

## Inducible chemical defences in primary producer – herbivore interactions: effects on population dynamics as a function of consumer trait diversity

In aquatic systems herbivory leads to three times higher consumption of primary production than in terrestrial system and is considered a strong selective agent for defensive mechanisms (Duffy and Hay, 2000). Accordingly, inducible chemical defences are widespread in marine and freshwater phytoplankton (von Elert, 2012); among these defences are chemical defences in phytoplankton that are induced in response to zooplankton grazers (e.g. Bergkvist et al., 2008; Jang et al., 2007). However, in no case the costs of enhanced defence have been analysed.

It is well known for long that the cellular content of such (defensive) secondary metabolites in algae and cyanobacteria is highly variable and depends on growth and resource conditions. As inducible chemical defences build on enhanced synthesis of specific secondary metabolites, which in many cases contain nitrogen or even phosphorus, it is reasonable to assume that the trade-off of the trait 'defence', which is equivalent to inhibitor content, is physiologically linked to resource ratios available to the primary producer.

The planktonic cyanobacterium *Microcystis aeruginosa* produces two known protease inhibitors (PIs) as anti-herbivore defence, and these PIs and thus the trait 'defence' can be quantified by measuring the inhibitor content of the cyanobacterium using HPLC coupled to high-resolution mass spectrometry. Here we have used this strain and investigated how resource availability and growth rate affect the cellular PI content and thus the defence of this cyanobacterium against the grazer *Daphnia*. The most important potentially limiting nutrients are nitrogen and phosphate. We investigated their effects on PI content in batch culture experiments with a range of initial concentrations of either nitrogen (as nitrate) or phosphate and determined growth curves, stoichiometry, and PI content, and from fitting of logistic growth curves, growth rates were derived. When related to the cyanobacterial biomass, the inhibitor content varied by more than 80% with  $\text{NO}_3^-$  concentration and time. The PI content increased with growth rate and N content of the cyanobacterium, which indicates that increased N availability supports higher cyanobacterial biomass with a higher content of defensive PIs (Burberg et al., 2019). Therefore, increased  $\text{NO}_3^-$  concentrations foster cyanobacterial blooms directly by providing more nutrients and indirectly by increasing the negative interference of cyanobacteria with their consumers due to an increased content of PIs.

Similar batch experiments with eight different initial phosphate concentrations resulted in PI-content normalized to biomass varying up to nineteen-fold. The PI-content increased with cyanobacterial C:P and was highest in the low-P treatments, especially during the mid-exponential growth phase. The results indicate that P-limitation supports a higher content of defensive PIs (Burberg et al., 2020).

In conclusion, the chemical defense of *M. aeruginosa* by these PIs is strongly but differently affected by P and N availability: while P-limitation supports higher PI content, N-limitation reduces the PI-content (Burberg et al., 2020; Burberg et al., 2019). The two PIs contain carbon and nitrogen but no phosphorus, and hence these findings are in good accordance with the Carbon Nutrient Balance Model (CNBM), which predicts the production of defence molecules to be determined by the relative availability of carbon and nutrients and which sees secondary metabolites largely as waste products (Reichardt et al., 1991). Our results show that the costs of increasing the PI content are low under P-limitation and under non-limiting N-supply. This strongly suggests that under these conditions the induction of PIs by chemical cues released by grazing will be strongest.

### References

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