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## Introduction

- Freshwater sponges are filter feeding organisms found in rivers and lakes.
- There are two common species in Ireland, *Ephydatia fluviatilis* and *Spongilla lacustris*.
- Freshwater sponges are seasonal and die back into seed-like structures called gemmules during the winter, from which they hatch again in spring.
- Previously it has been demonstrated that marine sponges contain antimicrobials, but it was unclear whether these properties were from the sponge or their microbiome.
- Riverine sponges exist with site specific microbial communities; in comparison microbial diversity is much lower in sponges which have been laboratory grown from gemmules allowing for the contribution of the microbiome for antimicrobials to be investigated.

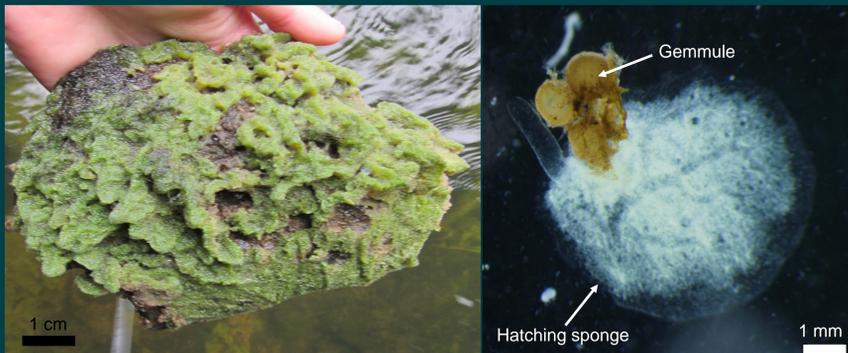


Figure 1: The freshwater sponge *Ephydatia fluviatilis* (left) and gemmule structure (right) including a hatching sponge

## Objectives

- Investigate if freshwater sponge extracts inhibited the growth of bacteria that are responsible for a range of healthcare associated infections.
- Determine if the antimicrobial effects were from the sponge or their microbial community.

## Methods

- The antimicrobial properties of *E. fluviatilis* and *S. lacustris* extracts were determined in a series of laboratory assays.
- Sponges of each species were collected from rivers (natural site specific microbiome) and laboratory grown in sterile water (low diversity microbiome).
- Sponge samples were dried and extracted in methanol.
- After evaporation of methanol, extracts were resuspended in DMSO.
- Antimicrobial properties were assessed by disc diffusion and recording of the minimal inhibitory zone (MIZ).
- The extracts were tested against the healthcare associated bacteria *Acinetobacter baumannii*, *Escherichia coli*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Staphylococcus epidermidis*.



Figure 2: Methanol extracts of different sponges (left) and an example of a disc diffusion assay of sponge extract to determine the strength of antimicrobial properties (right)

## Results

- None of the tested sponge extracts suppressed the growth of *P. aeruginosa*, *S. aureus* or *S. epidermidis*.
- Only *K. pneumoniae* was inhibited by all extracts, with the extracts from laboratory grown sponges resulting in significantly wider MIZs (U=73-89, p=0.004-0.019).
- *A. baumannii*, *E. coli* and *E. faecalis* were only inhibited by the extract from riverine *S. lacustris*.

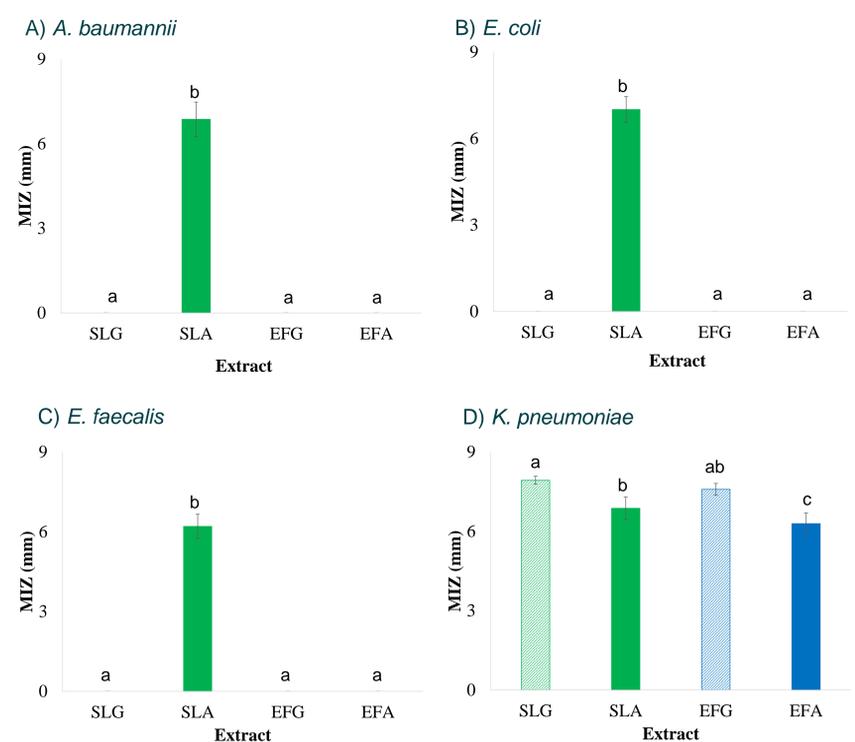


Figure 3: Arithmetic means and standard error values for the maximum inhibitory zone (MIZ) of bacterial growth around diffusion discs for laboratory grown and riverine sponges. SLG – laboratory *S. lacustris*, SLA – riverine *S. lacustris*, EFG – laboratory *E. fluviatilis* and EFA – riverine *E. fluviatilis*. Different letters represent a significant difference in pairwise comparisons with Mann Whitney U tests (p<0.05).

- The above results indicated that freshwater sponges and their microbiome can provide effective antimicrobial effects against selected bacterial strains.
- Figure 3 a-c indicated antimicrobial properties from the sponge microbiome.
- Figure 3 d indicated antimicrobial properties against *K. pneumoniae* from the sponge cells themselves.

## Conclusions

- Freshwater sponges are potential sources of antimicrobial compounds, particularly against *K. pneumoniae*.
- Inhibition of *K. pneumoniae* by riverine and laboratory grown sponges suggested that this effect originated from sponge cells themselves.
- The stronger antimicrobial effect of riverine *S. lacustris* against *A. baumannii*, *E. coli* and *E. faecalis* indicated a contribution of the sponge microbiome to inhibitory efficacy.

## References

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- Pejin, B, Talevska, A, Ciric, A, Glamoclija, J, Nikolic, M, Talevski, T (2014). Anti-quorum sensing activity of selected sponge extracts: a case study of *Pseudomonas aeruginosa*. *Natural Product Research*, 28(24), 2330-3.