

# A higher nitrate concentration leads to an elevated protein yield in the green seaweed *Ulva lactuca*

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## Background

This study is part of the RAAK-PRO project ZEEVIVO (seaweed in fish feed), where seaweed cultivation, seaweed bio-refinement and fish feed production are studied.

## Research question

What is the effect of an elevated nitrate concentration on the growth rate, photosynthetic efficiency and protein concentration of *U. lactuca*?

## Methods

*U. lactuca* was cut into discs of 3 cm  $\varnothing$  and incubated under low ( $0.2 \pm 0.1 \mu\text{M}$ ) ( $n=16$ ) and high nitrate ( $98.1 \pm 1.3 \mu\text{M}$ ) ( $n=16$ ) concentrations during 12 days. Nutrient concentrations were checked daily in order to prevent limitation. The photosynthetic efficiency (variable fluorescence (Fv) / maximal fluorescence (Fm)) was measured with a PAM Fluorometer every other day. On the same day pictures were taken in order to calculate the surface area with ImageJ software.



Figure 1. For a standardized starting surface area *U. lactuca* tissue was punched out in to circles of 3 cm  $\varnothing$  and pictures were taken to adjust for dissimilarities.

Relative growth rates were calculated using the formula:  $RGR = \frac{\ln(m_2 \div m_1)}{t} \times 100$

In which  $m_1$  and  $m_2$  are respectively initial and new surface area. Protein concentrations were analyzed using the Dumas method which results in the total nitrogen percentage. A conversion factor of 4,97 was used to calculate the total protein concentration in *U. lactuca* (Angell, 2015). The initial protein concentration was 10,18%. Prior to the experiment the *U. lactuca* thalli were starved for 13 days (Fujita, 1985; Lubsch, unpublished; Pedersen, 1994;), in order to enter the same physiological state and empty storage pigments, after starvation the protein level was 6,13%.



Figure 2. Experimental setup, punched out *U. lactuca* tissue in 200 ml medium on a rotating table (100 rpm) for mixing and gas exchange.

## Results

### 1 Growth rates under different nitrate concentrations

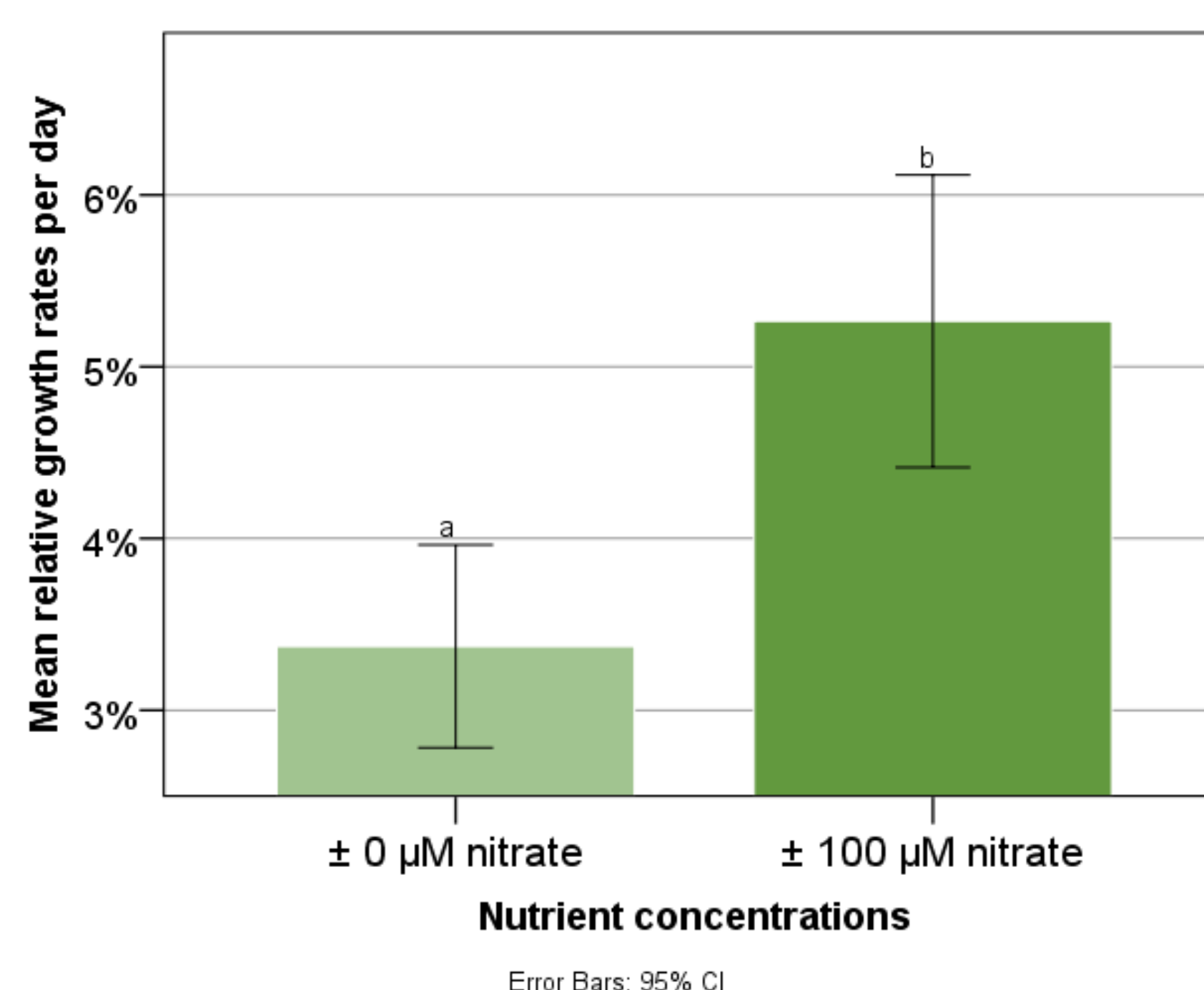


Figure 4. Mean relative growth rates per day (in %) during the experiment. An independent samples t-test shows a significant difference in relative growth rates per day between the  $0 \mu\text{M}$  and  $100 \mu\text{M}$  nitrate concentrations ( $p = 0,00$ ).

### 2 Relative growth rates and photosynthetic efficiency

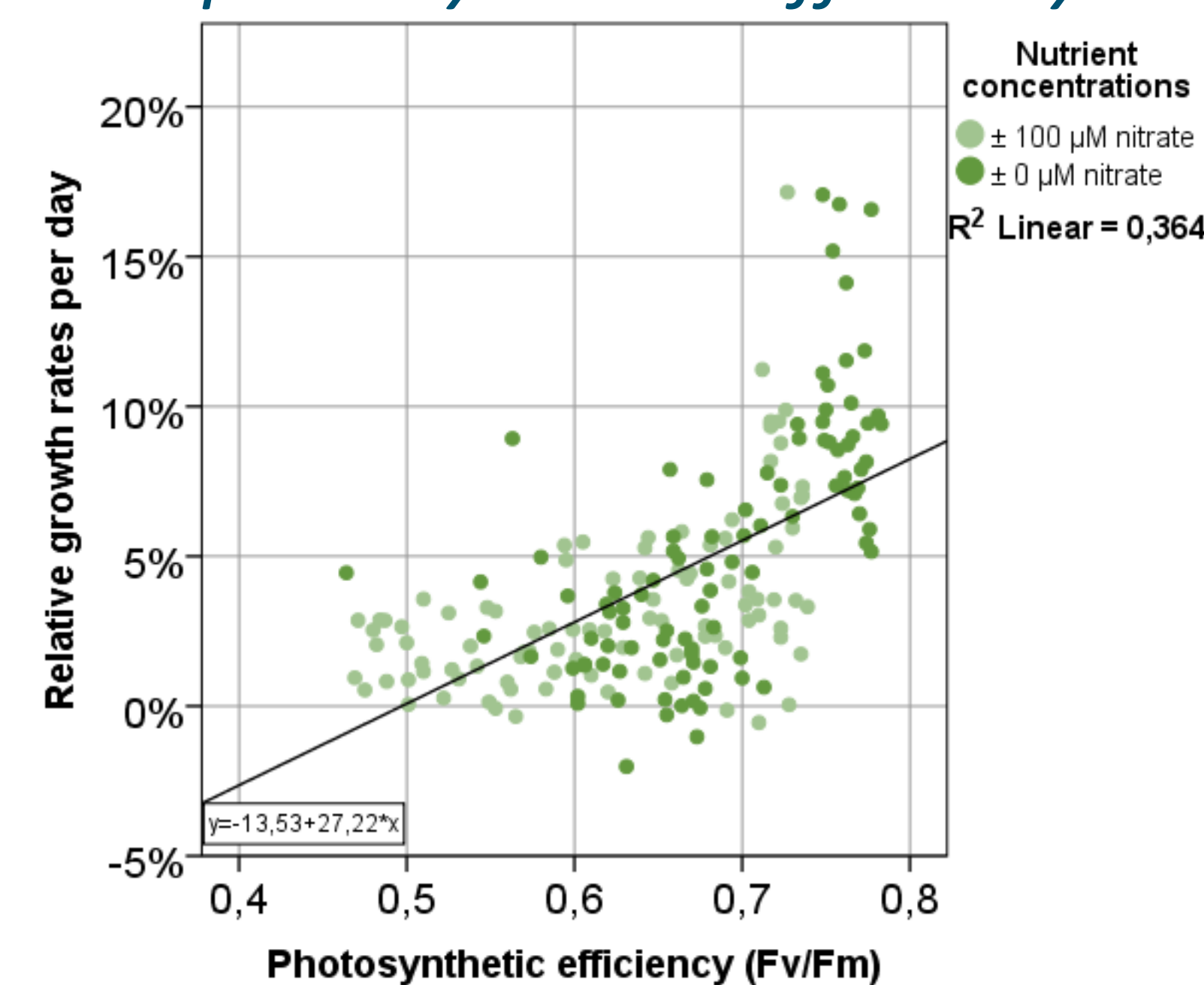


Figure 5. The Pearson correlation test shows a strong positive relationship ( $r=0,603$ ) between the relative growth rate per day (%) and the photosynthetic efficiency (Fv/Fm) ( $p = 0,00$ ).

### 3 Protein levels under different nitrate concentrations

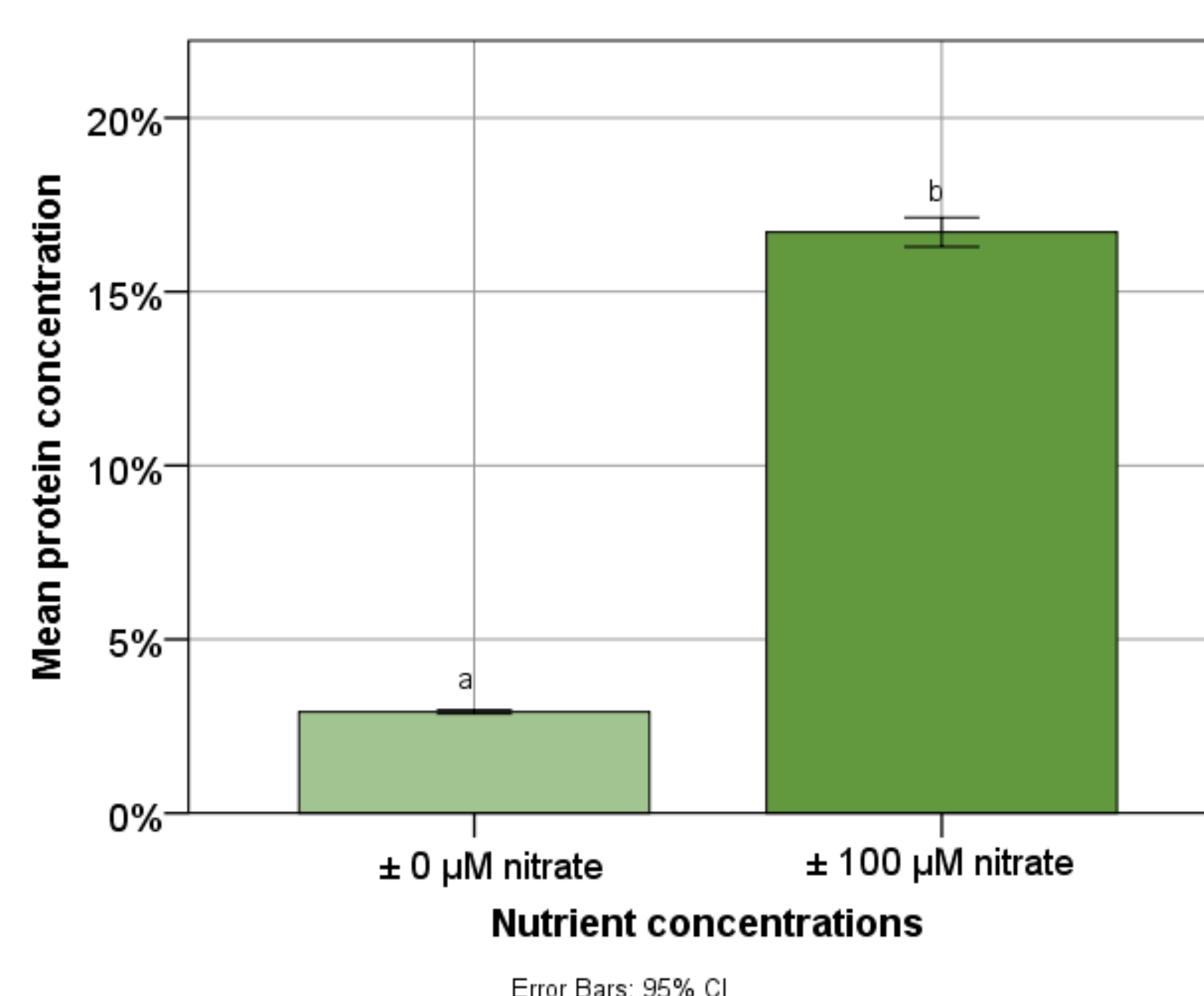


Figure 6. Average protein concentration of *U. lactuca* ( $N=32$ ) after 12 days of incubation in  $\pm 0 \mu\text{M}$  nitrate concentration ( $n=16$ ) and  $\pm 100 \mu\text{M}$  nitrate concentration ( $n=16$ ), an independent samples t-test shows a significant difference between the protein concentrations ( $p=0,00$ ).

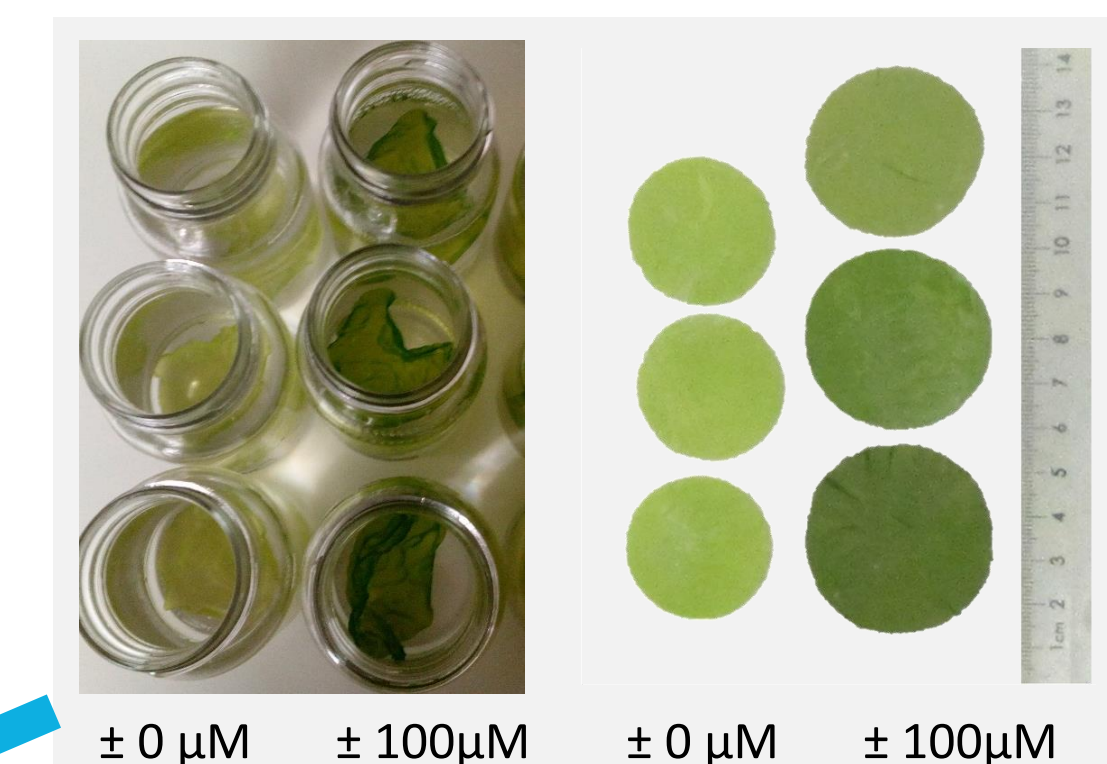


Figure 3. Punched out *U. lactuca* under different nitrate concentrations after 7 days.

## Conclusions

- In the  $\pm 100 \mu\text{M}$  concentration *U. lactuca* shows an 1.6-fold elevation in the average relative growth rate compared to the  $\pm 0 \mu\text{M}$  concentration.
- There is a positive correlation between the relative growth rate and the photosynthetic efficiency of *U. lactuca*.
- U. lactuca* shows an 1.6-fold elevation of protein concentration in the  $\pm 100 \mu\text{M}$  concentration compared to the initial protein concentration and it shows a 3.8-fold elevation in protein concentration compared to the  $\pm 0 \mu\text{M}$  concentration.

## Future research

Similar research as described here for *U. lactuca* is currently being conducted on the brown seaweed *Saccharina latissima* to give an estimation of achievable protein yields. For a better understanding of the applicability of the seaweed proteins the amino acid composition is analyzed for both species under different concentrations. Future research could focus on the combined effects of seasonal and nutrient influences on the protein concentration of seaweed.



Figure 7. Young *S. latissima* sporophytes incubated under relatively high nitrate concentrations.



Figure 8. Young *S. latissima* sporophytes incubated under relatively low nitrate concentrations.

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## References

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Picture of *U. lactuca* in headline, Biolib n.d., <https://www.biolib.cz/IMG/GAL/7080.jpg>

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