infections with metacercariae of *Diplostomum* will induce changes in the behaviour of their fish hosts. It was discovered that through parasite-host co-evolution, the parasite may change the hosts' behaviour by either reducing or increasing the risk to predation depending on the developmental stage of its life cycle or "readiness" of transfer to the next host. The present study concurs with the lattter statement since low intensity of infections with metacrcariae were recorded in *S. zambezensis* (1–12).

Conclusions

- This study provides the first statistical evidence of significant differences in behaviour between infected and uninfected *S. zambezensis*, a host not previously used in behavioural experiments.
- Additionally, our study established baseline data for future behavioural studies of fish infected with diplostomids in Africa.
- Finally, this study highlights the importance of future behavioural studies focusing on naturally infected hosts to depict an acurate representation of the behavioural changes induced by metacercariae of *Diplostomum*, a highly understudied area in Africa.

Acknowledgements

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