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### ► To cite this version:

Vincent Mériot, Gaëtan Herbette, Elnur Garayev, Noémie Coulombier, Loic Le Déan, et al.. Targeted and non-targeted metabolomic analyses of *Heterocapsa cf. bohaiensis* under nickel and iron high concentration and its photophysiology. 8th European Phycological Congress, Aug 2023, Brest, France. <hal-04211842>

**HAL Id: hal-04211842**

**<https://hal.science/hal-04211842v1>**

Submitted on 20 Sep 2023

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# Targeted and non-targeted metabolomic analyses of *Heterocapsa cf. bohaiensis* under nickel and iron high concentration and its photophysiology

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## Introduction

❑ **New Caledonian** coastal waters are naturally rich in **metallic trace elements**. (Biscéré et al. 2017; Merrot et al. 2022)

❑ An excess of metallic inputs induce an **oxidative stress**. (Pinto et al. 2016; Danouche et al. 2022)



*Heterocapsa cf. bohaiensis*

❑ ***Heterocapsa cf. bohaiensis***, a dinoflagellate isolated in New Caledonia, is associated with shrimp and crab mortalities. (Xiao et al. 2018)

❑ Might be a good candidate to study the production of **metabolites of interests** (e.g. carotenoids, biotoxins, fatty acids...)

❑ Does the species **adapted** to this particularly **metal-rich** environment and does it impacted the **photosystem** and **metabolome** by developing **adaptive strategies** ?

**Aim: Study the impact of nickel & iron on the photosystem and metabolome**

## Materials & Methods

### Culture in photobioreactors:

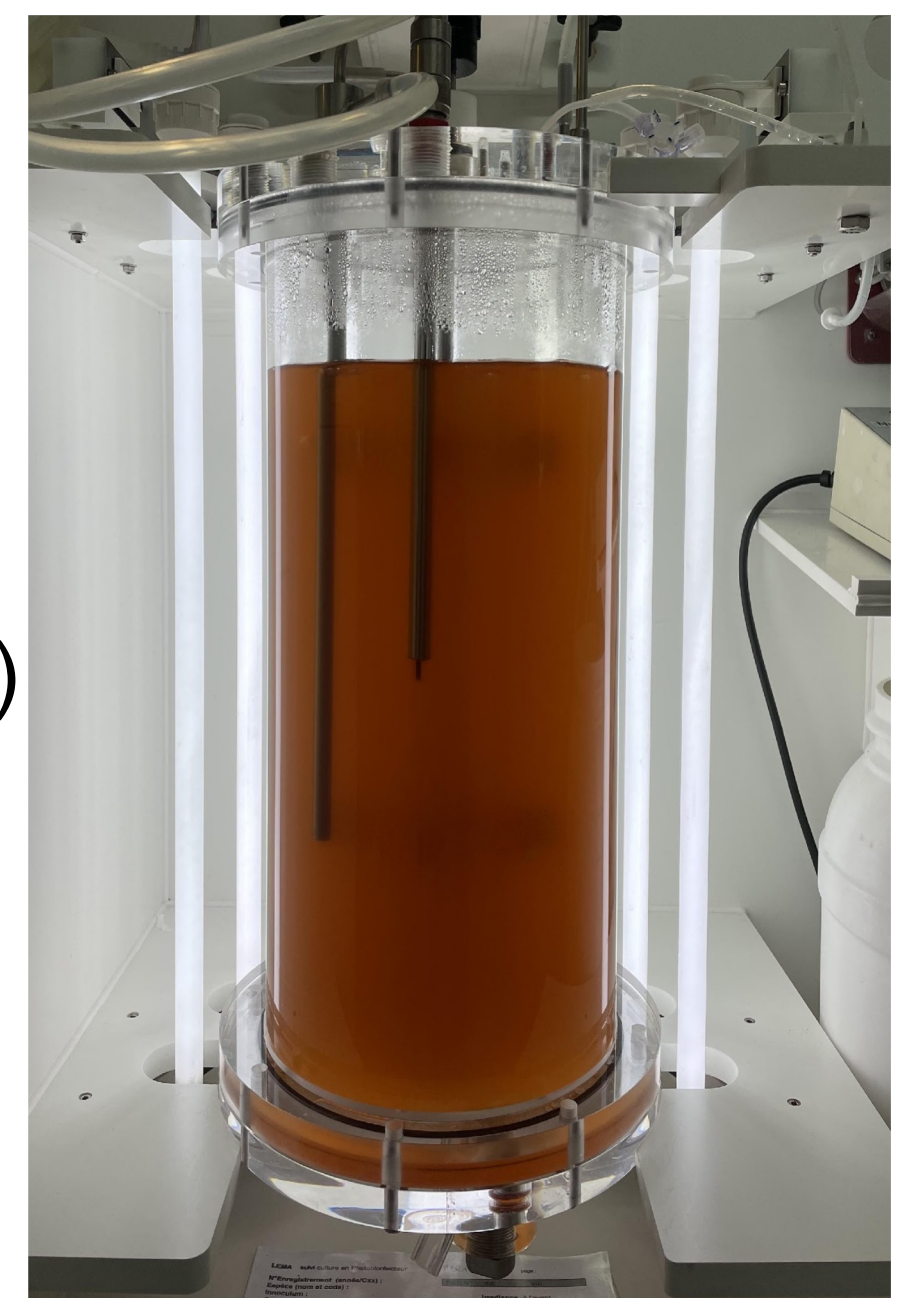
- Continuous culture of *H. cf. bohaiensis*
- Addition of **nickel** and/or **iron** after steady state

### Experimental conditions (in triplicate):

- **Metals:** Ni  $10^{-3}$  M ; Fe  $10^{-3}$  M ; Ni+Fe  $0.5 \cdot 10^{-3}$  M (of each)
- **Times:** Control  $t_0$  ;  $t_{24h}$  ;  $t_{72h}$  ;  $t_f$  (13 days)

### Analyses:

- **Chlorophyll a** fluorescence using PAM (Pulse Amplitude Modulated) fluorometry
- **Bioaccumulation** capacity by ICP-MS/OES
- **Pigment** analyses through HPLC-UV (450 nm)
- **Fatty acids** - GC-MS – (Transesterification)
- **Non targeted metabolites** - NMR



*H. cf. bohaiensis* in 10L photobioreactor

## Results

### Photosynthetic efficiency

#### Maximum Quantum yield

- Maximum photosynthetic efficiency **decreased** by **25%** and **15%** in Fe and Ni+Fe condition, respectively

#### Rapid Light Curves (RLC)

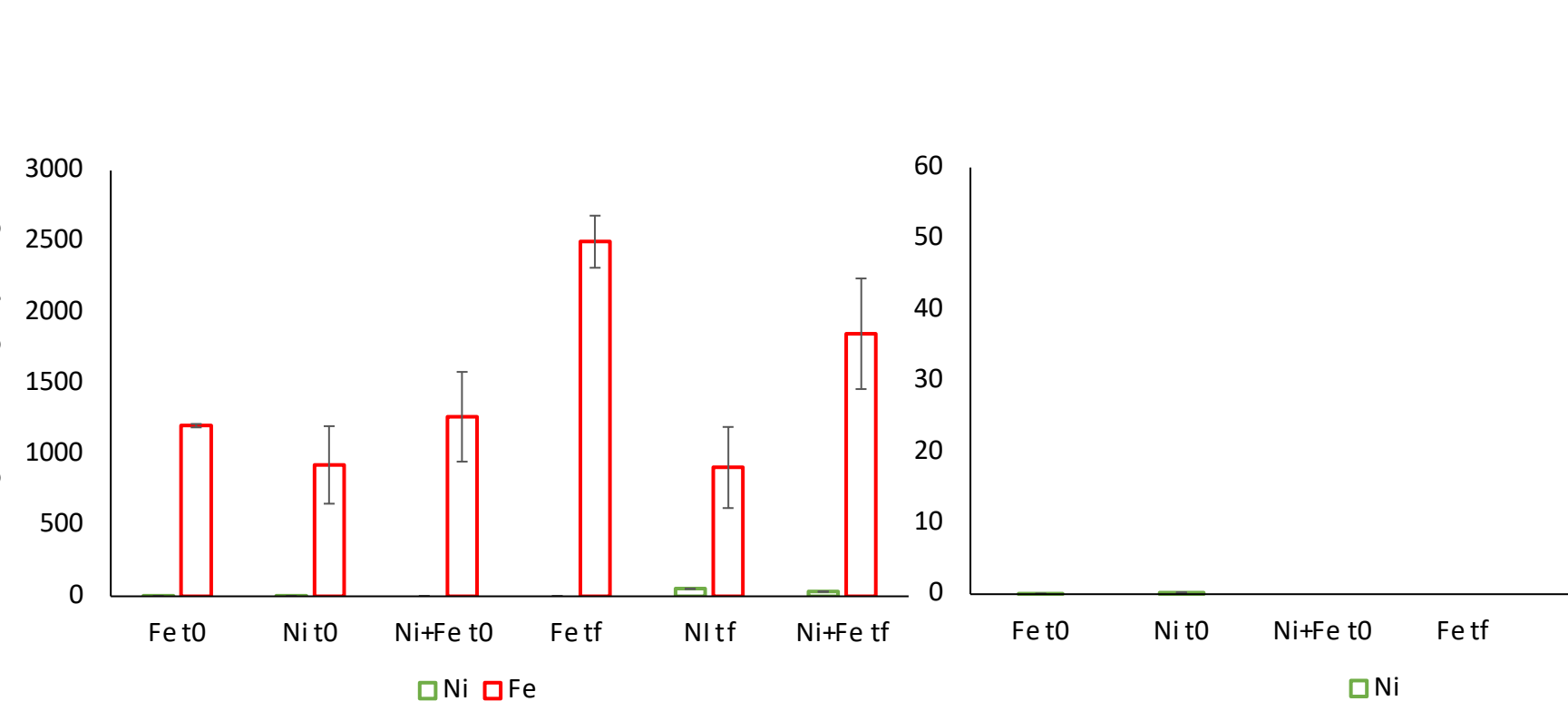
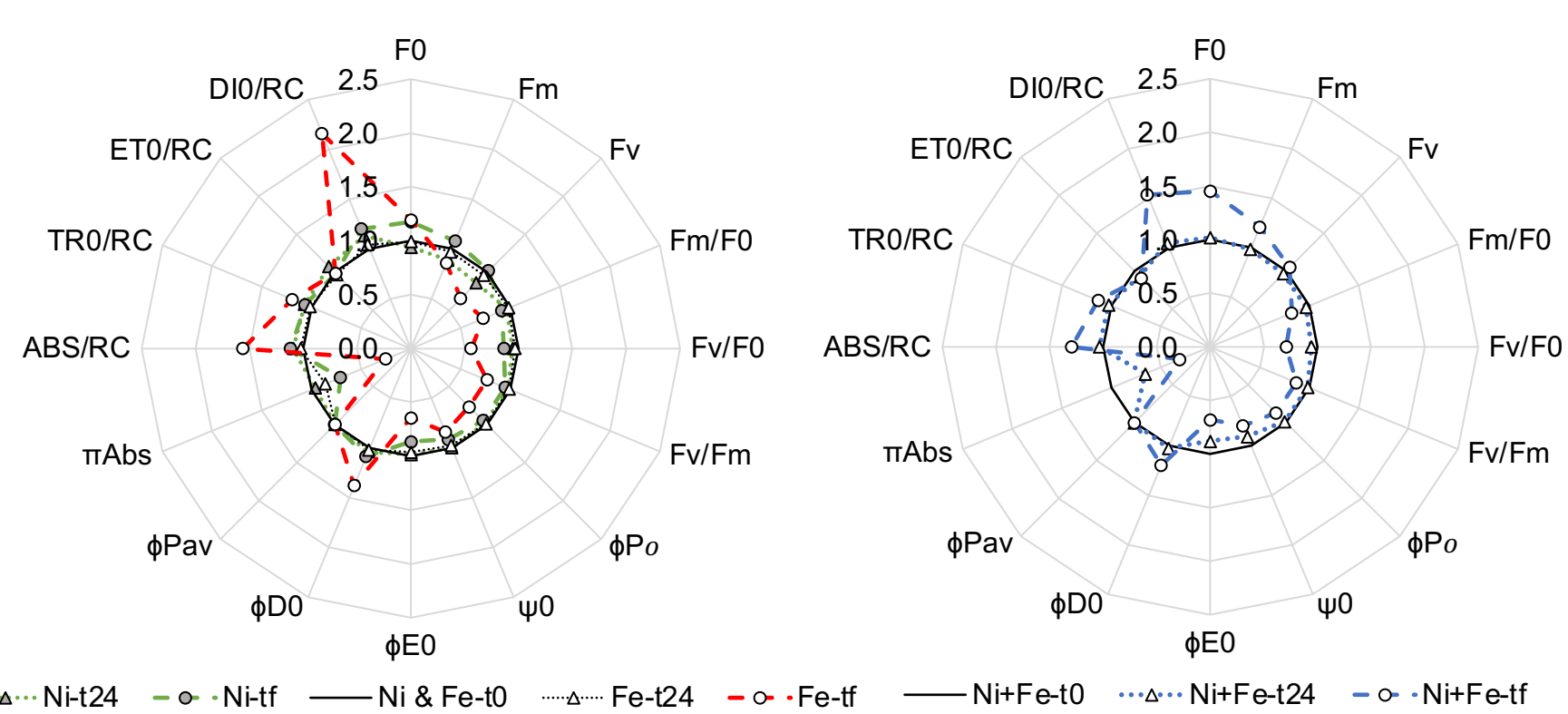
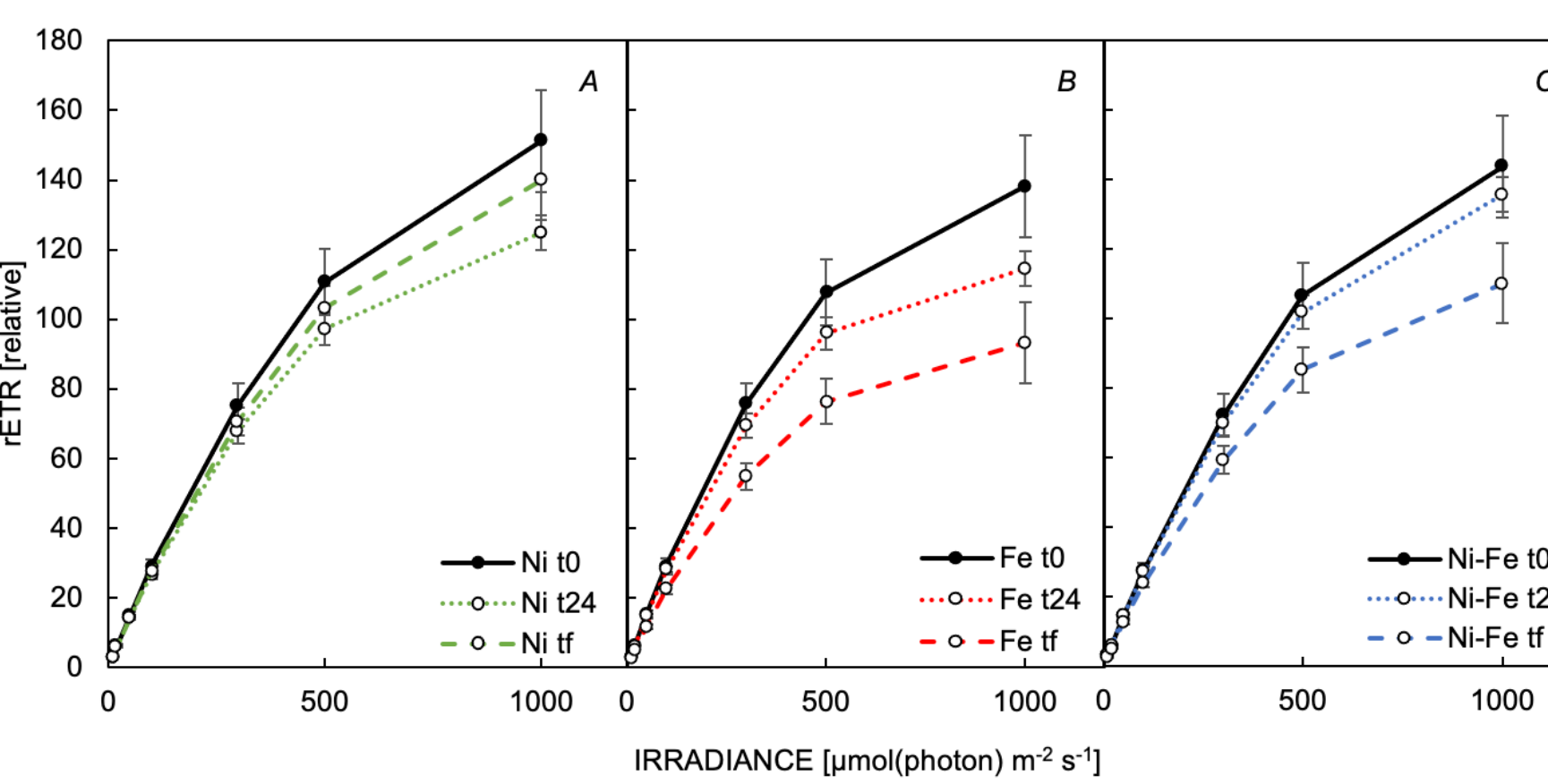
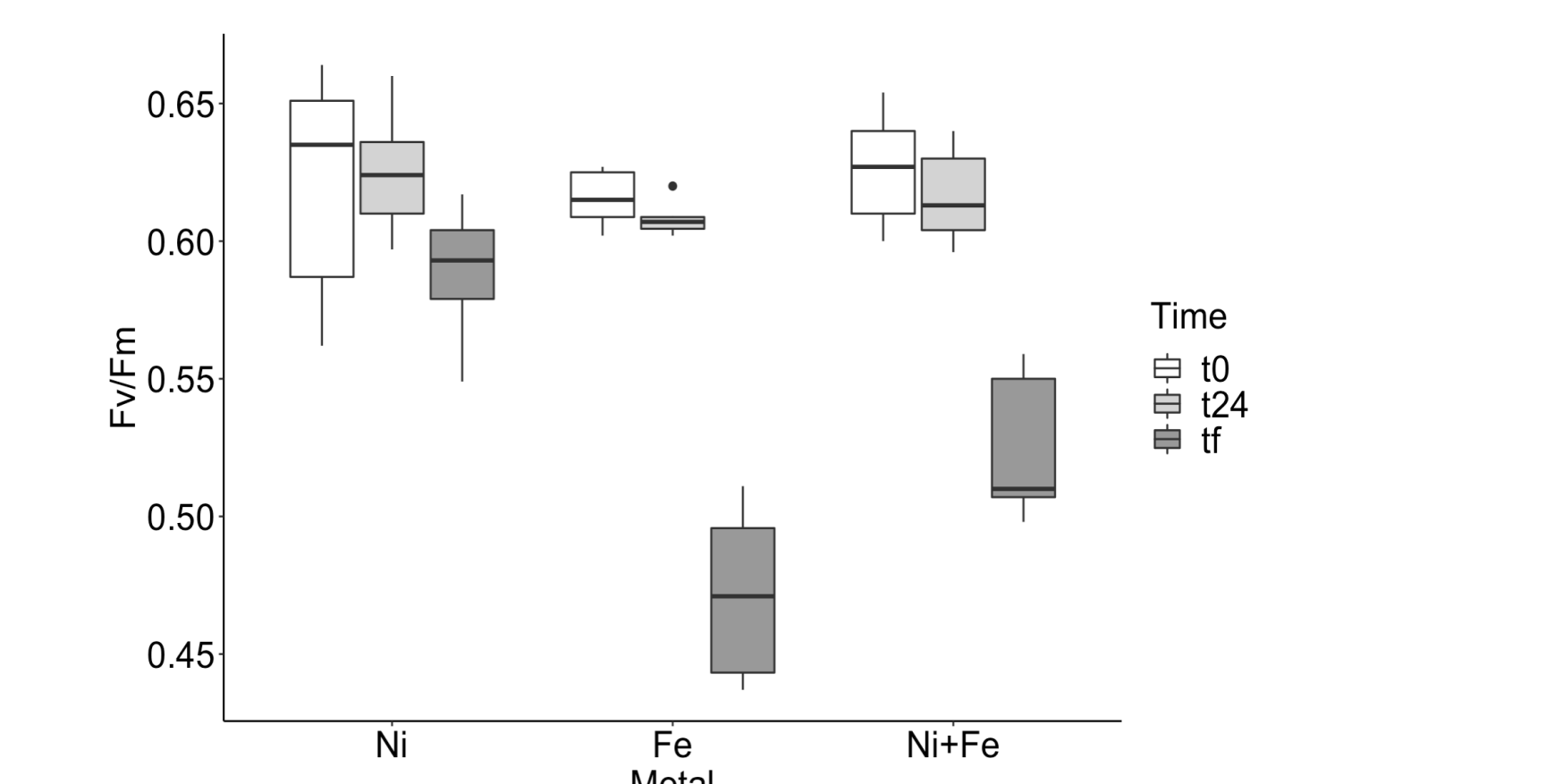
- **Negative** impact on photosynthetic activity due to high iron concentration

#### OJIP tests

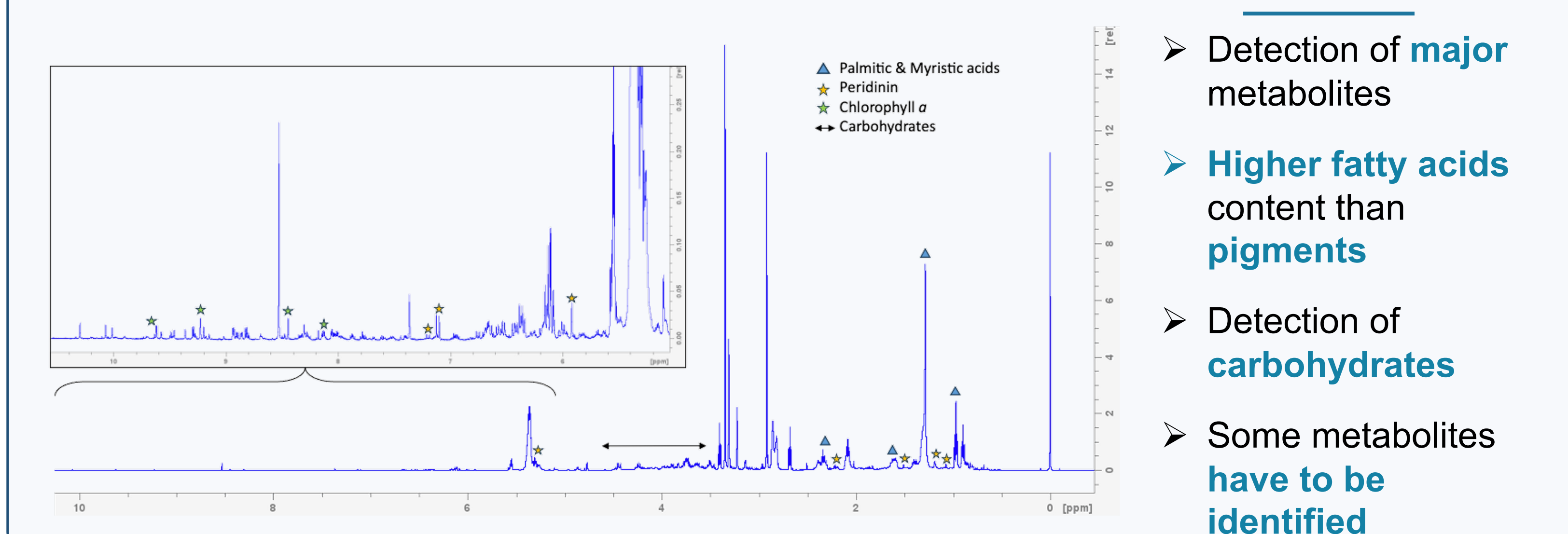
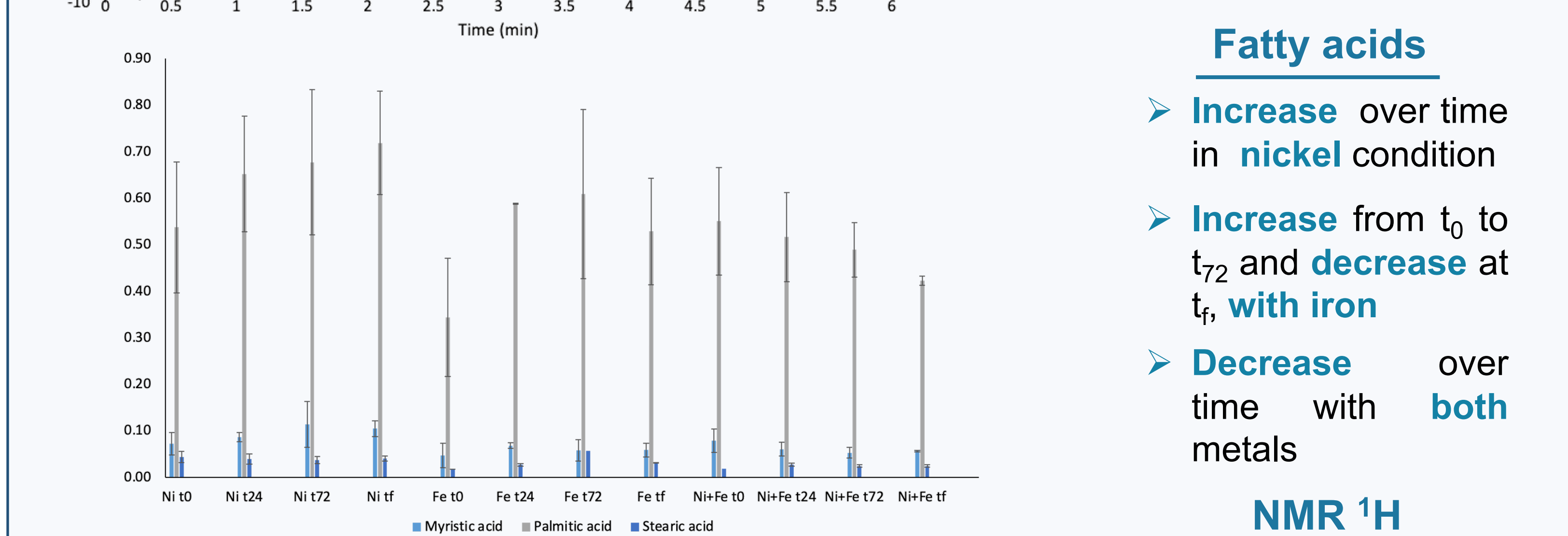
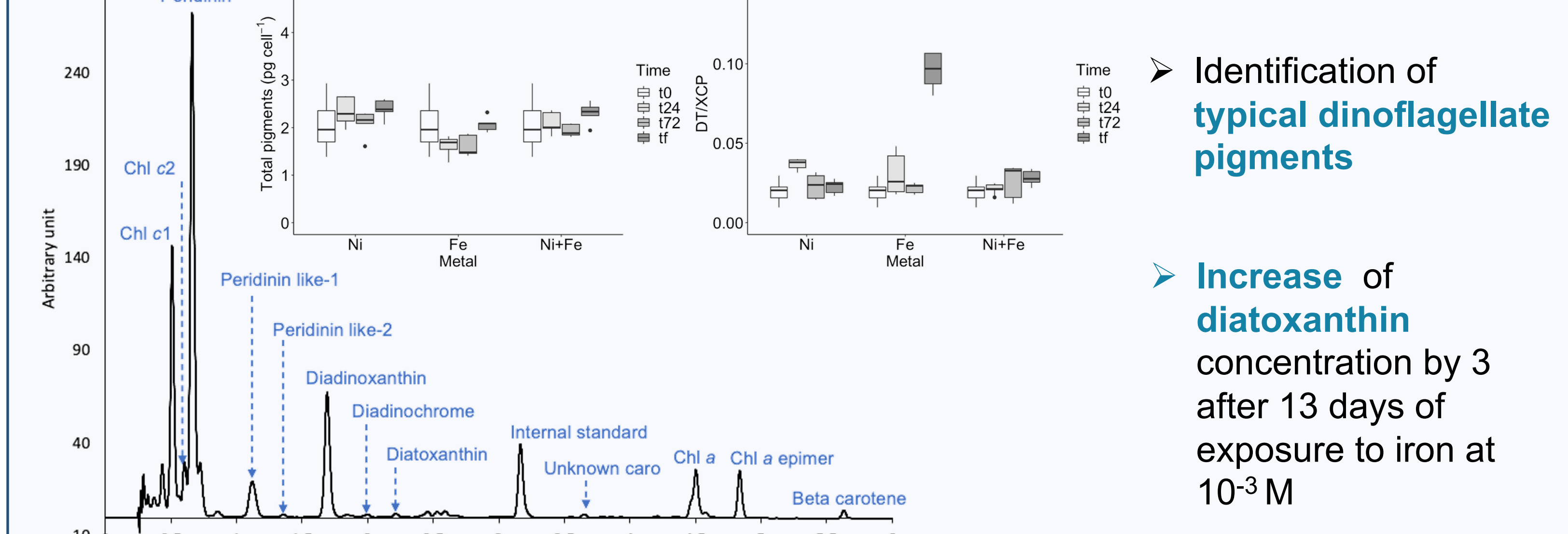
- **Decrease** of quantum efficiency parameters after 13 days with iron
- **Absorption, trapping and dissipation** were **higher** with addition of iron (concentration dependant) after 13 days

#### Bioaccumulation capacity

- **Higher** accumulation of the specific metal when added at high concentration in the culture



### Pigment analyses



➢ Identification of **typical dinoflagellate pigments**

➢ **Increase** of diatoxanthin concentration by 3 after 13 days of exposure to iron at  $10^{-3}$  M

#### Fatty acids

- **Increase** over time in **nickel** condition
- **Increase** from  $t_0$  to  $t_{72}$  and **decrease** at  $t_f$  **with iron**
- **Decrease** over time with **both** metals

#### NMR 1H

- Detection of **major metabolites**
- **Higher fatty acids** content than **pigments**
- Detection of **carbohydrates**
- Some metabolites **have to be identified**

## Discussion

### Photosynthetic efficiency

- *H. cf. bohaiensis* is **tolerant** to **nickel** at high concentrations
- In Iron conditions:**
  - **Fv/Fm** - *H. cf. bohaiensis* is impacted after a long term exposure
  - **RLC** - Iron reduced the ability to use light
  - **OJIP** - Iron stimulates capture and absorption of light energy & slows down the electron transport rate between photosystem II & I
  - **Increase of specific fluxes** (absorption, trapping and dissipation)

### Pigment analyses

- Increase of **diatoxanthin** in iron condition
- Responsible of **dissipation increase**
- **Photosystem protection** against iron induced ROS (Huang et al. 2017)

### Fatty acids

- Majority of 3 fatty acids more or less affected by the presence of metals
- NMR 1H**
  - Majority of **fatty acids**
  - Detection of **other metabolites** than in targeted methods

### Bioaccumulation

- Greater bioaccumulation of iron than nickel
- **Transfer in the food chain** (Hédouin et al. 2010)

## Conclusion

**Aim: Study the impact of nickel & iron on the photosystem and metabolome**

- ✓ **Toxic** effect of iron dependant of the concentration **on the photosystem**
  - ✓ Iron stress **adaptive strategies** to protect against ROS
  - ✓ Risk of **metal bioaccumulation** in the food chain
- ✓ Identification of specific metabolites by **targeted metabolomics**
- ✓ NMR results show metabolites previously metabolites and new ones
- ❑ **Untargeted metabolomic data** have to be statically analysed to evaluate the metabolome response to metals (NMR & UPLC-HRMS)

## References

Biscéré et al. (2017) Nickel and ocean warming affect scleractinian coral growth.  
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## Acknowledgments

The authors acknowledge the Province Nord, the Province SUD, the Government of New Caledonia, the Comité Interministériel de l'Outre-Mer (CIOM) and the CRESICA for financial support through the AMICAL and MICROCOMET projects.

