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Mélissa Eon, Mariem Fadhlou, Soizic Morin, Nicolas Creusot, Nicolas
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► To cite this version:

Mélissa Eon, Mariem Fadhlou, Soizic Morin, Nicolas Creusot, Nicolas Creusot. Combined effect of time, temperature and light intensity on the metabolome of the model green microalgae (*Scenedesmus costatus*) in the global change context. JS RFMF 2023, May 2023, Perpignan, France. hal-04172925

HAL Id: hal-04172925

<https://hal.science/hal-04172925>

Submitted on 28 Jul 2023

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Combined effect of time, temperature and light intensity on the metabolome of the model green microalgae (*Scenedesmus costatus*) in the global change context

Mélissa EON 1,2
Mariem FADHLAOUI 3
Soizic MORIN 1
Nicolas CREUSOT 1,2

1 INRAE Nouvelle-Aquitaine Bordeaux, UR EABX, 50 avenue de Verdun, 33612 Cestas, France

2 Bordeaux Metabolome, MetaboHUB

3 INRS-ETE, 490 rue de la Couronne, Québec, QC G1K 9A9, Canada

Introduction

- Aquatic ecosystems undergo an increase of temperature and light intensity that can affect their functioning [1]
- Increasing evidence about the effect of light and temperature on the physiology of micro-algae that play a key role in the aquatic trophic chain of both freshwater and marine ecosystems [2].
- Need to gain understanding of the molecular/biochemical mechanisms underlying the acclimation and adaptation of these organisms towards their resilience in a multi-stress environment
- **Untargeted metabolomics is a cutting-edge approach to provide a comprehensive picture of the molecular phenotype in response to changing environmental conditions [3]**

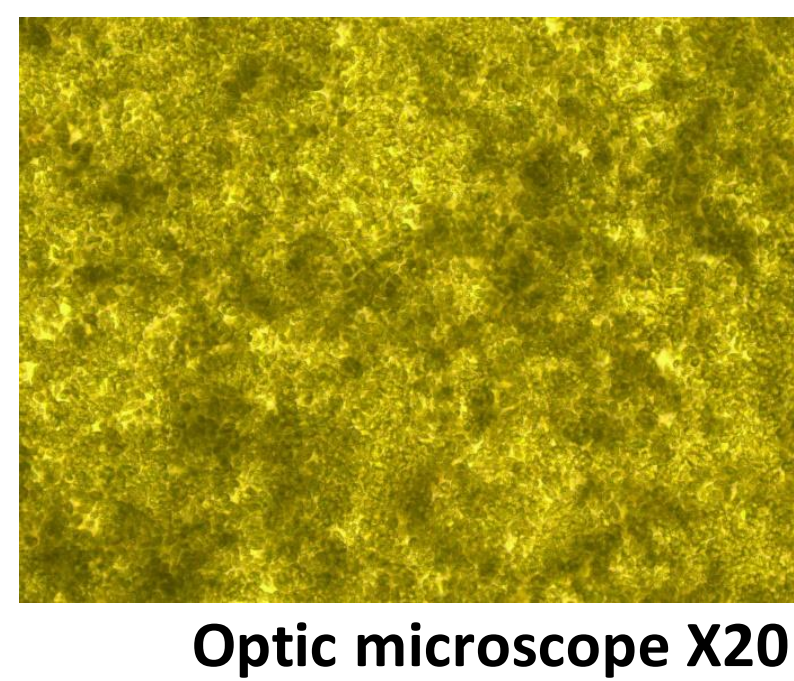
Aim: To unravel the molecular/biochemical responses of *Scenedesmus costatus* to increasing temperature and light intensity and their combination along the time through the implementation of untargeted metabolomics

Materials & Methods

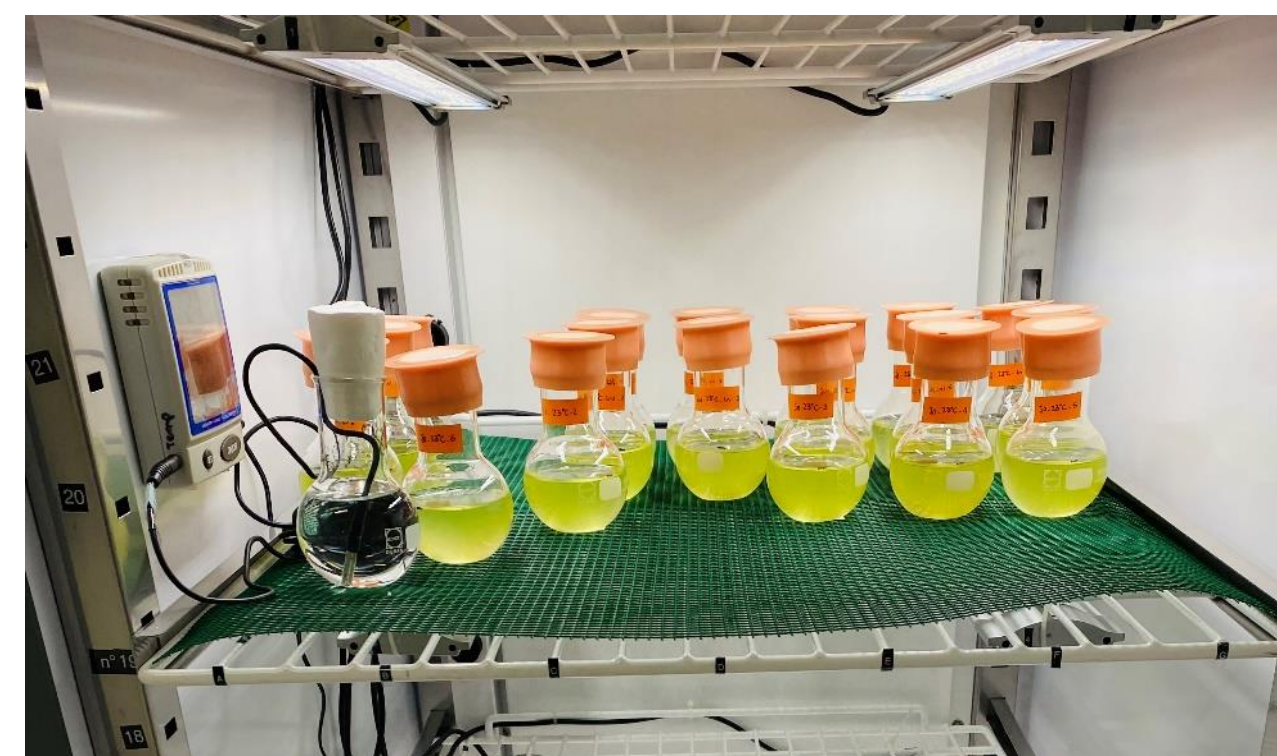
Biological model

freshwater chlorophyte

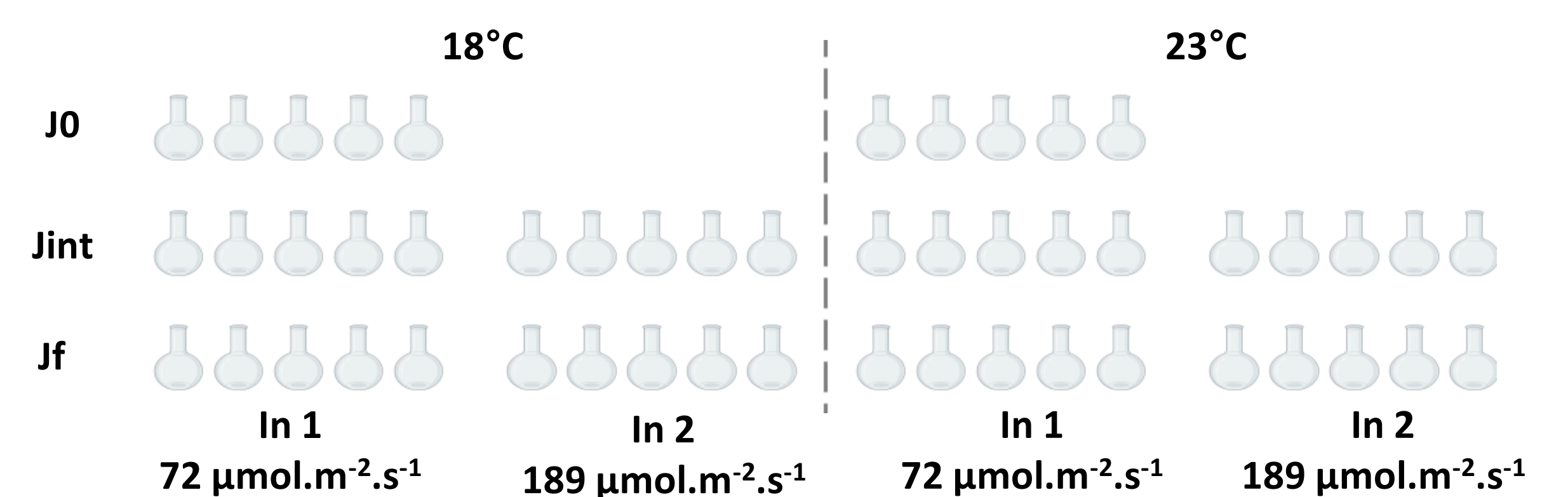
Scenedesmus costatus



Optic microscope X20



Experimental design: 2 T°C vs 2 Light intensity (In) at 3 times

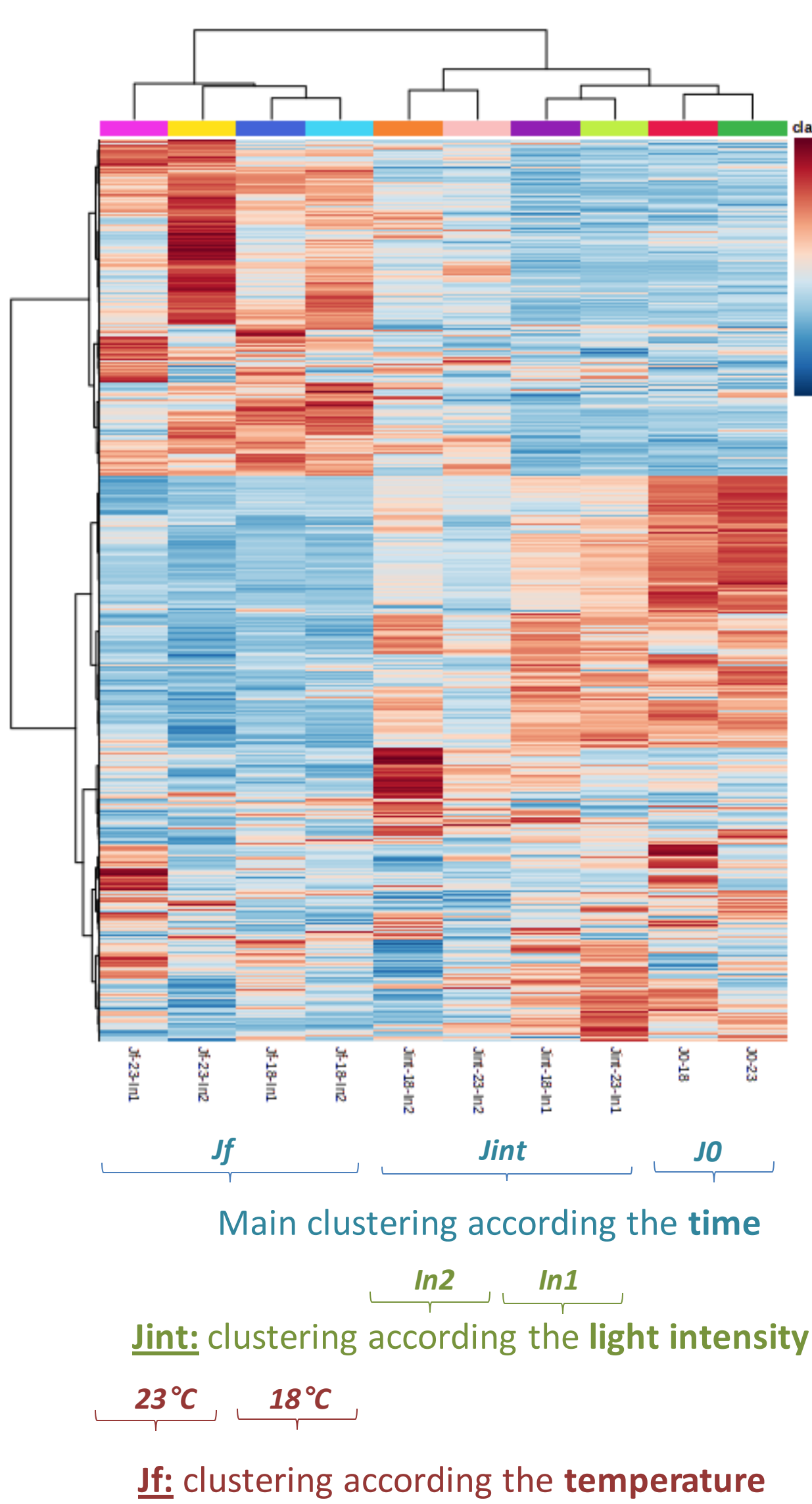


Metabolomics workflow

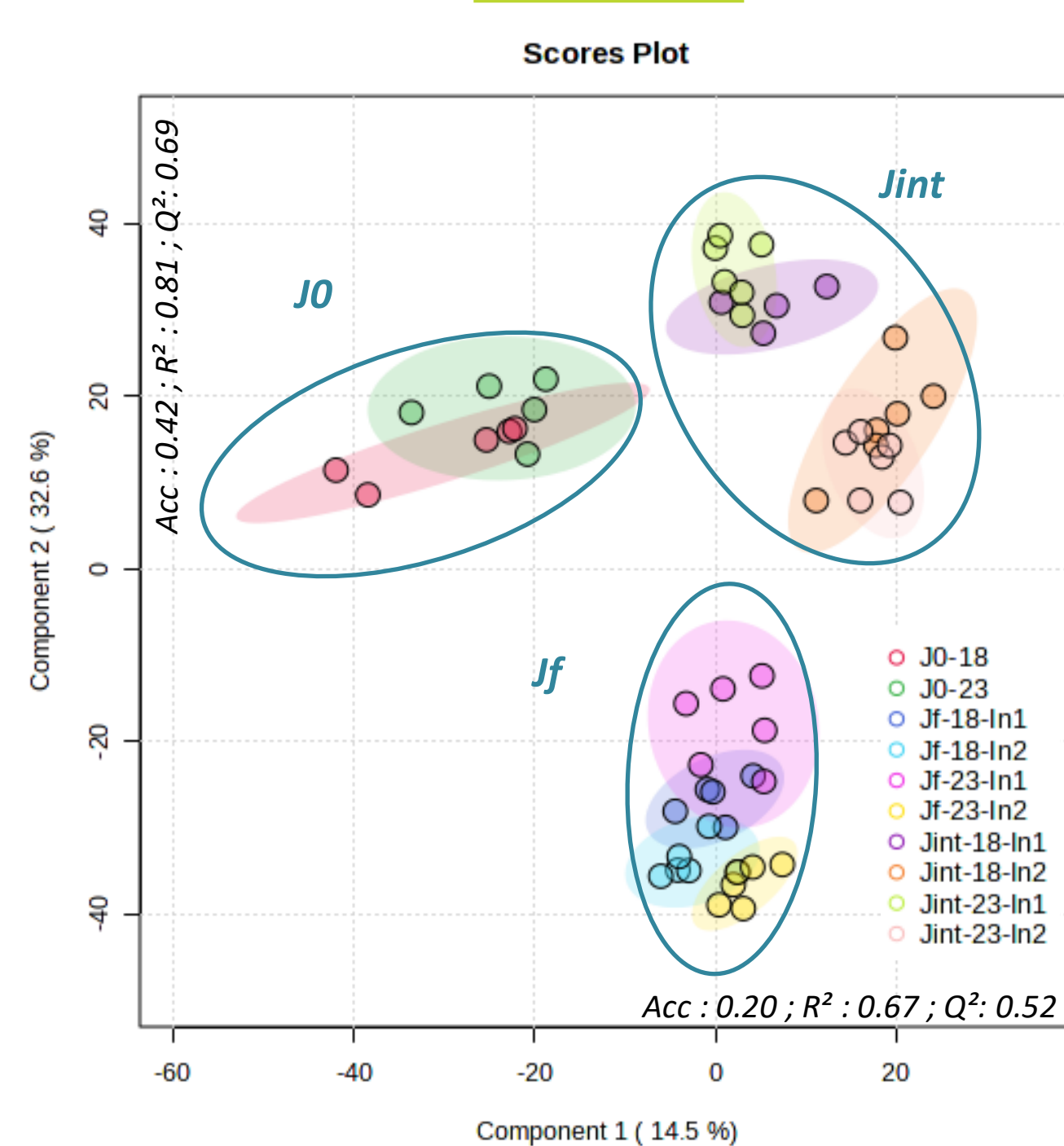


Results

HCA*



PLS-DA*



Discrimination mainly according to the time

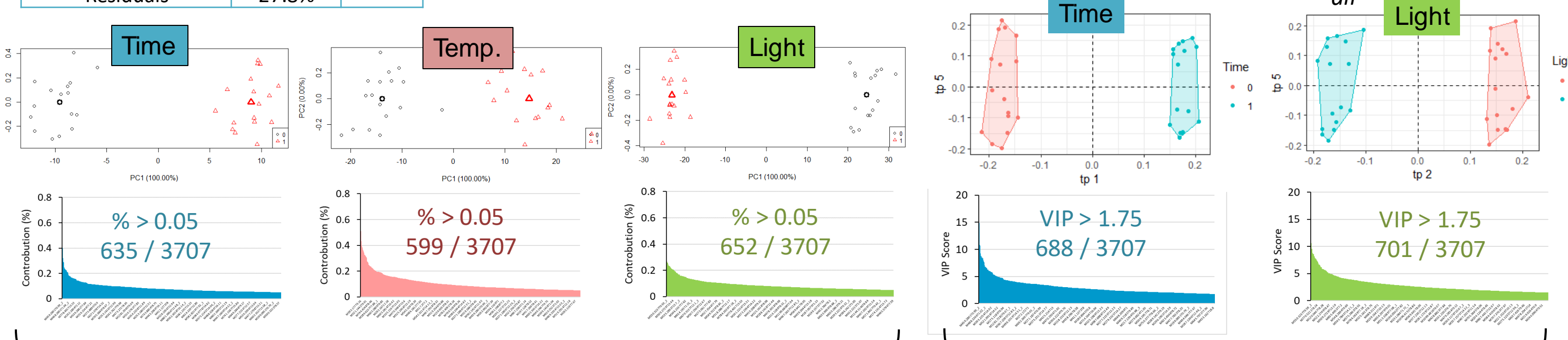
Difficulty to isolate the most contributing features to the discrimination of the various factors

ANOVA-simultaneous component analysis (ASCA) vs Analysis of Variance Multiblock Orthogonal Partial Least Squares (AMOPLS) [4]

ASCA*

Factor	% contrib.	p-val
Time	37.3%	0.001
Temp	5.2%	0.001
Light	13.7%	0.001
Time x Temp	4.5%	0.138
Time x Light	6.1%	0.077
Temp x Light	2.9%	0.115
Time x Temps x Light	2.50%	0.551
Residuals	27.8%	

- ✓ Significant effect of time > light > temperature
- ✓ No significant combined effect



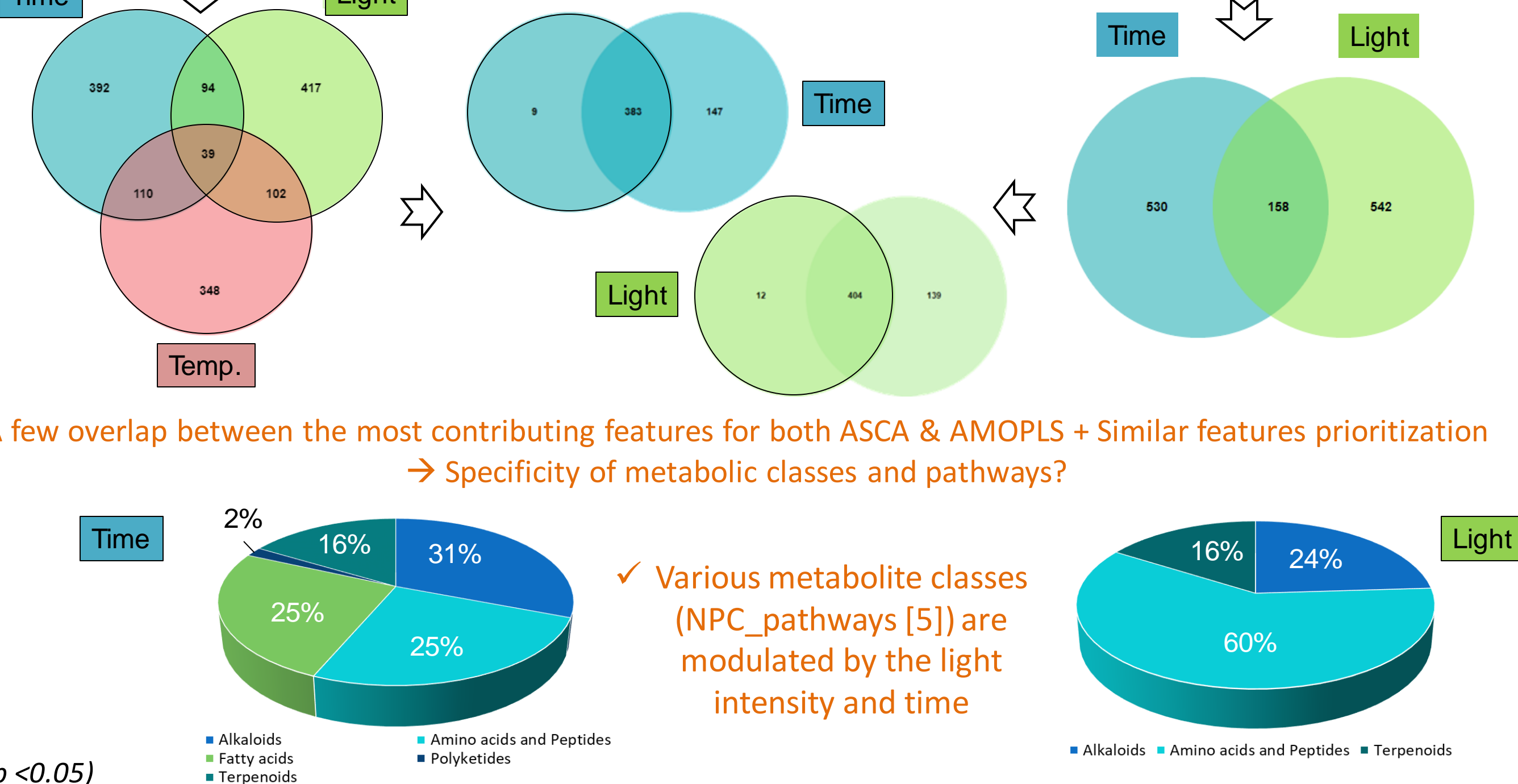
AMOPLS*

Factor	RSS ^a	p-val	RSR ^b	p-val
Time	0.373	0.01	5.175	0.01
Temp	0.052	1.00	1.240	1.00
Light	0.137	1.00	2.273	0.05
Time x Temp	0.045	1.00	1.213	1.00
Time x Light	0.061	1.00	1.350	1.00
Temp x Light	0.029	1.00	1.104	1.00
Residuals	0.303	NA	1.000	NA

- ✓ Significant effect of time > light
- ✓ No significant combined effect

^aRelative Sum of Squares (RSS), ^bResidual Structure Ratio (RSR). Permutation = 100, R²Yp-val = 0.01 for all

ASCA vs AMOPLS



A few overlap between the most contributing features for both ASCA & AMOPLS + Similar features prioritization → Specificity of metabolic classes and pathways?

- ✓ Various metabolite classes (NPC_pathways [5]) are modulated by the light intensity and time

Conclusions

- ✓ Strong effect of the time on the metabolome de *Scenedesmus costatus* compared to light intensity and temperature
- ✓ No significant combined effect of time, light intensity and temperature
- ✓ ASCA overestimates the significance of the temperature effect
- ✓ ASCA & AMOPLS prioritize the same features
- ✓ The most contributing features are factor specific:
 - Metabolite Class specific → Functional pathways specificity?
 - Relevant potential biomarkers ?

Next step :

- Identity of the metabolites and associated pathways?
- Natural fluctuation of *S. costatus* metabolome along its life cycle? Consequence on its « sensibility » to environmental stressors?
- Actual influence of temperature vs light following acclimation?

References :

- [1] Cavicchioli, R., Ripple, W.J., Timmis, K.N. et al. (2019) Scientists' warning to humanity: microorganisms and climate change. *Nat Rev Microbiol* 17, 569–586. <https://doi.org/10.1038/s41579-019-0222-5>
- [2] John Beardall & John A. Raven (2004) The potential effects of global climate change on microalgal photosynthesis, growth and ecology, *Phycologia*, 43:1, 26-40, DOI: [10.2216/00031-8884-43-1-26-1](https://doi.org/10.2216/00031-8884-43-1-26-1)
- [3] Bedia et al. (2018) Applications of Metabolomics Analysis in Environmental Research in *Comprehensive Analytical Chemistry Volume 82*, 2018, <https://doi.org/10.1016/bs.coac.2018.07.006>
- [4] Boccad J, Rudaz S. (2016) Exploring Omics data from designed experiments using analysis of variance multiblock Orthogonal Partial Least Squares. *Anal Chim Acta*. DOI: [10.1016/j.aca.2016.03.042](https://doi.org/10.1016/j.aca.2016.03.042)
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Acknowledgments: ISC XPO ; MetaboHUB (ANR-11-INBS-0010)

