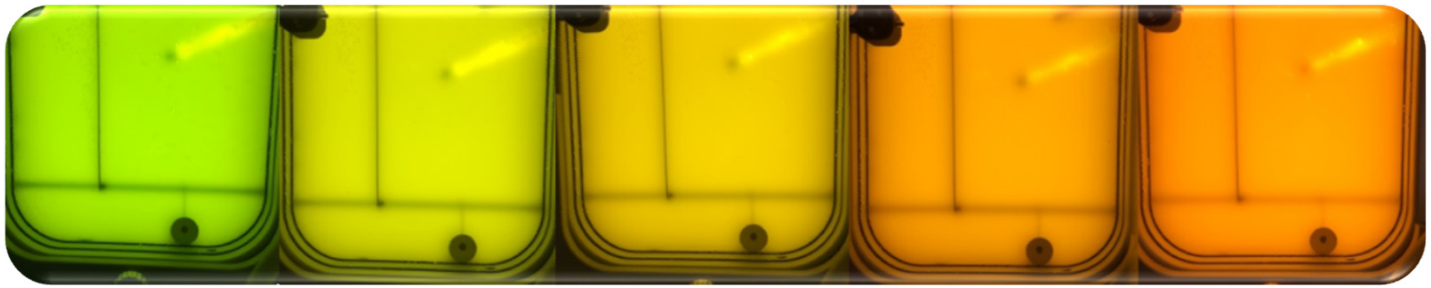
Translocation and *de novo* synthesis of eicosapentaenoic acid (EPA) during nitrogen starvation in *Nannochloropsis gaditana*

Jorijn H. Janssen, Packo P. Lamers, Ric C. H. de Vos, René H. Wijffels and Maria J. Barbosa

AlgaEurope 2018, Amsterdam

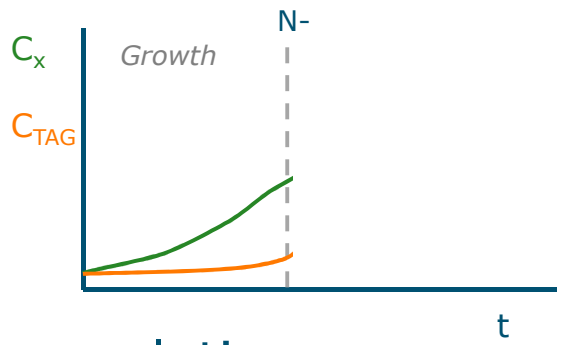


WAGENINGEN
UNIVERSITY & RESEARCH

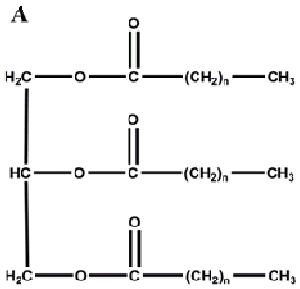
Contact: jorijn.janssen@wur.nl

Lipids in *Nannochloropsis gaditana*

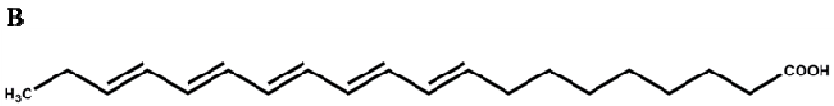
- Lipid fractions
  - Triacylglycerol (TAG)
  - Polar lipids (PL)



- Nitrogen starvation (N-) induces TAG accumulation
- Produce omega-3 fatty acid eicosapentaenoic acid (EPA)



TAG

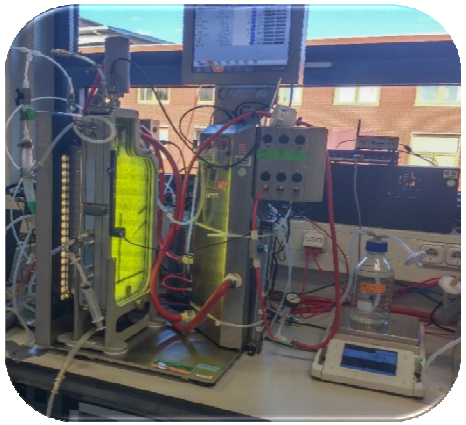


EPA
(C20:5)

Aim

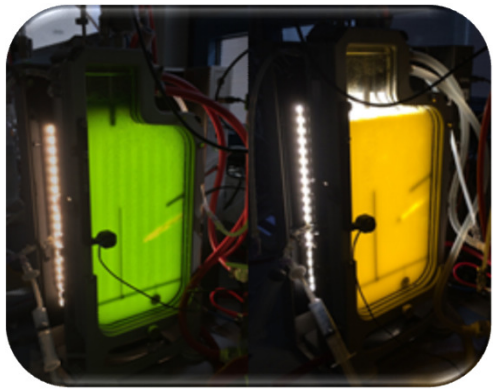
Identify origin of fatty acids accumulated in TAG during nitrogen starvation

→ **Follow ^{13}C- fatty acids**

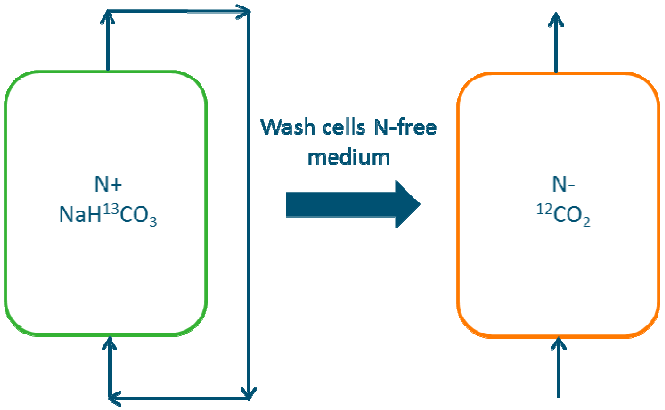


Experimental setup

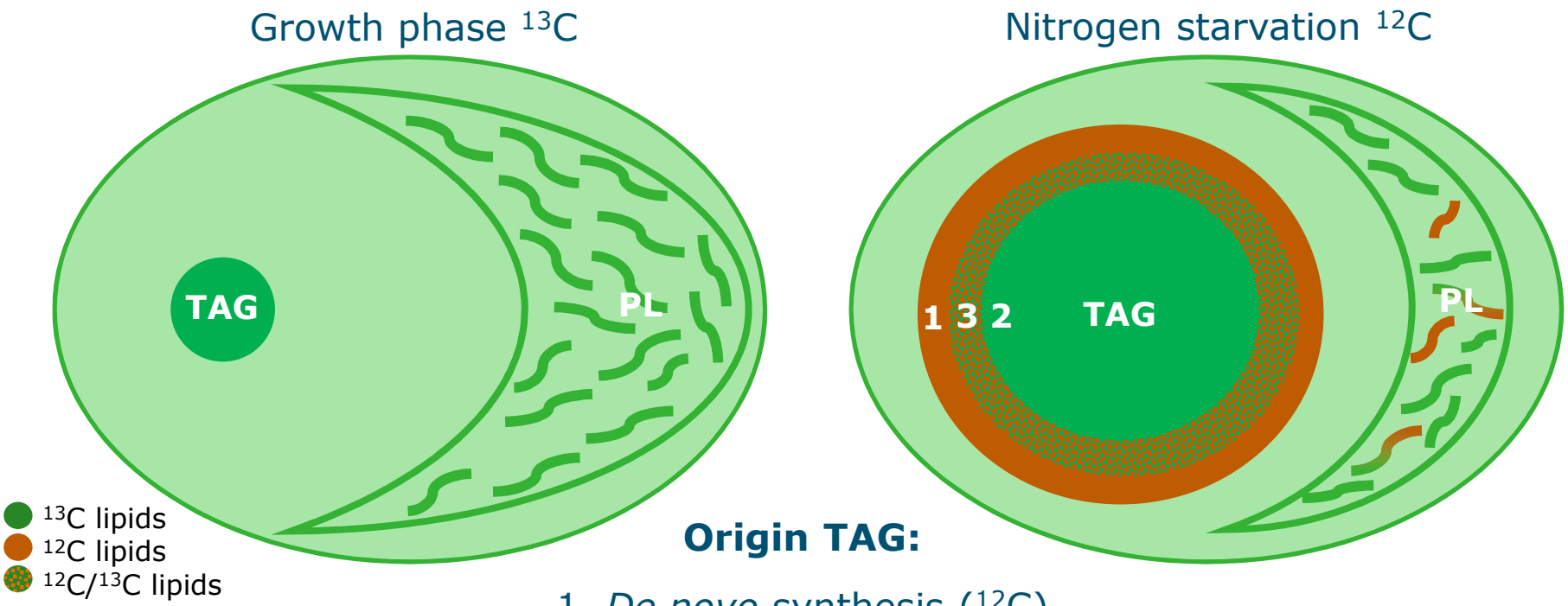
- ^{13}C isotope
  - Measure mass difference
    → Follow carbon flow



- Photobioreactor setup
  - Growth phase ^{13}C
  - Nitrogen starvation ^{12}C



Experimental setup



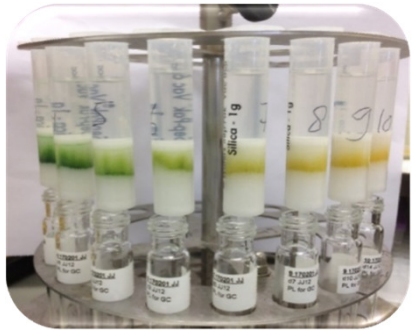
Origin TAG:

1. *De novo* synthesis (^{12}C)
2. Translocation from polar lipids (^{13}C)
3. Carbon recycling combined with *de novo* synthesis ($^{12}C/^{13}C$)

Fatty acid analysis

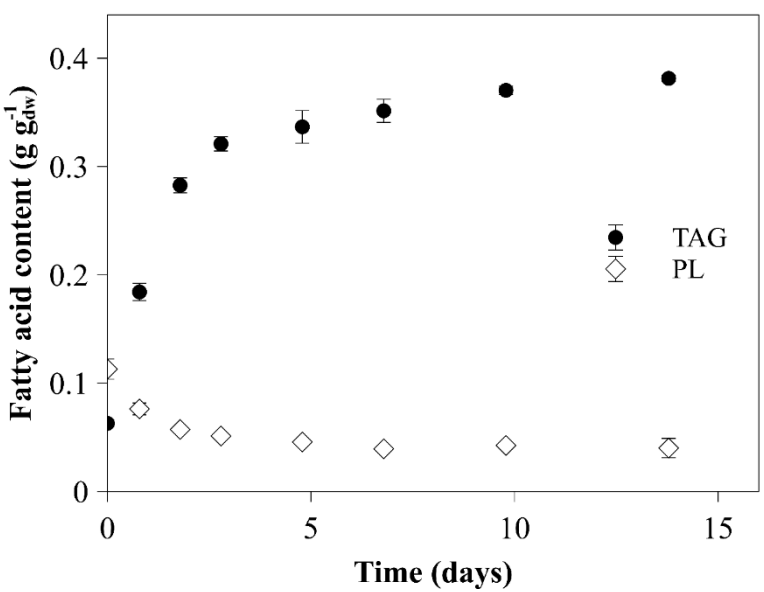
- Extraction lipids from *Nannochloropsis gaditana*
- Separate TAG and polar lipid fraction on SPE column

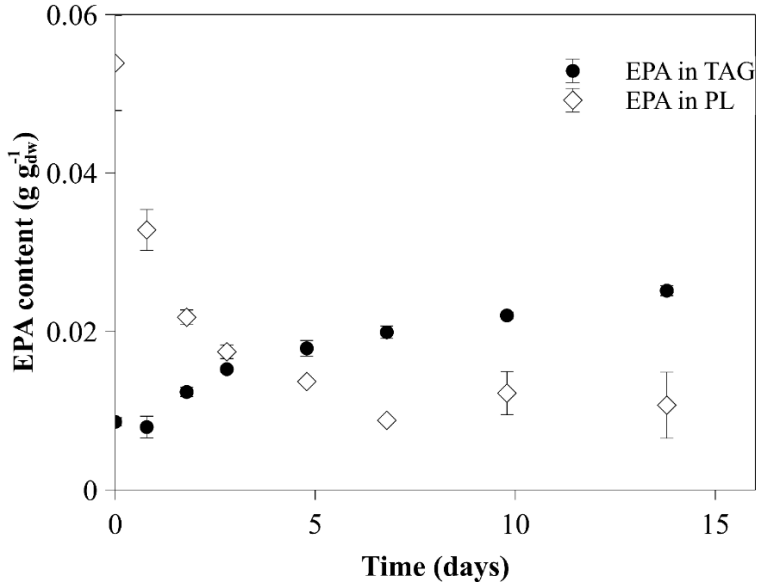
- Quantification FAMES with GC-FID
- Distinguish ^{12}C and ^{13}C fatty acids by UHPLC-MS



WAGENINGEN
UNIVERSITY & RESEARCH

Fatty acid and EPA content in TAG and PL during N-

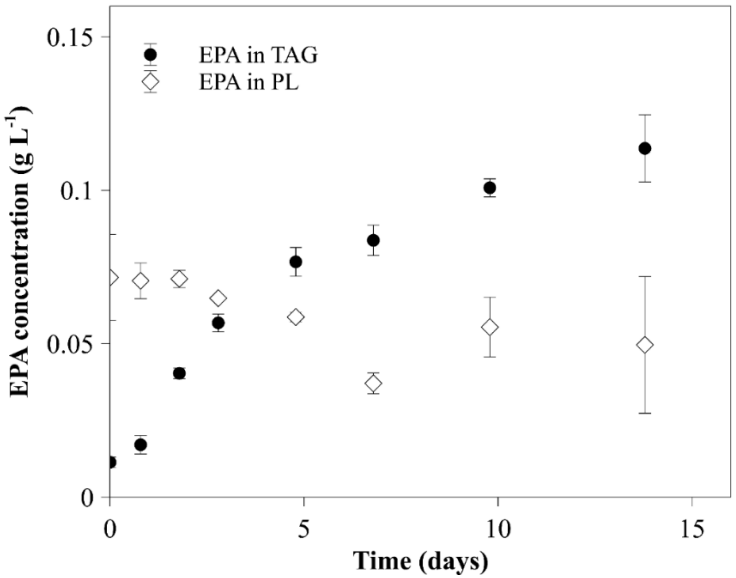


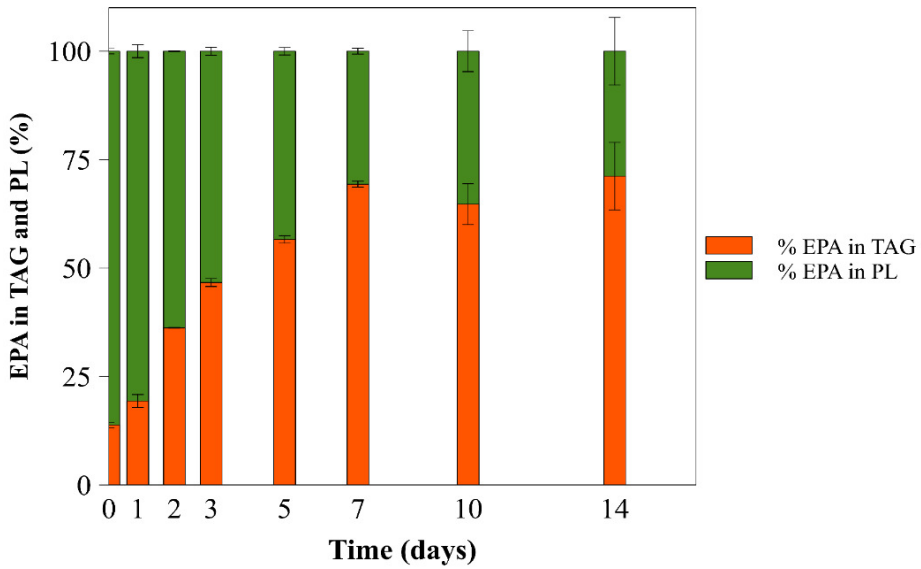


- TAG content increased during nitrogen starvation
- EPA content in TAG increased and in PL decreased
  - Translocation from PL to TAG?

Janssen et al. 2018, Algal research

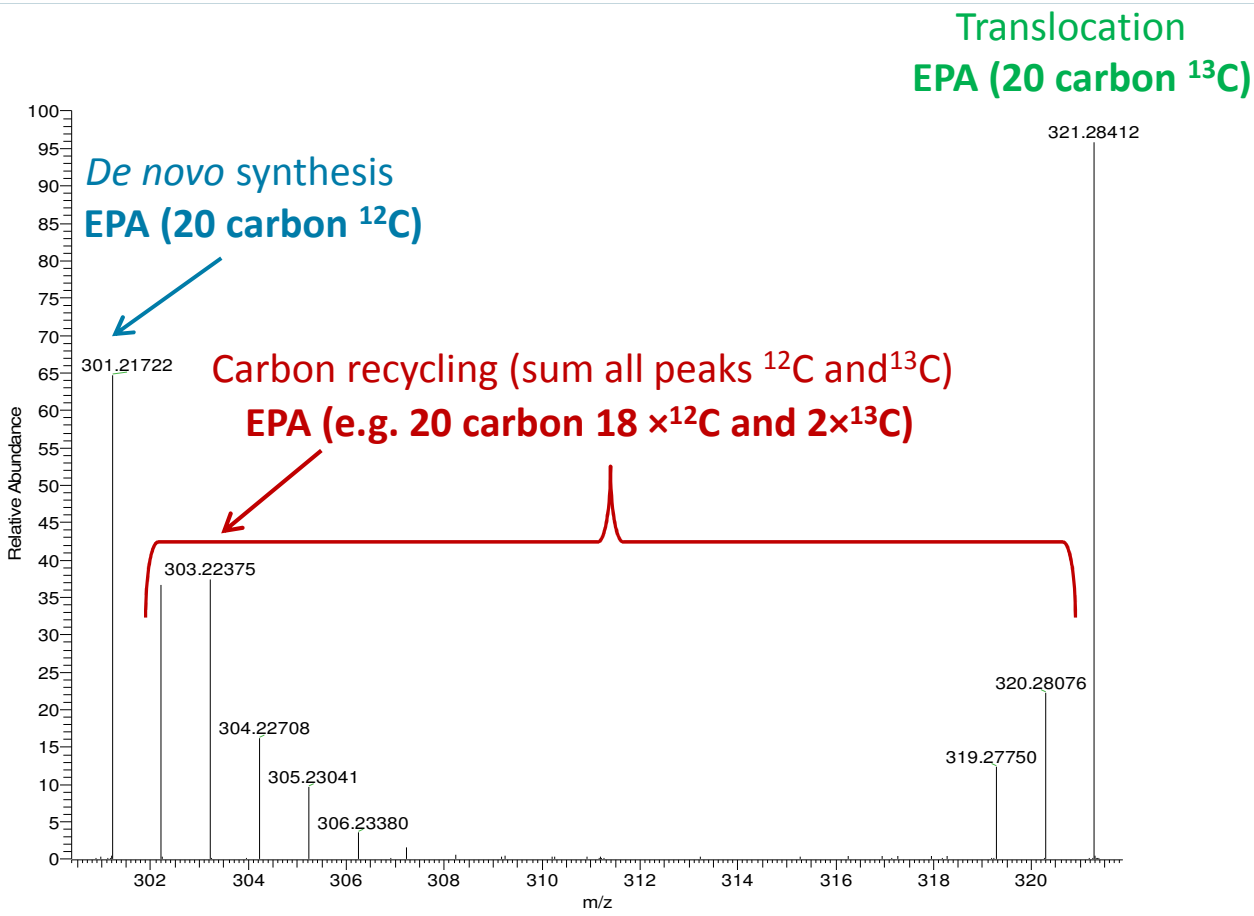
EPA concentration and lipid fraction localization



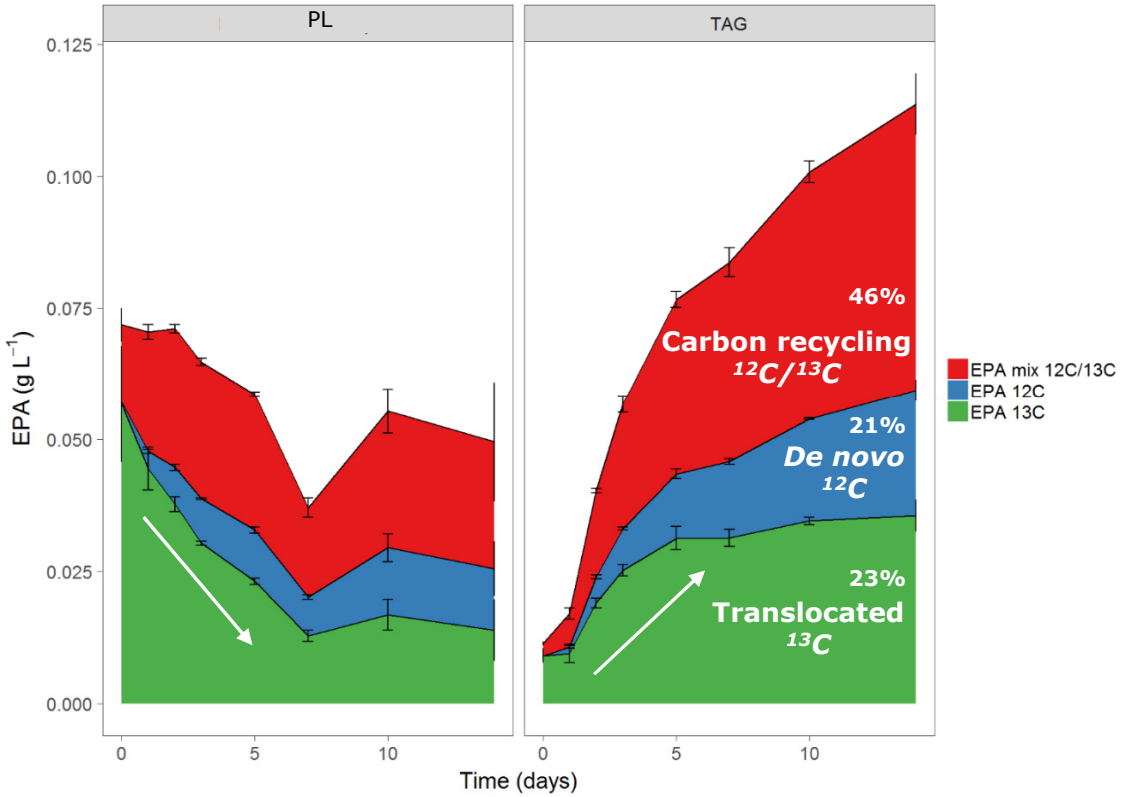


- EPA concentration increased due to increase biomass
- Increase fraction of EPA in TAG
- *De novo* synthesis and translocation → **^{13}C fatty acid analysis**

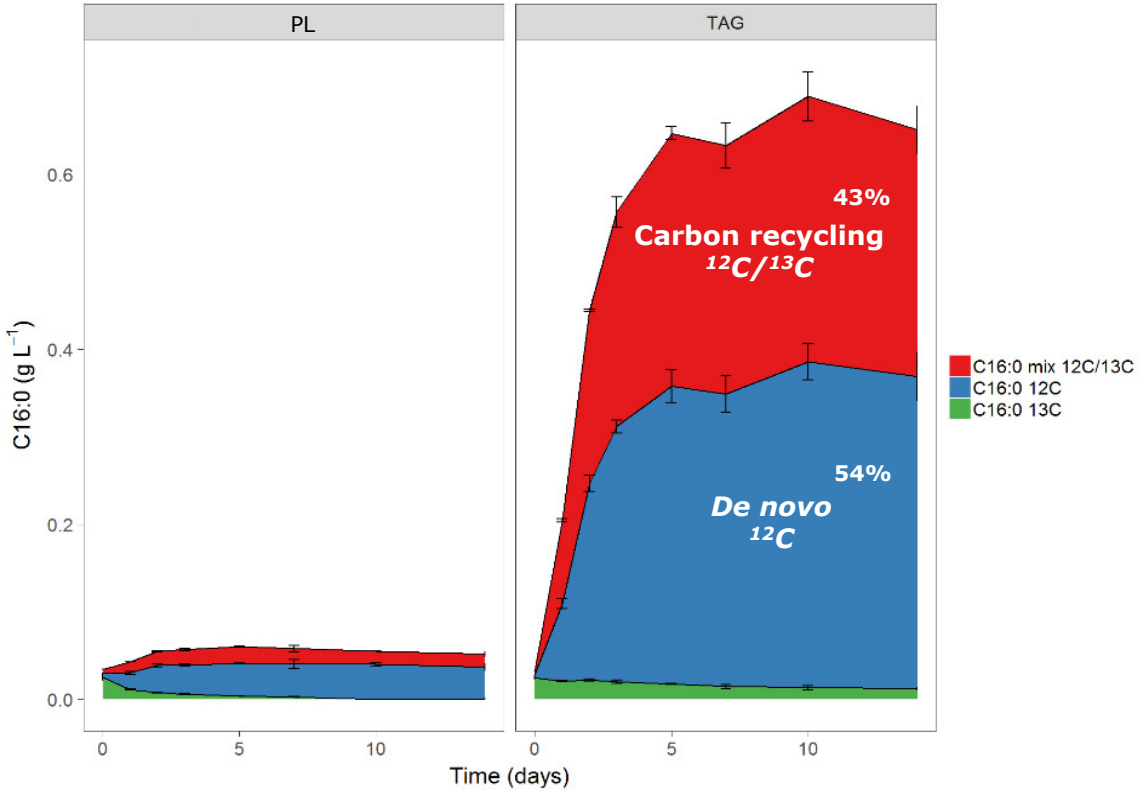
Mass spectrum EPA during nitrogen starvation



EPA (C20:5) was translocated, *de novo* synthesized and made from carbon recycling during N-



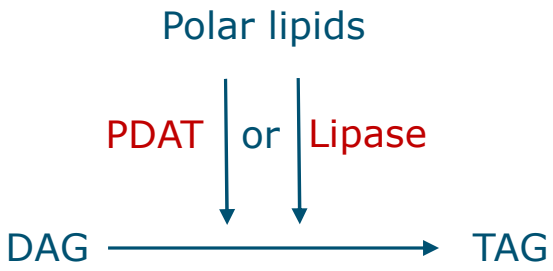
Palmitic acid (C16:0) was *de novo* synthesized and made from carbon recycling during N-



WAGENINGEN
UNIVERSITY & RESEARCH

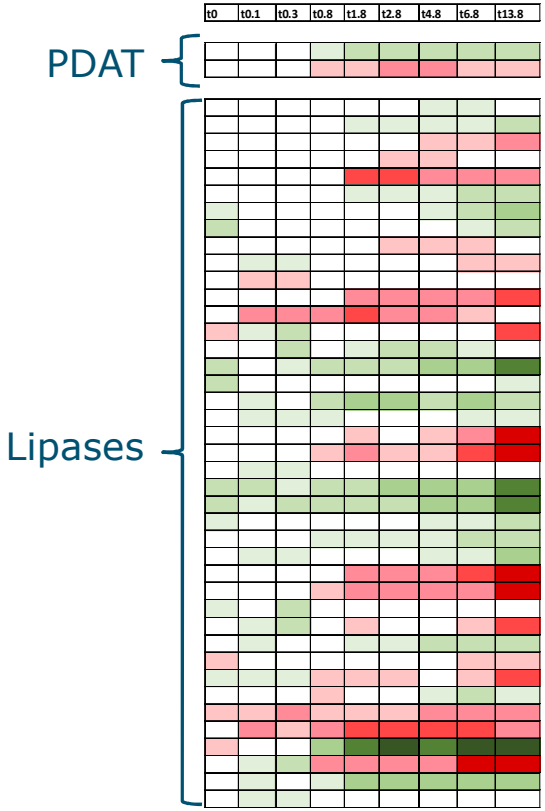
TAG synthesis pathways

- Acyl-CoA dependent pathway (Kennedy pathway)- *de novo* synthesis
- Acyl-CoA independent pathways – translocation fatty acids
  - Lipases
  - Phospholipid: diacylglycerol acyltransferase (PDAT)



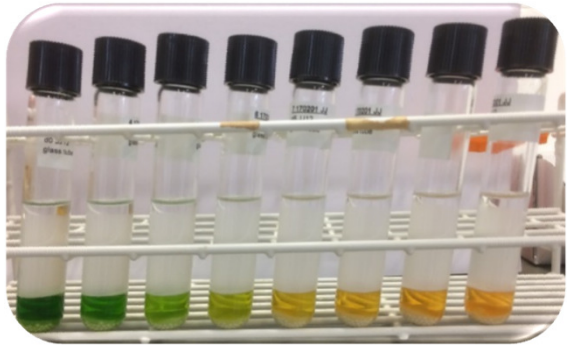
Possible pathways translocation

- Transcriptional regulation during N-
- Multiple copies identified
    - Different expression patterns



Conclusions: origin fatty acids in TAG during N-

- EPA originated from:
  - Translocation of polar lipids
    - Possibly by lipases and/or PDAT
  - *De novo* synthesis
  - Carbon recycling within the cell

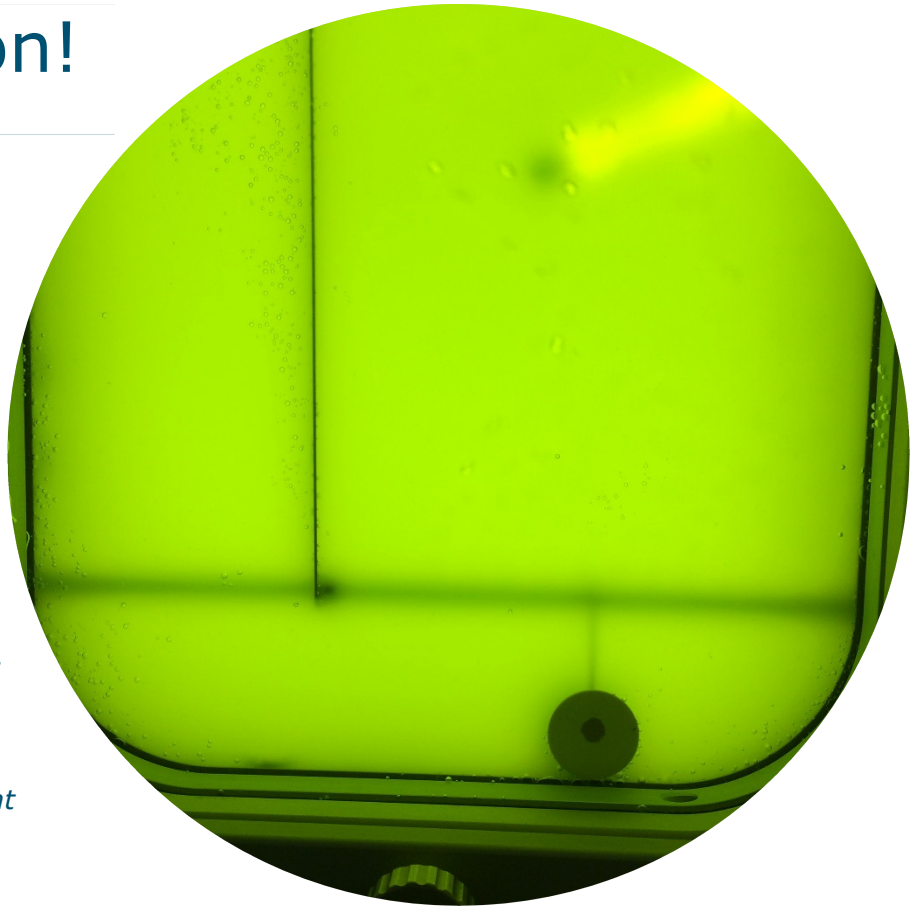


- Palmitic acid (C16:0) originated from:
  - *De novo* synthesis
  - Carbon recycling within the cell

WAGENINGEN UNIVERSITY & RESEARCH

Janssen et al. 2018, Algal research

Thanks for your attention!



Contact: jorijn.janssen@wur.nl

The work was performed in the framework of the MIRACLES project which is supported by the European Commission through the 7th Framework Program under Grant Agreement No. 613588 www.miraclesproject.eu

WAGENINGEN UNIVERSITY & RESEARCH

MIRACLES SPECIALTIES FROM ALGAE

AlgaePARC™