

Nudibranchs and their chemical defence mechanism through sequestration

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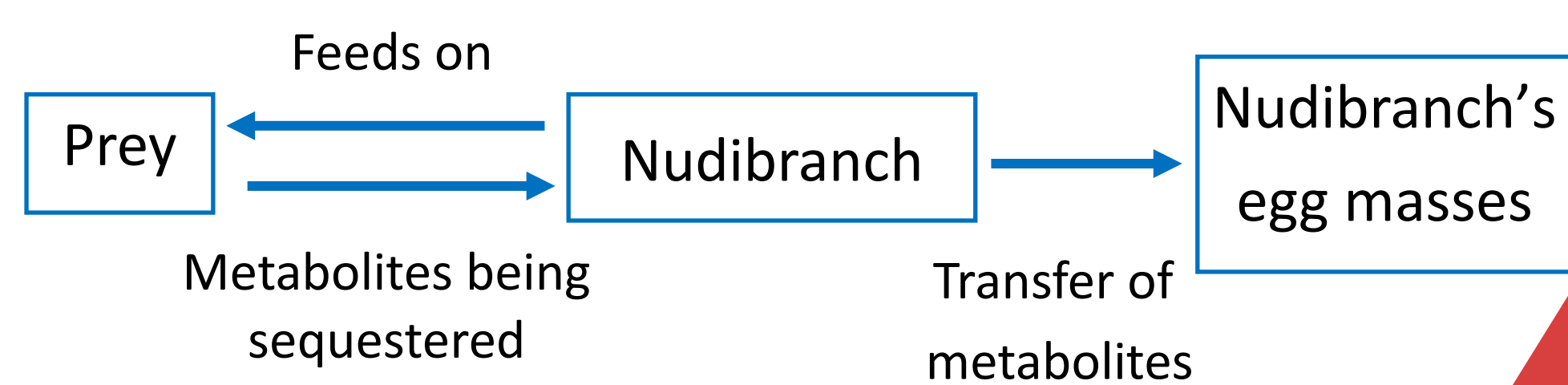
Introduction

Nudibranchs are fascinating soft-bodied marine gastropod molluscs often referred as sea-slugs.¹ They are found throughout the world's oceans, but are most abundant in shallow and tropical waters. Almost all nudibranchs live in the benthic zone, from shallow water to a depth of 30 metres and their size rarely exceeds 5 cm.²

There is an incredible diversity of shapes among nudibranchs and their fascinating colours are derived from the food they eat, which aids in camouflage. Nudibranchs are carnivorous and are most often found around their favourite prey: sponges, bryozoans, cnidarians and even other nudibranchs.² They are essentially blind and perceive their environment, as well as their prey, through chemosensory interactions with their two highly sensitive tentacles, called rhinophores.³

Chemical defence mechanism

Nudibranchs are slow moving creatures that lack physical protection because they shed their shells as larvae.⁴ Considering that they have few documented predators, they have a particularly effective defence mechanism, which relies partly on their chemical unpalatability.^{2,5} This unpalatability comes from their toxic and distasteful secondary metabolites (natural products) which cause a nauseating effect or even kill potential predators.⁶ These natural products are, in most of nudibranch species, obtained directly from their prey by sequestration.⁷ In some nudibranch species, these natural products, once sequestered by the nudibranch, are transferred to the nudibranch's egg masses for protective purposes.^{8,9}

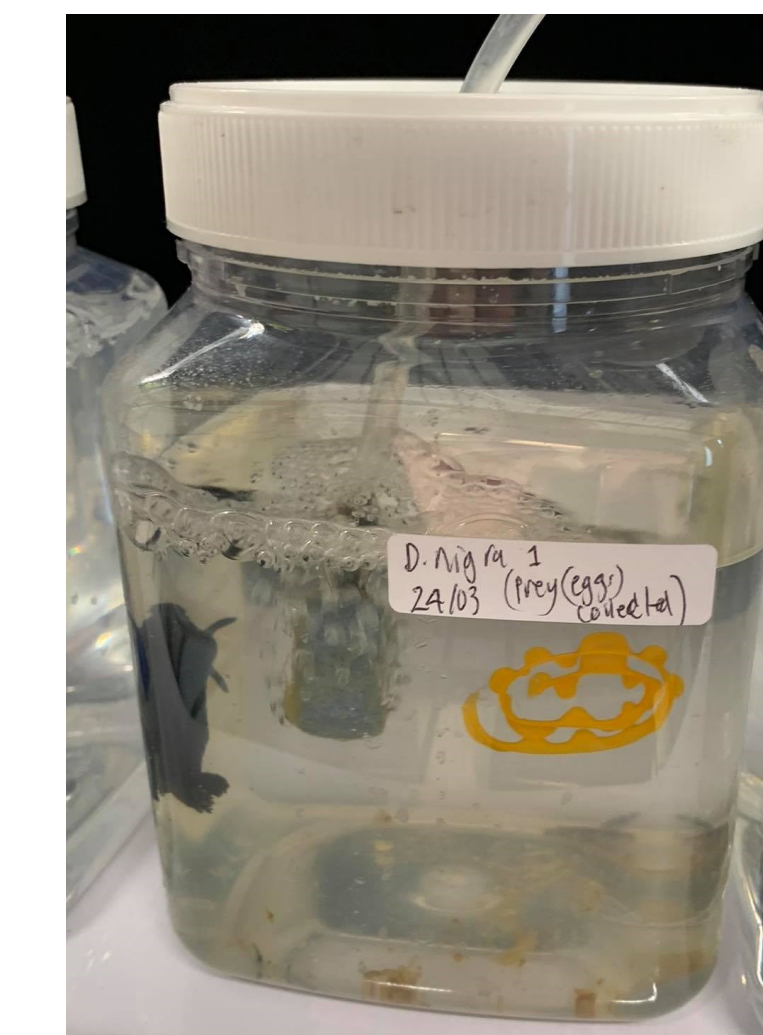


Research goals

- Establishing links between nudibranchs, eggs and prey species to better understand nudibranch defence mechanisms. To do so, the research focuses mainly on characterisation of natural products and on their origin.
- Exploring the natural products properties and possible applications of isolated natural products so that they can become lead compounds for the development of new drugs and therefore help improve human health and well-being.

Methodology

- Sample collection** : the nudibranch, prey and egg samples are collected by hand either by snorkeling or scuba-diving.
- Nudibranch spawning experiment** : nudibranchs are kept in tanks for a few days while waiting for them to spawn.
- Chemical analysis** : Organic solvent extraction, liquid chromatography-mass spectrometry (LC-MS) screening of crude extract, purification of the extract, structural elucidation mostly using nuclear magnetic resonance (NMR) spectroscopy and LC-MS.

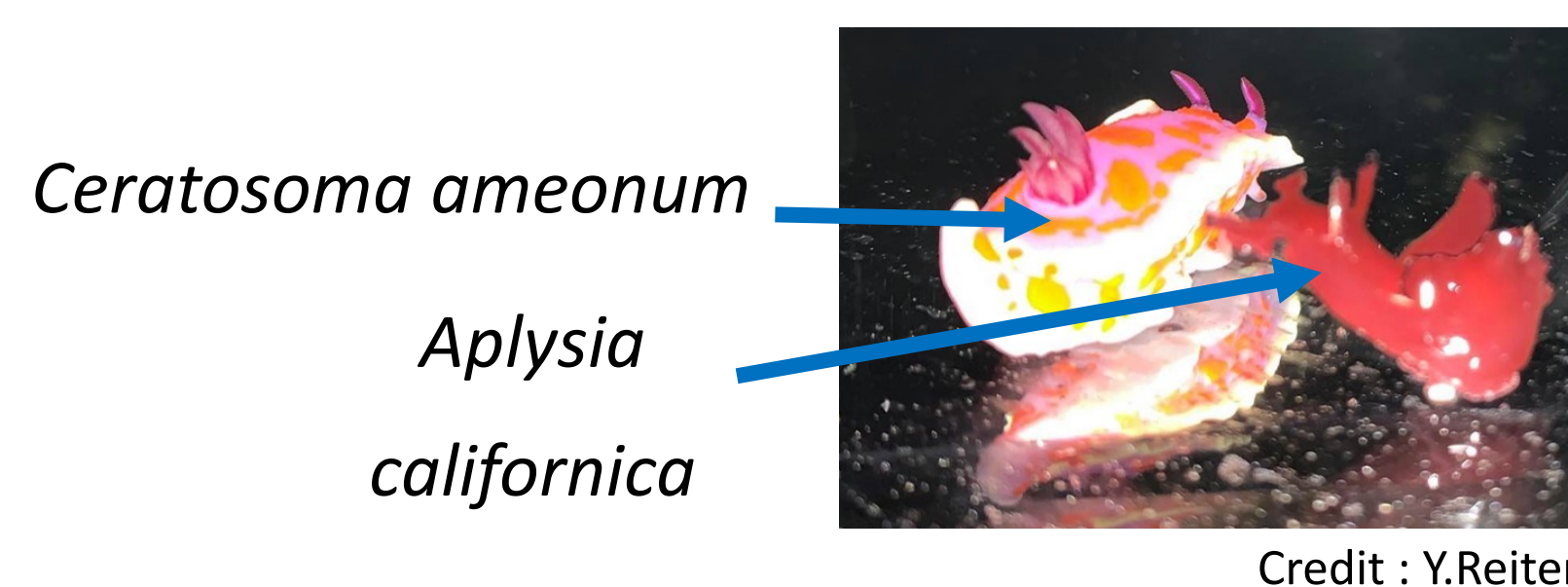


LC-MS screening results — *Ceratosoma amoenum*

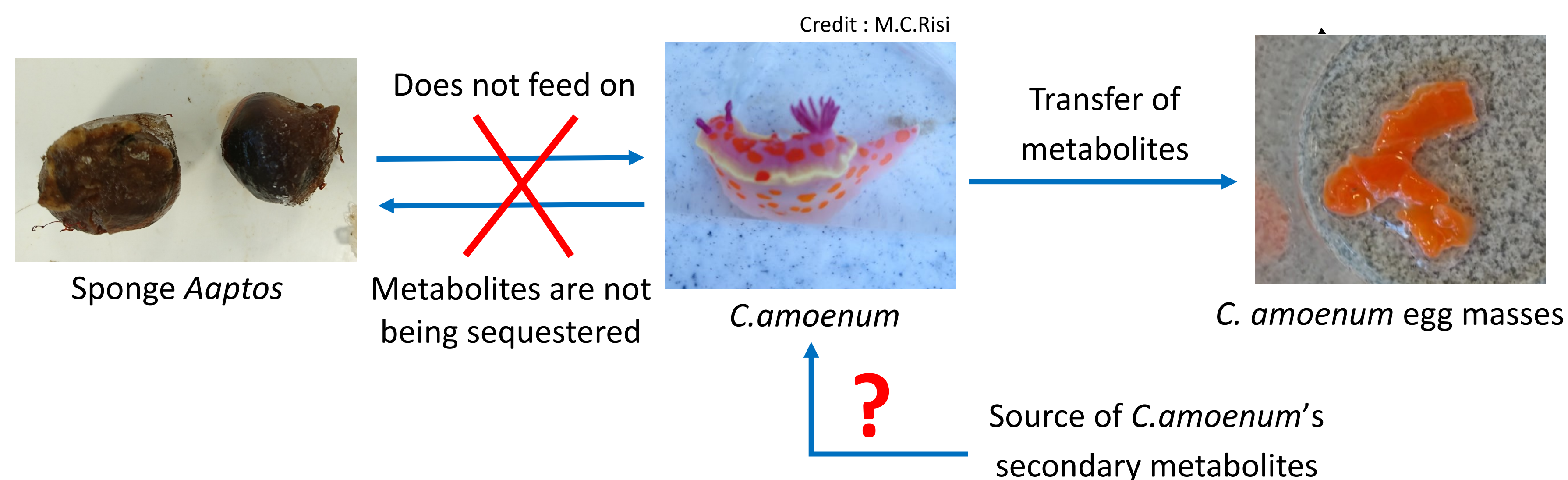
- Two specimens of *Ceratosoma amoenum* and a sponge *Aaptos* found near the nudibranch were collected in June 2020 in Tauranga (New Zealand). The nudibranchs were kept in captivity for several days and laid eggs. The crude extracts of the two nudibranchs, the sponge and the eggs were screened by LC-MS.

Further work

The results presented here are preliminary and further work is needed to elucidate the structure of the metabolites. Some more specimens of juvenile *Aplysia californica* will be collected and studied to possibly assign the origin of the metabolites. Also, the biological properties of the compounds will be investigated to understand if the nudibranch transfers these compounds to the eggs for protective purposes.

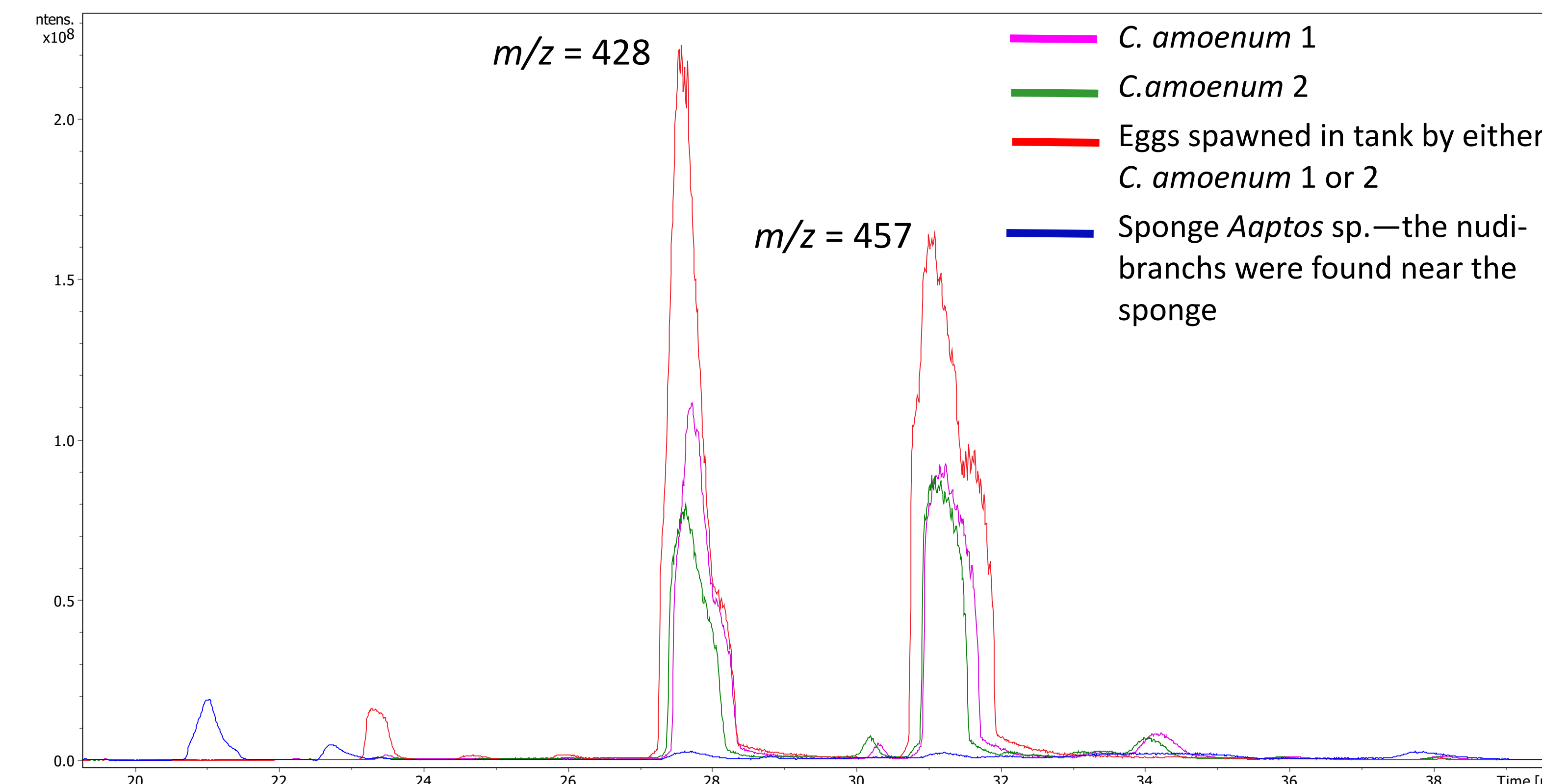


- The two nudibranchs were kept in tanks with a juvenile sea hare *Aplysia californica* and they ate it. It is possible that the nudibranchs sequestered these metabolites from the sea hare.



- The LC-MS chromatograms show similar profiles for the two nudibranchs and the egg mass samples. It can be concluded that some metabolites, corresponding to the two main peaks, are transferred to the eggs.

- The profile of the sponge does not match the nudibranch profiles, so it is unlikely that the nudibranchs were feeding on the *Aaptos* sponge and sequestering metabolites from it.



LC-MS screening of 2 crude extracts of clown nudibranch (*C. amoenum*), their eggs and the sponge they were collected on.

References

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Picture in the center of the poster by Emmy-Ahlen