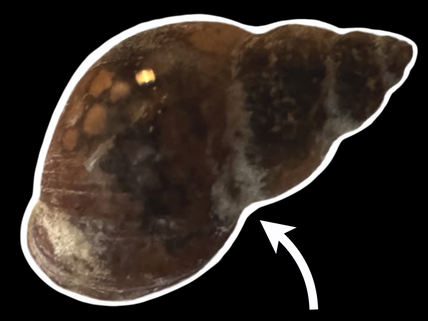
Mapping liver fluke risk areas and the future of diagnostics: are novel environmental biomarkers the key?

Mia R. Ley[1*], Peter M. Brophy[1], Paul McVeigh[2], Maggie Fisher[3], Beverley Hopkins[4] , Rhys A. Jones[1], & Russ M. Morphew[1]

[1]Department of Life Sciences, Aberystwyth University; [2]School of Biological Sciences, Queen's University Belfast; [3]Ridgeway Research; [4]Wales Veterinary Science Centre

Fasciola hepatica is a species of parasitic flatworm of global concern, with an estimated 90 million humans and 700 million ruminants at risk of liver fluke infection[1]. At present, no vaccine is available on the market and current treatment relies on the use of triclabendazole (TCBZ), though concern is growing over TCBZ-resistant *F. hepatica* populations[2]. The aim of this study is to identify novel environmental biomarkers that can be used to improve field diagnostics and to map risk areas for *F. hepatica* infection. Environmental DNA, RNA, and proteins (eDNA/eRNA/eProteins) collected from the habitat of the intermediate host *Galba truncatula,* a freshwater snail and the primary host of *F. hepatica* in Europe, will be key in identifying potential biomarkers and developing new diagnostic methods.



G. truncatula, the preferred snail host of *F. hepatica* in Europe[3].

STAGE ONE — Generating the transcriptome for the snail host, *Galba truncatula:*

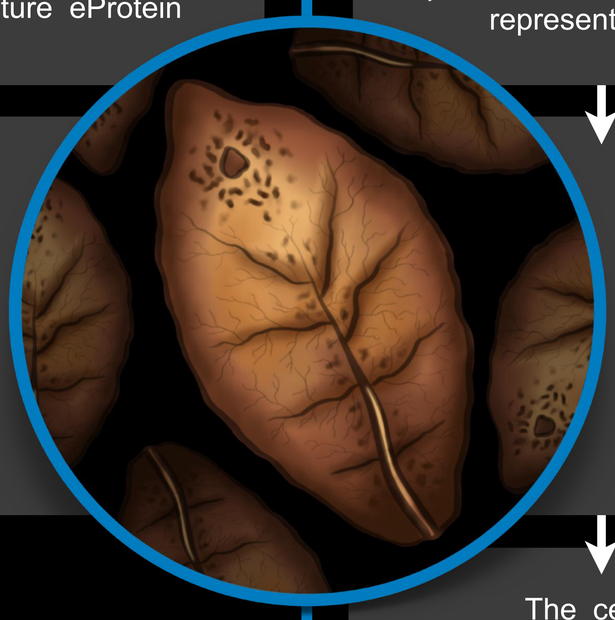
Generating a first transcriptome for *G. truncatula* using time points of infection:

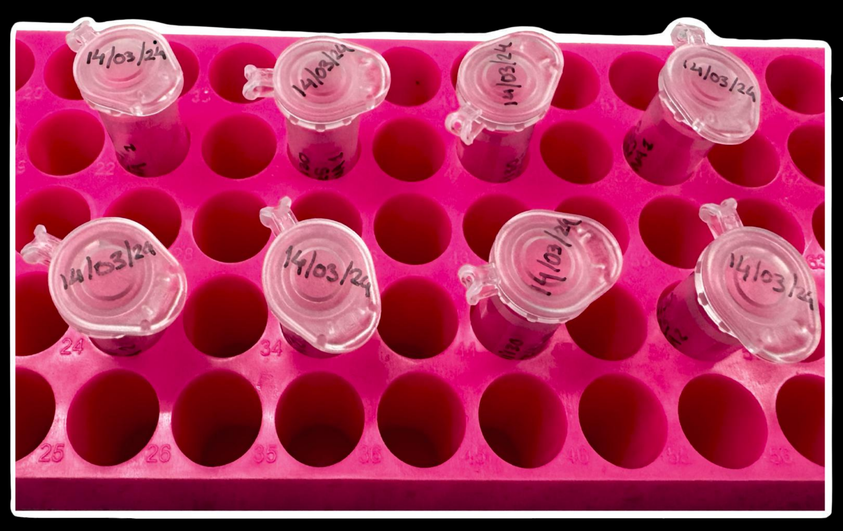
- **Day 0** (uninfected snails that have not been exposed to miracidia).
- **Day 10** post-infection, when infection is well established in the host[4].
- **Day 42** post-infection, around the time when cercarial shedding begins[4,5].

Outcome: 1) Generation of *Galba* transcriptome to support future eProtein analysis, and **2)** Insight into *Galba* infection from *F. hepatica.*

DNA and RNA will be collected from 15 *G. truncatula* snails (5 from each time point). DNA will be used for *Galba* species PCRs and confirmation of *F. hepatica* infection. RNA will be sequenced and the transcriptome of the snail will be analysed to highlight the key differences between control and infected snails.

Following identification of any notable differences in the transcriptome, the study will then investigate the suitability of these as potential novel biomarkers for use in diagnostics when testing water samples taken from the snail's environment.





Despite its importance in the life cycle of *F. hepatica,* the significance of *G. truncatula* in liver fluke control is often overlooked. Unlocking the transcriptome of the snail host is a vital first step towards the development of future diagnostic methods.

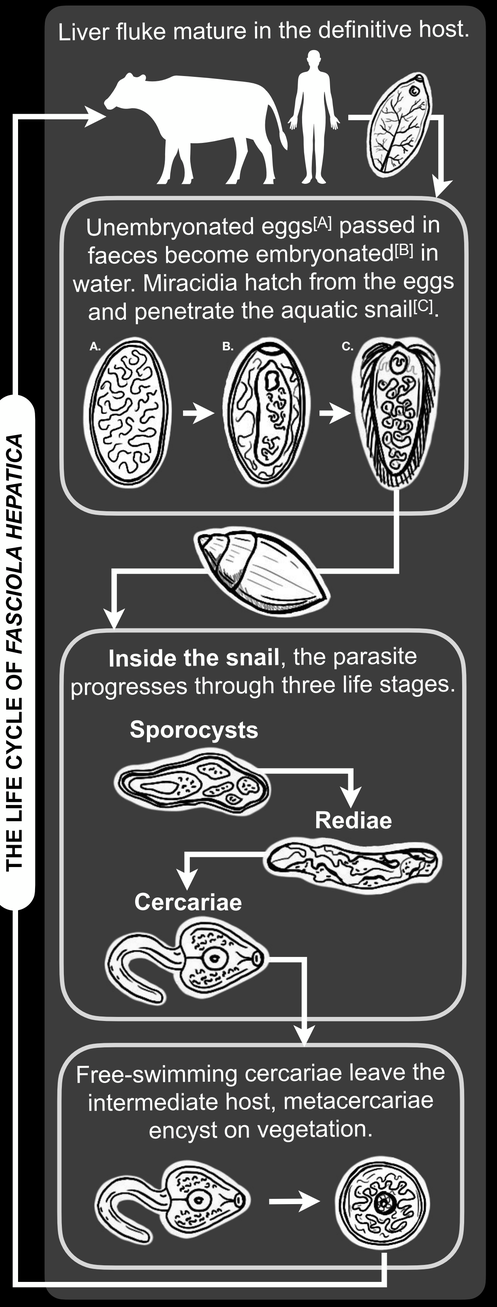
STAGE TWO — Identifying novel environmental biomarkers and their potential:

Potential environmental biomarkers may originate from a range of materials shed by *F. hepatica* during free-living stages in the environment, or by the intermediate snail host *G. truncatula* during a state of infection. Stage specific markers in protein or miRNA profiles represent suitable markers to target for environmental detection.

Following embryonation of liver fluke eggs in water, ciliated larvae known as miracidia hatch and live in the environment for up to 24 hours as they seek out the snail[6]. Once inside the snail, asexual reproduction occurs giving rise to sporocysts, rediae, and cercariae[6].

The cercariae are eventually shed from the host and encyst on vegetation as metacercariae before being ingested by mammals, the definitive hosts[7].

The physiological changes that occur in both *F. hepatica* and *G. truncatula* during this period in the life cycle will result in the accumulation of materials such as eDNA and eRNA in the environment. Water samples collected from contaminated sources will then be analysed to assess the presence of specific biomarkers, forming the basis of future diagnostic tests.



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[3] Image adapted from: Evans, S (2024) *Galba truncatula.* [Photograph]. (Private collection).

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Acknowledgements:

This work was supported by the Natural Environment, Biotechnology and Biological Sciences and Medical Research councils (NERC, BBSRC and MRC) [grant number: NE/X016714/1] as part of the One Health for One Environment: an A-Z Approach for Tackling Zoonoses ('OneZoo') Centre for Doctoral Training. Many thanks to the British Society for Parasitology for the Meeting Travel Award that made attendance to the conference possible. Thank you to my lead supervisor Dr Russ Morphew for his continued support and guidance during my PhD and for always answering my questions, no matter how many there are! Finally, thanks also go to my team of co-supervisors, to Ridgeway Research, and to my lab group.

***Further correspondence welcomed at mil30@aber.ac.uk**