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# Metabolic interactions between algae and bacteria in changing conditions

Simon M. Dittami

► **To cite this version:**

Simon M. Dittami. Metabolic interactions between algae and bacteria in changing conditions. Biodiversity and Ecology. Sorbonne Université, 2022. tel-04093436

**HAL Id: tel-04093436**

**<https://hal.science/tel-04093436>**

Submitted on 10 May 2023

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Station Biologique de Roscoff  
UMR 8227 - Integrative Biology of Marine Models  
Algal Biology and Interactions with the Environment team



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Mémoire d'habilitation à diriger des recherches

## **Metabolic interactions between algae and bacteria in changing conditions**

**Simon M. Dittami**

Soutenu 2022-01-27

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## Summary / Resumé

**English:** Brown algae form key marine ecosystems and live in tight relationships with microbes essential to their growth and development. Environmental changes can cause imbalances in these biological systems, leading to shifts in bacterial communities and sometimes giving rise to pathogenic interactions. Hence, considering both the algae and their microbiome (*i.e.* the holobiont) is essential to better understand their reactions to environmental challenges. In this memoir, I will outline the research I have conducted over the past nine years to explore this topic and highlight some of my ideas and challenges for the future. I will first touch on my work establishing the small filamentous brown alga *Ectocarpus* and in particular, a freshwater strain of the species *Ectocarpus subulatus* that we have shown to depend on a specific microbiome for freshwater tolerance, as a model system for algal-bacterial interactions. This includes the coordination of the sequencing and annotation of the *E. subulatus* genome, the characterization of its microbiome both in the field and in laboratory samples, and the establishment of a collection of cultivated bacteria representative of its microbiome now comprising ca. 400 strains from nearly 100 different species or genera, many of which have been genome-sequenced. Using multi-omics analyses we have furthermore put forward new hypotheses on the role of bacteria during the algal stress response, notably, the continued provision of bacterial services, most importantly vitamin K production, and the induction of quorum sensing pathways (potentially indicative of a change in the bacterial lifestyle towards dysbiosis). To identify key services provided to the algae by bacteria without strong a priori assumptions, we have, in collaboration with Anne Siegel's team from the IRISA Rennes, turned to metabolic complementarity as a predictor for potential mutualistic exchanges. The underlying idea is that beneficial metabolic exchanges between symbionts most likely occur where metabolic functions have been lost by one of the symbiotic partners, or vice versa functions may be lost once they have been replaced by external sources. Such points of intersection can be identified *in silico* using genome-scale metabolic networks. We have recently been able to prove the utility of this concept to predict beneficial metabolic interactions as well as the metabolic capacities of simplified holobiont systems *in vitro*, *i.e.* the algal host in co-culture with selected bacterial strains. Parallel to this work on *Ectocarpus*, we are starting to expand our research to the economically and ecologically more relevant kelp species *Saccharina latissima*. In this context, we have recently characterized the microbiome associated with both healthy and diseased *S. latissima* from different seasons and regions and generated a large collection of cultivated *Saccharina*-derived bacterial strains. These form a solid basis for both more targeted work with this model, such as more detailed studies on the role of Quorum Sensing (QS) in governing algal-bacterial interactions. Most recently we also commenced work on the *S. latissima* virome. The most important challenges in my future research will be, on one hand, to improve the reproducibility of experiments and thus to better control model systems, and the possible use of reverse genetics to gain precise functional insights into specific interactions. In parallel, I intend to expand our view of brown algal holobionts beyond bacteria and lately viruses to include fungi. Lastly, I hope to link work carried out in the laboratory to its potential applications, e.g. by assessing the impact of the microbiome on the defense-stimulating effect of *Ascophyllum nodosum* extracts in terrestrial plants.

**Français:** Les algues brunes sont des espèces clés dans les écosystèmes marins. Elles vivent en relation étroite avec des microbes essentiels à leur croissance et à leur développement. Les changements environnementaux peuvent provoquer un déséquilibre dans ces systèmes, entraînant la modification des communautés bactériennes et donnant parfois lieu à des interactions pathogéniques. Il est donc essentiel de considérer les algues et leur microbiome comme un ensemble (c'est-à-dire l'holobionte) pour mieux comprendre leurs réponses aux changements environnementaux. Dans ce mémoire, je décrirai mes recherches menées au cours des neuf dernières années pour explorer ce sujet et j'évoquerai mes idées ainsi que les défis clefs pour l'avenir. Je commencerai par mes travaux visant à faire de la petite algue brune filamenteuse *Ectocarpus* un système modèle pour les interactions algues-bactéries, et en particulier d'une souche de l'espèce *Ectocarpus subulatus*, dont nous avons montré qu'elle dépendait d'un microbiome spécifique pour sa tolérance à l'eau douce. Cela comprend la coordination du séquençage du génome d'*E. subulatus*, la caractérisation de son microbiome à la fois sur le terrain et dans des échantillons de laboratoire et l'établissement d'une collection de bactéries cultivées de son microbiome – à ce jour environ 400 souches de près de 100 espèces ou genres différents, dont beaucoup de génomes ont été séquencés. En parallèle, en utilisant des analyses multi-omiques, j'ai pu émettre de nouvelles hypothèses sur le rôle des bactéries pendant la réponse au stress de d'*E. subulatus*, notamment pour la production de vitamine K, mais également sur l'induction de voies de Quorum Sensing (QS), potentiellement à la base d'un changement du mode de vie bactérien vers une dysbiose. Pour identifier les services clés fournis aux algues par les bactéries sans *a priori* fort, nous avons utilisé, en collaboration avec l'équipe d'Anne Siegel de l'IRISA Rennes, la complémentarité métabolique comme prédicteur d'échanges mutualistes potentiels. L'idée est que les échanges métaboliques bénéfiques entre symbiotes se produisent probablement lorsque des fonctions métaboliques ont été perdues par l'un des partenaires symbiotiques, ou inversement des fonctions peuvent être perdues après avoir été remplacées par des sources externes. Ces points d'intersection peuvent être identifiés *in silico* grâce à des réseaux métaboliques. Nous avons récemment pu prouver l'utilité du concept de complémentarité métabolique pour prédire les interactions métaboliques bénéfiques ainsi que les capacités métaboliques de systèmes holobiontes simplifiés *in vitro*, c'est-à-dire l'hôte algal en co-culture avec des souches bactériennes sélectionnées. Parallèlement à ces travaux sur *Ectocarpus*, nous commençons à étendre nos recherches à l'espèce *Saccharina latissima*, qui est plus importante sur le plan économique et écologique. Dans ce contexte, nous avons récemment caractérisé le microbiome associé à des individus de *S. latissima* sains et malades, ainsi que provenant de différentes saisons et régions. Nous avons également généré une large collection de souches bactériennes isolées de *Saccharina*. Ces travaux constituent une base solide pour des travaux ciblés, tels que des études plus détaillées sur le rôle du QS dans la gestion des interactions algues-bactéries. Plus récemment, nous avons même commencé à travailler sur le virome de *S. latissima*. Les défis importants de mes futures recherches seront, d'une part, d'améliorer la reproductibilité des expériences et donc de mieux contrôler les systèmes modèles, y compris l'utilisation éventuelle de la génétique inverse pour obtenir des informations fonctionnelles précises sur des interactions spécifiques. En parallèle, j'ai l'intention d'élargir notre vision des holobiontes au-delà des bactéries et des virus pour inclure les champignons. Enfin, j'espère pouvoir relier mes recherches fondamentales à des applications potentielles, par exemple en évaluant l'impact du microbiome sur l'effet immunostimulant des extraits d'*Ascophyllum nodosum*.

## Curriculum vitae

Date of birth: 06.11.1978  
Place of birth: Munich, Germany  
Current occupation: CNRS researcher (CRCN)  
Present address: 321 Rue du Guillec, 29250 Plougoum  
Email: simon.dittami@sb-roscoff.fr

### Key scientific question:

How do algae react to, and interact with their abiotic and biotic environment, and in particular what is the role of host-microbe interactions during the processes of acclimation and adaptation?

### Science-related work experience:

2014-present            Researcher (CNRS), Laboratory of Integrative Marine Biology, Station Biologique de Roscoff  
2012-2014            Associate researcher (CNRS), Laboratory of Integrative Marine Biology, Station Biologique de Roscoff  
2010-2012            Post-doc position at the University of Oslo within the MIDTAL Project (<http://www.midtal.com>)  
2000 – 2005           Student assistant in the Zoology as well as Plant Physiology departments at the University of Kiel, Germany

### Education:

2006-2009            Ph.D. Thesis: “Abiotic Stress in *Ectocarpus siliculosus*: a global approach” at the Station Biologique de Roscoff, degree obtained from the University of Rennes 1, Date of diploma Feb 22<sup>nd</sup>, 2010  
2006                  Diploma in biology (Major: Ecology; Minors: Zoology and Computer Sciences, University of Kiel, Germany). Diploma thesis: “The influence of different culture conditions on the production of aldehydes by diatoms” at the Alfred Wegner Institute (Helgoland, Germany)  
2004                  Student exchange (Erasmus) at the Università della Calabria, Italy  
2000 – 2006           Student of biology at the University of Kiel, Germany

### Key skills:

Molecular biology:    Hands-on experience with many methods including nucleic acid extraction, quantitative RT-qPCR, microarrays, and sequencing  
Biochemistry:        Basic experience with spectrophotometry, liquid- (pigment analysis), and gas chromatography (fatty acid profiling)  
Culture work:         Culturing of micro- and macroalgae, measurements of quantum yield using PAM fluorometry; culturing of bacteria  
Statistics:             Standard parametric and non-parametric tests, R, analysis of large datasets - transcriptomic data, diversity indices

Bioinformatics:	Experience with automatic and manual genome annotation, BLASTs, phylogenetic analyses, transcript- and genome-assembly, probe design, and meta-barcoding, reconstruction and analysis of metabolic networks
Computer sciences:	Programming in (Bio)Java; basic knowledge of Pascal and Delphi; administration of Windows- and Unix-based systems
Management:	Course "De l'encadrement du projet doctoral au devenir professionnel du docteur", Sorbonne University, Paris, Dec 11-12 2018.
Language skills:	German (native), English (excellent), French (good), Italian, Norwegian, Latin (basic)

**Scientific output:** In total 53 publications in peer-reviewed journals or books, 32 as (co)-first or last author (H-index: 24 with Google), see detailed list in appendix 1.

**Conferences and Workshops:** I have been a co-organizer of the Marine Genomics Foresight workshop "Marine Holobionts - Concepts and challenges" (March 2018, Roscoff). I also regularly participate in national and international conferences such as the the European Phycological Congress (Zagreb, Croatia, Aug 2019, oral presentation), the International Seaweed Symposium (Jeju, Korea, April/May 2019, invited talk), or the International Phycological Conference (online only, March 2021, symposium organizer)

#### Recent teaching, student supervision, and other voluntary activities

- Teaching:
  - Part of "Projets de génomique marine" Master's course (2016-present, 4h/yr, lecture).
  - Part of the "Physiologie Moléculaire et Adaptations aux Stress" Master's course, University of Rennes 1, (2017-2019, 2h/yr, Lecture).
- (Co-)supervision of Master's and Bachelor's students:
  - Jamine Laugier (M2, 2021, main supervisor)
  - Bertille Burgunter-Delamare (M2, 2018, main supervisor):
  - Noe Pontoizeau (M2, 2015, co-supervisor with Erwan Corre and Thierry Tonon, 33%)
  - Enora Geslain (M1, 2019, co-supervisor with Erwan Corre and Elham Karimi, 33%)
  - Nahema Hecht (L3, 2020, co-supervisor with Aga Lipinska and Olivier Godfroy, 33%)
  - Ulysse Flandrian (L3, 2019, co-supervisor with Bertille Burgunter-Delamare and Sylvie Rousvoal, 33%)
  - Maeva Harrivel et Lolita Lecompte (Projet Tuteuré, L1, 2014, main supervisor).
- (Co-)supervision of two theses (60%) together with Catherine Boyen (40%):
  - Hetty KleinJan (2016-2018), "The influence of bacteria on the adaptation to changing environments in *Ectocarpus*: a systems biology approach", successful defense Sept. 24<sup>th</sup>, 2018.
  - Bertille Burgunter-Delamare (2018-2021) "Études des interactions hôte-microbiote chez l'algue brune *Saccharina latissima*".
- Participation in thesis committees:
  - Zujaila Nohemy Qui Minet (2014-2016, UMR7144, Station Biologique de Roscoff),
  - Charlotte Nef (2017-2019, Ifremer Nantes)
  - Mariana Camara dos Reis (2018-2020, UMR7144, Station Biologique de Roscoff).

- Member of the Thesis jury:
  - Srilakshmy Lakshminarayanapuram Hari Krishna, Ghent University, May 7<sup>th</sup> 2020.
- Co-responsible for the unit seminar series (2013-present)
- Participation in the organization of the transversal axis “Symbiosis” at the level of the Station Biologique de Roscoff.
- Participation in the organization of the transversal axis “Microbiology” within the UMR8227.
- Regular participation in the “Fete de la Science”.

### Current project involvement

- ANR project IDEALG (10MEur, 2011-2021, Co-responsible WP4: systems biology since 2015, PI Philippe Potin): Generating fundamental knowledge to support the growth and development of the French algae industry.
- ARC Project “Probiotics for the ocean” (2019-2022, 500 KAud, Partner, PI Thorsten Thomas, University of New South Wales): use of bacteria to improve macroalgal growth and health.
- ARED Project HOSALA, Brittany Region (2018-2021, 48 kEur, Co-PI, Co-supervision of the thesis of Bertille Burgunter-Delamare): “Host-microbe interactions in the kelp *Saccharina latissima*”.
- France Genomics sequencing project PHAEOEPLORER (2018, in-kind contribution only, PI Mark Cock, project participation with a focus on freshwater adaptation): Exploring brown algal genomic diversity
- Moore foundation aquatic symbiosis call (525 kEur, 2020-2023, partner working on bacteria associated with *Ectocarpus*, PI Suhelen Egan, UNSW;): development of model systems to study alga-symbiont interactions.
- ANR project “SEABIOZ” (500 kEur, 2021-2025, participation in WP1 and WP2, PI Philippe Potin,) - ANR (PCRE call): the impact of the microbiome on the production of bioactive compounds in seaweed extracts
- HIGHNESS – intra unit call for collaborations (5kEur, co-PI with Agnieszka Lipinska and Olivier Godfroy), Hi-C assembly for the genome of *Ectocarpus subulatus*.
- VIRALG – Conseil Departemental 29 (30 kEur, 2020-21, PI): The virome of *Saccharina latissima*.

### Previous projects

- ITN Alff (3.7 MEur, 2015-2018, participation and co-supervision of one early-stage researcher, PI Claire Gachon)
- Intra unit project “Revisiting *Ectocarpus subulatus* from Hopkins River falls” (3 kEur, 2016-2017, PI): sampling campaign for *Ectocarpus* in Hopkins River, Victoria Australia.
- CNRS momentum project (160MEur, 2017-2020, PI): Algal bacterial metabolic complementation and impact of abiotic stressors. Supervision of post-doctoral researcher Elham KARIMI (2018-2020)

**Reviewing:** I have peer-reviewed articles for a range of journals and have furthermore evaluated research projects for the ANR, the Czech Science Foundation, the DFG (Germany), the FWO (Belgium), and the FONDECYT (Chile).

Roscoff, August 27<sup>th</sup>, 2021



## Complete list of publications

### Preprints:

52. KleinJan H.<sup>\*</sup>, Caliafano G, Aite M, ..., **Dittami SM.** 2021. Changing microbial activities during low salinity acclimation in the brown alga *Ectocarpus subulatus*. bioRxiv 2021.04.13.

### Peer-reviewed articles:

51. Robla, J., García-Hierro, J., Alguacil, F., **Dittami, S.M.**, Marie, D., Villa, E., et al. (2021) Determination of the Efficiency of Filtration of Cultures from Microalgae and Bacteria using Hollow Fiber Filters. *Environ Sci Water Res Technol.* 7: 1230-1239

50. Girard, J., Lanneau, G., Delage, L., Leroux, C., Belcour, A., Got, J., **Dittami, S.M.**, ... (2021) Semi-Quantitative Targeted Gas Chromatography-Mass Spectrometry Profiling Supports a Late Side-Chain Reductase Cycloartenol-to-Cholesterol Biosynthesis Pathway in Brown Algae. *Front Plant Sci* 12: 648426.

49. Karimi, E.\*<sup>\*</sup>, Geslain, E., Belcour, A., Frioux, C., Aite, M., Siegel, A., Corre, C., **Dittami, S.M.** \*(2021) Robustness analysis of metabolic predictions in algal microbial communities based on different annotation pipelines. *PeerJ* 9: e11344.

48. Burgunter-Delamare, B.<sup>\*</sup>, Boyen, C. & **Dittami, S.M.**<sup>\*</sup> 2021. Effect of essential oil- and iodine treatments on the bacterial microbiota of the brown alga *Ectocarpus siliculosus*. *J. Appl. Phycol.* 33: 459-470.

47. Burgunter-Delamare, B., KleinJan, H., Frioux, C., Fremy, E., Wagner, M., Corre, E., Le Salver, A., Leroux, C., Leblanc, C., Boyen, C., Siegel, A. & **Dittami, S.M.** 2020\*. Metabolic Complementarity Between a Brown Alga and Associated Cultivable Bacteria Provide Indications of Beneficial Interactions. *Frontiers in Marine Sciences* 7:813686

46. Frioux, C., **Dittami, S.M.** & Siegel, A. 2020. Using automated reasoning to explore the metabolism of unconventional organisms: a first step to explore host–microbial interactions. *Biochem. Soc. Trans.* 48:901

45. **Dittami, S.M.**<sup>\*</sup>, Arboleda, E., Auguet, J.-C., Bigalke, A., Briand, E., Cardenas, P., Cardini, U., Decelle, J., Engelen, A.H., Eveillard, D., Gachon, C.M.M., Griffiths, S.M., Harder, T., Kayal, E., Kazamia, E., Lallier, F.H., Medina, M., Marzinelli, E.M., Morganti, T., et al. 2020, 2021. A community perspective on the concept of marine holobionts: current status, challenges, and future directions. Peer-reviewed and recommended by *PCI Ecology*. *Zenodo*:3696771. *PeerJ* 9: e10911.

44. **Dittami, S.M.**<sup>\*</sup>, Corre, E., Brillet-Gueguen, L., Lipinska, A.P., Pontoizeau, N., Aite, M., Avia, K., Caron, C., Cho, C.H., Collen, J., Cormier, A., Delage, L., Doubleau, S., Frioux, C., Gobet, A., Gonzalez-Navarrete, I., Groisillier, A., Herve, C., Jollivet, D. et al. 2020. The genome of *Ectocarpus subulatus* - a highly stress-tolerant brown alga. *Mar. Genomics.* 52:100740.

44. Belcour, A., Girard, J., Aite, M., Delage, L., Trottier, C., Marteau, C., Leroux, C., **Dittami, S.M.**, Sauleau, P., Corre, E., Nicolas, J., Boyen, C., Leblanc, C., Collén, J., Siegel, A. & Markov, G. V. 2020. Inferring biochemical reactions and metabolite structures to understand metabolic pathway drift. *iScience.* 23:100849

43. **Dittami, S.M.**<sup>\*</sup>, Peters, A.F., West, J., Cariou, T., KleinJan, H., Burgunter-Delamare, B., Prechoux, A., Egan, S. & Boyen, C. 2020. Revisiting Australian *Ectocarpus subulatus* (Phaeophyceae) from the Hopkins River: distribution, abiotic environment, and associated microbiota. *J. Phycol.* 56:719

42. Karimi, E.\*<sup>\*</sup>, Geslain, E., KleinJan, H., Tanguy, G., Legeay, E., Corre, E. & **Dittami, S.M.**<sup>\*</sup>. 2020. Genome sequences of 72 bacterial strains isolated from *Ectocarpus subulatus*: a resource for algal microbiology. *Genome Biol. Evol.* 12:3647–55.

41. Thomas, F., **Dittami, S.M.**, Brunet, M., Le Duff, N., Tanguy, G., Leblanc, C. & Gobet, A. 2020. Evaluation of a new primer combination to minimize plastid contamination in 16S rDNA metabarcoding analyses of alga-associated bacterial communities. *Environ. Microbiol. Rep.* 12:30.

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\* Students directly supervised by me are underlined

40. Nègre, D., Aite, M., Belcour, A., Frioux, C., Brillet-Guéguen, L., Liu, X., Bordron, P., Godfroy, O., Lipinska, A.P., Leblanc, C., Siegel, A., **Dittami, S.M.**, Corre, E. & Markov, G. V. 2019. Genome-scale metabolic networks shed light on the carotenoid biosynthesis pathway in the brown algae *Saccharina japonica* and *Cladosiphon okamuranus*. *Antioxidants*, 8:564.
39. Gobet, A., Mest, L., Perennou, M., **Dittami, S.M.**, et al. (2018). Seasonal and algal diet-driven patterns of the digestive microbiota of the European abalone *Haliotis tuberculata*, a generalist marine herbivore. *Microbiome*, 6:60.
38. KleinJan, H.\*, Jeanthon, C., Boyen, C., & **Dittami, S. M.\*** (2017). Exploring the cultivable *Ectocarpus* microbiome. *Frontiers in Microbiology*, 8:2456.
37. Teng, L., Han, W., Fan, X., Xu, D., Zhang, X., **Dittami, S. M.**, & Ye, N. (2017). Evolution and Expansion of the Prokaryote-Like Lipoxxygenase Family in the Brown Alga *Saccharina japonica*. *Frontiers in Plant Science*, 8:2018.
36. **Dittami, S. M.\***, & Corre, E. (2017). Detection of bacterial contaminants and hybrid sequences in the genome of the kelp *Saccharina japonica* using Taxoblast. *PeerJ*, 5:e4073.
35. Brawley, S. H., Blouin, N. A., Ficko-Blean, E., Wheeler, G. L., Lohr, M., Goodson, H. V., ..., **Dittami, S.M.**, ... Prochnik, S. E. (2017). Insights into the red algae and eukaryotic evolution from the genome of *Porphyra umbilicalis* (Bangiophyceae, Rhodophyta). *Proceedings of the National Academy of Sciences*, 114(31):E6361–E6370.
34. **Dittami, S. M.\***, Heesch, S., Olsen, J. L., & Collén, J. (2017). Transitions between marine and freshwater environments provide new clues about the origins of multicellular plants and algae. *Journal of Phycology*, 5:731.
33. Prigent, S., Frioux, C., **Dittami, S. M.**, Thiele, S., Larhlimi, A., Collet, G., ... Siegel, A. (2017). Meneco, a Topology-Based Gap-Filling Tool Applicable to Degraded Genome-Wide Metabolic Networks. *PLOS Computational Biology*, 13(1):e1005276.
32. McNamee, S. E., Medlin, L. K., Kegel, J., McCoy, G. R., Raine, R., Barra, L.,... **Dittami, SM.**, ... Campbell, K. (2016). Distribution, occurrence, and biotoxin composition of the main shellfish toxin producing microalgae within European waters: A comparison of methods of analysis. *Harmful Algae*, 55,:112–120.
31. Olsen, J. L., Rouzé, P., Verhelst, B., Lin, Y.-C., Bayer, T., Collen, J., ..., **Dittami S.M.**, ... Van de Peer, Y. (2016). The genome of the seagrass *Zostera marina* reveals angiosperm adaptation to the sea. *Nature*, 530(7590): 331–335.
30. **Dittami, S. M.\***, Duboscq-Bidot, L., Perennou, M., Gobet, A., Corre, E., Boyen, C., & Tonon, T. (2016). Host–microbe interactions as a driver of acclimation to salinity gradients in brown algal cultures. *The ISME Journal*, 10(1):51–63.
29. Ahmed, S., Cock, J. M., Pessia, E., Luthringer, R., Cormier, A., Robuchon, M., ... Coelho, S. M. (2014). A Haploid System of Sex Determination in the Brown Alga *Ectocarpus* sp. *Current Biology* 24:1945
28. Prigent, S., Collet, G., **Dittami, S. M.**, Delage, L., Ethis de Corny, F., Dameron, O., ... Tonon, T. (2014). The genome-scale metabolic network of *Ectocarpus siliculosus* (EctoGEM): a resource to study brown algal physiology and beyond. *Plant Journal*, 80(2):367–381.
26. **Dittami, S. M.\***, Barbeyron, T., Boyen, C., Cambefort, J., Collet, G., Delage, L., ... Tonon, T.\* (2014). Genome and metabolic network of “*Candidatus Phaeomarinobacter ectocarpus*” Ec32, a new candidate genus of *Alphaproteobacteria* frequently associated with brown algae. *Frontiers in Genetics*, 5:241.
25. Ritter, A., **Dittami, S. M.**, Goulitquer, S., Correa, J. A., Boyen, C., Potin, P., & Tonon, T. (2014). Transcriptomic and metabolomic analysis of copper stress acclimation in *Ectocarpus siliculosus* highlights signaling and tolerance mechanisms in brown algae. *BMC Plant Biology*, 14(1), 116.
24. **Dittami, S. M.\***, Eveillard, D., & Tonon, T. (2014). A metabolic approach to study algal-bacterial interactions in changing environments. *Molecular Ecology*, 23(7), 1656–60.
23. Stüken, A., **Dittami, S. M.**, Eikrem, W., McNamee, S., Campbell, K., Jakobsen, K. S., & Edvardsen, B. (2013). Novel hydrolysis-probe-based qPCR assay to detect saxitoxin transcripts of dinoflagellates in environmental samples. *Harmful Algae*, 28, 108–117. <https://doi.org/10.1016/j.hal.2013.06.003>

22. Collén, J., Porcel, B., Carré, W., Ball, S. G., Chaparro, C., Tonon, T., ..., **Dittami, S.M.**... Boyen, C. (2013). Genome structure and metabolic features in the red seaweed *Chondrus crispus* shed light on evolution of the Archaeplastida. *Proceedings of the National Academy of Sciences of the United States of America*, 110(13), 5247–52.
21. Edvardsen, B., **Dittami, S. M.**, Groben, R., Brubak, S., Escalera, L., Rodríguez, F., ... Medlin, L. K. (2013). Molecular probes and microarrays for the detection of toxic algae in the genera *Dinophysis* and *Phalacroma* (Dinophyta). *Environmental Science and Pollution Research International*, 20(10), 6733–50.
20. **Dittami, S. M.\***, Riisberg, I., & Edvardsen, B. (2013). Molecular probes for the detection and identification of ichthyotoxic marine microalgae of the genus *Pseudochattonella* (Dictyochophyceae, Ochrophyta). *Environmental Science and Pollution Research International*, 20(10), 6824–37.
19. **Dittami, S. M.**, Pazos, Y., Laspra, M., & Medlin, L. K. (2013). Microarray testing for the presence of toxic algae monitoring program in Galicia (NW Spain). *Environmental Science and Pollution Research International*, 20(10), 6778–93.
18. **Dittami, S. M.\***, Hostyeva, V., Egge, E. S., Kegel, J. U., Eikrem, W., & Edvardsen, B. (2013). Seasonal dynamics of harmful algae in outer Oslofjorden monitored by microarray, qPCR, and microscopy. *Environmental Science and Pollution Research International*, 20(10), 6719–32.
17. **Dittami, S. M.**, & Edvardsen, B. (2013). GPR-Analyzer: a simple tool for quantitative analysis of hierarchical multispecies microarrays. *Environmental Science and Pollution Research International*, 20(10), 6808–15.
16. McCoy, G., Raine, R., Medlin, L., Chen, J., Kooistra, W., Barra, L., ... **Dittami, S.M.**, ... Pazos, Y. (2012). Field testing for toxic algae with a microarray: initial results from the MIDTAL project. In P. Pagou & G. Hallegraeff (Eds.), *Proceedings of the 14th International Conference on Harmful Algae. International Society for the Study of Harmful Algae and Intergovernmental Oceanographic Commission of UNESCO 2012* (pp. 209–211). UNESCO.
15. Cock, J. M., Sterck, L., Ahmed, S., Allen, A. E., Amoutzias, G., Anthouard, V., ... **Dittami, S.M.**, ... Wincker, P. (2012). Genomic Insights into the Biology of Algae. In *Advances in Botanical Research Vol 64: Genomic Insights into the Biology of Algae* (Vol. 64, pp. 141–184). Elsevier.
14. **Dittami, S. M.**, & Tonon, T. (2012). Genomes of extremophile crucifers: new platforms for comparative genomics and beyond. *Genome Biology*, 13(8), 166.
13. **Dittami, S. M.\***, & Edvardsen, B. (2012). Culture conditions influence cellular RNA content in ichthyotoxic flagellates of the genus *Pseudochattonella* (Dictyochophyceae). *Journal of Phycology*, 48, 1050–1055.
12. **Dittami, S. M.**, Gravot, A., Goulitquer, S., Rousvoal, S., Peters, A. F., Bouchereau, A., ... Tonon, T. (2012). Towards deciphering dynamic changes and evolutionary mechanisms involved in the adaptation to low salinities in *Ectocarpus* (brown algae). *Plant Journal*, 71(3), 366–377.
11. **Dittami, S. M.\***, Riisberg, I., John, U., Orr, R. J. S., Jakobsen, K. S., & Edvardsen, B. (2012). Analysis of expressed sequence tags from the marine microalga *Pseudochattonella farcimen* (Dictyochophyceae). *Protist*, 163(1), 143–161.
10. **Dittami, S. M.**, Aas, H. T. N., Paulsen, B. S., Boyen, C., Edvardsen, B., & Tonon, T. (2011). Mannitol in six autotrophic stramenopiles and *Micromonas*. *Plant Signaling & Behavior*, 6(8), 1237–9.
9. **Dittami, S. M.**, Proux, C., Rousvoal, S., Peters, A. F., Cock, J. M., Coppee, J.-Y., ... Tonon, T. (2011). Microarray estimation of genomic inter-strain variability in the genus *Ectocarpus* (Phaeophyceae). *BMC Molecular Biology*, 12(1), 2.
8. **Dittami, S. M.**, Gravot, A., Renault, D., Goulitquer, S., Eggert, A., Bouchereau, A., ... Tonon, T. (2011). Integrative analysis of metabolite and transcript abundance during the short-term response to saline and oxidative stress in the brown alga *Ectocarpus siliculosus*. *Plant, Cell & Environment*, 34(4), 629–642.
7. Rousvoal, S., Groisillier, A., **Dittami, S. M.**, Michel, G., Boyen, C., & Tonon, T. (2011). Mannitol-1-phosphate dehydrogenase activity in *Ectocarpus siliculosus*, a key role for mannitol synthesis in brown algae. *Planta*, 233(2), 261–73.
6. **Dittami, S. M.\***, Michel, G., Collén, J., Boyen, C., & Tonon, T.\* (2010). Chlorophyll-binding proteins revisited-- a multigenic family of light-harvesting and stress proteins from a brown algal perspective. *BMC Evolutionary Biology*, 10(1), 365.

5. Gravot, A.<sup>#</sup>, **Dittami, S. M.<sup>#</sup>**, Rousvoal, S., Lukan, R., Eggert, A., Collén, J., ... Tonon, T. (2010). Diurnal oscillations of metabolite abundances and gene analysis provide new insights into central metabolic processes of the brown alga *Ectocarpus siliculosus*. *New Phytologist*, 188(1):98–110. <sup>#</sup>equal contribution.
4. **Dittami, S. M.\***, Wichard, T., Malzahn, A. M., Pohnert, G., Boersma, M., & Wiltshire, K. H. (2010). Culture conditions affect fatty acid content along with wound-activated production of polyunsaturated aldehydes in *Thalassiosira rotula* (Coscinodiscophyceae). *Nova Hedwigia Beiheft, Suppl. 136*, 231–248.
3. Cock, J. M., Sterck, L., Rouzé, P., Scornet, D., Allen, A. E., Amoutzias, G., ... **Dittami, S.M.**, ..., Wincker, P. (2010). The *Ectocarpus* genome and the independent evolution of multicellularity in brown algae. *Nature*: 617–21.
2. **Dittami, S. M.\***, Scornet, D., Petit, J.-L., Ségurens, B., Da Silva, C., Corre, E., ... Tonon, T.\* (2009). Global expression analysis of the brown alga *Ectocarpus siliculosus* (Phaeophyceae) reveals large-scale reprogramming of the transcriptome in response to abiotic stress. *Genome Biology* 10:R66.
1. Le Bail<sup>#</sup>, A., **Dittami, S. M.<sup>#</sup>**, de Franco, P. O., Rousvoal, S., Cock, J. M., Tonon, T., & Charrier, B. (2008). Normalization genes for expression analyses in the brown alga model *Ectocarpus siliculosus*. *BMC Molecular Biology*, 9:75. <sup>#</sup>equal contribution.

**Book chapters:**

2. **Dittami, S. M.**, & Tonon, T. (2014). Gene mining for environmental studies and applications: examples from marine organisms. In S. La Barre & J.-M. Kornprobst (Eds.), *Outstanding Marine Molecules* (pp. 435–456). WILEY-BLACKWELL PUBLISHING.
1. **Dittami, S. M.**, & Edvardsen, B. (2012). Analysis of GPR files. In J. Lewis, L. K. Medlin, & R. Raine (Eds.), *MIDTAL (Microarrays of the Detection of Toxic Algae): A protocol for a successful microarray hybridisation and analysis* (pp. 260–270).

## 1. Overview of past research

### 1.1 Background

Brown algae are multicellular, mainly marine organisms that live along the coastlines of all continents, where they form the dominant vegetation in the intertidal zone and constitute important primary producers (Mann 1973). They comprise over 1,700 known species worldwide (<http://www.algaebase.org>), several of which are economically and ecologically important as they provide habitats for thousands of other species (Kelly 2005). Many brown algae are strongly impacted by human activities and climate change, underlining the importance of understanding how these organisms interact with their environment and tolerate changes. This is a question I started to address in 2006 with the start of my Ph.D. thesis on the small filamentous model alga *Ectocarpus siliculosus*. *E. siliculosus* had been chosen as a model for brown algae mainly because it is easy to culture and has a small genome while it is closely related to the economically and ecologically important kelps (*Laminariales*) (Peters *et al.* 2004). Its genome was sequenced by an international consortium comprising 34 laboratories under the leadership of the UMR 7139 at the Station Biologique de Roscoff (Cock *et al.* 2010 #3) and it is one of the models selected within the ESFRI project “European Marine Biological Resource Centre” (EMBRC), which aims to promote the development of new approaches and models to study the biology and ecology of marine organisms. Hence numerous molecular tools for *E. siliculosus* have been and are being developed. In this context, during my thesis, I put into place microarray approaches to study the response of the brown algal model species *Ectocarpus* to different stressors (Dittami *et al.* 2009 #2), studied its metabolism (Gravot *et al.* 2010 #5), its genome (Cock *et al.* 2010 #3), and I also tried to exploit the natural variability found within the genus *Ectocarpus* to answer questions of adaptation and acclimation to abiotic change (Dittami *et al.* 2011 #9; Dittami, Corre, *et al.* 2020 #44). In particular, the adaptation to low salinity caught my interest, as brown algae have probably originated in a marine environment, and unlike green plants, and to a lesser extent red algae, have rarely been able to colonize freshwater let alone terrestrial environments (Dittami *et al.* 2017 #34). One strain of the species *Ectocarpus subulatus*, has, however, recently made the transition to fresh water, and hence attracted my curiosity. It was, at the time, the only available freshwater isolate of *Ectocarpus* and originated from Hopkins River falls, Victoria, Australia, where it was isolated in 1995 (West and Kraft 1996). What I found particularly fascinating about this strain was that it was able to grow in both freshwater (Dittami *et al.* 2012 #12) and highly saline water, while the model strain of *E. siliculosus* already displayed strong signs of stress at a salinity of 6.4 ppt.

It was during my work with this strain of *E. subulatus* that, triggered by a small observation, I started to become interested in the alga-associated microbiome: in addition to profound changes in the transcriptome, the algal morphology, and the metabolome depending on salinity, I observed the appearance of a peak corresponding to sucrose specifically in high salinity conditions (Dittami *et al.* 2012 #12). Sucrose is known to function as an osmolyte in bacteria but had not been described in brown algae. Furthermore, I could not find the genes considered necessary for the synthesis of this compound in the algal genome. Although a bacterial origin of sucrose immediately came to mind, at that time the gold standard for experiments with algae was ‘axenic’ cultures, which were usually simply cultures submitted to treatments with antibiotics. ‘Axenicity’, was usually proven by plating of parts of the algae that did not result in the growth of any bacterial colonies. Questioning

this paradigm based on one single compound, that the time seemed like too much of a stretch – despite the studies of Pedersen from the 1960s and 70s demonstrating the dependence of *Ectocarpus* on bacteria-produced morphogens (Pedersén 1968, 1973).

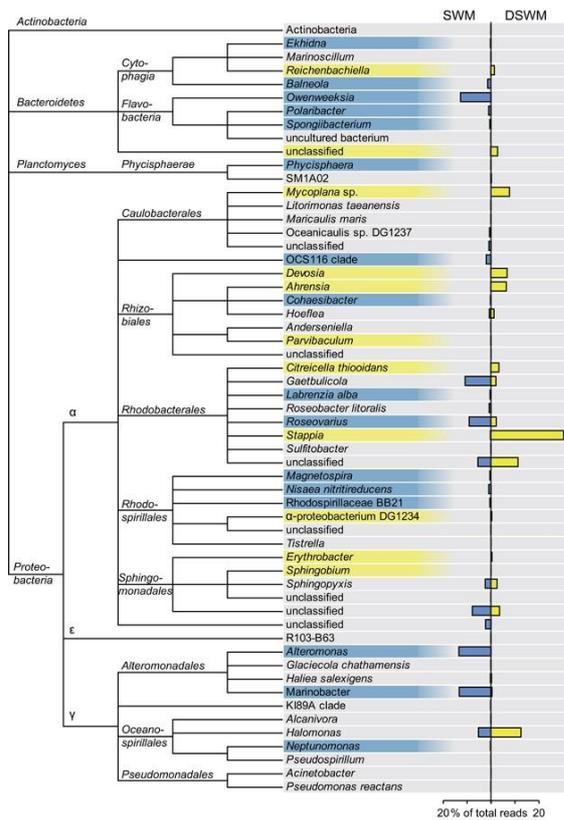
By the time I returned to Roscoff after a two-year post-doctoral position in Norway working on molecular methods to detect harmful algal blooms, this isolated view of macroorganisms was rapidly changing. Driven probably by the exponential increase in sequencing capacities and the concomitant increase in awareness of the omnipresence and diversity of microbial symbionts, several publications, including papers by Zilber-Rosenberg and Rosenberg (Zilber-Rosenberg and Rosenberg 2008; Rosenberg *et al.* 2010) had started to revive the view of organisms as “holobionts”, *i.e.* as an ecological unit of different species living together in symbiosis and this concept had also been extended to seaweeds (Wahl *et al.* 2012). Furthermore, it was becoming increasingly clear that this new understanding of organisms also impacted the way we needed to view their stress response and that even small imbalances in symbiotic relationships could have profound consequences for the meta-organisms. A prominent example of this is the red alga *Delisea pulchra* and the associated bacterium *Ruegeria* sp. R11. Using this model system Case *et al.* (2011) demonstrated that temperature changes may induce a shift from commensal to pathogenic interactions between the host and the bacterium depending on the production of algal defense compounds. Considering this gradual shift of awareness, the time seemed right to revisit the question of freshwater tolerance in *Ectocarpus*, this time tackling the question of host-microbiome interactions and their impact on the *Ectocarpus* stress tolerance. I started this work within the context of a two-year postdoctoral position at Roscoff and continued via my position as a CNRS researcher starting in 2014. In the following memoir, I will give an overview of my research in the last nine years, starting with my still ongoing work on the holobiont freshwater strain of *Ectocarpus subulatus* that had initially captured my interest and gradually moving to other more iconic models.

## 1.2. Fresh water tolerance in *E. subulatus* and the impact of bacteria.

One of the first experiments upon my return to Roscoff as a post-doctoral researcher was to attempt to eliminate microbial factors that could have biased my examination of the freshwater response of *E. subulatus* during my thesis. I did this by exposing the algal to standard antibiotic treatments in our laboratory (one-month exposure to 10 different antibiotics) before transferring it to various salinities. Primarily, I wanted to determine if this would result in the same profound effects on the algal transcriptome, metabolome including the sucrose peak, and morphology as observed during my thesis. To my surprise, *E. subulatus* entirely lost its ability to grow in low salinities even several months after the treatment. My first intuition was that this phenomenon likely was a direct long-term effect of the antibiotic treatment, but when inoculated with a small quantity of medium from a non-treated culture, presumably restoring its microbiome, treated cultures regained their capacity to grow in fresh water (Dittami *et al.* 2016 #30). Understanding this phenomenon became a driving question in my research. To address this question, I have commenced work in parallel on the algal host and on its associated microbiome. Both lines of work are described in more detail below, and although I still have not satisfactorily resolved this initial question, significant progress has been made towards a better understanding of this phenomenon.

**On the algal side**, a transcriptomic study I carried out during my thesis had already revealed profound transcriptomic changes during freshwater acclimation, but this transcriptomic plasticity was bound to have genetic bases, and thus it seemed essential to me to study its genome. Together with Thierry Tonon and in collaboration with the Center for Genomic Regulation (CRG, Barcelona, Spain) we therefore initiated the *E. subulatus* genome project in 2013. By comparing the genome of *E. subulatus* to that of its close relative *E. siliculosus* (Cock *et al.* 2010 #3), which is unable to grow in freshwater, I was hoping to identify candidate genes and processes that may be related to the fresh water tolerance of *E. subulatus*, but also form a basis for further research to be carried out in this latter species. First, only the freshwater strain of *E. subulatus* was sequenced, but as this strain was diploid and no longer reproduced sexually, we later also included a related haploid brackish water strain to facilitate the assembly process. Over several years I worked on the assembly of both genomes, organized the structural annotation and the production of a genome browser in collaboration with the ABIMS platform at Roscoff, put together a group of annotators of genes of interest, and performed extensive analyses of the predicted proteins (identification of orthologs, lineage-specific genes, identification of genes under positive selection in collaboration with Didier Jollivet from the UMR7144 at the Station Biologique de Roscoff and Agnieszka Lipinska from our research unit). These analyses yielded a surprisingly low number of confirmed differences between the two species. Most of the sequences unique to one species or the other were related to recent transposon activity, viral genes, or in 1436 cases algal genes with yet unknown function. Similarly, 82 of 83 proteins that were found to have been under positive selection since the separation of *E. subulatus* and *E. siliculosus* were of unknown function, the 83rd corresponding to a Mannosyl-oligosaccharide 1,2- $\alpha$ -mannosidase with no currently known role in salinity tolerance. Among the most interesting results was the fact that an expansion of the Light-Harvesting Complex (LHC)R/F protein family in *E. subulatus* may have been related to a recent burst of LARD transposon activity. LHCR/F proteins are frequently induced in response to stress and may be involved in non-photochemical quenching and thus the prevention of oxidative damage under stress (Dittami *et al.* 2010 #6). These analyses were finally published in 2020 (Dittami, Corre, *et al.* 2020 #44), although improvements of the *E. subulatus* genome (Nanopore sequencing, HiC sequencing) are still ongoing. Although in the end, none of the few genomic differences *per se* could convincingly explain the difference in freshwater tolerance between the two strains, this genome formed a basis for downstream work in this model system including on its microbiome.

**On the bacterial side**, no information on the *E. subulatus* microbiome was available when we started this research. The first step towards addressing the role of bacteria during the freshwater response was therefore to generate an inventory of bacteria in *Ectocarpus subulatus* cultures under different salinity conditions. This was done by putting into place a 16S metabarcoding study. It demonstrated that culture media strongly affect the bacterial community composition (Figure 1). Furthermore, by comparing the microbiome composition of a range of other *E. subulatus* cultivates and correlating this data with their respective capacity to tolerate low salinity, we observed that the presence of two bacterial operational taxonomic units (OTUs), one *Haliae* sp. and one unclassified *Sphingomonadales*, correlated significantly with an increase in low salinity tolerance. The actual implication of these bacteria in salinity tolerance, however, remained to be proven.

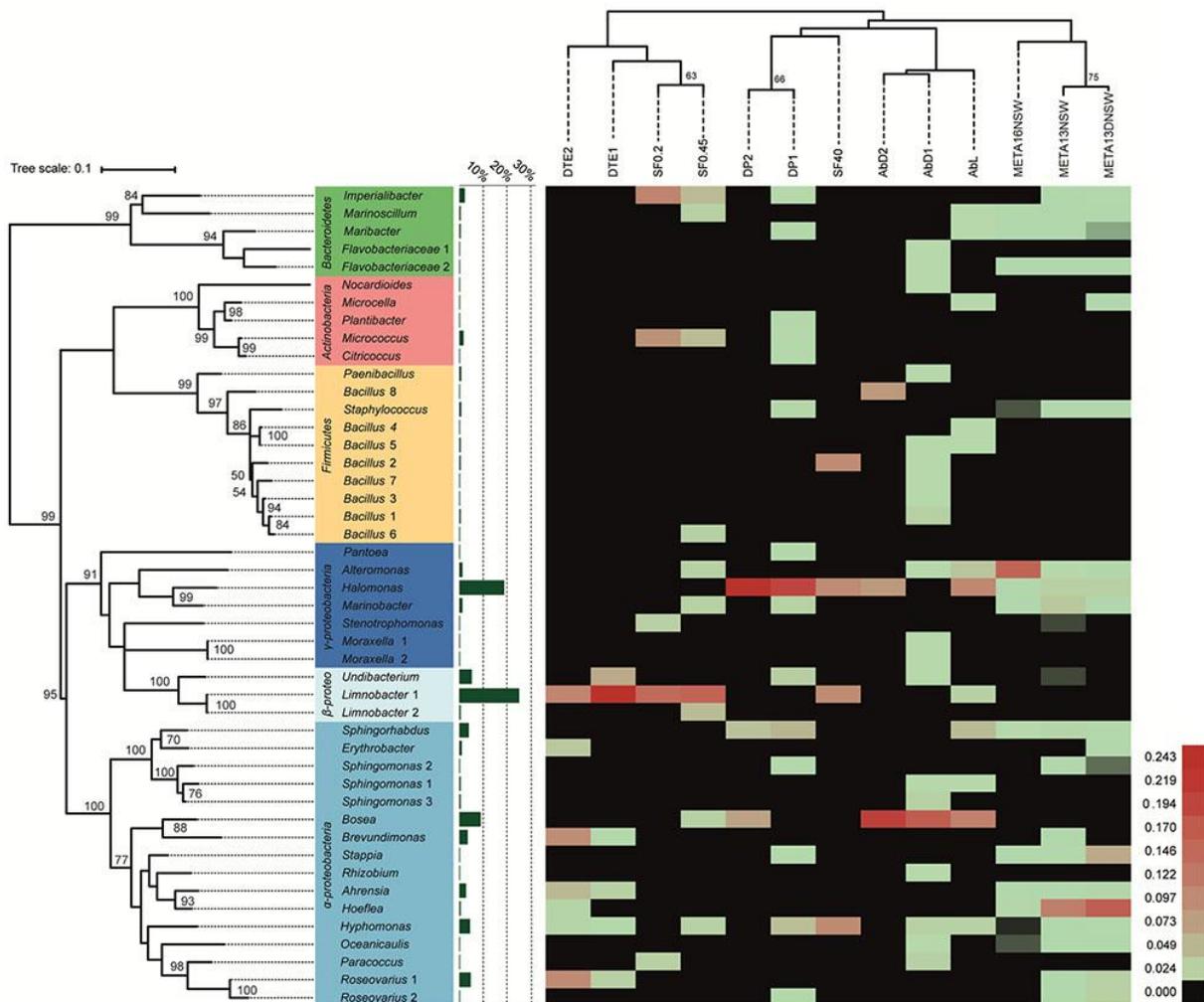


**Figure 1:** Bacterial community composition associated with the freshwater strain of *E. subulatus* grown in low salinity medium (DSWM) and full salinity medium (SWM). Taxonomic groups significantly overrepresented in DSWM or SWM (false discovery rate < 5%, n=3) are highlighted in yellow or blue, respectively. Only taxonomic groups represented by at least 10 sequences were considered. From Dittami *et al.* (2016 #30).

Based on these results, the next steps, at the time, seem straightforward. We needed to obtain bacterial cultures *E. subulatus*, and ideally isolate the two specific bacterial strains. This would enable us to perform targeted co-culture experiments and to pinpoint the bacteria responsible for the observed phenomenon. This task was started by myself using a wide range of culture media. Later Hetty KleinJan, a Ph.D. student whom I co-supervised in the context of a Marie Curie Innovative Training Network coordinated by Claire Gachon (SAMS, Oban), continued this work putting into place dilution-to-extinction cultivation techniques, and expanding the different media tested (KleinJan *et al.* 2017 #38). In addition to algal tissue, Mrs. KleinJan used algal growth media as inoculum and tested pre-treatments with antibiotics or filtration to remove part of the (fast-growing) bacteria. She further tested the effects of digesting the algal cell walls to release bacteria in that compartment. Together, we generated and identified a total of 388 isolates falling into 33 genera (Figure 2; 46 distinct strains based on their 16S sequences). *Halomonas* (*Gammaproteobacteria*), *Bosea* (*Alphaproteobacteria*), and *Limnobacter* (*Betaproteobacteria*) were the most abundant, but the two aforementioned candidate OTUs were not obtained in culture. Direct comparisons of the diversity of cultivated strains with 16S rRNA gene metabarcoding data showed that ~50% of total reads were accounted for by at least one isolate. Although this number is high compared to natural environments, where only about 1-10% of microbes are considered cultivable (Kellenberger 2001), it proved to be insufficient to identify the strains that facilitated freshwater tolerance.

Indeed, we tested all of the isolated strains individually in co-culture experiments with the alga, and we also selected several bacterial communities. Co-cultures were then screened for their ability to grow in fresh water. Unfortunately, none of the isolated bacteria, nor any of the tested communities were able to restore freshwater tolerance in

antibiotic-treated algal hosts. This means that either we were not able to isolate the right bacteria, or we did not test them in the right combinations or concentrations. Furthermore, at some point in time our previously used positive control, *i.e.* the addition of a small quantity of filtered culture medium from a non-treated culture, no longer restored growth in the freshwater strain. This suggests that a change in the associated microbiome of our culture had taken place over time, eliminating the possibility of including a positive control in the experiments.

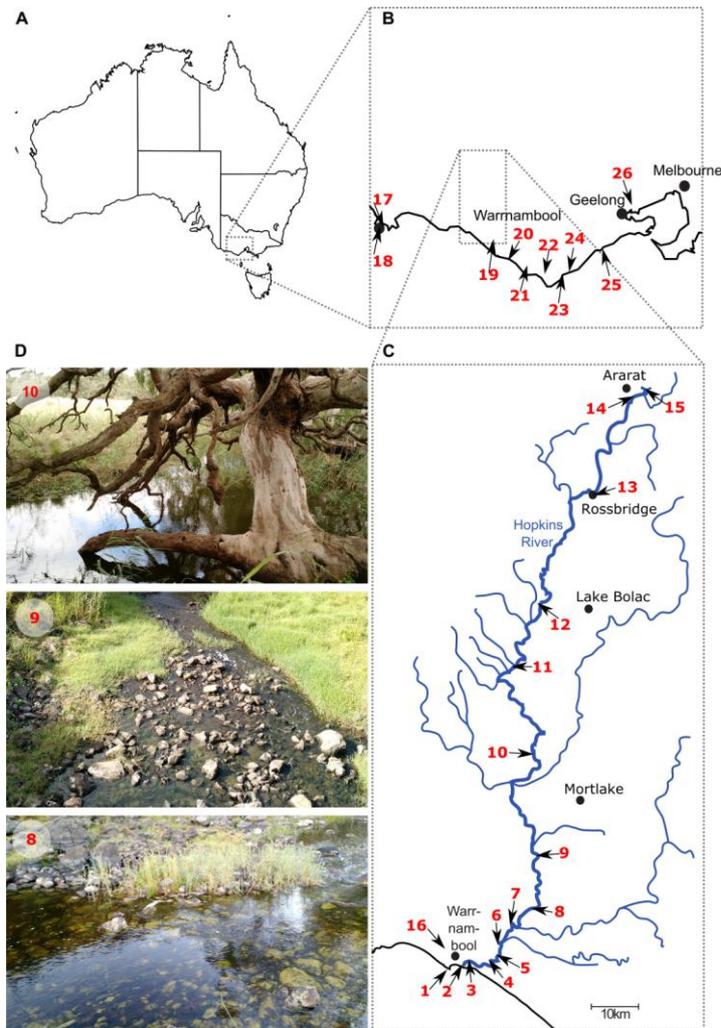


**Figure 2:** Heat-map of the number of cultivated *E. subulatus* strains per genus together with metabarcoding data. The number of sequences was normalized and  $\log(x+1)$ -transformed for each unique cultivable strain and each experimental treatment (DP, direct plating without pretreatment; AbD, DP with pretreatment with antibiotic disks; Abl, DP with pretreatment with liquid antibiotics; SF, DP with pretreatment by size-fractionation; DTE, dilution-to-extinction cultivation). Red colors indicate high abundance, while green corresponds to relatively low abundance. Black indicates taxa/strains that were not retrieved/isolated with the corresponding technique. Experimental treatments are grouped (top dendrogram) using hierarchical clustering (Euclidean distance, average linkage method), and the phylogenetic tree (left) was calculated using the Maximum-Likelihood method and the GTR+G+I model. Bootstrap analysis for both trees was carried out using 500 replications. Only bootstrap values  $\geq 50$  are shown. The bar graph (green) shows the proportion of isolates per unique strain obtained in the whole cultivation dataset. From KleinJan *et al.* (2017 #38).

Further investigating the processes potentially involved in the freshwater acclimation of *E. subulatus* and the microbial inputs thus required a new strategy. Rather than attempting to fully control the microbiome, we decided to try investigating this phenomenon merely by modifying the microbiome of laboratory-grown *E. subulatus* using mild antibiotic treatments that affected its ability to grow in low salinity. Then the acclimation of fresh water-tolerant and intolerant holobionts to sub-lethal low salinity stress was compared using a multi-omics approach integrating metabolomics, metatranscriptomics of both the alga and the associated bacteria, as well as metagenomics and metabolic networks to merge these data sets (KleinJan *et al.* 2021 preprint #52). Contrary to our initial expectations, these experiments did not bear evidence of the bacterial production of any compound that we suspect may directly improve algal stress tolerance. However, we identified vitamin K synthesis as one possible bacterial service missing specifically in the fresh water-intolerant holobiont in low salinity. We also noticed an increase in bacterial transcriptomic activity and the induction of microbial genes involved in the biosynthesis of the autoinducer AI-1, a compound that regulates quorum sensing (QS). QS is important in bacteria, for instance for biofilm formation, but has also been related to virulent behavior and the occurrence of dysbiosis (Zhou *et al.* 2020). This would fit well with the observed strong proliferation of bacteria in this condition. Our meta-omics approach thus generated two promising working hypotheses on the mechanisms by which uncultivated bacteria may influence their brown algal host's fresh water tolerance. It is also a first step towards bridging the gap between fully controlled, cultivable model systems, of which only very few are available, and purely environmental studies which frequently remain correlative.

Parallel to these laboratory approaches, and with the aim of at least partially relating our findings to observations in the field, I sought to gain more information about the *E. subulatus* freshwater strain in its natural environment. Indeed, this strain did not only constitute the only known freshwater isolate of *Ectocarpus* but it had been described only at one single location on one single date in 1995 and with very little background information on the abiotic parameters in the region. Therefore, I decided to search for *Ectocarpus* in the Hopkins River as well as surrounding rivers and the coastline. This had several aims. First and foremost, when putting so much effort into understanding this phenomenon, it seemed important to know if the finding of *E. subulatus* in this river was purely coincidental, or if *E. subulatus* has durably colonized this and possibly also other rivers in the region. Secondly, the aforementioned problems with changes in the alga-associated microbiome over time made me curious to sample the microbiome of naturally occurring *Ectocarpus*. At the time we only had little unpublished data available suggesting a profound change of the *E. siliculosus* microbiome upon transfer to the laboratory (Tapia and Potin, personal communication). Lastly, this sampling opened an opportunity to isolate additional alga-associated bacteria from the field. Funded by an intra-unit call for proposals, I therefore traveled to Victoria, Australia, and for two weeks, sampled for *Ectocarpus* at 15 sites along the Hopkins River as well as 10 neighboring sites (Dittami, Peters, *et al.* 2020 #43; Figure 3). I did not find any *Ectocarpus* at the original sampling site, but the campaign yielded several individuals with ITS and *cox1* sequences identical to the original isolate at three sites upstream of Hopkins River Falls. The salinity of the water at these sites ranged from 3.1 to 6.9, and it was unusually rich in sulfate (1–5 mM), possibly due to the volcanic origin of the rock in this region. Since sulfate has recently been demonstrated to be a key factor regulating cell differentiation in *Ectocarpus* (Siméon *et al.* 2020), this may constitute one possible reason why *E. subulatus* has successfully colonized particularly this river. I also

found that the diversity of bacteria associated with the algae in situ (1312 operational taxonomic units) was one order of magnitude higher than in previous studies of the original laboratory culture, and isolated 95 alga-associated bacterial strains from algal filaments on site. In particular, species of *Planctomycetes* were abundant in situ but rare in laboratory cultures. Overall, this expedition confirmed that *Ectocarpus* was still present in the Hopkins River, thus underlining the relevance of the model.



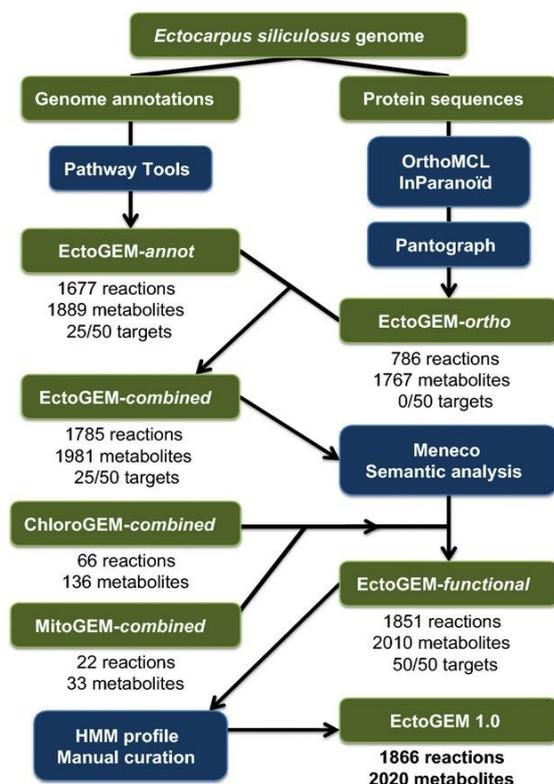
**Figure 3:** Sites sampled searching for *E. subulatus* in the Hopkins River area and 2017. Panels A-C: Map of sampling sites at an increasing scale. Each sampling site is numbered (1–26). *Ectocarpus* individuals were found at sites 8, 9, and 10, and Panel D contains photos taken at these sites. Hopkins River Falls, the original site of isolation, corresponds to site 7. From Dittami, Peters, *et al.* (2020 #43)

### 1.3 A focus on metabolic interactions between *Ectocarpus* and its associated bacteria

While elucidating the bacterial contributions to freshwater tolerance in *E. subulatus* was the primary objective of my work, even at the beginning of the efforts mentioned above, it was clear that both host and microbiome are likely to interact in multiple and complex ways. Thus, to understand the impact of microbes on the host stress tolerance, I also needed to develop a better understanding of the interactions between these components under non-stressed conditions. The key question for me was how such complex interactions could be addressed with minimal *a priori* assumptions. The time I was first confronted with this question, coincided with increased efforts at the Roscoff Biological Station to move towards an integrative view of algal biology and to establish systems biology tools to achieve this. One outcome of these efforts was the initiation of collaboration with

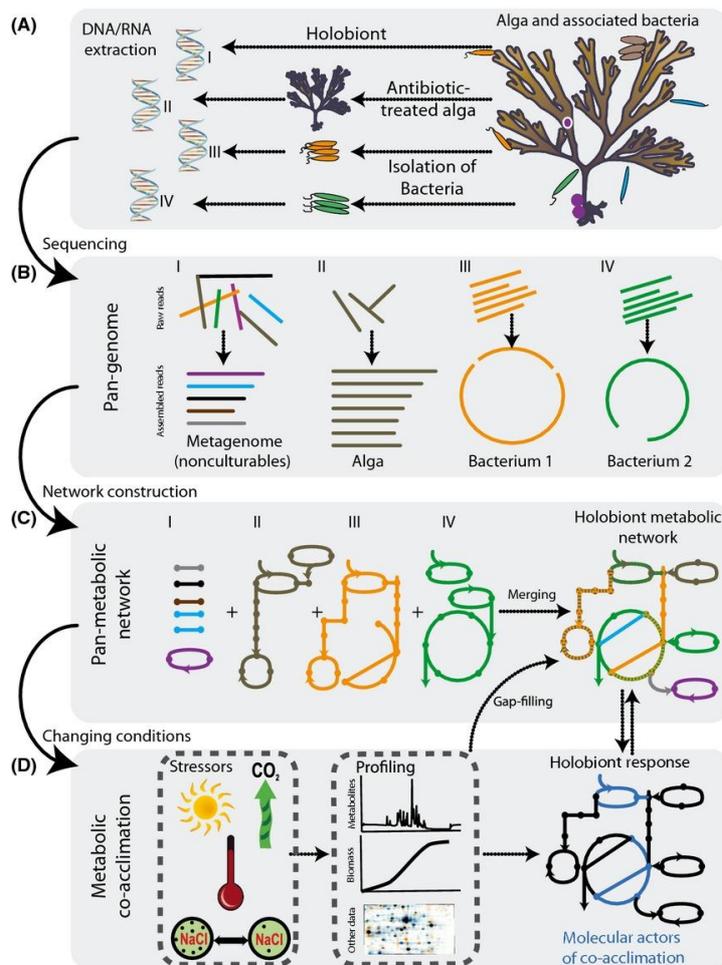
the Dyliss team led by Anne Siegel at the IRISA Rennes focused on the modeling of algal metabolism. This collaboration was reinforced via the joint 10-year PIA ANR project IDEALG led by P. Potin, which has just terminated.

The early work carried out in the context of IDEALG, led by the Dyliss team, did not yet include symbionts but was focused exclusively on generating metabolic models of algae, and notably the model *E. siliculosus*. While bacterial, fungal, and even plant metabolic models had a solid basis of physiological studies and pipelines to build on, at the time we started working the *E. siliculosus* metabolic model, only a single similar effort had been made for another stramenopile, the diatom *Phaeodactylum tricornutum* (Fabris *et al.* 2012). This and the fact that stramenopiles are phylogenetically distant from well-studied model organisms implied that many genes in their genomes were still of unknown function and hence the genome-derived metabolic networks contained a large number of gaps. Extensive work was necessary to develop a suitable workflow to transfer information available from other lineages (Figure 4), to identify and fill gaps in the network, and to finally generate the first published genome-scale metabolic network of a brown alga (EctoGEM; Prigent *et al.* 2014 #28). It consisted of 1866 reactions and 2020 metabolites and it was functional, *i.e.* it was able to produce biomass in flux balance analyses. Over the years, the pipeline used for reconstructing EctoGEM was further improved, numerous tools were added, and an entire analysis environment was created and distributed via docker and singularity images. Today, these efforts are still ongoing, notably via an extension that enables the simultaneous reconstruction and comparison of >40 different eukaryote genomes, while minimizing the impact of differences in sequencing and annotation quality on the results (Belcour *et al.* in prep).



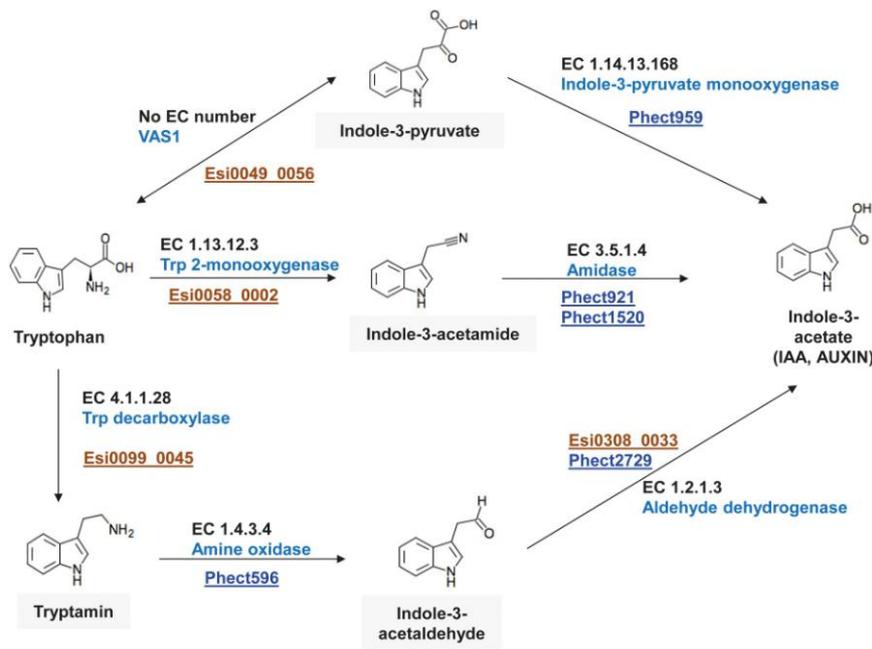
**Figure 4:** Summary of the integrative approach implemented to construct EctoGEM. From Prigent *et al.* (2014 #28)

However, even with the initial pipeline used to generate EctoGEM, it quickly became clear during our discussions with Anne Siegel's team that systems biology approaches and in particular metabolic networks also held great promise for the study of algal bacterial interactions: the underlying idea was to use the tools developed in Anne Siegel's team to work with the metabolic models of the alga, to be able to deal with the complexity of "holobiont" system comprising both algae and bacteria. We first proposed this approach in an opinion paper (Dittami, Eveillard, *et al.* #24) in 2014. Concretely, we suggested that metabolic models of both the host and the associated bacteria could be merged giving rise to a holobiont metabolic model (Figure 5). With this merged model we could monitor, for instance, stress responses of the whole holobiont (see also the aforementioned multi-omics study of *E. subulatus* acclimation), but also predict necessary exchanges of metabolites between the subnetworks (those of the host and the individual bacteria) for the holobiont network to function according to our expectations. Going one step further we then proposed that beneficial interactions between an algal host and its symbiotic bacteria, may, at least partially, be based on such metabolite exchanges and thus on the metabolic complementarity between the host and the symbiont metabolic networks. This resulted in a fundamental change of our view of "gaps" in metabolic networks – from something that caused problems during the analysis and was to be avoided and filled if possible, to an important piece of information that may or may not carry information on the biology of interactions between the examined organisms. In the following paragraphs, I will outline how we have worked towards developing this idea, which has become the second large axis of my research over the last nine years.



**Figure 5:** Studying co-acclimation in algal holobionts using an integrative metabolic approach. (A) The isolation of the different components of the holobiont and their DNA, (B) the sequencing of these components, (C) the reconstruction of individual metabolic networks and their merging, and (D) the use of the merged network to study acclimation processes in the holobiont. Some of the symbols used in this figure were provided courtesy of Tracey Saxby and Jane Thomas, IAN Image Library ([ian.umces.edu/imagelibrary/](http://ian.umces.edu/imagelibrary/)). From Dittami, Eveillard, *et al.* (2014 #24).

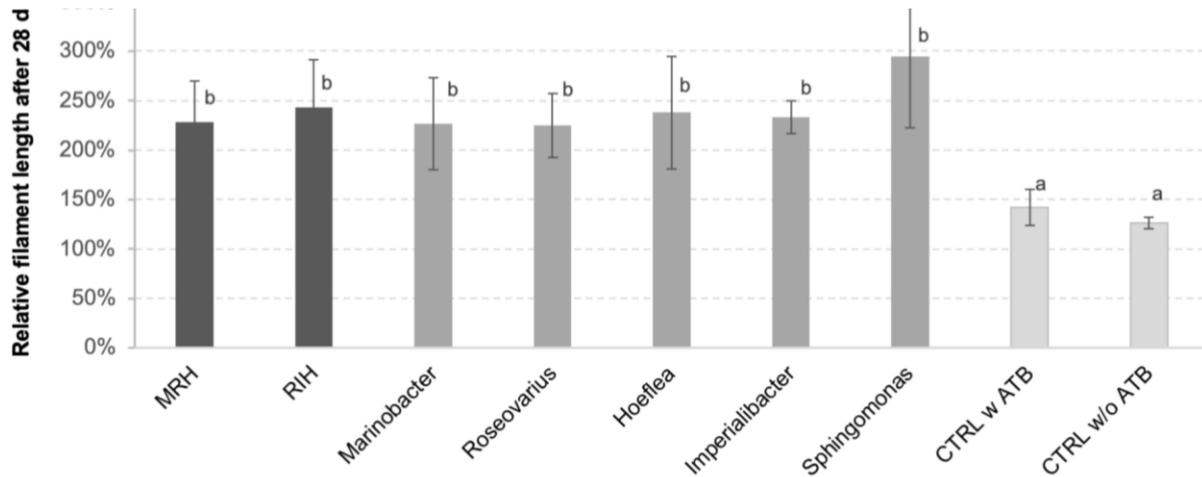
As a first test case of these metabolic network approaches, and before I started to obtain the first genomes of bacteria I had isolated from *Ectocarpus*, I decided to use contaminant bacterial sequences generated in the course of the *E. siliculosus* genome project (Cock *et al.* 2010 #3). These contaminant bacterial sequences comprised of a 3.4 Mbp circular contig likely corresponding to the complete genome of an entirely unknown genus of *Rhizobiales*, which we named "*Candidatus Phaeomarinobacter ectocarpi*" (Dittami, Barbeyron, *et al.* 2014 #26). Together with collaborators from the Station Biologique de Roscoff, we found this genome to encode numerous metabolic pathways that may be relevant for this bacterium to interact with algae. Notably, it possessed a large set of glycoside hydrolases and transporters, which may serve to process and assimilate algal metabolites. It also harbored several proteins likely to be involved in the synthesis of algal hormones such as auxins and cytokinins, as well as the vitamins pyridoxine, biotin, and thiamine. Finally, a metabolic model we generated for this bacterium was used with the algorithms designed to fill gaps in the algal metabolic network to identify potential complementarities and points of interaction between the bacterium and its algal host. As exemplified in Figure 6 for auxin, there were numerous cases where algal and bacterial predicted metabolic capacities could complement each other to enhance the metabolic capacity of the holobiont, even though, at this point, we had no proof that these metabolic exchanges actually occur.



**Figure 6.** Genes involved in tryptophan-dependent auxin synthesis in “*Ca. Phaeoamarinobacter ectocarpi*” Ec32 (blue loci) and *E. siliculosus* (brown loci). Together, both the alga and the bacterium may be able to produce auxin if intermediates are exchanged between both partners. From Dittami, Barbeyron, *et al.* (2014 #26).

Building on these preliminary analyses and ideas, our collaborators in Rennes and notably Clemence Frioux, a former Master’s and Ph.D. student in the Dyliss team, continued to improve their bioinformatic toolkit tailoring it more specifically to the question of metabolic interactions. The outcome of this work was a dedicated and highly scalable tool to explore metabolic interactions named MisCoTo (Frioux *et al.* 2018). MisCoTo was not only able to handle large symbiont communities with over 100 metabolic networks and to predict potential metabolic complementarities. It could also select minimal sub-sets of symbionts that would fulfill the metabolic needs of the host metabolic network. The tool thus provided a possible criterion to select bacterial communities for co-culture experiments - a key question which we had addressed so far relying much on “intuition”, and testing random associations or associations previously described in the literature. However, a key question that remained to be addressed was if or to what extent predicted potential interactions based on MisCoTo and more generally metabolic complementarity also occurred *in vivo*.

a



b

Target	Exchange	MRH	RIH	Marinobacter	Roseovarius	Hoeflea	Imperialibacter	Sphingomonas	CTRL w ATB
Spermidine	Dehydrospermidine	+/-	+/-	+/-	+/+	+/-	+/-	+/-	-/-
Putrescine	Agmatine	+/-	+/+	+/+	+/+	+/-	+/-	+/-	-/-
Nicotinic acid	Nicotinic acid	+/-	+/+	+/-	+/-	+/-	-/+	+/-	-/-
Folic acid	dihydrofolate	+/-	+/-	-/-	-/-	-/-	-/-	-/+	-/-
Auxine	Indole-3-acetaldehyde	+/+	+/+	-/-	-/-	-/+	+/+	-/+	-/-
L-histidine	Histidinol	+/-	+/-	+/-	+/-	+/+	+/+	+/+	-/-
Beta-Alanine	3-ureidopropanoate	+/-	+/+	+/+	+/-	+/+	+/-	-/-	-/-

**Figure 7:** a) Relative length of *E. siliculosus* filaments after 28 days of (co-)culture compared to the starting point. The bars represent the means of 3 replicate co-cultures  $\pm$  SD. The letters above the columns indicate the results of TUKEY HSD pairwise comparisons ( $p < 0.05$ ). b) Comparison of predicted production of target metabolites in co-cultures based on metabolic networks (symbol before the slash) and results from targeted UPC2-MS analyses of algal filaments after 28 days (symbol after the slash). The column “Exchange” indicates one possible compound provided by the microbiome leading to the production of the compound in the column “Target” in the algal metabolome; it was these target metabolites that were tested for using UPC2-MS. All experiments were carried out in triplicate, each replicate of the same condition yielding identical results. (-): a target metabolite was not predicted/detected (+): a metabolite was predicted/detected. Green highlights conditions where predictions correspond to the in vivo observations, red highlights compounds that were detected although no pathway was predicted. Yellow indicates compounds potentially producible via bacterial exchanges that were not detected. MRH, consortium 1: *Marinobacter*–*Roseovarius*–*Hoeflea*; RIH, consortium 2: *Roseovarius*–*Imperialibacter*–*Hoeflea*; CTRL, control; ATB, antibiotic treatment. From Burgunter-Delamare *et al.* (2020 #47).

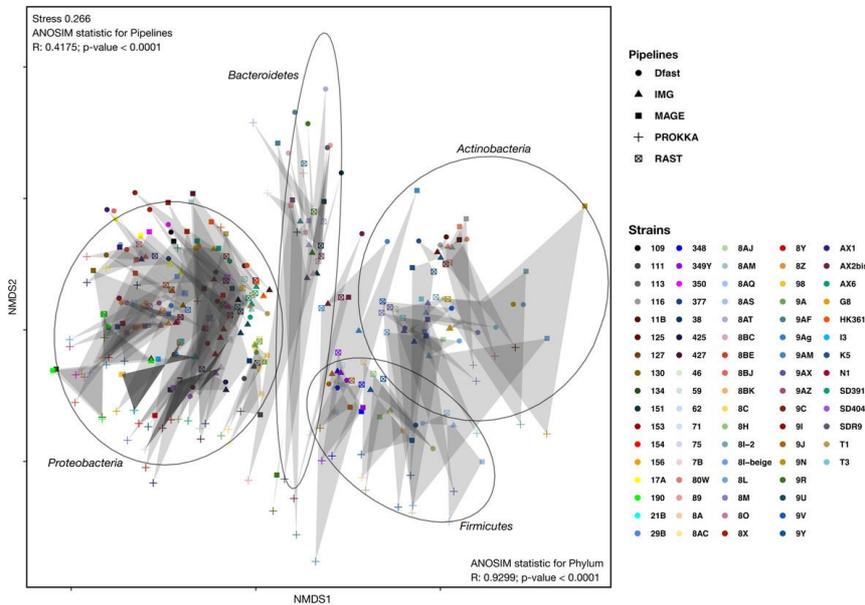
We first addressed this question in the context of the Master’s thesis of Bertille Burgunter-Delamare. To this means we sequenced, assembled, and annotated the genomes of 10 bacterial strains isolated from *Ectocarpus*, reconstructed their metabolic networks, and ran MisCoTo to predict minimal communities that would best complement the algal metabolic network. These communities were projected to facilitate the production of 205 new compounds by the algal host. Finally, we selected two of the predicted minimal communities comprising three bacteria each for in vitro co-culture experiments with

*Ectocarpus*. Co-cultures with each of the bacteria individually as well as a negative control without added bacteria were included as well. Over 28 days, we then monitored algal growth as well as the presence of 8 selected key algal metabolites that were predicted to become producible as a result of the interaction (Burgunter-Delamare *et al.* 2020 #47). Although we did not fully control but only modified the bacterial communities in these experiments, these data demonstrated a significant increase in algal growth in cultures inoculated with the selected consortia. In several cases, we also detected the presence of key metabolites predicted to become producible via an exchange of metabolites between the alga and the microbiome, while none of these compounds were detected in the negative control (green fields in Figure 7). These experiments thus strongly suggest that metabolic complementarity is a good indicator of beneficial metabolite exchanges in holobiont systems.

Despite these promising results these experiments also underlined the necessity of further methodological developments to be able to routinely and reliably use MisCoTo for the prediction of metabolic interactions. One key problem for the predictions was the reliability and completeness of the metabolic networks. Even a single missing reaction in a metabolic network will create a gap that may need to be filled and this could be falsely interpreted as a potential metabolic interaction. In the work mentioned above manual curation has been used to validate most of the reactions/interactions that were predicted by the models, but this is not feasible if predictions are upscaled to larger consortia. A simple solution towards improving the quality of the metabolic networks is to improve the quality of the genome annotations that were used to reconstruct them and this depends, in turn, on the annotation pipeline.

Before starting to apply MisCoTo to even larger data sets and in an attempt to optimize these analyses while minimizing the need for manual curation, we, therefore, decided to systematically test the impact of the annotation pipeline. This work was carried out primarily by Elham Karimi, a post-doctoral researcher working in the context of a CNRS momentum project, and Enora Geslin, a talented M1 student of bioinformatics. Concretely, we decided to compare five commonly used pipelines (Prokka, MaGe, IMG, DFAST, and RAST) at several different levels from predicted annotation features (coding sequences, Enzyme Commission numbers, hypothetical proteins) to the metabolic network-based analysis of symbiotic communities (biochemical reactions, producible compounds, and selection of minimal complementary bacterial communities) (Karimi *et al.* 2021 #49). While Prokka and IMG produced the most extensive networks, RAST and DFAST networks produced the fewest false positives and the most connected networks with the fewest dead-end metabolites. We observed differences between the outputs of the tested pipelines at all examined levels, with small differences in the draft metabolic networks, still allowing for separation by phylum regardless of the annotation pipeline use (Figure 8), resulting in the selection of different microbial consortia to expand the metabolic capabilities of the algal host. However, the different consortia predicted yielded similar sets of anticipated producible compounds and could therefore be considered functionally interchangeable. Although we were not able to identify one pipeline that was superior to the others, manual curation allowed us to conclude that most of the errors in the networks were found among reactions that were predicted only by a single pipeline. This suggests that in the future, running several annotation pipelines and then basing downstream analyses on a consensus, may improve the accuracy of predictions and reduce the need for manual curation. In the

same vein, this work also highlighted the delicate nature of these analyses – although the fundamental principle of metabolic complementarity appears to produce solid predictions, there may be multiple possible interactions, and it is the noise in the network that will lead MisCoTo to select e.g. one consortium over the other.



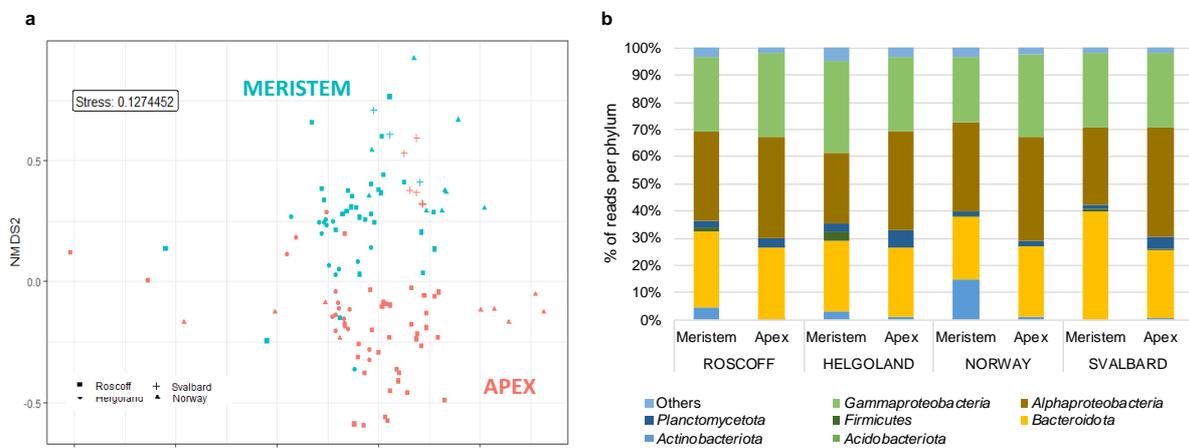
**Figure 8:** Non-metric Multi-Dimensional Scaling (NMDS) plot of the metabolic networks of the different bacterial strains and pipelines. The Bray-Curtis index was used as a dissimilarity measure; ellipses show clusters of strains based on bacterial phyla; grey polygons connect annotations of the same genome performed with different pipelines. From Karimi *et al.* 2021 #49)

#### 1.4 New model systems

Overall, the work described in sections 1.2 and 1.3 has contributed to our understanding of algal-bacterial interactions in *Ectocarpus* and also helped establish metabolic networks as a tool that can be used to disentangle these interactions in complex systems. I consider this work on model systems important and it will certainly be continued as described below, especially considering the establishment of genetic tools for this model (Badis *et al.* 2021). However, despite the importance of fundamental research, it seems important not to lose sight entirely of economic and ecological questions that are relevant for society. For example, in virtually all of my project proposals, I argued that the work carried out on *Ectocarpus* could eventually be transferred to the more relevant kelp species. Yet, for a long time, I made no effort to move in this direction. This changed in 2017/2018 due to the Master’s thesis of Bertille Burgunter-Delamare, who, as described above, performed key experiments validating the metabolic complementarity approach for the study of algal-bacterial interactions. At the end of her internship, Mrs. Burgunter-Delamare wished to continue her work via a Ph.D. thesis, but the direct follow-up work to her Master’s was already covered by a post-doctoral grant (CNRS momentum project). This finally opened up an opportunity to start testing to what extent similar work could be carried out in kelps. We thus decided to prepare a Ph.D. project focused on the sugar kelp *Saccharina latissima* – one of the dominant kelp species in Europe. Its current distribution ranges from Spain to Spitzbergen, but several populations are in decline (Araújo *et al.* 2016). Natural populations of *S. latissima* are harvested in several regions and it is also one of the brown algal species used in aquaculture in Europe. Furthermore, the genome of *S. latissima* was being sequenced in the context of Phaeoexplorer, a project financed by France Génomique to sequence 40 brown algal genomes coordinated by Mark Cock, in which I am also

participating. Despite this background, at the time, very little information was available on the composition of the *S. latissima* microbiome. Together with Mrs. Burgunter-Delamare, we obtained a Ph.D. scholarship from the Brittany region ED227 the doctoral school (Sorbonne University), thus starting the third axis of my research described in this memoir.

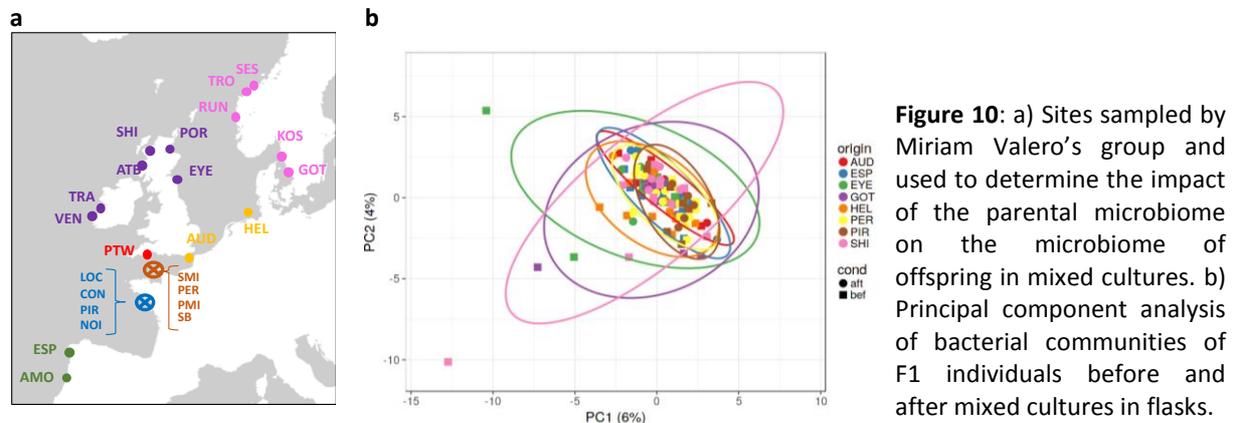
Despite the groundwork carried out for *Ectocarpus*, working on a new system required substantial preparation and reestablishing some foundations. Thus, as the first activity of her thesis, Mrs. Burgunter-Delamare started to characterize the microbial communities associated with *S. latissima*, looking at latitudinal changes (Roscoff to Svalbard), changes in the microbiome between healthy and diseased individuals (twisted stipes, bleaching, degradation of the blade), seasonal changes, and differences between the apex and meristem tissues (Figure 9). This gave us an overview of the *S. latissima* microbiome, which was subject to regional variability and dependent on the tissue of the alga sampled. However, there was no clear separation between diseased and healthy individuals – only a few specific strains that were significantly more abundant in either of the conditions.



**Figure 9:** a) Non-metric Multi-Dimensional Scaling (NMDS) plot shows a clear separation of apex and meristem samples and a weak separation of individuals from different geographical origins b) Global taxonomic composition of the microbiome of the *S. latissima* individuals sampled.

The next question we sought to address, was to what extent regional differences in the microbiome were merely a reflection of differences in the environment, or, on the contrary, due to microbiome “inheritance” and the genetic background of the individuals. Understanding the importance of horizontal (*i.e.* from the environment) vs. vertical (from the parents) transmission of the microbiome is essential for evaluating the impact of microbiome changes on the physiology of their algal hosts and vice versa. To address this question, we benefitted from an ongoing experiment in Myriam Valero’s team et the Roscoff Biological Station. This team had conducted an extensive sampling campaign of *S. latissima* from a wide range of different locations (Figure 10), which were brought to the laboratory and genotyped. Offspring of these algae originating from different locations were then co-cultured together in 10L culture flasks. After one month, individuals were genotyped and attributed to their original populations. We had the opportunity to assess the microbiome of these samples by 16S metabarcoding and compared it to the microbiome of the parents and the offspring before co-culture. These analyses revealed that, once put into culture, the microbiome of *S. latissima* quickly decreases in diversity, and that after one generation, even before co-culture experiments, the bacterial communities of algae of different origins could no longer be statistically differentiated. Thus, we can conclude that the microbial

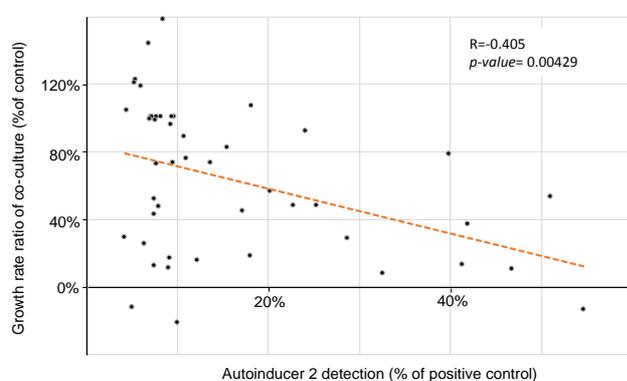
composition of *S. latissima* is strongly influenced by the environment and largely acquired horizontally, although this does not exclude the possibility of a few “marker” strains that may be transmitted over generations.



Once an overview of the composition, stability, and acquisition of the *S. latissima* microbiome had been established, we moved towards co-culture experiments, and started looking into the interactions between the algal host and specific bacteria. For this purpose, and in addition to using bacterial strains that had been isolated from *Ectocarpus*, Mrs. Burgunter-Delamare undertook extensive cultivation efforts. Over one year, she isolated >300 strains from *S. latissima* individuals, corresponding to 62 distinct 16S sequences. She then selected a subset of strains, based primarily on their occurrence either specifically with healthy or specifically with diseased individuals, and tested them in co-culture experiments with the host. Such co-culture experiments are, however, limited by the fact that *S. latissima* does not support our standard antibiotic treatments well, and usually, shortly after the treatment, the alga is overgrown by surviving bacterial strains. Our co-culture experiments, therefore, consisted merely of adding selected bacterial strains to an already existing bacterial community and observing the effect this may have. In total Mrs. Burgunter-Delamare performed over 400 such co-culture experiments, revealing both negative effects of some bacterial strains as well as the possible mitigation of these effects by other bacteria. Unfortunately, however, the results generated in these experiments were not or only partially reproducible when experiments were repeated at a later point in time. Our current hypothesis is that this was likely due to differences in the microbiome of the algal hosts before bacterial inoculation – a factor which, as described above, we cannot control: these different basal microbiomes may lead to different microbe-microbe interactions in the cultures and thus to different experimental outcomes.

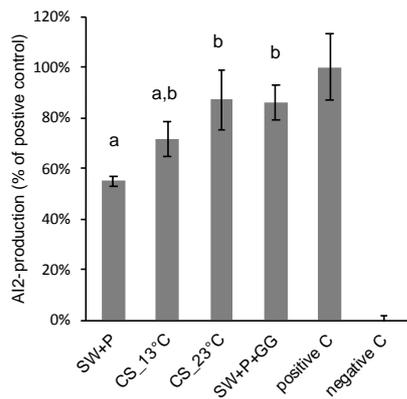
The lack of repeatability is a general problem of microbiome manipulations, especially in not fully controlled environments, and possible long-term solutions to this problem are discussed in detail in the Perspectives section. In the context of a Ph.D. thesis, however, medium- or short-term solutions are required, that will allow the student to write up and publish the data obtained in a meaningful way. In this specific case, our approach was to exploit the variability found in this dataset, to examine if other variables may explain the different experimental outcomes. One such variable, which we have been interested in since Mrs. KleinJan’s results on *Ectocarpus*, is quorum sensing (QS). QS compounds may

prompt some bacteria to modify their behavior and sometimes lead to dysbiosis. Via a collaboration with Soizic Prado's team at the Natural History Museum, Paris, we started using biosensors to evaluate the production of QS compounds. These biosensors are cultivated with filtered media obtained from algal-bacterial-cocultures, and the presence of QS compounds induces bioluminescence or Green Fluorescent Protein (GFP) production in the biosensors, making the presence of QS compounds visible with a spectrophotometer. As shown in Figure 11, a metanalysis of the co-culture experiments carried out by Mrs. Burgunter-Delamare revealed a significant negative correlation between the production of Autoinducer 2 compounds and algal growth (no AI-1 compounds were detected in any of the co-cultures). We are still in the course of analyzing the microbiome composition in these samples to search for microbial signatures that may be correlated with either the reduction in growth or the production of QS compounds. Moreover, what remains to be established is if the production of these QS compounds is the reason for the poor host health or rather its consequence.



**Figure 11:** Negative correlation between the observation of AI2-type quorum sensing (QS) compounds over a range of different co-culture experiments, and the growth rate observed in the algal host. We hypothesize that QS may mediate a switch to dysbiotic behavior in bacteria at least in some of the observed cases of reduced growth.

In a first attempt towards answering this last question, Jamie Laugier, a Master's student in our team, started testing if the supernatants of algae may induce the production of QS signals in bacterial cultures, and if QS was induced at lower cell densities, or if stronger signals were observed when algae were exposed to environmental stress (increased temperature in this case). Among the 7 bacterial cultures tested, two were observed to produce QS compounds, and one of them showed increased QS activity when cultivated with extracts of temperature-stressed algae, or oligo-guluronate, *i.e.* fragments of algal cell wall polysaccharides that are frequently released upon grazer attacks (Figure 12). This suggests that at least some bacteria are capable of sensing markers of algal health and possibly modify their behavior as a function of this.



**Figure 12:** Induction of bioluminescence in *Bacillus* sp. 181 displayed as a percentage of positive control (mean of three replicates  $\pm$  SD). Bacterial cultures were inoculated 1:1 with either Provasoli-enriched seawater (SW+P = algal culture medium), SW+P enriched with oligo-guluronate (GG), or filtered spent culture medium (CS) of *S. latissima* at 13 (control) or 23°C (temperature stress). Supernatants of bacterial cultures were harvested after 3h with the tested media. Letters above the graph indicate statistically different conditions (Tukey HSD test following an ANOVA,  $p=0.003$ )

## 2. Perspectives

Overall, the research I have presented in the two sections above has contributed to our understanding of algal bacterial interactions in brown algae and supports the use of metabolic networks in this context, but for every result obtained, new questions arose, frequently alongside technical and conceptual challenges. In the following section, I will highlight three key challenges which I have already started to work on, and which will likely occupy me for several years to come. They are related to 1) improving the controllability and reproducibility of experiments with improved model systems and improved experimental designs to gain more precise functional insights into specific interactions; 2) expanding our view of brown algal holobionts beyond bacteria and including also viruses and fungi; and 3) building bridges between work carried out in the laboratory and potential applications of this work.

### 2.1 Work on improved model systems and experimental designs

One of the primary motivations of commencing work on host-microbe interactions in *Ectocarpus* was its status as a model system for brown algae. It is easy to cultivate in the laboratory and has a quick growth rate and a short life cycle. In particular, the availability of strains that can be maintained indefinitely in the laboratory was a factor that I expected to provide a solid basis for reproducible experiments. The long list of available tools was complemented by the work described above to establish a collection of *Ectocarpus*-derived bacterial strains as well as the sequencing of numerous of their genomes (Karimi *et al.* 2020 #42). However, despite these favorable conditions, one key limitation encountered in our experiments with *Ectocarpus* was the control of the alga-associated microbiome, and in line with this the repeatability of results. Notably, the protocols used to generate “axenic” individuals (e.g. Tapia *et al.* 2016) are limited. Treated algae no longer produce visible bacterial colonies when plated on Petri dishes with standard culture media, but, as repeatedly seen in metabarcoding dormant bacteria are still present and frequently start developing during co-culture experiments. This reduces the reproducibility of results – the effect of e.g. adding a particular bacterium may depend on interactions with the other bacteria currently present and makes the interpretation of the results complex. We have repeatedly attempted to optimize protocols, and even looked into alternative methods to control the microbiome, notably essential oils and iodine (Burgunter-Delamare *et al.* 2021 #48), but so far without obtaining truly axenic host cultures.

Over time I have come to accept this inability to produce axenic cultures, and view it as a likely inherent property of host-symbiont systems: the tighter the physiology and metabolism of a host are interwoven with the associated symbionts, the lower the chances are that this host will be able to grow independently, as any treatment that efficiently eliminates the microbiome is bound to, at least indirectly, affect the host. To overcome this dilemma, it would be necessary to remove the alga-associated microbiome and at the same time substitute the microbial functions. One possibility here would be to use metabolic networks to predict the 'hidden' metabolic needs of the host and to substitute these metabolites, but due to their large number, this is impractical. Together with Elham Karimi, we have therefore started to put into place an alternative approach, during which alga-derived bacteria identified as important based on their metabolic networks were made resistant to the antibiotics Neomycin and Chloramphenicol by gradually exposing them to increased concentrations. We then substituted the natural host-microbiome with these resistant bacterial cultures and the antibiotics. Preliminary results are promising, showing only a low number of contaminant bacterial strains that appear, and none of these strains are abundant. However, further optimization of this approach notably, testing a wider range of antibiotics and bacterial strains is likely to improve our ability to control the microbiome and will certainly be part of my research in the years to come. The better we can control the microbiome, the higher the repeatability of experiments, and the higher the chances that we will be able to move from observing correlations to determining cause-effect relationships.

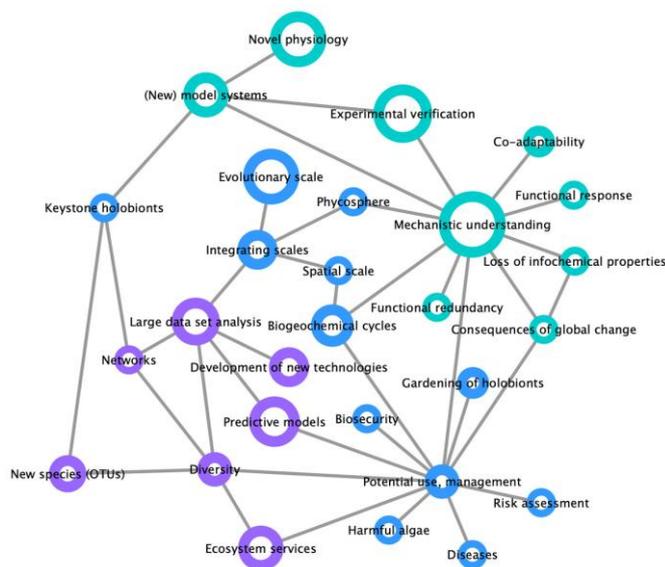
The second big development towards improved model systems will be the genetic modification of the different components of the algal holobiont. Genetic modification of *Ectocarpus* has recently been established at the Roscoff Biological station using the CRISPR/CAS9 system (Badis *et al.* 2021), opening the door to a new era of targeted experiments. In the same vein, and in the context of a Moore foundation-funded project led by S. Egan from the University of New South Wales, Sydney, similar genetic tools will be developed for alga-associated bacteria, including two bacteria isolated from *Ectocarpus* by me and Hetty KleinJan as mentioned above. These were the bacteria suspected to induce dysbiosis in *E. subulatus* via the activation of QS signaling, and we have observed similar results for one of the strains also in co-cultures with *S. latissima*. Possessing genetic tools for both the host and selected symbionts in the years to come, in combination with better control of co-cultures will therefore open new possibilities in my research and allow us to test hypotheses on the role of individual genes in the functioning of holobiont systems.

For questions where we have no suitable and highly controlled model system, as for *S. latissima*, we will need to choose a different approach. Here high levels of replication (true replicates from independent experiments), seem to be the most promising tactic. Even if the response of each holobiont depends on its specific associated microbiome, if replication is sufficient, we can estimate the probability of certain events such as dysbiosis or reduced or increased growth in response to the inoculation with a bacterium. Such data could then be used to deduce general trends in the holobiont response to e.g. the addition of a specific microbe or environmental stress. The approach could furthermore be adapted to *in silico* analyses of host-microbe interactions, where noise has also been an important limitation.

A dataset of particular interest to me in this context is a set of 901 bacterial genomes that were extracted from the over 40 brown algal genomes sequenced in the context of the Phaeoexplorer project coordinated by Mark Cock at the Roscoff Biological Station. Most of the genomes are (nearly) complete and highly suitable for genomic as well as metabolic

complementarity analyses. For some of these bacteria, their presence within the algal cultures, frequently despite antibiotic treatment, may be purely coincidental; for the majority, however, we can assume that they are well-adapted to life with brown algae. My idea is to constitute a parallel data set of 901 closely related genomes not derived from algal cultures, and then to perform systematic comparisons both at the gene and the metabolic network level. Unlike the analyses described above for one host and a few bacteria, due to the high level of replication (each pair comprising an alga-associated bacterium and the related non-associated bacterium is one replicate) noise in the networks and the composition of the microbiomes can be separated from the biologically relevant signals using statistics. Any systematic differences that prevail are thus likely to represent general bacterial adaptations to life with brown algae and thus promising candidates to refine or view and understanding of algal bacterial interactions.

In the end, I believe that the combination of both of these approaches is likely to generate the most biologically relevant insights. This is also what we proposed as one of the outcomes of a foresight workshop on the holobiont concept in marine sciences organized at the Roscoff Biological Station in 2018. During this workshop, we distinguished between predictive and mechanistic modeling and also concluded that it was their interface that was beneficial for our understanding of holobiont systems and potential applications of the holobiont concept (Dittami *et al.* 2021 #45; Figure 13)

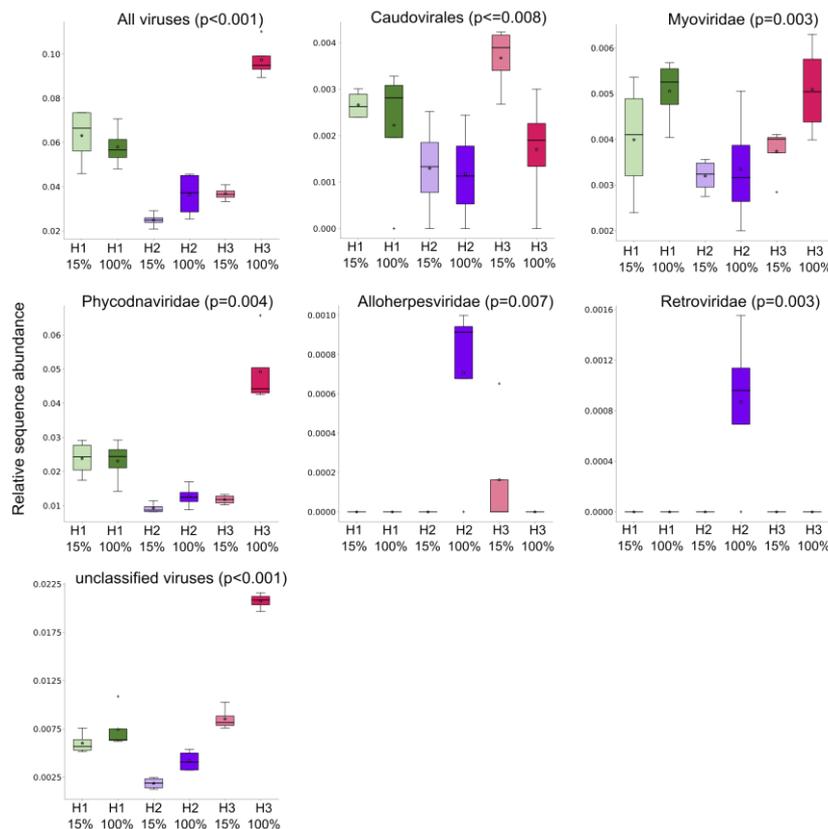


**Figure 13:** Mind map of key concepts, techniques, and challenges related to marine holobionts. The basis of this map was generated during the Holomarine workshop, Roscoff in 2018. The size of the nodes reflects the number of “importance” votes each keyword received from the participants of the workshop (a total of 120 votes from 30 participants). The two main clusters corresponding to predictive modeling and mechanistic modeling, are displayed in purple and turquoise, respectively. Among the intermediate nodes linking these disciplines (blue) “potential use, management” was the most connected. From Dittami *et al.* (2021 #45)

## 2.2 Expanding our view of holobionts to include viruses and fungi

The second key challenge for the years to come will be the further expansion of our view of algal holobionts. My initial argument for moving into the field of host-microbiome research was that if we only looked at the response of the host, we would be missing half of what is going on in the organism. Bacteria were the obvious first choice to expand our view of algae, as they are abundant and easily accessible, however, it is now clear that, depending on the alga we are working with, additional groups of organisms need to be considered. This became particularly evident when analyzing the metatranscriptome data for *E. subulatus* described in section 1.2.. In addition to algal and bacterial reads, this dataset also contained viral transcripts, and although these transcripts make up less than 0.001% of the reads, as

shown in Figure 14, the relative abundance of viruses was strongly altered depending on the bacterial composition (holobiont 1-3) and on the salinity (15% vs. 100%). These changes concerned virus families generally associated with algae (*Phycodnaviridae*), or more generally eukaryotes (*Alloherpesviridae*, *Retroviridae*), and families that have specialized on bacteria (*Caudovirales* including *Myoviridae*). This implies that changes in the microbiome and changes in the environmental conditions will not only affect the host and its microbiome directly but also indirectly via the virome, which, in turn, may interact with both the host and microbiome.



**Figure 14:** Box plot of relative abundances of viral RNA sequences belonging to different families associated with algae with different bacterial communities (H1-H3: holobiont 1-3) and in different salinities (100% seawater, vs 15% seawater diluted with distilled water). Values are given in % of the total number of RNAseq reads for each sample. P-values correspond to the results of an ANOVA across all six conditions (4 replicates each). From KleinJan *et al.* (2021 preprint #52)

Furthermore, viruses are likely not only relevant in *Ectocarpus*. A recent genomic study in *Laminaria digitata* has revealed the presence of *Phaeoviruses* in this species (McKeown *et al.* 2017), and a range of different viruses was also detected in the Australian kelp *Ecklonia radiata* (Beattie *et al.* 2018). In *S. latissima*, however, no knowledge about the associated virome is available yet. Considering the high levels of variability between replicates we observed in the co-culture experiments above, and in light of the results obtained for *E. subulatus*, I recently proposed a small project to start exploring the virome of *S. latissima* (VIRALG, one-year post-doctoral position financed by the Finistère department). In collaboration with Anne-Claire Baudoux from the UMR7144 at the Roscoff Biological Station we are currently attempting to isolate viral particles from field-collected *Saccharina* individuals, to purify them, and to sequence both RNA and DNA viruses at our local sequencing platform. While this is still far from fully integrating viruses into our concept of algal holobionts, it will constitute a starting point. Based on these sequence data, we will, for instance, be able to design qPCR probes that could be used to rapidly monitor the most

abundant viruses in our experiments and also during our field samplings, thus at least providing us with a rough estimation of the changes that occur in this compartment.

In addition to bacteria and viruses, fungi constitute a third compartment that will be incorporated into our future considerations. While our *Ectocarpus* cultures are generally free of fungi (as confirmed via past and ongoing genome projects), *S. latissima* has recently been shown to harbor a wide variety of fungi in the field (Tourneroche *et al.* 2020). These fungi cannot be detected by classical 16S metabarcoding approaches, but an additional primer pair targeting fungal Internal Transcribed Spacer (ITS) sequences, in the future, will allow us to monitor also the variability of this component in our samples. Lastly, it should be noted that *Archaea* and eukaryotes other than fungi do not appear to be tightly associated with brown algae (again based on our genomic data), or at least not in the genomic samples we have analyzed so far. I am aware that the study of fungi and algal associated viruses each may constitute an entire research career on its own. This implies that I will not be able to reach the same depth of analysis as I intend to obtain for some bacteria. Nevertheless, I believe that collecting these data much in the way I also register basic environmental variables, *i.e.* as background information which may or may not be useful to explain unexpected behavior, and which could be correlated with other variables, may prove highly valuable during the interpretation of our results and also for the research community.

### 2.3 Links to the economy

The third major line of my work in the next few years will consist in connecting fundamental research on holobionts with concrete applications. In this context, I have participated in the proposal of an Innovative Training Network (ITN) led at first by Ute Hentschel at the Geomar (Kiel), and later taken over by Rodrigo Costa (CMS, Faro), in the context of which we proposed to explore the suitability of our bacterial isolates from *S. latissima* to improve growth, stress, and disease resistance in *S. latissima* in hatcheries. This research was intended to be carried out in collaboration with Algolesco, a company based in the south of Brittany that specialized in the cultivation of seaweed. Unfortunately, this ITN proposal was not funded, but I plan to prepare a new, more targeted, project proposal once the work described above on *S. latissima* has been published. While I am convinced that we will be able to improve the growth and resistance of *S. latissima* in hatcheries by the addition of “probiotic” bacteria, the environmental risks of such approaches will require particular attention, such as the limitation of treatments to closed systems, and/or the use of exclusively endemic bacterial strains. Furthermore, the effect of microbial (pre-) treatments on other components of the ecosystem could also be tested in controlled mesocosm experiments.

Parallel to my intentions to work on *S. latissima* I have been part of the ANR proposal SEABIOZ led by P. Potin. In this project, I will start to work on a third algal model system, the wrack *Ascophyllum nodosum*. *A. nodosum* is a prominent source material for the production of plant biostimulants (Shukla *et al.* 2019). Extracts of *A. nodosum* are commercialized by the Roullier Group, the industrial partner of this ANR project, and have been demonstrated to improve plant growth and abiotic and biotic stress response (Jannin *et al.* 2013). Candidate bioactive molecules of these extracts were proposed in the literature *e.g.* polysaccharides (laminarin, fucoidans, alginates), mannitol, betaines, polyamines, and polyphenolic compounds, but their activity remains to be proven. Furthermore, one unique feature of *A.*

*nodosum* is its mutualistic association with the fungal endophyte “*Mycophycias ascophylli*” and other fungi and bacteria. These symbionts have been shown to protect *A. nodosum* germlings against desiccation and benefit its fitness (Garbary and London 1995). Currently, it is unclear if and to what extent the bioactive compounds in the *A. nodosum* commercialized extracts are derived directly from the algal host or associated symbionts. Furthermore, intermediate scenarios with metabolic cooperation as described in section 1.3 are possible. The idea behind the SEABIOZ project is to study the *A. nodosum* holobiont using a combined multi-omics approach as described above for *Ectocarpus* to identify potential active metabolites and to assess their origin (microbial, algal, or both). These will be complemented by monitoring the geographic and temporal variations of the holobiont composition via metagenome sequencing coupled with an assessment of the efficiency of the *A. nodosum* extracts. On the academic side, the hope behind this project is to improve our understanding of the interactions within the *A. nodosum* holobionts. In line with this on the industrial side, the hope is to be able to improve the efficiency of seaweed extracts, e.g. by sampling at particular seasons, by modifying the microbiome composition of the seaweed, by enriching or purifying specific fractions, or by developing new extracts derived directly from cultivated symbionts rather than the algal holobionts. To me, this makes this project, for which starting December 1<sup>st</sup> 2021, I will co-supervise a new Ph.D. student, Coralie Rousseau, a very nice example of how fundamental and applied research can not only coexist but benefit each other even in our current funding/and research landscape. I, therefore, hope that that this project will be successful, but likewise that I will be able to create similar links also in my future research projects.

## Acknowledgments

I would like to thank all of my friends, co-workers, students, and collaborators without whom this work would never have been possible, I would like to explicitly thank Catherine Boyen, Catherine Leblanc, and Thierry Tonon for their continued guidance throughout my career, and for successfully creating and maintaining a research environment in which solidary outweighs individualism and competition.

## Literature cited

- Araújo RM, Assis J, Aguillar R, et al. 2016.** Status, trends and drivers of kelp forests in Europe: an expert assessment. *Biodiversity and Conservation* **25**: 1319–1348.
- Badis Y, Scornet D, Harada M, et al. 2021.** Targeted CRISPR-Cas9-based gene knockouts in the model brown alga *Ectocarpus*. *New Phytologist* **231**: 2077–2091.
- Beattie DT, Lachnit T, Dinsdale EA, Thomas T, Steinberg PD. 2018.** Novel ssDNA viruses detected in the virome of bleached, habitat-forming kelp *Ecklonia radiata*. *Frontiers in Marine Science* **4**: 441.
- Burgunter-Delamare B, Boyen C, Dittami SM. 2021.** Effect of essential oil- and iodine treatments on the bacterial microbiota of the brown alga *Ectocarpus siliculosus*. *Journal of Applied Phycology* **33**: 459–470.
- Burgunter-Delamare B, KleinJan H, Frioux C, et al. 2020.** Metabolic complementarity between a brown alga and associated cultivable bacteria provide indications of beneficial interactions. *Frontiers in Marine Science* **7**: 813683.
- Case RJ, Longford SR, Campbell AH, et al. 2011.** Temperature induced bacterial virulence and bleaching disease in a chemically defended marine macroalga. *Environmental Microbiology* **13**: 529–537.
- Cock JM, Sterck L, Rouzé P, et al. 2010.** The *Ectocarpus* genome and the independent evolution of multicellularity in brown algae. *Nature* **465**: 617–21.

- Cormier A, Avia K, Sterck L, et al. 2017.** Re-annotation, improved large-scale assembly and establishment of a catalogue of noncoding loci for the genome of the model brown alga *Ectocarpus*. *New Phytologist* **214**: 219–232.
- Dittami SM, Arboleda E, Auguet J-C, et al. 2021.** A community perspective on the concept of marine holobionts: current status, challenges, and future directions. *PeerJ* **9**: e10911.
- Dittami SM, Barbeyron T, Boyen C, et al. 2014.** Genome and metabolic network of “Candidatus Phaeomarinobacter ectocarpus” Ec32, a new candidate genus of Alphaproteobacteria frequently associated with brown algae. *Frontiers in Genetics* **5**: 241.
- Dittami SM, Corre E, Brillet-Guéguen L, et al. 2020.** The genome of *Ectocarpus subulatus* – A highly stress-tolerant brown alga. *Marine Genomics* **52**: 100740.
- Dittami SM, Duboscq-Bidot LL, Perennou M, et al. 2016.** Host–microbe interactions as a driver of acclimation to salinity gradients in brown algal cultures. *ISME Journal* **10**: 51–63.
- Dittami SM, Eveillard D, Tonon T. 2014.** A metabolic approach to study algal-bacterial interactions in changing environments. *Molecular Ecology* **23**: 1656–60.
- Dittami SM, Gravot A, Goulitquer S, et al. 2012.** Towards deciphering dynamic changes and evolutionary mechanisms involved in the adaptation to low salinities in *Ectocarpus* (brown algae). *Plant Journal* **71**: 366–377.
- Dittami SM, Heesch S, Olsen JL, Collén J. 2017.** Transitions between marine and freshwater environments provide new clues about the origins of multicellular plants and algae. *Journal of Phycology* **53**: 731–745.
- Dittami SM, Michel G, Collén J, Boyen C, Tonon T. 2010.** Chlorophyll-binding proteins revisited—a multigenic family of light-harvesting and stress proteins from a brown algal perspective. *BMC Evolutionary Biology* **10**: 365.
- Dittami SM, Peters AF, West JA, et al. 2020.** Revisiting Australian *Ectocarpus subulatus* (Phaeophyceae) From the Hopkins River: Distribution, Abiotic Environment, and Associated Microbiota (M Roleda, Ed.). *Journal of Phycology* **56**: 719–729.
- Dittami SM, Proux C, Rousvoal S, et al. 2011.** Microarray estimation of genomic inter-strain variability in the genus *Ectocarpus* (Phaeophyceae). *BMC Molecular Biology* **12**: 2.
- Dittami SM, Scornet D, Petit J-L, et al. 2009.** Global expression analysis of the brown alga *Ectocarpus siliculosus* (Phaeophyceae) reveals large-scale reprogramming of the transcriptome in response to abiotic stress. *Genome biology* **10**: R66.
- Fabris M, Matthijs M, Rombauts S, Vyverman W, Goossens A, Baart GJE. 2012.** The metabolic blueprint of *Phaeodactylum tricorutum* reveals a eukaryotic Entner-Doudoroff glycolytic pathway. *Plant Journal* **70**: 1004–14.
- Frioux C, Fremy E, Trottier C, Siegel A. 2018.** Scalable and exhaustive screening of metabolic functions carried out by microbial consortia. *Bioinformatics* **34**: i934–i943.
- Garbary DJ, London J. 1995.** The *Ascophyllum* / *Polysiphonia* / *Mycosphaerella* symbiosis. V. *Mycosphaerella* protects *A. nodosum* from desiccation. *Botanica Marina* **38**: 529–533.
- Gravot A, Dittami SM, Rousvoal S, et al. 2010.** Diurnal oscillations of metabolite abundances and gene analysis provide new insights into central metabolic processes of the brown alga *Ectocarpus siliculosus*. *New Phytologist* **188**: 98–110.
- Jannin L, Arkoun M, Etienne P, et al. 2013.** *Brassica napus* growth is promoted by *Ascophyllum nodosum* (L.) Le Jol. seaweed extract: microarray analysis and physiological characterization of N, C, and S metabolisms. *Journal of Plant Growth Regulation* **32**: 31–52.
- Karimi E, Geslain E, Belcour A, et al. 2021.** Robustness analysis of metabolic predictions in algal microbial communities based on different annotation pipelines. *PeerJ* **9**: e11344.
- Karimi E, Geslain E, KleinJan H, et al. 2020.** Genome sequences of 72 bacterial strains isolated from *Ectocarpus subulatus*: a resource for algal microbiology (H Ochman, Ed.). *Genome Biology and Evolution* **12**: 3647–3655.
- Kellenberger E. 2001.** Exploring the unknown. *EMBO reports* **2**: 5–7.

- Kelly E (Ed.). 2005.** *The role of kelp in the marine environment. Irish Wildlife Manuals, No. 17. National.* Dublin, Ireland.: Parks and Wildlife Service, Department of Environment, Heritage and Local Government,.
- KleinJan H, Caliafano G, Aite M, et al. 2021.** Changing microbial activities during low salinity acclimation in the brown alga *Ectocarpus subulatus*. *bioRxiv* **2021.04.13**.
- KleinJan H, Jeanthon C, Boyen C, Dittami SM. 2017.** Exploring the cultivable *Ectocarpus* microbiome. *Frontiers in Microbiology* **8**: 2456.
- Mann KH. 1973.** Seaweeds: Their Productivity and Strategy for Growth: The role of large marine algae in coastal productivity is far more important than has been suspected. *Science* **182**: 975–981.
- McKeown DA, Stevens K, Peters AF, et al. 2017.** Phaeoviruses discovered in kelp (Laminariales). *The ISME Journal* **11**: 2869–2873.
- Pedersén M. 1968.** *Ectocarpus fasciculatus*: marine brown alga requiring kinetin. *Nature* **218**: 776–776.
- Pedersén M. 1973.** Identification of a Cytokinin, purine, in sea water and the effect of cytokinins on brown algae. *Physiologia Plantarum* **28**: 101–105.
- Peters AF, Marie D, Scornet D, Kloareg B, Cock JM. 2004.** Proposal of *Ectocarpus siliculosus* (Ectocarpales, Phaeophyceae) as a model organism for brown algal genetics and genomics. *Journal of Phycology* **40**: 1079–1088.
- Prigent S, Collet G, Dittami SM, et al. 2014.** The genome-scale metabolic network of *Ectocarpus siliculosus* (EctoGEM): a resource to study brown algal physiology and beyond. *Plant Journal* **80**: 367–381.
- Rosenberg E, Sharon G, Atad I, Zilber-Rosenberg I. 2010.** The evolution of animals and plants via symbiosis with microorganisms. *Environmental Microbiology Reports* **2**: 500–506.
- Shao Y, Forster SC, Tsaliki E, et al. 2019.** Stunted microbiota and opportunistic pathogen colonization in caesarean-section birth. *Nature* **574**: 117–121.
- Shukla PS, Mantin EG, Adil M, Bajpai S, Critchley AT, Prithiviraj B. 2019.** *Ascophyllum nodosum*-based biostimulants: sustainable applications in agriculture for the stimulation of plant growth, stress tolerance, and disease management. *Frontiers in Plant Science* **10**.
- Siméon A, Kridi S, Kloareg B, Hervé C. 2020.** Presence of exogenous sulfate is mandatory for tip growth in the brown alga *Ectocarpus subulatus*. *Frontiers in Plant Science* **11**.
- Tapia JE, González B, Goulitquer S, Potin P, Correa JA. 2016.** Microbiota influences morphology and reproduction of the brown alga *Ectocarpus* sp. *Frontiers in Microbiology* **7**: 197.
- Tourneroc A, Lami R, Burgaud G, et al. 2020.** The bacterial and fungal microbiota of *Saccharina latissima* (Laminariales, Phaeophyceae). *Frontiers in Marine Science* **7**.
- Wahl M, Goecke F, Labes A, Dobretsov S, Weinberger F. 2012.** The second skin: ecological role of epibiotic biofilms on marine organisms. *Frontiers in Microbiology* **3**: 292.
- West J, Kraft G. 1996.** *Ectocarpus siliculosus* (Dillwyn) Lyngb. from Hopkins River Falls, Victoria - the first record of a freshwater brown alga in Australia. *Muelleria* **9**: 29–33.
- Zhou J, Lin Z, Cai Z, Zeng Y, Zhu J, Du X. 2020.** Opportunistic bacteria use quorum sensing to disturb coral symbiotic communities and mediate the occurrence of coral bleaching. *Environmental Microbiology* **22**: 1944–1962.
- Zilber-Rosenberg I, Rosenberg E. 2008.** Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution. *FEMS Microbiology Reviews* **32**: 723–35.