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Doctoral Thesis External Examiner Report,
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Netíková K., 2018: Regulation of photosynthesis and primary production of phytoplankton under nutrient and light stress. Ph.D. Thesis Series, No.XX. University of South Bohemia, Faculty of Science, School of
Doctoral Studies in Biological Sciences, České Budějovice, Czech Republic, XX pp.

General:
I greatly enjoyed reading the thesis, and found the wide-ranging introduction useful.
I congratulate (Dr.) Netíková on having already published two of the three thesis chapters as peer-reviewed manuscripts in a reputable, peer-reviewed international journal, 'Photosynthesis Research'.

General Questions for Discussion:
1) In papers I & III you use changing Nitrogen chemostat levels to control growth rate over a ~3X range.
Would you expect comparable results if you controlled growth by varying some other limiting factor; ex. light (as in Paper II) or Phosphate, or Fe?

Are the responses driven by the change in growth rate? Or by the change in Nitrogen supply/status?
How could you (in principle) answer the question experimentally?

2) Did your analyses include organic carbon lost from cells to the media?
Would such loss alter your interpretations?
Could such loss account for some of the decline from GPC to NPC?

3) Not all your cultures were axenic.
How could that affect your interpretations of O2, CO2 and ETR patterns?

Recommendation:
I recommend acceptance and approval of this quality thesis.

Specific comments & suggestions:
Chapter 1.1
"...such a high cell density makes it the major contributor..."
The cell densities of Prochlorococcus are not generally high, but the total population is high, given the vast extent of the niche.

The distinctions among cell density (cells ml-1) and population (cells) is important.

Units:

global net primary productivity: 105 petagrams C y-1 = 105 X 10^15 g C y-1
aquatic primary producers: 45-57 petagrams C y-1 = 50 X 10^15 g C y-1
Prochlorococcus 4 gigatons C y-1 = 4 x 10^9 tons = 4 x 10^12 kg = 4 x 10^15 g C y
Why change units? Make it easier for the reader and do the unit conversions.

Chapter 1.3
"Interestingly, the absorption spectrum of DV-Chl a is red-shifted and therefore the red peak of DV-Chl a is shifted approx. 7 nm towards the
blue region (i.e. to longer wavelengths) in comparison to classic Chl a giving to the P. marinus the advantage when living in deep waters
enriched with blue light (Partensky et al. 1999, Ting et al. 2002)."

This text is confused. Recheck the references.
The description of peak shifts is worded wrongly.
I think extracted DV-Chl a has a red peak almost identical to Chl a.
But DV-Chl a bound to Pcb has a red peak shifted to shorter wavelengths than Chl a bound to typical chl-protein complexes.
And DV-Chl a has a blue peak shifted as well.

Chapter 1.4
"Members of the Prochlorococcus genus belong to the most diverse group
of phytoplankton on the Earth."

This is not a useful sentence; how to define 'diverse"? Cell size range (diatoms), genotypic complexity (dinoflagellates), evolutionary age (cyanobacteria), antenna absorption profiles (cyanobacteria), nutritional modes .....etc. etc.

"In this environment, strains have to cope with changing
conditions such as irradiance, temperature optima or nutrient availability."

I think, rather:
"Across these environments, strains have to cope with a wide range of conditions such as irradiance, temperature optima or nutrient availability...."

Individual Prochlorococcus strains actually inhabit relatively stable niches, but the strains inhabit a wide range of niches.

"...revealed differences within the members of specific clades and also revealed closely related isolates with different levels of fitness..."

I think Rather:
"revealed differences within the members of specific clades and also revealed closely related isolates with different adaptations..."

P.10 more confused wording about positions of absorbance maxima

Chapter 1.6

"As a result, nitrogen (N) forms are reduced and N concentration can be extremely low in comparison to the deeper layer, where mostly nitrates are present"

Reserve 'reduced' for the redox sense.
In this sentence, do you mean N is a low levels, or near the surface the forms of N are in a reduced state? Both are likely true.

P.16
"Once glucose is assimilated, it could be fully oxidized providing either twelve molecules of NADPH or two molecules of ATP."

This statement is wrong.
Full oxidation of $(CH_2O)_6$ to 6 CO_2 releases 24 electrons, sufficient to reduce 12 molecules of $NADP^+$ to NADPH.
But metabolically 4 of the electrons are extracted at the redox level of FADH, insufficient to reduce $NADP^+$.

In the parallel pentose phosphate path all electrons are passed to NADPH, but in the final cycle an equivalent of 1 CH_2O is 'left over', so again, 4 e^- are not available to pass to $NADP^+$.

The two molecules of ATP are just the byproduct of the rearrangement steps of glycolyses.
Full oxidation of glucose can generate 32-38 ATP, depending upon assumptions and redox shuttles use.

P.17
"bis-fosfate" (bis-phosphate)

'carboxysome' (not carboxyzome)

P.19
The discussion of carbon concentration mechanisms is confusing.

"According to comparative genomic analyses, it was revealed that Prochlorococcus lacks genes encoding
BCT1 and both CO_2 uptake systems (Giordano et al. 2005, Ting et al. 2014)."

Which CO_2 uptake systems? BCT1 is not previously explicitly mentioned.
It is difficult to follow the logic of the paragraph with names missing.

Also, bicarbonate can diffuse through the cytoplasm, but cannot diffuse across a plasma membrane.

Fig. 8: Very helpful.

P.25 "LOV domain" (define?)

P.32
Given the wide genomic diversity across Prochlorococcus marinus, it is important to specify the strain(s) or clade(s) ecotype(s) included in your analyses. We would expect different results from different strains.

Fig. 10, Better to replace this figure with the more complete version from Fig. 3 of the introduction?

Table 1: Very helpful.
I suggest moving OEC section upward to just below 'Photobiology'.

Fig. 11: Orbital shaker? Or magnetic stirrer?
Difficult to imagine a complicated continuous culture flask connected up on an orbital shaker?

P.41 negative net O2 evolution rates implies net heterotrophy, which is an important point.

P.43:
again, 'reduced concentrations of nitrogen'
replace with 'lowered concentrations of nitrogen' etc.

P45:
"...and also to the environmental conditions, i.e. higher concentration of nutrients in the natural environment, ability to take up more N sources and glucose uptake...."

This is an important point, but needs elaboration.
In your cultures you did not provide any glucose.
So if the low light strain displays 8% higher NPC than the low light strain, this means that, in the absence of supplied glucose, it retains more fixed C.
Is this consistent with a reliance upon exogenous C from the environment?

Paper I
"Carbon use efficiencies and allocation strategies in Prochlorococcus marinus strain PCC 9511 during nitrogen limited
growth"

ETR:
was estimated using the fixed ratio of 500 mol chl a/1 mol RCII.
But Table 3 shows changing nPSII (~3X) and sigmaPSII.

Will this discrepancy affect your interpretation of Fig. 1 in the paper?
Did you account for changing nPSII in the estimates in Fig. 1?

"It should be noted here that despite many trials, we have not yet been able to

quantitatively measure the rates of oxygen
evolution in Prochlorococcus that match the measured rates of ETR or C assimilation. For some unknown reason,
the maximal gross rates of O2 evolution [110–145 μmol O2 (mg DV-chl a h)−1] measured either using a Clark electrode
or MIMS were several times lower than the rates of ETR or GPCb. Further experiments are under way to elucidate
whether this is due to the mechanical stress caused by unavoidable concentrating and stirring of Prochlorococcus cell
suspensions, or whether it is inherently caused by some structural or functional modification of Photosystem II in
this organism with highly streamlined genome (e.g., Rocap et al. 203)"

This is a major finding, and it strongly suggests a large pseudocyclic flow from PSII oxygen evolution back to oxygen or some form of kinetic discrepancy between the time spans of different measures, or MIMS calibration issues?

In Table 4, can you include estimates of Ek derived from PSII ETR data?

Paper II

"Comparison of photosynthetic performances of marine picocyanobacteria with different configurations of the oxygen evolving
complex"

"Measurements were carried out on 2 mL aliquots of concentrated cultures placed into a cuvette, homogenized with a magnetic stirrer and maintained at 22 C by circulation of thermostated water from a MultiTempIII temperature-controlled bath (GE Healthcare, Amersham Biosciences, Uppsala, Sweden)."

Is is possible that the cell concentration step provokes an acceleration of O2 consumption?
And/or, that in vivo boundary effects lower the achieved O2 consumption?

Chl assays: Are their specific assays for DV chl?

Continuous light was lethal at >50 umol photons m-2 s-1 in paper 1, but here is tolerable up to 75 or even 163 umol photons m-2 s-1; differences in culturing conditions?

Fig.1
maximal net O2 evolution rates (Pm)
were normalized per (DV-)Chl a, per cell and per PSII, in
order to ease comparisons (Fig. 1a–c).

Although we developed the D2 antibody you used, I am cautious about normalizing rates to the protein content.
Many (most?) phytoplankton can carry large, and variable, pools of PSII protein that is

not part of active PSII complexes.
(ex. Wu et al., 2011, 2012, Plant Physiology; Bonisteel et al., 2018 submitted, PLoSOne).
I strongly suspect that some of the differences in Fig. relate to different ratios of D2: PSIIactive; that is, differences in the content of PSIIinactive. We find that MIT 9313 has very low capacity to turn over PSIIinactive, and thus the inactivated complexes accumulate.

"Although PChlm values obtained here for O2 release were systematically higher than Moore and co-workers' for CO2 assimilation, these discrepancies might be due in part to the different light conditions used
in the two studies"

This appears to contradict, or at least resolve the discrepancy noted in Chapter II?

Subject to caveats about using D2 as a proxy for PSII content:
Fig. S3 show, by subtracting dark respiration, dividing by 3600 s/h and multiplying by 4 e- per O2:
Prochlorococccus 9511, ~72 e- PSII-1 s-1 at light saturation after high light growth.
For Synechococcus, ~56 e- PSII-1 s-1 at light saturation after high light growth.

Prochlorococccus 9511, ~189 e- PSII-1 s-1 at light saturation after high light growth.
For Synechococcus, ~100 e- PSII-1 s-1 at light saturation after high light growth.

Prochlorococccus 9511, ~189 e- PSII-1 s-1 at light saturation after high light growth.
For Synechococcus, ~100 e- PSII-1 s-1 at light saturation after high light growth.

How did the concentration and measurement procedures vary between Paper II (O2 evln lower than C uptake?) and Paper III (O2 evln higher than C uptake?)

Paper III Manuscript in preparation)
"Carbon metabolism differ in low-light and high-light ecotypes of Prochlorococcus marinus"

Materials & Methods;
"Time-dependence of ^{14}C uptake"

There is something wrong with the description of timing.
"...were added to the culture in 2 min interval. For another 2 minutes, sampling for ^{14}C uptake rate started and has continued regularly over 24h by taking 1.1 ml samples at each time point and transferring the sample"...

Something is wrong here. I think you mean:

"...were added to the culture. After 2 minutes sampling for ^{14}C uptake rate started and continued repeatedly over 24h by taking 1.1 ml samples at each time point and transferring the sample..."

(based upon looking at Fig. 1)

p. 120, 'spun', not 'spinned'

Results, Fig. 1
"523 ± 36 µmol C (mg DV-Chl a h)$^{-1}$"

~44 umol C mg DV-Chl a h-1;
0.012 umol C mg DV Chl a s-1

Fig. 2: I think the PCC9511 data is taken from Paper I?
That is fine, but should be cited in the Figure Legend 2.

Fig. 3 same comment.
Fig. 3 Legend needs more words.
I think the X axis is growth rate normalized to mu max for the particular strain?
Or to maximum measured growth rate?

Otherwise, the data does not line up with Fig. 2, which shows only small differences among the strains at 20 min.

Fig. 5 same comments; what is 'relative growth rate'
Figure + Legend need to stand alone without reference to the text.

Discussion:
p. 124 and elsewhere:
reserve 'reducing' for the redox sense; use 'lowered', 'declining', 'drop' etc. for the general sense.

"PbEg"
does not appear to be defined anywhere.

P.127
Repeat of incorrect description of metabolic equivalents of NADPH and ATP from glucose.

Ph.D.
24 Oct 2018

Review of the Ph.D. thesis by Kristina Felcmanová „Regulation of photosynthesis and primary production of phytoplankton under nutrient and light stress"

The dissertation deals with a characterization of selected physiological and namely photosynthetic parameters of various *Prochlorococcus* strains under various light conditions and in nitrogen-limited environment. Even though *Prochlorococcus* strains are probably the most abundant oxygen-evolving photosynthetic organisms on Earth, their regulation processes connected with photosynthesis are mostly unknown. As *Prochlorococcus* strains are also the most abundant phototrophs in nutrition-limited sea areas, their study has high eco-physiological impact. This dissertation is based on two papers published in the journal Photosynthesis Research (Kristina Felcmanová is the first author in one of them) and one manuscript. At the beginning of the thesis, there is a statement indicating her contribution in particular papers or the manuscript. I admit that I would have expected more than "a participation in writing" in the paper where Kristina Felcmanova is the first author. **Did you write at least a whole draft of this manuscript?** I am also a bit surprised that the in title of the thesis, there is a general term "phytoplankton", although the whole thesis is clearly focused on *Prochlorococcus*.

The thesis consists of a general introduction (31 pages), focusing on the *Prochlorococcus* discovery, radiation and variability of genetic and physiological characteristics. Special attention was paid to light-harvesting complexes and associated pigments, nitrogen assimilation, carbon-concentrating mechanism, oxygen evolution, and mechanisms of photoprotection. This part is well written and easy to follow. The last part of the general introduction (1.7.) deals with more general methodological problems connected to the estimation of oceanic primary production. The citation of author's own papers is an organic part of this general introduction, however, the papers are missing in the part References. I would also prefer the author's papers denoted as Paper I – III in the text.

The text continues with "Aim and hypothesis" part, in which four specific hypotheses are clearly stated, and "Methods", with a brief description of the methods used in the papers/manuscript with references to the attached papers/manuscript.

The results and discussion of the thesis are presented as two chapters "Overview of my research" (4 pages) and "Conclusions and future prospects" (5 pages). In the first chapter, there are more or less the abstracts of the supplemented papers/manuscript with a paragraph about a connection of the results with the hypotheses presented in the previous part "Aim and hypothesis". The second chapter represents a discussion of the results and the conclusions. In this part of the thesis I would expect a more thorough discussion of the results. Although detailed discussions are in the papers/manuscript, I prefer a more comprehensive discussion part in a PhD thesis, which should put the obtained results in the wider context of the present knowledge in the field.

I have several questions/notes to be addressed during the defense:

1) How can the results presented in this thesis improve the estimation of primary production of oceanic ecosystems by satellites? What parameters of the mathematical models could be changed using the results?
2) In page 5, it is written that "... absorption spectrum of DV-chla is red-shifted and therefore the red peak of od DV-chl a is shifted approx.. 7 nm towards the blue region (i.e to longer wavelengths)...". This is not correct. It is correct to write that the bands in the blue part of the spectrum are red shifted in comparison to Chl a and that the bands in the red part of the spectrum are slightly blue-shifted (i.e. to shorter wavelengths) in comparison to Chl a. More precisely, this is correct for DV-Chl a bound to proteins in

Prochlorococcus sp. The absorption bands of DV-Chl a and Chl a in organic solvents in the red region are almost identical.

3) It is interesting that in many *Prochlorococcus* strains, PsbU and PsbV proteins are missing in the oxygen evolving complex (OEC). However, the PsbP is present in OEC (Table 1). On page 40 you wrote that "PsbO alone is seemingly sufficient to ensure proper oxygen evolution". I do not think so. In my opinion, PsbP is important for a proper function of PSII when PsbU and PsbV proteins are missing. What is a role of PsbP in this complex? Can you predict what would happen with PSII in the absence of PsbP? How the thermoluminescence bands (after one single turnover flash at about 0°C) will change?
4) *Prochlorococcus* strains live frequently also in deep sea, e.g. 100 m below water surface. In such depth there is a high pressure (cca 10 atm) and solubility of gasses dramatically increases (according to Henry's law). How these pressure conditions change *Prochlorococcus* physiology in comparison to normal pressure conditions?

In summary, the thesis is written in good English and author's results represent a considerable scientific contribution to the knowledge of regulatory mechanisms in *Prochlorococcus* species and, **in case of successful oral defense, I recommend the award of Ph.D. to Kristina Felcmanová.**

In Olomouc, 17/10/2018

Prof. RNDr. Petr Ilík, Ph.D.